Cape Peninsula University of Technology

Sand based system for physiochemical and biological treatment of winery wastewater

by

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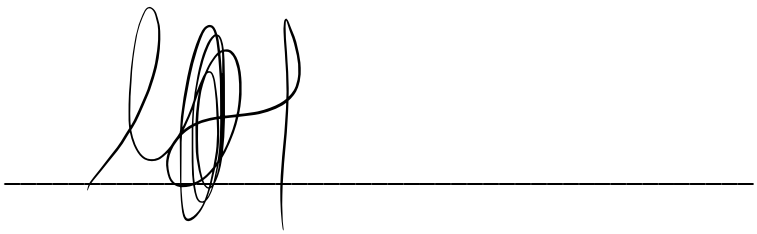
Declaration

I declare that this research dissertation is my own unaided work. It is being submitted for the Doctor of Engineering Degree at Cape Peninsula University of Technology, Cape Town. It has not been submitted before for any degree or examination in any other University.



Signed in Durbanville this 10 day of October 2023

Outputs

The following outputs reflect the contributions by the candidate to scientific literacy and progress during his doctoral candidacy.

The following research articles accredited by the Department of Higher Education and Training (DHET) and conference proceedings were published from the studies reported in this thesis:

Holtman, G.A., Haldenwang, R., Welz, P.J., 2023. Biosand reactors for remediation of winery effluent in support of a circular economy and the positive effect of sand fractionation on hydraulic and operational performance. Journal of Water Process Engineering. 53, 103849 https://doi.org/10.1016/j.jwpe.2023.103849

Holtman, G.A., Haldenwang, R., Welz, P.J., 2022. calcite dissolution and bioneutralization of acidic wastewater in biosand reactors. Water 14, 3482. https://doi.org/10.3390/w14213482

Holtman, G.A., Haldenwang, R., Welz, P.J., 2022. Comparison of continuous and pulse mode of operation of pilot biosand reactors treating winery effluent. Ecological Engineering. 182, 106706. https://doi.org/10.1016/j.ecoleng.2022.106706

Holtman, G.A., Haldenwang, R., Welz, P.J., 2022. Effect of particle character and calcite dissolution on the hydraulic conductivity and longevity of biosand filters treating winery and other acidic effluents. Water 14, 2603. https://doi.org/10.3390/w14172603

Kaira, W.M., Kimpiab, E., Mpofu, A.B., Holtman, G.A., Ranjan, A., Welz, P.J., 2022. Anaerobic digestion of primary winery wastewater sludge and evaluation of the character of the digestate as a potential fertilizer. Biomass Convers. Biorefinery. https://doi.org/10.1007/s13399-022-03087-8

Mader, A.E., Holtman, G.A., Welz, P.J., 2022. Treatment wetlands and phyto-technologies for remediation of winery effluent: Challenges and opportunities. Science of The Total Environment. 807, 150544. https://doi.org/10.1016/j.scitotenv.2021.150544

Offprints are available in Appendices

The following international conference presentations related to this thesis were delivered:

Holtman, G., Haldenwang, R., Welz P.J. Treatment of winery wastewater using a biological sand filtration system. (Oral presentation at WISA biennial conference and exercitation 2018, Cape Town, South Africa, 2018) [National Conference]

Holtman, G., Haldenwang, R., Welz P.J. Treatment of winery wastewater using a biological sand filtration system. (Oral Presentation at IWA World Water Conference and Exercitation 2018, Tokyo, Japan, 2018) [International Conference]

Holtman, G., Haldenwang, R., Welz P.J. Treatment of winery wastewater using a biological sand filtration system. (Oral Presentation at SASEV-WINETECH 41st International Conference, Somerset West, South Africa, 2018) [International Conference]

The following DHET-accredited research articles, book chapter and conference proceedings were published from previous studies related to this thesis:

Welz PJ, Mbasha W, Smith I, Holtman G, Terblanche G, Le Roes-Hill M, et al. The influence of grain physicochemistry and biomass on hydraulic conductivity in sand-filled treatment wetlands. Ecological Engineering. 2018;116(February):21–30. available from, https://doi.org/10.1016/j.ecoleng.2018.02.017

Holtman GA, Haldenwang R, Welz PJ. Biological sand filter system treating winery effluent for effective reduction in organic load and pH neutralisation. Journal of Water Process Engineering. 2018;25(July):118–27. available from, https://doi.org/10.1016/j.jwpe.2018.07.008

Welz PJ, Holtman G, Haldenwang R, Le Roes-Hill M, Roes-hill M. Characterisation of winery wastewater from continuous flow settling basins and waste stabilisation ponds over the course of 1 year: Implications for biological wastewater treatment and land application. Water Science & Technology. 2016;74(9):2036–50. available from, https://doi.org/10.2166/wst.2016.226

Abstract

In South Africa, many wineries use cellar effluent for irrigation after primary settling and pH adjustment. This poses an environmental risk as winery effluent can contain high concentrations of organic and inorganic pollutants. Most vine growing countries, including South Africa, are water-stressed, so it is important that winery effluent is remediated to the extent that it is safe for reuse as irrigation water. In order to align the South African wine industry with global views to achieve sustainable development, there is a need for low maintenance, low cost and effective solutions for treating winery wastewater which can be easily integrated into the existing infrastructure. This body of work presents the results of a number of fundamental and applied studies used to determine the validity of biosand reactors as a potential solution to fulfil this winery wastewater treatment function in small to medium sized cellars.

In the first study, chapter 3, a pilot biosand reactor with a nodal design was installed, operated and monitored for 2 years at a medium sized winery (crushing 1600 tons of grapes per annum) in the Stellenbosch area, Western Cape. Two reactors were operated in alternating continuous and pulse modes, with COD removal efficiencies of 70% achieved in each mode. Higher hydraulic and organic loading rates were achieved in continuous mode (113 $L.m^{-3}sand.day^{-1}$ and 279 $gCOD.m^{-3}sand.day^{-1}$, respectively) than in pulse mode (90 $L.m^{-3}sand.day^{-1}$ and 192 $gCOD.m^{-3}sand.day^{-1}$). In comparison to other passive systems treating winery effluent, the biosand reactor system achieved significantly higher loading rates with a smaller spatial footprint.

This applied study was followed by three fundamental studies to elucidate the biodegradation, neutralization and hydraulic mechanisms involved in remediating winery wastewater in biosand reactors. These studies were conducted using a series of columns containing raw sand and various sized fractions of sand from the Philippi quarry site in Cape Town.

Biosand reactors have been shown to effectively neutralize winery wastewater, and it was assumed that the major neutralization was via dissolution of calcite sand particles. The first flow-through column experiment was conducted to determine whether there may be a biotic contribution to pH adjustment. Sand cores containing functional microbial communities were extracted from an operational biosand reactor system. One half of the cores were sterilized by irradiation and used to determine the abiotic contribution, while the biotic contribution was

calculated by deducting the results obtained from the irradiated columns from those obtained from the non-irradiated columns. The cores were dosed with synthetic winery wastewater and hydrochloric acid at the same pH. Using the hydroxide ion concentration in the effluent as a proxy, it was found that the major neutralization mechanism was abiotic (average 95.5±0.16%) due to the dissolution of calcite, with a small biotic contribution (average 4.5±0.13%).

The second series of column experiments was conducted in order investigate calcite dissolution kinetics and the effect of calcite dissolution on the hydraulic conductivity of the Philippi sand. The results were used in conjunction with those obtained from an operational pilot biosand reactor system to determine the temporal abiotic neutralization capacity of biosand reactors. Flow-through experiments using hydrochloric acid were conducted using columns containing sand with a variety of particles sizes. The larger particles (>0.425 mm) contained lower amounts of calcite (using Ca as a proxy), but exhibited higher hydraulic conductivities before and after calcite dissolution (3.0 ± 0.05 %Ca and 2.57 to 2.75 $mm \cdot s^{-1}$, respectively) compared to the fractions containing smaller particles and/or raw sand (4.8 ± 0.04 to 6.8 ± 0.03 %Ca and 0.19 to 1.25 $mm \cdot s^{-1}$, respectively). By measuring the amount of Ca removed from the sand as a proxy for calcite dissolution, a temporal abiotic neutralization capacity of 37 years was calculated for biological sand reactor systems containing Philippi sand with 5.4 % wt.wt Ca and an influent with a pH ranging from 2 to 3 and a hydraulic loading rate of 150 $L \cdot m^{-3}$ of $sand.d^{-1}$.

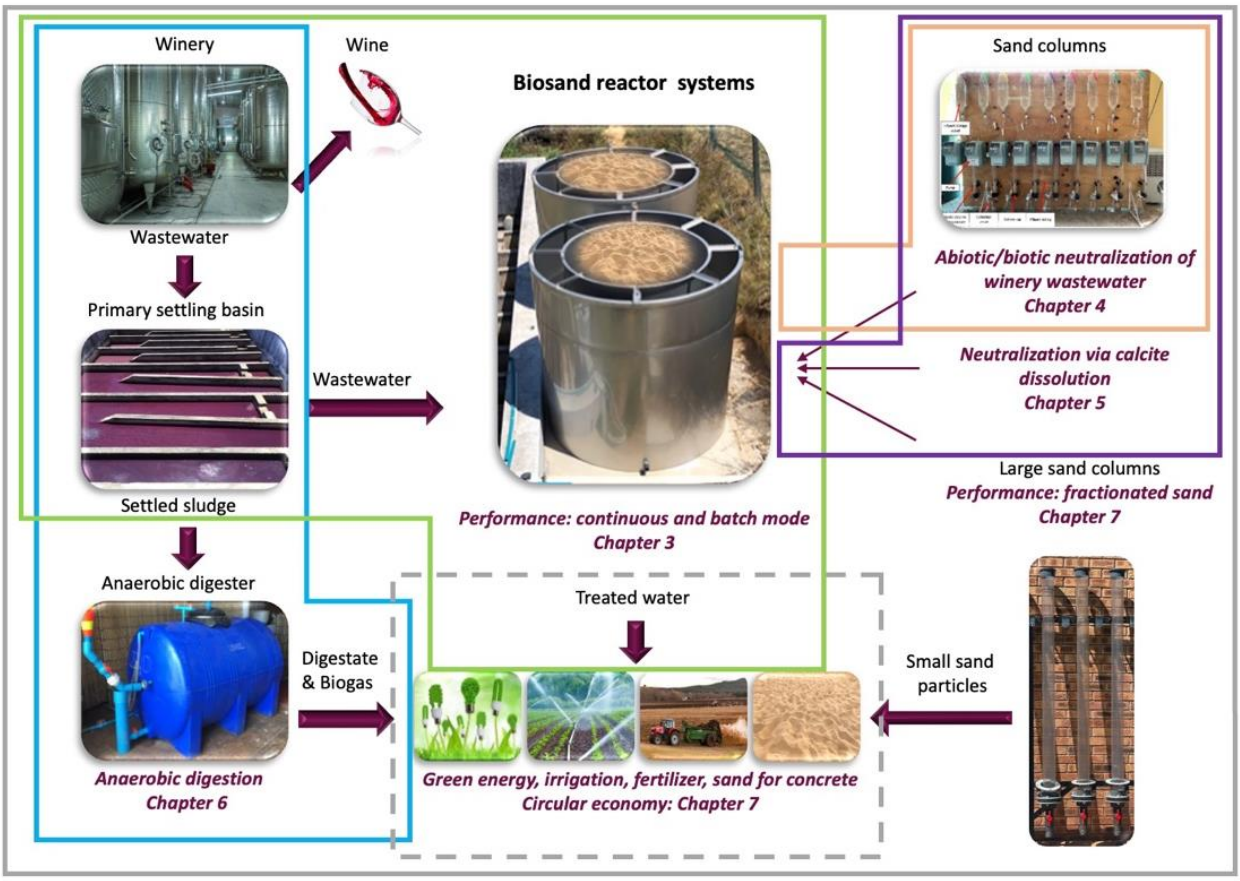
Based on the promising results obtained with the larger sand particles in the second series of columns experiments, a third set of column experiments was used to compare the raw (unfractionated) sand to fractionated sand (<0.425 mm removed) in terms of operational performance. The flow in biosand reactors slows down after start-up due to the growth of functional biomass within the sand pores that decreases the hydraulic conductivity. In this study, initial hydraulic conductivities of 0.285 $mm.s^{-1}$ and 2.504 $mm.s^{-1}$, were measured in the columns containing raw and fractionated sand, respectively. After operating the columns by feeding with winery effluent for 3 months, the hydraulic conductivities reduced to 0.129 $mm.s^{-1}$ and 1.116 mm $mm.s^{-1}$, respectively. Similar organic removal efficiencies were obtained with raw (94%) and fractionated (95%) sand, and both sands were able to effectively neutralize the acidic (pH 4.9) winery wastewater. It was calculated that one 5.6 m^3 biosand reactor containing the fractionated sand can potentially treat 8102 $L.d^{-1}$ of winery wastewater, which surpasses the design flow rate of raw sand of 1000 $L.d^{-1}$.

During the applied pilot study, it was noted that copious amounts of primary wastewater sludge is generated at the study site. Currently, the winery contacts a commercial company to remove this waste to landfill which is an economic and environmental burden. Biochemical methane potential tests were used to determine the potential for valorisation of this organic-rich waste-stream via anaerobic digestion. The highest specific methane yield of sludge harvested during the crush season (206 ± 2.7 $mLCH_4/gVS_{added}$) was obtained under mesophilic (37°C) conditions. The composition of the digestate compared favorably with commercial organic agricultural fertilizers.

Finally, building on the results obtained, a zero-waste model is presented for treatment of winery effluent based on the use of biosand filters. This includes: (i) using fractionated sand in the filters and the sifted smaller sand particles in the concrete industry, (ii) remediating the winery wastewater in biosand filters, and using the treated effluent for safe irrigation, (iii) valorizing the primary wastewater sludge via anaerobic digestion and using the digestate as an agricultural fertilizer.

Preface

This thesis is submitted via publication and each chapter delineates inter-related bodies of work pertaining to this thesis, the chapters and outline of the thesis is explained in Chapter 1 Section 1.9 entitled “Thesis outline”. The Graphic below shows the flow of the thesis and how the different chapters are connected and will be repeated at the start of each chapter to refresh the reader.



Ledger

Chapter 3 – Paper 2

Chapter 4 – Paper 3

Chapter 5 – Paper 4

Chapter 6 – Paper 5

Chapter 7 – Paper 6

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While the process of compiling this body of work has been tedious at times I have learnt a great deal about myself and others. The highs and lows of this body of work has been an intangible experience with great personal growth and I would suggest to anyone to study further and challenge themselves both intellectually, mentally and all the other …llys.

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Abbreviations and Acronyms

Abbreviation	Description
AD	Anaerobic digestion
AFFR	Anaerobic fixed film reactor
AMD	Acid mine drainage
B	Bottom
BMP	Biochemical methane potential
BOD	Biological oxygen demand
BOD_5	Five-day biological oxygen demand
BSF	Biological sand filter
BSR	Bio-sand reactor
BV	Ball valve
C	Crush season
CAS	Continuous activated sludge
CHNS	Carbon, hydrogen, nitrogen and sulfur
COD	Chemical oxygen demand
Cont.	Control
CW	Constructed wetland
CWs	Constructed wetlands
D	Design
DWW	domestic wastewater
EC	Electronic conductivity
E_h	Redox potential
EPS	Extracellular polymeric substances
Eq	Equation
ET	Evapotranspiration
FSA	Functional surface area
FV	Functional Volume
FWS	Free water surface
GAE	Gallic acid equivalent
GDP	Gross domestic product
GFM	Glucose + fructose + maltose
HC	Hydraulic conductivity
HF	Horizontal (subsurface) flow
HLR	Hydraulic loading rate
HLR_{FSA}	Hydraulic loading rate, Functional surface area
HLR_{VLF}	Hydraulic loading rate, Volume of liquid portion
HLR_{Vol}	Hydraulic loading rate, Volumetric
HPLC	High pressure liquid chromatography
HRT	Hydraulic retention time
HTC	Hydraulic treatment capacity
HUSB	Hydraulic upflow sludge blanket reactor
IC	Inhibitory concentration
IPW	Integrated production of wine scheme
IR	Irradiated
ISR	Inoculum to substrate
M	Middle
MFR	Measured flow rate

N	Number
NA	Not applicable
NC	Non-crush season
ND	Not determined
NG	Not given
OC	Organic carbon
OLR	Organic loading rate
OLR_{FSA}	Organic loading rate, Functional surface area
OLR_{VLF}	Organic loading rate, Volume of liquid portion
OLR_{Vol}	Organic loading rate, Volumetric
ORR	Organic removal rates
ORR_{VLF}	Organic removal rate, Volume of liquid portion
ORR_{Vol}	Organic removal rate, Volumetric
PCA	Principal component analyses
pH	Potential of hydrogen
PLC	Programmable logic controller
PRB	Permeable reactive barrier
PS	Primary settling
PVC	Polyvinyl chloride
PWWS	Primary winery wastewater sludge
QEMSCAN®	Quantitive Evaluation of Minerals by SCANning Electron Microscopy
Ref	Reference
ROS	Reactive oxygen species
RSA	Root system architecture
RT	Retention time
S	Siemens
SAR	Sodium adsorption ratio
SCC	Self-compacting concrete
SD	Standard deviation
SLR	Surface loading rate
SMPs	Soluble microbial products
spAlk	Specific Alkalinity
spCa	Specific Ca
spCOD	Average specific chemical oxygen demand
spEC	Specific electronic conductivity
$spOH^-$	specific OH^- concentrations
ST	Settling tank
SWW	Synthetic winery wastewater
SWW cont.	Control with new, unused sand
SWWS	Secondary winery wastewater sludge
T	Top
T	Holding tank
TANC	Temporal abiotic neutralization capacity
TEA	Techno-economic analysis
TKN	Total kjeldahl nitrogen
TN	Total nitrogen
TOC	Total organic carbon
TP	Total phosphorous
TPhen.	Total phenolics
TS	Total solids

TS	Trickling filter
TSS	Total suspended solids
TVS	Total volatile solids
TW	Treatment wetland
TWs	Treatment wetlands
UASB	Upflow anaerobic sludge blanket reactor
UTC	Unable to calculate
V	Solenoid valve
VF	Vertical (subsurface) flow
VFA	Volatile fatty acids
VLP	Volume of liquid portion
VOA	Volatile organic acid
VS	Volatile solids
VSS	Volatile suspended solids
VUF	Vertical up-flow
WAS	Waste activated sludge
WT	Weight
WW	Wastewater/ Winery wastewater
WWW	Winery wastewater
XRF	X-ray fluorescence

Nomenclature

Symbol	Description	Unit
A	Area	m^2
h_1	Initial start head	m
h_2	Ending head	m
hr	Hour	h
Hrs	Hours	H
k	Permeability	
K	Hydraulic conductivity	$mm.s^{-1}$
l	Length	m
L	Liters	L
Q	Volumetric flow rate	$m^3.hr$
t	Time	t
V	Volume	m^3

Chemical formula

Symbol	Description
Al	Aluminum
Al^{3+}	Aluminum ion
As	Arsenic
Ba	Boron
C	Carbon
C_2H_5OH	Ethanol
Ca	Calcium
Ca^{2+}	Calcium ion
$CaCO_3$	Calcium carbonate
$CaSO_4$	Gypsum
Cd^{2+}	Cadmium
CH_3COO^-	Acetate
CH_3Hg	methylmercury
CH_4	Methane
Cl^-	Chloride ion
Co	Cobalt
CO_2	Carbon dioxide
Co^{2+}	Cobalt
Cr	Chromium
Cr^{2+}	Chromium ion
Cu^{2+}	Copper
dH_2O	distilled water
Fe	Iron
Fe^{2+}	Ferrous
Fe^{3+}	Ferric ion
H^+	Hydrogen ion
H_2CO_3	Carbonic acid
H_2O	Dihydrogen oxide (Water)
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulfuric acid
H_3PO_4	Phosphoric acid
HCl	Hydrochloric acid
HCO^-	bicarbonate
HCO_3^-	Bicarbonate
Hg	Mercury
HS^-	Bisulfide
K	Potassium
K^+	Potassium ion
KOH	Potassium hydroxide
Mg	Magnesium
Mg^{2+}	Magnesium ion
Mn	Manganese
Mo	Molybdenum
N	Nitrogen
N_2	Nitrogen gas
Na^+	Sodium

Na_2CO_3	Sodium carbonate
Na_2SiO_3	Sodium metasilicate
Na_3PO4	Trisodium phosphate
NaCl	Sodium cloride
NaOH	Sodium hydroxide or caustic soda
NH_3	Ammonia
NH_4^+	Ammonium
NH_4^+-N	Ammonia as nitrogen
Ni	Nickel
Ni^{2+}	Nickel
NO_3^-	nitrate
O_2	Oxygen
P	Phosphorus
Pb	Lead
Pb^{2+}	Lead
PO_4^{3-}	Phosphate
S	Sulfur
Se	Selenium
Si	Silica
Sn	Tin
SO_3^{2-}	Sulfite
SO_4^{2-}	Sulphate
Sr	Strontium
Ti	Titanium
Zn^{2+}	Zinc

Glossary

Abiotic	Non-biological in nature
Anaerobic	In the absence of oxygen
Berries	Berries are referred to the grapes used for wine making
bio-hydrogeochemical	
Biogas	The gaseous mixture continuing methane, carbon dioxide and hydrogen sulfide which is formed from the bacterial decomposition of organic matter
Biotic	Biological in nature (living)
Cellar	Premises at winery where berries are crushed, fermented, matured and usually bottled
Crush	The crush is generally a 3-month period in which harvested berries are crushed to produce grape juice which is then processed
Digestate	The residue from anaerobic digestion of a substrate
Halophyte	A salt-tolerant plant that is capable of growing and thriving in salty or high salinity waters
Macrophytes	Plants that live in aquatic environments, either emerged, submerged or floating in the water.
Phytoremediation	The use of plants to remove contaminants
Salinity	This is the saltness or amount of salt in a body of water or substrate
Sludge	A semi-solid slurry which generally contains organic matter which is collected by gravitational selling
Treatment wetland	A treatment wetland or constructed wetland is a manmade wetland which mimics and takes advantage of the natural process found in wetlands in order to remove pollutants.

Chapter 1

Introduction

[Provides a brief introduction of the research, background, aims and objectives and rationale for this doctoral work.]

1.1 Introduction and background

In addition to the pertinent literature provided in each chapter, a short introduction to the subject is provided here.

In the process of wine making, the raw product (berries), are crushed and converted to wine which results in the generation of by-products and wastes. Within the context of South Africa, there are 536 cellars and 2 613 primary wine producers, harvesting 1.46 million tonnes of grapes a year which are disseminated throughout the country (South African Wine Industry statistics, 2021). These cellars produce approximately 1.13 billion litres of wine-related products of which 824 million litres are wine (South African Wine Industry statistics, 2021). There are several waste streams generated at wineries and in the context of South Africa winery wastewater (WWW) is classified as a "biodegradable industrial wastewater" and is regulated by the (Department of Water Affairs, 2013, 1998). At smaller wineries, this WWW is generally settled, pH adjusted and irrigated to pastures which can cause an negative environmental impact on the receiving environment (Hirzel et al., 2017; Kaira et al., 2022; Ngwenya et al., 2022).

As there are many different processes involved in winemaking and different practices from cellar to cellar, the quantities and compositions of WWW are highly variable (Contreras et al., 2022; Perra et al., 2021; Welz et al., 2016). In addition, WWW is generated at different times of the year with the greatest quantity occurring during the 3-month crush period (Contreras et al., 2022; Perra et al., 2021; Welz et al., 2016). During the non-crush period, flows may fluctuate between no flows to high flows, for example, during cleaning operations (Conradie et al., 2014; Mosse et al., 2012). During the crush period, the WWW is readily biodegradable due to the high organic loading with sugars from the grapes (Calheiros et al., 2018). Although organics such as alcohols and volatile organic acids (VOA) are often present in WWW in non-crush periods, high concentrations of inorganics from cleaning products are also often present (Conradie et al., 2014). Conventional biological treatment systems are often unable to adapt to the rapid changes in the quality and quantity of WWW which can lead to poor treatment performance, especially during periods of heavy loading (Devesa-Rey et al., 2011; Giacobbo et al., 2017; Mosse et al., 2012).

This is more prevalent in small to medium sized wineries as large co-operatives can usually afford to install and operate advanced wastewater remediation systems (Ioannou et al., 2015). In

contrast, small to medium sized wineries do not have fiscal liberties or WWW flow rates for these types of treatment systems (Holtman et al., 2022; Ioannou et al., 2015; Mader et al., 2022). Smaller wineries generally use settling ponds or oxidation ditches to reduce the settleable solids. In some cases, treatment wetlands/constructed wetlands (TWs/CWs) are then used to improve the quality of the WWW as they have minimal energy requirements, are low maintenance and are relatively economical to operate (Johnson and Mehrvar, 2020; Sánchez et al., 2021). However, one of the drawbacks of using CWs for remediation of WWW is that the (poly)phenolics in WWW may be phytotoxic, and plants are most vulnerable during the crush season (Howell et al., 2018; Masi et al., 2015; Sirohi et al., 2020). During this period, cellars are extremely busy, which makes maintenance and replanting difficult.

Biosand reactors (BSR) are similar to an unplanted sand filled CWs, as they provide a low cost, low maintenance, sustainable and energy efficient system for treating WWW.

1.2 Research problem statement

Currently, within the South African context, 71% of wineries crush less than 500 tonnes of grapes a year and mostly dispose of their cellar effluent via 'beneficial' irrigation after primary settling and pH adjustment (Hirzel et al., 2017; Kaira et al., 2022; Ngwenya et al., 2022; South African Wine Industry statistics, 2021). This poses an environmental risk because of the high organic load, potential phytotoxicity and microbial toxicity of the (poly)phenolics, low pH and high SAR of WWW. Although there are several treatment options available for WWW, there is no reliable, low maintenance and cost-effective treatment system available in South Africa. In addition to WWW, primary wastewater sludge (PWWS) is generated from settling of WWW, and generally disposed of via landfill which ignores the potential for valorization of this organic-rich waste-stream.

1.3 Research rationale and novelty

This body of work focuses on passive treatment of WWW using a vertical flow BSR system with novel design features that allow hydraulic flexibility. This is the first time that such a system has been tested for treatment of WWW. While continuous operation theoretically allows higher hydraulic capacity, studies using other passive treatment systems (CWs) have shown that biodegradation may be increased when employing pulse mode of operation as oxygen is drawn

into the substratum during periods of drainage. The performance of two BSR systems operated in alternating continuous and pulse modes of operation were therefore compared.

In addition to the applied study, three series of flow-through column experiments were conducted to ascertain the effects of sand particle size, sand particle mineralogy and functional biomass accumulation on the hydraulic conductivity, biotic and abiotic neutralization and performance of BSRs containing Phillipi sand and treating WWW. These studies added significantly to the body of knowledge about BSR operation and performance, and the results were applied to assist with design criteria for full-scale systems.

1.4 Hypothesis

The overall hypothesis of the applied study was that BSRs may reduce the organic load, reduce the SAR and neutralize acidic winery effluent, rendering it compliant with the Department of Water and Sanitation General guidelines for disposal of biodegradable industrial wastewater. The hypotheses for each fundamental study are provided in the relevant chapters.

1.5 Significance of research

This doctoral work was conducted to fill the knowledge gaps surrounding core functional mechanisms, longevity and real life applications of BSR systems treating WWW and (potentially) other acidic effluents. The results were used to provide a blueprint for future low cost, low maintenance and effective treatment systems that overcome shortfalls of existing systems for treatment of WWW. Based on BSRs containing fractionated sand for remediation of WWW and AD of PWWS, a zero-waste model is presented for valorization of WWW within the context of a circular economy.

1.6 Research aims and objectives

This overall aim of this doctoral work was to advance BSR technology for small to medium sized wineries within South Africa and abroad in order to minimize the potential environmental impact of irrigation with WWW.

1.6.1 Research objectives

1. To review the potential opportunities and challenges of treatment wetlands and phyto-technologies for the treatment of WWW

2. To design, install and operate a pilot BSR plant containing locally available sand with adjustable operational parameters at a medium-sized winery in the Western Cape, South Africa.
3. To monitor the physicochemical parameters of the influent and effluent of the pilot scale BSR system to determine treatment performance and compliance with legislation and validate against previous studies and other passive treatment systems.
4. To compare the effects of different modes of operation (continuous and pulse fed), on the treatment performance, and determine guidelines for a full-scale treatment plant.
5. To compare the relationship between biotic and abiotic neutralization mechanisms in BSRs using a series of irradiated/non-irradiated sand column experiments.
6. To model and determine the longevity of abiotic neutralization of WWW or other acidic WW in BSRs in terms of calcite dissolution kinetics using a series of sand column experiments.
7. To determine the link between size grading, available calcite and HC in different fractions of sand before and after calcite dissolution.
8. To determine the effect of functional biomass on the HC of fractionated (>0.425 mm grains only) and unfractionated (raw) sand using a series of column experiments.
9. To compare the organic removal efficiency and HC of fractionated and raw sand using a series of column experiments.
10. To determine the potential for anaerobic digestion of PWWS and to characterize the digestate in terms of suitability as an agricultural fertilizer.

1.7 Research questions

1. What are the challenges and opportunities for treating WWW using CWs and phyto-remediation?
2. Is there a suitable pilot BSR design which has adjustable operational parameters and uses local sand?
3. Is a BSR a feasible treatment system for the treatment of WWW?
4. Is a vertical flow BSR system operating in continuous or pulse fed mode a viable solution for the treatment of WWW in terms of performance, maintenance, operation and sustainability?
5. What is the relationship between abiotic and biotic neutralization of WWW in BSR?

6. What is the longevity of the sand in BSR in terms of abiotic pH neutralization?
7. What is the effect of grain size of the HC of sand before and after calcite dissolution?
8. What effect does biomass accumulation have on HC of raw and fractionated sand?
9. Is a larger fraction of sand comparable in terms of treatment performance to raw sand?
10. Is there a potential for the anaerobic digestion of WWW sludge and is it feasible for digestate to be used as an agricultural fertiliser?

1.8 Delineation

The study of the onsite treatment system has the following limiting factors (i) to run at a constant flow rate which was expected to gradually reduce due to the build-up of functional biomass (ii) to treat only a portion of the WWW from a medium size cellar, and (iii) to recirculate effluent back into the head of an existing solids settling basin. The system was not designed to remove sodium and potassium and the effects of the effluent on the environment were not monitored.

The laboratory and desktop studies were limited to the investigation of fundamental operational principles. Further testing and investigation of the effect on the greater environment was not investigated.

The literature of each chapter was focused on the specific topic of the chapter and providing in-site to the chapter, in chapter 2 the literature review of the thesis focus on passive treatment systems used to treat winery wastewater and not specifically BSR due to the gap in the knowledge base.

1.9 Thesis outline

This thesis is presented as six published inter-related manuscripts that each form separate chapters of the thesis (Chapters 2-7). The first manuscript, presented in Chapter 2, is a literature review, while the rest are research manuscripts (Chapters 3-7). The literature background, conclusions and recommendations for each study are included in the manuscripts/Chapters, and a summation of the work is presented in the final publication (Chapter 7). Additional separate literature review and conclusion chapters are therefore not included in the thesis.

Chapter 1 provides a brief introduction of the research, background, aims and objectives and rationale for this doctoral work.

Chapter 2 provides a literature review of WWW and passive WWW treatment systems, including phytoremediation systems.

Chapter 3 presents the findings of a study comparing the performance of a novel pilot BSR system operated in continuous and pulse mode and compares the results with other passive treatment systems in terms of loading rates and spatial footprints. The novel design allowed for drastic manipulation of potential head across the BSR and an outlet design mitigating the risks of clogging at the outlet.

Chapter 4 presents the findings of a study to determine the contribution of biotic (microbial) and abiotic (calcite dissolution) mechanisms on neutralization of WWW in BSRs.

Chapter 5 presents the findings of an investigation to determine the role that particle size plays on the hydraulic performance and longevity in terms of calcite dissolution in BSRs.

Chapter 6 presents the findings of a study to determine the BMP of PWWS, and to assess the character of the digestate for potential as an agricultural fertilizer.

Chapter 7 presents the findings of a study comparing the hydraulic and organic removal performance of raw sand and fractionated sand (>0.425 mm grains). Furthermore, a synopsis of the doctoral work is provided in the form of a zero-waste model for the remediation of winery wastewater based on the use of BSRs.

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Chapter 2

Literature review - Treatment wetlands and phyto-technologies for remediation of winery effluent: Challenges and opportunities

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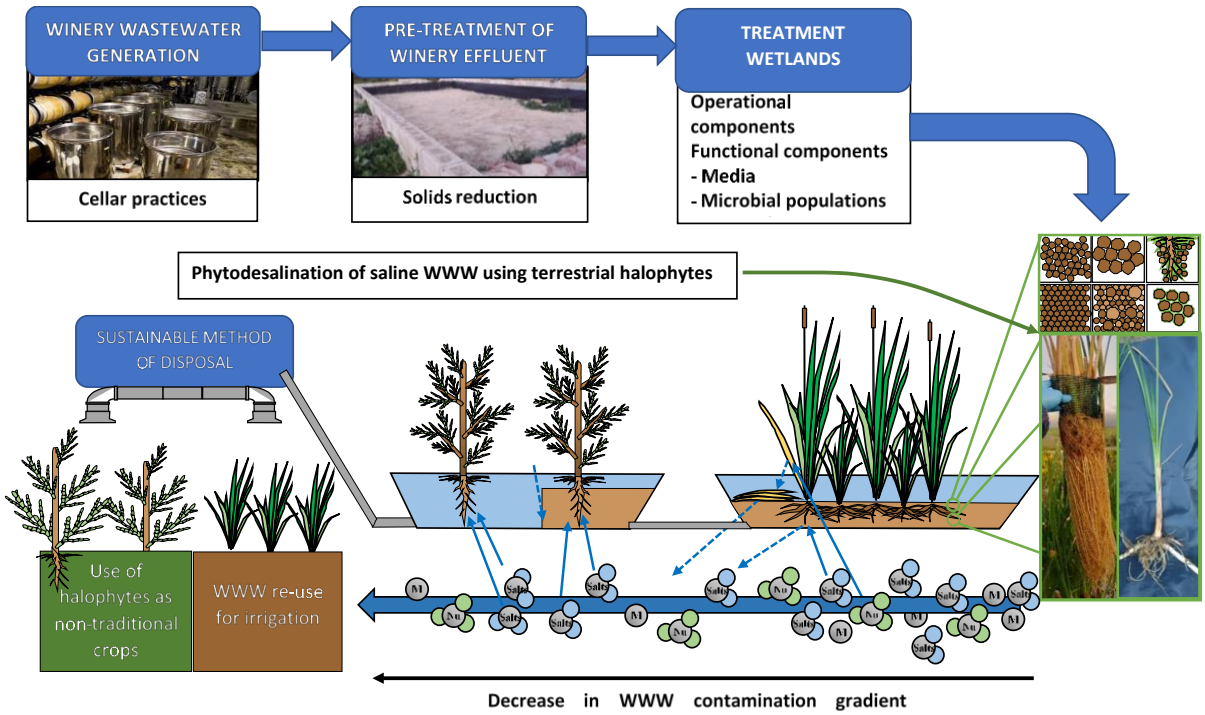
Offprint available in Appendix 1

[Provides a literature review of WWW and passive WWW treatment systems, including phytoremediation systems.]

Graphical Abstract



Highlights

- Review of WWW remediation by TWs relative to bio-hydrogeochemical components.
- Salinity impacts inorganic remediation by TWs and wastewater reuse.
- Review presents case for novel application of terrestrial halophytes.
- Halophytes are viable alternatives to macrophytes for saline wastewater treatment.
- Review describes solutions to challenges impacting sustainability of wine industry.

Abstract

The composition and concentration of contaminants present in winery wastewater fluctuate through space and time, presenting a challenge for traditional remediation methods. Bio-hydrogeochemical engineered systems, such as treatment wetlands, have been demonstrated to effectively reduce contaminant loads prior to disposal or reuse of the effluent. This review identifies and details the status quo and challenges associated with (i) the characteristics of winery wastewater, and the (ii) functional components, (iii) operational parameters, and (iv) performance of treatment wetlands for remediation of winery effluent. Potential solutions to challenges associated with these aspects are presented, based on the latest literature. A particular emphasis has been placed on the phytoremediation of winery

wastewater, and the rationale for selection of plant species for niche bioremediatory roles. This is attributed to previously reported low-to-negative removal percentages of persistent contaminants, such as salts and heavy metals that may be present in winery wastewater. A case for the inclusion of selected terrestrial halophytes in treatment wetlands and in areas irrigated using winery effluent is discussed. These are plant species that have an elevated ability to accumulate, cross-tolerate and potentially remove a range of persistent contaminants from winery effluent via various phytotechnologies (e.g., phytodesalination).

Keywords

Biological sand filter, halophyte, phytoremediation, treatment wetland, winery wastewater

2.1 Introduction

The wine industry contributes significantly to the global economy, with a steady increase in the trade value of this product of 25.8 to 31.4 billion € between 2014 and 2018, and a production volume ranging between 2.49 and 2.92 $x10^{10}$ L over the same period [1]. Concurrent with wine-making, the industry generates large amounts of potentially hazardous solid and liquid 'wastes' from various cellar activities [2, 3]. In the move towards a circular economy, a biorefinery approach has been adopted in many countries for the valorization of grape pomace and other forms of solid residues [4, 5]. However, apart from the beneficial use of winery wastewater (WWW) of adequate quality for crop irrigation (i.e. beneficial irrigation), it is still largely viewed as a form of waste rather than a resource [4]. Consumer pressure is driving wineries to adopt best practice measures in cellars, including those that simultaneously reduce the quantity and mitigate the toxicity of WWW [6]. Cellars can reduce potable water usage and the amount of solids in the effluent by introducing best available technologies and cleaner production methods (e.g. re-using treated effluent for basic cleaning activities and installing filters to retain solids) [2, 7]. Despite these efforts, WWW generally requires some form of primary physicochemical and/or secondary treatment in order to improve the quality to comply with legislated standards before discharge.

The effluent produced by wineries contains a range of organic and inorganic constituents, with considerable inter- and intra-site qualitative and quantitative variability [2, 7, 8]. Although the volume of WWW differs for each winery, studies conducted between 2003 and

2016 provide a rough estimate of 2.86 L (average) of effluent generated from each L of wine produced [2, 9, 10], translating to a global total of approximately 7.5 x 10^{10} L per annum.

The extent and type of treatment that is required depends on the means of discharge (i.e. directly to sensitive environments, subsurface discharge, beneficial irrigation, or discharge to municipal sewage systems) [2, 4, 11]. Multiple factors need to be considered when opting for any particular WWW treatment system. These include the (i) volume and site-specific character of the effluent that is generated, (ii) availability and value of land, (iii) size of the winery, (iv) availability of skilled operators, and (iv) capital and operational costs [2, 8].

For smaller wineries that have sufficient land available, but do not have the skills, time, or capital to install and operate more sophisticated systems, treatment wetlands (TWs) are a viable option. Data is available for systems that have been piloted or operated at full scale for WWW remediation in a number of countries, including Greece [12], Portugal [13], Spain [14, 15], Italy [16], France [17], the USA [18-20], Mexico [21], South Africa [4, 22], and Canada [11, 23].

This review identifies the status quo and challenges associated with the (i) characteristics of WWW, (ii) functional components of TWs, (iii) operational parameters of TWs, and (iv) TW performance. Furthermore, opportunities for overcoming these challenges are discussed. A particular emphasis is placed on phytoremediation of WWW, and the rationale for selection of plant species for niche bioremediatory roles.

2.2 Winery wastewater

Winery wastewater is generated from wine-making processes, including pressing, fermenting, clarification, storage, and bottling [2, 10, 24]. In terms of quality, many studies report on basic WWW characteristics as an adjunct to the primary aim/s of the research, such as wastewater treatment system performance. Such data may be skewed as criteria are typically limited to once-off batches and/or one site. Results from four comprehensive seasonal WWW characterization studies from multiple wineries in wine-making areas in Spain, France, Portugal, the USA and South Africa are summarized in Table 2-1. The variability in organic concentration in WWW is reflected in the chemical oxygen demand (COD) measurements, with inter-study averages of around 3500 mg L^{-1} to 7700 mg L^{-1} both in and

out of the crush (vintage) periods, but with minimum and maximum values ranging from < 100 mg L^{-1} to > 70 000 mg L^{-1} being reported (Table 2-1).

The composition and concentrations of organics, which may include alcohols, organic acids, sugars and phenolics, are dependent on the seasonal wine-making processes: for example, ethanol constitutes the highest fraction in the non-crush period, while significant concentrations of grape sugars are generally only produced during periods of crushing [7, 25]. In terms of amenability to secondary remediation technologies, the organics range from being highly biodegradable (e.g. sugars) to more slowly biodegradable or even recalcitrant (e.g. phenolics) [7, 26]. Phenolics also differ in their degree of toxicity to microbes and plants, a factor that requires consideration, particularly for WWW treatment options such as planted TWs [27, 28].

In general, if the ratio of COD to nitrogen to phosphorus (COD:N:P) in WWW (Table 2-1) is higher than 100:5:1, wastewater may be supplemented with nutrients to enhance the biodegradation of organics for efficient secondary wastewater treatment [7, 29]. The WWW may also be treated concurrently with domestic wastewater [30], but the potential introduction of pathogens, such as multi-drug resistant bacteria, may render the effluent unsuitable for land application.

Inorganics in WWW emanate from (i) cleaning and sanitizing agent/s [including sodium hydroxide (NaOH), potassium hydroxide (KOH), sodium metasilicate (Na_2SiO_3), trisodium phosphate (Na_3PO4), sodium carbonate (Na_2CO_3), phosphoric acid (H_3PO_4) quaternary ammonium compounds, peracetic acid, hydrogen peroxide (H_2O_2), and sulfur (S)], (ii) filtration and clarification aids (e.g. diatomaceous earth, bentonite clay, perlite), and (iii) grapes [6, 9, 31, 32]. Grape residues and juice can contribute appreciably to the potassium (K^+) concentration whereas the use of cleaning and sanitizing products are the major contributing factors to the introduction of sodium (Na^+) in WWW as NaOH is still the agent of choice for many wineries due to its effectiveness and low cost [6, 9]. Salts are not removed during secondary treatment processes, and monovalent cations, particularly Na^+, can lead to soil degradation (via increasing soil sodicity) if the WWW is used for irrigation purposes [33]. Best practice therefore advocates the use of K-based, or ideally, organic cleaning and sanitizing products [33].

2.3 Operation and performance

A literature search (SCOPUS) using the keywords “winery, wastewater, effluent, treatment, wetland, constructed” was used to identify studies pertaining to treatment of synthetic and/or real winery effluent in treatment wetlands. Selected data obtained from all the studies that assessed and monitored the physical set-up, operation, and performance of TWs for the remediation of WWW are provided in Table 2-2 and Table 2-3. Data may be cross-referenced between these tables using the numbers in the left hand (ID) column. Additional information for these studies is provided in Supplementary Table A2-1.

2.3.1 Lack of data and experimental flaws

It is difficult to holistically evaluate which of the existing TW systems are the most effective options for remediation of WWW by comparing the published data. While it is not possible to standardize factors such as the character of WWW or climatic variables, other fundamental data required to allow meaningful comparisons between systems are often omitted from manuscripts (Table 2-2). These include the functional surface area (FSA), functional depth, hydraulic loading rate (HLR), organic loading rates (OLR), and measured flow rates (MFR). These can exhibit notable variations, for example, the data available for the HRT range from 1.8 to 24 days. In addition, although the FSA is an important parameter for calculating the spatial footprint of a TW, the standard use of this parameter to describe the HLR in TWs should ideally be augmented by the use of the functional volume (FV), which is more informative when performing inter- and intra-study comparisons of systems containing, for example, different types of media. This is because the saturation level (depth) also varies between systems, in this instance from 0.3 to 1.2 m (Table 2-2). For example, if a shallow system is compared with a deep system using only the FSA to calculate the HLR and/or OLR, it will give a skewed picture of the contribution of the media to the overall remediation potential of the studied TW [4].

In order to account for the random variability inherent in biological systems, experimental replication is required to ensure the statistical validity of studies comparing different factors [34]. Replication is more feasible at laboratory-scale, but most studies reported in literature have been conducted using full-scale TWs (Table 2-3). For TWs treating WWW, a limited number of laboratory studies have been used to compare the presence/absence of plants [12,

21] and/or media [35]. However, in all but one instance [21], experiments were not replicated. This compromises the reproducibility and practical application of studied TWs as the 'snapshot' of the TW's remediation efficacy may be an anomaly compared with a standardized and expected outcome. Going forward, this highlights the need to consider performing well-replicated laboratory studies to improve the performance of TWs treating WWW.

It has been suggested that because of the relatively large spatial footprint of TWs, they are not suited to large wineries that produce copious amounts of effluent, particularly if land values are high. However, the connection between the size of a winery (small, medium, large) and the capacity of a TW system is difficult to establish. Ideally, the volume of effluent should be measured. For a number of reasons, this is practically difficult, which is evidenced by the fact that the effluent volume was only provided in one of the twenty studies included in this review (Table 2-3). Wineries are alternatively described by the amounts of grapes crushed or wine produced (bottles/cases or volume), but it is difficult to define the size of a winery based on these criteria as some wineries do not bottle, while others do not crush.

Table 2-1 Seasonal composition of winery effluent (adapted from [14][a] [7][b] [24][c] [25][d])

Parameter	Crush			Non-crush		
	Minima	Maxima	Averages	Minima	Maxima	Averages
pH	4[a] 4[b] 4[c] 4[d]	7[a] 12[b] 13[c] 5[d]	-	7[a] 2[b] 4[c]	8[a] 12[b] 11[c]	-
EC ($mS.cm^{-1}$)	0.37[c]	9.7[c]	1.3[c]	0.14[c]	3.3[c]	0.97[c]
TSS ($mg.L^{-1}$)	523[a]	2190[a]	1237[a]	<200[a] 267[b]	<1000[a] 21697[b]	2382[b]
COD ($mgO_2.L^{-1}$)	1031[a] 54[b] 320[d]	16825[a] 13900[b] 5760[d]	7684[a] 5951[b] 3449[d]	<500[a] 28[b]	<2000[a] 76900[b]	5489[b]
BOD_5 ($mgO_2.L^{-1}$)	650[a] 6[c]	10300[a] 15400[c]	3542[a], 1790[c]	<250[a] 4[c]	<1000[a] 4100[c]	1390[c]
VFA ($mg.L^{-1}$)	7[b] 1[d]	308[b] 799[d]	123[b] 216[d]	0[b]	11254[b]	830[b]
Ethanol ($mg.L^{-1}$)	0[b]	1240[b]	338[b]	0[b]	21000[b]	1693[b]
Sugars (GFM$mg.L^{-1}$)	0[b] 0[d]	1474[b] 4940[d]	195[b] 2006[d]	0[b]	733[b]	33[b]
TPhen. ($mg.L^{-1}$)	5[b] 0[d]	86[b] 27[d]	46[b] 9[d]	3[b]	273[b]	50[b]
Sodium ($mg.L^{-1}$)	8.6[b] 7.8[c]	105[b] 3060[c]	31[b] 137[c]	5.9[b] 5.4[c]	574[b] 714[c]	61[b] 87[c]
Potassium ($mg.L^{-1}$)	5.8[b] 3.2[c]	387[b] 772[c]	187[b] 176[c]	1.7[b] 1.3[c]	393[b] 1270[c]	162[b] 133[c]
TN ($mg.L^{-1}$)	9.7[a] 0.7[b]	109[a] 34[b]	43[a] 10[b]	<20[a] 0.4[b]	<100[a] 176[b]	14[b]
TP ($mg.L^{-1}$)	1.5[a] 0.4[b]	188[a] 31[b]	6.9[a] 11[b]	<10[a] 0.5[b]	<50[a] 280[b]	19[b]
COD:N	-	-	146:1[a] 595:1[b]	-	-	392:1[b]
COD:P	-	-	912:1[a] 541:1[b]	-	-	289:1[b]

Data from studies using respectively unknown [a], 168[b], 360[c], and 9[d] samples from 6[a], 4[b], 18[c], and 9[d] wineries

Note: EC = electrical conductivity TSS = total suspended solids COD = chemical oxygen demand BOD_5 = five-day biological oxygen demand VFA = (short chain) volatile fatty acids GFM = glucose + frutose + maltose TPhen. = total phenolics SAR = sodium adsorption ratio TN = total nitrogen TP = total phosphorous

2.3.2 Performance evaluation

Most studies have focused on the universal objectives of removal/reduction of organics and solids from WWW, and pH neutralization (Table 2-2 and Table 2-3). For reference, the influent COD (COD_{in}) and OLR in terms of FSA and FV, as well as the organic removal rates (ORR) are given in Table 2-2. These results are not discussed further because the organic removal performances of most of the TWs described in this manuscript have already been adequately reviewed [11, 36]. In summary, the authors of these reviews concluded that TWs are able to reduce the organic load sufficiently, provided that the HRT is sufficient to maintain the OLR within design parameters, and that pre-treatment for solids reduction is satisfactory. In addition, TWs have proven to be effective at reducing the total suspended solids (TSS) concentration, increasing the pH of acidic WWW, and decreasing the pH of alkaline WWW [4, 36, 37]. The pH buffering mechanism/s of WWW in TWs has not yet been elucidated.

Due to potential environmental toxicity, removal of (poly)phenolics from WWW in TWs is an important consideration. However, studies typically limit analysis of organic removal to COD and/or BOD removal, and to our knowledge there are only four studies describing removal of phenolics or tannins from WWW in TWs or simulated TWs. Grismer et al. [18] found higher removal rates during the non-crush season (average 78%; influent = 55 ± 16 $mg.L^{-1}$) compared with the crush season (average 46%; influent 55 ± 22 $mg.L^{-1}$). The reduced removal rates correlated with a decreased HRT during the crush season. In another system operated with a more consistent HRT, no seasonal trend in phenolic removal rates was noted, and an average removal rate of 77% (range 5.1-44 $mg.L^{-1}$) was obtained. Other studies were conducted using WW with low phenolic concentrations: WWW mixed with domestic WW (9.7 ± 3.0 $mg.L^{-1}$) and synthetic WWW (13 $mg.L^{-1}$) [38, 39]. Studies have also been conducted using other forms of agri-industrial wastewaters at moderate influent phenolic concentrations. Although the plant-based phenolic profiles would be expected to differ from those of WWW, these studies can still provide some valuable insights. In gravel-based horizontal flow systems, Rossman et al. [40] found that pre-aeration and inclusion of rye grass increased the removal of total phenolics from coffee processing wastewater from 54% to 72%, while Gomez et al. [41] found that phenolic removal rates decreased when loads in cork boiling effluent exceeded 0.4 $mg/L.day^{-1}$, and that some phenolic molecules were more readily removed than others.

Typically, less importance is placed on the removal of inorganics in biological systems treating WWW [42]. The need to remove nutrients and other inorganic elements and molecules from WWW is incumbent on local legislative requirements, place/type of discharge, and WWW character, and is therefore site-specific [11, 14, 37, 38]. If the effluent is discharged into a sensitive aquatic environment or municipal sewer where nutrient discharge limits are set, then removal mechanisms such as nitrification/denitrification will be important and must be considered. Conversely, the essential plant nutrients, namely N and P, may be seen as such if the effluent is to be re-used for irrigation [14]. In the case where treated WWW is utilized for irrigation purposes, it will be important to limit the Na^+ concentration and sodium adsorption ratio (SAR) within permissible limits to prevent soil sodicity and salinization, and associated loss of soil structure [42].

It has been clearly demonstrated that N removal takes place over the long-term operation of full-scale TWs treating WWW. However, as with the character of WWW itself, removal rates vary from site to site and seasonally. Masi *et al*. [36] reported an elevated average total nitrogen (TN) removal in three full scale systems with influent concentrations of 15 $mg.L^{-1}$, 27 $mg.L^{-1}$, and 65 $mg.L^{-1}$, and respective removal rates of 81%, 90% and 58%. As with tannins, Grismer *et al*. [18] found lower nitrate (NO_3^-) removal rates in the crush compared with non-crush seasons (17% vs 73%, respectively), which could be attributed to reduced HRT during the crush season. Similarly, the authors also reported lower average NH_4^+ removal rates in samples taken during the crush period (average 29% of 37 $mg.L^{-1}$ v/s 62% of 118 $mg.L^{-1}$). Apart from the shorter HRT during the crush season, diminished nitrification may have been exacerbated by a more unfavorable C:N ratio (200:1 v/s 15:1), as nitrification rates decrease with increasing C:N ratios in TWs [43]. De la Varga *et al*. [37] reported low NH_4^+ removal rates (≤ 29%) in a system treating combined WWW and domestic WW with influent concentrations of 0.6 to 74 $mg.L^{-1}$ in Europe. In a Canadian system designed to treat the combination of WWW and domestic WW by enhancing nitrification-denitrification processes within the TW, elevated NH_4^+ removal rates (>99%) were achieved during the warmer half of the year (6-month season) for three years but decreased to as low as 19% thereafter. The removal rates were consistently >99% for the rest of the year. This result appears to be anomaly, as given the cold winter climate in Canada, it would be expected that nitrification would decrease during this period. The result may be attributable to low sampling frequencies (*n*= 2 per

season) that may not have presented a true reflection of overall performance for each season, along with low influent concentrations, particularly in the colder seasons (average 2.2 $mg.L^{-1}$ and 0.9 $mg.L^{-1}$ in the warmer and colder seasons, respectively). Grismer *et al*. [18] also measured removal of sulfur (S) species, and found 95% removal of sulfates (SO_4^{2-}) and 78% removal of sulfites (SO_3^{2-}) in a HF gravel-based system with influent concentrations of 35±19 $mg.L^{-1}$ and 0.56±0.20 $mg.L^{-1}$, respectively.

TWs do not appear to effectively remove cations from WWW. For example, Mulidzi *et al*. [22] found marginal, but erratic removal of Na^+ (1-43%, average 12%; influent 101-282 $mg.L^{-1}$; *n*=23) and K^+ (1-43%, average 8%; influent 141-615 $mg.L^{-1}$; *n*=23) in HF gravel-based systems containing plants (*Typha, Scirpus* and *Phragmites* spp.). Removal was attributed to plant uptake, which is supported by the fact that no removal of Na^+, K^+, or magnesium (Mg^{2+}) was found by Holtman *et al*. [4] in an unplanted sand-based WWW treatment system. The selection of certain plant species with the ability to remove elevated concentrations of such contaminants may therefore be utilized in TWs to improve the quality of WWW used for irrigation and is further discussed in Section 2.4.3.

For interested readers, more detailed data on specific (poly)phenolic and inorganic concentrations and removal efficiencies in TWs, treating WWW, are included in Supplementary Table A2-1. The biotic and abiotic mechanisms, present in TWs, for removal of important WWW organics and inorganics are described later in this manuscript.

Table 2-2 Selected operational and performance data of studies pertaining to treatment wetlands used to remediate winery wastewater.

ID	FSA (m^2)** [Depth (m)]	HRT [D] (days)	HLR – FSA** ($mm.day^{-1}$)	HLR – FV** ($L.m^{-3}.day^{-1}$)	COD_{in} ($mg\ L^{-1}$)	OLR - FSA ($g\ COD.m^2.d^{-1}$)	OLR - FV ($g\ COD.m^3.d^{-1}$)	ORR (%)	MFR ($m^3.day^{-1}$)	Ref
1	0.48 [0.35]	2-4	UTC	UTC	788-2985	UTC	UTC	81-92	NG	[12]
2	1.2 [0.6]	NG	UTC	UTC	1258	UTC	UTC	NG	NG	[13]
3	350* [0.3-1.4]	NG	20±7 (VF&HF) 13-25 ($HF_{1,2,3}$)	14 (VF) 43-82 (HF_1) 22-41 ($HF_{2,3}$)	1558±1023	30.4±19.3 (all) 213 (VF) 16.2 ($HF_{1,2,3}$)	152* (VF) 54* (HF_1) 27* ($HF_{2,3}$)	71*	5.2	[37, 38]
4	350* [0.3-1.4]	3	77-215 (VF) 13-36 ($HSSF_{1,2,3}$)	55-154* (VF) 43-120* (HF_1) 22-60* ($HF_{2,3}$)	NG	30.4 (all) 43-466 (VF) 3.6-55 ($HF_{1,2,3}$)	31-333* (VF) 12-183* (HF_1) 6.0-92* ($HF_{2,3}$)	73 (all) 29-70 (VF) 23-79 (HF)	NG	[15]
5	60 [1-1.2]	NG	25.7 (VF&HF)	25.7 VF* 15.4 HF*	1665 VF NG (HF)	162 VF 65 HF	162.0 VF* 39.2 HF*	47-96 VF 7* HF	1.28±1.18	[14, 164]
6	14.9 [0.9]	9.7	34	28*	993-4720	35-164*	37-176*	97-99	0.5	[19,20,60]
7	4400 [1.2]	5.5 [10]	31*	26*	7406 ±2090 (C) 1721±439 (NC)	120-270	100-225*	49 (C) 79 (NC)	137	[18]
8	304 [1.2]	[5]	UTC	UTC	290	553	465*	98	137	[18]
9	NG	2.5-5.0	UTC	UTC	1183	UTC	UTC	49-70	NG	[21]
10	120* [0.9]	6±1.6 [14]	UTC	UTC	72965 ±29066	UTC	UTC	94-97	NG	[21]
11	144* [0.9]	18-24 [14]	UTC	UTC	5080 ±1211 (C)	UTC	UTC	98-99	NG	[21]
12	7.3* [NG]	1.8	109*	150 (12-313)	1138	110*	152 (23-469)	79 (28-98)	0.41	[4, 59]
13	75-164 [1.2]	NG	119-160 (all) 357-480 (HF_1)	99-133*	NG	NG	UTC	>98	10-23	[11]
14	190-404 [1.2]	NG	39-53 (all) 133-164 (HF_1)	33-44*	NG	NG	UTC	>98	10-17 (C)	[11]

ID	FSA (m^2)** [Depth (m)]	HRT [D] (days)	HLR – FSA** ($mm.day^{-1}$)	HLR – FV** ($L.m^{-3}.day^{-1}$)	COD_{in} ($mg\ L^{-1}$)	OLR - FSA ($g\ COD.m^2.d^{-1}$)	OLR - FV ($g\ COD.m^3.d^{-1}$)	ORR (%)	MFR ($m^3.day^{-1}$)	Ref
15	NG	3-5	38 (all) 76 (HF_1)	32*	NG	NG	UTC	>98	< 10.0	[11]
16	1330* [0.7]	NG	26	18*	4045	329	230*	98	35	[52]
17	752* [0.7]	NG	23	16*	1003	236	165*	93	10	[52]
18	215 [0.7]	NG	37	26*	722	352	246*	88	8	[52]
19	3034* [0.4-0.85]	[2.5]	60-80	71-94* (VF)	1159±432 (VF)	160-230 (D)	188-271*	98 (all) 70 (VF)	61±28 (max. 118)	[16]
20	1.63 [0.6]	9.6	31* (SA)	51*	171±14 184±11	5	8.8* 9.4*	69-93 86-93	0.050	[39]
21	180 [0.9]	1) 14 2) 7	1) 23* 2) 45*	1) 25* 2) 50*	1) 14000 2) NG	1) 315* 2) NG	1)350* 2) NG	1) 77-80 (NC) 1) 83-88 (C) 2) 60	1) 4.1 2) 8.1	[22, 166]
22	404 [0.4-0.8]	NG	22.3	56* ($VF_{1,2,4}$) 28* (VF_3)	3043 (C) 2117 (NC)	34	85*($VF_{1,2,4}$) 43*	99	7.3* (C) 11* (NC)	[23]
23	NA [0.75]	14	6.9	9.2*	4997-6189	5.18	6.9*	>99	NG	[35]

* Calculated from literature data **FWS data not included

FSA = functional surface area D = design FV = functional volume HRT = hydraulic retention time HLR = hydraulic loading rate COD_{in} = influent chemical oxygen demand OLR = organic loading rate ORR = organic removal rate MFR = measured flow rate UTC = unable to calculate (from literature data) NG = not given C = crush season NC = non-crush season

Table 2-3 Selected operational data and details of functional components of treatment wetlands treating winery wastewater (plants excluded).

ID	Study type & duration	Wine/effluent production per annum	Wastewater type/discharge	Pre-treatment	Configuration & Mode	Media			HC ($mm.s^{-1}$)	Ref.
						Material	Diameter (mm)	Porosity (%)		
1	Lab study 2 years	NA	Diluted WWW NA	PS → TF	HF	Igneous fine gravel Bulgarian zeolite	6 (D_{50}) 4 (D_{50})	35 25	-	[12]
2	Pilot study 2 weeks	6000 bottles	WWW NG	-	HF	Cork stoppers	3-7	44	-	[13]
3	Full-scale 2 years	315 m^3	WWW & DWW Municipal sewer	UASB	VF → $HF_{1,2,3}$	Granitic gravel Gravel	8-16 (T,B) 3-6 (M) 6-12	NG 40	- 1.5-1.6	[37,38]
4	Full-scale 2 years	315 m^3	WWW & DWW Municipal sewer	UASB	VF → $HF_{1,2,3}$	Granitic gravel Gravel	8-16 (T,B) 3-6 (M) 6-12	NG 40	- -	[15]
5	Pilot 2 years	368 m^3/ 1398 m^3	WWW Recirculated	Homogenization tank HUSB	VF → HF →	Granitic gravel Sand Gravel	6-12 (B), 2-4 (M) 1-2 (T) 6-12	NG	-	[14, 164]
6	Pilot study 2 years	18200 m^3	WWW NG	Sand pre-filter	HF	Pea gravel	4.7 (D_{50})	36	6.0	[19,20,60]
7	Full-scale 1 year	Moderately sized winery	WWW Irrigation	Settling pond	HF	Pea gravel	4.0	NG	-	[18]
8	Full-scale 1 year	Small winery	WWW Irrigation	Settling pond	HF	Rock	NG	NG	-	[18]
9	Lab study 30 days	NA	WWW NA	NA	HF & FWS	Volcanic rock	40	50	-	[21]
10	Full-scale NG	14000 cases	WWW Irrigation	Septic tank	HF	Pea gravel	<8	NG	-	[21]
11	Full-scale NG	14000 cases	WWW Irrigation	Septic tank	HF	Pea gravel	<8	NG	-	[21]
12	Pilot 3 years	Small winery	WWW Irrigation	Primary settling →balancing tank	HF	Dune sand	0.4 (D_{50}) 0.2-1.0	29	0.04-0.20	[4, 59]
13	Full-scale (4) NG	NG	WWW & sewage Sub-surface	Septic tank →AFFR	HF_1 (anoxic) $HF_{2,3}$ (aerobic)	Wood chips Gravel & sand	NG NG	NG NG	-	[11]
14	Full-scale (3) NG	NG	WWW & sewage Sub-surface	Septic tank →AFFR	$HF_{1,2,4}$ (aerobic) HF_3 (anoxic)	Gravel & sand Wood chips				[11]

ID	Study type & duration	Wine/effluent production per annum	Wastewater type/discharge	Pre-treatment	Configuration & Mode	Media			HC ($mm.s^{-1}$)	Ref.
						Material	Diameter (mm)	Porosity (%)		
15	Full-scale (20) NG	NG	WWW Sub-surface	Septic tank	$HF_{1,2}$	Gravel & sand				[11]
16	Full-scale 1 year	NG	WWW Irrigation	Imhoff tank	HF → FWS	Gravel	5-10	NG	-	[52]
17	Full-scale 1 year	NG	WWW & DWW Irrigation	Imhoff tank	$VF_{1,2}$ → HF → FWS → pond	Gravel & sand Gravel (HSSF)	8-40 (T-B) 8-12	NG	-	[52]
18	Full-scale 1 year	NG	WWW Water body	Imhoff tank	HF	Gravel	5-10	NG	-	[52]
19	Full-scale 2 years	Bottling and aging only	WWW (no crush) River	Equalisation tank	VF → HF → FWS	Gravel NG	2-40 (T-B)	NG 35 (HSSF)	- 5.8	[16]
20	Pilot 5 months	NG	SWW	NA	HF	Gravel	6-9	NG	-	[39]
21	Pilot 2 years	NG	WWW Cabbage irrigation	NG	HF	Dolomitic gravel	20-30	35	-	[22, 166]
22	Full-scale 6 years	NG	WWW & DWW Subsurface	Dosing tank	$VF_{1,2,3}$ VF_4 (anoxic)	Gravel Wood chips Peat moss (T) 0.3 m	5-10 NG	NG NG	-	[23]
23	Lab column 6 months	Lab study	WWW NA	Primary settling	VF replicates	Gravel Clinopatilolite Tyre chips Oyster shells	6.4 1.2-2.4 10-15 1.2-2.4	NG	NG	[35]

HC = hydraulic conductivity PS = primary settling TF = trickling filter HF = horizontal (subsurface) flow WWW = winery wastewater NA = not applicable NG = not given VF = vertical (subsurface) flow FWS = free water surface UASB = upflow anaerobic sludge blanket reactor AFFR = anaerobic fixed film reactor T = top M =middle B = bottom DWW = domestic wastewater HUSB = hydraulic upflow sludge blanket reactor FWS = free water surface flow SWW = synthetic winery wastewater

2.4 Functional significance and interactions of major biotic and abiotic components

2.4.1 Background

As alluded to previously, the performance of TWs treating WWW has been reviewed by Masi *et al.* [36] in 2015. However, in order to continually improve the efficiency of these systems, there is a need to critically analyse the current fundamental knowledge pertaining to the types of abiotic substrate (media) and plants, as well the microbial community structure and function which may be utilized in these TWs. The selection of plants is particularly important because of the potential phytotoxicity of WWW [27].

To address this, the relevance and characteristics of (i) the media, microbes, and media-microbe interactions, and (ii) the plants, and plant-media and plant-microbe interactions, are discussed generically, and as they specifically pertain to the treatment of WWW in the following two sub-sections.

2.4.2 Media and microbial populations

2.4.2.1 Types of media used in winery wastewater treatment wetlands

Various types of gravel, with diameters ranging from 3-40 mm, have historically been the preferred media used in TWs remediating WWW. Sand has also been extensively used, in combination with or without gravel (Table 2-2). Other substrates have also been used to achieve particular removal functions. For example, Johnson & Mehrvar [11] used wood chips as a carbon (C) source for denitrification of WWW mixed with sewage, Calheiros *et al*. [13] used cork for its general adsorptive capacity, while Skornia *et al*. [35] added oyster shells as a buffering agent in a laboratory study.

2.4.2.2 Biotic and abiotic removal mechanisms

The primary biotic removal mechanisms in TWs are microbial biodegradation, biotransformation, and bioprecipitation (Table 2-4), and a series of different phytoremediatory mechanisms (Table 2-4, 2.4.3). These are accompanied by physicochemical (abiotic) mechanisms, most notably adsorption and precipitation [42, 43]. Degradation of organic molecules can also be facilitated by physicochemical catalysis. For example, the oxidation of phenolic acids may be coupled to the reduction of iron and/or manganese present in the substrate particles [46, 47].

Although adsorption and precipitation may be the primary removal mechanisms for some molecules and elements, there is a danger that these may later desorb or re-mineralize due to changes in physicochemical conditions, and/ or the saturation of substrate (media) binding sites. In cases where concurrent biotic and abiotic removal takes place, good long-term removal rates may still be achieved, as demonstrated with the phenolics *viz* - gallic acid and vanillin present in synthetic WWW [47]. This combined biotic/abiotic approach was also used by Skornia *et al*. [35], where media was supplemented with clinopatilolite to adsorb ammonium ions (NH_4^+) (average 13.7 $mg.L^{-1}$) and tyre chips to adsorb NO_3^- (average 4.2 $mg.L^{-1}$) from WWW in gravel columns. The rationale for the experiment was that in colder climates where nitrification rates are low in winter, these forms of N could be adsorbed, and then mineralized to nitrogen gas (N_2) by nitrification-denitrification with the advent of warmer weather. While the approach had merit, the short-term study failed to show significant differences in removal rates between the amended columns and the gravel controls in this instance. In laboratory TW simulations testing systems containing different plants and media, Akratos *et al*. showed increases in NH_4^+ removal rates from domestic wastewater [48] and WWW [12]. In the latter, rates increased from 30% to 57% to 78% in unreplicated laboratory HF systems containing gravel, gravel and plants, and zeolite and plants, respectively. The authors attributed the increased nitrification to the presence of plants and greater NH_4^+ adsorption of zeolite when compared to igneous gravel.

In contrast to N, where primary removal in TWs is microbially mediated (nitrification-denitrification), and discounting removal by plants (discussed in Section 2.4.3), removal of P is abiotic (adsorption and/or precipitation) [43]. Adsorption rates are dependent on the chemistry and morphology of the substrate particles, and can be increased by using highly reactive media such as apatite or steel slag [12, 48-51]. Although many studies have looked at P removal rates in gravel and/or sand filled TWs, in reality, unless significant plant uptake occurs and the plants are subsequently harvested, negative P removal rates may occur at some point due to plant senescence, saturation of binding sites and/or desorption or mineralization due to changes in physicochemistry (e.g. pH, redox, increased sulfate concentrations) [45, 49]. While Masi *et al*. [52] reported excellent total P removal rates over one (1) year in two (2) full scale systems in Europe: average 73% (influent 4.9 $mg.L^{-1}$, n=5) and average 94% (influent 1.9 $mg.L^{-1}$, n = 10), Rozema *et al*. [23] measured negative P “removal” after 5 years of operation (range: -115% to >99% n=23) in a full-scale TW remediating WWW combined with domestic WW. Over the first 3

years of operation, >99% removal was achieved, suggesting that the primary reason for the decreased performance was saturation of P binding sites. It is therefore recommended that if P removal is seen as a priority, and in order to recapture this limited resource, specific downstream removal processes are applied. A promising example is provided by Skornia *et al*. [35], whom used a proprietary iron-oxide based commercial P adsorbent (sponge) as a polishing step to precipitate and recover P from WWW. Such technologies that support a circular economy could be viable options going forward and should be investigated further.

2.4.2.3 Biofilm, solids and hydraulic conductivity

The main advantage of using sand instead of gravel in TWs is that the particles provide a larger surface area for biofilm attachment and surface chemistry [12, 48, 53]. The major comparative disadvantage is the increased risk of clogging because of the smaller matrix pores in the sand milieu [54, 55]. The term 'clogging' in TWs is applied when the hydraulic conductivity (HC) decreases to the extent that the system can no longer function effectively. The risk of clogging can be ameliorated by the (i) popular use of WWW pre-treatment (Table 2-2) to remove suspended solids, (ii) intermittent operation to allow solids and excess biofilm degradation during the resting period, and/or (iii) application of low organic loading rates such as effluent recycling [53, 54, 56, 57].

While clogging with non-biodegradable solids can negatively impact the long-term operation of TWs, it should be noted that some critical decrease in the HC is expected to occur after start up due to the gradual, and necessary build-up of a functional biofilm [58, 59]. Operational HC values of 1.5 $mm.s^{-1}$ [37], 5.8 $mm.s^{-1}$ [16] and 6.0 $mm.s^{-1}$ have been reported in gravel-based systems, and values ranging from 0.04-0.20 $mm.s^{-1}$ (calculated from flow rates measured *in-situ*) were reported in a sand-based system [59]. It has been shown that predicted HC measurements based on grain size distribution may be inaccurate due to most models assuming particle sphericity [59, 60]. For example, Grismer *et al*. [60] found an order of magnitude difference in the HC of poorly graded pea gravel between predicted values and column experiment measurements (6.0 $mm.s^{-1}$).

Temporal changes in the HC of TWs treating WWW are rarely described in literature, making comparisons between systems difficult. Akratos *et al*. [12] reported a 15% loss in porosity over 2 years in a HF system containing igneous fine gravel and Bulgarian zeolite, however the HC was

not measured. De la Varga *et al*. [37] studied the accumulation of solids in a gravel-based system and found that, although the accumulated TSS and volatile suspended solids (VSS) increased annually to reach maximum values of 8.6±3.0 kg and 1.01±0.78 kg.m^2, respectively after 2.8 years of operation, the average reduction in HC (1.64 mm.s^{-1} to 1.49 mm.s^{-1}) measured by the falling head method was insignificant over three measurement periods (1.6, 2.2, and 2.8 years of operation). In contrast, reductions in HC of up to 50% have been measured in sand-based treatment systems treating WWW [59], highlighting the need to monitor the HC in studies where sand is used as a physical substrate. If HC reductions are factored into the design capacity, and realistic flow rates can still be achieved, the use of sand as a medium may still be a viable option [59]. Furthermore, it has been shown that biofilm-related decreases in HC are inversely related to the organic loading rate, and are reversible [57, 59]. Similarly, clogging due to accumulation of organic sludge formed during the crush season is also degraded during the non-crush period, restoring the HC [36, 52].

2.4.2.4 Microbial community structure and function

The microbial community structure and function in CWs is influenced by the plant species, climatic variables, and physicochemical variables related to the substrate, mode of operation and type of wastewater [61-63]. In TWs, sedimentary and epiphytic bacteria, as well as planktonic bacteria (in FWS systems) are principally responsible for nitrification, denitrification, SO_4^{2-} oxidation/reduction and hydrocarbon degradation, as well as transformation and mineralization, which follow natural principles for the biogeochemical cycling of C, N and sulfur (S). Studies suggest that it takes around 100 days from start-up for the microbial communities to become established (equilibrated) in TWs [61, 63-65]. This is an important factor that is not always considered by researchers. In literature reports on TWs treating WWW, full-scale studies have been conducted over ≥ 2 years. However, the results of many lab- and pilot-scale experiments may not provide an accurate assessment of the remediation potential of the systems because the duration of the experiments was too short to allow effective establishment of the functional microbial communities (Table 2-3).

It has been shown that under the same climatic conditions and influent characteristics, even small physicochemical differences in the substrate play a significant selective role on the microbial community structure in unplanted TWs (biological sand filters) [66]. Even in particles

with similar geochemistry, the shape of sand particles can affect biofilm attachment/abundance and consequent C and N removal performance; for instance, it has been demonstrated that natural sand presents a superior biofilm attachment surface to crushed sand [53]. Significant differences in the microbial community structure have also been noted in systems containing the same substrate, but with different WWW influent [62, 67, 68]. For example, in high rate biological contact reactors treating WWW and operated in series, de Beer *et al*. [67] found that the higher pH and lower organic load achieved in the (identical) second reactor allowed the preferential selection of different bacterial and fungal species to the first reactor, including nitrifying and denitrifying bacteria. Similarly, different microbial communities are selected within spatial niches of TWs, with redox status and influent degradation gradients thought to be the primary selective drivers [62, 68, 69]. In biological sand filters, and, by inference, TWs receiving high C:N WWW, nutrient limitation may be naturally mitigated by selection of N-fixing bacteria such as *Azotobacter* spp. [62, 70]. These results are supported by a recent study by Ospina-Betancourth *et al*. [71], whom enriched high C:N effluent with *Azotobacter vinelandii* and achieved appreciable rates of N-fixation. The authors proposed the use of the treated waste as a high N organic fertilizer, which is potentially a novel option for beneficiation of WWW which supports circular economy principles.

2.4.3 Plants, and plant-microbe plant-media interactions

Phytoremediation (plant-based) and microbial bioremediation (e.g. bacteria- and fungi-based) processes in wetland systems have been demonstrated to effectively remediate a range of inorganic and organic contaminants present in wastewater originating from different sources (e.g. acid mine drainage, sewage, aquaculture, and to a lesser degree, WWW) [72]. Plants and/ or microbes, used as tools for phyto- and biotechnologies, have the ability to degrade, take up, transform (e.g. methylate), volatilize, and/or stabilize (i.e. immobilize) contaminants (Table 2-4) (see Yan *et al.* [73] for review). However, various factors, including plant, microbe, and plant-microbe interactions (e.g. plant-assisted bioremediation), physicochemical properties of WWW (e.g. bioavailability, octanol-water partition coefficient, competitive ion adsorption), and environmental factors (*viz* – pH, redox potential (E_h), salinity, and temperature/ solar radiation) influence the efficacy of these remediation processes [73, 74].

Table 2-4 Phyto- and biotechnologies, used as tools for the biological remediation of various contaminants present in winery wastewater (WWW).

Bio- and Phytotechnology	Description	Mechanism(s)
Microbial biotechnologies		
Bioprecipitation	Contaminants are precipitated out of solution by various mechanisms including the release of complexing agents by microorganisms, rendering the contaminant biologically inert.	Bioprecipitation of contaminants by reduction-oxidation (redox) reactions, intracellular assimilation, complexation, sorption, release of complexing agents (e.g. ligands), and biosorption to membranes
Microbial biodegradation	Bacteria release extracellular biodegradation enzymes that breakdown contaminants resulting in the release of water-soluble intermediates, CO_2, H_2O, and other metabolites.	Enzymatic biodegradation and mineralization. Extracellular biodegradation enzymes are released by bacteria to break down contaminants. Degradation products (water-soluble intermediates) are released into the environment and/or assimilated into bacterial cells. Bacteria (present within the biofilm) attach to the surface of the contaminant resulting in contaminant degradation and the release of CO_2, H_2O and other metabolic products.
Biomethylation	Transformation of contaminants by microbes (aerobic and anaerobic bacteria, and fungi) into volatile derivatives and can be removed from the TW system via evaporation.	Enzymatic transfer of methyl groups to metals (e.g. As, Hg, Pb, Se, and Sn). For example, microbes may methylate Hg (forming methylmercury, CH_3Hg) which accounts for one of the removal pathways from TWs. It must be noted that the volatile derivative, e.g. CH_3Hg, may be more toxic due to its lipophilic nature and therefore, its bioavailability for plant and animal uptake.
Plant-assisted biotechnologies		
Rhizodegradation	Plant-assisted bioremediation involving the complex interplay between plant roots, plant exudates, rhizosphere chemistry, and microbe species/ communities to render contaminants biologically inert via microbial degradation processes.	Plant roots provide surface for microbe colonization. Plants stimulate the growth of microbial species/ communities (via release of exudates such as organic acids), enhancing microbial bioremediation pathways (e.g. biodegradation and bioprecipitation).
Phytotechnologies		
Rhizofiltration	Sorption (ad- and absorption), bioconcentration, and precipitation of contaminants from wastewater by plant roots of aquatic and terrestrial plant species.	Phytoextraction and (hyper)accumulation of contaminants (e.g. heavy metals) in roots.
Phytodesalination	Use of halophytic plant species to remove salts, and/or their constituents, from contaminated land or water.	Halophytes hypertolerate (and cross-tolerate) and accumulate elevated concentrations of salts, and their constituents, due to their effective ion homeostasis networks [strict and integrated cross-talk between genetic (e.g. upregulation of similar genes), molecular (e.g. reactive oxygen species, ROS), cellular (e.g. compartmentalization of contaminants), and physiological (e.g. contaminant secretion from specialized glands on the surface of leaves) mechanisms].

Phytostabilization	Establishing plant cover to physically and chemically stabilize soil contaminants thereby mitigating soil and aeolian erosion, and contaminant leaching.	Release of exudates into the rhizosphere to chelate metals which may subsequently be ad/absorbed by plant roots and/or sequestered into the plant's rhizosphere (phytosequestration).
Phytoextraction	Use of plants to sequester (phytosequestration), take up, and accumulate elevated concentrations of contaminants in planta.	Once contaminants have been sequestered (via the release of chelating compounds) and taken up by plant roots, the plant may utilize avoidance (root-to-shoot restriction/ shoot-to-root re-translocation) and/or tolerance (such as excretion from leaves, contaminant complexation, and translocation of contaminants to storage sites such as vacuoles) strategies to render toxic contaminants biologically inert. The number, and efficiency, of these strategies enable elevated concentrations of contaminants to be accumulated by particular plant species.
Phytohydraulics	Use of plants to intercept and prevent the horizontal migration of contaminants within surface and groundwater (including phytofiltration, and more specifically rhizofiltration).	Rhizosphere reverses the hydraulic gradient forming zone of stagnation which 'captures' contaminants thereby mitigating the transport of contaminants downstream (surface water) and within groundwater.

It must be noted that the type and efficacy of phyto- and biotechnologies are mediated by the interplay between the nature of WWW [i.e. contaminant behavior (e.g. competitive ion adsorption, composition and concentration of contaminants)], functional components [media, biological (e.g. plant and microbe factors), and environmental conditions] and operational parameters. References: [63, 73, 74, 83, 120, 121, 128, 156, 160, 168-174].

2.4.3.1 Plant factors

Plant species, as well as their genotypes, differ in their ability to remove inorganic and organic contaminants. This is attributed to the number, type, and efficacy of genetic, molecular, cellular, and physiological mechanisms, as well as the interplay between these mechanisms [75, 76]. Phytotoxic concentrations of contaminants present in WWW have been demonstrated to impede plant growth and survival [77]. However, as plants are sessile organisms, they have developed various strategies to adapt to abiotic and biotic stresses [75]. This enables certain plant species with elevated phytoremediation potentials to be utilized in TWs to remediate targeted contaminants. Plants selected for TWs must possess elevated (i) growth rates, (ii) biomass production (especially well-developed root systems), (iii) degree of stress cross-tolerance, and (iv) ability to render targeted contaminants biologically inert via the extraction, volatilization, or sequestration of contaminants [78, 79]. Based on these criteria, commonly selected plant species for TWs include *Phragmites australis* (common Reed), *Typha* spp. (cattails), and *Cyperus* spp. (sedges) (Table 2-5).

Table 2-5 Commonly selected plant species for use in TW for the remediation of organic and inorganic contaminants present in winery wastewater (WWW).

Plant species	Reason for selection for WWW remediation	References
Phragmites australis	- Fast growth rate and high biomass productivity (ranges from 0.413 to 9.890kg dry mass.m^{-2} per annum); - Tolerant to elevated organic and nutrient levels (determined thresholds include COD: 14000mg.L^{-1}, TKN: 506mg.L^{-1}, and TP: 95mg.L^{-1}); - Elevated rate of evapotranspiration (e.g. implications for WWW dewatering); - Flood, salinity-, and metal-tolerant (e.g. Fe^{2+}, Zn^{2+}, and Ni^{2+}) and effectively removes certain metals such as chromium (Cr^{2+}); - Tolerant to range of pH levels (e.g. used for remediation of acid mine drainage and industrial effluent); - Cosmopolitan species with a widespread distribution (i.e. the ability to growth and survive in a range of environmental conditions) - Extensive root system	[109, 175-178]
Typha spp. (including *T. latifolia*)	- Fast growth rate and high biomass productivity (exceeds 5.00kg dry mass.m^{-2} per annum); - Tolerant to a range of abiotic stresses (salinity, organic matter, heavy metals, and nutrients); - Effectively remove suspended solids; - Effectively removes Cu^{2+} and Cd^{2+} and is more effective at removing certain contaminants (such as P, Na^{+}, Ca^{2+}, Mg^{2+}, Cu^{2+} and Fe^{2+}), compared with *P. australis;* - Tolerant to a range of pH levels; - Widespread distribution and colonizes anthropogenically-affected habitats.	[179-183]
Cyperus spp. (e.g. *C. alternifolius*)	- Fast-growing plant species with a dense root system and is easily propagated; - Utilized in TWs for remediation of organics, pathogens, nutrients, and heavy metals (e.g. Al^{3+}, Cd^{2+}, Cu^{2+}, Pb^{2+}, and Zn^{2+}); - Demonstrated to effectively reduce COD, BOD, TSS, NO_3^-, NH_3, PO_4^{3-}, as well as total coliforms and fecal coliforms.	[184-187]

Unlike inorganic contaminants present in WWW, plants can metabolize organic contaminants. The metabolism of organics involves the transformation (e.g. redox reactions which alters the species of contaminant), conjugation (e.g. complexation with other molecules such as low-molecular weight thiols), compartmentalization (e.g. storage of contaminants in vacuoles), and/or volatilization (via evapotranspiration processes) of contaminants [73, 80]. These metabolic processes may render toxic organics biologically inert. Various studies have investigated the role of plants in TWs, for the removal of organic constituents from WWW, especially COD (*refer to Section 2.2; see* Supplementary Table A2-1 *for extensive list of studies investigating the remediation of organics, present in WWW using TWs*).

Although numerous inorganic constituents, including salts and their constituents (e.g. Na^+, Cl^-, and heavy metals), have been investigated (Supplementary Table A2-1), TWs are generally ineffective in remediating the inorganic fraction of WWW due to its inhibitory effect on biological processes. For example, Na^+ removal efficiency in wetland system ranges from -78 to 43%, of which Na^+ and other salt constituents (e.g. Cl^-) are considered as the most persistent and challenging contaminants to remove from wastewater [11, 12, 36, 37]. The removal of other inorganics, namely N and P, is also mediated by wastewater salinity, where the removal efficiency significantly decreases as salinity increases [81]. This highlights the need to desalinate wastewater in order to effectively reduce the remaining inorganic fraction present in WWW [82]. Moreover, the presence of these inorganics at elevated, phytotoxic concentrations generally requires expensive forms of treatment, *viz*. - dilution or physicochemical treatment methods (e.g. electrodialysis, reverse osmosis, and/or ion exchange treatments), creating other environmental challenges such as the production of a concentrated brine requiring disposal. Furthermore, these treatment methods have various disadvantages which include the method's low resistance to fluctuating contaminant loading, inhibiting effect of salts on the microbial component present in biological reactors, and inability to effectively treat non-point source pollution [78].

The concentrations and composition of inorganics present in WWW, which fluctuates through space (e.g. geographic locations) and time (e.g. according to seasonal cellar activities), creates a selective pressure to utilize cross-tolerant plant species with an elevated ability to remove targeted inorganics. Moreover, WWW must be treated before its disposal as per regulatory guidelines where legislation on permissible inorganic concentrations for disposal differ between countries.

The use of halophytic plants for the remediation of the inorganic fraction of WWW presents an inexpensive and effective alternative to the use of dilution and physicochemical treatment methods. Halophytes are generally characterized as plants which can complete their life cycles in saline environments where salt concentration is greater than 200mM NaCl [83, 84]. The use of halophytes has gained increasing attention over the past decades due to their elevated ability to cross-tolerate a range of stresses [85, 86]. Elevated Na^+ and Cl^- concentrations in saline soils induce osmotic and ionic stresses in plants – leading to secondary stresses (Figure 2-1). Halophytes have evolved various mechanisms to tolerate salinity stress, and are associated with the controlled uptake and assimilation of Na^+, K^+, and Cl^- [87, 88]. These tolerance mechanisms

(described below) enable halophytes to maintain homeostasis, preventing the toxic build-up of Na^+ ions, and potential antagonistic interactions between Na^+ and K^+ due to these cations sharing similar physicochemical properties [89, 90]. Although Na^+ is preferentially taken up over K^+ by plants, the elevated capacity of halophytes to accumulate elevated concentrations of these persistent inorganics (consequently removing these cations from saline WWW) indicates a potential application of halophytes for use in TWs. Moreover, heavy metal hyperaccumulation has been a long reported phenomenon where plants accumulate elevated concentrations of a particular metal *in planta* above a threshold (e.g. 1000 mg/kg in leaves for nickel (Ni) - see van der Ent *et al.* [91] for review). However, the hyperaccumulation threshold for other elements, such as Na^+, has not been defined. A study, conducted by Levinish *et al.* [92], investigated the accumulation of Na^+ in leaves by 102 plant taxa across 77 sampling sites. Based on leaf Na^+ concentrations, the "Na^+-hyperaccumulation threshold" was set at 18 – 30 g/kg dry mass. It must however be noted that further research on the hyperaccumulation threshold of Na^+ (as well as elements other than heavy metals) must be conducted to validate these thresholds. This further suggests the potential application of halophytes for use in TWs for the removal of persistent inorganics, namely Na^+ and K^+, from saline WWW.

Halophytes may be divided into different categories based on the mode of salt transport, storage, and potential excretion. These categories include recretohalophytes [divided into exo- (excrete excess salts from specialized salt glands) and endo-recretohalophytes (store salts in glands)], euhalophytes (compartmentalize salts into leaf/ stem vacuoles), and pseudohalophytes (accumulate salts in the vacuoles of the wall or parenchyma of xylem present in roots) [88, 93, 94]. Halophytes can be further characterized by their dependence on salt (i.e. facultative vs obligate halophytes) and the environments they inhabit (e.g. hydro-halophytes, xero-halophytes). Halophytes may tolerate a range of inorganic contaminants by effectively detoxifying metal ions via heavy metal exclusion (i.e. selective restriction of the uptake and translocation of metals by roots), excretion (i.e. phytoexcretion, where salt glands excrete excess ions thereby contributing to the maintenance of the plant's metal homeostasis network), and/ or accumulation (i.e. compartmentalization of metal ions into vacuoles) [86, 95, 96]. These halophytic characteristics have important implications for the removal of inorganics as the use of exo-recretohalophytes may re-introduce extracted contaminants into the TW water body, resulting in negative inorganic removal percentages. For example, the presence of salt glands in

the photosynthetic plant parts of some exo-recretohalophytes are used to detoxify accumulated metal ions where excess salts (and heavy metal constituents) are excreted onto the surface of the leaves [96, 97]. However, exo-recretohalophytes may also desalinate environments by haloconduction - a process whereby salt excreted onto the leaf surface is mobilized by wind and deposited onto surrounding areas [98, 99]. The deposition of salts away from the contaminated site may enable the dispersal of salts over large areas, diluting the impact of salt accumulation within the receiving environment [99]. Moreover, the dispersal of salts (where some salts are considered macro- and micro-nutrients) at low concentrations over large areas may improve the nutrient content of salt-deficient soils [99]. Although this provides an additional application of terrestrial exo-recretohalophytes, this phytotechnology is a relatively novel concept requiring further investigation. The implications for treatment of WWW in TWs is that during rainfall events secreted salts may be washed off plant leaves and subsequently re-introduced into the WWW, consequently increasing the concentration of salts in WWW, resulting in negative removal efficiencies [96, 99]. Another important implication was demonstrated by Matinzadeh *et al.* [100] where euhalophytes accumulated elevated concentrations of Na^+ compared with K^+. This was attributed to the role of Na^+ in mediating the plant's ion homeostasis network whereas elevated concentrations of K^+ were accumulated by facultative and pseudo-halophytes (essential macronutrient) thereby differing in their ability to accumulate salts. This study elucidates the importance of identifying viable halophyte candidates with desirable halophytic traits for targeted contaminant removal in TWs. Moreover, the composition and concentration of contaminants present in WWW (refer to Table 2-1) must be characterized prior to the selection of halophytic plant species.

In many countries, the main means of disposal of treated WWW is via land irrigation, presenting one of the major environmental challenges associated with the wine production industry [8]. Land irrigation with WWW containing inorganics may result in land salinization, increase in soil sodicity, potential eutrophication of receiving water bodies, and assimilation of other inorganics (e.g. heavy metals) in the soil [8, 101, 102].

Therefore, to mitigate the negative impacts associated with disposal practices, after secondary treatment, WWW must be effectively managed with a focus on the removal of salts, and their constituents. The management of WWW includes, but is not limited to, (i) treatment of saline WWW by phytodesalination before its disposal (Table 2-4), and/ or (ii) irrigation of cash crops

tolerant of saline WWW (i.e. beneficial irrigation). These management options must be considered as they directly impact socioeconomic development (e.g. food security) and the receiving environment. To implement either of these management strategies, halophytes with an elevated degree of cross-tolerance and extraction potential should be utilized in TWs to reduce salt load, as well as their constituents, present in WWW.

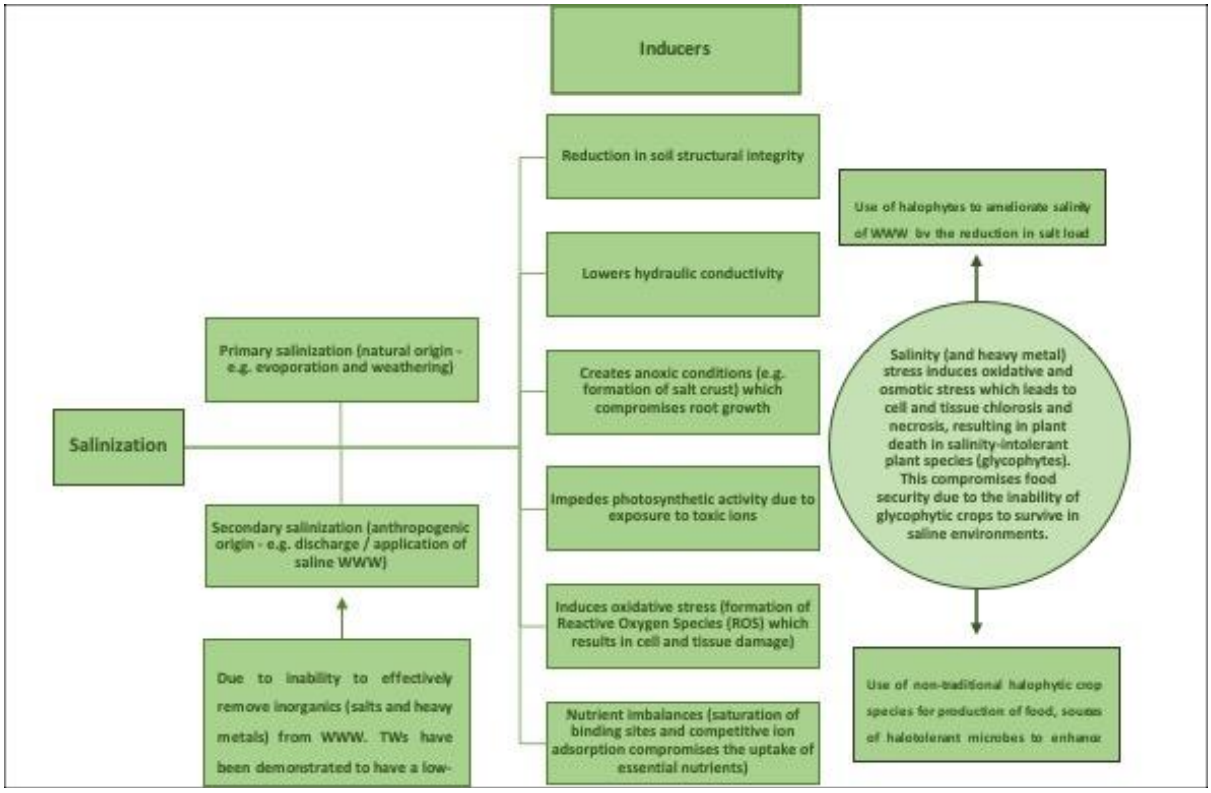


Figure 2-1 Pathways of salt (and heavy metal) phytotoxicity. Phytotoxic effects of salinity on plant growth and survival, where salinity stress may induce the combination of ionic and osmotic stress leading to secondary stresses. References: [24, 94, 1885, 189].

2.4.3.2 Phytoremediation of saline winery wastewater before disposal

Although numerous studies have investigated the phytoremediation of saline and heavy metal contaminated land by halophytes, limited research has been conducted on the phytodesalination and phytoextraction of inorganics present in wastewater originating from various sources [103-105]. To the knowledge of the authors, studies investigating the phytoremediation potential of halophytes have been restricted to studies on wastewater originating from aquaculture [106, 107]] where salt concentrations have been reported in the range of 17 000 - 46 000 mg NaCl/L), domestic (e.g. [108]), tannery (e.g. salt concentrations up to 80 000 $mg.L^{-1}$) [109-111], tool factory [45], oil production [112], and textile [113] saline wastewater. The wine, aquaculture, and other agricultural industries generate relatively similar key contaminants (namely organics, salts,

N, and P) compared with the mining, tannery, textile, and petrochemical industries – comprised of relatively complex contaminants and solution chemistry [78, 112, 114]. Although, the concentration and composition of these inorganics differ within and between industries, limited studies have investigated the use of halophytes for the treatment of saline wastewater originating from various industries. The majority of these studies have been restricted to the use of hydro-halophytes, such as *P. australis* [115] and *Typha* spp. [116] which cross-tolerate saline and heavy metal stresses under flooded conditions (which they naturally inhabit) and have therefore received notable attention for their use in TWs.

Halophytes have been reported to effectively (hyper)accumulate a range of inorganics including salts and their constituents (Na^+, K^+, Mg^{2+}, Cl^-, NaCl, and SO_4^{2-}), as well as heavy metals (e.g. cadmium (Cd^{2+}), copper (Cu^{2+}), lead (Pb^{2+}), and zinc (Zn^{2+}) – ecotoxicologically important heavy metals present in WWW from soil and water [103-105]. For example, Fountoulakis *et al*. [108] demonstrated the ability of the terrestrial halophyte, *Atriplex halimus*, to effectively remove elevated salt load from TWs along with high biomass production compared with the *Juncus acutus* and *Sarcocornia perennis*. This highlights the possible role of halophytes, and the need to select certain halophytes with an elevated ability to remediate and cross-tolerate a range of inorganics present in WWW.

The novel application of terrestrial halophytes

Flooding in combination with salinity stress are common environmental variables. To survive, plant species must tolerate the combination of these stresses (and secondary stresses such as the reduction in uptake and translocation of nutrients, such as K^+, to aboveground biomass) where the inability to do so results in plant mortality [117]. Although tolerance to the combination of these stresses typically involves adventitious root production (maintaining an internal O_2 content), halophytes possess the ability to effectively maintain their ion homeostasis network - regulating shoot ion concentrations independent of anoxic environmental conditions [118]. The ability of some halophytes to cross-tolerate these abiotic stresses is elucidated by the elevated productivity of salt marshes [119]. Although no link has been provided, various studies have indicated the ability of terrestrial halophytes to cross-tolerate these stresses. For example, Farzi *et al*. [120] demonstrated the use of three terrestrial halophytes, namely *Salicornia europaea, Salsola crassa,* and *Bienertia cycloptera*, in a TW under a salinity dose-dependent

experiment. These halophytes completed their life cycles under TW conditions and reduced measured elemental parameters to permissible levels. Aquatic plant species have typically been investigated for their rhizofiltration potential (i.e. the sorption (adsorption / absorption), concentration, and precipitation (onto the root surface) of contaminants from wastewater by plant roots), suggesting the possible role of terrestrial plant species, and halophytes in particular, for rhizofiltration and selection for use in TWs. A study conducted by Lee and Yang, [121] demonstrated that the terrestrial plant, *Helianthus annuus* (sunflower), effectively removed 80% of the uranium (U) present in a hydroponic solution via rhizofiltration. Moreover, terrestrial xerophytes and halophytes (such as *Atriplex halimus* and *Bassia indica*) have been successfully propagated in TWs [108, 122]. Another study demonstrated that terrestrial halophytes from the genus *Salicornia* possess aerenchyma within their roots, a flooding-tolerance mechanism present in hydrophytes, promoting redox conditions within the rhizosphere. This creates microenvironments conducive to nitrification/ denitrification processes within TW systems [123-125]. Furthermore, the growth, survival, and phytoremediation potential of terrestrial halophytes may be enhanced through the control of TW operational and functional components. For example, substrate-less TWs, such as floating (FTW, utilizing larger, emergent hydrophytes planted in a buoyant mat) or vertical up-flow (VUF) TW, modifies the plant's root system architecture (RSA). This may enhance the halophyte's rhizofiltration potential by increasing root growth and root surface for contaminant sorption, concentration, and/or precipitation, biofilm development (see Section 2.4.3.3), and/or root-contaminant contact time as roots are directly exposed to contaminants present within the WWW (Figure 2-2) [126-128]. As terrestrial halophytes have been demonstrated to grow under TW conditions, the phytoremediation potential of terrestrial halophytes can therefore be enhanced by modifying TW design.

The management of WWW is a major sustainability challenge within the wine industry. Although TWs generate multi-purpose by-products (e.g., harvesting leaf biomass for biofuel or fertilizer, re-use of treated WWW for irrigation, etc.), WWW is still largely viewed as a waste product [129]. Moreover, global climate change is expected to negatively impact the quality and quantity of treated WWW available for re-use [2, 7]. This predicted impact on water scarcity is also expected to intensify the reliance on re-using treated wastewater – forming a key role in sustainable water management practices [130]. Thus, WWW sustainability challenges within the wine industry must be addressed in the context of global climate change.

Various climatic drivers (e.g., temperature, evapotranspiration (ET), and wind) place a selective pressure on plants and microbes that can tolerate various hazards associated with the combination of the effects of these drivers, such as environmental salinization [131, 132]. Macrophytes, traditionally used in TWs, possess a low water use efficiency. These inefficient water users have an elevated water loss potential due to elevated ET processes, processes which are expected to be exacerbated by climate change [133]. Elevated water loss potential directly impacts the quality (i.e., concentration of contaminants within the TW system) and quantity (due to change in hydrologic regime, decreasing the volumetric flow of WWW passing through the system) of treated wastewater available for reuse [133, 134]. Thus, it is envisaged that climate change is likely to decrease the sustainability and performance of traditional biological treatment systems utilizing salinity-intolerant macrophytes [130].

This sustainability challenge may be addressed by the selection of efficient water use plant species and/or decreasing the hydraulic retention time to minimize ET [136]. Some terrestrial halophytes possess the ability to cross-tolerate a combination of environmental conditions, such as salt and drought stresses [137]. This is attributed to various adaptations including physiological processes such as the (i) density, size, and location of stomata (small pores regulating gaseous exchange), (ii) waxed epidermal layer in leaves (reducing transpiration water loss from the surface of the leaves), and /or (iii) small-sized leaves/ scales (reduction in surface area) which result in reduced transpiration and thus, water loss [138-140]. This further strengthens the case for utilizing terrestrial halophytes in the treatment of saline WWW, where these adaptations promote water use efficiency by halophytes while simultaneously tolerating a range of abiotic stresses affected by climatic drivers.

Thus, the criteria for selecting terrestrial halophytes in TWs for saline WWW treatment should be expanded to include the selection of species which (i) are easily propagated, (ii) are able to grow and survive under TW conditions, (iii) are able to (hyper)accumulate salts and their constituents (Na^+), (iv) are efficient water users with low ET, (v) have desirable modes of salt transport, accumulation and/or excretion (i.e., haloconduction), and (vi) simultaneously contribute to the removal of other inorganics such as N and/or P [18]. Thus, the potential use of terrestrial halophytes should be incorporated as an additional plant selection criterion in future studies for the treatment of saline wastewater by TWs, contributing to sustainable wastewater resource management within the wine industry [141].

This review highlights a gap in knowledge and proposes the novel, non-traditional use of terrestrial halophytes (which have been extensively studied for their phytoremediation potential in highly saline and heavy metal-contaminated terrestrial environments) for the remediation of saline WWW. Ultimately, the reduction of WWW salt load, along with other targeted inorganics, may reduce the negative impacts associated with the irrigation of land using treated WWW. This would ameliorate the negative impacts associated with the salinization and increase in soil sodicity – advancing the survivable conditions of numerous glycophytic plant species, including crops, and promoting food security in semi- and arid environments [142]. Solutions to these challenges have been described above and illustrated in Figure 2-2, a process diagram presenting potential solutions to challenges negatively impacting the TW remediation process and the sustainability of the wine industry.

Irrigation of cash crops tolerant of saline winery wastewater.

Globally, secondary salinization by irrigation has contributed to the salinization of approximately 20% of total cropland, which is predicted to increase to 50% by 2050 [143, 144]. Two themes to improve crop production under saline conditions have been researched, namely the (i) introduction of salt tolerance traits into glycophytic crops (i.e. genetic engineering), and (ii) domestication of halophytes as non-traditional crops [145, 146]. The use of halophytes for the non-traditional production of food (e.g. *Chenopodium quinoa* (quinoa)), proposed sources of halotolerant microorganisms (to increase tolerance of glycophytic crops), biofuels, and chemicals in arid areas (i.e. saline water with poor soil quality and high solar radiation) has received notable attention in the last few decades [147, 148]. Halophytes can therefore be grown in non-arable land under saline WWW irrigation without competing for nutrients and resources required by glycophytic crop species, ultimately promoting food security in arid and semi-arid areas where climatic drivers (e.g. evapotranspiration and extreme temperatures) are expected to increase amid global climate change [149].

The practical implications of selecting halophytes for use in phytoremediation or as a cash crop must consider the origin of the plant species. For example, *Eichhornia crassipes* (water hyacinth), a halophyte originating from South America, has been demonstrated to tolerate highly saline wastewater and accumulate various heavy metals and nutrients [150]. However, *E. crassipes* is considered one of the world's most prevalent invasive aquatic plant species, negatively impacting

socioeconomic development and the ecosystems they inhabit [151]. This has led to the legislated prohibition (e.g. South African National Environmental Management: Biodiversity Act – Alien Invasive Species Regulations, Act 10 of 2004) of growing and utilizing alien invasive plant species, such as *E. crassipes*, in phytoremediation trials or for recreational purposes. This highlights the need to select indigenous plant species, where the plant's potential spread outside of the TW would not negatively impact the receiving environment. It must be noted that *P. australis* is considered a cosmopolitan species with an elevated genetic diversity and phenotypic variation (Table 2-5) [152]. This suggests that *P. australis* rapidly adapts to various geographic regions (i.e. different environmental conditions) across the world, making the case to use *P. australis* as a model plant species to investigate the remediation potential of TWs across geographical ranges.

2.4.3.3 Plant-assisted bioremediation (plant-microbe interactions)

Plant-microbe interactions can enhance the remediation of inorganic and organic contaminants in wetland systems. The use of plants, in combination with rhizosphere and/or endophytic bacteria enhances the remediation of organic and inorganic contaminants compared with using phytoremediation in isolation. Plant roots provide (i) a surface for microbial adhesion, (ii) protection of microbial communities from environmental conditions, such as desiccation, (iii) a C source for microbial growth and activity (i.e. release of exudates, such as organic acids, sugars, and amino acids), and (iv) aerobic microenvironments for aerobic (heterotrophic and autotrophic) microbial activities – where plants release O_2 into the rhizosphere via aerenchyma adventitious roots [153-155]. In turn, microbes associated with the rhizosphere promote plant growth and survival by increasing the bioavailability of nutrients, such as P and N, for plant uptake, N fixation, protection against plant pathogens, as well as promoting resistance (i.e. avoidance or tolerance) to phytotoxic elements [78, 156, 157]. The composition of the microbial community is directly influenced by the plant species due to variation in (i) root system architecture, and (ii) rhizosphere pH (influenced by the release of exudates) [78, 157].

Rhizodegradation, or plant-assisted bioremediation, is a primary contributor to the remediation of organic contaminants [158]. By-products, produced by rhizodegradation processes may be taken up into the plant, translocated to aboveground biomass via the transpiration stream, and subsequently volatilized by evapotranspiration (i.e. phytovolatilization). This process enhances the plant's ability to remove elevated concentrations of contaminants and/or their transformed/

degraded species from TWs [159, 160]. Moreover, plant-assisted bioremediation has long been demonstrated to significantly increase the assimilation of contaminants within wetland substrate [161] when compared with non-planted wetlands. This reduces the mobility, and thus the toxicity, of contaminants within aquatic and terrestrial (e.g. via treated WWW irrigation) environments, decreasing contaminants entering the food chain through plant uptake and accumulation in aboveground biomass.

Complex biological interactions associated with plant-assisted bioremediation of WWW limit the comparability and reproducibility of studies. This is attributed to the inability to effectively distinguish the role of plants (phytoremediation component), microbes (bioremediation component), microbe-microbe/ plant-plant interactions (i.e. intra- and interspecies competition), as well as the plant-assisted bioremediation components utilized in TWs. Moreover, these biological, along with hydrogeochemical, contaminant removal pathways occur simultaneously and fluctuate through space and time [162]. The degree to which plants contribute to the overall remediation potential of TWs differ between studies. For example, Zhang *et al*. [163] demonstrated that plants play a significant role in the remediation of certain pharmaceutical contaminants whereas other studies have demonstrated no significant difference between contaminant removal efficiencies between planted and unplanted TWs. This further highlights the complexity of determining the efficacy of TWs treating WWW as the fate of contaminants (e.g. competitive ion adsorption, composition of contaminants), functional components (media, plant species, and environmental conditions) and operational parameters differ between studies (Supplementary Table A2-1). In order to ensure that the role of plants is not masked, studies should ideally consider a range of relevant parameters that may affect the speciation, bioavailability, and toxicity of WWW contaminants on plants. These include environmental factors such as pH, electrical conductivity, redox potential, temperature, and O_2 availability of WWW.

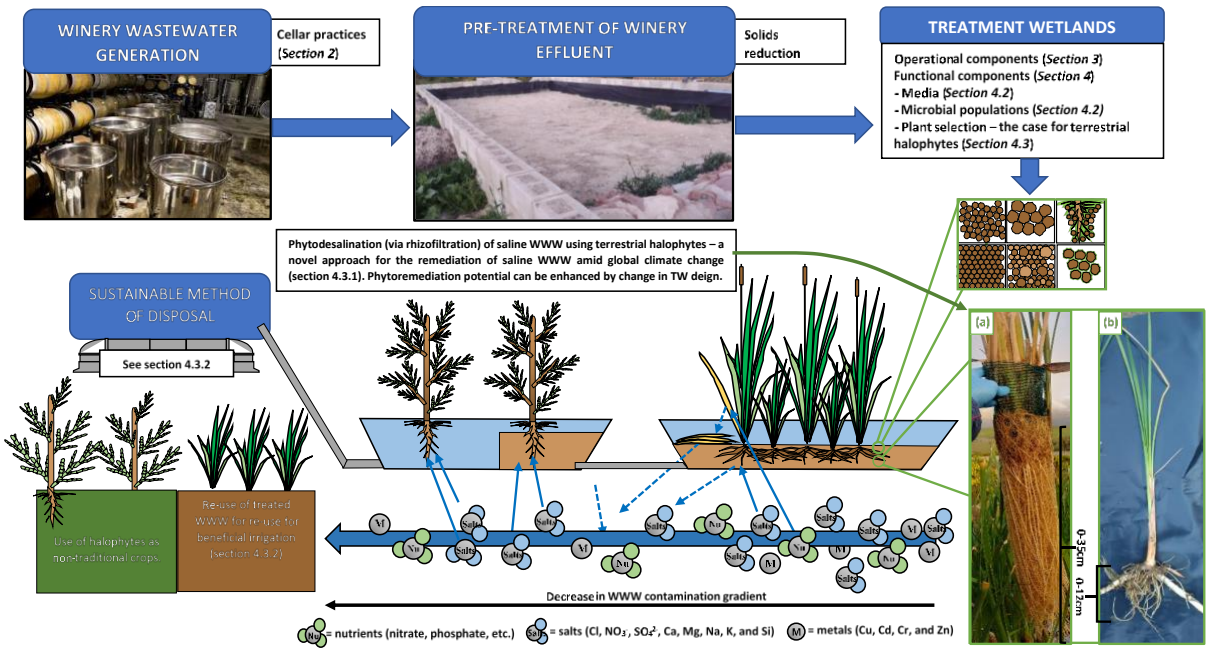


Figure 2-2 Schematic depicting the contents of this review

2.5 Conclusion

Treatment wetlands, comprised of biological and hydrogeochemical components, have been used to remediate wastewater originating from various anthropogenic sources. The systems have proven effective for reducing the organic load and neutralising the pH of WWW, but concurrent or downstream removal of inorganics remains a challenge. Removal of inorganic macronutrients (N,P,K) is important if the treated WWW is discharged into aqueous environments, but not if the effluent is to be used for irrigation. While N compounds can be mineralised, P is removed chiefly by media adsorption, so negative removal rates can be found once the binding sites are saturated. In line with circular economy principles, there is a need for studies aimed at capturing P from WWW for re-use. Other inorganics found in high concentrations in WWW (Na^+, K^+) are not removed by microbial action, nor adsorbed by the TW substrate. This creates a selective pressure for the use of halophytes which have the elevated ability to (hyper)accumulate these inorganics (effectively removing these cations from WWW) and cross-tolerate a range of stresses. This review also presents the case for the use of terrestrial halophytes in TWs. Terrestrial halophytes have the ability to (hyper)accumulate and cross-tolerate a range of contaminants, along with the ability to control operational parameters of TWs (i.e. artificial aeration) - creating viable alternatives to the selection of macrophytes currently used world-wide. Salinization is a growing issue compromising food security due to the inability

of glycophytes to survive under saline conditions. The non-traditional use of halophytic crops which can be grown in non-arable, saline land under WWW irrigation, is a viable solution ultimately promoting food security in arid and semi-arid environments, where fresh water for irrigation is limited.

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N/A

Supplementary data Appendix 2

Reference List

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Chapter 3

Comparison of continuous and pulse mode of operation of pilot biosand reactors treating winery effluent

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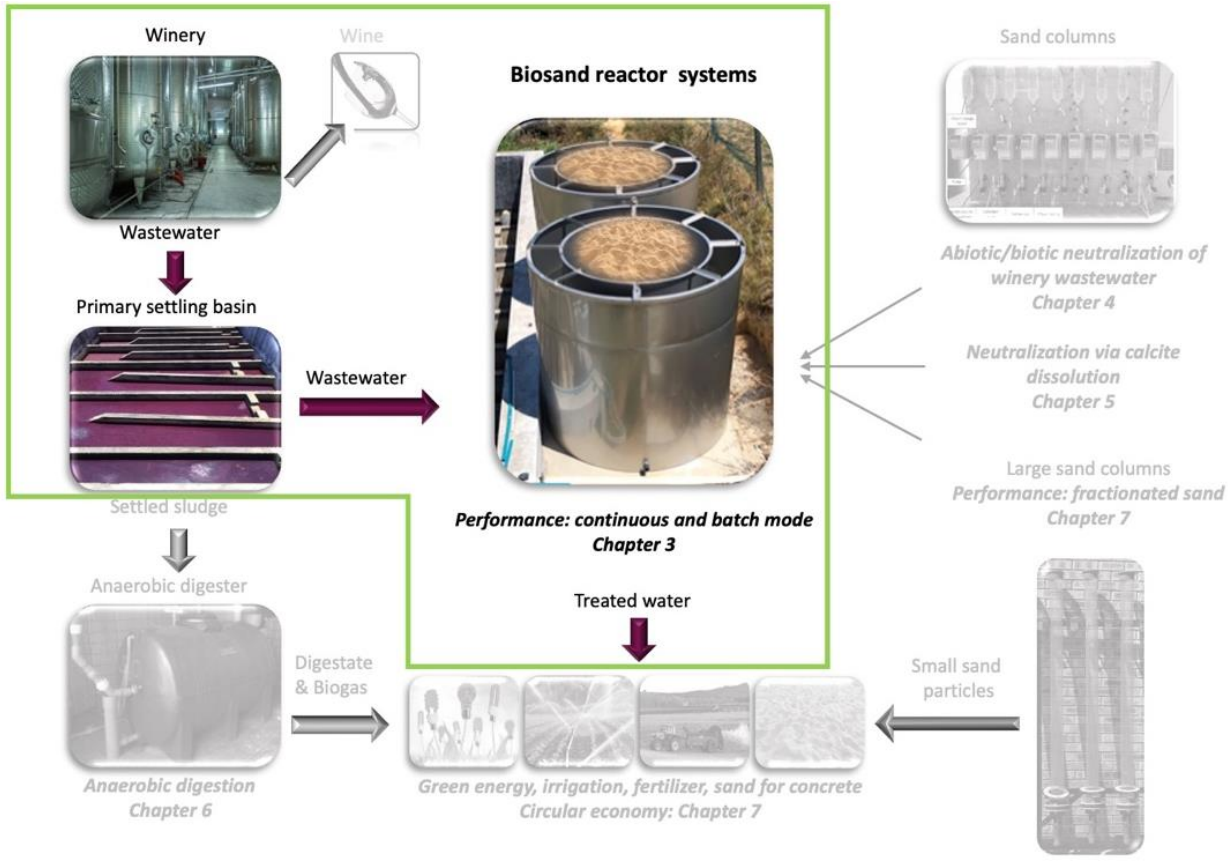
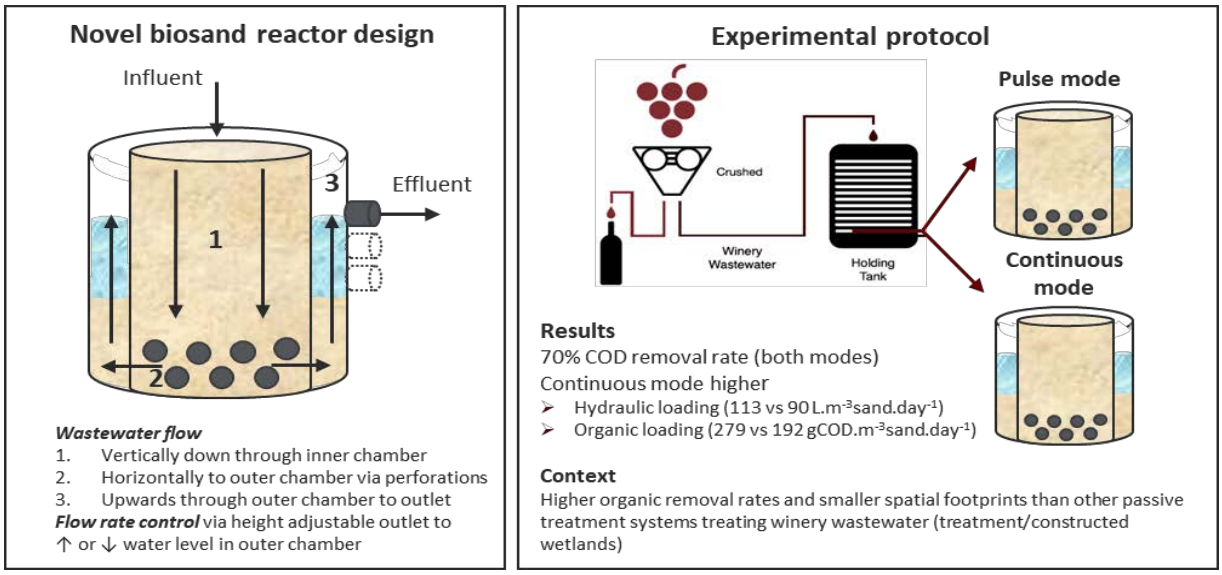
Offprint available in Appendix 3



[Presents the findings of a study comparing the performance of a novel pilot BSR system operated in continuous and pulse mode, and compares the results with other passive treatment systems in terms of loading rates and spatial footprints.]

Graphical abstract



Abstract

In 2020 there was approximately 260 million hectolitres of wine produced across the world. Many winemaking and cellar cleaning activities generate winery wastewater. In vine growing areas which are water stressed, this wastewater is often used for irrigation and if it is inadequately treated it can be detrimental to land and aquatic environments. It has been shown at pilot scale that horizontal flow biosand filters are suitable for treating winery effluent to comply with irrigation standards. In this two year study, vertical flow biosand reactors with novel design features were operated in both continuous and pulse modes of operation. It was envisaged that (i) the loading rates (organic and hydraulic) could be increased by changing the flow from horizontal to vertical flow, and that (ii) higher organic biodegradation rates could be achieved consequent to the increased redox potential from draw-down of atmospheric oxygen during system drainage in pulse mode in comparison to continuous mode. It was found that system performance was higher in continuous mode, attaining a hydraulic loading rate of 113 $L.m^{-3}$ of sand a day^{-1}, organic loading rate of 279 $gCOD.m^{-3}$ of $sand.day^{-1}$ and COD removal efficiency of 70% compared to pulse mode with 90 $L.m^{-3}$ of sand a day^{-1}, 192 $gCOD.m^{-3}$ of $sand.day^{-1}$ and 70%, respectively. In comparison to other passive winery wastewater treatment systems (constructed/treatment wetlands), these biosand filters are able to treat winery wastewater at higher loading rates with smaller spatial footprints.

Keywords: chemical oxygen demand, constructed wetland, hydraulic loading, organic loading, pH neutralisation

3.1 Introduction

In 2020 there were approximately 7.3 million hectares under vine across the world, producing 260 million hectolitres of wine. Of this, 105.8 million hectolitres, valued at 29.6 billion Euros were exported [1]. The practice of wine making relies on the beneficial processes which turn sugars into ethanol, as well as the formation of organic compounds which enhance the aroma and flavour of the end-product. Winemaking generates different waste streams, including winery wastewater (WWW). Due to different seasonal cellar activities, the quantity and composition of WWW fluctuates, not only seasonally, but also from cellar to cellar. Conventional secondary wastewater (WW) treatment systems require a consistent influent in both quality and quantity. Inconsistency may result in poor treatment performance during periods of heavy hydraulic and/or organic loading. In the case of WWW, treatment systems may also be redundant during several months of a year due to low flows, requiring repeated start-ups. For these reasons, WWW may not be adequately remediated if treatment systems are unable to adapt to rapid changes.

In water stressed areas such as South Africa, Australia and parts of the United States, WWW is often reused for irrigation of pastures or crops. Inadequately treated WWW can pose a threat to the soil and/or groundwater safety and security [2,3].

Biosand reactors (BSRs), alternatively designated as biological sand filters, are similar to unplanted sand filled constructed/treatment wetlands (CW/TW). They are low cost, low maintenance, sustainable and energy efficient systems that can be used for treating WWW [4,5]. Biosand reactors have shown promising results for remediation of WWW, from laboratory-based experiments [6,7] to a pilot-scale reactor system [4]. They have proven capable of achieving high levels of treatment and providing safe effluent for irrigation while being able to readily adapt to the temporal changes of WWW and protracted shut down periods. A gravity-fed horizontal pilot system was able to effectively reduce the organic load and neutralise acidic WWW while increasing the sodium adsorption ratio [4]. The system was however only suited to very small wineries because the achievable flow rates were low (402 $L.day^{-1}$). The low flow rates translate into large spatial footprints for the system.

In order to increase the flow rates in comparison to the original pilot BSR system while maintaining high organic removal rates (ORR), in-depth studies were conducted on more sophisticated, vertical flow BSRs with a novel design *in-situ* at a local winery in South Africa. These

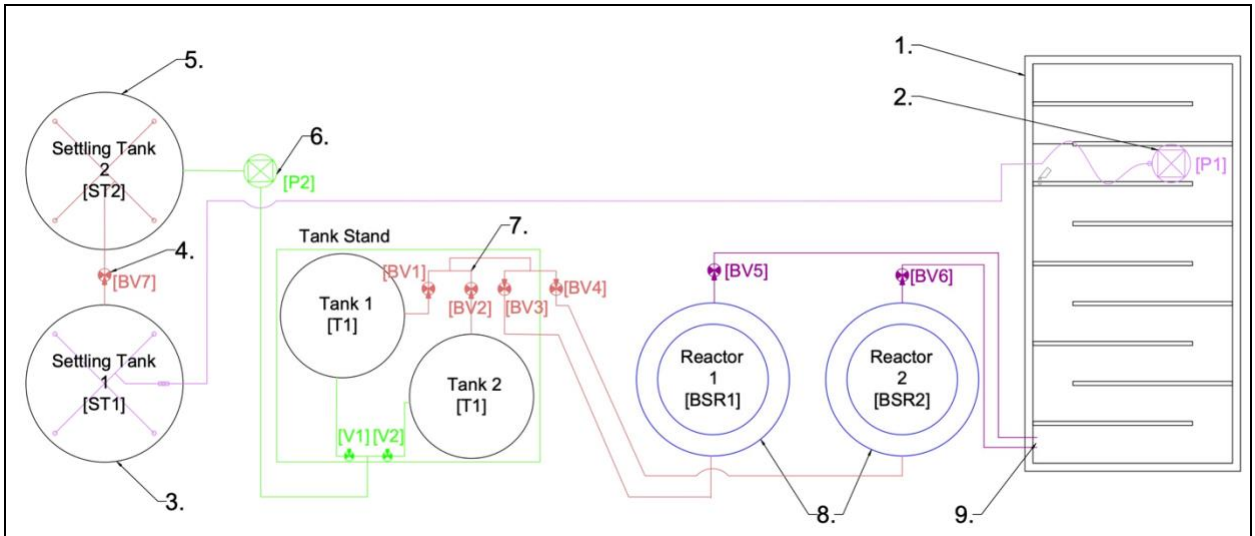
were operated alternately in either continuous or pulse mode over two crush seasons to ascertain which mode of operation yielded the most efficient performance. The operational and performance results are presented in the manuscript and results are compared with other passive systems treating WWW in terms of the hydraulic loading rate (HLR), organic loading rate (OLR) and ORR.

3.2 Materials and methods

3.2.1 Set-up and operation of pilot scale biosand reactor system

The vertical flow BSR treatment system was a pilot system treating a fraction of the WWW. At full-scale, it is intended to treat a significant portion of the WWW generated by the winery in order to improve the overall quality of the effluent for irrigation, thereby protecting the soil environment. The treatment system was designed around the extraction of WWW from an existing baffled concrete solids settling delta to a series of holding tanks which acted as additional settling tanks and rudimentary anaerobic digestors (Figure 3-1). Each BSR was inoculated evenly at the top with 500 g of sand from an existing horizontal flow BSR used to treat winery effluent. The sand was then further acclimated by intermittent feeding with WWW 3 months before the start of the crush season in year 1 of the study. Due to technical problems with the control system during start-up, the BSRs were not feed as often as planned. The BSRs were fed with the settled, pre-digested WWW and the final effluent flowed via gravity back into the settling delta from where the effluent was fed into a holding dam used for irrigation (Figure 3-1). More specifically, the WWW was extracted from the delta via a submersible sludge pump into a 5000 L settling tank (ST1) via 4 upward facing inlets when the liquid level in ST1 dropped to 50%. To reduce disturbance of solids, the inlets were located 1 m meter from the bottom of the tank. After a settling period of 120 minutes, the settled WWW was fed by gravity via a floating outlet pipe controlled by a 1” full bore electronic ball valve into a second 5000 L settling tank (ST2). The outlet was located 10 cm below the liquid surface level of ST2 to prevent extraction of floating biomass or foam. After a settling period of 120 minutes, the WWW was extracted via a centrifugal pump to two 2500 L holding tanks (T1, T2) elevated on a steel tank stand. T1 and T2 were fed and emptied alternatively, the flows to T1 and T2 being controlled by two solenoid valves (V1 and V2). The two BSRs (BSR1, BSR2) were fed from T1 outlet controlled via BV1 or T2 outlet controlled via BV2 which were connected via a manifold. BSR1 inlet was controlled via BV3 and

BSR2 controlled via BV4 while BSR1 outlet was controlled by BV5 and BSR2 by BV6. The system was set up so that BSR1 and BSR2 could be operated in continuous/pulse or continuous mode of operation. The entire system's logic was controlled via a RievTech micro PLC (PR-14DC-DA-R) and two expansion units (PR-E-16DC-DA-R) and a series of analogue and digital inputs and relay outputs together with basic remote control via an Accentronix Infinity Cellswitch.



1. Existing concrete settling delta 2. Submersible sludge pump [P1] extracts wastewater from the existing delta to [ST1]. 3. [ST1] fills via 4 upward facing inlets 1 m above the bottom. Solids settling time = 120 min. 4. A 1" ball valve [BV7] controls the gravity flow from 5000 L [ST1] to identical [ST2]. 5. [ST2] is filled after the rest period of [ST1]. Both [ST1] & [ST2] have floating outlet pipes 100 mm below the surface.	6. Centrifugal pump [P2] extracts the settled supernatant from [ST2] and fills two 2500 L tanks [T1] & [T2] on tank stand. The flow direction is controlled by two solenoid valves [V1] & [V2]. 7. Water flows alternatively via gravity from [T1] and [T2]. Flow and mode of operation is controlled by ball valves [BV1] and [BV2]. 8. The Biosand reactors [BSR1 and [BSR2] can be operated in continuous or pulse fed mode of operation. 9. Treated wastewater is fed back into the settling delta

Figure 3-1. Design layout schematic of the treatment system

3.2.2 Sampling and characterisation of influent and effluent

Grab samples were extracted from sampling ports in ST1. Influent and effluent samples were taken from the influent lines to and outlet points to/from BSR1 and BSR2, respectively using the schedule provided in Table 3-1. Intense sampling (Table 3-1) and monitoring was conducted during the crush season only (Day 1 to 72 year 1 and day 1 to 58 year 2) because (i) approximately 61% of winery effluent is generated during the crush season at this winery (ii) the organic load is highest during the crush season [8], and (iii) it has already been shown that performance of BSRs is only under stress during the crush season [4]. Start-up commenced on 13th February in year 1

and sampling began on the 20th of February (day 1). BSR1 was operated in pulse mode and BSR2 in continuous mode from day 1 to day 43, after which the order was reversed until day 72. Similarly, in year 2 BSR1 was operated pulse mode and BSR2 in continuous mode from day 1 to day 36, after which the order was reversed. Sampling and monitoring were terminated early in year 2 (day 58) because winery operations were interrupted due to an enforced hard lockdown as a result of the COVID-19 pandemic. Nevertheless, sufficient results were obtained in order to assess system performance. The system continues to operate effectively to date (year 4).

Table 3-1 Sampling schedule for the study period

Influent and effluent to BSR1 and BSR2: COD, VOA, Total phenolics, pH, Electrical conductivity, TP, TN, Alkalinity, Na, Ca, Mg, K, P, Al	Influent and effluent to BSR1 and BSR2: COD, VOA, Total phenolics, pH, Electrical conductivity, Alkalinity	Settling tank: COD, VOA, Total phenolics, pH, Electrical conductivity, TP, TN, Alkalinity, Na, Ca, Mg, K, P, Al
Year 1 crush BSR1 pulse BSR2 in continuous mode		
Days; 1,10,14,16,22,29,34,37,43	Days: 6,24,27,31,38,41,45	Days: 16,37,43
Year 1 crush BSR1 continuous BSR2 in pulse mode		
Days: 50,57,64,72	Days: 48,55,59	Days: 50,57,64,72
Year 2 crush BSR1 pulse BSR2 in continuous mode		
Days: 1,8,22,36	Days: 3,6,8,13,17,20,24,27,31,34	Days: 1,8,22,36
Year 2 crush BSR1 continuous BSR2 in pulse mode		
Days: 43,52,57	Days: 38,41,45,55,58	Days: 43,57

BSR= biosand reactor, COD = chemical oxygen demand, VOA = volatile organic acid, TP = total phosphorous, TN = total nitrogen, ALK = total alkalinity, SALTS = Calcium, magnesium, potassium, sodium, aluminium and phosphorous

3.2.2.1 Analytical procedures

The concentrations of COD, total phosphorus (TP), total nitrogen (TN), alkalinity and volatile organic acids (VOA), were determined using a Merck (Merck®, Whitehouse Station, USA) Spectroquant® Pharo instrument and Merck Spectroquant® cell tests or kits according to manufacturer's instructions as previously described [4]. The total phenolic concentrations were determined using the Folin Ciocalteau method as previously described [4].

3.2.2.2 Determination of pH, temperature and electrical conductivity

Probes were placed inside ST1 and ST2 to monitor electrical conductivity (EC, B&C C7335, K = 1.0, Carnate), temperature (PT100), pH (van London co. P822, Houston) and connected to an analog converter (Acdc Dynamics TRT-PT100). Data was recorded to an SD card connected to a micro PLC (RievTech PR-14DC-DA-R and PR-E-16DC-DA-R, Nanjing).

The pH of the lab samples was determined according to the manufacturer's instructions using a CyberScan pH300 meter and appropriately calibrated pH probe PHWP300/02K (Eutech instruments, Singapore).

3.2.3 Calculation of operational parameters

3.2.3.1 Flow rates, volume of wastewater treated and electricity consumption

The flow rate data was logged via two Kamstrup Multical 21 (02146VO1N94) 20 mm ultrasonic flow meters. This data was recorded by a Kamstrup Omnipower single phase electricity meter which also monitored the system's electricity consumption.

3.2.3.2 Hydraulic conductivity, hydraulic loading rate, organic loading rate

To counter biases from flow variability, the flow rate (Section 3.2.3.1) was converted to a 3-day lagging average for calculation of the HLR and OLR.

In CWs, the HLR or surface loading rate (SLR) and OLR are typically only calculated using the surface area of a wetland. In this study, the HLR and OLR were also determined using the entire volume of the reactor as previously described [4,5]. The ORR was determined by multiplying the OLR by the removal efficiency in terms of the COD.

3.3 Results and discussion

3.3.1 Performance of biosand reactors

The BSRs were evaluated in terms of (i) organic removal efficiency (COD, VOA, total polyphenolics), discussed in Section 3.3.1.1, (ii) inorganics and pH changes (pH, EC, alkalinity), discussed in Section 3.3.1.2, and, the (iii) hydraulic performance, discussed in Section 3.3.1.3. This is followed by (iv) a critical evaluation of the OLR, HLR and ORR that were achieved in the BSRs when operated in continuous and pulse modes, and comparison of the results to other passive systems treating WWW, discussed in Section 3.3.1.4.

3.3.1.1 Organic removal performance

The COD of samples for year 1 and year 2 are shown in Figure 3-2A. Since an ideal pre-crush start-up/acclimation period could not be achieved in year 1 (Section 3.2.1), the COD removal efficiency

was low (<50% for the first 3 weeks of operation). However, performance increased significantly over time. It can be seen in Figure 3-2A that the COD concentration in the final effluent decreased with length of operation of the system during year 1. Overall, there was an average 88% (54–97%) COD removal efficiency in year 1 and 73% (33-93%) in year 2. The lower efficiency in year 2 may be attributed to differences in WWW characteristics and sampling periods related to variances in year-on-year cellar activities. The original vertical flow on-site pilot BSR system [4] operated with a notably lower influent COD than the system described in this study over the two years of operation, however, COD removal rates were similar (70% vs. 79%), indicating notably superior COD removal performance with the new system. In terms of VOAs, in some instances they were formed within the BSRs as metabolic products, as previously demonstrated with WWW at laboratory scale and within settling basins [8,9].

Polyphenolics in WWW need to be reduced before discharge of the effluent because they may be toxic to microbes and plants [10,11]. In this study, the average removal efficiency of total polyphenolics during the monitoring period was 75%. The results compared favourably with the 77% (influent 18.4 mgGAE.L^{-1}) achieved with original horizontal flow system [4] but at higher flow rates (Section 3.3.1.3). These results confirmed the ability of BSRs to effectively reduce polyphenolics in WWW.

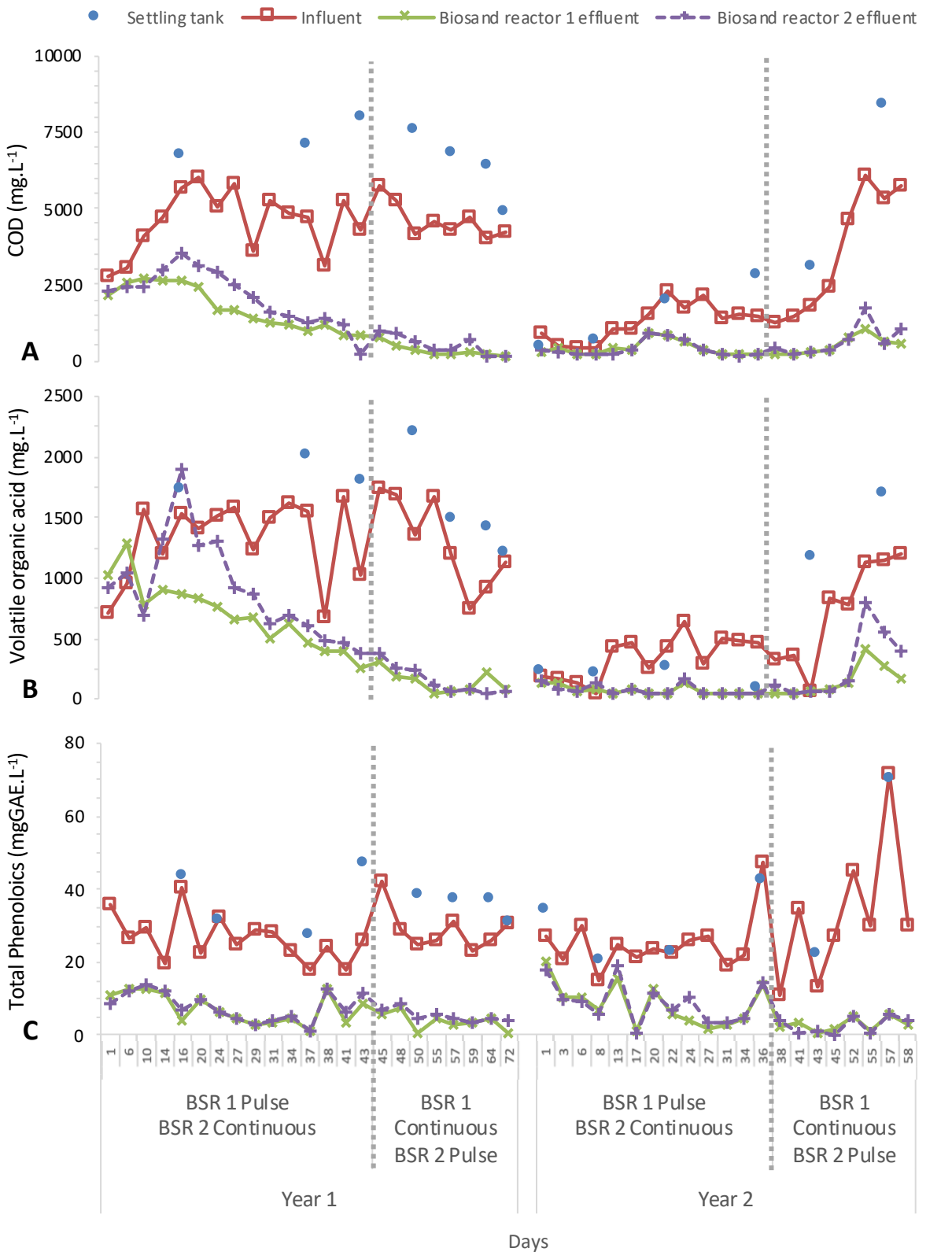


Figure 3-2. The A) chemical oxygen demand, B) volatile organic acids, C) total phenolics samples taken from the biosand reactor treatment system during the pre-crush and crush period for year 1 and year 2

3.3.1.2 Assessment of pH and inorganic changes

The pH of the WWW depends on the on the seasonal activities taking place in the cellar [8], but it is typically acidic [12,13]. In this study, the pH in ST1 ranged from 4.5 to 5.1 and 4.8 to 8.4 in year 1 and year 2, respectively (Figure 3-3. A). Overall, the BSRs increased the pH of the acidic effluent to > 6 throughout the sampling periods. Similar results were found previously in a system containing sand from the same quarry site [4].

The average electrical conductivity (EC) from ST1 for both periods was 1735 us.M^{-1} with respective averages for year 1 and 2 of 1690 us.M^{-1} and 1779 us.M^{-1} (Figure 3-3C). Over the 2-year sampling period, the average EC increased by 75% from influent to effluent mainly due to the dissolution of calcite in the sand which was also responsible for pH buffering as previously described [4,14]. The dissolution of calcite was also largely responsible for the increase in alkalinity from BSR inlet to outlet in year 1 and year 2 (Figure 3-3C), also as previously described [4].

The respective average influent TN and TP concentrations for year 1 were 1.4 mg.L^{-1} and 10.1 mg.L^{-1} and 31.8 mg.L^{-1} and 16.1 mg.L^{-1} in year 2 (data not shown). The average effluent concentrations from the BSRs in year 1 were 9.6 mg.L^{-1} TN and 2.4 mg.L^{-1} TP (ranges: 0 to 52 mg.L^{-1} and 0.2 to 24.4 mg.L^{-1} for TN and TP, respectively). In year 2, the average effluent TN from was 149.6 mg.L^{-1} (7.3-710 mg.L^{-1}) and average TP was 29.5 mg.L^{-1} (range: 4.8-67.5 mg.L^{-1}) (results not shown). The intermittent negative removal rates of TN may be attributed to microbial atmospheric N_2 fixation as previously described in BSRs [15]. From a practical perspective, the high effluent TP, and to a lesser extent high TN results may be seen as problematic in cases where the treated effluent is disposed directly to aquatic environments because of the risk of eutrophication. However, if the WWW is being used for irrigation purposes, as intended with the BSR systems, the presence of N and P is seen as beneficial as they are essential plant nutrients [5]. Therefore, unlike many secondary wastewater treatment systems, removal of N and P is not a treatment objective.

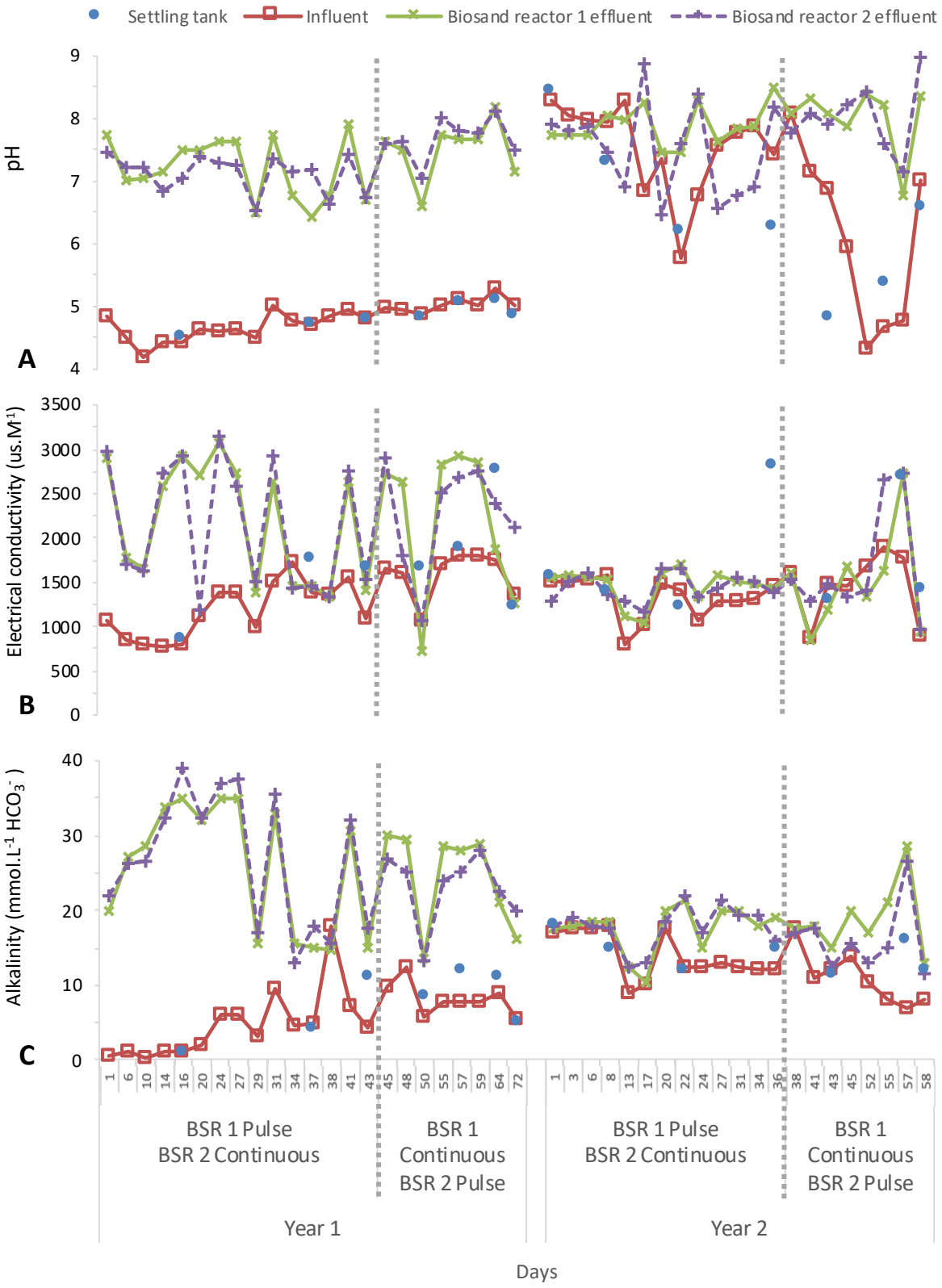
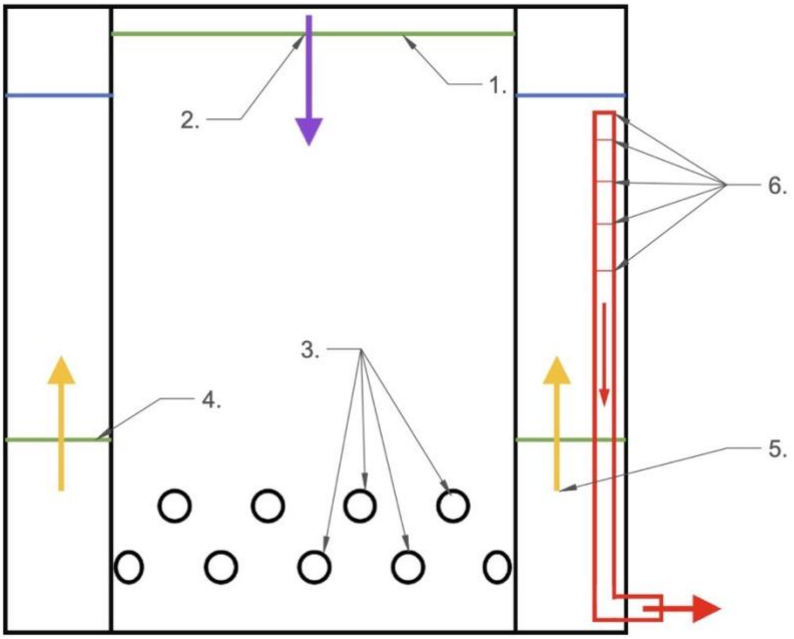


Figure 3-3. The A) pH, B) electrical conductivity, C) alkalinity of samples taken from the biosand reactor treatment system during the pre-crush and crush period for year 1 and year 2

3.3.1.3 Hydraulic capacity and performance

The BSRs used a novel design to increase the hydraulic performance while maintaining good pH neutralisation and organic removal performance, which was the primary objective of this study. It is envisaged that under normal operational circumstances, the fall across the BSRs (from the inside to the outside chamber) will be adjusted according to operational needs to increase or decrease flow rates, making the systems hydraulically versatile. In the study systems, the fall can be increased to up to 1700 mm during general operations to increase flow rates during high biomass and/or organic matter build-up (Figure 3-4). However, for the purposes of the study, changes made to the fall during either of the two monitoring periods (year 1 and year 2 crush seasons) would have introduced an experimental variable, so no adjustments were made during either year. Nevertheless, no permanent clogging occurred in the BSRs and the temporal reduction in hydraulic conductivity (HC) was attributed to accumulation of functional biomass (microbial growth). The novel design and unrestricted flow from the inner to outer core ensured no clogging within or at the outlets of the BSRs.

In year 1, the fall was maintained at 200 mm, with a sand height of 1500 mm. Over the year 1 crush period, the BSRs used on average 0.97 $Kw.day^{-1}$ of electricity or 437 $L.Kw^{-1}$, to treat 30.2 m^3 of WWW at a flow rate of 425.3 L of WWW per day (205.5 $L.day^{-1}$ and 219.7 $L.day^{-1}$ in BSR1 and BSR2, respectively), with hydraulic flows of 659 $L.day^{-1}$ and 483 $L.day^{-1}$, respectively over the first two weeks (results not shown). This reduced to a flow of < 100 $L.day^{-1}$ at the end of the crush season. It was theorised that the low flow rates were due to either (i) inorganic solids that may have entered the BSRs before the settling tanks were installed, and/or (ii) a lack in fall across the BSRs. Due to the unique design of the BSRs (Figure 3-4), assumptions were made during the calculation of the fall that were not validated at a practical level. A new model was therefore applied that included a reduction in height of the sand in the outer chambers and reduction of the outlet height of the BSRs to increase the overall fall. It was calculated that this would result in an increase in treatment capacity to 1000 $L.day^{-1}$ for each BSR.



1.	Level of sand within the central core
2.	Wastewater is fed onto the surface of the biosand reactor via four float valves and flows vertically downwards within the central core
3.	The wastewater flows laterally from the central core to the outside chamber via peripheral holes
4.	Level of sand within the outside chamber
5.	The wastewater flows vertically upwards within the outer core to the outlet pipe
6.	The outlet pipe: the height can be adjusted to increase or decrease the flow rate by manipulating the hydraulic head

Figure 3-4 Section view of the novel design of the biosand reactors

The operation of the system was suspended at the end of the year 1 monitoring period. At this point, the flow of incoming WWW into the delta had reduced to an impractical rate, and the surface of the delta was covered with organic sludge that required removal. Some adjustments were made, and the fall was increased to 800 mm in year 2 with a sand height of 300 mm in the outside chamber. Over the year 2 crush period, the combined BSRs treated an average of 1017 L WWW.day^{-1}, and a total volume of 66.0 m^3 WWW and used on average 1.44 Kw.day^{-1} or 705 L.Kw^{-1}. The initial two-week flow rates were 790 L.day^{-1} and 1057 L.day^{-1} for BSR1 and BSR2, respectively. However, there was a reduction in flow rate over the treatment period to 500 L.day^{-1}. A build-up of organic matter was noted on the surface which was reducing the ingress of WWW into the top layer of sand, thereby reducing the overall HC of the BSRs. As with previous studies, the organic matter (including some microbial biomass) in the BSRs degraded during the off-crush period, restoring good flow rates [4]. This however takes time and does not rectify the problem experienced during the crush period itself. It is therefore suggested, as a simple remedy, that the organic layer is scarified in the affected areas on a weekly basis during the crush season to mitigate flow reduction in BSRs.

In the off-crush (non-monitoring) period of year 2, the system was re-started with both BSRs operated in continuous mode. Over the first week, the average flow rate was 3854 L.day^{-1} for both BSRs combined, showing the effect of increased fall on the initial rates and the potential recovery of HC due to biomass degradation. The rates then dropped to 871 L.day^{-1} and

426 L.day^{-1} or BSR1 and 2, respectively by the third week and reduced further thereafter. It was hypothesized that this was an artefact attributable to a partial blockage in the influent line entering the ultrasonic flow meters - for experimental purposes, the influent WWW flow rates were monitored by ultrasonic meters which have small pre-filters. The hypothesis was validated by the fact that electricity was still consumed by the pump and the PLC logged events during periods when the ultrasonic meter recorded no flow.

3.3.1.4 Hydraulic and organic loading rates

The treatment capacity of CWs, and by inference BSRs, is critical. Low HLRs translate into large spatial footprints with a larger area to source, operate and maintain. In CWs, the HLR/SLR and OLR are typically calculated using the surface area of the CW because they are limited by O_2 transfer to the root systems of the wetland plants. BSRs have no such limitations, and the loading rate calculations can be based on the cross-sectional area in the direction of flow, the functional surface area (FSA) (HLR_{FSA} and OLR_{FSA}) or the entire volume of the reactor (HLR_{Vol} and OLR_{Vol}). The latter are more accurate efficiency indicators, especially when comparing the efficiencies obtained with different types of media [4,14].

The performance of the BSR system was compared with previously published data on other passive systems (CW/TWs) treating WWW specifically focusing on systems with reported porosities, which ranged from 25 to 40% and in the different studies and this study 29% (Table 3-2). If provided, the OLR and HLR in these studies were reported as HLR_{FSA} and OLR_{FSA} in the cited manuscripts. Wherever possible, the HLR_{Vol} and OLR_{Vol} were also calculated from available data and included in Table 3-2. Loading rates in terms of the volume of reactors allow a more accurate comparison of the treatment performance of different media and operation modes [4,5]. However, this still includes the volume taken up by the inert media and actual biodegradation of the WWW takes place within the pore spaces. In order to more accurately compare the actual biodegradation rates (i.e. microbial activity) from system to system, this was calculated using the porosity of the media to calculate the volume of voids $V_{Voids} = V_{VLF} = V \times Porosity$ (Eq. 1) and this value to calculate the OLR in the void liquid fraction (OLR_{VLF}) $OLR_{VLP} = OLR_{Vol}/V_{VLF}$ (Eq. 2) (Table 3-2). The same equation (Eq. 2) was used to determine the HLR_{VLF} and ORR_{VLF} for comparison of the different systems.

In terms of the $HLR_{Vol,}$ results obtained during year 1 compared favourably with most other studies, while in year 2, the average HLR_{Vol} was higher than the other passive systems with the exception of other BSRs [4,14] and a gravel-filled horizontal flow CW [16]. The highest OLR_{Vol} applied was in a horizontal flow gravel-filled CWs at a large winery/distillery in South Africa [17,18]. The primary reason for the high OLR_{Vol} was that the average influent COD was highest at this site (14000 $mgCOD.L^{-1}$). Due to the average influent COD to the study BSRs being either higher (year 1: 4568 $mgCOD.L^{-1}$) or comparable (year 2: 2148 $mgCOD.L^{-1}$) to the remaining systems that were included in the comparison (535 – 4720 $mgCOD.L^{-1}$), and the relatively high HLR_{Vol}, comparatively high OLR_{Vol} were applied in the BSRs.

Most notably, in terms of the average $ORR_{Vol,}$ the BSRs substantially outperformed all of the other passive systems included in the comparison over the 2-year study period (Table 3-2). When the ORR considered only the void fraction of the different media (ORR_{VLF}), the difference in magnitude of the results obtained in the BSRs and the other systems increased even further (Table 3-2). These results strongly suggest that the sand used in the BSRs provides a superior matrix for attachment and activity of the functional microbes responsible for biodegradation of organics in WWW, a significant finding. The HLR, OLR and ORR of the BSRs during different modes of operation are provided in Table 3-3.

The BSRs were also compared with other passive treatment systems terms of the hydraulic treatment capacity (HTC) (Table 3-2), as systems that occupy large tracts of valuable land that can potentially be used for viticulture are not desirable [19]. The HTC was calculated as the area of land required to treat 1 m^2 of wastewater d^{-1} $HTC = Area/Flow$ (Eq. 3). The spatial footprints of the BSRs were notably lower than all the other systems. Some of these systems consisted of more than one TW in series [2,6,7,23], each having their own spatial footprint. The spatial footprint of the study BSRs of 13.3 $m^2.m^{-3}$ $WW.day^{-1}$ and 5.6 $m^2.m^{-3}$ $WW.day^{-1}$ for year 1 and 2 respectively was notably lower that for the other systems (22.2 to 150 $m^2.m^{-3}$ $WW.day^{-1}$).

Table 3-2 Comparison of operational and design parameters and performance of wetlands treating winery wastewater

Area	Functional volume			Void liquid fraction			Reactor	Refs.
HLR	HLR_{Vol}	OLR_{Vol}	ORR_{Vol}	HLR_{VLF}	OLR_{VLF}	ORR_{VLF}	HTC	
mm.d^{-1}	L.m^{-3}.d^{-1}	g COD.m^3.d^{-1}	g COD.m^3.d^{-1}	L.m^{-3}.d^{-1}	g COD.m^3.d^{-1}	g COD.m^3.d^{-1}	m^2.m^{-3} WW.d^{-1}	
14.6*	41.7*	32.9 to 124.5	26.6 to 114.5*	131.7*	103.9 to 393.2*	84.2 to 361.7*	50.0	[20]
7.3*	20.8*	16.4 to 62.1	13.3 to 57.1*	66.4*	52.3 to 198.2*	42.4 to 182.3*	100	
14	14 (VF)	152* (VF)	NG	UTC	UTC	UTC	9.6	[21,22]
24.8	43-82 (HF_1)	54* (HF_1)	38.3*	107.5-205* (HF_1)	135* (HF_1)	95.9* (HF_1)	57.7	
36.3	22-41 ($HF_{2,3}$)	27* ($HF_{2,3}$)	19.1	55-102.5* ($HF_{2,3}$)	67.5* ($HF_{2,3}$)	47.9* ($HF_{2,3}$)	57.7	
77-215	55-154* (VF)	31-333* (VF)	NG	UTC	UTC	UTC	9.6	[16]
13-36	43-120* (HF_1)	12-183* (HF_1)	3.5 to 128.1*	107.5-300* (HF_1)	107.5 to 457.5* (HF_1)	29-320.3* (HF_1)	57.7	
13-36	22-60* ($HF_{2,3}$)	6.0-92* ($HF_{2,3}$)	1.4 to 64.4*	55-150* ($HF_{2,3}$)	50.0-230* ($HF2_{,3}$)	3.5-161.0* ($HF_{2,3}$)	57.7	
34	28*	37-176*	35.9 to 174.2*	77.8*	102.8 to 488.9*	99.7 to 484*	29.8	[23–25]
333*	150	152	120.1*	517.2*	524.1*	414*	17.8	[4,14]
23	1) 25*	1)350*	19.3 to 22*	65.7*	71.4 to 71.4*	55 to 62.8*	43.9	[17,18]
45	2) 50*	2) NG	UTC	142.9*	UTC	UTC	22.2	
78.3	57.0	260.4	177.0	196.6	897.9	610.3	13.3	This study Year 1
206.5	150.3	322.9	232.3	518.4	1113.4	801.1	5.6	This study Year 2

HF = horizontal (subsurface) flow COD = chemical oxygen demand VF = vertical (subsurface) flow, VLF = void liquid fraction. UTC = Unable to calculate, * = Calculated HLR_{Vol} = volumetric hydraulic loading rate, OLR_{Vol} = volumetric organic loading rate, ORR_{Vol} = volumetric organic removal rate HLR_{VLF} = void liquid fraction hydraulic loading rate, OLR_{VLF} = void liquid fraction organic loading rate, ORR_{VLF} = void liquid fraction organic removal rate, HTC = hydraulic treatment capacity, HLR = Surface loading rate

3.3.2 Comparison of pulse and continuous mode of operation

The BSR systems were designed to allow different modes of operation. While continuous operation is simple, some researchers have shown that increased ORR may be achieved with intermittent or pulse operation modes. In order to definitively establish the preferential mode of operation, each BSR was operated alternatively in continuous and pulse mode each year during the crush periods (Table 3-1) and results were compared (Table 3-3).

3.3.2.1 Hydraulic and organic loading rates

On average continuous mode had greater HLR, OLR and ORR, outperforming pulse mode by average magnitudes of 21%, 31% and 30% respectively over the 2-year experimental period (Table 3-3). The ORR_{Vol} of VOA was 28% higher in continuous mode, and there was no difference in total phenolic removal efficiencies between the two modes (results not shown).

Table 3-3 Comparison of parameters in different modes of operation, averages

Year	Filter	Mode	Period	Influent COD	RE	HLR_{Vol}	OLR_{Vol}	ORR_{Vol}
				$mg.L^{-1}$	%	$L.m^{-3}$ $sand.day^{-1}$	$gCOD.m^{-3}$ $sand.day^{-1}$	$gCOD.m^{-3}$ $sand.day^{-1}$
1	BSR1	Pulse	First	4541 (2800-5990)	59% (17-84)	74 (24-44)	288 (82-1236)	133 (33-294)
	BSR2	Continuous			52% (19-95)	91 (0-526)	433 (0-3151)	180 (0-1510)
1	BSR1	Continuous	Second	4619 (3990-5710)	93% (87-96)	13 (3-22)	59 (15-124)	54 (14-108)
	BSR2	Pulse			89% (83-96)	7 (0.1-15)	32 (0.4-67)	28 (0.3-62)
2	BSR1	Pulse	First	1265 (441-2310)	63% (20-87)	177 (52-540)	190 (42-394)	114 (22-257)
	BSR2	Continuous			67% (42-89)	208 (3-717)	216 (4-985)	130 (4-310)
2	BSR1	Continuous	Second	3583 (1217-6060)	85% (80-90)	102 (29-207)	311 (101-638)	266 (87-536)
	BSR2	Pulse			81% (64-89)	61 (0.5-176)	176 (3-591)	149 (2-502)

BSR = biosand reactor, RE = removal efficiency, HLR_{Vol} = volumetric hydraulic loading rate, OLR_{Vol} = volumetric organic loading rate, ORR_{Vol} = volumetric organic removal rate, () = range

While the organic degradation of WWW relies on the microbial populations in BSRs and other similar systems, the HC decreases, and the HRT increases as a consequence of the functional

microbial biomass which attaches to the inert medium and reduces the porosity. This retards the flow, but the increased HRT typically results in higher ORR [14]. There is a critical balance between HLR (and HRT) and system operation and performance. Systems operated with low HLR require large treatment plants with associated capital and operational costs and large spatial footprints, while the performance of those operated with high HLR (and low HRT) may be inefficient [4,26,27]. In BSRs, higher HLR can be achieved by manipulating the hydraulics within the system by: (i) increasing the fall, (ii) adjusting the loading rates, and/or (iii) intermittent operation [26–29]. Generally, the top layer of media or area around the inlet of TWs/CWs has the greatest accumulation of biomass. Several operational methods have been used to negate excessive biomass build-up, namely: (i) back washing, (ii) scarifying the surface layer, (iii) applying higher HLR to flush the solids out, and (iv) changing the direction of flow or areas of ingress to allow accumulation of biomass to dissipate during the rest period [30]. Then there are methods which involve the total or partial removal and replacement or offsite cleaning of the media which does have significant financial constraints [30]. Some of these methods involve the degradation of the biomass within the systems themselves. In this study, it was envisaged that the intermittent aerobic/anoxic conditions created by fill and drain cycles during pulse mode operation would result in reduced biomass and concomitant increased HC within the BSRs as described by Nivala et al. (2012). In addition, the drain cycle introduces air into the media which exposes the biomass to O_2. The O_2 is used as a terminal electron acceptor for aerobic heterotrophic metabolic processes, which are energetically more favourable than anoxic or anaerobic metabolic processes [30–32]. However, when the BSRs were operated in pulse mode, the sand remained saturated after the drain cycle. A significant increase in drain cycle time was required to achieve a meaningful aerobic period. It was decided that this was not warranted as the hydraulic treatment capacity would be severely impacted. In addition, it has previously been shown that, contrary to expectations, higher degradation of ethanol and phenolics in synthetic WWW takes place in lower redox environments in sand-filled treatment systems, albeit with accumulation of VOAs which are formed as metabolic by-products [7–9]. The higher accumulation of VOAs in continuous mode of operation supports these previous findings.

Overall, it is recommended that due to the comparative operational simplicity greater volumetric hydraulic loading, organic loading, and organic removal rates, that the systems are operated in continuous mode.

3.4 Conclusion

This study showed that continuous mode of operation of BSRs out-performs pulse mode of operation in terms of HLR, OLR, ORR by 21%, 31% and 30% respectively. The novel BSR systems provide a small spatial footprint compared to similar passive treatment systems with a reduction in the reactors special footprint ranging from a 40% to 96%. The systems provide a conducive environment for functional microbial growth and activity, allowing treatment of WWW at high ORL_{VLF} with no evidence of permanent clogging. These systems require minimal outside interference and do not require skilled labour for operation. Future designs should have outlets at different heights spaced 200 mm apart to allow more simple manipulation of flow rates.

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Chapter 4

Calcite dissolution and bioneutralization of acidic Wastewater in biosand reactors

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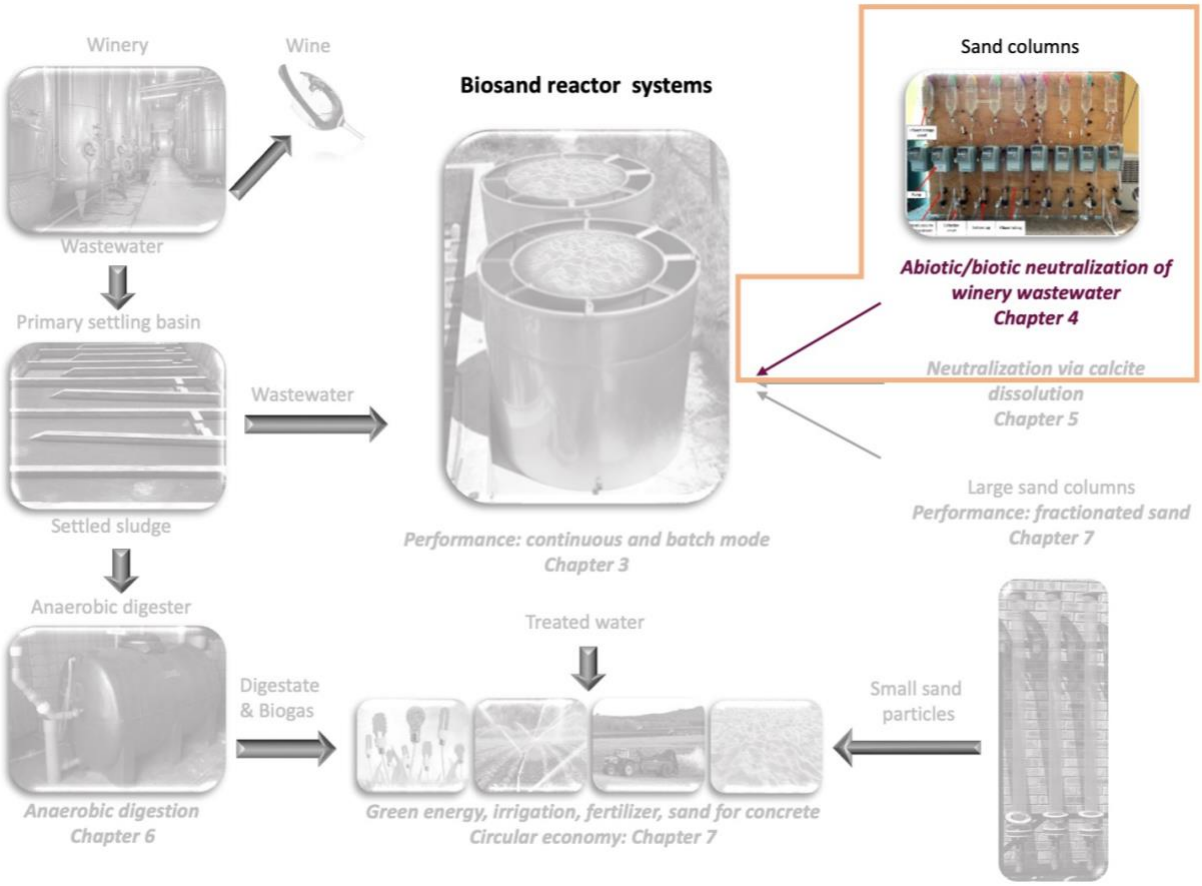
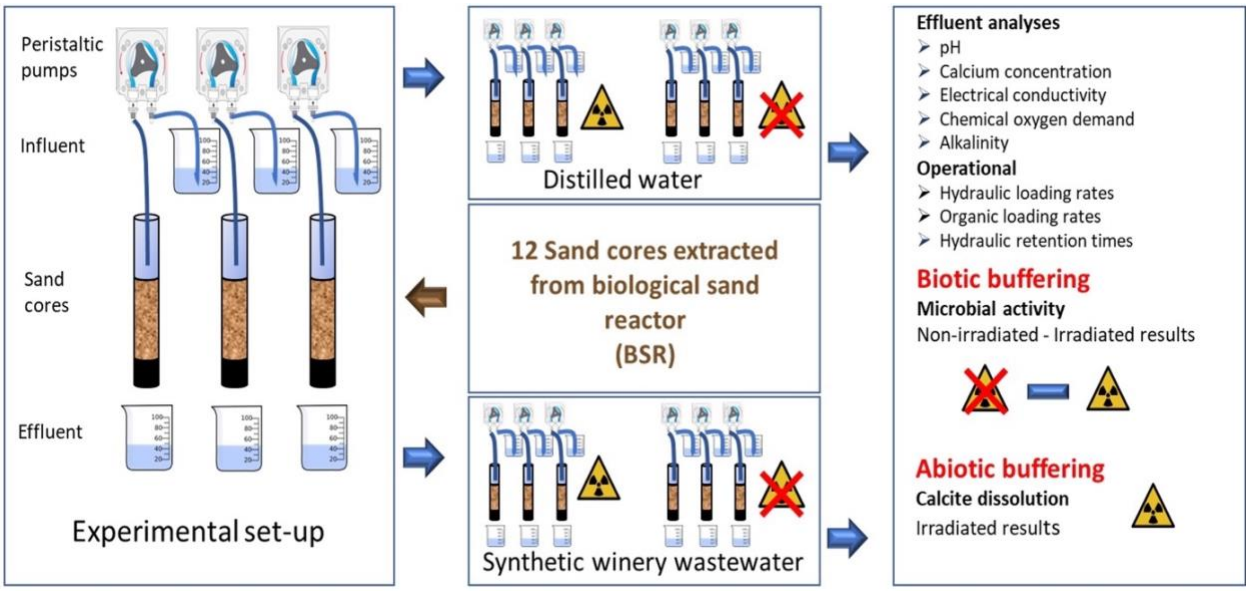
Offprint available in Appendix 4



[Presents the findings of a study to determine the contribution of biotic (microbial) and abiotic (calcite dissolution) mechanisms on neutralization of WWW in BSRs.]

Graphical abstract



Abstract

Acidic wastewaters such as winery wastewater require treatment to increase the pH before discharge into the environment. Biosand filters have been shown to reduce the organic load while simultaneously providing a buffering function. Previous research has shown increases in pH which was assumed to mainly take place via dissolution of calcite from the sand particles. This study investigated the possible role of biotic mechanisms for pH adjustment in sand column experiments by comparing results obtained from irradiated (biotic) and non-irradiated (biotic and abiotic) sand columns extracted from biosand filters used to treat winery wastewater. The columns were fed with either synthetic winery wastewater or filtered water (control). It was shown that the specific hydroxide concentrations in the eluant from the non-irradiated columns was significantly ($p<0.05$) higher than in the eluant from the irradiated columns ($1.1x10^{-5}$ versus $4.0x10^{-6}$ M/kgsand^{-1}), indicating the presence of both biotic (average 4.5±0.13%) and abiotic (average 95.5±0.16%) pH increases. Using multivariate statistical tools to analyze a combination of parameters linked with biotic and abiotic pH adjustment, significant differences (ANOVA, $p<0.05$) were found between the four treatment groups (irradiated/non-irradiated SWW and control) and the groups showed good clustering in cluster plots (group average) linkages, and principal component analysis plots.

Keywords: Abiotic; Biotic; Carbonate; Dissolution; Microbial; Sand; Acid mine drainage

4.1 Introduction

The process of making wine results in the generation of 0.2 to 14 L of typically organic rich, acidic, and sometimes saline winery wastewater (WW) for each litre of wine produced [1–3]. It has been shown that biosand reactors (BSRs) containing locally available dune sand have higher organic removal rates (ORR) and spatial footprints than other passive systems treating WW [4]. These systems are inexpensive to install and maintain and are well suited for remediation of WW for irrigation purposes because they are able to reduce the organic load while simultaneously increasing the pH and sodium adsorption ratio (SAR) of acidic WW [1,4,5].

Calcite and aragonite are mineralised forms of calcium carbonate ($CaCO_3$) that make up limestone. In a previous study conducted with BSRs, it was assumed that dissolution of calcite was the primary abiotic WW buffering mechanism [1] because the sand particles consisted of quartz (81%) and calcite (18%) as the dominant minerals [5,6]. Apart from buffering acidic WW, irrigation with WW containing calcium (Ca^{2+}) from $CaCO_3$ dissolution can potentially improve the quality of sodic soils by increasing the SAR [7,8].

Calcite dissolution reactions (Equations 4-1, to 4-3) are reversible and take place at the solid-liquid interface [9]. The reactions are mediated by the pH and the partial pressure of carbon dioxide (CO_2). The first reaction (Equation 4-1) involves protonation of $CaCO_3$ to Ca^{2+} and bicarbonate (HCO_3^-). In the other reactions, carbonic acid (H_2CO_3) and water (H_2O) are the reactants responsible for $CaCO_3$ dissolution (Equations 4-2 & 4-3, respectively).

$$CaCO_3 + H+ \leftrightarrow Ca^{2+} + HCO_3^- \quad (4\text{-}1)$$

$$CaCO_3 + H_2CO_3 \leftrightarrow Ca^{2+} + 2HCO_3^- \quad (4\text{-}2)$$

$$CaCO_3 + H_2O \leftrightarrow Ca^{2+} + HCO_3^- + OH^- \quad (4\text{-}3)$$

More traditionally, the addition of limestone, calcite, or other $CaCO_3$-rich residues such as eggshells, seashells or concrete aggregates have been applied for passive remediation of acid mine drainage (AMD) [10,11], with various buffering and metal precipitation reactions taking place [12,13]. Calcite dissolution is also used for the mineralisation of desalinated potable water [9]. This is usually achieved by the addition of sulfuric acid (H_2SO_4) or by flushing with CO_2 to form

H_2CO_3, but it has recently been shown that dissolution using acetate (CH_3COO^-) results in superior potable water quality [9]. The excellent dissolution kinetics achieved with CH_3COO^- [9] suggest that WW may be an ideal calcite dissolution agent because (i) volatile fatty acids (VFAs) constitute up to 60% of the organic fraction of WW [2] and (ii) VFAs are formed during organic biodegradation in BSRs, with CH_3COO^- being the major contributor to the chemical oxygen demand (COD) in the final effluent [14].

The rate of $CaCO_3$ dissolution is also correlated with particle size as smaller particles provide larger overall reaction surface areas [5,11] . In the case of AMD, the Ca^{2+} can react with sulfate (SO_4^{2-}) to form gypsum ($CaSO_4$) on the particle surfaces, slowing dissolution reaction kinetics [13]. In addition, efficiency of passive calcite-based systems for treatment of iron (Fe)-rich AMD can be restricted by coating of the particles with Fe oxides [11]. These reactions should theoretically be limited in the case of WW because Fe and SO_4^{2-} concentrations are lower and acidity is more likely associated with the presence of organic acids [2].

Changes in pH can also be microbially mediated (bioneutralization). For example, generation of alkalinity (Alk.) and increased pH has been associated with consumption of H^+ by microbial denitrification, SO_4^{2-} reduction, and reduction of metals stimulated by the addition of organic carbon (OC) as an electron donor [15]. Concurrent biotic and abiotic neutralisation of acidic saline waters has been demonstrated in bioreactors containing either compost or municipal organic waste and limestone, where limestone dissolution accounted for 78%-91% of Alk., and bacterial SO_4^{2-} reduction for 9%-22% [16]. In alkaline leachates and other higher pH waters, reverse reactions (Equations 4-1 to 4-3) can lead to $CaCO_3$ precipitation, with concomitant pH decreases [17]. The reaction rates can be increased by aeration and microbial release of CO_2 from organic substrates *viz*. concurrent biotic and abiotic mechanisms [17]. Haloalkalophilic bacterial fermentative generation of organic acids has also been associated with bioneutralization of alkaline bauxite residues [18,19].

Passive biochemical reactors (PBRs) for remediation of AMD typically contain organic microbial electron donors such as wood chips, chicken manure, leaf compost, and lignocellulosic waste and inorganic buffering agents such as calcite [20]. Such systems operate best at pH values between 5 and 8 [20]. If some cases, Fe reducing bacteria may compete for substrate with the SO_4^{2-} reducing bacteria, retarding SO_4^{2-} reduction rates [20]. Similar principles have been applied for

the rehabilitation of degraded soils with high metal concentrations by re-saturating dried sulfuric soils [21]. The reduced conditions lead to the formation of metal sulfides, which is enhanced by the action of SO_4^{2-} reducing bacteria in the presence of electron donors from lignocellulosic organic matter [21].

In lab-scale BSRs containing sand with no detectable calcite, the pH of acidic WW increased from inlet to outlet, strongly suggesting that biotic neutralization mechanisms exist in BSRs [22]. It is plausible that electron donors for reduction reactions are supplied by the major organics ethanol (C_2H_5OH), VFAs, sugars, (poly)phenolics and other minor organics, the quantities of which vary on a seasonal basis and from winery to winery [2].

In order to verify the hypothesis that both biotic and abiotic buffering of WW (and other acidic organic wastewaters) occur in BSRs, sand columns were extracted from pilot scale BSRs that had been operational for >2 years and contained functionally adapted microbial communities. Half of the columns were irradiated, and both irradiated and non-irradiated columns were fed with either (i) filtered water (controls), or (ii) synthetic WW (SWW). Buffering in irradiated columns was assumed to be completely abiotic, while biotic buffering was determined by comparing the results obtained from the non-irradiated with those obtained from the irradiated columns.

4.2 Materials and methods

4.2.1 Column experiment set-up

Twelve core sand samples were extracted from pilot vertical flow BSRs that had been operational at a winery in the Western Cape, South Africa for over 2 years [5] (Figure 4-1). The composition of the dune sand from the quarry site (approximately 81% quartz and 18% calcite) has been described previously in detail [5,6]. Cores were extracted from the surface of the BSRs using acrylic pipes (40 mm OD and 30 mm ID) which were sharpened on one end to promote penetration into the sand. The pipes were pushed carefully into the sand with as little disruption to the structure of the material as possible. After extraction of approximately 350 mm sand, the cores were capped. Nine of the 12 cores were sterilized by irradiation at 30 kGy at a commercial facility as previously described [23].

The cores were then set-up and used in flow-through column experiments (see graphical abstract for set-up). They were fitted with end pieces that retained the sand but did not impede the flow

of liquid through the columns. Influent was pumped using individual infusing pumps via tubing ending in T-pieces onto the top of the sand and allowed to flow passively through the sand via gravity.

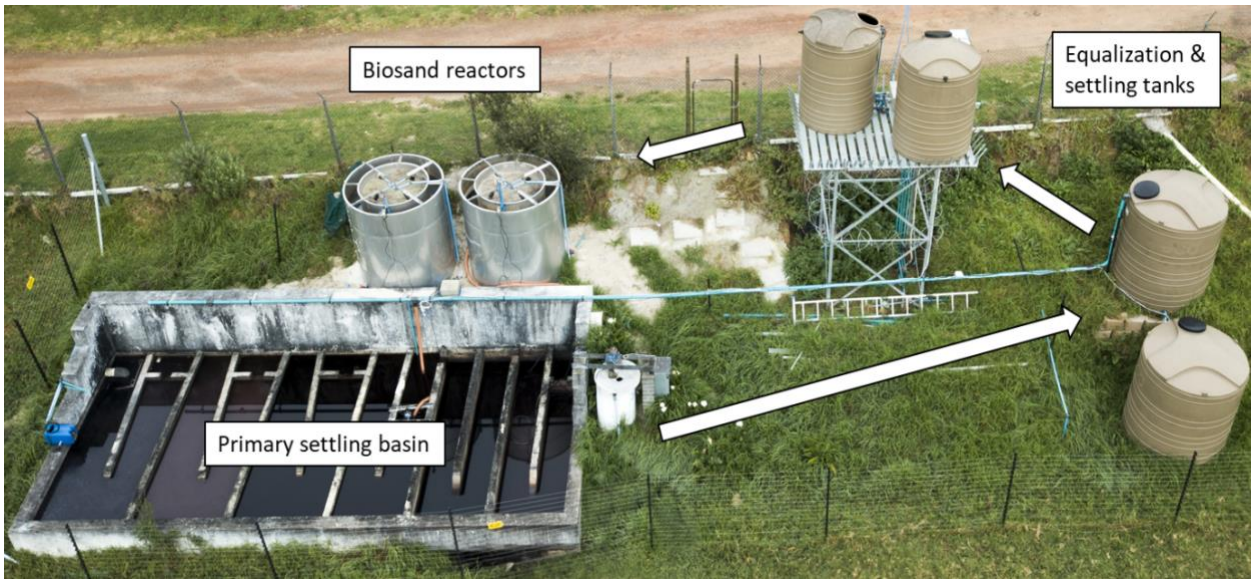


Figure 4-1. Set up of pilot biosand reactor system treating winery wastewater

4.2.2 Operation of column experiments

In order to determine whether buffering (neutralization) of WW and possibly other acid wastewaters in BSRs is due to abiotic (notably via calcite dissolution), biotic (microbial) or combined biotic/abiotic factors, the irradiated and non-irradiated column replicates were fed each 24 hrs for 48 hrs with 400 mL of either: (i) filtered water (control), or (ii) synthetic WW with a pH 3.07. In addition, secondary negative controls consisting of fresh sand were fed with SWW (as per ii). The SWW components were added to give total COD (COD) concentrations of 1000 mg/L, made up of 500 mgCOD/L C_2H_5OH, 400 mgCOD/L acetic acid and 50 mgCOD/L gallic acid, 50 mgCOD/L vanillin, as previously described [23,24]. Each experiment was conducted in triplicate. In order to ensure robust microbial activity, the non-irradiated column experiments commenced within 4 hrs of coring. Each set of experimental replicates (n=3) was fed with autoclaved influent from the same receptacle to ensure influent consistency. In order to reduce downstream microbial activity, the eluant was collected into separate autoclaved sealed beakers held within cooler boxes containing dry ice. The design operational parameters are provided in Table 4-1. The flow rate was set to mimic the hydraulic loading rate (HLR) of the BSRs from which they were extracted, which allowed full saturation of the sand in the columns without excessive pooling. The design HLR was calculated as previously described [1].

Table 4-1 Percentage calcium in sand from cores taken from biosand reactors treating winery wastewater

Influent	Flow rate (mL/hr)	HLR ($L/m^3_{sand}.day^{-1}$)	OLR ($gCOD/m^3_{sand}.day^{-1}$)	HRT (hrs)
Control	8.3	808	NA	8.7
SWW	8.3	808	NA	8.7

SWW = synthetic winery wastewater HLR = hydraulic loading rate OLR = organic loading rate HRT = hydraulic retention time NA = not applicable

4.2.3 Eluant sampling and analytical procedures

Composite eluant samples were taken after 24 hrs (0-24 hr) and after 48 hrs (24-48 hr). The pH was determined according to the manufacturer's instructions using a CyberScan pH300 meter and appropriately calibrated pH probe PHWP300/02K (Eutech instruments, Singapore). The pH was converted into OH^- concentrations before calculating the means and standard deviations from the mean. The electrical conductivity (EC) was determined using a hand-held Oakton ECTestr 11+ multi-range, cup-style pocket conductivity meter (Eutech Instruments, Singapore Cat No: 35665-35). This instrument is capable of reading conductivity with a range of 0 µS/m to 20.00 mS/m. The COD concentrations were determined on the same day of the sampling using a Merck (Merck®, Whitehouse Station, USA) Spectroquant® Pharo instrument and Merck Spectroquant® cell tests (cat. no. 1.14895.0001). Total Alk. was measured using the Merck titrimetric method with titration pipette MQuant catalogue number (1.111109.0001), according to the manufacturer's instructions. The organic composition of the eluants was determined using high pressure liquid chromatography (HPLC) as previously described [23,24]. At the end of the experiments, the sand in each column was allowed to drain for 24 hr, dried and weighed (range 310-404 g) and the physicochemical results were specifically adjusted to account for the unavoidable variability in the amount of sand within each column to give specific values (per kg of sand).

4.2.4 Statistical analysis

T-tests were performed using Microsoft Excel (Microsoft, Redmond, Washington, USA). Paired 2 sample for means T-tests were used to determine (i) significant differences between irradiated and non-irradiated experimental results, and (ii) correlations between physicochemical parameters for each column replicate. Two sample T-tests assuming unequal variances were

used to determine significant differences between treatments (control and SWW). Differences were deemed significant if t-crit<t-stat and p<0.05. Standard deviations (SD) were calculated as SD from the mean. Multivariate statistical analyses (principal component analyses (PCA), analysis of similarity (ANOSIM), and cluster analyses (group average linkage) on normalised physicochemical data using Primer 7®software (Primer-e, Auckland, New Zealand).

All statistical differences were deemed significant if p<0.05 and p≥0.001 and highly significant if p<0.001. These criteria are applied throughout the manuscript when referring to "significant" or "highly significant".

4.3 Results and discussion

4.3.1 Operational parameters

In order to closely mimic the physicochemical and microbial structures within the BSRs in the column experiments, the content of each column was kept intact from coring through experimentation. Although every effort was made to retrieve similar amounts of sand in each column (height approximately 350 mm), some variability in the weight of sand in each column was unavoidable (Table 4-2). This translated into some differences in the HLR and HRT in the columns, albeit in a relatively narrow range (Table 4-2). The measured HLR and HRT (Table 4-2) that were achieved were close to the design values (Table 4-1) based on the actual flow rates in the BSR from which the cores were extracted. The cores therefore provided a good approximation of the 'real world' situation.

Table 4-2 Measured operational parameters (average ± SD and range, n=3)

	Sand height (mm)	**Sand weight (g)**	**HLR ($L/m^3_{sand}.day^{-1}$)**	**HRT (hrs)**
Control	356±8.4	383±9.7	796±19	8.81±0.21
	(346-361)	(375-394)	(784-818)	(8.57-8.94)
Control IR	352±6.8	380±8.7	805±16	8.71±0.17
	(344-357)	(370-386)	(793-822)	(8.52-8.84)
SWW	358±22	373±30	792±48	8.87±0.53
	(337-380)	(344-404)	(745-840)	(8.35-9.41)
SWW IR	363±4	396±4.5	779±8.6	8.99±0.10
	(359-367)	(391-400)	(771-788)	(8.89-9.09)
SWW cont.	316±5	376±0	896±14.2	7.83±0.15
	(311-321)	(376-376)	(881-910)	(7.70-7.95)

HLR = hydraulic loading rate; HRT = hydraulic loading rate; IR = irradiated; SWW = synthetic winery wastewater; SWW cont. = control with new, unused sand

4.3.2 Organic biodegradation in irradiated and non-irradiated columns

Residual organics and inorganics were unavoidably present in the cores extracted from the working BSRs. Consequently, although no organics were added to the columns, residual COD leached into the column eluants (Figure 4-2a), adding to the experimental complexity. This can be seen by: (i) the high average specific COD concentrations (spCOD) measured in the eluants from the control columns collected in the first 24 hrs, and (ii) the fact that the average spCOD was higher in the eluants collected over first 24 hrs (2527 mgCOD/L.kg_{sand}^{-1}) than provided in the influent (2831 mgCOD/L.kg_{sand}^{-1}) over the same period in the columns fed with SWW (Figure 4-2a).

The large and highly significant differences in average spCOD in the eluant collected from the irradiated columns of 1049 and 1804 mgCOD/L.kg_{sand}^{-1}, from the control columns and those fed with SWW, respectively, proved that good biodegradation rates were achieved in all the non-irradiated columns. However, no significant differences were found in the eluants that were collected after the first 24 hrs (24-48 hrs). It was therefore assumed that there was a temporal loss of sterility in the irradiated columns. Radiation is the preferred method for sterilizing sand, soil and sediment as it has minimal effects on the physicochemical properties of the substrate compared with other methods such as autoclaving [23,25,26]. Although sterile procedures were used and the radiation levels (30 kGy) were theoretically sufficient, factors such as shading and the presence of radiation resistant bacteria or spores can result in renewed microbial growth over time in substrates such as sand [23,25,26]. In summary, the highly significant differences in the spCOD measurements in the eluants from all the irradiated and non-irradiated columns over the first 24 hrs confirmed biotic activity and the relevance of the biotic/abiotic experiment, but indicated that interpretation of the physicochemical results are less relevant after 24 hrs.

For the columns fed with SWW, the OLR for the irradiated and non-irradiated columns were 772±46 (range 726-819) gCOD/L.kg_{sand}.day^{-1} and 760±8.4 (range 752-768) gCOD/L.kgs_{and}.day^{-1}, respectively, providing a reasonable approximation of the design values (831 gCOD/L.kg_{sand}.day^{-1}) calculated for the operational BSR. Selected organics were measured in the eluant samples to substantiate organic biodegradation. Samples were screened for sugars, glycerol, C_2H_5OH, VFAs (CH_3COO^-, propionate, butyrate), and selected phenolics (vanillin, gallic acid, vanillic acid, catechol). If present, the concentrations were measured. In the eluant from the control columns,

random and negligible amounts of fructose and glycerol were detected (<8 mg/L average per experimental triplicate and CH_3COO^- was also detected (0-32 mg/L), but only in the eluants collected during the first 24 hrs. No particular trends were discernible between replicates. This was not unexpected as the columns had been extracted from different spatial locations within the BSRs and some variability was inevitable.

For the columns fed with SWW, mass balances were determined for the amounts of C_2H_5OH and CH_3COO^- that were added in the influent and collected in the eluants. Over the first 24 hrs, all of the C_2H_5OH was removed in the non-irradiated columns, with 92% spCOD removal, while only 26% of the C_2H_5OH was removed in the irradiated columns, with only 37% spCOD removal (Figure 4-2b). These removal rates are likely over-estimated as existing WW from the BSR system would have eluted out first from the columns. The C_2H_5OH and CH_3COO^- biodegradation rates were similar in the eluants collected from the irradiated and non-irradiated columns between 24 and 48 hrs (overall 54% and 59%, respectively). By way of comparison, when operated in continuous mode, the COD removal rates achieved during the crush periods in the vertical flow BSR system that the cores were extracted from ranged from 37% to 95% in year 1 of operation (including the start-up period), and 42% to 90% in year 2 of operation (Figure 4-3a).

The C_2H_5OH/CH_3COO^- mass balances therefore proved unequivocally that biodegradation was taking place preferentially within the non-irradiated columns during the first 24 hrs and that there was a temporal loss of sterility in the irradiated columns.

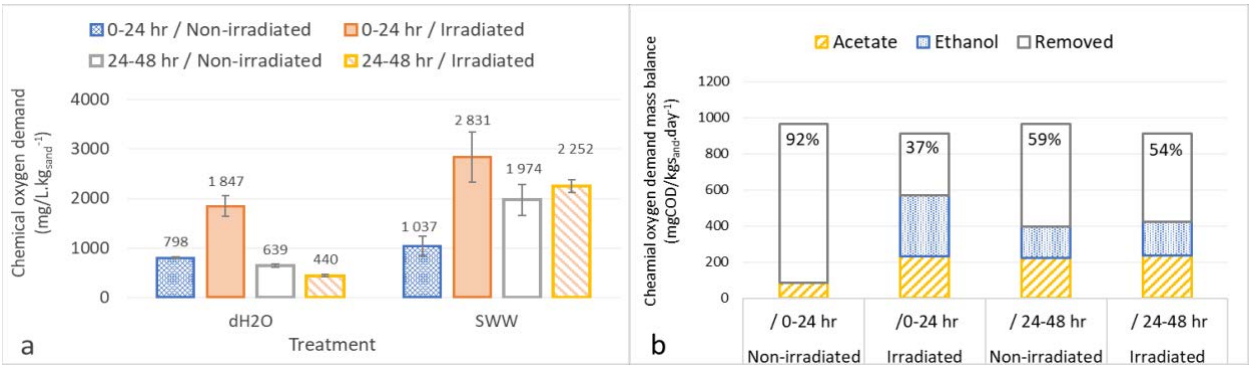


Figure 4-2. Specific chemical oxygen demand measurements for all the column replicates (a) and mass balance for ethanol and acetate added to the columns feed with synthetic winery wastewater (b)

It was not possible to identify the exact biotic neutralization mechanisms that took place within the cores due to: (i) the complexity and variability of the substrate (WW residuals) already existing within the pore spaces of the extracted cores, (ii) the possible presence of multiple biotic neutralization mechanisms, and (iii) the fact that elucidation of these mechanisms would require addition and monitoring of a range of different chemicals such as sulfates and nitrates which was not feasible in the context of this study.

4.3.3 Analysis of eluant hydroxide ion, alkalinity and calcium concentrations and electrical conductivity

In the pilot BSR system, the influent pH ranged from 4.2 to 8.3, but the eluant pH was maintained in the range of 6.5 to 8.9 (Figure 4-3b). In comparison, the eluant pH values from the columns ranged from 7.9 to 8.8 with influent pH values of 6.7 and 3.07 for the control (filtered water) and SWW, respectively, demonstrating similar functionalities between the BSR and columns.

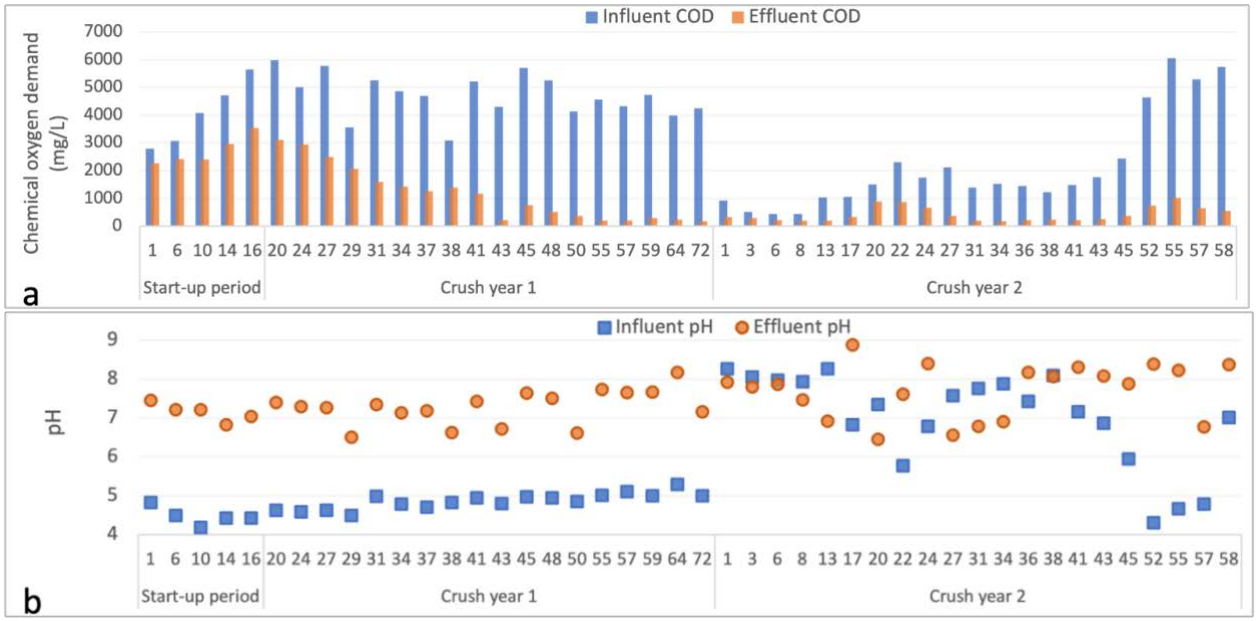


Figure 4-3. Chemical oxygen demand (a) and pH (b) measurements from the pilot biological sand reactor system (adapted from [4])

Due to the loss of sterility in the columns after 24 hrs and the fact that the focus of the study was on determining the presence of biotic pH adjustment mechanisms, the discussion on the physicochemical analyses is mainly concentrated on the results obtained within the first 24 hrs. Details on the primary abiotic neutralization mechanism, namely, calcite dissolution, as well as the changes in the sizes and shapes of the calcite particles has previously been described in detail [5].

The specific OH^- concentrations ($spOH^-$) (Figure 4-4a) were significantly higher in the eluants collected from the non-irradiated than the irradiated columns for the control columns as well as those fed with SWW during the first 24 hrs of the experiment, clearly demonstrating a biotic component to increased pH. This was substantiated by the fact that there was no significant difference in the $spOH^-$ in the eluants from the irradiated and non-irradiated samples collected between 24 and 48 hrs. It was hypothesized that the temporal loss of sterility in the irradiated columns allowed both biotic and abiotic pH adjustments to take place simultaneously in all columns after 24 hrs (in contrast to only abiotic mechanisms in the irradiated columns initially). The average biotic and abiotic contributions to $spOH^-$ increases were calculated as 4.5±0.13% and 95.5±0.16%, respectively. This is likely an over-estimate as some microbial growth in the irradiated columns may have commenced before 24 hrs.

With the exception of spCa and spAlk. (Pearsons' = 0.821), there were no significant correlations between the inter-related $spOH^-$, spAlk. spEC and specific Ca (spCa), the parameters most likely to reflect pH adjustment via calcite dissolution and/or biotic factors. There were no significant differences in the specific alkalinity (spAlk.), but highly significant differences in the specific EC (spEC) measurements in the 0-24 hr eluants from the irradiated and non-irradiated columns fed with SWW (Figure 4-4b,c). In the case of spAlk. and specific SpCa, the measurements were significantly higher in the eluants from columns fed with SWW than from the control columns, indicating good solubilisation of calcite by the SWW as previously shown with CH_3COO^- [9].

It has previously been shown that with fresh sand, abiotic calcite dissolution kinetics and mass balances can be accurately calculated using the Ca concentrations in the eluant from sand columns as a proxy for $CaCO_3$ dissolution [5]. Using the calculated mass-balances from the column experiments in conjunction with historical calcium loss data obtained from the sand in a pilot BSF system *in-situ* at a winery, it was also shown the calcite would not be expended within the feasible lifespans of such BSF systems treating WW [5]. However, in this study, the eluant spCa concentrations were highly variable, and did not exhibit a distinct pattern (Figure 4-4d), indicating the complex nature of this 'real world' study using sand extracted from pilot BSRs, necessitated by the need to obtain functional microbial communities acclimated to WW.

It has also been previously been shown that laboratory-scale BSRs containing river sand with minimal calcite (<0.3% Ca) were able to increase the pH of diluted WW from 4.2 to 7.7 [6,22].

The lack of calcite in this system suggested that biotic pH adjustment was the primary WW neutralization mechanism, but this was not substantiated and only a limited number of samples were taken. This study has shown unequivocally that biotic pH adjustment can occur in BSRs treating WW. This is noteworthy because increases in the pH of acidic WW (and possibly other acidic organic effluents) may continue once calcite has been expended or can occur in instances where calcite-poor sands are employed. However, the use of calcite-containing sand is still recommended because the addition of Ca to the final effluent reduces the SAR and protects the receiving environment from becoming sodic if the effluent is used for irrigation purposes [1]. In such systems, the SAR and effluent pH should be monitored to the ensure that the receiving soils are protected, as previously described [5].

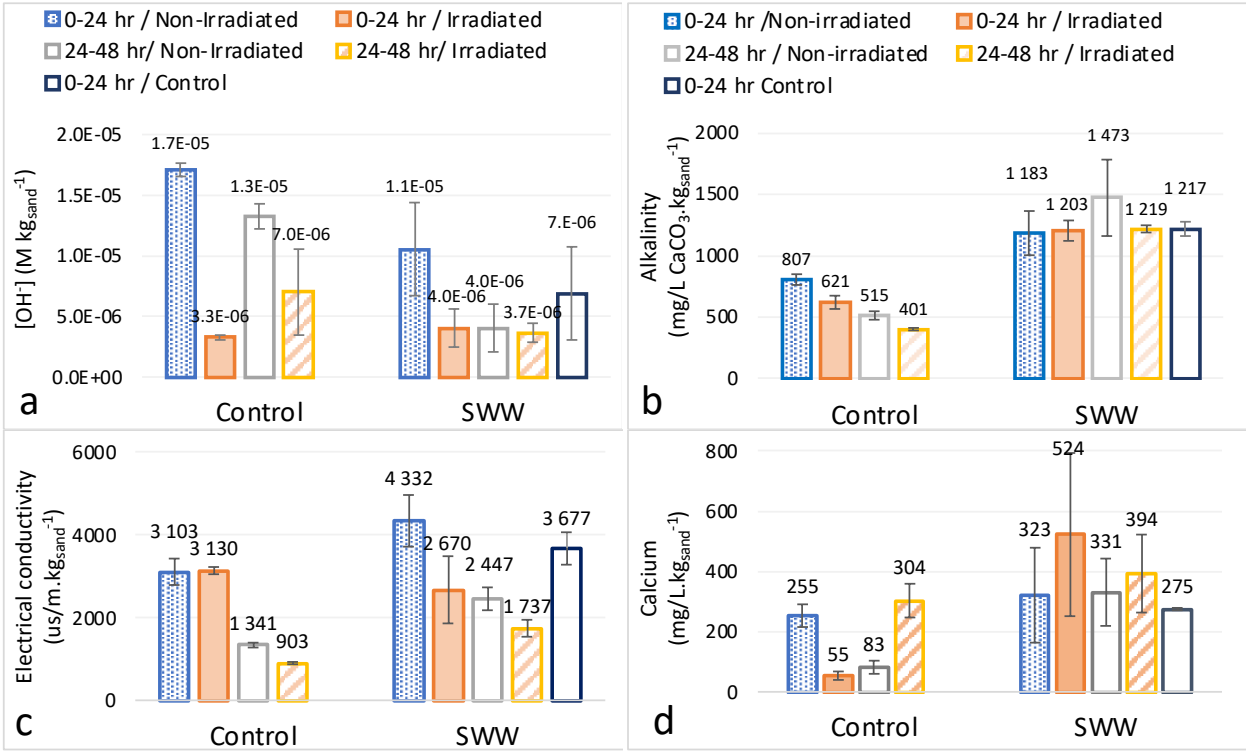


Figure 4-4 Average specific concentrations of hydroxide ions (a), alkalinity (b), electrical conductivity (c) and calcium (d) in column eluent (n=3 replicates, error bars represent standard deviation from the mean)

Not only were residual organics present in the pore water of the columns, but also other dissolved solids, most notably Na and K, which are commonly found in high concentrations in WW. By examining the Ca, Na and K concentrations measured in the eluant of all the replicates for the first (Figure 4-5a) and second (Figure 4-5b) 24-hour period, it is clear that there was considerable variation in the character of the interpore WW, and that complex biotic and abiotic interactions were responsible for leaching of inorganics from the columns. As alluded to

previously, univariate statistical analyses only showed a significant correlation between spCa and spAlk, demonstrating the effect of confounding variables on the study results.

In summary, the trends towards: (i) higher values of indicator parameters in the eluant from columns fed with SWW in comparison to those fed with filtered water and (ii) differences in values of indicator parameters in eluants from irradiated and non-irradiated columns, showed that: (i) SWW increased the solubilisation and leaching of Ca and other dissolved salts from the columns, (ii) solubilisation and/or leaching was increased by both biotic and abiotic interactions (iii) both biotic and abiotic mechanisms were responsible for pH increases in the eluant from inlet to outlet in non-irradiated columns.

However, due to the presence of confounding variables as a consequence of the organic and inorganic residuals within the columns, results were not consistent for the indicator parameters used to demonstrate biotic and abiotic pH adjustment mechanisms. Multivariate statistical analyses were therefore used to assess the indicator parameters spAlk., spEC and $spOH^-$ simultaneously (Figure 4-6).

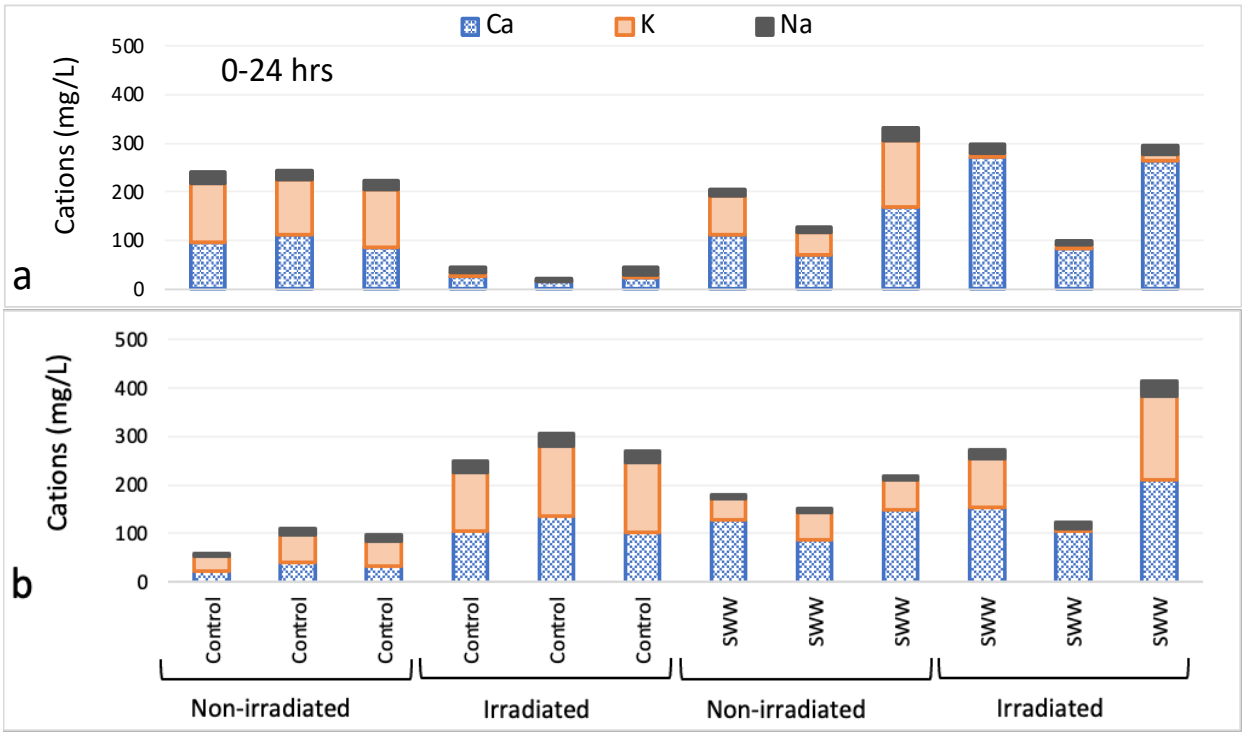


Figure 4-5 Major cations measured in the eluant from each column replicate for the first (a) and second (b) day of the experiment. Figure 'a' is vertically aligned with 'b'.

For combined $spOH^-$, spAlk. and spEC eluant measurements, there were significant (ANOVA) inter-group differences and intra-group similarities (irradiated SWW, non-irradiated SWW,

irradiated control, non-irradiated control). In samples taken during the first 24 hrs of the experiment, the replicates fell into four distinct clusters (Figure 4-6a). In samples taken between 24 and 48 hrs, the irradiated and non-irradiated groups formed two initial clusters, but there was less distinction between the different treatments (SWW or control) (Figure 4-6b). Despite the loss of sterility, it was expected that the irradiated and non-irradiated column eluants would have different characteristics over the course of the experiment because of the higher biotically induced leaching of residuals in the first 24 hrs from the non-irradiated columns (Figure 4-5). Overall, the multivariate analyses, including the PCA (Figure 4-6c), validated the assumptions made using the univariate analyses results.

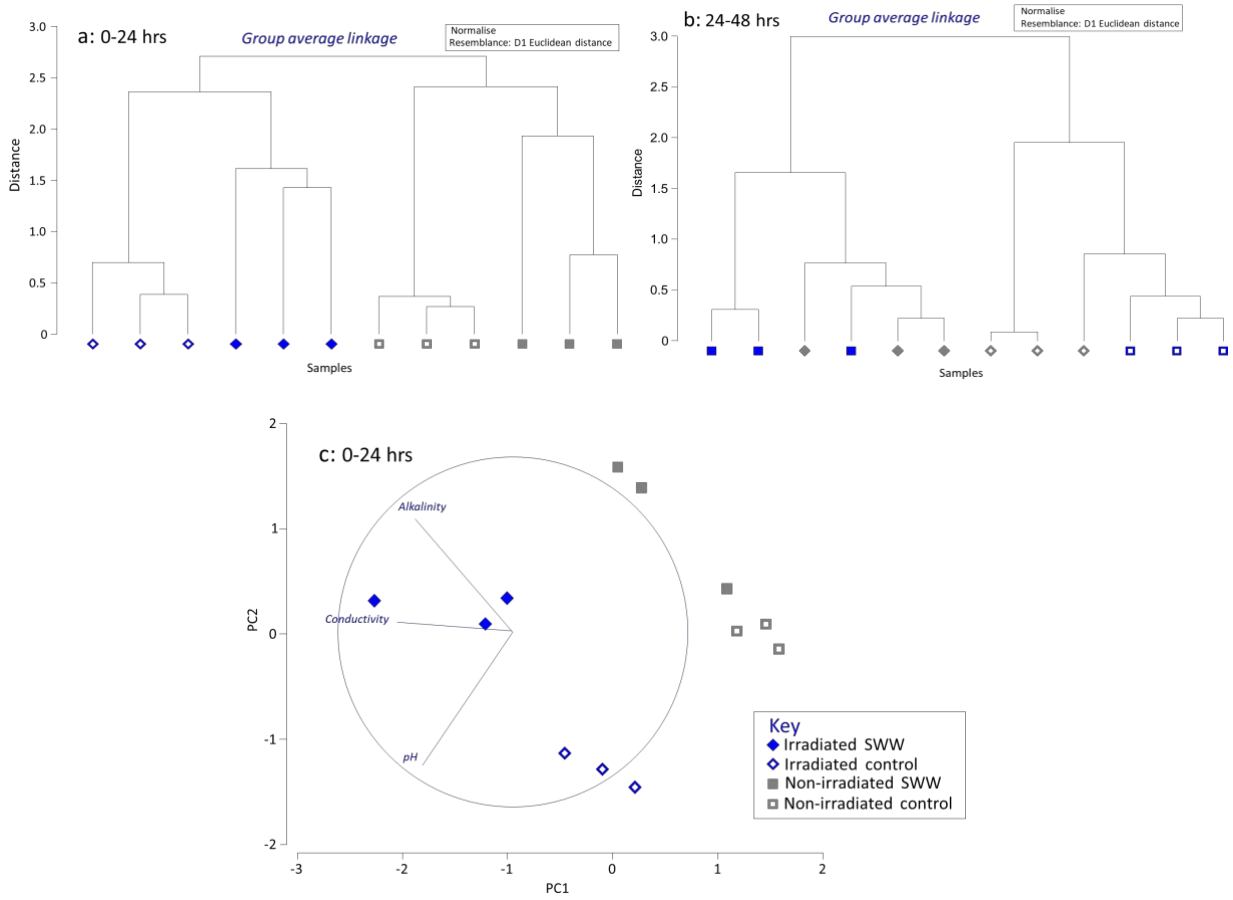


Figure 4-6 Cluster plots of selected physicochemical parameters showing the different treatment groups in eluant samples taken between 0-24 hrs (a) and 24-48 hrs (b), and principal component analyses of the same data from eluant samples taken between 0-24 hrs (c).

Author Contributions: Conceptualization, G.A.H., R.H. and P.J.W.; methodology, G.A.H., R.H. and P.J.W.; software, G.A.H and P.J.W; validation, G.A.H., and P.J.W.; formal analysis, G.A.H and P.J.W; investigation, G.A.H., R.H. and P.J.W; resources, R.H. and P.J.W; data curation, G.A.H. and P.J.W; writing—original draft preparation, G.A.H., and P.J.W; writing—review and editing, G.A.H., R.H. and P.J.W; visualization, G.A.H.; supervision, R.H. and P.J.W; project administration, G.A.H., and P.J.W; funding acquisition, G.A.H. and P.J.W All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest

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Chapter 5

Effect of particle character and calcite dissolution on the hydraulic conductivity and longevity of biosand filters treating winery and other acidic effluents

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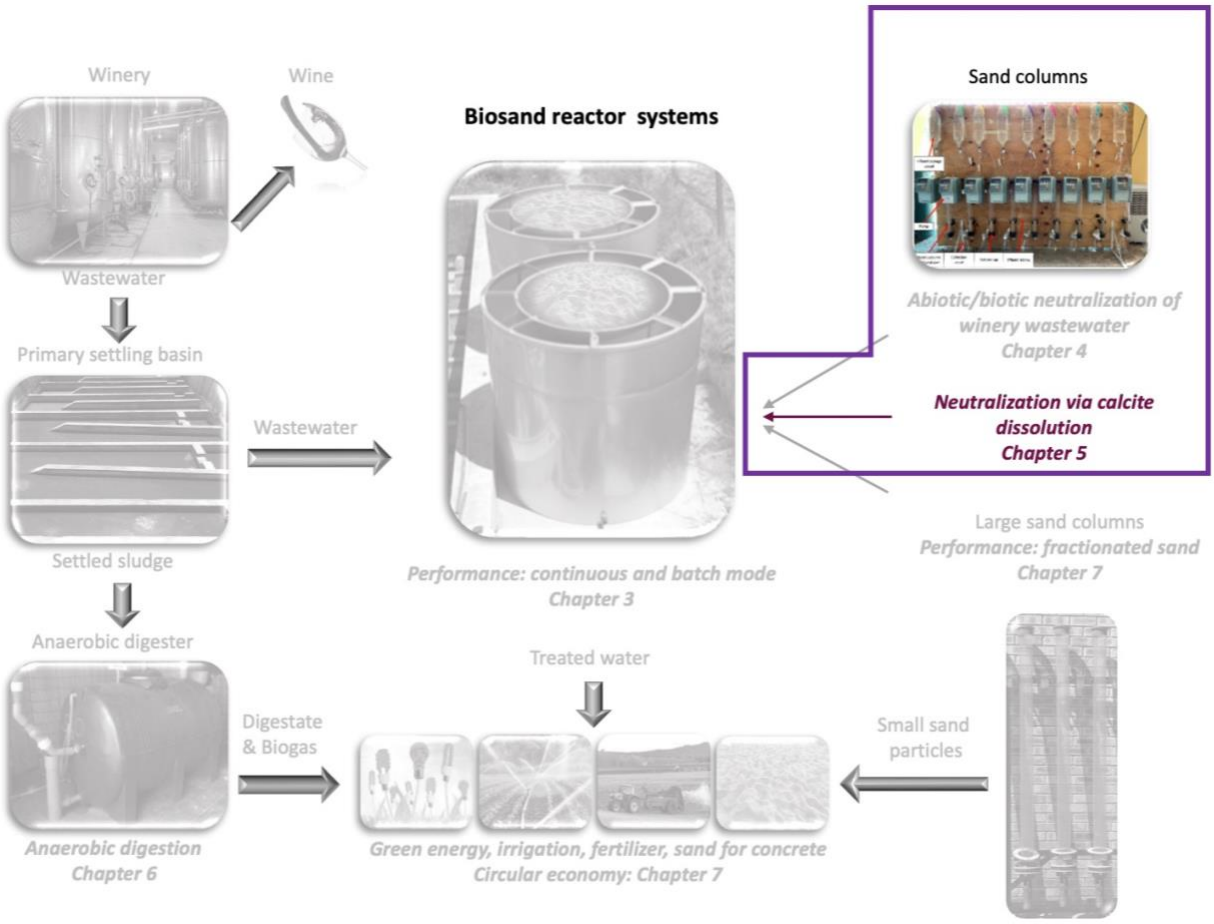
Offprint available in Appendix 5



[Presents the findings of an investigation to determine the role that particle size plays on the hydraulic performance and longevity in terms of calcite dissolution in BSRs.]

Abstract

Acidic effluent such as winery wastewater is challenging to remediate. Biological sand reactors can simultaneously remove organics and neutralize winery wastewater via biotic and abiotic mechanisms. The systems have been shown to be suitable for treating the intermittent flow of wastewater at small wineries. It has been shown that dissolution of calcite is the most important abiotic mechanism for increasing the pH of the influent. In this study, sand column experiments were used to determine the effects of (i) sand particle size distribution on calcite dissolution kinetics, and (ii) the effects of calcite particle dissolution on the hydraulic conductivity. The results were then used to calculate the theoretical temporal abiotic neutralization capacity of biological sand reactors with differently sized sand fractions, including unfractionated (raw) sand. The results were compared with those determined from a pilot system treating winery wastewater over a period of 3 years. Sand fractions with larger particles contained lower amounts of calcite (using Ca as a proxy), but exhibited higher hydraulic conductivities (3.0 ± 0.05 %Ca and 2.57 to 2.75 $mm \cdot s^{-1}$, respectively) than those containing smaller particles and/or raw sand (4.8 ± 0.04 to 6.8 ± 0.03 %Ca and 0.19 to 1.25 $mm \cdot s^{-1}$, respectively). The theoretical abiotic neutralization capacity of biological sand reactors was compared with a pilot system with the same flow rates, and a temporal abiotic neutralization capacity of 37 years was calculated for biological sand reactors, which compared favorably with the theoretical results obtained for wastewater with pH values between 2 (8.2 years) and 3 (82 years). It was concluded that biological sand filters with around 10% calcite will be able to abiotically neutralize winery wastewater and other wastewaters with similar acidities for the projected life span of the system. Future work should focus on determining the effect of sand grain size on the bioremediation capacity, as well as the use of biological sand reactors for treating other acidic organic wastewaters such as fruit processing, food production and distillery wastewater.

Keywords: mineralogy, neutralization, remediation, sand particles, wastewater, winery wastewater; water treatment; biological remediation

5.1 Introduction

In the cycle of production, development and processing of raw material to a final product, waste and/or wastewater (WW) is generated. The global shortage of clean water is aggravated by the rapid expansion of industries and the subsequent increase in the overall volume of WW, including

acidic WW, which has become a global environmental challenge [1–3]. If the acid WW is not sufficiently remediated, there is potential for adverse impacts on the environment and on human health [2,4]. If the WW is treated before discharge, the energy and cost requirements as well the quality and quantity of the acid WW inform the choice of treatment method. Physicochemical methods include adsorption, extraction, distillation, and membrane filtration [5–7]. However, these treatment systems require large capital outlays and skilled personnel which may not be economically feasible for some industries, especially those that do not generate large quantities of WW. Wineries typically generate acidic WW that is highly seasonal in quantity and quality, but most smaller wineries cannot afford to install, maintain and operate sophisticated WW treatment systems.

Biosand reactors (BSRs), otherwise known as biological sand filters (BSF) or unplanted constructed/treatment wetlands are cost-efficient, low maintenance treatment systems that are able to effectively biodegrade the organic fraction of winery wastewater (WWW). Operational results from a pilot horizontal flow system as well as a more advanced vertical flow system with a novel hydraulic design have shown that the systems are also effective in neutralizing acidic WWW while increasing the sodium adsorption ratio (SAR) of the effluent [8,9]. The neutralization mechanism has been attributed to abiotic dissolution of calcite present in the sand [8,9] and may be applicable to other forms of acidic WW, similar to passive systems for treatment of acid mine drainage [10,11].

While there are in depth reports in the literature on determining and modelling particle shapes in sand [12–14], and it has been shown that stress pressure is the major factor influencing the size and shape of sand particles, including calcareous particles [15], there is little information in the literature comparing the shape and size of particles in sand with mixed mineralogy. Using QEMSCAN® analyses [16,17], demonstrated differences in particle shape related to minerology in dune sand containing approximately 18% calcite and 81% quartz. In this case, it was found that the calcite particles were more angular and less round than the quartz particles. As shape plays a major role in particle packing, the hydraulic conductivity (HC) of the sand could not be predicted accurately using existing models which assume particle sphericity [17].

The dissolution kinetics of $CaCO_3$ from the calcite is affected by the pH and partial pressure of CO_2. Three heterogenous reactions usually take place simultaneously at the solid–liquid

interface, namely: solid surface protonation where the H^+ ions in solution diffuse to the solid surface (Equation (5-1)), surface interaction with carbonic acid (H_2CO_3) where it adsorbs to the surface of the calcite (Equation (5-2)), and surface hydration where the H_2O migrates to an active site (Equation (5-3)) [18]. The exothermic dissolution reaction (Equation (5-4)) results in an increase in temperature of the solution and a decrease in calcite solubility [19]. Once solubilised, the products desorb into solution and migrate away from the reaction sites into the bulk solution [18]. Solubilisation is driven at lower pH values, while reprecipitation can take place when the pH of the WWW increases. While BSRs are effective at neutralizing acidic WWW, the longevity of the abiotic calcite dissolution process is unknown and cannot be modelled using kinetics due to the seasonal variability in the quality and quantity of this effluent, both inter- and intra-winery [8,9,20]. In addition, flow rates are confounded by the attachment of functional microbial biomass to the sand particles creating ever-changing porosities in the sand matrix [17]. The pH of the liquid is the most important parameter affecting calcite dissolution. Although WWW generally has a low pH and high sodium (Na) concentration, the pH varies from acidic to alkaline depending on the seasonal cellar practices taking place at the time of WWW generation [21,22].

$$CaCO_3 + H^+ \leftrightarrow Ca^{2+} + HCO_3^- \quad (5\text{-}1)$$

$$CaCO_3 + H_2CO_3 \leftrightarrow Ca^{2+} + 2HCO_3^- \quad (5\text{-}2)$$

$$CaCO_3 + H_2O \leftrightarrow Ca^{2+} + HCO_3^- + OH^- \quad (5\text{-}3)$$

$$CaCO_3 \leftrightarrow Ca^{2+} + CO_3^{2-} \quad (5\text{-}4)$$

This study was conducted in order to ascertain: (i) How BSR systems may function over time in terms of calcite removal *viz* how long before the abiotic neutralizing capacity is expended, (ii) Whether calcite dissolution kinetics can be improved by using different sand size fractions in BSRs, (ii) Whether calcite dissolution positively or negatively affects the HC of fractionated and/or raw sand, (iii) How results obtained from *ex-situ* column experiments may relate to field data from operational BSRs.

5.2 Materials and Methods

5.2.1 Column experiments: set-up and operation

The experimental set-up (Figure 5-1) consisted of a series of identical clear acrylic columns with internal diameters of 30 mm and lengths of 500 mm. The particle size distribution of the raw sand was determined (Section 5.2.4.2), and the sand was then partitioned into six different size fractions. Each column was filled with 100 g of Dune quarry sand from Philippi, Cape Town, South Africa with either raw (unfractionated) sand or sand with different size fractions (Table 5-1). The bottom caps of the columns consisted of stainless-steel screens and open cell polyurethane that retained the sand but did not impede the flow rate. The sand was saturated and allowed to settle for 24 h and the HC was determined by the falling head method using tap water as described in Section Calculation of hydraulic conductivity by the falling head method. The columns were then placed in a 37 °C constant environment room overnight to allow the sand to dry.

Figure 5-1 Experimental setup

Each column was dosed continuously with 2.5 L of either 0.1M HCl (tests) or distilled water (dH_2O) (negative control) using IVAC volumetric pumps (Model 597) at a flow rate of 10 $mL.h^{-1}$ for a total of 10.4 days at a hydraulic retention time (HRT) of approximately 1.8 h and a hydraulic loading

rate (HLR) of ±3850 $L.m^{-3}$ of $sand.day^{-1}$. The volume of influent was theoretically calculated according to Equation (5-1) to ensure complete dissolution of calcite in the original fraction of sand. The effluent was collected in enclosed Erlenmeyer flasks which were emptied daily, and the contents from each column was pooled and stored at 3 °C until the end of each respective column experiment. After the dosing period, the final HC was measured and the sand was allowed to dry at 37 °C and then weighed. Each experiment was conducted in triplicate.

Table 5-1 Sand particle size fractions in columns (100 g per column)

COLUMN (*N*=3)	PARTICLE SIZE (MM)	AMOUNT (% WT.WT)	CA IN SAND (%WT.WT)	COMMENTS
1 & 2	>1.00-2.00	10.0	5.4±0.01	Raw (unfractionated) sand
	>0.600-1.00	24.3		
	>0.425-0.600	18.2		
	>0.300-0.425	19.7		
	>0.150-0.300	25.3		
	>0.075-0.150	2.1		
	<0.075	0.4		
3	>1.00-2.00	29.2	3.0±0.05	Homogenized fractions
	>0.600-1.00	70.8		
4	0.425-0.600	48.0	6.7±0.07	Homogenized fractions
	>0.300-0.425	52.0		
5	0.150-0.300	91.0	6.8±0.03	Homogenized fractions
	>0.075-0.150	7.6		
	<0.075	1.4		
6	1.00-2.00	13.9	4.8±0.04	Homogenized fractions
	>0.600-1.00	13.7		
	>0.425-0.600	25.2		
	>0.300-0.425	27.3		
7	1.00-2.00	10.3	5.6±0.019	Homogenized fractions
	>0.600-1.00	24.9		
	>0.425-0.600	18.7		
	>0.300-0.425	20.2		
	>0.150-0.300	25.9		

5.2.1.1 Calculation of operational parameters

Calculation of hydraulic conductivity by the falling head method

The HC or co-efficient of permeability (k) of a porous media is the ease of which water passes through it and is defined by Darcy's Law. This can be rewritten in terms of the falling head conditions as in Equation (5-5):

$$k = (2.303aL/At)\ Log_{10}(h_1/h_2) \qquad (5\text{-}5)$$

where a is the cross-sectional area of the standpipe, L is the length of the porous media, A is the cross-sectional area of the porous media, t is the time the liquid takes to drop from h_1 to h_2, and h_1 and h_2 are the start and stop levels above the outlet. In this study, in the HC experiment, h_1 and h_2 were 470 mm and 145 mm above the stainless-steel sieve, respectively. The times taken for the water to drop from h_1 to h_2 were recorded with a stopwatch. Each measurement was conducted in triplicate.

5.2.1.2 Hydraulic loading rate

The HLR was calculated in terms of the volume of sand within the column described by Holtman et al. (2018) and was on average 2244 $L.m^{-3}$ of $sand.day^{-1}$.

5.2.2 Biosand filters: set up and operation

A novel pilot scale BSR/BSF was installed and operated at a small cellar within the Western Cape, South Africa. The system consisted of four units (1.73 × 1.05 × 0.42 m) filled with a total volume of 7.26 m^3 sand which were gravity fed and operated in horizontal subsurface flow mode (Figure 5-2). The system treated approximately 402 $L{\cdot}day^{-1}$ or 137 $L{\cdot}m^{-3}$ of $sand{\cdot}day^{-1}$, and the performance results have been published elsewhere [8].

5.2.3 Sampling

For the column experiments, the effluent from each column was collected and analysed daily, then pooled and stored at 3 °C until the end of each experimental run. Samples of homogenised fractions and raw unfractionated sands (Table 5-1) were set aside before the start of the experiments. At the end of the experimental period, the contents of each column were homogenised and sampled.

In order to compare the *ex-situ* experimental data obtained with the columns with *in-situ* operational data, core samples were extracted from each of the 4 BSR modules each year for three years. At each sampling instance, six sand cores were extracted from each module (three from the inlet and three from the outlet, as shown in Figure 5-2). The contents of each core were partitioned into samples from the top (0–5 cm below the surface) and bottom (25–30 cm below the surface) of the BSRs. A total of 144 samples were collected (48 each year). All the sand samples were dried thoroughly before further analyses.

An additional set of samples were taken at 3.2 years at the inlet, middle and outlet from one filter at (0–5 cm below the surface) and middle (25–30 cm below the surface) bottom (45–50 cm below the surface) of the BSRs.

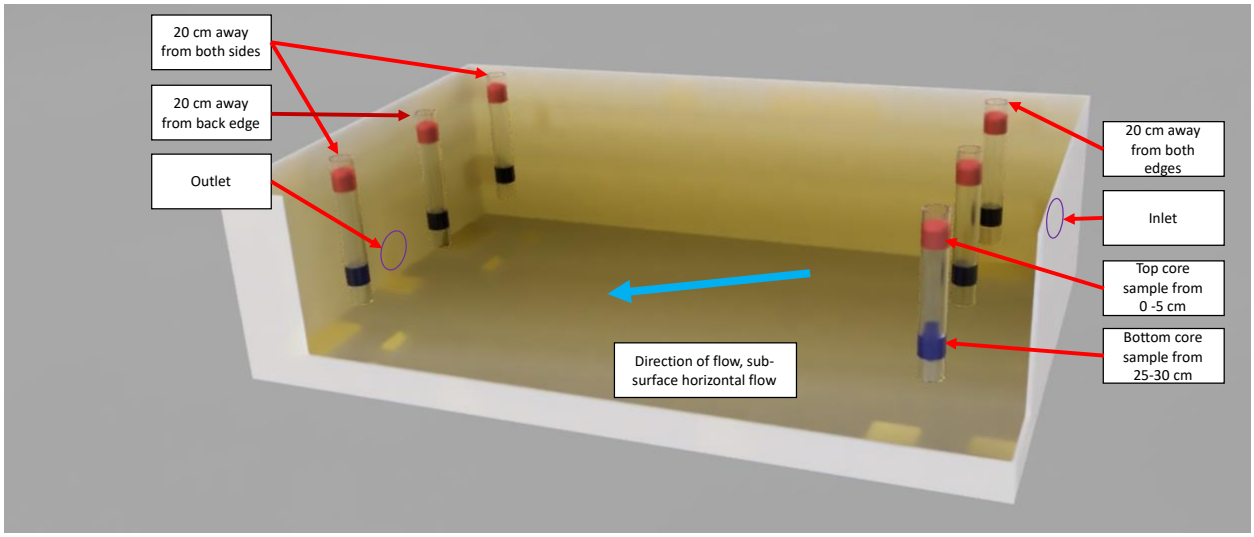


Figure 5-2. Sample locations of onsite treatment system

5.2.4 Analytical procedures

5.2.4.1 Effluent

The pH of the daily and pooled effluent was determined according to the manufacturer's instructions using a CyberScan pH300 meter and appropriately calibrated pH probe PHWP300/02K (Eutech instruments, Singapore). Concentrations of Ca in the pooled effluent samples were determined using a Thermo ICap 6200 ICP-AES plasma optical emission spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) at the Central Analytical Facility at Stellenbosch university (Stellenbosch, South Africa) according to the manufacturers' instructions.

5.2.4.2 Sand

The particle size distribution of the sand was performed according to SANS 3001 (Method AG1, PR5, AG21) by Cetlab, South Africa.

The sand samples were ground to a fine powder by swing milling and the elemental composition was determined by the Central Analytical Facility by X-ray fluorescence (XRF) spectrometry on a PANalytical (Almelo, The Netherlands) wavelength dispersive spectrometer according to the manufacturer's instructions.

Fractionated samples of sand were analysed by automated scanning electron microscopy using a FEI QEMSCAN® (Quantitive Evaluation of Minerals by SCANning Electron Microscopy) instrument as previously described [23] (Thermo Fisher Scientific, Waltham, MA, USA) and iDiscover™ software. The roundness and aspect ratios of the different mineral fractions determined using the iDiscover™ software were used to compare the shapes and sizes of particles with different mineral compositions according to the method described by [16].

5.3 Results and discussion

5.3.1 Calcite dissolution in raw sand and fractionated sand: column experiments

The concentration of Ca was measured as an equimolar proxy for dissolution of $CaCO_3$ from the calcite particles (Equations (5-1)–(5-3)). In order to cross check $CaCO_3$ solubilisation, the total amount of Ca captured in the column effluent was compared with the total amount of Ca lost from the sand in the columns (Figure 5-3A). Results were generally in good agreement with one another, with no significant differences between the amount of Ca lost and Ca captured ($p > 0.05$, paired T-test).

Only negligible amounts (<1%) of $CaCO_3$ were solubilised from the negative control columns containing raw unfractionated sand treated with dH_2O (column 1, Figure 5-3). In the case of the test columns, less $CaCO_3$ was mineralised in the columns containing the larger sand particles (column 3) than in the other columns. This was an anomaly unique to column 3 because: (i) the initial Ca concentration in the sand (3% wt.wt) was lower than in the other columns (4.8–6.8% wt.wt, Table 5-1), (ii) 99% of the Ca was removed during the experimental period, and (iii) the neutralization endpoint was achieved between day 6 and day 7 of the experiment (Figure 5-4). In comparison, neutralization endpoints were achieved after 10 days in the columns containing raw sand (column 2), and those with medium/large particles sizes only (columns 6 and 7), with 91%, 96% and 95%, respectively, of the Ca being solubilised. In contrast, only 84–85% of the Ca was solubilised in the columns that contained only small sand particles (columns 4 and 5), and no neutralization endpoint was achieved after 11 days. However, these columns contained the highest Ca concentrations (≥6.7 g), and the amount of HCl solution added was theoretically calculated to solubilise 5.00 g $CaCO_3$.

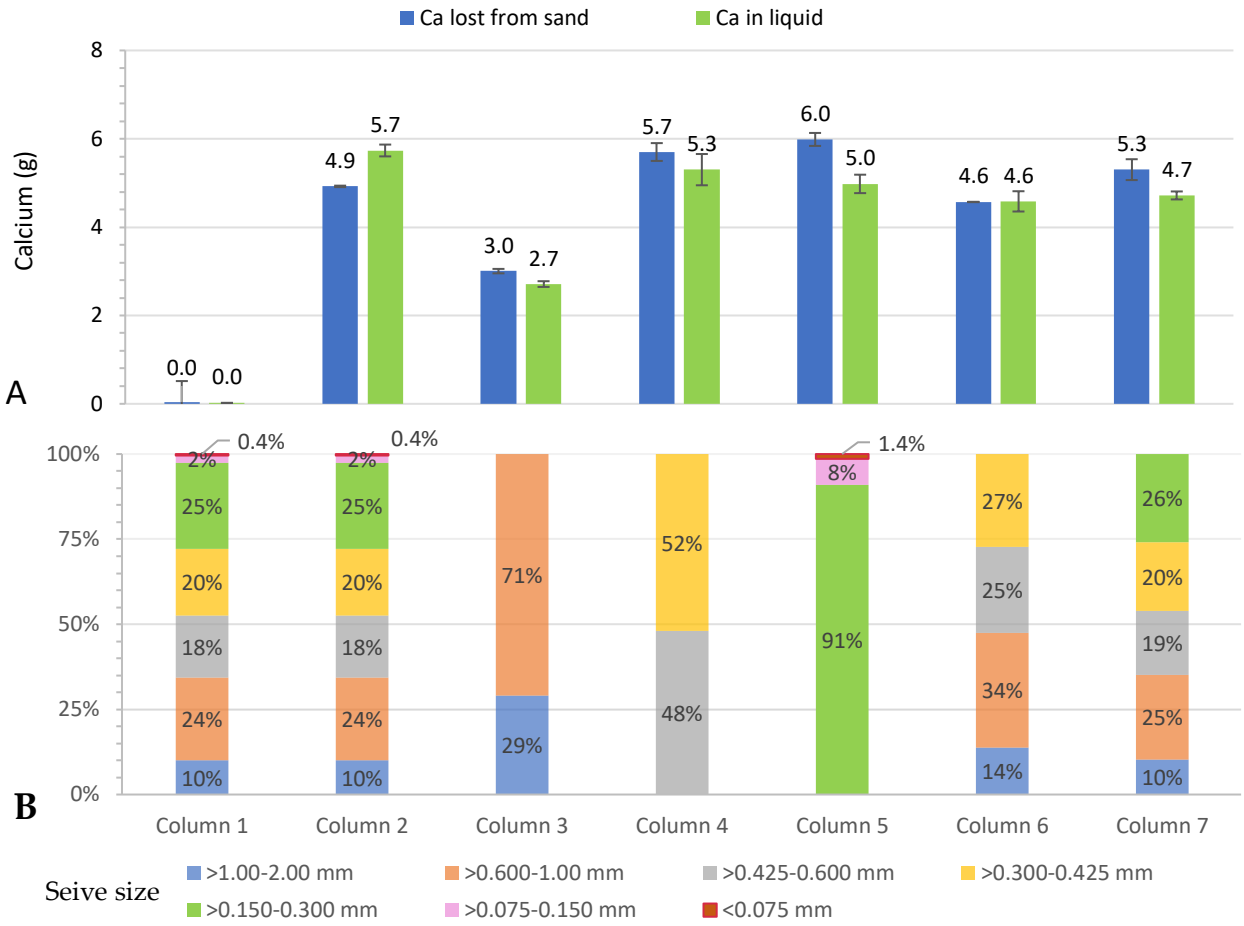


Figure 5-3. Mass balance of the calcium captured in the effluent and the calcium lost from the sand (A), and the distribution of grain size fractions in the columns (B). The bars from plot A are aligned vertically with those from plot B, both representing columns 1 to 7.

Overall, as shown in Figure 5-4 there were no significant differences in the average pH values measured in the effluent from the test columns (2–7) over the first 5 days of the experimental period (ANOVA: F crit > F, $p > 0.05$), indicating that $CaCO_3$ solubilisation may not be significantly affected by particle size provided sufficient calcite is available. The results clearly demonstrate that from a long-term neutralization perspective, it is important to include sand particles with higher calcite concentrations in BSRs. In this study, more of the smaller sand particles were composed of calcite, as discussed in more detail in Section 5.3.3. This may also be beneficial in terms of dissolution kinetics because the overall reaction surface area is larger with smaller particles [10].

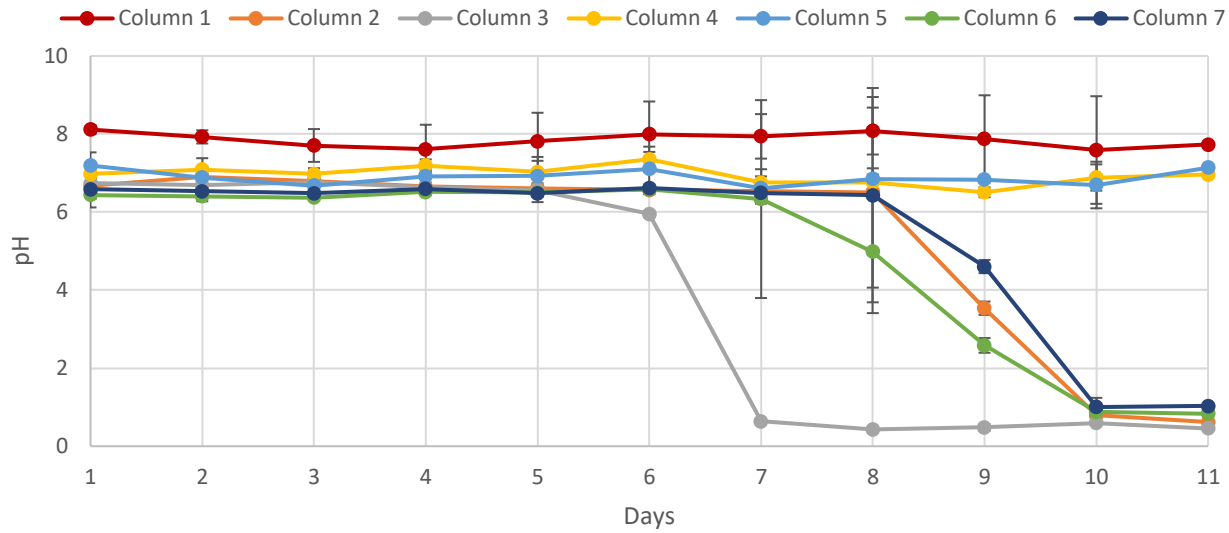


Figure 5-4. Daily effluent pH measurements used to compare neutralization efficiencies and endpoints

5.3.2 Hydraulic conductivities of raw and fractionated sand: column experiments

The column $CaCO_3$ dissolution experiments (Section 5.3.1) provided insight into which fractions of sand and combinations thereof can maximise the capacity of BSRs to neutralize acidic WW such as WWW. However, the HC of BSRs needs to be maintained at rates that will allow sufficient HRT for treatment but not impede the flow to the extent where the treatment capacity becomes limited [9,24]. For spherical sand particles, the HC increases as the particles increase in size and/or become less uniform in size [25,26]. More holistically, the particle size, particle size distribution (PSD) and particle morphology all influence the manner in which the particles physically pack together [27–29]. The porosity as well as the intrapore distribution space are key parameters influencing the HC, and these are dependent on particle packing [27–29].

To determine whether particular fractions of sand particles, or combinations thereof could offer both good flow properties as well as neutralization efficiencies, the HCs of the sand-containing columns were experimentally determined (Figure 5-5). There were no significant differences ($p > 0.05$, paired T-test) in the HC measurements taken before and after $CaCO_3$ solubilisation, indicating that the loss of the calcite particles did not have a negative effect on particle packing. The HC measurements in column 3 containing the largest particles (>0.6 to 2 mm) were more than 10-fold higher than those in the columns 1,2 and 5 containing the smallest particles (≤0.150 mm). In all the columns containing fractionated sand mixes without particles ≤0.150 mm (columns 3, 4, and 6), the HC was higher than that in the columns containing raw (unfractionated)

sand. The second highest HC was measured in the columns containing all fractions of sand >0.150 mm (column 6); however, this was only half the HCs measured in the columns containing the larger particles (column 3) but double the HC of the raw sand (columns 1 and 2).

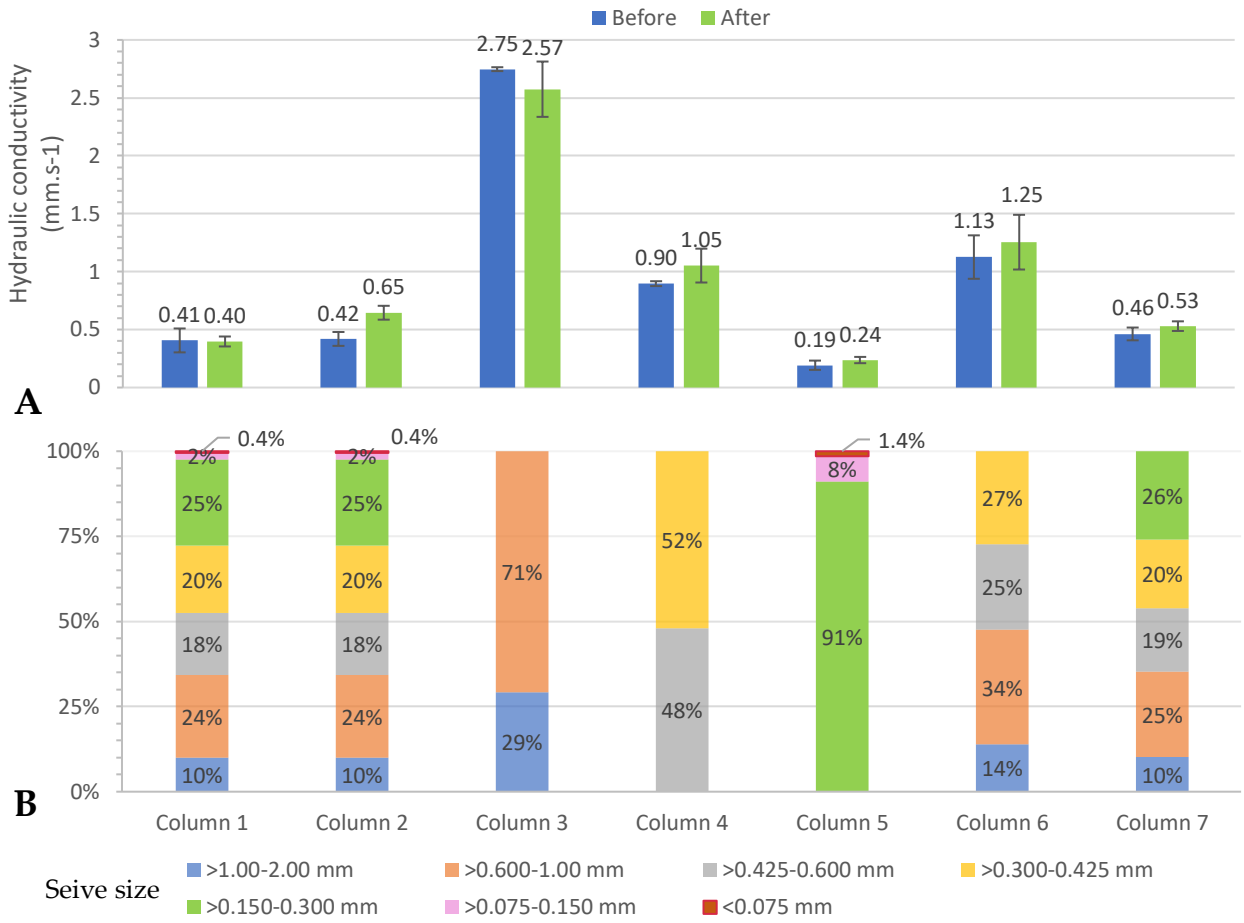


Figure 5-5. Measured hydraulic conductivity in sand columns before and after calcite dissolution (A), and the distribution of grain size fractions in the columns (B). The bars from plot A are aligned vertically with those from plot B, both representing columns 1 to 7.

In summary, the column experiments showed that prudent fractionation of sand particles can increase the HC of sand-based treatment systems significantly. As expected, the columns with the most and least efficient HCs were those containing the largest and smallest particles, respectively. The converse was true when considering the neutralization capacity (Section 5.3.1). When designing BSR systems, if the HC of raw sand is too low to achieve design HLRs, it may justify the cost of fractionating the sand. Following this, it should be considered which fractions of sand afford the best treatment performance. In this study, there was a trade-off between achievable flow rates and neutralization capacity. It must also be noted that while neutralization of acidic WW was emphasised in this study, other important factors such as the bioremediation capacity may also differ between sand fractions.

5.3.3 Temporal abiotic neutralization capacity (TANC) of biological sand reactors

5.3.3.1 Theoretical values based on data obtained from column experiments

To date, operational BSR systems have been filled with raw (unfractionated sand) [8,9]. In the column replicates containing raw sand (5.4 ± 0.01 g), the neutralization endpoint was reached after 10 days, and 91% of the Ca in the columns was solubilised and washed out after 11 days. These results indicated that 9% of the calcite in the raw sand was recalcitrant to solubilisation and/or that preferential flow paths existed within the column intra-pores so that some calcite particles were not exposed to the acidic influent. The latter is more likely, as smaller particles can fill the void spaces afforded by larger particles, hampering flow on a spatial level, as evidenced by low HC values previously measured using sand with mixed grain sizes [17].

The longevity of BSRs in terms of the temporal abiotic neutralization capacity (TANC) is theoretically affected by three main factors: the available calcite, the HC (or flow rate), and the influent WW composition. The pH is the most important WW parameter affecting calcite dissolution kinetics. Although the calcite in a particular batch of sand is finite, it is anticipated that the neutralization capacity may be supplemented in a dedicated upstream or downstream process similar to a permeable reactive barrier (PRB) once the calcite within BSRs is expended [30].

Simplified reaction kinetics (Equation (5-6)) using the major overall chemical reaction were used to determine the TANC of BSR systems containing the raw sand and the different sand fractions used in the column experiments. Based on Equation (5-4), Equation (5-7) was formulated to calculate the longevity of BSRs per cubic meter of sand by using the mass of available calcium concentration with the sand and dividing it by the amount of H^+ applied to the sand due to the influent pH values (Ca as a proxy for $CaCO_3$).

$$CaCO_3 + 2HCl \leftrightarrow CaCl_2 + CO_2 + H_2O \qquad (5\text{-}6)$$

$$T_{years} = \%Ca \times \rho \div (731.4235 \times Q \times (10^{-pH} - 10^{-7})) \qquad (5\text{-}7)$$

In Equation (5.7) the %Ca is the percentage calcium available in the sand, ρ is the density of the sand, Q is the flow rate of a reactor expressed in $L{\cdot}m^{-3}$ of $sand{\cdot}day^{-1}$, and 731.4235 is a constant

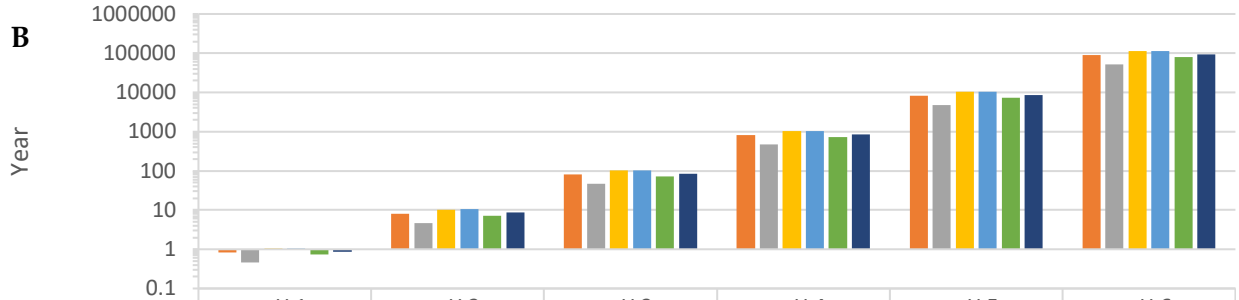
for converting the molar masses of Ca to H^+ required per annum at a given Q. The pH is converted into the concentration of H^+, and effluent pH values > 7 are ignored.

The theoretical TANCs based on a pilot BSR system containing raw sand from the same quarry site and operated in horizontal subsurface flow mode [8] were calculated for the raw sand and different sand fractions used in the column experiments. The experimental HCs measured in the column experiments (Figure 5-5A) were notably higher than the actual HC (0.040 $mm \cdot s^{-1}$) calculated from the *in-situ* flow rates (150 $L \cdot m^{-3}$ of $sand.d^{-1}$) in the pilot BSR system [8].

When flow rates were extrapolated from the experimental HCs determined in the column experiments to the scale (0.735 m^3) of the pilot BSR system, the TANC ranged from around 2 months to over 15.8 centuries for influent with pH values ranging from 2 to 6 (Figure 5-6A). The HC values for the columns containing raw sand (column 2 replicates) and the columns with the highest HC and least amount of Ca (column 3 replicates) would translate into theoretical flow rates of 2400 $L \cdot m^3$ of $sand \cdot day^{-1}$ and 15600 $L \cdot m^3$ of $sand \cdot day^{-1}$, respectively, in the BSR system. Although such high flow rates can increase treatment capacity, the RTs of 10.4 and 1.5 h, respectively, would be insufficient for effective WW bioremediation. In reality, BSRs can be designed with mechanisms to retard flow rates/RTs and, to a lesser extent, to increase flow rates [9].

The HC values obtained in the column experiments did not account for the accumulation of functional biomass, which was shown to reduce the HC by up to 80% without problematic clogging [8]. The microbial biomass in sand-based systems promotes not only biodegradation, but also flocculation, adsorption and interception [31].

Taking all these factors into account, the TANCs were re-calculated using the actual HC achieved in the pilot BSR system (Figure 5-6B). In each case, the values increased. For example, the theoretical TANC of a BSR containing the same sand as column 2 increased from around 6 months to 8 years and from 5 to 90 centuries at influent pH values from 2 to 6, respectively.



	pH 1	pH 2	pH 3	pH 4	pH 5	pH 6
Column 2	0.8	8.2	82	818	8251	90762
Column 3	0.5	4.6	46	464	4678	51460
Column 4	1.0	10.1	101	1015	10239	112629
Column 5	1.0	10.4	104	1039	10487	115358
Column 6	0.7	7.3	73	728	7343	80771
Column 7	0.9	8.6	86	857	8645	95095

Figure 5-6. Log graphs and tables showing the theoretical abiotic neutralization capacity values of a horizontal flow gravity-fed biological sand reactor system with flow rates based on: (A) Colum experiments (this study), and (B) in-situ flow rates in a pilot biological sand reactor system as described by Holtman et al. (2018)

5.3.3.2 Validation of theoretical results with data obtained from operational biological sand reactors

In order to validate the TANC results obtained using Equation (5-7) (Figure 5-6), a three-part approach was adopted using the results from the on-site BSR system and the measured flow rates of that system. Three chemical parameters were assessed: (i) the Ca concentrations in sand cores taken annually over a three-year period, (ii) The difference in Ca concentrations between influent and effluent (Ca solubilised), and (iii) the average H^+ of the influent calculated from the pH values [8].

5.3.3.2.1 Results based on the calcium concentrations in core samples

The Ca was preferentially removed from the top inlet of the BSR modules, with negligible removal in the other core samples (Figure 5-7A). The different spatial results were anticipated as the WWW is expected to become gradually less acidic as it passes through the BSR modules, perhaps even precipitate again as $CaCO_3$ in some instances towards the outlet. Despite the spatial variation, it was estimated from averaged inlet, middle and outlet core results taken after 3.2 years of operation that 8.6% of the calcite or a 5.3 kg Ca·m^{-3} of sand·year^{-1} had been solubilised (Table 5-2), translating into a TANC of approximately 37 years with an influent of the same strength and composition.

Table 5-2 Percentage calcium in sand from cores taken from biosand reactors treating winery wastewater

Position	Inlet (Ca %WT.WT)	Middle (Ca %WT.WT)	Outlet (Ca %WT.WT)
Top (0-5 cm)	4.5	9.0	9.2
Middle (25-30 cm)	9.2	9.3	9.4
Bottom (45-50 cm)	9.4	9.2	9.5

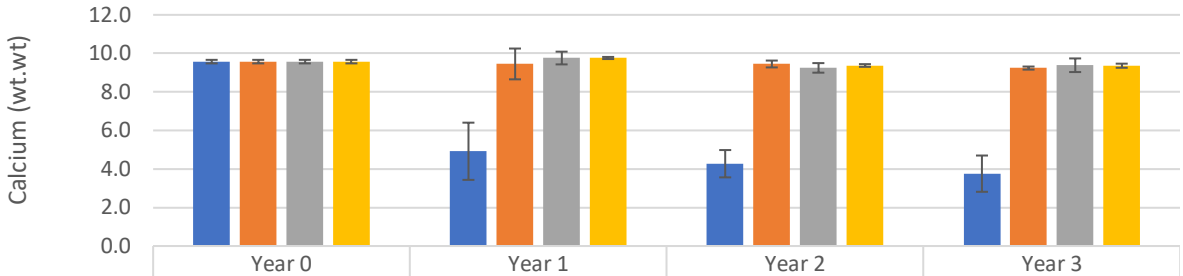
5.3.3.2.2 Results based on influent and effluent calcium concentrations

The Ca concentration increased by an average of 24 ± 32 $mg \cdot L^{-1}$ from influent to effluent, which translates into a removal of 1.3 kg ± $Ca \cdot m^{-3}$ of $sand \cdot year^{-1}$ as previously published [8]. The initial Ca concertation in the sand was 159 $kgCa \cdot m^{-3}$ of sand (9.6% wt.wt), which would result in a TANC of 132 years.

5.3.3.2.3 Results based on influent pH values

The pH measurements in the WWW influent ranged from 4.55 to 7.95 (n = 33) as previously published by [8]. The amount of Ca that could theoretically be solubilised for the amount of H^+ added was 0.003 $mol \cdot year^{-1}$, indicating a TANC of 17,652 years using Equation (7).

In summary, the three approaches provided notably different results ranging from 37 years to 17,652 years. Amongst other factors, the accuracy of the results based on the influent and effluent values was compromised by the variability of WWW and temporal sampling, while those based on Ca removal from the BSRs were compromised to a lesser extent by the spatial variation in calcite dissolution. Nonetheless, the preservation of calcite within the middle and outlet of the BSRs after 3.2 years provided unequivocal evidence of system longevity. The TANC of 37 years based on temporal changes in the %Ca in the extracted cores compared favorably with the theoretical TANC based on the results obtained from column 2 containing raw sand, with the actual BSR flow rates for influent with pH between 2 (8.2 years) and 3 (82 years).



	Year 0	Year 1	Year 2	Year 3
Inlet top	9.6	4.9	4.3	3.8
Inlet bottom	9.6	9.5	9.4	9.2
Outlet top	9.6	9.8	9.2	9.4
Outlet bottom	9.6	9.8	9.4	9.4

Figure 5-7. Percentage calcium in sand from cores taken from biosand reactors treating winery wastewater for three years.

5.3.4 Changes in the character of the sand particles before and after calcite dissolution in columns and biological sand reactors

The changes in the character of the sand particles after calcite dissolution were assessed using two batches of raw sand from the same quarry site, namely, the sand used in the column experiments, and the sand used in the BSR system. Only sand taken from the top inlet area of the BSRs was used to assess changes in the BSR system sand as negligible calcite dissolution was observed in the other spatial niches (Table 5.2). In the case of the columns, the calcite was dissolved artificially using HCl, while in the BSRs, real WWW was responsible for any dissolution that occurred.

5.3.4.1 Chemical composition of sand

The major minerals and accompanying elements in both batches of sand were calcite and Ca, and quartz and Si, respectively. In both batches of sand, decreases in Ca and calcite were accompanied by relative increases in Si and quartz (Table 5-3, Table 5-4). Other elements and minerals were only present in very low concentrations, and only negligible relative or actual increases or decreases occurred (Table 5-3, Table 5-4).

In the columns, the %Ca and calcite were reduced by 91%, and 99.9%, respectively (Table 5-3, Table 5-4), indicating almost complete dissolution of calcite, but there was some residual Ca, either alone or complexed with other anions in the sand. In contrast, in the BSRs, the %Ca and calcite were reduced by 57% and 29.7%, respectively, after 3 years of operation. The absence and presence of calcite in the column and BSR sands at the end of the respective experimental

periods were determined by QEMSCAN® using thousands of automated digital images, an example of which is shown in Figure 5-8 The anomalous result between the %calcite and Ca reduction in the BSFs can be explained by the addition of Ca from the influent WWW which contained an average of 36.2 mg·L^{-1} Ca (range 6.6 to 104.0 mg·L^{-1}) [8].

Table 5-3 Average major elemental composition (% wt.wt) of the raw sand determined using X ray diffraction (n=4)

	Al	Ca	Cr	Fe	K	Mg	Mn	Na	P	Si	Ti
COLUMN BEFORE	0.15	5.39	bdl	0.05	0.11	0.01	bdl	0.06	0.01	43.0	0.03
COLUMN AFTER	0.16	0.54	bdl	0.04	0.12	bdl	0.01	0.02	0.00	49.0	0.02
BSR YEAR 0	0.18	9.57	bdl	0.06	0.13	0.06	bdl	0.10	0.01	37.7	0.03
BSR YEAR 3	0.24	4.26	bdl	0.09	0.17	0.06	0.01	0.04	0.02	43.9	0.03

bdl = below detectable limit

Table 5-4 Mineral composition of the raw sand determined using QEMSCAN®

	COLUMN BEFORE				**COLUMN AFTER**			
SIZE FRACTION (µM)	Combined	1180>	>425	<425	Combined	600>	>300	<300
QUARTZ	81.3	87.8	80.0	82.5	87.49	97.30	99.36	97.91
FELDSPAR	0.8	0.1	0.9	0.8	1.25	2.46	0.50	1.56
MICA	ND	ND	ND	ND	0.07	0.14	0.02	0.09
OTHER SILICATES	ND	ND	ND	ND	0.06	0.03	0.04	0.13
CALCITE	17.6	11.9	19.0	16.2	0.02	0.01	0.02	0.06
FE-OXIDES /HYDROXIDES	0.1	0.1	0.1	0.1	0.08	0.05	0.05	0.18
OTHERS*	0.1	0.0	0.1	0.2	0.03	0.01	0.01	0.08

ND = not detected

5.3.4.2 Size and shape of sand particles

After respective dissolution with HCl and WWW, the sand particle sizes between 0.1 and 1 mm in both the columns and the BSR sands decreased, but the differences were negligible in the BSR sand (Figure 5-9). This could be related to some extent to the lower calcite dissolution in the BSR and/or physical effects of the HCl on the non-calcite particles in the column sand.

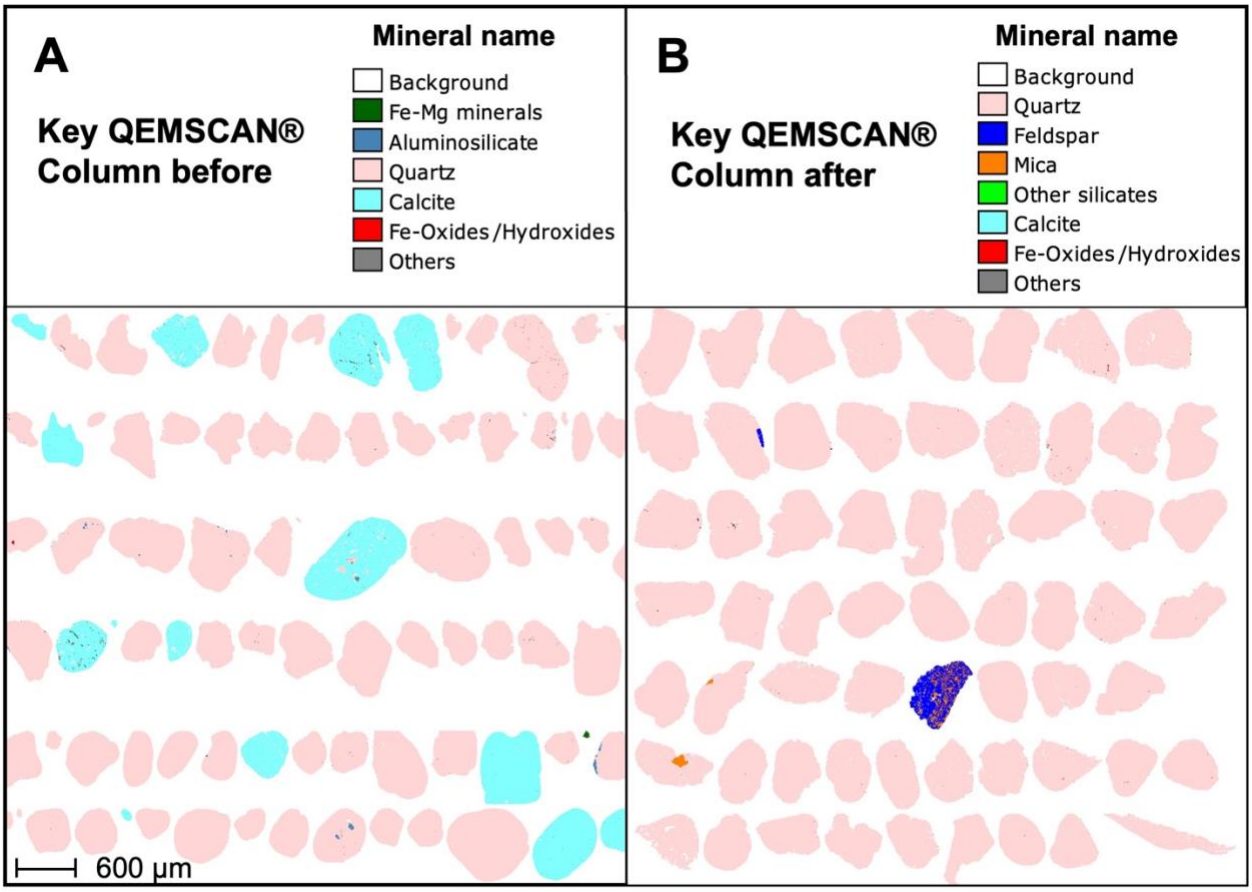


Figure 5-8. Particle image of column sand before (A) and after (B) calcite dissolution

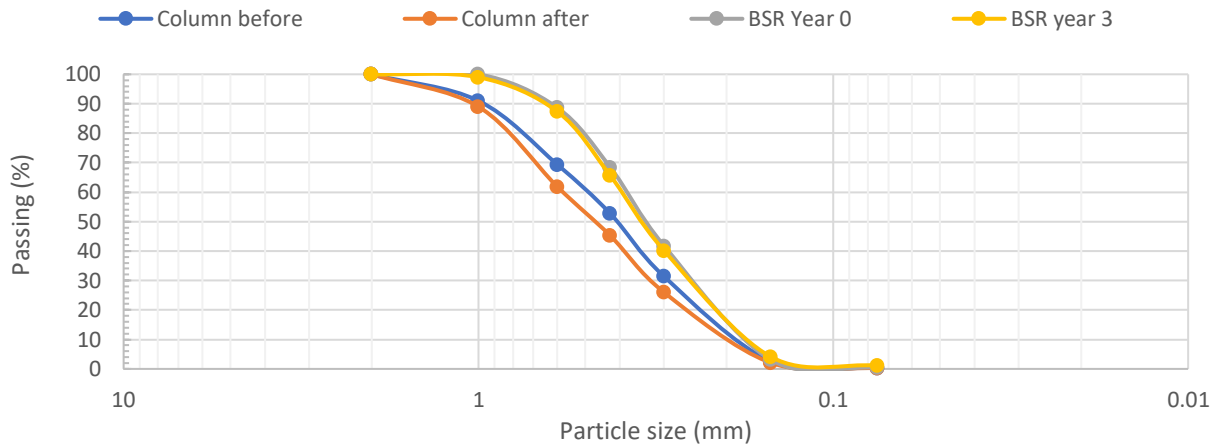


Figure 5-9. Particle size distribution curves for the batches of sand used in the column 2 experiments and the biological sand reactors at the beginning and end of the respective experimental periods

The shape of particles in terms of roundness and elongation can be described using roundness and aspect ratios [16]. Roundness is the ratio of the area of the particle to the smallest perfect circle able to fit around the particle outline, with rounder particles having higher roundness ratios. The aspect ratio is a ratio of the lengths of the long and short particle axes, so that higher ratios equate to more elongated particles [16]. In this study, the mineral composition of the

grains was relatively pure and the quartz particles of both batches of raw sand before dissolution were generally rounder and more elongated than the calcite quartz particles (Table 5-5, Figure 5-8, Figure 5-10)

In the columns, only 9% of the original calcite particles remained after dissolution. Although the roundness and aspect ratio mass balances of these particles showed marked temporal differences, no trends were noted (Table 5-5). The quartz particles, however, became less round as well as less elongated, possibly due to the action of the HCl on the particle surfaces.

In the BSRs, there were no significant temporal changes in the roundness or aspect ratios of either the calcite or quartz particles ($p > 0.05$, paired T-test). It was hypothesized that the less acidic WWW allowed the particles to maintain their structure in comparison with the particles subjected to HCl in the columns. In addition, less calcite was solubilised in the BSRs.

Table 5-5 Relationship of mass % distribution of quartz and calcite particles in terms of aspect ratio and roundness

COLUMN									
ASPECT RATIO	<0.2	<0.3	<0.4	<0.5	<0.6	<0.7	<0.8	<0.9	<1.0
%QUARTZ BEFORE (N= 2572)	0.00	0.02	0.15	2.19	8.24	18.19	28.93	26.88	15.40
%QUARTZ AFTER (N=5191)	0.00	0.18	0.96	4.40	12.48	23.30	27.44	23.03	8.15
%CALCITE BEFORE(N=253)	0.53	1.81	4.60	15.85	13.77	21.58	18.48	15.10	8.28
%CALCITE AFTER(N=478)*	0.00	0.00	0.79	2.33	33.39	16.38	1.76	3.28	42.07
ROUNDNESS	<0.2	<0.3	<0.4	<0.5	<0.6	<0.7	<0.8	<0.9	<1.0
%QUARTZ BEFORE (N= 2572)	0.00	0.10	1.17	8.13	18.86	33.26	31.47	7.00	0.00
%QUARTZ AFTER=5191)	0.10	0.66	3.99	14.72	29.13	32.06	16.69	2.66	0.01
%CALCITE BEFORE (N=253)	0.53	1.81	4.60	15.85	13.77	21.58	18.48	15.10	8.28
%CALCITE AFTER(N=478)*	0.00	4.78	6.95	9.26	32.95	5.38	0.00	1.17	39.50
BSR									
ASPECT RATIO	<0.2	<0.3	<0.4	<0.5	<0.6	<0.7	<0.8	<0.9	<1.0
%QUARTZ YEAR 0 (N=5267)	0.00	0.08	1.17	5.35	14.22	21.47	29.24	20.33	8.13
%QUARTZ YEAR 3 (N=5472)	0.00	0.12	1.12	5.34	13.90	23.78	25.59	20.11	10.04
%CALCITE YEAR 0 (N=4768)	0.96	4.20	16.69	18.99	22.18	14.48	11.20	7.87	3.42
%CALCITE YEAR 3 (N=2999)	0.52	5.88	17.11	16.90	17.31	19.77	11.89	7.53	3.07
ROUNDNESS	<0.2	<0.3	<0.4	<0.5	<0.6	<0.7	<0.8	<0.9	<1.0
%QUARTZ YEAR 0 (N=5267)	0.06	0.89	5.12	17.78	30.59	31.48	12.00	2.03	0.06
%QUARTZ YEAR 3(N=5472)	0.02	0.92	5.86	16.82	32.08	26.09	15.51	2.66	0.05
%CALCITE YEAR 0 (N=4768)	1.67	11.11	24.11	27.00	18.75	11.48	4.50	1.16	0.22
%CALCITE YEAR 3 (N=2999)	1.50	11.01	25.34	25.14	19.25	13.40	3.46	0.70	0.18

* = calcite in too low quantities for reliable shape data

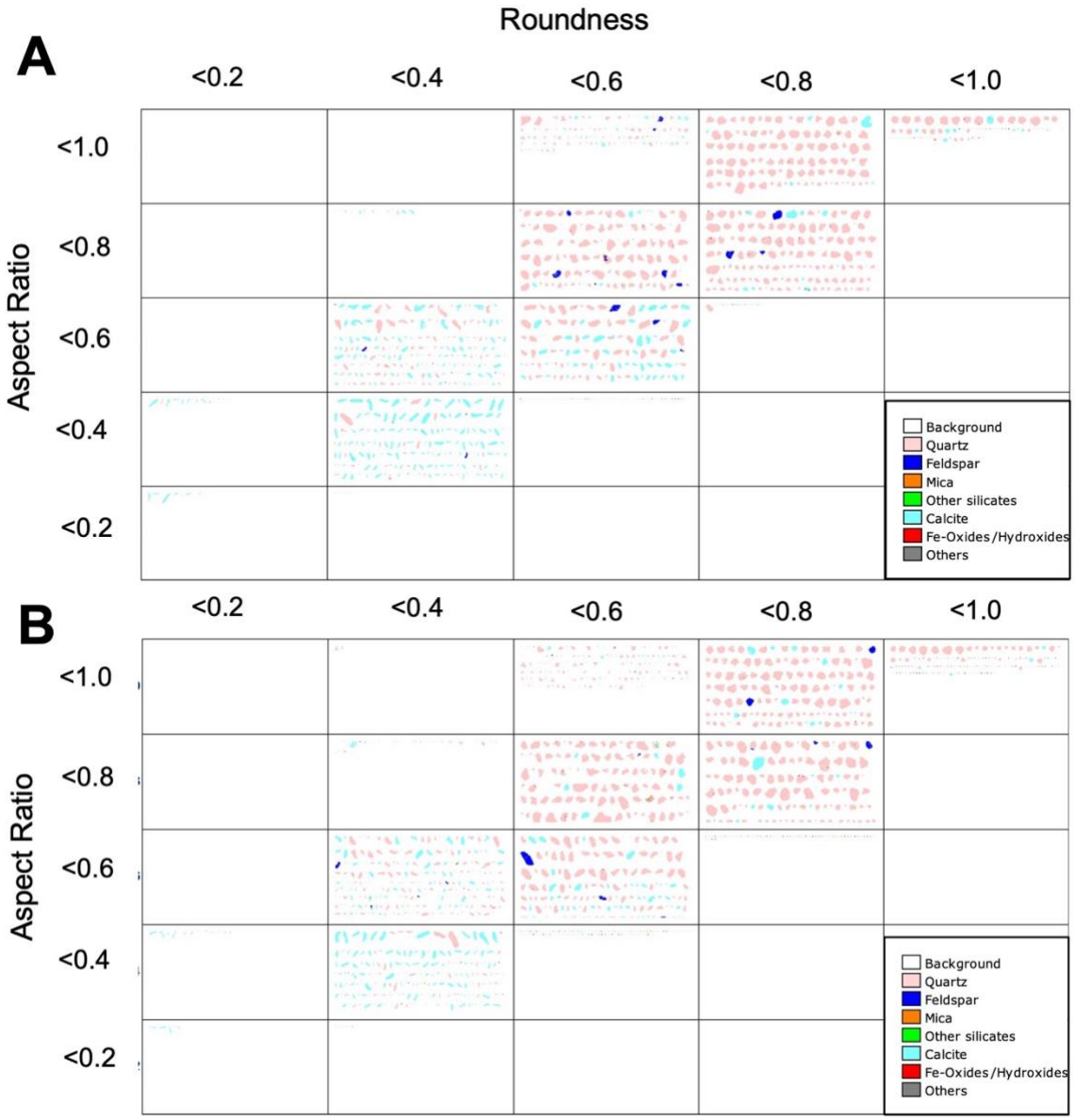


Figure 5-10. Image showing the mineralogy and relative roundness and aspect ratios of the different sizes of sand particles from the biological sand reactors of core samples taken at year 0 (A) and year 3 (B)

5.4 Conclusions

Biosand reactors containing unfractionated sand have a TANC longer than the projected lifespan of the related infrastructure. The hydraulic conductivity is unaffected by dissolution of calcite particles but can be increased by removing smaller sand particles. In real world situations, it is suggested that the sand is replaced every 10 to 15 years or when the effluent pH is no longer increased through the filters. Based on the results of this study, future research should focus on the bioremediation performance of BSRs containing selected fractions of sand, as well as changes in HC that occur due to build-up of functional biomass in these systems. It is suggested that future

BSRs should operate in vertical flow conditions which allows for sufficient head across the filter together with the ability to manipulate flow rates, and the systems should be operated with an HRT of no less than 24 h to allow sufficient microbial interaction with the wastewater. It is also suggested that the optimal grading would be sand particles > than 0.450 mm which would be a combination of the sand used in column 3 and column 4 in this study. For the sand used in this study, this would constitute 54% of the entire grading and would provide the 46% fraction with smaller particles for use in concrete mixes where smaller particles are more desirable. The projected TANC of such a system would be 68 years. In addition, the use of BSRs for the remediation of other acidic organic WW should be investigated.

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Chapter 6

Anaerobic digestion of primary winery wastewater sludge and evaluation of the character of the digestate as a potential fertilizer

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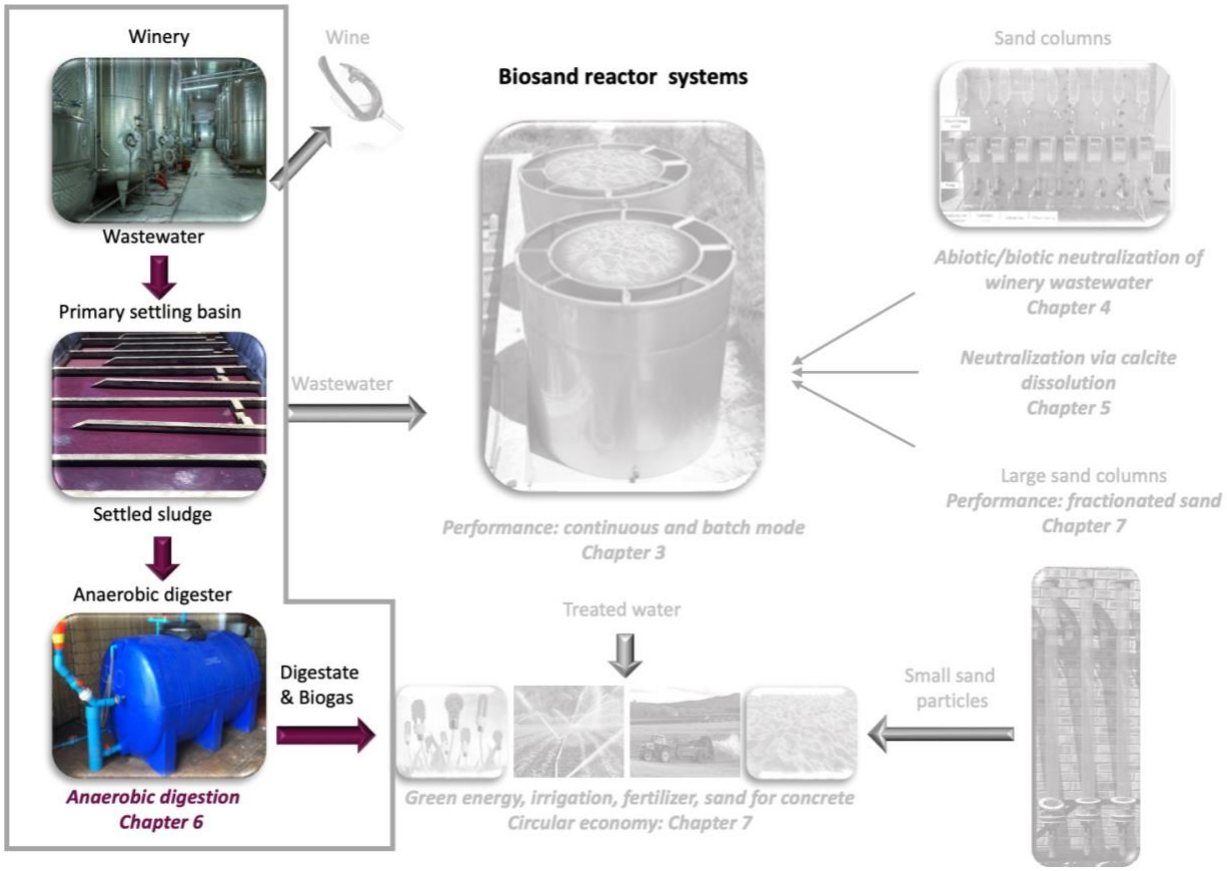

[Presents the findings of a study to determine the BMP of PWWS, and to assess the character of the digestate for potential as an agricultural fertilizer.]

Abstract

Sludge is generated from settling of winery wastewater from seasonal cellar activities. Most primary winery wastewater sludge is generated during the crush season and is often disposed to landfill. This practice is an economic and environmental burden and ignores the potential for valorization. In this study, a series of biochemical methane potential tests were conducted using seasonal batches of sludge with and without the addition of selected micronutrients (Co, Cu, Ni) at different inoculum to substrate ratios and temperatures (ambient vs 37°C). The highest specific CH_4 yields were obtained at an inoculum to substrate ratio of 4 with the addition of micronutrients from sludge collected during (206 ± 2.7 $mLCH_4/gVS_{added}$) and after (177 ± 1.4 $mLCH_4/gVS_{added}$) the crush season at 37°C and ambient temperatures (15.3°C to 27.1°C), respectively. In some instances, digestion at 37°C appeared to promote inhibited steady state conditions leading to lower CH_4 yields than at ambient temperatures. This suggested that the sludge could be easily digested without heating, particularly during the warmer months of grape harvesting and crushing. The composition of the digestate indicated that it may be suitable as an agricultural fertilizer, with high concentrations of N (21.5 to 27.7 g/kg dry weight) and C (229 to 277 g/kg dry weight), as well as the presence of all essential micronutrients.

Keywords: Anaerobic digestion, fertilizer, irrigation, reuse, valorization, waste

6.1 Introduction

Solid and liquid wastes are generated by the wine industry through wine-making processes. Solid wastes from winemaking include lees, fining agents, filtration aids, and agricultural waste known as grape marc or pomace (skins, seeds and stems). Winery wastewater (WWW) is generated when cellar equipment and floors are washed and/or sterilized and contains solid grape residuals, chemicals and filter aids in variable quantities [1]. Over the last three decades, a number of industrial biorefineries have been set up to extract value-added products from grape pomace and lees including bioactive compounds such as phenolics, dietary fibre, cellulose, grapeseed oil, and tartaric acid [2-8]. Valorization approaches that have been explored on a more theoretical level include fermentation of pomace for bioethanol production, energy production from gasification or pyrolysis of pomace, and extraction of condensed tannins for use as wood adhesives [9-13]. Grape pomace has also been composted, vermi-composted or co-composted with substrates such as green waste, municipal waste activated sludge (WAS), olive mill waste,

and spent winery filter material as a lower value valorization option [14-18]. Co-composting may enhance the quality of the compost [14,16]. Application of such compost to agricultural land may increase plant yield, as in the case of fertilizer obtained from co-composting of pomace, WWW, olive mill sludge, olive mill wastewater and green waste, which was shown to increase radish yield [17].

Primary winery wastewater sludge (PWWS) and secondary winery wastewater sludge (SWWS) are indirect solid wastes that are generated by the wine industry. Unlike direct forms of solid winery wastes, WWS is not suitable for higher value valorization. Indeed, some wineries contract commercial companies to remove WWS and dispose it to landfill, which is: (i) an economic burden on the industry, (ii) a burden on landfill sites, and (iii) ignores the potential for valorization of the organic-rich waste.

In water-stressed countries, including South Africa, Australia and some areas of the United States, cellar effluent is often used for beneficial irrigation after primary settling and pH adjustment if required. This results in the generation of copious amounts of PWWS. Conversely, in many wineries, especially those in Europe, WWW is either discharged into municipal sewers for concurrent treatment with domestic wastewater or it undergoes secondary treatment *in-situ* generating SWWS that differs in character from PWWS [19,20]. Although SWWS and PWWS differ in nature, both are malodorous and potentially toxic to the environment, mainly due to high concentrations of (poly)phenolics and volatile fatty acids (VFA). It has been shown that stabilized SWWS from constructed wetlands can be used directly as a soil conditioner [19], and that SWWS from a continuous activated sludge (CAS) process is a suitable substrate for anaerobic digestion (AD) [21].

From economies of scale, composting and AD are generally preferred for decentralized and centralized biodegradation of organic matter, respectively [22]. However, composting can be labor-intensive because compost piles need regular turning and stalks may need to be ground [14]. In addition, a large spatial footprint is required as the process is slow. Anaerobic digestion occurs at a faster rate, requires a smaller spatial footprint and emits less greenhouse gases than composting, provided the biogas from AD is utilized [22]. Promising results have been obtained from AD of grape pomace, lees and grape marc [21,23-25,53]. Arguably, in contrast to WWS, these are already being used for extraction of more valuable products than biogas. Nonetheless,

these studies indicate that valorization of WWS via AD may be a viable remediation approach. Previous studies of AD of primary and secondary WWS were ineffective (<30 $mLCH_4/gVS$) in relation to other winery wastes like grape marc [58]. To the best of our knowledge, no other studies have been conducted on the AD of PWWS, including the addition of micronutrients and the use of digestate as a fertilizer.

The aims of this study were therefore to determine the biochemical methane potential (BMP) of PWWS from a winery during and after the crush season at different inoculum to substrate (ISR) ratios, with and without the addition of micronutrients at different temperatures (ambient and 37°C). Furthermore, the digestates were characterized and compared with commercial organic fertilizers in terms of their macro- and micronutrient compositions.

6.2 Materials and Methods

6.2.1 Biochemical methane potential experiments

The inoculum was prepared by randomly feeding the digestate obtained from previous AD experiments [26] with PWWS for > 6 months before the start of the first experiments. The composition of the biogas produced by the inoculum was monitored on a weekly basis. The inoculum was deemed ready for use once the CH_4% of the biogas was > 60%.

Approximately 100 L of PWWS was collected from during and after the crush season. The PWWS was transported to the laboratory immediately and thoroughly homogenised using a blender. The PWSS was fully characterized together with the inoculum. As the microbial communities are naturally dynamic in nature, the inoculum for each of the four experiments were characterized separately.

Two experiments were conducted with both the crush and the post-crush PWSS, giving a total of four sets of experiments: (i) Crush Experiment 1 (ii) Crush Experiment 2 (iii) Post-crush Experiment 1 (iv) Post-crush Experiment 2. Experiment 1 for both PWWS substrates was conducted at ambient temperature (20.7°C ± 2.0°C) using a combination of two variables inoculum to substrate ratio (ISR) (2:1, 3:1, 4:1) and nutrient addition (nutrients/no nutrients) (Table 6-1). The character of the PWSS taken during the crush season informed the choice of (micro)nutrients (5 mg/L copper (Cu^{2+}), 20 mg/L nickel (Ni^{2+}), 20 mg/L cobalt (Co^{2+})). These metabolic co-factors were deficient in both batches of PWWS [32,57]. Three controls were

included in each experiment: inoculum only, substrate only, and inoculum + acetate, all with and without nutrients. All BMP experiments, including controls, were conducted in duplicate.

The experimental set-ups for the variable combination resulting in the most efficient performance in terms of specific biogas and CH_4 generation in Experiment 1 were repeated at ambient (19.4°C ± 1.77°C) and mesophilic (37°C) temperatures simultaneously to determine the effect of these temperatures on AD efficiency (Experiment 2). The sets of experiments were first performed during the crush season using the crush season sludge, followed by the post-crush season sludge.

For reference, the results for characterization of all of the reactor contents at the beginning and end of the experiments are provided in the Supplementary material (Appendix 7; Supplementary Table A7-1, Supplementary Table A7-2).

Table 6-1 Parameters used for Experiments 1 and 2

	Crush season PWWS			Post-crush season PWWS		
	Experiment 1	Experiment 2		Experiment 1	Experiment 2	
Temperature	Ambient	Ambient	37°C	Ambient	Ambient	37°C
ISR 2	✓	×	×	✓	×	×
ISR 2 + N	✓	×	×	✓	×	×
ISR 3	✓	×	×	✓	×	×
ISR 3 + N	✓	×	×	✓	×	×
ISR 4	✓	✓	✓	✓	✓	✓
ISR 4 + N	✓	✓	✓	✓	✓	✓

PWWS = primary winery wastewater sludge; ISR = inoculum to substrate ratio; N = nutrients (5 mg/L Cu2+, 20 mg/L Ni2+, 20 mg/L Co2+)

6.2.2 Biogas measurements

Biogas was collected directly from the reactors into 2 L Supelco (Bellefonte, USA) foil gas sampling bags. The volume of biogas was determined three times per week on designated days by extracting the gas from each bag into graduated gas tight syringes. Volumes were measured at 20.7°C ± 2.0°C and pressure of 1008.7 ± 2.7 hpa. The composition of the biogas was determined using a Geotech (Warwickshire, UK) Biogas 5000 instrument. The performance of AD was assessed using the specific total biogas and CH_4 yields based on volatile solids added (mL/gVS).

6.2.3 Physicochemical analyses

Total solids (TS) and total volatile solids (TVS) concentrations were determined by standard methods (weight loss on ignition at 105°C and 550°C), respectively. A Merck (Darmstadt, Germany) Spectroquant Prove® 600 spectrophotometer together with Merck cell tests or kits were be used to determine chemical oxygen demand (COD) (cat no: 14555), volatile fatty acids (VFA) measured as total volatile organic acids (VOAt) (cat no: 01763), total organic carbon (TOC) (cat no: 14879), total nitrogen (TN) (cat no: 14537), total alkalinity (TAlk) (cat no: 101758), total phosphorous (TP) (cat no: 14729), ammonia as NH_4^+ (cat no: 00683), sulfate SO_4^{2-} (cat no: 14791), sulfide (S^{2-}) (cat no: 14779), and chloride (Cl^-) (cat no: 14789) concentrations, according to manufacturers' instructions. Quantification of carbon, hydrogen, nitrogen and sulfur (CHNS) concentrations (%wt.wt) were determined at the Central Analytical Facility of Stellenbosh University using an Elementar (Hamburg, Germany) Vario EL cube Elemental analyser according to the manufacturers' instructions. Major and minor elements were quantified at the same facility using a Thermo ICap 6200 ICP-AES instrument for trace analyses, while ultra-trace analyses were performed on an Agilent (Santa Clara, USA) 7900 ICP-MS instrument, according to manufacturers' instructions.

6.3 Results and discussion

6.3.1 Characterization of wastewater, primary winery wastewater sludge and inoculum and theoretical suitability of sludge for anaerobic digestion

6.3.1.1 Organic fractions, ammonia and sulfides

Due to the seasonal nature of winery operations, there is considerable intra- and inter-site variation in the characteristics of WWW, and by inference in PWSS [1]. Samples of WWW taken from the same winery over the crush season exhibited a high COD:N ratio of 226 (Table 6-2). High COD:N ratios are typical of WWW, and a source of N is often added to secondary wastewater treatment systems to assist bioremediation [1]. Notably lower COD:N ratios were measured in the PWWS (12.9 and 24.1 for the crush and post-crush season PWWS, respectively). It was therefore noted that N partitioned preferentially into the PWWS and indicated that the character of the PWWS cannot be extrapolated directly from that of the WWW.

In this study, the C content from the (CHNS) elemental analysis was notably higher in the post-crush than the crush season PWWS, but the converse was true for the TOC. The post-crush PWWS exhibited significantly higher (ANOVA, p>0.05) concentrations of TS, TVS and COD than the crush PWWS (Table 6-2). The higher TOC in the crush season PWWS was due to the presence of fresh pomace residues from washing equipment and floors during the crushing. The post-crush PWWS still contained some pomace residues and solubilised degradation products because the settling delta at the winery had not been completely de-sludged after the crush period. Overall, the organic character of the PWSS adequately reflected seasonal operational variabilities.

Theoretically, the C:N ratio of the crush season PWWS (11.6) and post-crush season PWWS (7.1) were both below optimal for AD of carbonaceous substrates, which has been widely reported as 20–30 [27]. The AD process for carbonaceous substrates with lower C:N ratios may be susceptible to instability due to the potential accumulation of NH_4^+ and VFAs. High concentrations of VFAs can inhibit methanogenesis leading to bioreactor instability or failure [28]. In this study, The VFAs contributed 21% and 32% to the crush and post-crush season PWWS CODs, respectively, based on the theoretical COD yield of acetic acid [29]. Similarly, the stoichiometric contribution of VFAs to the TOC for the crush and post-crush PWWS was 40% and 62%, respectively. In this case, the total VFA concentrations of the PWWS and inocula (Table 6-2) were above previously reported inhibitory thresholds of 5.80–6.90 g/L [30].

Ammonia in WWW emanates from cellar cleaning products and/or from hydrolysis of proteins from lees and grapes. The pH, temperature, C:N ratio, and elemental concentrations influence the degree of toxicity of NH_4^+ on the sensitive methanogenic archaea during AD [31]. Hence, a range of inhibitory concentrations (IC) have been reported in literature, from 2–14 g/L as total NH_3-N and 0.053–1.45 g/L as NH_3 [32]. In this study, the NH_4^+ concentrations in the PWWS fell within these previously reported ranges for inhibition of AD (Table 6-2).

The S^{2-} concentration measured in the post-crush PWWS was 89% higher than the concentration measured in the crush season PWWS (0.75 g/L and 0.08 g/L, respectively). For hydrogenotrophic and aceticlastic methanogens, IC_{50} ranges reported in literature for H_2S are 0.043–0.125 g/L and 0.014–0.060 g/L, respectively [33]. In this study, the measured equivalent H_2S concentrations for the PWWS substrates were within the inhibitory range, particularly for aceticlastic methanogens. However, HS^- is likely to ppt. as metal sulfides during AD. This can alleviate toxicity by reducing

the HS^- concentration, and in some instances, reducing the bioavailability of concentrations of toxic cations by co-ppt. Conversely, AD can be retarded if the bio-availabilities of essential metabolic co-factors are reduced to below functional microbial requirements.

Although the measured VFA, NH_4^+ and HS^- concentrations were above inhibitory values reported in literature, this does not necessarily translate into poor performance as each substrate and inoculum is unique. Indeed, it has been shown that substrates that appear unsuitable for AD may yield good AD results after long-term acclimation of the functional microbial communities to the particular physicochemical milieu provided by the substrate [34].

Table 6-2 Characteristics of primary winery wastewater sludge and inoculum used in the biochemical methane potential experiments as well as parameters measured in the wastewater over the same period (average and standard deviation from the mean, n =3)

Parameter (*n*=3)	Crush PWWS	Crush Inoculum 1	Crush Inoculum 2	Post-crush PWWS	Post-crush Inoculum 1	Post-crush Inoculum 2	Winery effluent
Elemental CHNS analysis (g/kg dry weight)							
C	29.4 ± 2.45	32.5 ± 2.2	25.2 ± 2.9	40.1 ± 1.3	26.5 ± 0.3	20.4 ± 3.9	NA
H	0.55 ± 0.05	4.86 ± 0.55	4.85 ± 0.42	6.61 ± 0.43	3.62 ± 0.52	2.28 ± 0.64	NA
N	2.54 ± 0.04	2.86 ± 0.16	1.89 ± 0.25	5.67 ± 0.14	2.25 ± 0.20	1.78 ± 0.39	NA
S	0.38 ± 0.04	0.89 ± 0.05	0.57 ± 0.15	0.34 ± 0.03	0.38 ± 0.07	0.38 ± 0.08	NA
C:N	11.6	11.6	13.3	7.1	11.8	11.5	NA
Characterisation (g/L wet weight homogenised sludge, g/L wastewater)							
COD	149 ± 5	35.5 ± 1.0	206 ± 14	346 ± 26	95.2 ± 8.8	71.7 ± 5.6	6.79 ± 1.20
TP	0.70 ± 0.01	0.17 ± 0.01	0.82 ± 0.20	1.65 ± 0.13	0.49 ± 0.09	0.44 ± 0.06	0.20 ± 0.07
TN	11.5 ± 0.5	2.3 ± 0.4	2.5 ± 1.07	14.3 ± 0.5	2.4 ± 0.8	4.5 ± 0.1	0.03 ± 0.02
VFA	28.5 ± 0.7	8.98 ± 0.35	44.6 ± 10.4	104 ± 5	26.1 ± 1.1	23.3 ± 2.36	1.37 ± 0.15
HS^-	0.08 ± 0.01	0.06 ± 0.01	0.08 ± 0.01	0.75 ± 0.02	0.11 ± 0.01	0.13 ± 0.02	ND
NH_4^+-N	1.89 ± 0.04	1.3 ± 0.17	5.39 ± 0.06	1.18 ± 0.35	1.02 ± 0.21	1.16 ± 0.13	ND
TS	173 ± 10	257 ± 7	62.3 ± 4.1	301 ± 14	145 ± 2	117 ± 1	ND
VS	91.9 ± 11	97.8 ± 8.7	32.8 ± 22	247 ± 9	67.9 ± 1.5	85.3 ± 1.4	ND
COD:VS	1.62	0.36	6.28	1.40	1.40	0.84	ND
VS:TS	0.53	0.38	0.53	0.82	0.49	0.72	ND

PWWS = Primary winery wastewater sludge; Inoculum 1 = inoculum for ambient experiments (Experiment 1); NA = not applicable; ND = not determined; Inoculum 2 = inoculum used for ambient vs mesosphilic experiments (Experiment 2)

6.3.1.2 Elemental composition of primary winery wastewater sludge and inoculum

The elemental composition of grapes varies according to the soil geochemistry, the agricultural practices and the grape varietal and rootstock. Potassium (K^+), the element found in the highest concentrations in the crush season PWWS is found in high concentrations in grapes and can also be present in cellar cleaning products. Sodium is also typically present in high concentrations in

WWW because caustic soda (NaOH) is the main cleaning and sterilising agent used in most cellars [1]. However, Na^+ tends to remain in the soluble fraction of WWW [35], while much of the K remains in the PWWS because it is a constituent of the pomace. This is reflected by the relatively low concentrations of Na compared with K in the PWWS, particularly in the crush season PWWS (Table 6-3).

Table 6-3 Elemental cation concentrations in primary winery wastewater sludge and inoculum used in the biochemical methane potential experiments

	Crush PWWS	Crush Inoculum 1	Crush Inoculum 2	Post-crush PWWS	Post-crush Inoculum 1	Post-crush Inoculum 2
Major elemental parameters (g/kg dry weight)						
Fe	3.83 ± 0.13	3.59 ± 0.11	4.23 ± 0.95	2.96 ± 0.12	4.85 ± 0.85	5.02 ± 0.36
Al	11.1 ± 0.14	7.18 ± 0.24	10.2 ± 2.23	10.7 ± 0.22	13.0 ± 2.03	10.0 ± 1.16
Ca	7.37 ± 0.45	22.1 ± 0.19	10.4 ± 2.07	0.91 ± 0.12	10.3 ± 1.15	14.4 ± 3.03
K	51.8 ± 4.70	43.2 ± 1.39	29.5 ± 4.17	3.73 ± 0.12	61.5 ± 24.7	39.8 ± 15.7
Mg	1.60 ± 0.23	3.74 ± 0.19	2.32 ± 0.44	1.21 ± 0.41	2.19 ± 0.21	1.99 ± 0.13
Na	1.03 ± 0.16	30.9 ± 0.99	3.40 ± 0.09	0.18 ± 0.05	4.72 ± 1.61	3.75 ± 1.69
P	2.85 ± 0.12	5.24 ± 0.15	4.14 ± 0.93	5.40 ± 0.11	2.91 ± 0.05	3.80 ± 0.31
Si	0.91 ± 0.01	1.39 ± 0.12	0.65 ± 0.11	2.52 ± 0.53	1.57 ± 0.27	1.78 ± 0.35
Minor elemental parameters (mg/kg dry weight)						
B	68.1 ± 48.3	54.6 ± 5.60	40.5 ± 7.58	BDL	45.3 ± 2.00	46.4 ± 2.50
Mn	39.7 ± 1.60	333 ± 3.80	93.0 ± 17,0	23.4 ± 2.20	74.5 ± 13.5	80.3 ± 11.3
Co	1.30 ± 0.50	16.9 ± 0.83	26.3 ± 5.84	BDL	20.4 ± 8.90	BDL
Ni	36.9 ± 4.9	11.6 ± 0.70	52.9 ± 12.1	12.9 ± 3.10	49.5 ± 5.40	36.0 ± 6.30
Cu	147 ± 12.7	168 ± 8.10	157 ± 34.5	120 ± 3.00	208 ± 30.3	197 ± 79.4
Zn	300 ± 15.0	1443 ± 71	449 ± 100	354 ± 2.4	511 ± 82.6	385 ± 35.2
Sr	21.6 ± 1.70	90.4 ± 0.70	51.14± 9.67	67.1 ± 11.9	39.1 ± 3.00	67.1 ± 11.9
Mo	3.01 ± 1.30	5.0 ± 0.2	1.70 ± 0.47	ND	ND	ND
Pb	34.2 ± 6.40	19.1 ± 6.70	24.3 ± 7.30	34.7 ± 8.60	29.7 ± 6.30	35.1 ± 10.5
Ba	133 ± 8.60	42.8 ± 4.90	72.9 ± 19.2	69.8 ± 2.50	114 ± 29.6	114 ± 13.1

BDL = below detection limit; PWWS = primary winery wastewater sludge; Inoculum 1 = inoculum for ambient experiments (experiment 1); Inoculum 2 = inoculum used for ambient vs mesosphilic experiments (experiment 2); ND = not determined

In the case of metals, a large range of concentrations expected to inhibit AD have been reported in literature. This is due to variabilities in substrates, the degree of functional microbial acclimation to the intended substrates, substance synergism and antagonism and operational differences [32]. Acclimated microbial communities may adapt to prevent metal toxicity or deficiency by altering their rate limiting flux and/or protecting key enzymes from reactive metals [36]. Soluble microbial products (SMPs) and extracellular polymeric substances (EPS) also play significant roles in chelating metals, thereby reducing their bioavailability to microbes in anaerobic digesters [36]. Metals are not only inhibitory - many are also essential metabolic co-

factors, and it is common practice to supplement AD reactors with a co-factor cocktail to enhance AD. In this study, the metabolic co-factors Ni, Zn, Co, and Cu were either within or below the optimal range for AD [32], hence the addition of Ni, Co and Zn as micronutrients in the experiments.

6.3.2 Biochemical methane potential tests

The quality and quantity of CH_4 generated from AD is fundamentally dependent on the oxidation state of the organic C. Higher CH_4 yields are associated with more reduced substrates [37]. Sludge characteristics are often used to infer CH_4 generation potential [38]. Buswell's equation has been used for decades to estimate the theoretical BMP of organic substrates assuming 100% conversion into carbon dioxide (CO_2) and CH_4 [39]. However, the theoretical yield is usually overestimated as the methanogenic archaea are highly susceptible to inhibition [31,32]. The yields were therefore determined experimentally in this study.

6.3.2.1 Anaerobic digestion of primary winery wastewater sludge from the crush season

The first set of BMP experiments were conducted using PWWS taken during the crush season. Although it is well known that stable mesophilic (30-38°C) and thermophilic (40-60°C) temperatures typically increase the rate of AD, digestion still occurs under ambient conditions in some temperate and tropical climates [40]. In order to ascertain whether AD of PWWS could feasibly be conducted without having to install and operate sophisticated heating equipment, the BMP experiments were first conducted in the laboratory under ambient conditions in a non-temperature-controlled environment. Most WWW (and hence PWWS) is generated during the crush period, which falls within the warm summer/autumn months in wine-producing countries. As a cost-effective option, it is feasible that PWWS could be pumped from existing settling tanks/deltas into simple digesters. These could be off-the-shelf polyethylene tanks, for example. Black containers exposed to sunlight could absorb heat and assist with increasing the temperatures of the reactor contents. Alternatively, specialized insulated reactors used in combination with solar heating could be employed.

Promising results were obtained at ambient temperatures (Experiment 1). Good specific biogas (Fig. 1A), and CH_4 generation (Fig. 1B) was achieved within 40 days (Fig. 2). In terms of ISR and nutrient addition, both biogas and CH_4 generation increased with increased ISR and nutrient

addition. In practice, reactor capacity is reduced at lower ISRs as less digestate needs to be retained between batches (for batch reactors), and more solids can be wasted in the case of continuous reactors. In this study, the comparatively low efficiency at ISR2 obtained during (ambient) Experiment 1 would not support the use of this ISR for piloting a system to treat PWWS. Although reasonable absolute cumulative CH_4 generation was achieved at ISR2, the quantities were masked when corrected for the contribution by the inoculum control as prescribed in the standard method applied [41]. This led to some anomalous negative generation rates at ISR2. Although the inoculum had been pre-acclimated to a different batch of PWWS, it was hypothesized that the sensitive microbial populations needed to adjust to the slight change in substrate, especially when present in lower concentrations (ISR2). Many researchers do not pre-acclimate inocula to the intended substrate, which can reduce AD efficiency as acclimation then needs to take place during the experimental period [32]. This may lead to long lag phases, and in some cases, the microbial consortia may completely fail to adapt to the new substrate. In an attempt to standardize testing to allow inter- and intra-study comparisons, some standardized methodologies prescribe the use of inocula from municipal wastewater sludge digesters for all AD testing [41]. While this may be acceptable if a similar substrate is being tested, the sudden exposure of sensitive functional microbial species (such as methanogens) to completely different physicochemical environments may not be prudent. For example, it has been shown that methanogenesis was completely inhibited using a non-acclimated inoculum from digesters treating abattoir effluent [42], while good methanogenesis was obtained using a specifically pre-acclimated inoculum to digest the same tannery effluent [43].

In this study, the advantage of using acclimated inocula was jeopardized by the presence of residual substrate in the inoculum at the start of the experiments. Ideally, the substrate should be depleted in inocula used for BMP experiments [41]. The residual substrate was due to the nature of the PWWS that contained slowly biodegradable lignocellulosic material from the grape pomace, making substrate exhaustion impossible without compromising on the viability of the sensitive methanogenic populations via starvation. The presence of residual substrate resulted in biogas and CH_4 generation in the inoculum control. However, the origin of the substrate in the inoculum was the same as the experimental substrate, and the specific yields were corrected to include the biogas contribution from the inoculum in the results (Fig. 1). Notwithstanding the

biogas contribution from the inoculum substrate, around 1.5 L CH_4 was generated from 1 L reactor contents at ISR2 during Experiment 1 (Fig. 2A).

To assess the degree to which AD could be enhanced at under stable mesophilic conditions, the optimal CH_4 generation conditions (ISR4 with nutrient addition) was applied under both ambient and controlled mesophilic (37°C) conditions simultaneously (Experiment 2, Fig. 1). Under ambient conditions, the biogas and CH_4 yields were lower than those attained during Experiment 1, once again demonstrating the variability in biogas yields that can be obtained when even slightly different inocula are used. In this case, all of the other experimental factors remained consistent between Experiment 1 and Experiment 2. The same inoculum had been maintained by feeding with the same PWWS. The results suggest that it is impossible to avoid temporal variations in the microbial community structure and function when maintaining inocula. However, the 2.6-fold higher specific CH_4 generation obtained at 37°C clearly demonstrated that, as expected, performance efficiency could be increased significantly by increasing and controlling the temperature within an optimal range.

In comparison to the theoretical yield (530 $mLCH_4/gVS_{added}$ and 58% CH_4) calculated from the COD:VS ratio [37], the maximum yield (206 ± 2.7 $mLCH_4/gVS_{added}$) of the crush season PWWS was only 39% of the theoretical, but the CH_4 composition of the biogas (56%) was close to the theoretical value.

6.3.2.2 Anaerobic digestion of primary winery wastewater sludge from the post-crush season

In contrast to results obtained with crush season PWWS, no clear trend on the effect of ISR and nutrient addition on AD efficiency of post-crush PWWS was demonstrated (Fig. 1). However, in terms of absolute cumulative CH_4 generation (Fig. 2B), the highest yield was obtained at ISR2 with nutrient addition, and the lowest at ISR4, strongly suggesting that there were sufficient active functional microbes present at ISR2 for efficient methanogenesis. At ambient temperature (Experiment 1), the yields attained were all higher with the post-crush season PWWS than with the crush season PWWS. This may have been related to the inoculum, and/or the presence of higher concentrations of readily biodegradable organics, including VFAs in the post-crush PWWS (COD 346 g/L, VFA 104 g/L) than in the crush PWWS (COD 149 g/L, VFA 29 g/L) and/or the use of a more robust inoculum.

The results of Experiment 2 (ambient vs 37°C) showed a similar trend to those obtained during AD of crush season PWWS in that substantially lower specific biogas and CH_4 yields were obtained at ambient temperature (ISR4 with nutrients) during Experiment 2 than during Experiment 1. In contrast to the results obtained with crush season PWWS, the amount of biogas was only negligibly higher (27 mL/gVS_{added}) at 37°C than under ambient conditions during Experiment 2. However, due to a promisingly high CH_4 contribution at 37°C (79%) when compared to ambient conditions (59%), there was a greater difference in the specific CH_4 yield (45 mL/gVS_{added}). In comparison to the theoretical yield (496 mLCH_4/gVS_{added}) calculated from the COD:VS ratio [37], the maximum yield (177 ± 1.4 mLCH_4/gVS_{added}) was only 36% of the theoretical maximum, but the CH_4 composition of the biogas fell within the theoretical range (50-71%) under ambient conditions and was notably higher than the theoretical yield at 37°C.

The differences in yields in Experiments 1 and 2 with the same substrate at ambient temperatures could be explained by the fact that the average ambient temperatures during Experiment 1 (20.7 ± 2.0°C) were higher than those during Experiment 2 (19.8 ± 1.87). However, greater temperature variations (range of temperatures) occurred during Experiment 1 (16.8 to 27.1°C) in comparison with Experiment 2 (15.3 to 22.8°C), which could theoretically lead to greater methanogenic community instability and reduced AD efficiency [44]. Furthermore, in comparison to lower temperatures, mesophilic and thermophilic temperatures typically improve reaction kinetics and promote methanogenesis [44-46]. Therefore, temperature differences and fluctuations do not appear to explain why the yields were also less at 37°C than at ambient temperatures during Experiment 2, even though higher temperatures can promote some reactor instability due to an increased rate of dissociation/solubilization of inhibitors as previously described [26].

Apart from the complexity of the physicochemical and biochemical reactions, these results appear to confirm that that the inoculum (as the only other variable) was less active during Experiment 2, once again highlighting: (i) the importance of having a suitable functional microbial consortium for BMP experiments and for larger scale reactor start-up, and (ii) the inherent difficulty of comparing intra- and inter-study results. Typically, although BMP studies often include multiple variables, they do not account for temporal microbial variations as they are conducted as singular experiments.

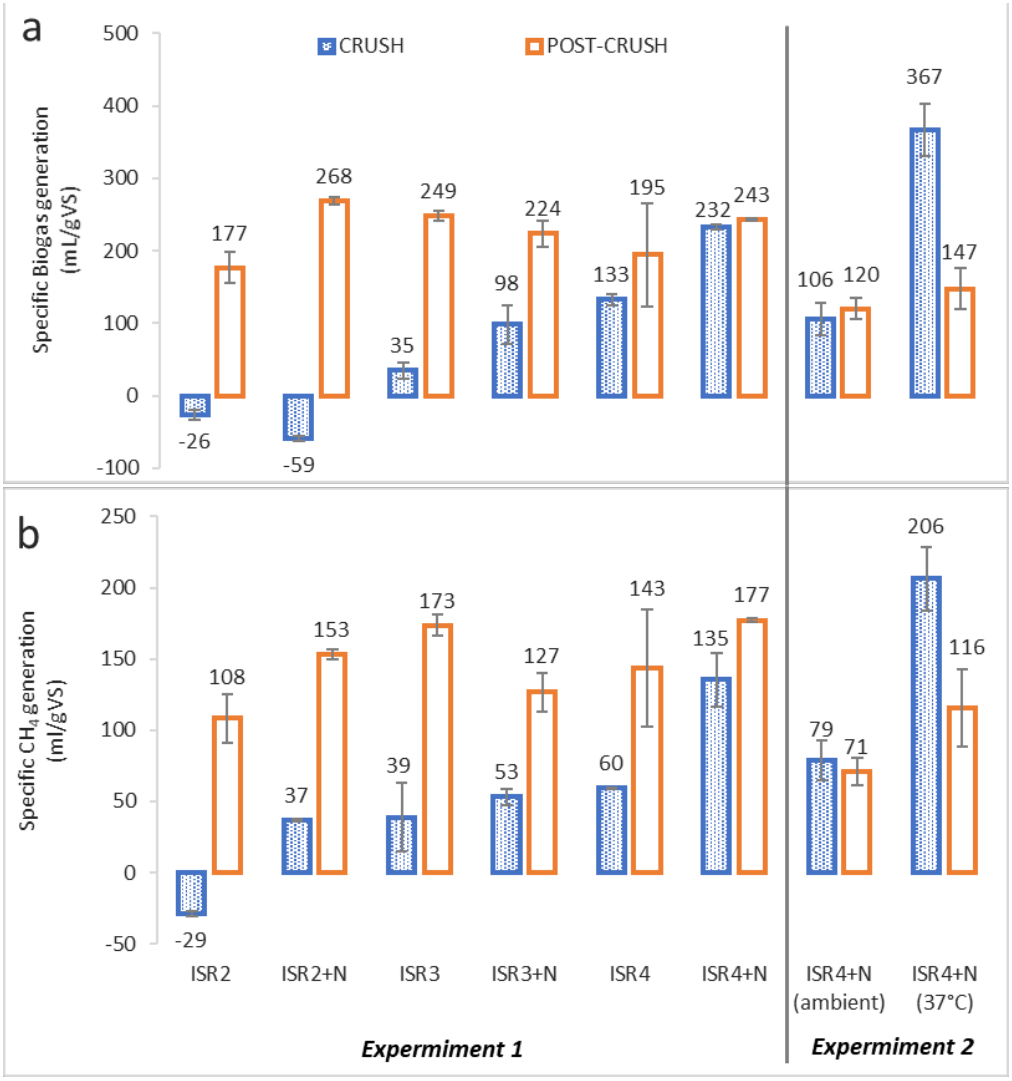

Figure 6-1 Corrected specific biogas (A) and methane (B) measured in reactors. ISR = inoculum to substrate ratio; N = (micro)nutrients (Co, Cu, Ni). Experiment 1 at ambient temperature and Experiment 2 at ambient and 37°C.

Notwithstanding the inherent difficulties already alluded to regarding inter- and intra-study comparisons, results were compared with those reported in literature for AD of winery solid wastes under mesophilic conditions. All but one study used sludge from municipal wastewater treatment plants as the inoculum. Higher specific CH_4 yields (365 ± 20 $mLCH_4/gVS$) were obtained digesting pomace in 250 mL semi-continuous reactors [25]. However, the hydraulic retention time of 104 days was considerably longer than with PWWS where most biogas was generated within 30 days (Fig. 2). The highest specific CH_4 yields were obtained for batch AD of lees (876 ± 45 $mLCH_4/gVS$), grape must (838 $mLCH_4/gVS$), SWWS (690 ± 25 $mLCH_4/gVS$) in 500 mL digesters with 57 days RT [21, 58]. These substrates have also been successfully co-AD at pilot scale in 230 L continuously stirred tank reactors, but only the overall biogas measurements were reported in this case (average 0.450 $m^3/kg\ COD_{fed}$) [23]. It has been calculated that sufficient biogas could be

generated from co-AD of these substrates to support winery operations [21]. The lowest yields (6.45 $mLCH_4/gVS$) were obtained with co-AD of dried spent grape pomace and cheese whey (100 mL digesters, 58 days), despite using a well-acclimated inoculum from a laboratory digester [24]. Similarly, the AD of stems, PWWS and secondary WWS was stunted (<30 $mLCH_4/gVS$) likely due to poor acclimation of inoculum to substrate [58]

Overall, this study demonstrated that PWWS is a suitable substrate for AD, with no lag phase and complete digestion taking place within 30-40 days even under ambient conditions. In general, nutrient addition and controlled higher temperatures (37°C) enhanced biogas and CH_4 generation at higher ISRs, indicating the increased metabolic co-factor demand associated with higher concentrations and activities of the functional microbial communities (higher ISR).

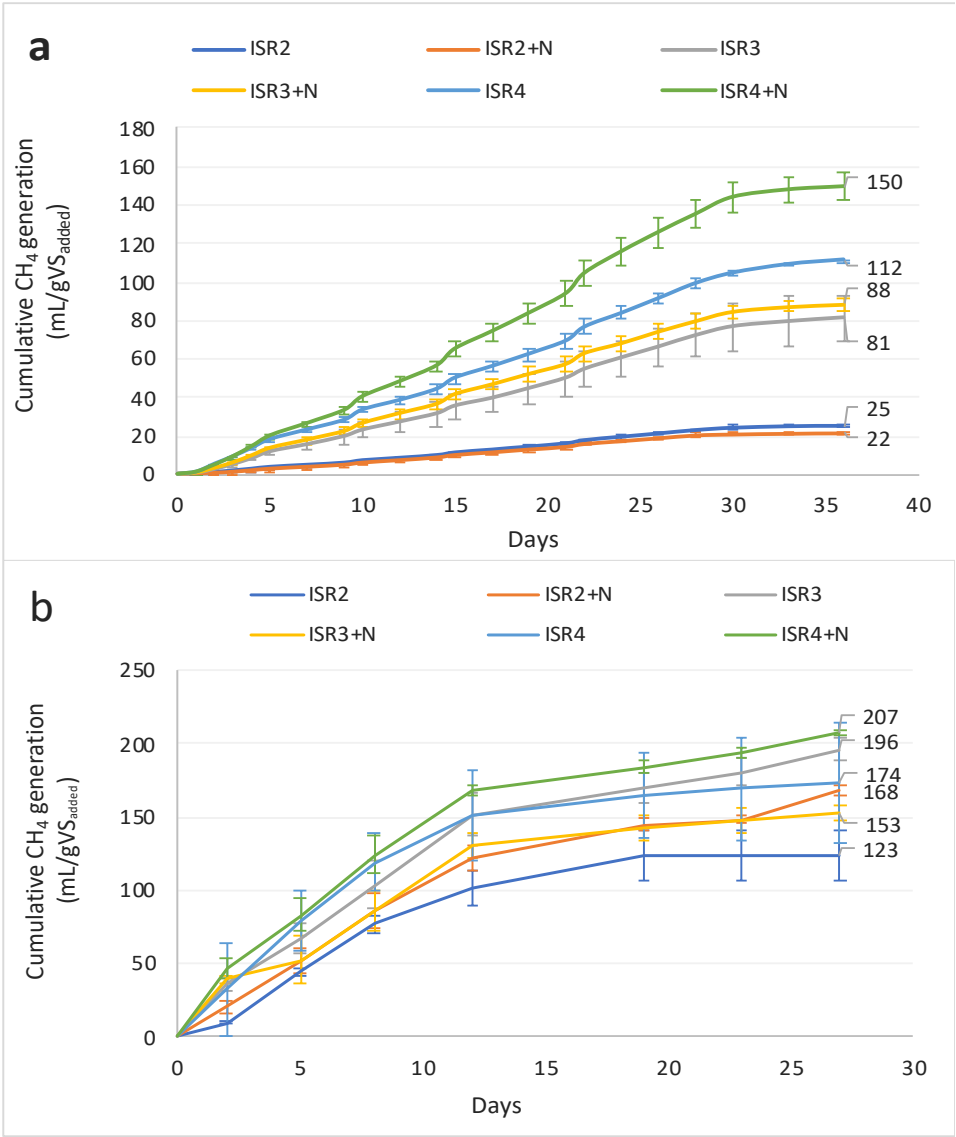

Figure 6-2 Cumulative methane generation with crush season sludge (A) and post-crush season (B) primary winery wastewater sludge at ambient temperatures.

6.3.2.3 Evaluation of methane generation kinetic models

To evaluate the effectiveness of kinetic models for predicting the AD of PWWS, commonly applied models (Cone, Logistic, modified Gompertz, and First order) were fitted to the cumulative CH_4 production from Experiment 1 for both crush and post crush PWWS. In both cases, the Cone, Logistic and First order models fitted the experimental data with high precision (adjusted R^2 > 0.958). For optimal reactors (ISR4 + N), the models predicted the experimental data in the order: Logistic > Cone > First order > modified Gompertz according to AIC (Akaike information criterion) for the crush season PWWS, and predicted maximum CH_4 yields of 151.5, 142.0, 144.1 and 121.2 $mlCH_4/gVS_{added}$, respectively. For the post crush PWWS, the model fit order was Cone > First order > Logistic > modified Gompertz, with predicted maximum methane yields of 199.4, 203.8, 195.2, and 155.7 $mlCH_4/gVS_{added}$ respectively. For reference, the complete set of calculated kinetic parameters have been provided in the Supplementary Material (Supplementary Table A7-2, Supplementary Table A7-3).

6.3.3 Evaluation of digestate of crush and post-crush primary winery wastewater sludge as a soil conditioner/fertilizer

In depth theoretical analyses of fertilizers need to account for a variety of different soil types and plants, ideally including pot and/or field experiments. Such analyses were beyond the scope of this study which was instead limited to a brief evaluation of the PWWS as a 'proof of concept' to determine whether it may be suitable as a fertilizer. This was achieved by (i) comparing the macronutrient and micronutrient composition of the digestates with typical organic fertilizers, and (ii) evaluating whether any elements were present in quantities that may be detrimental to plant and/or soil health.

The PWWS digestate composition was compared with a range of four commercial agricultural organic fertilizers based on composted chicken manure. Two of these were enriched, one with kelp and fishmeal, and the other with Ca and P. Both inorganic and organic forms of C are important as much C is being lost from the soil due to agriculture, other anthropogenic land use practices and climate change across the globe [50,51]. The total C concentrations were 2.0 to 2.6-fold higher in the PWWS than in the commercial fertilizers, indicating that would be a valuable source of C as a fertilizer. In terms of organic C, decayed organic material (humus), increases the water retention capacity and the cation exchange capacity of soils, assists with soil

aeration and provides a reservoir for gradual release of plant nutrients [47,48]. All the PWWS digestates contained notable amounts of organic carbon (measured as TOC), and the amount was higher in the crush season PWWS digestate (average 261 g/kg) than in the post-crush season PWWS digestate (average 104 g/kg) (Table 6-3). Given the worldwide average soil organic carbon (SOC) concentration of 15.5 g/kg, even the post-crush PWWS could be a valuable contributor of humic material, especially in the case of sandy soils [49].

Different plants require macro-nutrients in different quantities, and some soils themselves contain sufficient elements. For example, as the name suggests, calcareous soils have high Ca concentrations, but have limited P and Zn availability [52]. To cater for different requirements, fertilizers contain variable ratios of the macronutrients N, P and K, among other elements. The PWWS digestates contained similar amounts of N and K to the commercial fertilizers but were deficient in P, Ca and S (Table 6-4). Depending on the soil type and crop, the PWWS may require supplementation with a source of these elements. Waste gypsum is an example of a sustainable source of Ca and S. In terms of micronutrients, both batches of PWWS digestates contained reasonable concentrations in comparison to the commercial organic fertilizers (Table 6-4), but both the commercial fertilizers and the PWWS contained high concentrations of Fe. Although Fe deficiency can be detrimental to plant growth, it can be toxic to plants in highly acidic or hypoxic soils (usually formed via waterlogging) as Fe^{3+} is reduced to Fe^{2+} which is more bioavailable than the oxidized form [53]. In well-aerated less acidic to alkaline soils, relatively high Fe concentrations are generally not problematic [53].

Unlike K, Na is not a plant nutrient and can negatively affect the soil structure by binding with negatively charged soil particles. This can be offset to some extent by the presence of divalent cations (Ca^{2+} and Mg^{2+}) that have more than one binding site and replace Na^{+} [54]. Although the Na concentrations in the digestates were high (Table 6-4), the concentrations in the PWSS before AD were relatively low in comparison to the inocula (Table 6-4), clearly demonstrating that most of the Na in the digestates emanated from the inocula. The original starter culture was taken from AD of highly saline tannery effluent, and thereafter the inoculum was fed constantly with WWW which also resulted in accumulation of Na over time. In reality, Na remains soluble in WWW, and is not expected to accumulate to high concentrations in PWWS [1,35] as evidenced by the results in Table 6-3. Even with the anomalously high concentrations measured in the PWWS digestates, any sodicity risk was offset by high concentrations of Ca and Mg that reduced

the sodium adsorption ratio (SAR) to ≤ 1.3. To put this in perspective, soils with and SAR > 13 are considered sodic [54,55] and those with SAR < 5 pose a low risk [56]. Nevertheless, the Na levels will be closely monitored in future studies.

For reference, the character of the digestates from all of the reactor contents is included in the Supplementary Material (Supplementary Table A7-5).

Table 6-4 Concentrations of essential plant nutrients, sodium and sodium adsorption ratio in the digestates from optimal anaerobic digestion at 37°C and ambient temperatures

	Crush PWWS		Post-crush PWWS		Commercial*
	37 °C	Ambient	37°C	Ambient	
MACRONUTRIENTS (g/kg dry weight)					
Elemental CHNS analysis					
C	256.9±8.1	277.8±1.5	271.7±.9.1	229.2±4.9	106-115
H	40.9±3.7	43.8±2.6	30.7±1.1	51.0±1.9	-
N	25.5±1.4	25.6 ±0.8	27.7±0.1	21.5±0.5	26-34
S	3.0±0.2	3.0±0.02	5.1±0.5	3.6±1.1	9-10
XRF analyses					
Ca	10.5±0.24	9.8±1.57	10.36±0.83	9.69±1.66	35-60
K	38.3±2.11	37.9±3.47	38.42±1.91	27.67±6.63	30-33
Mg	2.20±0.07	2.00±0.01	2.93±0.09	2.38±0.55	6-7
P	3.4±0.01	3.3±0.51	5.15±0.43	4.47±0.69	17-27
MICRONUTRIENTS (mg/kg dry weight, **g/kg dry weight)					
XRF analyses					
Fe**	5.1±0.01	5.0±0.02	5.72 ±0.04	4.76±1.57	5
B	62±11	57±1.4	50±0.1	40±11	50
Mn	80±1.1	70±1.6	90±1.0	80±22	570-610
Cu	25±2.2	23±1.1	250±17	180±56	60
Zn	57±2.8	49±1.8	620±7.9	450±133	500-540
Mo	2.0±0.1	4.0±0.0	ND	ND	5
OTHER (g/kg dry weight)					
TOC	282±13.0	241±17.1	98.7±10.6	110±2.82	ND
Na	3.20±0.17	3.10±0.19	3.26±0.08	2.28±0.57	ND
RATIOS					
C:N	10.08	10.87	9.82	10.66	3.73
SAR	1.3	1.3	1.2	0.9	ND

*Range of organic fertilizers based on composed chicken manure (Bio ganic® Bio ganic crumble®), enriched with kelp and fishmeal (Bio ocean®), enriched with calcium and phosphate (Bio rock®) TOC = total organic carbon; SAR = sodium adsorption ratio; ND = not determined

Overall, the composition of the PWWS showed excellent potential for addition of C, N and micronutrients to soils. Further work will be conducted using pot experiments to determine the effects of PWSS digestates on crop growth in different soils.

6.4 Conclusions

Concurrent AD of PWWS for bioenergy and biofertilizer supports a circular economy. During the warmer post-crush months, good biogas and CH_4 yields from AD may be feasible without the need for heating, making is economically viable for smaller wineries. The digestate is a promising agricultural fertilizer, especially for addition of C, N and micronutrients to soils. To prevent potential Fe toxicity, it is recommended that it should not be used in highly acidic or waterlogged soils. Future studies will focus on conducting pot experiments with different batches of digestates and comparing the results

6.5 Statements and declarations

Competing interests

There are no competing interests to declare

Funding declaration

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Author contributions

The authors confirm contribution to the manuscript as follows: *Study conception and design* PJ Welz (PJW), AB Mpofu (ABM), GA Holtman (GAH); *Data collection* ABM, E Kimpiab (EK), A Ranjan (AR), WM Kaira (WMK); *Analysis and interpretation of results* PJW, ABM, EK, WMK; *Manuscript preparation* PJW, WMK.

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Chapter 7

Biosand reactors for remediation of winery effluent in support of a circular economy and the positive effect of sand fractionation on hydraulic and operational performance

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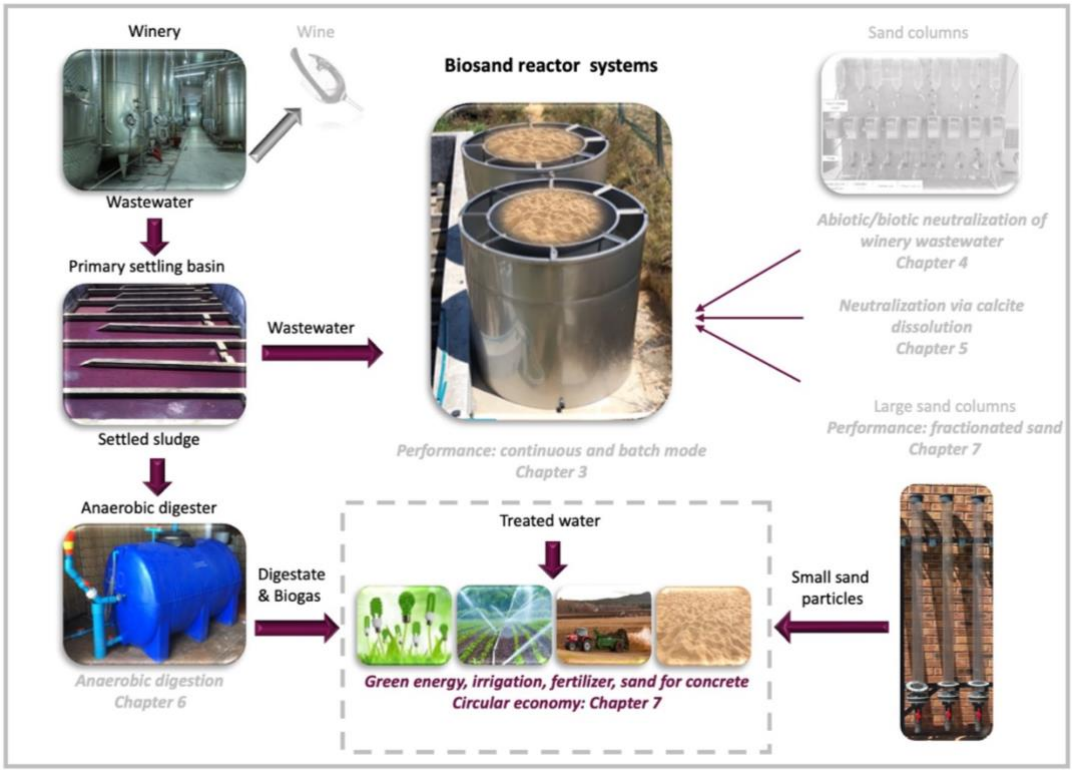
Offprint available in Appendix 8



*[*presents the findings of a study comparing the hydraulic and organic removal performance of raw sand and fractionated sand (>0.425 mm grains). Furthermore, a synopsis of the doctoral work is provided in the form of a zero-waste model for the remediation of winery wastewater based on the use of BSRs*]*

Abstract

There is an extensive body of knowledge pertaining to the treatment of winery wastewater and other acidic effluents in biosand reactors. This manuscript compares the performance of biosand reactors containing raw sand and fractionated sand. Fractionation of sand (particles <0.425 mm removed) increased the hydraulic conductivity of the sand matrix 9-fold when compared to raw sand: from 0.285 $mm.s^{-1}$ to 2.50 $mm.s^{-1}$ and 0.129 $mm.s^{-1}$ to 1.11 mm $mm.s^{-1}$ before and after start-up, respectively. Similar results for both sands were obtained in terms of organic removal performance (94% and 95%, respectively) and neutralization of acidic (pH 4.9) winery wastewater. Results indicate that one 5.6 m^3 biosand reactor module containing 2.9 m^3 of fractionated sand can theoretically treat 8102 $L.d^{-1}$, which is the approximate volume generated from wineries crushing 329 to 547 tonnes of grapes per annum. This is notably higher than the design flow rate of 1000 $L.d^{-1}$ in used in a pilot system containing raw sand. Furthermore, a zero-waste biosand reactor model is presented for treatment of winery wastewater. The strategy includes reuse of treated effluent for irrigation, anaerobic digestion of primary winery wastewater sludge and use of the digestate as an agricultural fertilizer, and re-purposing of the residual sand.

Keywords: biological sand filter, circular economy, constructed wetland, irrigation, neutralization, treatment

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7.1 Introduction

Fermentation of grape juice to wine relies on the microbially mediated conversion of grape sugars to ethanol with the concurrent formation of other metabolites which add to the complexity of the final product [1–4]. This winemaking process and ancillary operations generate solid wastes, by-products, and winery wastewater (WW). Many solid waste streams, including lees and marc, are valorised on-site or in dedicated biorefineries [5], but due to its nature, WW has little beneficial use. Winery wastewater fluctuates in physiochemical quality, with the highest organic and volumetric loads occurring during the crush/harvest period [6–8]. After primary settling and pH adjustment, 'beneficial' irrigation is often used as a means of WW disposal, particularly in water stressed countries [9]. Depending on cellar activities and practices,

the WW can contain high concentrations of organic molecules and inorganics. For example, high sodium (Na) loads can emanate from the ubiquitous use of the relatively inexpensive and effective cleaning product, sodium carbonate (Na_2CO_3) [6]. Polyphenolics in WW can be phytotoxic, and highly saline WW may cause some soils to become sodic [6,10–12]. Irrigation with untreated WW can therefore pose a threat to the receiving environment. It is therefore prudent to remediate WW before discharge on land or to aquatic environments.

There are several WW treatment options available which vary in cost, complexity, and efficiency. The challenge for designers of WW treatment systems is the seasonal variability in the quality and quantity of WW [13–15]. Depending on the local legislation, the amount of WW generated, and the location of the cellar, different levels of remediation may be required. While complex treatment systems may be effective, they are generally not applicable for smaller wineries due to cost and staff constraints [16]. Constructed/treatment wetlands have been extensively used as passive WW treatment systems, but typically have large spatial footprints and the WW may be phytotoxic to the plants [17]. Biosand reactors (BSRs), alternatively known as biological sand filters or unplanted constructed/treatment wetlands, are cost effective, require minimal maintenance and provide an environment for biodegradation of the organic fraction of WW and neutralization of acidic WW. After start-up, the hydraulic conductivity (HC) of the BSRs decreases due to the formation of functional biomass. This is inversely correlated with organic loading and therefore reversible, so that the systems do not require backwashing after the crush season even after long-term operation [18–21]. When operated in vertical mode, BSRs have smaller spatial footprints and higher specific organic removal rates (ORR) than other passive treatment systems, and can be retrofitted to existing treatment systems [21].

One of the challenges that has previously been associated with pilot BSRs is the large (up to 95%) reduction in the hydraulic conductivity (HC) of the sand due to build-up of biomass during WW treatment [21]. The biomass is a necessary functional component of BSRs and is most abundant during periods of high organic loading. During this time, it retards the flow of WW, causing a transient reduction in the hydraulic capacity of BSRs [18,21,22]. The size of sand particles is a critical factor governing the HC of soil and sand environments, with liquid flow rates increasing as particles increase in size [22,23]. It was therefore hypothesized that: (i) the hydraulic performance of BSRs during the crush period could be improved by fractionating the sand and using only the larger sand particles in the systems, and (ii) the smaller sand particles could be

used for another purpose. For example, smaller sand particles are more desirable in self-compacting concrete (SCC) mixes [24]. The proposed dual usage of the sand feeds into the principles of a circular economy.

In passive treatment systems, there is theoretically a balance between hydraulic retention time (HRT) and remediation efficiency [25,26] and functional microbial communities are influenced by the character of sand particles in BSRs [27]. It was therefore recognized that the fractionation strategy could have a negative impact on BSR performance. Column replicates containing raw or fractionated sand (particles >0.425 mm) were therefore used to compare (i) The HC before and after build-up of biomass consequent to feeding with WW, (ii) organic removal rates, and (iii) WW neutralization rates. This study is the final in a series of studies pertaining to remediation of WW in BSRs. The manuscript therefore contains a section exploring the holistic use of BSRs and proposed ancillary methods for treating WW and WW sludge in the context of resource utilization and reuse for a bio-circular economy.

7.2 Materials and methods

7.2.1 Experimental set-up and operation

The experimental set-up consisted of six identical clear 2000 mm tall polyvinyl chloride (PVC) columns with internal diameters of 105 mm (Figure 7-1A). Each column contained approximately 20 kg of sand to a height of 1450 mm (Table 7-1). The columns were operated in triplicate with one set containing raw (unfractionated) sand and the second containing sand with all particles < 0.425 mm removed (fractionated sand), (Figure 7-2). The raw sand has an effective size, uniformity coefficient and fineness modulus of D_{10}=0.36, 2.3 and 1.67 respectively and D_{10}=0.36, 2.0 and 2.78 respectively for the fractionated sand. To prevent sand washout and to ensure permanent saturation but unimpeded flow when required during operation, a complex sealing system consisting of gaskets, mesh screens, spigots, backing rings and ball valves were installed at the bottom outlet of each column. To increase the column lengths for sand HC measurements, spigots and backing rings were also installed at the top of the columns (Figure 7-1B,C).

For the HC tests, the outlet piping was removed from the columns so that the flow was only restricted by the 50 mm ball valves. To increase the hydraulic head, temporary 2000 mm column lengths were joined to the top of the columns above the mesh and a stopwatch was used to

measure the time it took for the water level to drop each 200 mm, with the final measurement being taken at a hydraulic head of 1520 mm. HC measurements for each column were conducted in triplicate.

For the performance evaluation experiments, each column was fed with WW. The hydraulic loading rate (HLR) was calculated in terms of the volume of sand within the column as previously described [18] (Table 7-1). The columns were fed via IVAC volumetric pumps (Model 597) at a rate of 2.4 L.day^{-1} with operational parameters as shown in Table 7-1. All the pumps were fed from the same holding vessel to ensure influent consistency.

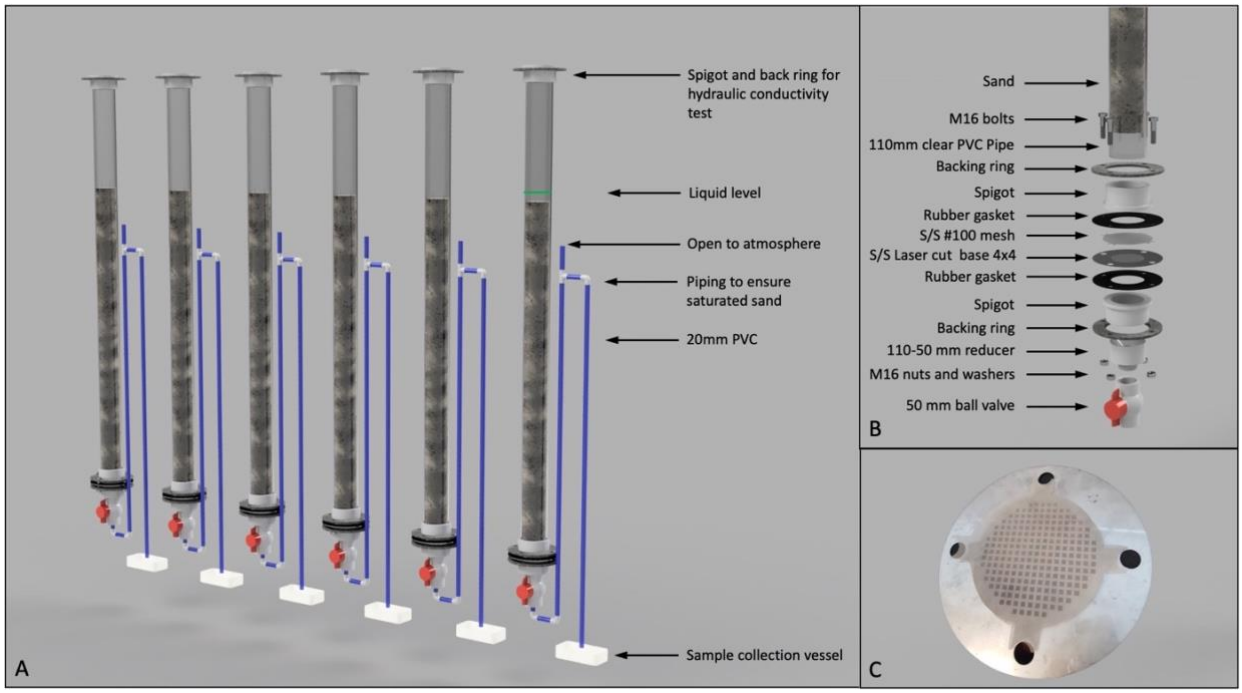

Figure 7-1. Layout of experimental set-up of column experiment together with outlet piping (A), exploded view of outlet filter of column (B), stainless steel number 100 mesh and the stainless-steel laser cut base with 4x4 mm square openings (C)

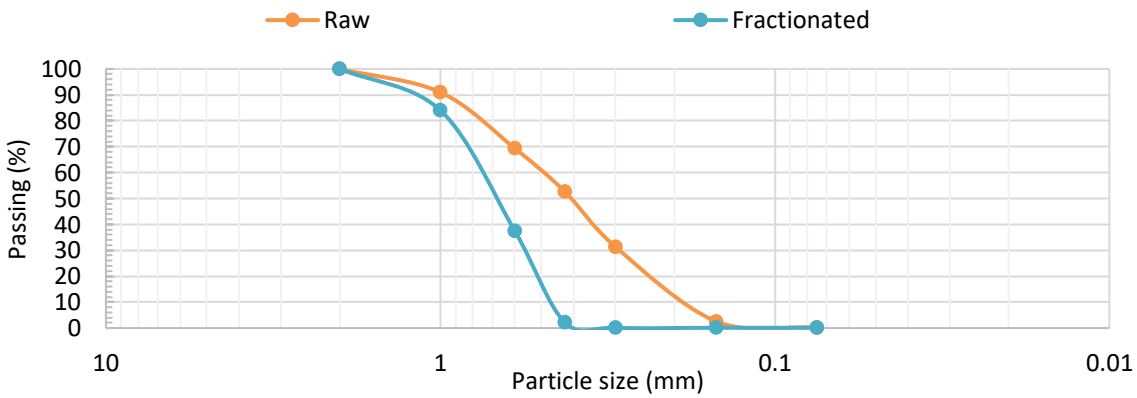

Figure 7-2. Particle size distribution curves for raw and fractionated sand

Approximately 3 kg of sand was extracted from an operational BSR system containing functional biomass [21]. Distilled water was added to the extracted sand and sonicated. After the initial HC test, the columns were allowed to drain for 48 hours then inoculated with 1 L of the supernatant fluid from the sonicated sand to the top of each column, which were then allowed to acclimate for 14 days.

After inoculation, starting at days 0, 21, 42, 63, 84, 105 and 126, the columns were fed with different batches of WW at 100 $mL.h^{-1}$ for the allotted volume, sampled and outlet was closed till the next dosing (Table 7-2). During the start-up period (day 0 to 83), the WW was diluted with filtered water and the columns were fed with WW in increasing concentrations to allow the functional microbial communities to gradually acclimate to the WW. This 'incremental priming' has been shown to increase system performance in BSRs [28]. The systems were considered operational from day 84 (Table 7-2).

Table 7-1. Operational parameters of sand columns

Sand Type	Sand height (mm)	Sand weight (g)	HRT (hrs)	HLR ($L.m^3$ sand.day^{-1})
Raw	1450±10.8	20000±0	36.7±0.3	191.2±1.4
	[1435-1460]	[20000-20000]	[36.3-36.9]	[189.9-193.1]
Fractionated	1444±7.4	21000±408	37.6±0.3	191.9±1.0
	[1435-1453]	[20500-21500]	[37.4-37.9]	[190.8-193.1]

[] = Range, HRT = hydraulic retention time, HLR = hydraulic loading rate

Table 7-2. Feeding schedule and physicochemical analysis of influent winery wastewater

Period (day)	Volume (L)	COD ($mg.L^{-1}$)	TN ($mg.L^{-1}$)	TP ($mg.L^{-1}$)	TPP ($mgGAE.L^{-1}$)	pH (pH)	Alk ($mgHCO^{-}.L^{-1}$)	Con ($\mu S.m^{-1}$)
Day 0-21 [1]	4*	101	0.0	0.0	3.6	6.8	92	161
Day 21-42 [1]	4*	206	0.0	0.0	2.4	6.8	67	153
Day 42-63 [1]	8*	492	0.5	1.6	10.3	6.8	116	230
Day 63-84 [1]	8*	961	2.6	5.8	16.4	6.6	134	438
Day 84-105 [1]	13	1013	20.8	21.1	17.7	6.8	183	470
Day 105-126 [2]	21	2538	73.1	9.2	55.3	5.6	409	1286
Day 126-130 [3]	8	4681	89.3	29.6	38.2	4.9	311	1551

COD = chemical oxygen demand, TN = total nitrogen, TP = total phosphate, TPP= total poly-phenolics, GAE = gallic acid equivalent, Alk = Alkalinity, HCO^{-} = bicarbonate, Con = electrical conductivity, S = siemens, 1,2,3 = batch number of the winery wastewater, * = wastewater diluted with filtered water

7.2.2 Sampling and characterisation of influent and effluent

From the third feeding period (day 42-63), effluent samples were taken over the last hour of each feeding period and analysed immediately. The pH was determined according to the manufacturer's instructions using a CyberScan pH300 meter and appropriately calibrated pH probe PHWP300/02K (Eutech instruments, Singapore). The electrical conductivity (EC) was determined using a hand-held Oakton ECTestr 11+ multirange, cup-style pocket conductivity meter (Eutech Instruments, Singapore Cat No: 35665-35) with a range of 0 µS/m to 20 mS/m. The COD concentrations were determined using a Merck (Merck®, Whitehouse Station, USA) Spectroquant® Pharo instrument and Merck Spectroquant® cell tests (cat. no. 1.14895.0001, 1.14541.0001 and 1.14691.0001) according to manufactures instructions. The total phenolic concentrations were determined using the Folin-Ciocalteu micro method [29] using a Merck®Folin–Ciocalteu reagent (Cat No: 1.09001.0500). Total Alkalinity was measured using the Merck titrimetric method with titration pipette MQuant catalogue number (1.111109.0001), according to the manufacturer's instructions.

7.2.3 Characterisation of sand

The particle size distribution was performed using the SANS 3001 (Method AG1, PR5, AG21) by Cetlab, South Africa.

Fractionated samples of sand analysed by automated scanning electron microscopy using a FEI QEMSCAN® (Quantitive Evaluation of Minerals by SCANning Electron Microscopy) instrument (Thermo Fisher Scientific, Waltham, USA) and iDiscover™ software. The roundness and aspect ratios of the different mineral fractions determined using the iDiscover™ software were used to compare the shapes and sizes of particles with different mineral composition as previously described [23,30].

7.3 Results and discussion

7.3.1 Hydraulic performance

The saturated HC is the intrinsic value which describes the rate at which fluid moves though a saturated porous media and is affected by the porosity of the medium, particle packing, particle size [23] and uniformity of the particles [22]. In this study, indices obtained via QEMSCAN® were

used to compare the morphologies of the raw and fractionated sand [23,30]. For the raw sand, the quartz and calcite contributions were 81.3% (wt.wt.) and 17.6% (wt.wt.), while for the fractionated sand, they were 74.0% (wt.wt.) and 9.6% (wt.wt.) respectively. Computer generated images of the morphology and mineralogy of the raw and fractionated sand obtained using via QEMSCAN are shown in Figure 7-3. Additional information of the character of the raw sand can be found in literature [22,23].

It was previously established that the HC of the sand with the smaller fractions (>0.6 mm) removed exhibited a significantly higher ($p<0.05$) HC (2.75 ± 0.02 $mm.s^{-1}$) than the raw sand (0.41 ± 0.10 $mm.s^{-1}$), but the relative losses in HC due to the formation of functional biomass were not determined [19,22]. In this study, the HC results before and after biomass formation were very similar to those obtained in an earlier experiment conducted using columns containing a batch of sand from the same quarry site fed with synthetic WW for 14 weeks [22]. The initial comparative HC values of the raw sand batches were: 0.275±0.05 $mm.s^{-1}$ (this study) and 0.284 $mm.s^{-1}$ [22], with respective reductions in HC due to the growth of functional biomass of 52.3±6% and 52%. For the fractionated sand, the initial HC of 2.504±0.20$mm.s^{-1}$ reduced by 55.4±2% after feeding with WW for 19 weeks, but the HC (average 1.146±0.081 $mm.s^{-1}$) was still >4 fold higher than the initial HC of the raw sand (Figure 7-4).

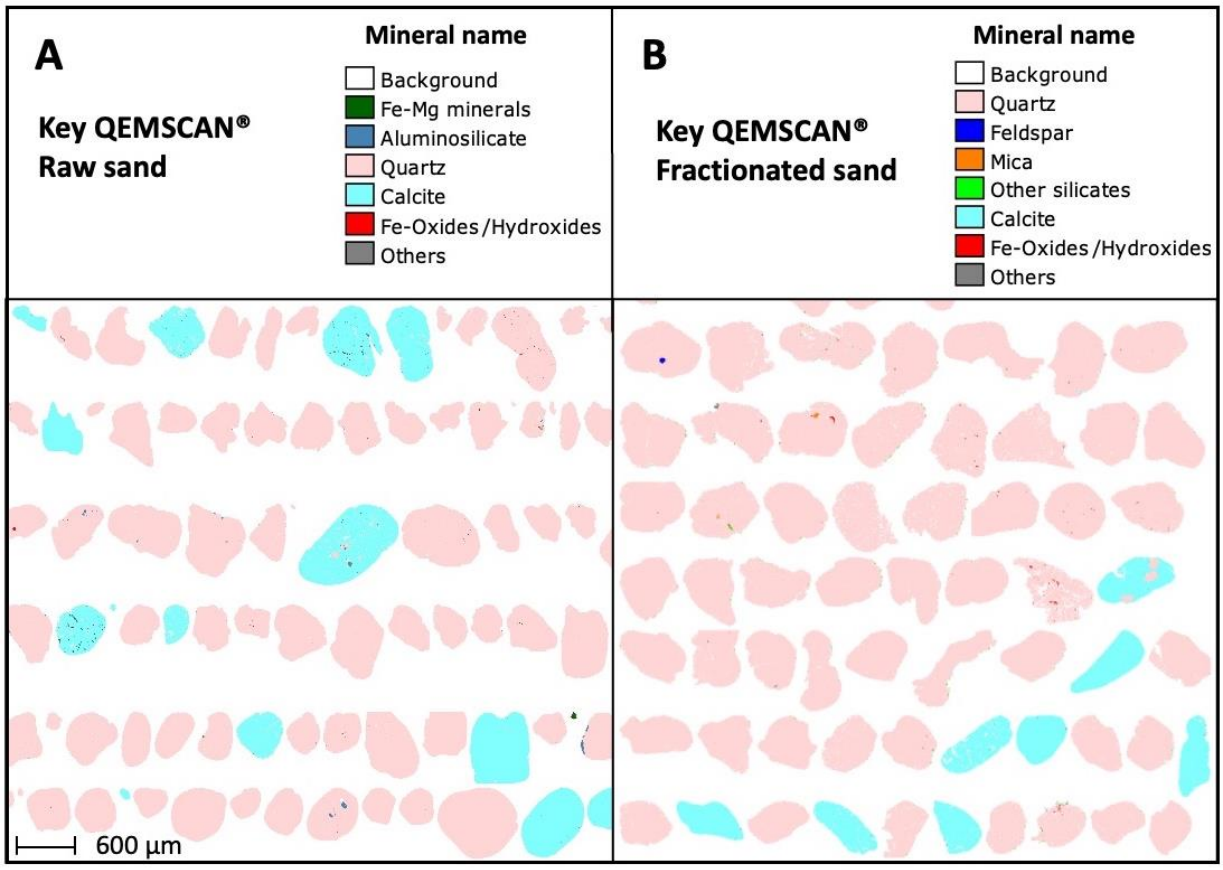

Figure 7-3. Computer generated (QEMSCAN®) images of the raw (A) and fractionated (B) sand particles

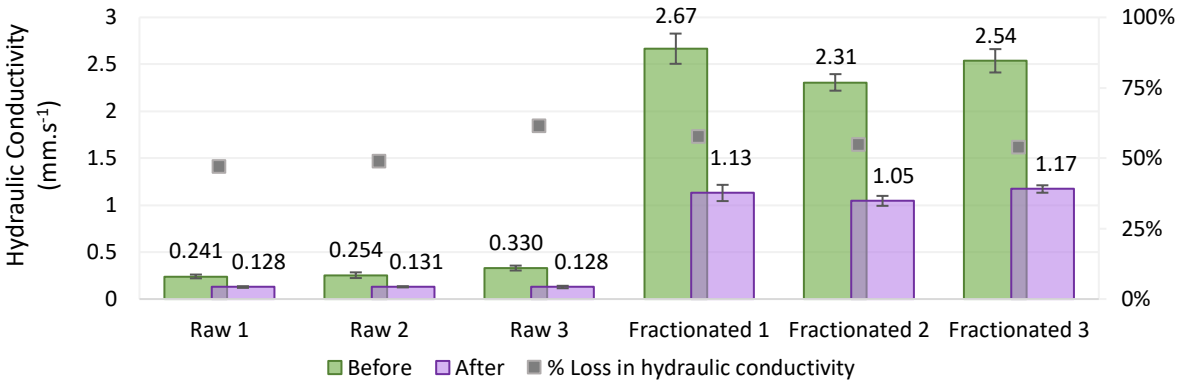

Figure 7-4. The hydraulic conductivity of raw and fractionated sand measured in experimental columns before and after feeding with winery wastewater

The HC results were applied to a pilot BSR system with a novel design [21] shown in Figure 7-5. In the system, pre-settled WW is applied to the surface of the BSR modules and flows passively through the system as indicated in the diagram. The adjustable outlets allow the HLR to be manipulated by changing the hydraulic head to the maximum rate dictated by the HC. It was found that the range of possible flow rates was considerably greater when using fractionated sand in comparison to raw sand in the BSR modules (Figure 7-6).

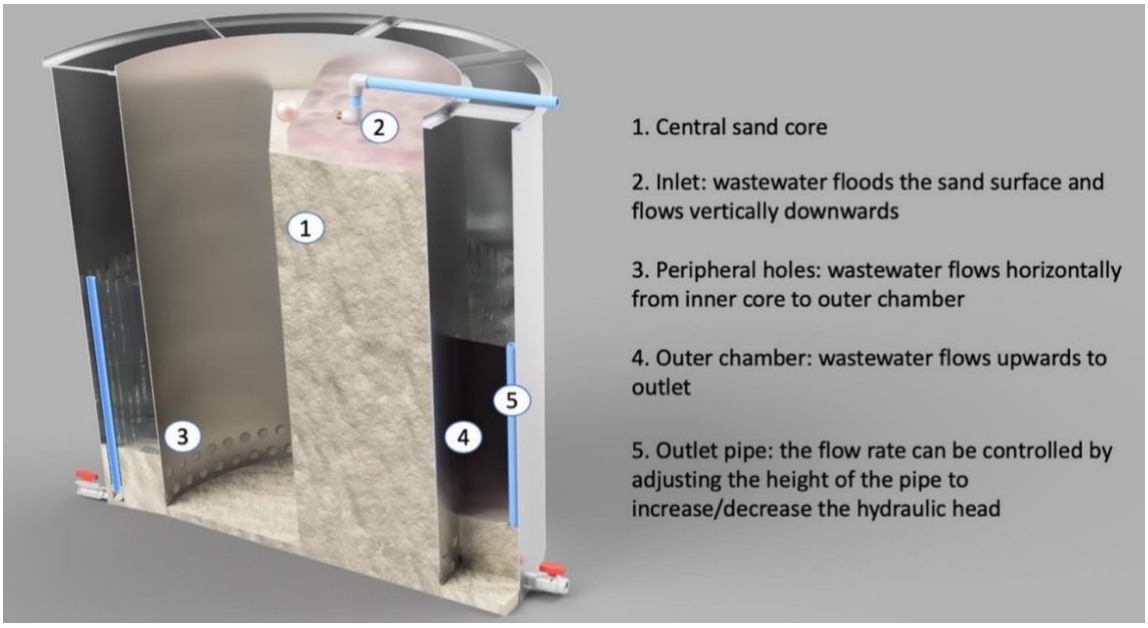

Figure 7-5. Cross-section of a novel biosand filter module [21]

When the pilot system was operated with raw sand and a hydraulic head of 0.8 m, the maximum achievable flow rate during the crush season was <250 $L.d^{-1}$ per module [21]. By applying the HC results obtained in the column experiments containing fractionated sand, it was calculated that this rate could theoretically be increased to 114681 $L.d^{-1}$ (before) and 5455 $L.d^{-1}$ (after) biomass accumulation during the crush season. This would reduce the HRT for the reactor module to 17.8 hours during the crush season (Figure 7-6). In reality, the maximum operational flow, with a hydraulic head of 0.8 m, would be limited to 8102 $L.d^{-1}$ which equates to a HLR of 3212 $L.m^{-3}$of $sand.d^{-1}$ in order to provide sufficient HRT (12 hrs) for bioremediation of the WW. In other words, when calculating the WW volume generated per tonne of grapes crushed previously formulated [31], one BSR module containing fractionated sand would be required for wineries that crush 547 tonnes or 329 tonnes of grapes, respectively, producing 50% or 80% of their WW during the crush season. In comparison, a single BSR module would be capable of treating the WW from wineries crushing approximately three times as many grapes when applying the WW generation volume of 3.05 m^3 WW per tonne of grapes crushed formulated by another group of researchers [32].

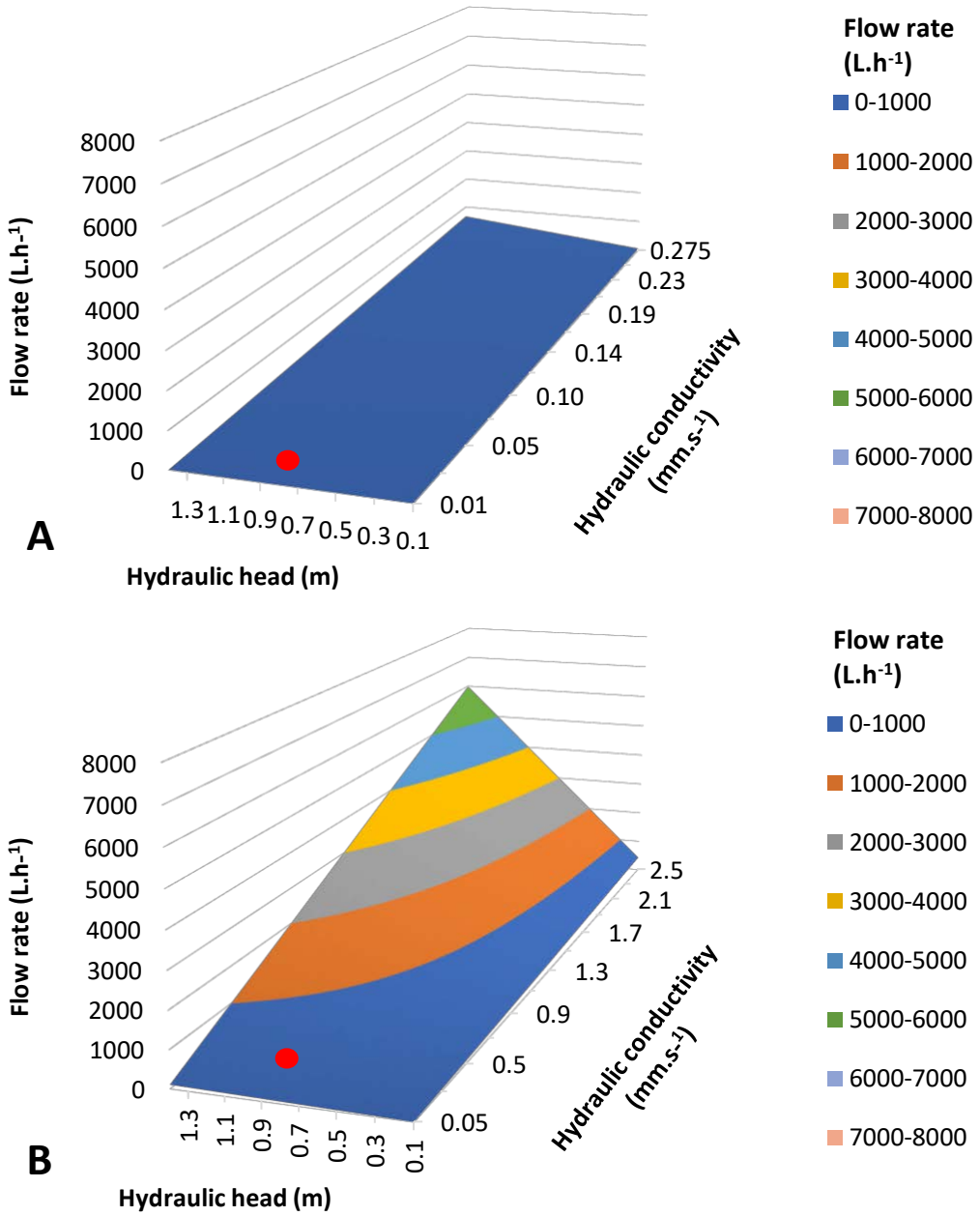

Figure 7-6. Potential ranges of flow rates that can be achieved in biosand reactors containing raw (A) and fractionated (B) sand before and after biomass accumulation. The theoretical flow rates, used in Table 7-3, after biomass accumulation are denoted with a red circle

7.3.2 Remediation performance of biosand filters with fractionated and raw sand

7.3.2.1 Organic removal rates

Due to the organic nature of WW, the organic fraction was measured using COD as a proxy [6]. It has been conclusively established that BSRs containing raw sand are capable of significantly reducing the COD of WW, including the concentrations of polyphenolics [18,21,33,34]. A pilot system containing raw sand was able to treat WW at higher organic loading rates (279 gCOD.m-

^{3} of sand.day^{-1}) with a smaller spatial footprint than reported for other passive systems (constructed/treatment wetlands) [21].

In this study, the use of fractionated sand had a positive influence, not only on hydraulic performance, but on COD removal efficiency (Figure 7-7A). The COD removal efficiencies in the columns containing raw and fractionated sand ranged from 71% to 94%, and 74% to 95%, respectively. For the final sampling instance (with the most concentrated WW), the removal efficiency in the columns containing fractionated sand was 95% (±1%) with influent and effluent concentrations of 4681 mg.L^{-1} and 222 mg.L^{-1} (±43), respectively. This was comparable with the 94% (±1%) efficiency and effluent concentration of 298 mg.L^{-1} (±61) obtained with the raw sand (Figure 7-7A). The trend continued with the removal of total polyphenolics with the fractionated sand columns having slightly higher removal efficiencies (87% to 94%) than the raw sand columns (87% to 93%) with influent values ranging from 10.4 to 55.3 mgGAE.L^{-1} (Figure 7-7B).

7.3.2.2 Neutralization of acidic winery wastewater

Winery wastewater is typically acidic, which may have a negative impact on the receiving environment. It has been established that BSRs are capable of neutralizing acidic WW and increasing the sodium adsorption ratio (SAR) [18]. The main pH adjustment mechanism is via abiotic dissolution of calcite [19,23], but biotic mechanisms are also involved [19]. It is possible that some calcite solubilises and then re-precipitates when the WW flows through BSRs and becomes less acidic. However, QEMSCAN results have shown that no other Ca-based minerals such as anhydrite or gypsum are formed [19,23]. The longevity of BSR systems in terms of calcite dissolution capacity is beyond the projected life of the systems, with a lifespan of 37 years being calculated using data from column and in-situ experiments for systems containing approximately 10% calcite. Furthermore, it has been shown that loss of calcite particles does not have a deleterious effect ($p>0.05$) on the HC [23].

In this study, the pH in the final effluent from the columns containing both raw and fractionated sand was slightly alkaline, with no significant ($p>0.5$) differences between effluent values. In the final sampling instance, the pH of the acidic WW increased from 4.86 to 8.13 (±0.17) and 8.10 (±0.14) after treatment in the columns containing raw and fractionated sand, respectively (Figure 7-7C). There were temporal changes over the sampling period for both total alkalinity and EC of the effluent WW (Figure 7-7D,E). Both of these parameters increased when the columns were

fed with more concentrated WW with lower pH values, supporting previous results that showed that calcite dissolution increases with decreases in acidity [19,23]. Overall, the result showed that fractionation of sand did not have a negative impact on the WW pH neutralization mechanisms.

7.3.2.3 Comparison of hydraulic and organic loading of biosand reactors with other passive treatment systems treating winery wastewater

The theoretical organic loading rates (OLR) and HLRs loading rates measured in the pilot BSR system containing raw sand were notably higher than in other passive systems treating WW, translating into a comparatively lower spatial footprint [21]. Based on the results obtained from this study when using fractionated sand and applying a hydraulic head of 0.8 m (Figure 7-5), the theoretical OLRs and HLRs after biomass accumulation during the crush season were typically an order of magnitude higher than in other systems, including the pilot BSR system containing raw sand (Table 7-3).

7.3.3 Sustainable and circular economy approach for valorisation of winery wastewater: towards zero waste for wineries

Circular economy principles have been envisaged, evaluated, and/or applied in the wine industry for a range of products, services, and solid wastes/by-products. These include wine packaging [43], multiple external and internal stakeholder engagements and activities [44], biorefineries for the extraction of tartrates, antioxidants and other value added products from grape pomace and lees [7,8], and the use of solids wastes for animal feed and composting.

Passive systems such as constructed/treatment wetlands and BSRs are sustainable options for remediation of WW [17,18,20,21,45]. Building on the results obtained from previous studies, a circular economy and zero waste model for WW remediation and valorisation based on the use of BSRs is proposed (Figure 7-8). The intention is to promote the design and implementation of integrated sustainable systems for reuse and recycling of WW and primary winery wastewater sludge (PWWS) in order to contribute to minimizing waste formation and maximizing resource recovery in wineries [46].

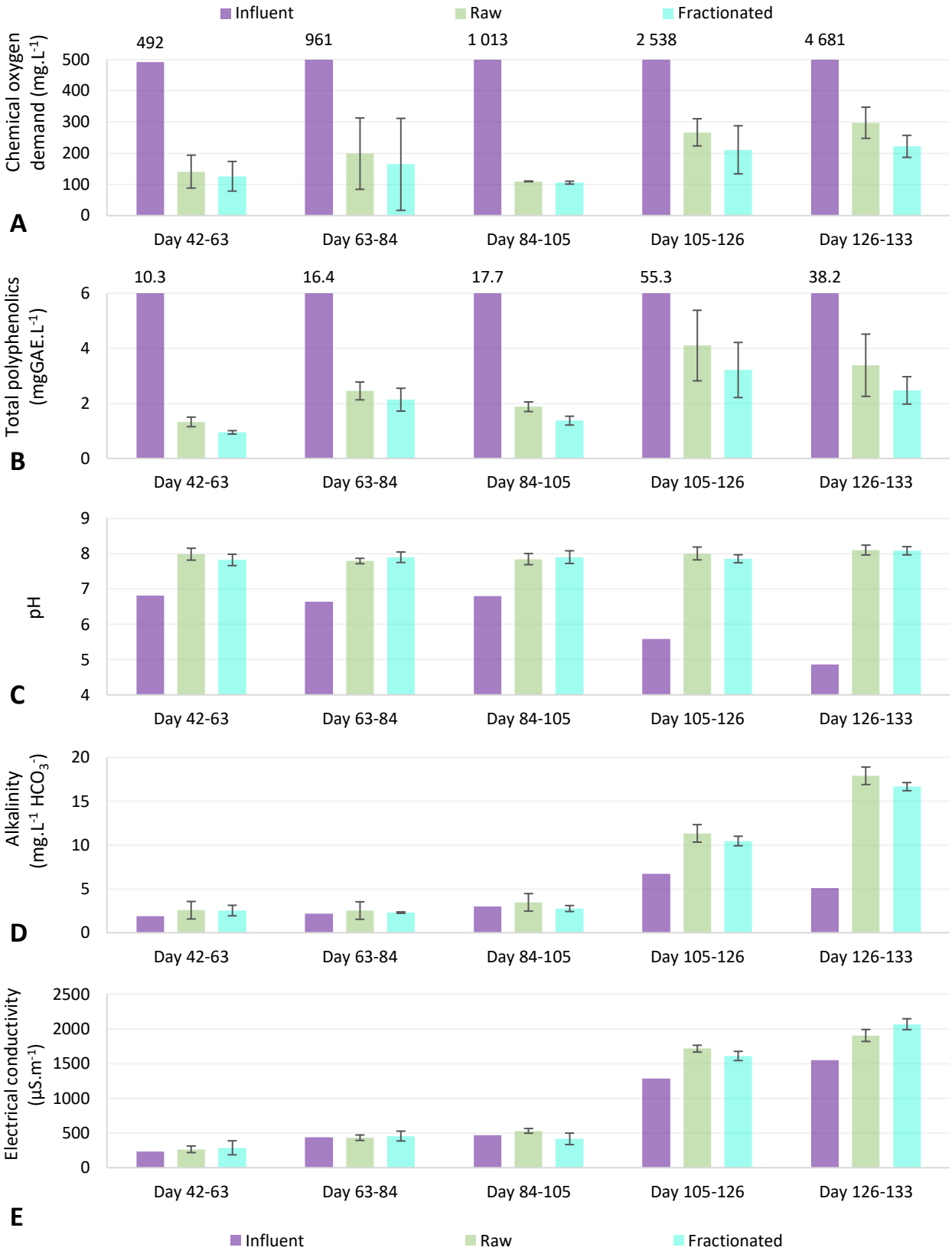

Figure 7-7. Results of A) chemical oxygen demand, B) total polyphenolics, C) pH, D) Alkalinity and E) Electrical conductivity of influent and samples taken from experimental columns containing raw and fractionated sand

Table 7-3. Comparison of hydraulic and organic loading rates of biological sand reactors and other passive systems treating winery wastewater (adapted from [21])

HLR $mm.d^{-1}$	HLR_{Vol} $L.m^{-3}.d^{-1}$	OLR_{Vol} $g\ COD.m^{3}.d^{-1}$	Ref
14.6* 7.3*	41.7* 20.8*	32.9 to 124.5 16.4 to 62.1	[34]
14 24.8 36.3	14 (VF) 43-82 (HF_1) 22-41 ($HF_{2,3}$)	152* (VF) 54* (HF_1) 27* ($HF_{2,3}$)	[35,36]
77-215 13-36 13-36	55-154* (VF) 43-120* (HF_1) 22-60* ($HF_{2,3}$)	31-333* (VF) 12-183* (HF_1) 6.0-92* ($HF_{2,3}$)	[25]
34	28*	37-176*	[38-40]
333*	150	152	[18,22]
23 45	1) 25* 2) 50*	1) 350* 2) NG	[40,41]
78.3	57.0	260.4	[21] Year 1
206.5	150.3	322.9	[21] Year 2
425	223	447#	This study: raw sand after biomass accumulation during the crush season
4110	2163	4326#	This study: fractionated sand after biomass accumulation during the crush season

HLR = Hydraulic (surface) loading rate, HF = horizontal (subsurface) flow, VF = vertical (subsurface) flow, * = Calculated, HLR_{Vol} = volumetric hydraulic loading rate, OLR_{Vol} = volumetric organic loading rate, # = theoretical value with assumed influent of 2 000 mg (COD).L-1

In comparison to other passive treatment systems, BSRs containing fractionated sand (particles >0.425 mm) are envisaged to have even smaller spatial footprints and higher OLRs than previous systems containing raw sand (Section 3.2.2, [19]). As alluded to in Section 7.3.1, it is feasible that these 'new' BSR systems can be implemented at wineries crushing around 547 tonnes of grapes and generating 1507 m^3 of WW or less per annum, taking into account 50% peak flows during the crush period. This amount of WW can theoretically be treated using just one 5.6 m^3 BSR module containing 2.9 m^3 of sand. The WW treatment capacity can be increased by adding additional modules where practically possible (Table 7-4). Alternatively, the size of the modules may be increased. While sand containing larger particles is more desirable for use in BSRs, the

cost of fractionating the sand can be offset by using the finer sand fractions, for example, SCC in the building industry [24].

Remediation of WW using BSRs renders it suitable for irrigation purposes, reducing water consumption which is important in water-stressed wine-producing areas such as South Africa, Australia and California, USA [47,48].

Before WW is remediated in BSRs, the solids are removed using primary settling. In a survey conducted in South Africa, 25% of wineries indicated that they use commercial companies to remove PWSS and dump it in landfills. This is an economic and environmental burden on the industry and is more likely to take place in medium and larger wineries than smaller wineries. In addition, medium-large wineries tend to dispose of PWWS more frequently than smaller wineries [49,50]. In another study, it was found that the Na in WW does not partition into the PWWS, and that it is amendable to anaerobic digestion (AD), generating up to 206 ± 2.7 $mLCH_4/gVS_{added}$ [5]. These results suggest that it may be feasible for biogas generated from co-digestion of PWWS and other available organic substates to be used as part of a renewable energy mix in wineries [5,51]. In addition, it was shown that the digestate from AD of PWWS has potential as an organic agricultural fertilizer as it contains micronutrients and high concentrations of N (21.5 to 27.7 g/kg dry weight) and C (229 to 277 g/kg dry weight) [5].

In summary, based on the results of this research and previous findings, the a zero waste model is proposed which consists of the following objectives: (i) to fractionate sand and use the fraction with larger particles (>0.425 mm) in BSRs and the smaller fraction in high performance concrete i.e. SCC, (ii) to remediate WW in BSRs and use the treated effluent for irrigation, (iii) to digest PWWS and use the biogas for energy and the digestate as an agricultural fertilizer (Figure 7-8). Data for different size categories of wineries generating different volumes of WW are provided in (Table 7-4).

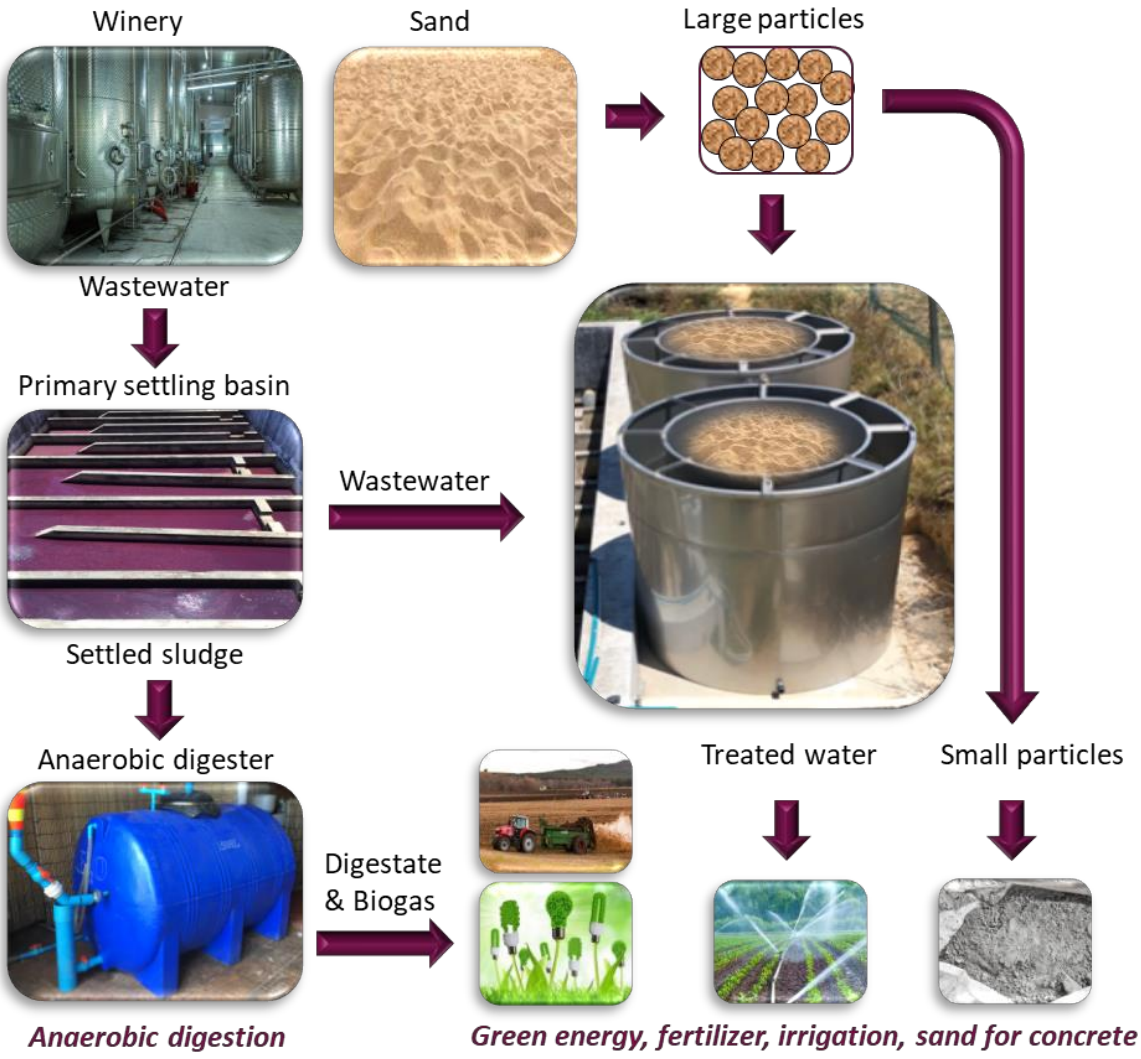

Figure 7-8. Schematic of proposed 'zero waste' biosand reactor-based system for valorisation of winery wastewater

The volume and character of WW is incumbent on cellar practices and differs from winery to winery [6,32,52]. In most cases, the highest volume of WW (up to 80%) is usually generated during the standard three-month crush period, but this figure can vary quite widely. For example, some wineries do not crush while other do not bottle on site and a number of wineries have bottling facilities which will result in greatly different flow rates with the latter generating considerable quantities outside the crush season [17,53,54] Table 7-4 provides data that can assist practitioners to capacitate integrated BSR systems for different sized wineries based on the amount of grapes crushed per annum and maximum peak flows (50-80% during crush period). The amount of methane generated can be estimated using the volume of total sludge (TS) 2261 $mgTS.L^{-1}$ of wastewater [49 in press], converting the TS to volatile solids (VS) TS:VS ratio of 0.675 and VF to methane 191.5 $mLCH_4.gVS^{-1}$ [5]. In addition the average calorific value of 6

kWh.m^3 and a conversion efficiency of 35% was used to calculate the electricity generation [55]. For example, a winery crushing 1000 tonnes of grapes would require 3 BSR modules with fractionated sand, if 80% of the WW is produced in the crush. The sludge would potentially yield 1266 kWh of electricity from the methane.

Table 7-4. Design criteria for biosand reactor systems treating winery wastewater and/or digesting primary winery wastewater sludge

Parameter	units	Tonnes of grapes crushed							
		10	100	500	1000	2000	5000	10000	20000
Calculated volume of wastewater produced yearly*	$m^3.year^{-1}$	37	313	1387	2633	4996	11654	22116	41971
# produced 80% of wastewater in crush	$m^3.day^{-1}$	0.3	2.7	12	23	43	100	190	361
# produced 50% of wastewater in crush	$m^3.day^{-1}$	0.2	1.7	7.5	14	27	63	119	226
# number of 5.6 m^3 BSR modules required for 80% wastewater in crush	each	1	1	2	3	6	13	24	45
# number of 5.6 m^3 BSR modules required for 50% wastewater in crush	each	1	1	1	2	4	8	15	28
Calculated volume of sludge produced per year	$kg.year^{-1}$	84	709	3137	5953	11297	26349	50004	94896
# produced 80% of sludge in crush	$kg.day^{-1}$	0.7	6.1	27	51	97	227	430	816
# produced 50% of sludge in crush	$kg.day^{-1}$	0.5	3.8	17	32	61	142	269	510
Calculated volume of methane produced per year	ML CH_4	11	92	405	769	1460	3406	6464	12266
# produced 80% of methane in crush	ML $CH_4.day^{-1}$	0.1	0.8	3.5	6.6	13	29	56	106
# produced 50% of methane in crush	ML $CH_4.day^{-1}$	0.1	0.5	2.2	4.1	7.9	18	35	66
Potential power produced per year	kW	23	192	851	1616	3066	7152	13574	25760
# produced 80% of electricity in crush	$kW.day^{-1}$	0.2	1.7	7.3	14	26	62	117	222
# produced 50% of electricity in crush	$kW.day^{-2}$	0.1	1.0	4.6	8.7	16	38	73	138

*calculated using Sheridan, 2003, ML = Mega litres

Conclusion

To conclude, a circular economy approach for the treatment and valorisation of waste gives a purpose and a value to waste. The BSR system containing raw sand has a proven track record in terms of treatment of WW. Previously, a drawback was associated with reduction in HC due to the accumulation of functional biomass during the crush season that reduced the BSR treatment capacity to below the design limit. This study showed that the HC and flow rate can be increase 9-fold by using fractionated sand with the smaller particles removed. With the experimental test setup, BSR columns containing fractionated sand also showed excellent organic removal efficiencies and increased the pH of acidic WW. With a zero-waste model, it is suggested that the larger fraction of sand (>0.425 mm) is used in BSRs and the smaller fraction in high performance concretes. The solid fraction of WW can be anaerobically digested producing biogas which may be used for energy and digestate used as an agricultural fertilizer. The quality of the remediated WW is acceptable for irrigation purposes.

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Conclusion and recommendation

Conclusion and recommendations

[Provides a brief conclusion for this doctoral work.]

8.1 Conclusion

In summery a conclusion for the entire body of works presenting the findings of the thesis.

The novel onsite BSR system had a smaller spatial footprint compared to other passive treatment system with a reduction ranged from 40 % to 96% and included higher hydraulic, organic and removal loading rates. The substrate provides a conductive environment for the accumulation of functional microbial growth and activity, without any permanent clogging, which allows for the effective treatment of WWW. The system was operated in both batch and continuous mode of operation and achieved better loading and removal rates in continues mode of operation. Both modes resulted in a 70% reduction in COD, however, the hydraulic and organic loading rate of 113 $L.m^{-3}sand.day^{-1}$ and 279 $gCOD.m^{-3}sand.day^{-1}$, respectively in continuous mode outperformed pulse mode with loading rates of 90 $L.m^{-3}sand.day^{-1}$ and 192 $gCOD.m^{-3}sand.day^{-1}$ respectively.

By performing column experiments it was determined that on average 95.5±0.16% of the neutralisation of the WWW was attributed to abiotic neutralisation due to the dissolution of calcite within the substrate and a small biotic contribution average of 4.5±0.13%. In addition, the effects of HC and the longevity of the abiotic neutralisation mechanism via calcite dissolution on different fractions showed that a BSR with Philippi sand containing 5.4 % wt.wt Ca would have a temporal abiotic neutralization of 37 years in treated an influent pH raging from 2 to 3 at a hydraulic loading rate of 150 $L{\cdot}m^{-3}$ of $sand.d^{-1}$.

Further investigating the effects on different fraction of sand the larger fractions contained a lower concentration of Calcite and higher HC compared to smaller fractions of sand. Using fractionated sand with partials >0.425 mm, which was approximately 50% of the grading envelope, the HC increased 9 fold compared to unfractionated sand thus intern resulted in an increase of theoretical hydraulic loading rate of 8102 $L.d^{-1}$ in a 5.6 m^3 BSR. The fractionated an unfractionated sand both neutralized acidic WWW and removed 95% and 94% influent COD. The <0.425 mm fraction of sand can be used in the concrete industry.

The primary WWW sludge that was observed during on site studies was analyzed and a biochemical methane potential tests was performed and achieved a highest specific methane yield of 206 ± 2.7 $mLCH_4/gVS_{added}$ which was from sludge harvested during the crush season, this

was achieved under mesophilic (37°C) conditions. The composition of the digestate compared favorably with commercial organic agricultural fertilizers.

The overreaching conclusion of the thesis presents the novel designed BSR as an effective passive treatment system which will have sufficient operational longevity, require minimal maintenance, is low cost, small special footprint with high effective loading rate and is an effective treatment solution for treating WWW with effluent suitable for irrigation and a system can easily be integrated into excising infrastructure. BSR can also be integrated with co-digestion AD to minimise disposal of sludge and potential digestate can be used as a soil conditioner creating a circular economy.

8.2 Recommendations

From the body of works the following recommendations are proposed for future studies or real world implementation.

The novel reactor design of the on-site BSR would require slight alteration by changing the outlets by having several outlets at different heights which will allow the system operator to simply manipulation of potential head across the system by opening a lower valve and thus increase the flow rates. These adjustments will be easier that the current internal adjustment. During higher loading and subsequent biomass accumulation the outlet can be dropped resulting in a higher potential head across the filter and a subsequent increase in flow rate. In lower loading conditions and off season the flow rate can be reduced by increasing the hight of the outlet in order to decrease the potential head across the system, this will also allow for the natural reduction of beneficial biomass due to low loading rates. In addition, it is recommended that future onsite treatment systems use >0.425mm sand in order to increase the intrinsic HC of the substrate and therefore increase operational parameters for the treatment system in order to operate at a wider range of loading rates and biomass accumulation. However the treatment systems must not have a HRT of less that 24 hours.

The TANC calculation should be used in conjunction with the physiochemical composition of WW in order to determine the theoretical longevity of BSR. This must be coupled with a practical service period of substrate of up to 10 to 15 years before replacing the sand and to determine the potentially the addition of other calcite sources if required.

Chapter 9 Appendices

Appendix 1 Journal Article 1 offprint

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Review

Treatment wetlands and phyto-technologies for remediation of winery effluent: Challenges and opportunities

Anthony E. Mader [a], Gareth A. Holtman [b,c], Pamela J. Welz [c,*]

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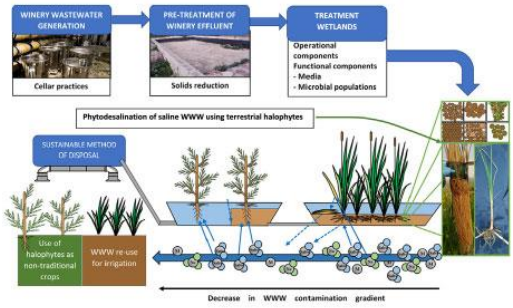
HIGHLIGHTS

- Review of WWW remediation by TWs relative to bio-hydrogeochemical components
- Salinity impacts inorganic remediation by TWs and wastewater reuse.
- Review presents case for novel application of terrestrial halophytes.
- Halophytes are viable alternatives to macrophytes for saline wastewater treatment.
- Review describes solutions to challenges impacting sustainability of wine industry.

GRAPHICAL ABSTRACT



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The composition and concentration of contaminants present in winery wastewater fluctuate through space and time, presenting a challenge for traditional remediation methods. Bio-hydrogeochemical engineered systems, such as treatment wetlands, have been demonstrated to effectively reduce contaminant loads prior to disposal or reuse of the effluent. This review identifies and details the status quo and challenges associated with (i) the characteristics of winery wastewater, and the (ii) functional components, (iii) operational parameters, and (iv) performance of treatment wetlands for remediation of winery effluent. Potential solutions to challenges associated with these aspects are presented, based on the latest literature. A particular emphasis has been placed on the phytoremediation of winery wastewater, and the rationale for selection of plant species for niche bioremediatory roles. This is attributed to previously reported low-to-negative removal percentages of persistent contaminants, such as salts and heavy metals that may be present in winery wastewater. A case for the inclusion of selected terrestrial halophytes in treatment wetlands and in areas irrigated using winery effluent is discussed. These are plant species that have an elevated ability to accumulate, cross-tolerate and potentially remove a range of persistent contaminants from winery effluent via various phytotechnologies (e.g., phytodesalination).

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Contents

1. Introduction

The wine industry contributes significantly to the global economy, with a steady increase in the trade value of this product of 25.8 to 31.4 billion € between 2014 and 2018, and a production volume ranging between 2.49 and 2.92×10^{10} L over the same period (OIV, 2019). Concurrent with wine-making, the industry generates large amounts of potentially hazardous solid and liquid 'wastes' from various cellar activities (Bolzonella et al., 2019; Bordiga et al., 2019). In the move towards a circular economy, a biorefinery approach has been adopted in many countries for the valorization of grape pomace and other forms of solid residues (Holtman et al., 2018; Khan et al., 2015). However, apart from the beneficial use of winery wastewater (WWW) of adequate quality for crop irrigation (i.e. beneficial irrigation), it is still largely viewed as a form of waste rather than a resource (Holtman et al., 2018). Consumer pressure is driving wineries to adopt best practice measures in cellars, including those that simultaneously reduce the quantity and mitigate the toxicity of WWW (Conradie et al., 2014). Cellars can reduce potable water usage and the amount of solids in the effluent by introducing best available technologies and cleaner production methods (e.g. re-using treated effluent for basic cleaning activities and installing filters to retain solids) (Bolzonella et al., 2019; Welz et al., 2016). Despite these efforts, WWW generally requires some form of primary physicochemical and/or secondary treatment in order to improve the quality to comply with legislated standards before discharge.

The effluent produced by wineries contains a range of organic and inorganic constituents, with considerable inter- and intra-site qualitative and quantitative variability (Bolzonella et al., 2019; Welz et al., 2016; Howell and Myburgh, 2018). Although the volume of WWW differs for each winery, studies conducted between 2003 and 2016 provide a rough estimate of 2.86 L (average) of effluent generated from each L of wine produced (Bolzonella et al., 2019; Mosse et al., 2011; Vlyssides et al., 2005), translating to a global total of approximately 7.5×10^{10} L per annum.

The extent and type of treatment that is required depends on the means of discharge (i.e. directly to sensitive environments, subsurface discharge, beneficial irrigation, or discharge to municipal sewage systems) (Bolzonella et al., 2019; Holtman et al., 2018; Johnson and Mehrvar, 2020). Multiple factors need to be considered when opting for any particular WWW treatment system. These include the (i) volume and site-specific character of the effluent that is generated, (ii) availability and value of land, (iii) size of the winery, (iv) availability of skilled operators, and (iv) capital and operational costs (Bolzonella et al., 2019; Howell and Myburgh, 2018).

For smaller wineries that have sufficient land available, but do not have the skills, time, or capital to install and operate more sophisticated systems, treatment wetlands (TWs) are a viable option. Data is available for systems that have been piloted or operated at full scale for WWW remediation in a number of countries, including Greece (Akratos et al., 2020), Portugal (Calheiros et al., 2018), Spain (Flores et al., 2019; Serrano et al., 2011), Italy (Rizzo et al., 2020), France (Kim et al., 2014), the USA (Grismer et al., 2003; Shepherd et al., 2001a; Shepherd et al., 2001b), Mexico (Grismer and Shepherd, 2011), South Africa (Holtman et al., 2018; Mulidzi, 2010), and Canada (Johnson and Mehrvar, 2020; Rozema et al., 2016).

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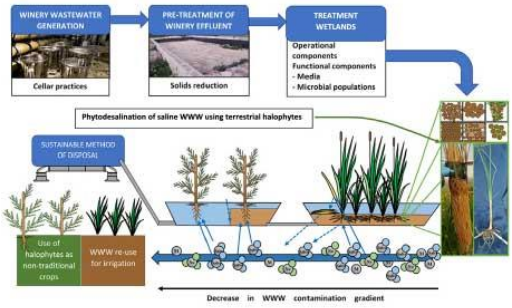

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Table 1
Seasonal composition of winery effluent (adapted from (Flores et al., 2019)[a] (Welz et al., 2016)[b] (Buelow et al., 2015)[c] (Malandra et al., 2003)[d]).

Parameter	Crush			Non-crush		
	Minima	Maxima	Averages	Minima	Maxima	Averages
pH	4[a] 4[b] 4[c] 4[d]	7[a] 12[b] 13[c] 5[d]	–	7[a] 2[b] 4[c]	8[a] 12[b] 11[c]	–
EC ($mS \cdot cm^{-1}$)	0.37[c]	9.7[c]	1.3[c]	0.14[c]	3.3[c]	0.97[c]
TSS ($mg \cdot L^{-1}$)	523[a]	2190[a]	1237[a]	<200[a] 267[b]	<1000[a] 21,697[b]	2382[b]
COD ($mgO_2 \cdot L^{-1}$)	1031[a] 54[b] 320[d]	16,825[a] 13,900[b] 5760[d]	7684[a] 5951[b] 3449[d]	<500[a] 28[b]	<2000[a] 76,900[b]	5489[b]
BOD_5 ($mgO_2 \cdot L^{-1}$)	650[a] 6[c]	10,300[a] 15,400[c]	3542[a] 1790[c]	<250[a] 4[c]	<1000[a] 4100[c]	1390[c]
VFA ($mg \cdot L^{-1}$)	7[b] 1[d]	308[b] 799[d]	123[b] 216[d]	0[b]	11,254[b]	830[b]
Ethanol ($mg \cdot L^{-1}$)	0[b]	1240[b]	338[b]	0[b]	21,000[b]	1693[b]
Sugars (GFM$mg \cdot L^{-1}$)	0[b] 0[d]	1474[b] 4940[d]	195[b] 2006[d]	0[b]	733[b]	33[b]
TPhen. ($mg \cdot L^{-1}$)	5[b] 0[d]	86[b] 27[d]	46[b] 9[d]	3[b]	273[b]	50[b]
Sodium ($mg \cdot L^{-1}$)	8.6[b] 7.8[c]	105[b] 3060[c]	31[b] 137[c]	5.9[b] 5.4[c]	574[b] 714[c]	61[b] 87[c]
Potassium ($mg \cdot L^{-1}$)	5.8[b] 3.2[c]	387[b] 772[c]	187[b] 176[c]	1.7[b] 1.3[c]	393[b] 1270[c]	162[b] 133[c]
TN ($mg \cdot L^{-1}$)	9.7[a] 0.7[b]	109[a] 34[b]	43[a] 10[b]	<20[a] 0.4[b]	<100[a] 176[b]	14[b]
TP ($mg \cdot L^{-1}$)	1.5[a] 0.4[b]	188[a] 31[b]	6.9[a] 11[b]	<10[a] 0.5[b]	<50[a] 280[b]	19[b]
COD:N	–	–	146:1[a] 595:1[b]	–	–	392:1[b]
COD:P	–	–	912:1[a] 541:1[b]	–	–	289:1[b]

Data from studies using respectively unknown [a], 168[b], 360[c], and 9[d] samples from 6[a], 4[b], 18[c], and 9[d] wineries.
EC = electrical conductivity TSS = total suspended solids COD = chemical oxygen demand BOD_5 = five-day biological oxygen demand VFA = (short chain) volatile fatty acids GFM = glucose + frutose + maltose TPhen. = total phenolics SAR = sodium adsorption ratio TN = total nitrogen TP = total phosphorous.

The composition and concentrations of organics, which may include alcohols, organic acids, sugars and phenolics, are dependent on the seasonal wine-making processes: for example, ethanol constitutes the highest fraction in the non-crush period, while significant concentrations of grape sugars are generally only produced during periods of crushing (Welz et al., 2016; Malandra et al., 2003). In terms of amenability to secondary remediation technologies, the organics range from being highly biodegradable (e.g. sugars) to more slowly biodegradable or even recalcitrant (e.g. phenolics) (Welz et al., 2016; Mutabaruka et al., 2007). Phenolics also differ in their degree of toxicity to microbes and plants, a factor that requires consideration, particularly for WWW treatment options such as planted TWs (Arienzo et al., 2009; Chen et al., 2009).

In general, if the ratio of COD to nitrogen to phosphorus (COD:N:P) in WWW (Table 1) is higher than 100:5:1, wastewater may be supplemented with nutrients to enhance the biodegradation of organics for efficient secondary wastewater treatment (Welz et al., 2016; O'Flaherty and Gray, 2013). The WWW may also be treated concurrently with domestic wastewater (Ganesh et al., 2010), but the potential introduction of pathogens, such as multi-drug resistant bacteria, may render the effluent unsuitable for land application.

Inorganics in WWW emanate from (i) cleaning and sanitizing agent/s [including sodium hydroxide (NaOH), potassium hydroxide (KOH), sodium metasilicate (Na_2SiO_3), trisodium phosphate (Na_3PO4), sodium carbonate (Na_2CO_3), phosphoric acid (H_3PO_4) quaternary ammonium compounds, peracetic acid, hydrogen peroxide (H_2O_2), and sulfur (S)], (ii) filtration and clarification aids (e.g. diatomaceous earth, bentonite clay, perlite), and (iii) grapes (Conradie et al., 2014; Mosse et al., 2011; Lucas et al., 2010; Versari et al., 2014). Grape residues and juice can contribute appreciably to the potassium (K^+) concentration whereas the use of cleaning and sanitizing products are the major contributing factors to the introduction of sodium (Na^+) in WWW as NaOH is still the agent of choice for many wineries due to its effectiveness and low cost (Conradie et al., 2014; Mosse et al., 2011). Salts are not removed during secondary treatment processes, and monovalent cations, particularly Na^+, can lead to soil degradation (via increasing soil sodicity) if the WWW is used for irrigation purposes (Rengasamy and Marchuk, 2011). Best practice therefore advocates the use of K-based, or ideally, organic cleaning and sanitizing products (Rengasamy and Marchuk, 2011).

3. Operation and performance

A literature search (SCOPUS) using the keywords "winery, wastewater, effluent, treatment, wetland, constructed" was used to identify studies pertaining to treatment of synthetic and/or real winery effluent in treatment wetlands. Selected data obtained from all the studies that assessed and monitored the physical set-up, operation, and performance of TWs for the remediation of WWW are provided in Tables 2 and 3. Data may be cross-referenced between these tables using the numbers in the left hand (ID) column. Additional information for these studies is provided in Table S1.

3.1. Lack of data and experimental flaws

It is difficult to holistically evaluate which of the existing TW systems are the most effective options for remediation of WWW by comparing the published data. While it is not possible to standardize factors such as the character of WWW or climatic variables, other fundamental data required to allow meaningful comparisons between systems are often omitted from manuscripts (Table 2). These include the functional surface area (FSA), functional depth, hydraulic loading rate (HLR), organic loading rates (OLR), and measured flow rates (MFR). These can exhibit notable variations, for example, the data available for the HRT range from 1.8 to 24 days. In addition, although the FSA is an important parameter for calculating the spatial footprint of a TW, the standard use of this parameter to describe the HLR in TWs should ideally be augmented by the use of the functional volume (FV), which is more informative when performing inter- and intra-study comparisons of systems containing, for example, different types of media. This is because the saturation level (depth) also varies between systems, in this instance from 0.3 to 1.2 m (Table 2). For example, if a shallow system is compared with a deep system using only the FSA to calculate the HLR and/or OLR, it will give a skewed picture of the contribution of the media to the overall remediation potential of the studied TW (Holtman et al., 2018).

In order to account for the random variability inherent in biological systems, experimental replication is required to ensure the statistical validity of studies comparing different factors (Casler et al., 2015). Replication is more feasible at laboratory-scale, but most studies reported in literature have been conducted using full-scale TWs (Table 3). For TWs treating WWW, a limited number of laboratory studies have been used to compare the presence/absence of plants (Akratos et al., 2020; Grismer and Shepherd, 2011) and/or media (Skornia et al., 2020). However, in all but one instance (Grismer and Shepherd, 2011), experiments were not replicated. This compromises the reproducibility and practical application of studied TWs as the 'snapshot' of the TW's remediation efficacy may be an anomaly compared with a standardized and expected outcome. Going forward, this highlights the need to consider performing well-replicated laboratory studies to improve the performance of TWs treating WWW.

Table 2
Selected operational and performance data of studies pertaining to treatment wetlands used to remediate winery wastewater.

ID	FSA (m^2)** [Depth (m)]	HRT [D] (days)	HLR – FSA** ($mm \cdot day^{-1}$)	HLR – FV** ($L \cdot m^{-3} \cdot day^{-1}$)	COD_{in} ($mg\ L^{-1}$)	OLR - FSA ($g\ COD \cdot m^{2} \cdot d^{-1}$)	OLR - FV ($g\ COD \cdot m^{3} \cdot d^{-1}$)	ORR (%)	MFR ($m^3 \cdot day^{-1}$)	Ref
1	0.48 [0.35]	2-4	UTC	UTC	788-2985	UTC	UTC	81-92	NG	(Akratos et al., 2020)
2	1.2 [0.6]	NG	UTC	UTC	1258	UTC	UTC	NG	NG	(Calheiros et al., 2018)
3	350* [0.3-1.4]	NG	20 ± 7 (VF&HF) 13-25 ($HF_{1,2,3}$)	14 (VF) 43-82 (HF_1) 22-41 ($HF_{2,3}$)	1558 ± 1023	30.4 ± 19.3 (all) 213 (VF) 16.2 ($HF_{1,2,3}$)	152* (VF) 54* (HF_1) 27* ($HF_{2,3}$)	71*	5.2	(De la Varga et al., 2013a; De la Varga et al., 2013b)
4	350* [0.3-1.4]	3	77-215 (VF) 13-36 ($HSSF_{1,2,3}$)	55-154* (VF) 43–120* (HF_1) 22–60* ($HF_{2,3}$)	NG	30.4 (all) 43–466 (VF) 3.6–55 ($HF_{1,2,3}$)	31–333* (VF) 12–183* (HF_1) 6.0–92* ($HF_{2,3}$)	73 (all) 29–70 (VF) 23–79 (HF)	NG	(Serrano et al., 2011)
5	60 [1–1.2]	NG	25.7 (VF&HF)	25.7 VF* 15.4 HF*	1665 VF NG (HF)	162 VF 65 HF	162.0 VF* 39.2 HF*	47–96 VF 7* HF	1.28 ± 1.18	(Flores et al., 2019; Pascual et al., 2021)
6	14.9 [0.9]	9.7	34	28*	993–4720	35–164*	37–176*	97–99	0.5	(Shepherd et al., 2001a; Shepherd et al., 2001b; Grismer et al., 2001)
7	4400 [1.2]	5.5 [10]	31*	26*	7406 ± 2090 (C) 1721 ± 439 (NC)	120–270	100–225*	49 (C) 79 (NC)	137	(Grismer et al., 2003)
8	304 [1.2]	[5]	UTC	UTC	290	553	465*	98	137	(Grismer et al., 2003)
9	NG	2.5–5.0	UTC	UTC	1183	UTC	UTC	49–70	NG	(Grismer and Shepherd, 2011)
10	120* [0.9]	6 ± 1.6 [14]	UTC	UTC	72,965 ± 29,066	UTC	UTC	94–97	NG	(Grismer and Shepherd, 2011)
11	144* [0.9]	18–24 [14]	UTC	UTC	5080 ± 1211 (C)	UTC	UTC	98–99	NG	(Grismer and Shepherd, 2011)
12	7.3* [NG]	1.8	109*	150 (12−313)	1138	110*	152 (23–469)	79 (28–98)	0.41	(Holtman et al., 2018; Welz et al., 2018a)
13	75–164 [1.2]	NG	119–160 (all) 357–480 (HF_1)	99–133*	NG	NG	UTC	>98	10–23	(Johnson and Mehrvar, 2020)
14	190–404 [1.2]	NG	39–53 (all) 133–164 (HF_1)	33–44*	NG	NG	UTC	>98	10–17 (C)	(Johnson and Mehrvar, 2020)
15	NG	3–5	38 (all) 76 (HF_1)	32*	NG	NG	UTC	>98	< 10.0	(Johnson and Mehrvar, 2020)
16	1330* [0.7]	NG	26	18*	4045	329	230*	98	35	(Masi et al., 2002)
17	752* [0.7]	NG	23	16*	1003	236	165*	93	10	(Masi et al., 2002)
18	215 [0.7]	NG	37	26*	722	352	246*	88	8	(Masi et al., 2002)
19	3034* [0.4–0.85]	[2.5]	60–80	71–94* (VF)	1159 ± 432 (VF)	160–230 (D)	188–271*	98 (all) 70 (VF)	61 ± 28 (max. 118)	(Rizzo et al., 2020)
20	1.63 [0.6]	9.6	31* (SA)	51*	171 ± 14 184 ± 11	5	8.8* 9.4*	69–93 86–93	0.050	(Mena et al., 2009)
21	180 [0.9]	1) 14 2) 7	1) 23* 2) 45*	1) 25* 2) 50*	1) 14000 2) NG	1) 315* 2) NG	1)350* 2) NG	1) 77–80 (NC) 1) 83–88 (C) 2) 60	1) 4.1 2) 8.1	(Mulidzi, 2010; Mulidzi, 2007)
22	404 [0.4–0.8]	NG	22.3	56* ($VF_{1,2,4}$) 28* (VF_3)	3043 (C) 2117 (NC)	34	85*($VF_{1,2,4}$) 43*	99	7.3* (C) 11* (NC)	(Rozema et al., 2016)
23	NA [0.75]	14	6.9	9.2*	4997–6189	5.18	6.9*	>99	NG	(Skornia et al., 2020)

FWS = free water surface. FSA = functional surface area D = design FV = functional volume HRT = hydraulic retention time HLR = hydraulic loading rate COD_{in} = influent chemical oxygen demand OLR = organic loading rate ORR = organic removal rate MFR = measured flow rate UTC = unable to calculate (from literature data) NG = not given C = crush season NC = non-crush season.

* Calculated from literature data

** FWS data not included.

Table 3
Selected operational data and details of functional components of treatment wetlands treating winery wastewater (plants excluded).

ID	Study type & duration	Wine/effluent production per annum	Wastewater type/discharge	Pre-treatment	Configuration & mode	Media			HC ($mm \cdot s^{-1}$)	Ref.
						Material	Diameter (mm)	Porosity (%)		
1	Lab study 2 years	NA	Diluted WWW NA	PS → TF	HF	Igneous fine gravel Bulgarian zeolite	6 (D_{50}) 4 (D_{50})	35 25	–	(Akratos et al., 2020)
2	Pilot study 2 weeks	6000 bottles	WWW NG	–	HF	Cork stoppers	3–7	44	–	(Calheiros et al., 2018)
3	Full-scale 2 years	315 m^3	WWW & DWW Municipal sewer	UASB	VF → $HF_{1,2,3}$	Granitic gravel Gravel	8–16 (T,B) 3–6 (M) 6–12	NG 40	- 1.5–1.6	(De la Varga et al., 2013a; De la Varga et al., 2013b)
4	Full-scale 2 years	315 m^3	WWW & DWW Municipal sewer	UASB	VF → $HF_{1,2,3}$	Granitic gravel Gravel	8–16 (T,B) 3–6 (M) 6–12	NG 40	- -	(Serrano et al., 2011)
5	Pilot 2 years	368 m^3/ 1398 m^3	WWW Recirculated	Homogenization tank HUSB	VF → HF →	Granitic gravel Sand Gravel	6–12 (B), 2–4 (M) 1–2 (T) 6–12	NG	–	(Flores et al., 2019; Pascual et al., 2021)
6	Pilot study 2 years	18,200 m^3	WWW NG	Sand pre-filter	HF	Pea gravel	4.7 (D_{50})	36	6.0	(Shepherd et al., 2001a; Shepherd et al., 2001b; Grismer et al., 2001)
7	Full-scale 1 year	Moderately sized winery	WWW Irrigation	Settling pond	HF	Pea gravel	4.0	NG	–	(Grismer et al., 2003)
8	Full-scale 1 year	Small winery	WWW Irrigation	Settling pond	HF	Rock	NG	NG	–	(Grismer et al., 2003)
9	Lab study 30 days	NA	WWW NA	NA	HF & FWS	Volcanic rock	40	50	–	(Grismer and Shepherd, 2011)
10	Full-scale NG	14,000 cases	WWW Irrigation	Septic tank	HF	Pea gravel	<8	NG	–	(Grismer and Shepherd, 2011)
11	Full-scale NG	14,000 cases	WWW Irrigation	Septic tank	HF	Pea gravel	<8	NG	–	(Grismer and Shepherd, 2011)
12	Pilot 3 years	Small winery	WWW Irrigation	Primary settling →balancing tank	HF	Dune sand	0.4 (D_{50}) 0.2–1.0	29	0.04–0.20	(Holtman et al., 2018; Welz et al., 2018a)
13	Full-scale (4) NG	NG	WWW & sewage Sub-surface	Septic tank →AFFR	HF_1 (anoxic) $HF_{2,3}$ (aerobic)	Wood chips Gravel & sand	NG NG	NG NG	–	(Johnson and Mehrvar, 2020)
14	Full-scale (3) NG	NG	WWW & sewage Sub-surface	Septic tank →AFFR	$HF_{1,2,4}$ (aerobic) HF_3 (anoxic)	Gravel & sand Wood chips				(Johnson and Mehrvar, 2020)
15	Full-scale (20) NG	NG	WWW Sub-surface	Septic tank	$HF_{1,2}$	Gravel & sand				(Johnson and Mehrvar, 2020)
16	Full-scale 1 year	NG	WWW Irrigation	Imhoff tank	HF → FWS	Gravel	5–10	NG	–	(Masi et al., 2002)
17	Full-scale 1 year	NG	WWW & DWW Irrigation	Imhoff tank	$VF_{1,2}$ → HF → FWS → pond	Gravel & sand Gravel (HSSF)	8–40 (T-B) 8–12	NG	–	(Masi et al., 2002)
18	Full-scale 1 year	NG	WWW Water body	Imhoff tank	HF	Gravel	5–10	NG	–	(Masi et al., 2002)
19	Full-scale 2 years	Bottling and aging only	WWW (no crush) River	Equalisation tank	VF → HF → FWS	Gravel NG	2–40 (T-B)	NG 35 (HSSF)	- 5.8	(Rizzo et al., 2020)
20	Pilot 5 months	NG	SWW	NA	HF	Gravel	6–9	NG	–	(Mena et al., 2009)
21	Pilot 2 years	NG	WWW Cabbage irrigation	NG	HF	Dolomitic gravel	20–30	35	–	(Mulidzi, 2010; Mulidzi, 2007)
22	Full-scale 6 years	NG	WWW & DWW Subsurface	Dosing tank	$VF_{1,2,3}$ VF_4 (anoxic)	Gravel Wood chips Peat moss (T) 0.3 m	5–10 NG	NG NG	–	(Rozema et al., 2016)
23	Lab column 6 months	Lab study	WWW NA	Primary settling	VF replicates	Gravel Clinopatilolite Tyre chips Oyster shells	6.4 1.2–2.4 10–15 1.2–2.4	NG	NG	(Skornia et al., 2020)

HC = hydraulic conductivity PS = primary settling TF = trickling filter HF = horizontal (subsurface) flow WWW = winery wastewater NA = not applicable NG = not given VF = vertical (subsurface) flow FWS = free water surface UASB = upflow anaerobic sludge blanket reactor AFFR = anaerobic fixed film reactor T = top M = middle B = bottom DWW = domestic wastewater HUSB = hydraulic upflow sludge blanket reactor FWS = free water surface flow SWW = synthetic winery wastewater.

It has been suggested that because of the relatively large spatial footprint of TWs, they are not suited to large wineries that produce copious amounts of effluent, particularly if land values are high. However, the connection between the size of a winery (small, medium, large) and the capacity of a TW system is difficult to establish. Ideally, the volume of effluent should be measured. For a number of reasons, this is practically difficult, which is evidenced by the fact that the effluent volume was only provided in one of the twenty studies included in this review (Table 3). Wineries are alternatively described by the amounts of grapes crushed or wine produced (bottles/cases or volume), but it is difficult to define the size of a winery based on these criteria as some wineries do not bottle, while others do not crush.

3.2. Performance evaluation

Most studies have focused on the universal objectives of removal/reduction of organics and solids from WWW, and pH neutralization (Tables 2 and 3). For reference, the influent COD (COD_{in}) and OLR in terms of FSA and FV, as well as the organic removal rates (ORR) are given in Table 2. These results are not discussed further because the organic removal performances of most of the TWs described in this manuscript have already been adequately reviewed (Johnson and Mehrvar, 2020; Masi et al., 2015). In summary, the authors of these reviews concluded that TWs are able to reduce the organic load sufficiently, provided that the HRT is sufficient to maintain the OLR within design parameters, and that pre-treatment for solids reduction is satisfactory. In addition, TWs have proven to be effective at reducing the total suspended solids (TSS) concentration, increasing the pH of acidic WWW, and decreasing the pH of alkaline WWW (Holtman et al., 2018; Masi et al., 2015; De la Varga et al., 2013a). The pH buffering mechanism/s of WWW in TWs has not yet been elucidated.

Due to potential environmental toxicity, removal of (poly)phenolics from WWW in TWs is an important consideration. However, studies typically limit analysis of organic removal to COD and/or BOD removal, and to our knowledge there are only four studies describing removal of phenolics or tannins from WWW in TWs or simulated TWs. Grismer et al. (2003) found higher removal rates during the non-crush season (average 78%; influent = 55 ± 16 mg·L^{-1}) compared with the crush season (average 46%; influent 55 ± 22 mg·L^{-1}). The reduced removal rates correlated with a decreased HRT during the crush season. In another system operated with a more consistent HRT, no seasonal trend in phenolic removal rates was noted, and an average removal rate of 77% (range 5.1–44 mg·L^{-1}) was obtained. Other studies were conducted using WW with low phenolic concentrations: WWW mixed with domestic WW (9.7 ± 3.0 mg·L^{-1}) and synthetic WWW (13 mg·L^{-1}) (De la Varga et al., 2013b; Mena et al., 2009). Studies have also been conducted using other forms of agri-industrial wastewaters at moderate influent phenolic concentrations. Although the plant-based phenolic profiles would be expected to differ from those of WWW, these studies can still provide some valuable insights. In gravel-based horizontal flow systems, (Rossman et al. (2012) found that pre-aeration and inclusion of rye grass increased the removal of total phenolics from coffee processing wastewater from 54% to 72%, while Gomes et al. (2018) found that phenolic removal rates decreased when loads in cork boiling effluent exceeded 0.4 mg L^{-1}·day^{-1}, and that some phenolic molecules were more readily removed than others.

Typically, less importance is placed on the removal of inorganics in biological systems treating WWW (Marchuk and Rengasamy, 2010). The need to remove nutrients and other inorganic elements and molecules from WWW is incumbent on local legislative requirements, place/type of discharge, and WWW character, and is therefore site-specific (Johnson and Mehrvar, 2020; Flores et al., 2019; De la Varga et al., 2013a; De la Varga et al., 2013b). If the effluent is discharged into a sensitive aquatic environment or municipal sewer where nutrient discharge limits are set, then removal mechanisms such as nitrification/denitrification will be important and must be considered. Conversely, the essential plant nutrients, namely N and P, may be seen as such if the effluent is to be re-used for irrigation (Flores et al., 2019). In the case where treated WWW is utilized for irrigation purposes, it will be important to limit the Na^+ concentration and sodium adsorption ratio (SAR) within permissible limits to prevent soil sodicity and salinization, and associated loss of soil structure (Marchuk and Rengasamy, 2010).

It has been clearly demonstrated that N removal takes place over the long-term operation of full-scale TWs treating WWW. However, as with the character of WWW itself, removal rates vary from site to site and seasonally. Masi et al. (2015) reported an elevated average total nitrogen (TN) removal in three full scale systems with influent concentrations of 15 mg·L^{-1}, 27 mg·L^{-1}, and 65 mg·L^{-1}, and respective removal rates of 81%, 90% and 58%. As with tannins, Grismer et al. (2003) found lower nitrate (NO_3^-) removal rates in the crush compared with non-crush seasons (17% vs 73%, respectively), which could be attributed to reduced HRT during the crush season. Similarly, the authors also reported lower average NH_4^+ removal rates in samples taken during the crush period (average 29% of 37 mg·L^{-1} v/s 62% of 118 mg·L^{-1}). Apart from the shorter HRT during the crush season, diminished nitrification may have been exacerbated by a more unfavorable C:N ratio (200:1 v/s 15:1), as nitrification rates decrease with increasing C:N ratios in TWs (Lai et al., 2020). De la Varga et al. (2013a) reported low NH_4^+ removal rates (≤ 29%) in a system treating combined WWW and domestic WW with influent concentrations of 0.6 to 74 mg·L^{-1} in Europe. In a Canadian system designed to treat the combination of WWW and domestic WW by enhancing nitrification-denitrification processes within the TW, elevated NH_4^+ removal rates (>99%) were achieved during the warmer half of the year (6-month season) for three years but decreased to as low as 19% thereafter. The removal rates were consistently >99% for the rest of the year. This result appears to be anomaly, as given the cold winter climate in Canada, it would be expected that nitrification would decrease during this period. The result may be attributable to low sampling frequencies ($n = 2$ per season) that may not have presented a true reflection of overall performance for each season, along with low influent concentrations, particularly in the colder seasons (average 2.2 mg·L^{-1} and 0.9 mg·L^{-1} in the warmer and colder seasons, respectively). Grismer et al. (2003) also measured removal of sulfur (S) species, and found 95% removal of sulfates (SO_4^{2-}) and 78% removal of sulfites (SO_3^{2-}) in a HF gravel-based system with influent concentrations of 35 ± 19 mg·L^{-1} and 0.56 ± 0.20 mg·L^{-1}, respectively.

TWs do not appear to effectively remove cations from WWW. For example, Mulidzi (2010) found marginal, but erratic removal of Na^+ (1–43%, average 12%; influent 101–282 mg·L^{-1}; $n = 23$) and K^+ (1–43%, average 8%; influent 141–615 mg·L^{-1}; $n = 23$) in HF gravel-based systems containing plants (*Typha, Scirpus* and *Phragmites* spp.). Removal was attributed to plant uptake, which is supported by the fact that no removal of Na^+, K^+, or magnesium (Mg^{2+}) was found by Holtman et al. (2018) in an unplanted sand-based WWW treatment system. The selection of certain plant species with the ability to remove elevated concentrations of such contaminants may therefore be utilized in TWs to improve the quality of WWW used for irrigation and is further discussed in Section 4.3.

For interested readers, more detailed data on specific (poly)phenolic and inorganic concentrations and removal efficiencies in TWs, treating WWW, are included in Table S1. The biotic and abiotic mechanisms, present in TWs, for removal of important WWW organics and inorganics are described later in this manuscript.

4. Functional significance and interactions of major biotic and abiotic components

4.1. Background

As alluded to previously, the performance of TWs treating WWW has been reviewed by Masi et al. (2015) in 2015. However, in order to

continually improve the efficiency of these systems, there is a need to critically analyse the current fundamental knowledge pertaining to the types of abiotic substrate (media) and plants, as well the microbial community structure and function which may be utilized in these TWs. The selection of plants is particularly important because of the potential phytotoxicity of WWW (Arienzo et al., 2009).

To address this, the relevance and characteristics of (i) the media, microbes, and media-microbe interactions, and (ii) the plants, and plant-media and plant-microbe interactions, are discussed generically, and as they specifically pertain to the treatment of WWW in the following two sub-sections.

4.2. Media and microbial populations

4.2.1. Types of media used in winery wastewater treatment wetlands

Various types of gravel, with diameters ranging from 3 to 40 mm, have historically been the preferred media used in TWs remediating WWW. Sand has also been extensively used, in combination with or without gravel (Table 2). Other substrates have also been used to achieve particular removal functions. For example, Johnson and Mehrvar (2020) used wood chips as a carbon (C) source for denitrification of WWW mixed with sewage, Calheiros et al. (2018) used cork for its general adsorptive capacity, while Skornia et al. (2020) added oyster shells as a buffering agent in a laboratory study.

4.2.2. Biotic and abiotic removal mechanisms

The primary biotic removal mechanisms in TWs are microbial biodegradation, biotransformation, and bioprecipitation (Table 4), and a series of different phytoremediatory mechanisms (Table 4, Section 4.3). These are accompanied by physicochemical (abiotic) mechanisms, most notably adsorption and precipitation (Marchuk and Rengasamy, 2010; Lai et al., 2020). Degradation of organic molecules can also be facilitated by physicochemical catalysis. For example, the oxidation of phenolic acids may be coupled to the reduction of iron and/or manganese present in the substrate particles (Polubesova et al., 2010; Welz et al., 2012).

Table 4
Phyto- and biotechnologies, used as tools for the biological remediation of various contaminants present in winery wastewater (WWW).

Bio- and phytotechnology	Description	Mechanism(s)
Microbial biotechnologies		
Bioprecipitation	Contaminants are precipitated out of solution by various mechanisms including the release of complexing agents by microorganisms, rendering the contaminant biologically inert.	Bioprecipitation of contaminants by reduction-oxidation (redox) reactions, intracellular assimilation, complexation, sorption, release of complexing agents (e.g. ligands), and biosorption to membranes
Microbial biodegradation	Bacteria release extracellular biodegradation enzymes that breakdown contaminants resulting in the release of water-soluble intermediates, CO_2, H_2O, and other metabolites.	Enzymatic biodegradation and mineralization. Extracellular biodegradation enzymes are released by bacteria to break down contaminants. Degradation products (water-soluble intermediates) are released into the environment and/or assimilated into bacterial cells. Bacteria (present within the biofilm) attach to the surface of the contaminant resulting in contaminant degradation and the release of CO_2, H_2O and other metabolic products.
Biomethylation	Transformation of contaminants by microbes (aerobic and anaerobic bacteria, and fungi) into volatile derivatives and can be removed from the TW system via evaporation.	Enzymatic transfer of methyl groups to metals (e.g. As, Hg, Pb, Se, and Sn). For example, microbes may methylate Hg (forming methylmercury, CH_3Hg) which accounts for one of the removal pathways from TWs. It must be noted that the volatile derivative, e.g. CH_3Hg, may be more toxic due to its lipophilic nature and therefore, its bioavailability for plant and animal uptake.
Plant-assisted biotechnologies		
Rhizodegradation	Plant-assisted bioremediation involving the complex interplay between plant roots, plant exudates, rhizosphere chemistry, and microbe species/communities to render contaminants biologically inert via microbial degradation processes.	Plant roots provide surface for microbe colonization. Plants stimulate the growth of microbial species/communities (via release of exudates such as organic acids), enhancing microbial bioremediation pathways (e.g. biodegradation and bioprecipitation).
Phytotechnologies		
Rhizofiltration	Sorption (ad- and absorption), bioconcentration, and precipitation of contaminants from wastewater by plant roots of aquatic and terrestrial plant species.	Phytoextraction and (hyper)accumulation of contaminants (e.g. heavy metals) in roots.
Phytodesalination	Use of halophytic plant species to remove salts, and/or their constituents, from contaminated land or water.	Halophytes hypertolerate (and cross-tolerate) and accumulate elevated concentrations of salts, and their constituents, due to their effective ion homeostasis networks [strict and integrated cross-talk between genetic (e.g. upregulation of similar genes), molecular (e.g. reactive oxygen species, ROS), cellular (e.g. compartmentalization of contaminants), and physiological (e.g. contaminant secretion from specialized glands on the surface of leaves) mechanisms].
Phytostabilization	Establishing plant cover to physically and chemically stabilize soil contaminants thereby mitigating soil and aeolian erosion, and contaminant leaching.	Release of exudates into the rhizosphere to chelate metals which may subsequently be ad/absorbed by plant roots and/or sequestered into the plant's rhizosphere (phytosequestration).
Phytoextraction	Use of plants to sequester (phytosequestration), take up, and accumulate elevated concentrations of contaminants *in planta*.	Once contaminants have been sequestered (via the release of chelating compounds) and taken up by plant roots, the plant may utilize avoidance (root-to-shoot restriction/shoot-to-root re-translocation) and/or tolerance (such as excretion from leaves, contaminant complexation, and translocation of contaminants to storage sites such as vacuoles) strategies to render toxic contaminants biologically inert. The number, and efficiency, of these strategies enable elevated concentrations of contaminants to be accumulated by particular plant species.
Phytohydraulics	Use of plants to intercept and prevent the horizontal migration of contaminants within surface and groundwater (including phytofiltration, and more specifically rhizofiltration).	Rhizosphere reverses the hydraulic gradient forming zone of stagnation which 'captures' contaminants thereby mitigating the transport of contaminants downstream (surface water) and within groundwater.

References: (Truu et al., 2019; Yan et al., 2020; Truu et al., 2015; Flowers and Colmer, 2015; Farzi et al., 2017; Lee and Yang, 2010; Saddhe et al., 2020; Chaudhry et al., 2005; Limmer and Burken, 2016; LeDuc and Terry, 2005; Dushenkov et al., 1995; Manousaki et al., 2008; McGrath and Zhao, 2003; Mendez and Maier, 2008; Pérez-Esteban et al., 2014; Sander and Ericsson, 1998)

Although adsorption and precipitation may be the primary removal mechanisms for some molecules and elements, there is a danger that these may later desorb or re-mineralize due to changes in physicochemical conditions, and/or the saturation of substrate (media) binding sites. In cases where concurrent biotic and abiotic removal takes place, good long-term removal rates may still be achieved, as demonstrated with the phenolics viz - gallic acid and vanillin present in synthetic WWW (Welz et al., 2012). This combined biotic/abiotic approach was also used by Skornia et al. (2020), where media was supplemented with clinopatilolite to adsorb ammonium ions (NH_4^+) (average 13.7 $mg \cdot L^{-1}$) and tyre chips to adsorb NO_3^- (average 4.2 $mg \cdot L^{-1}$) from WWW in gravel columns. The rationale for the experiment was that in colder climates where nitrification rates are low in winter, these forms of N could be adsorbed, and then mineralized to nitrogen gas (N_2) by nitrification-denitrification with the advent of warmer weather. While the approach had merit, the short-term study failed to show significant differences in removal rates between the amended columns and the gravel controls in this instance. In laboratory TW simulations testing systems containing different plants and media, Akratos et al. showed increases in NH_4^+ removal rates from domestic wastewater (Akratos and Tsihrintzis, 2007) and WWW (Akratos et al., 2020). In the latter, rates increased from 30% to 57% to 78% in unreplicated laboratory HF systems containing gravel, gravel and plants, and zeolite and plants, respectively. The authors attributed the increased nitrification to the presence of plants and greater NH_4^+ adsorption of zeolite when compared to igneous gravel.

In contrast to N, where primary removal in TWs is microbially mediated (nitrification-denitrification), and discounting removal by plants (discussed in Section 4.3), removal of P is abiotic (adsorption and/or precipitation) (Lai et al., 2020). Adsorption rates are dependent on the chemistry and morphology of the substrate particles, and can be increased by using highly reactive media such as apatite or steel slag (Akratos et al., 2020; Akratos and Tsihrintzis, 2007; Dell'Osbel et al., 2020; Korkusuz et al., 2005; Zhang et al., 2007). Although many studies have looked at P removal rates in gravel and/or sand filled TWs, in reality, unless significant plant uptake occurs and the plants are subsequently harvested, negative P removal rates may occur at some point due to plant senescence, saturation of binding sites and/or desorption or mineralization due to changes in physicochemistry (e.g. pH, redox, increased sulfate concentrations) (Maine et al., 2007; Dell'Osbel et al., 2020). While Masi et al. (2002) reported excellent total P removal rates over one (1) year in two (2) full scale systems in Europe: average 73% (influent 4.9 $mg \cdot L^{-1}$, $n = 5$) and average 94% (influent 1.9 $mg \cdot L^{-1}$, $n = 10$), Rozema et al. (2016) measured negative P "removal" after 5 years of operation (range: −115% to >99% $n = 23$) in a full-scale TW remediating WWW combined with domestic WW. Over the first 3 years of operation, >99% removal was achieved, suggesting that the primary reason for the decreased performance was saturation of P binding sites. It is therefore recommended that if P removal is seen as a priority, and in order to recapture this limited resource, specific downstream removal processes are applied. A promising example is provided by Skornia et al. (2020), whom used a proprietary iron-oxide based commercial P adsorbent (sponge) as a polishing step to precipitate and recover P from WWW. Such technologies that support a circular economy could be viable options going forward and should be investigated further.

4.2.3. Biofilm, solids and hydraulic conductivity

The main advantage of using sand instead of gravel in TWs is that the particles provide a larger surface area for biofilm attachment and surface chemistry (Akratos et al., 2020; Akratos and Tsihrintzis, 2007; Torrens et al., 2009). The major comparative disadvantage is the increased risk of clogging because of the smaller matrix pores in the sand milieu (Knowles et al., 2011; Pedescoll et al., 2011). The term 'clogging' in TWs is applied when the hydraulic conductivity (HC) decreases to the extent that the system can no longer function effectively. The risk of clogging can be ameliorated by the (i) popular use of WWW pretreatment (Table 2) to remove suspended solids, (ii) intermittent operation to allow solids and excess biofilm degradation during the resting period, and/or (iii) application of low organic loading rates such as effluent recycling (Torrens et al., 2009; Knowles et al., 2011; Prochaska et al., 2007; Tao et al., 2007).

While clogging with non-biodegradable solids can negatively impact the long-term operation of TWs, it should be noted that some critical decrease in the HC is expected to occur after start up due to the gradual, and necessary build-up of a functional biofilm (Truu et al., 2009; Welz et al., 2018a). Operational HC values of 1.5 $mm \cdot s^{-1}$ (De la Varga et al., 2013a), 5.8 $mm \cdot s^{-1}$ (Rizzo et al., 2020) and 6.0 $mm \cdot s^{-1}$ have been reported in gravel-based systems, and values ranging from 0.04–0.20 $mm \cdot s^{-1}$ (calculated from flow rates measured in-situ) were reported in a sand-based system (Welz et al., 2018a). It has been shown that predicted HC measurements based on grain size distribution may be inaccurate due to most models assuming particle sphericity (Welz et al., 2018a; Grismer et al., 2001). For example, Grismer et al. (2001) found an order of magnitude difference in the HC of poorly graded pea gravel between predicted values and column experiment measurements (6.0 $mm \cdot s^{-1}$).

Temporal changes in the HC of TWs treating WWW are rarely described in literature, making comparisons between systems difficult. Akratos et al. (2020) reported a 15% loss in porosity over 2 years in a HF system containing igneous fine gravel and Bulgarian zeolite, however the HC was not measured. De la Varga et al. (2013a) studied the accumulation of solids in a gravel-based system and found that, although the accumulated TSS and volatile suspended solids (VSS) increased annually to reach maximum values of 8.6 ± 3.0 kg and 1.01 ± 0.78 $kg \cdot m^2$, respectively after 2.8 years of operation, the average reduction in HC (1.64 $mm \cdot s^{-1}$ to 1.49 $mm \cdot s^{-1}$) measured by the falling head method was insignificant over three measurement periods (1.6, 2.2, and 2.8 years of operation). In contrast, reductions in HC of up to 50% have been measured in sand-based treatment systems treating WWW (Welz et al., 2018a), highlighting the need to monitor the HC in studies where sand is used as a physical substrate. If HC reductions are factored into the design capacity, and realistic flow rates can still be achieved, the use of sand as a medium may still be a viable option (Welz et al., 2018a). Furthermore, it has been shown that biofilm-related decreases in HC are inversely related to the organic loading rate, and are reversible (Tao et al., 2007; Welz et al., 2018a). Similarly, clogging due to accumulation of organic sludge formed during the crush season is also degraded during the non-crush period, restoring the HC (Masi et al., 2015; Masi et al., 2002).

4.2.4. Microbial community structure and function

The microbial community structure and function in CWs is influenced by the plant species, climatic variables, and physicochemical variables related to the substrate, mode of operation and type of wastewater (Ramond et al., 2012; Ramond et al., 2013a; Truu et al., 2019). In TWs, sedimentary and epiphytic bacteria, as well as planktonic bacteria (in FWS systems) are principally responsible for nitrification, denitrification, SO_4^{2-} oxidation/reduction and hydrocarbon degradation, as well as transformation and mineralization, which follow natural principles for the biogeochemical cycling of C, N and sulfur (S). Studies suggest that it takes around 100 days from start-up for the microbial communities to become established (equilibrated) in TWs (Ramond et al., 2012; Truu et al., 2019; Weber, 2016; Oopkaup et al., 2016). This is an important factor that is not always considered by researchers. In literature reports on TWs treating WWW, full-scale studies have been conducted over ≥2 years. However, the results of many lab- and pilot-scale experiments may not provide an accurate assessment of the remediation potential of the systems because the duration of the experiments was too short to allow effective establishment of the functional microbial communities (Table 3).

It has been shown that under the same climatic conditions and influent characteristics, even small physicochemical differences in the substrate play a significant selective role on the microbial community structure in unplanted TWs (biological sand filters) (Welz et al., 2014a). Even in particles with similar geochemistry, the shape of sand particles can affect biofilm attachment/abundance and consequent C and N removal performance; for instance, it has been demonstrated that natural sand presents a superior biofilm attachment surface to crushed sand (Torrens et al., 2009). Significant differences in the microbial community structure have also been noted in systems containing the same substrate, but with different WWW influent (Ramond et al., 2013a; De Beer et al., 2018; Ramond et al., 2013b). For example, in high rate biological contact reactors treating WWW and operated in series, De Beer et al. (2018) found that the higher pH and lower organic load achieved in the (identical) second reactor allowed the preferential selection of different bacterial and fungal species to the first reactor, including nitrifying and denitrifying bacteria. Similarly, different microbial communities are selected within spatial niches of TWs, with redox status and influent degradation gradients thought to be the primary selective drivers (Ramond et al., 2013a; Ramond et al., 2013b; Welz et al., 2014b). In biological sand filters, and, by inference, TWs receiving high C:N WWW, nutrient limitation may be naturally mitigated by selection of N-fixing bacteria such as *Azotobacter* spp. (Ramond et al., 2013a; Welz et al., 2018b). These results are supported by a recent study by Ospina-Betancourth et al. (2020), whom enriched high C:N effluent with *Azotobacter vinelandii* and achieved appreciable rates of N-fixation. The authors proposed the use of the treated waste as a high N organic fertilizer, which is potentially a novel option for beneficiation of WWW which supports circular economy principles.

4.3. Plants, and plant-microbe plant-media interactions

Phytoremediation (plant-based) and microbial bioremediation (e.g. bacteria- and fungi-based) processes in wetland systems have been demonstrated to effectively remediate a range of inorganic and organic contaminants present in wastewater originating from different sources (e.g. acid mine drainage, sewage, aquaculture, and to a lesser degree, WWW) (Muthusaravanan et al., 2018). Plants and/or microbes, used as tools for phyto- and biotechnologies, have the ability to degrade, take up, transform (e.g. methylate), volatilize, and/or stabilize (i.e. immobilize) contaminants (Table 4) (see Yan et al., 2020 for review). However, various factors, including plant, microbe, and plant-microbe interactions (e.g. plant-assisted bioremediation), physicochemical properties of WWW (e.g. bioavailability, octanol-water partition coefficient, competitive ion adsorption), and environmental factors (viz – pH, redox potential (E_h), salinity, and temperature/solar radiation) influence the efficacy of these remediation processes (Yan et al., 2020; Truu et al., 2015).

4.3.1. Plant factors

Plant species, as well as their genotypes, differ in their ability to remove inorganic and organic contaminants. This is attributed to the number, type, and efficacy of genetic, molecular, cellular, and physiological mechanisms, as well as the interplay between these mechanisms (Manara et al., 2020; Sytar et al., 2020). Phytotoxic concentrations of contaminants present in WWW have been demonstrated to impede plant growth and survival (Nagajyoti et al., 2010). However, as plants are sessile organisms, they have developed various strategies to adapt to abiotic and biotic stresses (Manara et al., 2020). This enables certain plant species with elevated phytoremediation potentials to be utilized in TWs to remediate targeted contaminants. Plants selected for TWs must possess elevated (i) growth rates, (ii) biomass production (especially well-developed root systems), (iii) degree of stress cross-tolerance, and (iv) ability to render targeted contaminants biologically inert via the extraction, volatilization, or sequestration of contaminants (Liang et al., 2017; Jesus et al., 2018). Based on these criteria, commonly selected plant species for TWs include *Phragmites australis* (common Reed), *Typha* spp. (cattails), and *Cyperus* spp. (sedges) (Table 5).

Unlike inorganic contaminants present in WWW, plants can metabolize organic contaminants. The metabolism of organics involves the transformation (e.g. redox reactions which alters the species of contaminant), conjugation (e.g. complexation with other molecules such as low-molecular weight thiols), compartmentalization (e.g. storage of contaminants in vacuoles), and/or volatilization (via evapotranspiration processes) of contaminants (Yan et al., 2020; Tripathi et al., 2020). These metabolic processes may render toxic organics biologically inert. Various studies have investigated the role of plants in TWs, for the removal of organic constituents from WWW, especially COD (*refer to Section 2; see Table S1 for extensive list of studies investigating the remediation of organics, present in WWW using TWs*).

Although numerous inorganic constituents, including salts and their constituents (e.g. Na^+, Cl^-, and heavy metals), have been investigated (Table S1), TWs are generally ineffective in remediating the inorganic fraction of WWW due to its inhibitory effect on biological processes. For example, Na^+ removal efficiency in wetland system ranges from −78 to 43%, of which Na^+ and other salt constituents (e.g. Cl^-) are considered as the most persistent and challenging contaminants to remove from wastewater (Johnson and Mehrvar, 2020; Akratos et al., 2020; Masi et al., 2015; De la Varga et al., 2013a). The removal of other inorganics, namely N and P, is also mediated by wastewater salinity, where the removal efficiency significantly decreases as salinity increases (Wu et al., 2008). This highlights the need to desalinate wastewater in order to effectively reduce the remaining inorganic fraction present in WWW (Li et al., 2018). Moreover, the presence of these inorganics at elevated, phytotoxic concentrations generally requires expensive forms of treatment, viz. - dilution or physicochemical treatment methods (e.g. electrodialysis, reverse osmosis, and/or ion exchange treatments), creating other environmental challenges such as the production of a concentrated brine requiring disposal. Furthermore, these treatment methods have various disadvantages which include the method's low resistance to fluctuating contaminant loading, inhibiting effect of salts on the microbial component present in biological reactors, and inability to effectively treat non-point source pollution (Liang et al., 2017).

The concentrations and composition of inorganics present in WWW, which fluctuates through space (e.g. geographic locations) and time (e.g. according to seasonal cellar activities), creates a selective pressure to utilize cross-tolerant plant species with an elevated ability to remove targeted inorganics. Moreover, WWW must be treated before its disposal as per regulatory guidelines where legislation on permissible inorganic concentrations for disposal differ between countries.

The use of halophytic plants for the remediation of the inorganic fraction of WWW presents an inexpensive and effective alternative to the use of dilution and physicochemical treatment methods. Halophytes are generally characterized as plants which can complete their life cycles in saline environments where salt concentration is greater than 200 mM NaCl (Flowers and Colmer, 2015; Flowers et al., 1986). The use of halophytes has gained increasing attention over the past decades due to their elevated ability to cross-tolerate a range of stresses (Manousaki and Kalogerakis, 2011; Nikalje and Suprasanna, 2018). Elevated Na^+ and Cl^- concentrations in saline soils induce osmotic and ionic stresses in plants – leading to secondary stresses (Fig. 1). Halophytes have evolved various mechanisms to tolerate salinity stress, and are associated with the controlled uptake and assimilation of Na^+, K^+, and Cl^- (Flowers and Colmer, 2008; Dassanayake and Larkin, 2017). These tolerance mechanisms (described below) enable halophytes to maintain homeostasis, preventing the toxic build-up of Na^+ ions, and potential antagonistic interactions between Na^+ and K^+ due to these cations sharing similar physicochemical properties (Wakeel, 2013; Flowers et al., 2019). Although Na^+ is preferentially taken up over K^+ by plants, the elevated capacity of halophytes to accumulate elevated concentrations of these persistent inorganics (consequently

Table 5
Commonly selected plant species for use in TW for the remediation of organic and inorganic contaminants present in winery wastewater (WWW).

Plant species	Reason for selection for WWW remediation	References
Phragmites australis	- Fast growth rate and high biomass productivity (ranges from 0.413 to 9.890 kg dry mass·m^{-2} per annum); - Tolerant to elevated organic and nutrient levels (determined thresholds include COD: 14000 mg·L^{-1}, TKN: 506 mg·L^{-1}, and TP: 95 mg·L^{-1}); - Elevated rate of evapotranspiration (e.g. implications for WWW dewatering); - Flood, salinity-, and metal-tolerant (e.g. Fe^{2+}, Zn^{2+}, and Ni^{2+}) and effectively removes certain metals such as chromium (Cr^{2+}); - Tolerant to range of pH levels (e.g. used for remediation of acid mine drainage and industrial effluent); - Cosmopolitan species with a widespread distribution (i.e. the ability to growth and survive in a range of environmental conditions) - Extensive root system	(Ayyappan et al., 2016; Bonanno and Giudice, 2010; Kumari and Tripathi, 2015; Peruzzi et al., 2009; Vymazal and Kröpfelová, 2011)
Typha spp. (including *T. latifolia*)	- Fast growth rate and high biomass productivity (exceeds 5.00 kg dry mass·m^{-2} per annum); - Tolerant to a range of abiotic stresses (salinity, organic matter, heavy metals, and nutrients); - Effectively remove suspended solids; - Effectively removes Cu^{2+} and Cd^{2+} and is more effective at removing certain contaminants (such as P, Na^{+}, Ca^{2+}, Mg^{2+}, Cu^{2+} and Fe^{2+}), compared with *P. australis;* - Tolerant to a range of pH levels; - Widespread distribution and colonizes anthropogenically-affected habitats.	(Maine et al., 2006; Sukumaran, 2013; Vymazal, 2020; Zingelwa and Wooldridge, 2009; Hadad et al., 2018)
Cyperus spp. (e.g. *C. alternifolius*)	- Fast-growing plant species with a dense root system and is easily propagated; - Utilized in TWs for remediation of organics, pathogens, nutrients, and heavy metals (e.g. Al^{3+}, Cd^{2+}, Cu^{2+}, Pb^{2+}, and Zn^{2+}); - Demonstrated to effectively reduce COD, BOD, TSS, NO_3^-, NH_3, PO_4^{3-}, as well as total coliforms and fecal coliforms.	(Sepúlveda et al., 2020; Cheng et al., 2002; Cui et al., 2009; García-Ávila et al., 2019)

removing these cations from saline WWW) indicates a potential application of halophytes for use in TWs. Moreover, heavy metal hyperaccumulation has been a long reported phenomenon where plants accumulate elevated concentrations of a particular metal *in planta* above a threshold (e.g. 1000 mg/kg in leaves for nickel (Ni) - see Van der Ent et al. (2013) for review). However, the hyperaccumulation threshold for other elements, such as Na^{+}, has not been defined. A study, conducted by Levinsh et al. (2021), investigated the accumulation of Na^{+} in leaves by 102 plant taxa across 77 sampling sites. Based on leaf Na^{+} concentrations, the "Na^{+}-hyperaccumulation threshold" was set at 18–30 g/kg dry mass. It must however be noted that further research on the hyperaccumulation threshold of Na^{+} (as well as elements other than heavy metals) must be conducted to validate these thresholds. This further suggests the potential application of halophytes for use in TWs for the removal of persistent inorganics, namely Na^{+} and K^{+}, from saline WWW.

Halophytes may be divided into different categories based on the mode of salt transport, storage, and potential excretion. These categories include recretohalophytes [divided into *exo-* (excrete excess salts from specialized salt glands) and *endo*-recretohalophytes (store salts in glands)], euhalophytes (compartmentalize salts into leaf/stem vacuoles), and pseudohalophytes (accumulate salts in the vacuoles of the wall or parenchyma of xylem present in roots) (Dassanayake and Larkin, 2017; Deinlein et al., 2014; Litalien and Zeeb, 2020). Halophytes can be further characterized by their dependence on salt (i.e. facultative vs obligate halophytes) and the environments they inhabit (e.g. hydrohalophytes, xero-halophytes). Halophytes may tolerate a range of inorganic contaminants by effectively detoxifying metal ions via heavy metal exclusion (i.e. selective restriction of the uptake and translocation of metals by roots), excretion (i.e. phytoexcretion, where salt glands excrete excess ions thereby contributing to the maintenance of the plant's metal homeostasis network), and/or accumulation (i.e. compartmentalization of metal ions into vacuoles) (Nikalje and Suprasanna, 2018; Christofilopoulos et al., 2016; Wei et al., 2020). These halophytic characteristics have important implications for the removal of inorganics as the use of *exo*-recretohalophytes may re-introduce extracted contaminants into the TW water body, resulting in negative inorganic removal percentages. For example, the presence of salt glands in the photosynthetic plant parts of some *exo*-recretohalophytes are used to detoxify accumulated metal ions where excess salts (and heavy metal constituents) are excreted onto the surface of the leaves (Wei et al., 2020; Sookbirsingh et al., 2010). However, exo-recretohalophytes may also desalinate environments by haloconduction - a process whereby salt excreted onto the leaf surface is mobilized by wind and deposited onto surrounding areas (Yensen and Biel, 2008; Yun et al., 2019). The deposition of salts away from the contaminated site may enable the dispersal of salts over large areas, diluting the impact of salt accumulation within the receiving environment (Yun et al., 2019). Moreover, the dispersal of salts (where some salts are considered macro- and micronutrients) at low concentrations over large areas may improve the nutrient content of salt-deficient soils (Yun et al., 2019). Although this provides an additional application of terrestrial exo-recretohalophytes, this phytotechnology is a relatively novel concept requiring further investigation. The implications for treatment of WWW in TWs is that during rainfall events secreted salts may be washed off plant leaves and subsequently re-introduced into the WWW, consequently increasing the concentration of salts in WWW, resulting in negative removal efficiencies (Wei et al., 2020; Yun et al., 2019). Another important implication was demonstrated by Matinzadeh et al. (2019) where euhalophytes accumulated elevated concentrations of Na^{+} compared with K^{+}. This was attributed to the role of Na^{+} in mediating the plant's ion homeostasis network whereas elevated concentrations of K^{+} were accumulated by facultative and pseudo-halophytes (essential macronutrient) thereby differing in their ability to accumulate salts. This study elucidates the importance of identifying viable halophyte candidates with desirable halophytic traits for targeted contaminant removal in TWs. Moreover, the composition and concentration of contaminants present in WWW (refer to Table 1) must be characterized prior to the selection of halophytic plant species.

In many countries, the main means of disposal of treated WWW is via land irrigation, presenting one of the major environmental challenges associated with the wine production industry (Howell and Myburgh, 2018). Land irrigation with WWW containing inorganics

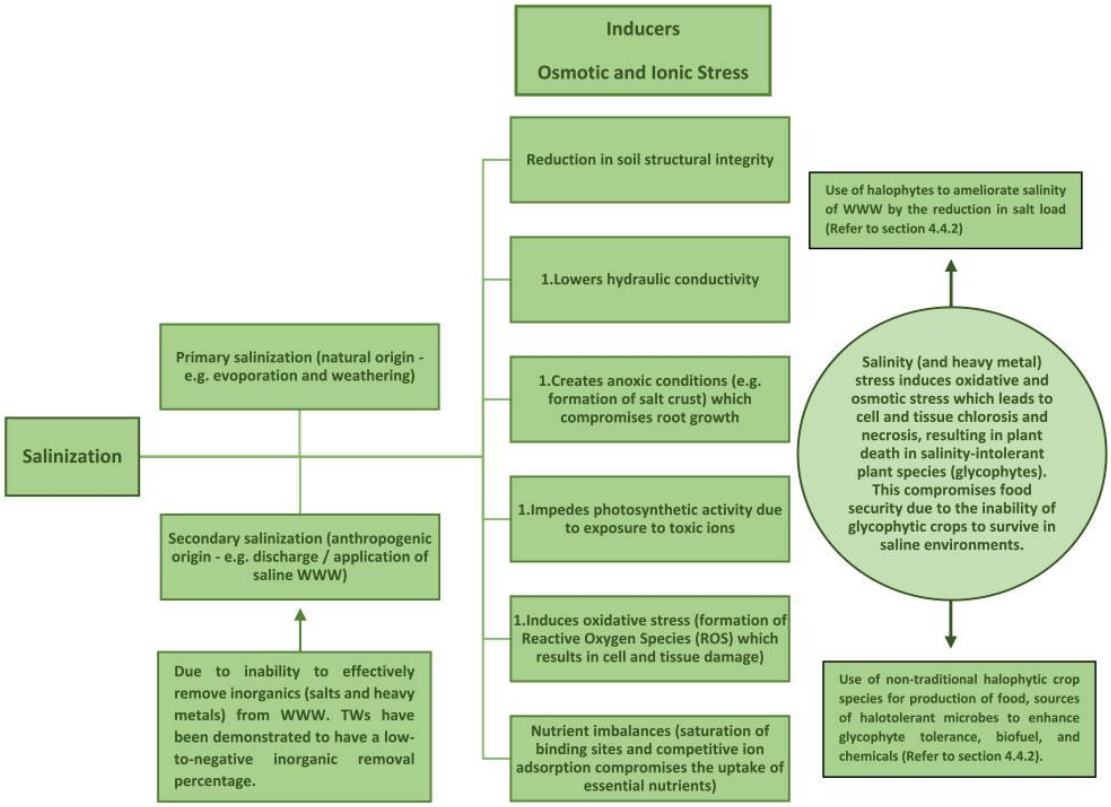

Fig. 1. Pathways of salt (and heavy metal) phytotoxicity. Phytotoxic effects of salinity on plant growth and survival, where salinity stress may induce the combination of ionic and osmotic stress leading to secondary stresses.
References: (Buelow et al., 2015; Litalien and Zeeb, 2020; Acosta et al., 2011; Butcher et al., 2016).

may result in land salinization, increase in soil sodicity, potential eutrophication of receiving water bodies, and assimilation of other inorganics (e.g. heavy metals) in the soil (Howell and Myburgh, 2018; Liang et al., 2021; Mulidzi et al., 2020).

Therefore, to mitigate the negative impacts associated with disposal practices, after secondary treatment, WWW must be effectively managed with a focus on the removal of salts, and their constituents. The management of WWW includes, but is not limited to, (i) treatment of saline WWW by phytodesalination before its disposal (Table 4), and/or (ii) irrigation of cash crops tolerant of saline WWW (i.e. beneficial irrigation). These management options must be considered as they directly impact socioeconomic development (e.g. food security) and the receiving environment. To implement either of these management strategies, halophytes with an elevated degree of cross-tolerance and extraction potential should be utilized in TWs to reduce salt load, as well as their constituents, present in WWW.

4.3.2. Phytoremediation of saline winery wastewater before disposal

Although numerous studies have investigated the phytoremediation of saline and heavy metal contaminated land by halophytes, limited research has been conducted on the phytodesalination and phytoextraction of inorganics present in wastewater originating from various sources (Khanlarian et al., 2020; Mujeeb et al., 2020; Wiszniewska et al., 2019). To the knowledge of the authors, studies investigating the phytoremediation potential of halophytes have been restricted to studies on wastewater originating from aquaculture (Lymbery et al., 2013; Diaz et al., 2020)] where salt concentrations have been reported in the range of 17,000 - 46,000 mg NaCl/L), domestic (e.g. (Fountoulakis et al., 2017)), tannery (e.g. salt concentrations up to 80,000 mg·L^{-1}) (Ayyappan et al., 2016; Calheiros et al., 2007; Ashraf et al., 2020), tool factory (Maine et al., 2007), oil production (Stefanakis, 2020), and textile (Saeed and Sun, 2013) saline wastewater. The wine, aquaculture, and other agricultural industries generate relatively similar key contaminants (namely organics, salts, N, and P) compared with the mining, tannery, textile, and petrochemical industries – comprised of relatively complex contaminants and solution chemistry (Liang et al., 2017; Stefanakis, 2020; Zhao et al., 2020). Although, the concentration and composition of these inorganics differ within and between industries, limited studies have investigated the use of halophytes for the treatment of saline wastewater originating from various industries. The majority of these studies have been restricted to the use of hydrohalophytes, such as *P. australis* (Nawaz et al., 2020) and *Typha* spp. (Meitei and Prasad, 2021) which cross-tolerate saline and heavy metal stresses under flooded conditions (which they naturally inhabit) and have therefore received notable attention for their use in TWs.

Halophytes have been reported to effectively (hyper)accumulate a range of inorganics including salts and their constituents (Na^+, K^+, Mg^{2+}, Cl^-, NaCl, and SO_4^{2-}), as well as heavy metals (e.g. cadmium (Cd^{2+}), copper (Cu^{2+}), lead (Pb^{2+}), and zinc (Zn^{2+}) – ecotoxicologically important heavy metals present in WWW from soil and water (Khanlarian et al., 2020; Mujeeb et al., 2020; Wiszniewska et al., 2019). For example, Fountoulakis et al. (Fountoulakis et al., 2017) demonstrated the ability of the terrestrial halophyte, *Atriplex halimus*, to effectively remove elevated salt load from TWs along with high biomass production compared with the *Juncus acutus* and *Sarcocornia perennis*. This highlights the possible role of halophytes, and the need to select certain halophytes with an elevated ability to remediate and cross-tolerate a range of inorganics present in WWW.

4.3.2.1. The novel application of terrestrial halophytes. Flooding in combination with salinity stress are common environmental variables. To survive, plant species must tolerate the combination of these stresses (and secondary stresses such as the reduction in uptake and translocation of nutrients, such as K^+, to aboveground biomass) where the inability to do so results in plant mortality (Sairam et al., 2008). Although tolerance to the combination of these stresses typically involves adventitious root production (maintaining an internal O_2 content), halophytes possess the ability to effectively maintain their ion homeostasis network - regulating shoot ion concentrations independent of anoxic environmental conditions (Colmer and Flowers, 2008). The ability of some halophytes to cross-tolerate these abiotic stresses is elucidated by the elevated productivity of salt marshes (Kelleway et al., 2017). Although no link has been provided, various studies have indicated the ability of terrestrial halophytes to cross-tolerate these stresses. For example, Farzi et al. (2017) demonstrated the use of three terrestrial halophytes, namely *Salicornia europaea, Salsola crassa,* and *Bienertia cycloptera*, in a TW under a salinity dose-dependent experiment. These halophytes completed their life cycles under TW conditions and reduced measured elemental parameters to permissible levels. Aquatic plant species have typically been investigated for their rhizofiltration potential (i.e. the sorption (adsorption/absorption), concentration, and precipitation (onto the root surface) of contaminants from wastewater by plant roots), suggesting the possible role of terrestrial plant species, and halophytes in particular, for rhizofiltration and selection for use in TWs. A study conducted by Lee and Yang (2010) demonstrated that the terrestrial plant, *Helianthus annuus* (sunflower), effectively removed 80% of the uranium (U) present in a hydroponic solution via rhizofiltration. Moreover, terrestrial xerophytes and halophytes (such as *Atriplex halimus* and *Bassia indica*) have been successfully propagated in TWs (Fountoulakis et al., 2017; Shelef et al., 2013). Another study demonstrated that terrestrial halophytes from the genus *Salicornia* possess aerenchyma within their roots, a flooding-tolerance mechanism present in hydrophytes, promoting redox conditions within the rhizosphere. This creates microenvironments conducive to nitrification/denitrification processes within TW systems (Brix, 1994; Haberl et al., 1995; Faulwetter et al., 2009). Furthermore, the growth, survival, and phytoremediation potential of terrestrial halophytes may be enhanced through the control of TW operational and functional components. For example, substrate-less TWs, such as floating (FTW, utilizing larger, emergent hydrophytes planted in a buoyant mat) or vertical up-flow (VUF) TW, modifies the plant's root system architecture (RSA). This may enhance the halophyte's rhizofiltration potential by increasing root growth and root surface for contaminant sorption, concentration, and/or precipitation, biofilm development (see Section 4.3.3), and/or root-contaminant contact time as roots are directly exposed to contaminants present within the WWW (Fig. 2) (Colares et al., 2020; Afzal et al., 2019; Saddhe et al., 2020). As terrestrial halophytes have been demonstrated to grow under TW conditions, the phytoremediation potential of terrestrial halophytes can therefore be enhanced by modifying TW design.

The management of WWW is a major sustainability challenge within the wine industry. Although TWs generate multi-purpose by-products (e.g., harvesting leaf biomass for biofuel or fertilizer, re-use of treated WWW for irrigation, etc.), WWW is still largely viewed as a waste product (Liu et al., 2012). Moreover, global climate change is expected to negatively impact the quality and quantity of treated WWW available for re-use (Bolzonella et al., 2019; Welz et al., 2016). This predicted impact on water scarcity is also expected to intensify the reliance on re-using treated wastewater – forming a key role in sustainable water management practices (Vo et al., 2014). Thus, WWW sustainability challenges within the wine industry must be addressed in the context of global climate change.

Various climatic drivers (e.g., temperature, evapotranspiration (ET), and wind) place a selective pressure on plants and microbes that can tolerate various hazards associated with the combination of the effects of these drivers, such as environmental salinization (Merloni et al., 2018; Zscheischler et al., 2018). Macrophytes, traditionally used in TWs, possess a low water use efficiency. These inefficient water users have an elevated water loss potential due to elevated ET processes, processes which are expected to be exacerbated by climate change (Headley et al., 2012). Elevated water loss potential directly impacts the quality (i.e., concentration of contaminants within the TW system) and quantity (due to change in hydrologic regime, decreasing the volumetric flow of WWW passing through the system) of treated wastewater available for reuse (Headley et al., 2012; Białowiec et al., 2007). Thus, it is envisaged that climate change is likely to decrease the sustainability and performance of traditional biological treatment systems utilizing salinity-intolerant macrophytes (Vo et al., 2014).

This sustainability challenge may be addressed by the selection of efficient water use plant species and/or decreasing the hydraulic retention time to minimize ET (Freedman et al., 2014). Some terrestrial halophytes possess the ability to cross-tolerate a combination of environmental conditions, such as salt and drought stresses (Nikalje et al., 2019). This is attributed to various adaptations including physiological processes such as the (i) density, size, and location of stomata (small pores regulating gaseous exchange), (ii) waxed epidermal layer in leaves (reducing transpiration water loss from the surface of the leaves), and/or (iii) small-sized leaves/scales (reduction in surface area) which result in reduced transpiration and thus, water loss (Shabala, 2013; Belkheiri and Mulas, 2013; Akcin et al., 2017). This further strengthens the case for utilizing terrestrial halophytes in the treatment of saline WWW, where these adaptations promote water use efficiency by halophytes while simultaneously tolerating a range of abiotic stresses affected by climatic drivers.

Thus, the criteria for selecting terrestrial halophytes in TWs for saline WWW treatment should be expanded to include the selection of species which (i) are easily propagated, (ii) are able to grow and survive under TW conditions, (iii) are able to (hyper)accumulate salts and their constituents (Na^+), (iv) are efficient water users with low ET, (v) have desirable modes of salt transport, accumulation and/or excretion (i.e., haloconduction), and (vi) simultaneously contribute to the removal of other inorganics such as N and/or P (Grismer et al., 2003). Thus, the potential use of terrestrial halophytes should be incorporated as an additional plant selection criterion in future studies for the treatment of saline wastewater by TWs, contributing to sustainable wastewater resource management within the wine industry (Knight et al., 2019).

This review highlights a gap in knowledge and proposes the novel, non-traditional use of terrestrial halophytes (which have been extensively studied for their phytoremediation potential in highly saline and heavy metal-contaminated terrestrial environments) for the remediation of saline WWW. Ultimately, the reduction of WWW salt load, along with other targeted inorganics, may reduce the negative impacts associated with the irrigation of land using treated WWW. This would ameliorate the negative impacts associated with the salinization and increase in soil sodicity – advancing the survivable conditions of numerous glycophytic plant species, including crops, and promoting food security in semi- and arid environments (Hayat et al., 2020). Solutions to these challenges have been described above and illustrated in Fig. 2, a process diagram presenting potential solutions to challenges negatively impacting the TW remediation process and the sustainability of the wine industry.

4.3.2.2. Irrigation of cash crops tolerant of saline winery wastewater. Globally, secondary salinization by irrigation has contributed to the salinization of approximately 20% of total cropland, which is predicted to increase to 50% by 2050 (Nachshon, 2018; Singh, 2021). Two themes to improve crop production under saline conditions have been researched, namely the (i) introduction of salt tolerance traits into glycophytic crops (i.e. genetic engineering), and (ii) domestication of halophytes as non-traditional crops (Mishra and Tanna, 2017; Nikalje

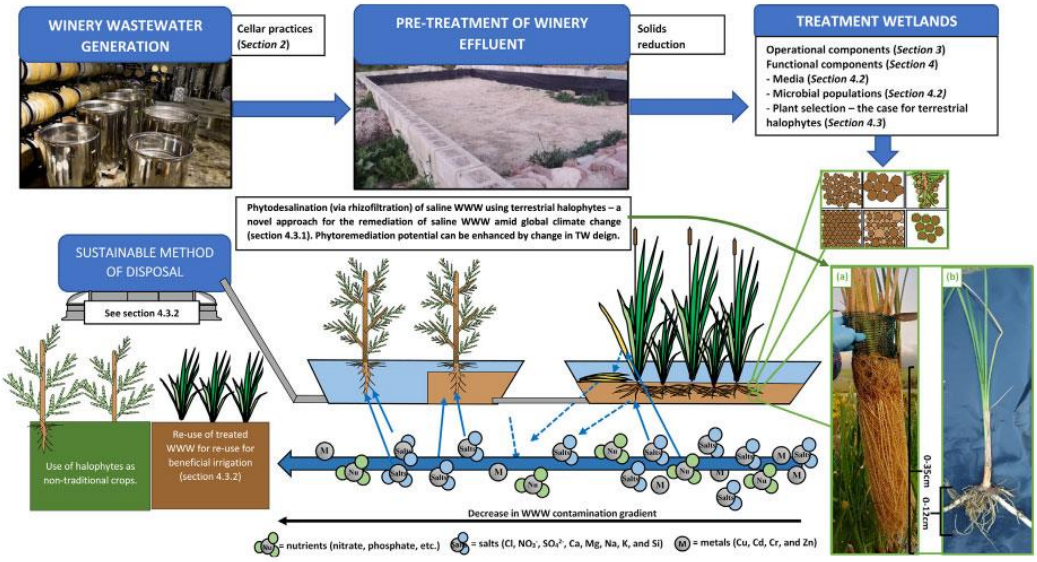

Fig. 2. Schematic depicting the contents of this review.

et al., 2018). The use of halophytes for the non-traditional production of food (e.g. *Chenopodium quinoa* (quinoa)), proposed sources of halotolerant microorganisms (to increase tolerance of glycophytic crops), biofuels, and chemicals in arid areas (i.e. saline water with poor soil quality and high solar radiation) has received notable attention in the last few decades (Debez et al., 2017; Etesami and Beattie, 2018). Halophytes can therefore be grown in non-arable land under saline WWW irrigation without competing for nutrients and resources required by glycophytic crop species, ultimately promoting food security in arid and semi-arid areas where climatic drivers (e.g. evapotranspiration and extreme temperatures) are expected to increase amid global climate change (Fisher et al., 2017).

The practical implications of selecting halophytes for use in phytoremediation or as a cash crop must consider the origin of the plant species. For example, *Eichhornia crassipes* (water hyacinth), a halophyte originating from South America, has been demonstrated to tolerate highly saline wastewater and accumulate various heavy metals and nutrients (Auchterlonie et al., 2021). However, *E. crassipes* is considered one of the world's most prevalent invasive aquatic plant species, negatively impacting socioeconomic development and the ecosystems they inhabit (Enyew et al., 2020). This has led to the legislated prohibition (e.g. South African National Environmental Management: Biodiversity Act – Alien Invasive Species Regulations, Act 10 of 2004) of growing and utilizing alien invasive plant species, such as *E. crassipes*, in phytoremediation trials or for recreational purposes. This highlights the need to select indigenous plant species, where the plant's potential spread outside of the TW would not negatively impact the receiving environment. It must be noted that *P. australis* is considered a cosmopolitan species with an elevated genetic diversity and phenotypic variation (Table 5) (Eller et al., 2017). This suggests that *P. australis* rapidly adapts to various geographic regions (i.e. different environmental conditions) across the world, making the case to use *P. australis* as a model plant species to investigate the remediation potential of TWs across geographical ranges.

4.3.3. Plant-assisted bioremediation (plant-microbe interactions)

Plant-microbe interactions can enhance the remediation of inorganic and organic contaminants in wetland systems. The use of plants, in combination with rhizosphere and/or endophytic bacteria enhances the remediation of organic and inorganic contaminants compared with using phytoremediation in isolation. Plant roots provide (i) a surface for microbial adhesion, (ii) protection of microbial communities from environmental conditions, such as desiccation, (iii) a C source for microbial growth and activity (i.e. release of exudates, such as organic acids, sugars, and amino acids), and (iv) aerobic microenvironments for aerobic (heterotrophic and autotrophic) microbial activities – where plants release O_2 into the rhizosphere via aerenchyma adventitious roots (Gagnon et al., 2007; Hussain et al., 2018; Abdullah et al., 2020). In turn, microbes associated with the rhizosphere promote plant growth and survival by increasing the bioavailability of nutrients, such as P and N, for plant uptake, N fixation, protection against plant pathogens, as well as promoting resistance (i.e. avoidance or tolerance) to phytotoxic elements (Liang et al., 2017; Chaudhry et al., 2005; Stefanakis et al., 2019). The composition of the microbial community is directly influenced by the plant species due to variation in (i) root system architecture, and (ii) rhizosphere pH (influenced by the release of exudates) (Liang et al., 2017; Stefanakis et al., 2019).

Rhizodegradation, or plant-assisted bioremediation, is a primary contributor to the remediation of organic contaminants (Donnelly and Fletcher, 1994). By-products, produced by rhizodegradation processes may be taken up into the plant, translocated to aboveground biomass via the transpiration stream, and subsequently volatilized by evapotranspiration (i.e. phytovolatilization). This process enhances the plant's ability to remove elevated concentrations of contaminants and/or their transformed/degraded species from TWs (Heaton et al., 1998; Limmer and Burken, 2016). Moreover, plant-assisted bioremediation has long been demonstrated to significantly increase the assimilation of contaminants within wetland substrate (Doyle and Otte, 1997) when compared with non-planted wetlands. This reduces the mobility, and thus the toxicity, of contaminants within aquatic and terrestrial (e.g. via treated WWW irrigation) environments, decreasing contaminants entering the food chain through plant uptake and accumulation in aboveground biomass.

Complex biological interactions associated with plant-assisted bioremediation of WWW limit the comparability and reproducibility of studies. This is attributed to the inability to effectively distinguish the role of plants (phytoremediation component), microbes (bioremediation component), microbe-microbe/plant-plant interactions (i.e. intra- and interspecies competition), as well as the plant-assisted bioremediation components utilized in TWs. Moreover, these biological, along with hydrogeochemical, contaminant removal pathways occur

simultaneously and fluctuate through space and time (Imfeld et al., 2009). The degree to which plants contribute to the overall remediation potential of TWs differ between studies. For example,Zhang et al. (2014) demonstrated that plants play a significant role in the remediation of certain pharmaceutical contaminants whereas other studies have demonstrated no significant difference between contaminant removal efficiencies between planted and unplanted TWs. This further highlights the complexity of determining the efficacy of TWs treating WWW as the fate of contaminants (e.g. competitive ion adsorption, composition of contaminants), functional components (media, plant species, and environmental conditions) and operational parameters differ between studies (Table S1). In order to ensure that the role of plants is not masked, studies should ideally consider a range of relevant parameters that may affect the speciation, bioavailability, and toxicity of WWW contaminants on plants. These include environmental factors such as pH, electrical conductivity, redox potential, temperature, and O_2 availability of WWW.

5. Conclusion

Treatment wetlands, comprised of biological and hydrogeochemical components, have been used to remediate wastewater originating from various anthropogenic sources. The systems have proven effective for reducing the organic load and neutralising the pH of WWW, but concurrent or downstream removal of inorganics remains a challenge. Removal of inorganic macronutrients (N,P,K) is important if the treated WWW is discharged into aqueous environments, but not if the effluent is to be used for irrigation. While N compounds can be mineralized, P is removed chiefly by media adsorption, so negative removal rates can be found once the binding sites are saturated. In line with circular economy principles, there is a need for studies aimed at capturing P from WWW for re-use. Other inorganics found in high concentrations in WWW (Na^+, K^+) are not removed by microbial action, nor adsorbed by the TW substrate. This creates a selective pressure for the use of halophytes which have the elevated ability to (hyper)accumulate these inorganics (effectively removing these cations from WWW) and cross-tolerate a range of stresses. This review also presents the case for the use of terrestrial halophytes in TWs. Terrestrial halophytes have the ability to (hyper)accumulate and cross-tolerate a range of contaminants, along with the ability to control operational parameters of TWs (i.e. artificial aeration) - creating viable alternatives to the selection of macrophytes currently used world-wide. Salinization is a growing issue compromising food security due to the inability of glycophytes to survive under saline conditions. The non-traditional use of halophytic crops which can be grown in non-arable, saline land under WWW irrigation, is a viable solution ultimately promoting food security in arid and semi-arid environments, where fresh water for irrigation is limited.

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Appendix A. Supplementary data

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Appendix 2 Journal Article 1 – Supplementary data

Supplementary Table A2-1 Operational and functional components of treatment wetlands (TW) for the remediation of winery wastewater (WWW). These studies exclude laboratory-based studies [ID 1, 9, and 23].

ID [ref]	**2** [12]	**3** [37,38]	**4** [15]	**5** [14]	**6** [19,20,60]	**7** [18]	**8** [18]	**10** [21]	**11** [21]	**12** [4]
Pre-treatment	None	UASB	UASB	HUSB	Sand pre-filter	Settling pond	Settling pond	Septic tank	Septic tank	Primary settling → Balancing tank
Configuration & mode	HF	VF → $HF_{1,2,3}$	VF → $HF_{1,2,3}$	VF → HF	HF	HF	HF	HF	HF	HF
HC ($mm.s^{-1}$)	-	1.5-1.6 ($HF_{1,2,3}$)	-	-	6.0	-	-	-	-	0.04-0.20
Media (type)	Cork stoppers	Granitic gravel	Granitic gravel	Gravel	Pea gravel	Pea gravel	Rock	Pea gravel	Pea gravel	Dune sand
FSA (m^2)	1.2	350	350	60	14.9	4400	304	120	144	7.3
Unit FSA (m^2)	VF=1.2	VF=50 $HSSF_{1,2,3}$=100 ea.	VF=50 $HF_{1,2,3}$=100 ea.	VF =30 m^2 HF =30m^2	HF=14.9	HF=4400	HF=304	Planted HF=60 Unplanted HF=60	Planted HF=72 Unplanted HF=72	$HF_{1,2,3,4}$=1.8 ea.
Unit FD (m)	VF=0.6	VF=1.4 HF_1=0.3 $HF_{2\&3}$=0.6	VF=1.4 HF_1=0.3 $HF_{2\&3}$=0.6	-	HF=14.9	HF=1.2	HF=1.2	Planted HF=0.9 Unplanted HF=0.9	Planted HF=0.9 Unplanted HF=0.9	-
HRT (days)	-	-	3	-	9.7	5.5 10 (design)	5 (design)	6.0±1.6	18-24 14 (design)	1.8
HLR – FSA ($mm.day^{-1}$)	-	19.5±6.9 (all) 12.9±24.5 ($HF_{1,2,3}$)	77-215 (VF) 13-36 ($HF_{1,2,3}$)	128	34	31	-	-	-	109
HLR – FV ($L.m^{-3}.day^{-1}$)	-	13.9 (VF) 43-82 (HF_1) 22-41 ($HF_{,2,3}$)	55-154 (VF) 43-120 (HF_1) 22-60 ($HF_{2,3}$)	-	28	26	-	-	-	150 (12-313)

ID [ref]	**2** [12]	**3** [37,38]	**4** [15]	**5** [14]	**6** [19,20,60]	**7** [18]	**8** [18]	**10** [21]	**11** [21]	**12** [4]
MFR	-	5.2	-	39.5	0.5	137	137	-	-	0.41

($m^3.day^{-1}$)										
COD_{in} ($mg\ L^{-1}$)	1258	1558±1023	-	1031* (Crush) <500* (Non-C)	993-4720	7406 ±2090 (Crush) 1721±439 (Non-C)	290	72965 ±29066	5080 ±1211 (Crush)	1138
OLR - FSA ($g\ COD.m^2.d^{-1}$)	-	30.4±19.3 (all) 213 (VF) 16.2 ($HF_{1,2,3}$)	30.4 (all) 43-466 (VF) 3.6-55 ($HF_{1,2,3}$)	132* (Crush) <64* (Non-C)	35-164	120-270	553	-	-	110
OLR - FV ($g\ COD.m^3.d^{-1}$)	-	152 (VF) 54 (HF_1) 27 ($HF_{2,3}$)	31-333* (VF) 12-183* (HF_1) 6-92* ($HF_{2,3}$)	-	37-176	100-225	465	-	-	152 (23-469)
ORR (%)	-	71	73 (all) 29-70 (VF) 23-79 (HF)	-	97-99	49 (Crush) 79 (Non-C)	98	94-97	98-99	79 (28-98)
Phenolics ($mg\ L^{-1}$)	-	9.7±3	-	-	-	55±11 (Crush) 55±22 (Non-C)	-	-	-	18 (5.1-44)
Phenolic RR (%)	-	30-50	-	-	-	78 (Crush) 46 (Non-C)	-	-	-	77 (16-100)
N_{in} ($mg.L^{-1}$)	-	-	-	-	-	NO_3^- 13±7 (Crush) 16±1.8 (Non-C)	-	-	-	-
N RR (%)	-	-	-	-	-	17 (Crush) 73 (Non-C)	-	-	-	-
$NH_3/NH_4^+{}_{in}$ ($mg.L^{-1}$)	0.6-74	-	-	-	-	37±28 (Crush) 118 (Non-C)	-	-	-	-
NH_3/NH_4^+ RR (%)	≤29	-	-	-	-	29 (Crush) 62 (Non-C)	-	-	-	-
TP_{in} ($mg.L^{-1}$)	0.9-6.3	-	-	-	-	-	-	-	-	-

ID [ref]	**2** [12]	**3** [37,38]	**4** [15]	**5** [14]	**6** [19,20,60]	**7** [18]	**8** [18]	**10** [21]	**11** [21]	**12** [4]

TP RR (%)	≤29	-	-	-	-	-	-	-	-	-
Na_{in} ($mg.L^{-1}$)	-	-	-	-	-	-	-	-	-	30 (11-71)
Na RR (%)										0
K_{in}	-	-	-	-	-	-	-	-	-	122 (17-285)
K RR (%)	-	-	-	-	-	-	-	-	-	0
Plant species	*Iris pseudocorus*	*Juncus effusus*	*VF: Phragmites australis* HF: *J. effusus*	Not stated	*Typha dominicus, Scirpus acutus Sagittaria latifolia*	*Typha* spp.	*Typha* spp.	*T. domingensis, S. acutus, S. latifolia*	*T. domingensis, S. acutus, S. latifolia*	unplanted
Plant spacing Number per m^2	8	3-4	3-4	-	2.8	-	-	-	-	-
Other						95% removal 35±19 mg L^{-1} SO_4^{2-} 78% removal 0.6±0.2 mg $L^{-1}SO_3^-$				0% Mg removal 4.5 (1.3-10 mg L^{-1})
Plant spacing Number per m^2	8	3-4	3-4	-	2.8	-	-	-	-	-

UASB = up-flow anaerobic sludge blanket reactor **HUSB** = hydrolytic up-flow sludge blanket reactor **HF** = horizontal subsurface flow **VSF** = vertical subsurface flow **FWS** = free water surface flow **FSA** = functional surface area **FD** = functional depth **HRT** = hydraulic retention time **HLR** = hydraulic loading rate **MFR** = measured flow rate **COD_{in}** = influent chemical oxygen demand concentration **OLR** = organic loading rate **FV** = functional volume **ORR** = organic removal rates **RR** = removal rate **N_{in}** = influent N concentration **TP** = total phosphorus concentration.

Supplementary Table A2-2 Operational and functional components of treatment wetland (TW) for the remediation of winery wastewater (WWW). These studies exclude laboratory-based studies [ID 1, 9, and 23].

ID [ref]	**13** [11]	**14** [11]	**15** [11]	**16** [52]	**17** [52]	**18** [52]	**19** [16]	**21** [22,166]	**22** [23]
Pre-treatment	Septic tank → AFFR	Septic tank → AFFR	Septic tank	Imhoff tank	Imhoff tank	Imhoff tank	Equalization tank	NG	Dosing tank
Configuration & mode	HF_1 (anoxic) $HF_{2,3}$ (aerobic)	$HF_{1,2,4}$ (aerobic) HF_3 (anoxic)	$HF_{1,2}$	HF → FWS	$VF_{1,2}$ → HF → FWS → pond	HF	VF → HF → FWS	HF	$VF_{1,2,3}$ VF_4 (anoxic)
HC ($mm.s^{-1}$)	-	-	-	-	-	-	5.8	-	-
Media	Wood chips (HF_1) Gravel & sand ($HF_{2,3}$)	Gravel & sand ($HF_{1,2,4}$) Wood chips (HF_3)	Gravel & sand	Gravel	Gravel & sand ($VF_{1,2}$) Gravel (HF)	Gravel	Gravel (VF) NG (HF)	Dolomitic gravel	Gravel ($VF_{1,2,3}$) Wood chips & peat moss (VF_4)
FSA (m^2)	75-164	190-404	-	1330	752	215	3034	180	404
Unit FSA (m^2)	25-55	38-101	-	HF=480 FWS=850	$VF_{1,2}$=90 ea. HF=86 FWS=148 Pond=338	HF=215	VF=1197 HF=987 FWS=850	HF=180	$VF_{1,2,3,4}$=404
Unit FD (m)	1.2	1.2	-	HF=0.7	$VF_{1,2}$=0.7 HF=0.9	HF=0.7	VF=0.85 HF=0.8 FWS=0.4	HF=0.9	$VF_{1,2,3}$=0.4 VF_4=0.8
HRT (days)	-	-	3-5	-	-	-	2.5 (design)	1) 14 20 7	-
HLR – FSA ($mm.day^{-1}$)	119-160 (all) 357-480 (HF_1)	39-53 (all) 133-164 (HF_1)	38 (all) 76 (HF_1)	26	23	37	60-80	1) 22.5 2) 45	22.3
HLR – FV ($L.m^{-3}.day^{-1}$)	99-133	33-44	32	18	16	26	71-94 (VF)	1) 25 2) 50	55.8* ($VF_{1,2,4}$) 27.9* (VF_3
MFR ($m^3.day^{-1}$)	10.4-22.5	10.0-16.6 (crush)	<10.0	35	10	8	60.5±28.3 (max. 118	1) 4.1 2) 8.1	7.26 (Crush) 11.0 (Non-C)
COD_{in} ($mg\ L^{-1}$)	-	-	-	4045	1003	722	1159±432 (VF)	1) 14000 2) -	3043 (Crush) 2117 (Non-C)
OLR - FSA ($g\ COD.m^2.d^{-1}$)	-	-	-	329	236	352	160-230 (design)	1) 315 2) -	34
OLR - FV ($g\ COD.m^3.d^{-1}$)	-	-	-	230	165	246	188-271	1) 350 2) -	85*($VF_{1,2,4}$) 43*

ID [ref]	**13** [11]	**14** [11]	**15** [11]	**16** [52]	**17** [52]	**18** [52]	**19** [16]	**21** [22,166]	**22** [23]
ORR (%)	>98	>98	>98	98	93	88	98 (all) 70 (VF)	1) 77-80 (Non-C) 1) 83-88 (Crush) 2) 60	99
N_{in} ($mg.L^{-1}$)	-	-	-	14.7	26.6	65.2	-	-	1-410 (TKN)
N RR (%)	-	-	-	81	90	58	85 (NO_2^-) 40 (NO_3^{2-})	-	≥89
$NH_3/NH_4^+{}_{in}$ ($mg.L^{-1}$)	-	-	-	-	-	-	-	-	0.2-5.4
NH_3/NH_4^+ RR (%)	-	-	-	-	-	-	-	-	≥73
TP_{in} ($mg.L^{-1}$)	-	-	-	4.9	1.9	-	-	-	-
TP RR (%)	-	-	-	73	94	-	45	-	-115-99
Na_{in} ($mg.L^{-1}$)	-	-	-	-	-	-	-	101-282	-
Na RR (%)								1-43	
K_{in} ($mg.L^{-1}$)	-	-	-	-	-	-	-	141-615	-
K RR (%)	-	-	-	-	-	-	-	1-43	-

ID [ref]	**13** [11]	**14** [11]	**15** [11]	**16** [52]	**17** [52]	**18** [52]	**19** [16]	**21** [22,166]	**22** [23]
Plant species	*Typha* spp. *Schoenoplectus tabernaemontani* (syn. *Scirpus validus*)	*Typha* spp. *S. tabernaemontani*	*Typha* spp. *S. tabernaemontani*	HF: "reeds" FWS*: J. effuses Typha latifolia, P. australis, Elodea canadensis, Ceratophyllum demersum, Nymphaea alba, Nymphea rustica, Caltha palustris, Epilobium hirsutum, Epatorium cannabium, Iris pseudacorus, Botumus umbellatus*	HF: "reeds" FWS*: J. effuses, T. latifolia, P. australis, Myriophyllum spicatum, E. cannabium, Ausmaplanatado aquatica, Iris pseudacorus, B. umbellatus, Mentha aquatica, Nymphea alba, N. rustica, Lythrum salicaria, Mentha aquatica, Ranunculus aquaticus, Nuphar lutem, Epilobum hirsutum, Typha minua*	HF: "reeds"	HF: *P. australis* FWS: *P. australis, T. latifolia, Myriophyllum spicatum, E. canadensis, C. demersum, L. salicaria, Iris pseudacorus, E. hirsutum, Alisma plantago aquatica, B. umbellatus*	*Typha* spp, *Scirpus* spp. *Phragmiters* spp	*T. latifolia, S. tabernaemontani*
Plant spacing	-	0.4	-	-	-	-	-	8-10	-
Other									

UASB = up-flow anaerobic sludge blanket reactor **HUSB** = hydrolytic up-flow sludge blanket reactor **HF** = horizontal subsurface flow **VSF** = vertical subsurface flow **FWS** = free water surface flow **FSA** = functional surface area **FD** = functional depth **HRT** = hydraulic retention time **HLR** = hydraulic loading rate **MFR** = measured flow rate **COD_{in}** = influent chemical oxygen demand concentration **OLR** = organic loading rate **FV** = functional volume **ORR** = organic removal rates **RR** = removal rate **N_{in}** = influent N concentration **TP** = total phosphorus concentration.

Appendix 3 Journal article 2 offprint

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Comparison of continuous and pulse mode of operation of pilot biosand reactors treating winery effluent

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ABSTRACT

In 2020 there was approximately 260 million hectolitres of wine produced across the world. Many winemaking and cellar cleaning activities generate winery wastewater. In vine growing areas which are water stressed, this wastewater is often used for irrigation and if it is inadequately treated it can be detrimental to land and aquatic environments. It has been shown at pilot scale that horizontal flow biosand filters are suitable for treating winery effluent to comply with irrigation standards. In this two-year study, vertical flow biosand reactors with novel design features were operated in both continuous and pulse modes of operation. It was envisaged that (i) the loading rates (organic and hydraulic) could be increased by changing the flow from horizontal to vertical flow, and that (ii) higher organic biodegradation rates could be achieved consequent to the increased redox potential from draw-down of atmospheric oxygen during system drainage in pulse mode in comparison to continuous mode. It was found that system performance was higher in continuous mode, attaining a hydraulic loading rate of 113 $L.m^{-3}$ of sand a day^{-1}, organic loading rate of 279 $gCOD.m^{-3}$ of $sand.day^{-1}$ and COD removal efficiency of 70% compared to pulse mode with 90 $L.m^{-3}$ of sand a day^{-1}, 192 $gCOD.m^{-3}$ of $sand.day^{-1}$ and 70%, respectively. In comparison to other passive winery wastewater treatment systems (constructed/treatment wetlands), these biosand filters are able to treat winery wastewater at higher loading rates with smaller spatial footprints.

1. Introduction

In 2020 there were approximately 7.3 million hectares under vine across the world, producing 260 million hectolitres of wine. Of this, 105.8 million hectolitres, valued at 29.6 billion Euros were exported (OIV, 2021). The practice of wine making relies on the beneficial processes which turn sugars into ethanol, as well as the formation of organic compounds which enhance the aroma and flavour of the end-product. Winemaking generates different waste streams, including winery wastewater (WWW). Due to different seasonal cellar activities, the quantity and composition of WWW fluctuates, not only seasonally, but also from cellar to cellar. Conventional secondary wastewater (WW) treatment systems require a consistent influent in both quality and quantity. Inconsistency may result in poor treatment performance during periods of heavy hydraulic and/or organic loading. In the case of WWW, treatment systems may also be redundant during several months of a year due to low flows, requiring repeated start-ups. For these reasons, WWW may not be adequately remediated if treatment systems are unable to adapt to rapid changes.

In water stressed areas such as South Africa, Australia and parts of the United States, WWW is often reused for irrigation of pastures or crops. Inadequately treated WWW can pose a threat to the soil and/or groundwater safety and security (Hirzel et al., 2017; Mosse et al., 2012).

Biosand reactors (BSRs), alternatively designated as biological sand filters, are similar to unplanted sand filled constructed/treatment wetlands (CW/TW). They are low cost, low maintenance, sustainable and energy efficient systems that can be used for treating WWW (Holtman et al., 2018; Mader et al., 2021). Biosand reactors have shown promising results for remediation of WWW, from laboratory-based experiments (Ramond et al., 2013; Welz and le Roes-Hill, 2014) to a pilot-scale reactor system (Holtman et al., 2018). They have proven capable of achieving high levels of treatment and providing safe effluent for irrigation while being able to readily adapt to the temporal changes of WWW and protracted shut down periods. A gravity-fed horizontal pilot system was able to effectively reduce the organic load and neutralise acidic WWW while increasing the sodium adsorption ratio (Holtman et al., 2018). The system was, however,r only suited to very small wineries because the achievable flow rates were low (402 $L.day^{-1}$). The

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low flow rates translate into large spatial footprints for the system.

In order to increase the flow rates in comparison to the original pilot BSR system while maintaining high organic removal rates (ORR), in-depth studies were conducted on more sophisticated, vertical flow BSRs with a novel design in-situ at a local winery in South Africa. These were operated alternately in either continuous or pulse mode over two crush seasons to ascertain which mode of operation yielded the most efficient performance. The operational and performance results are presented in the manuscript and results are compared with other passive systems treating WWW in terms of the hydraulic loading rate (HLR), organic loading rate (OLR) and ORR.

2. Materials and methods

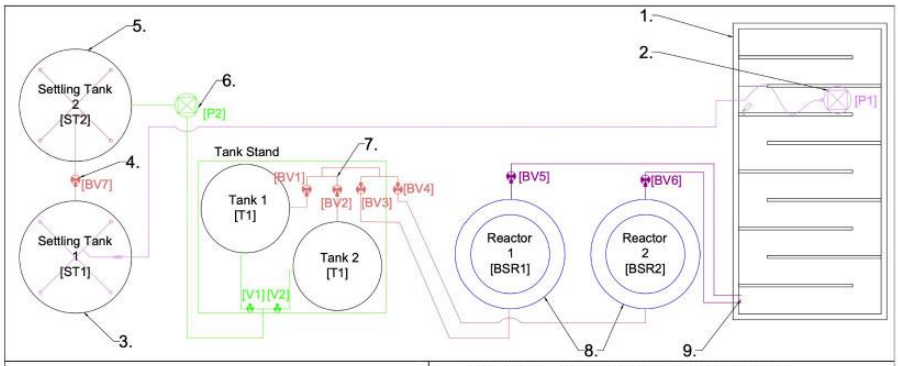
2.1. Set-up and operation of pilot scale biosand reactor system

The vertical flow BSR treatment system was a pilot system treating a fraction of the WWW. At full-scale, it is intended to treat a significant portion of the WWW generated by the winery in order to improve the overall quality of the effluent for irrigation, thereby protecting the soil environment. The treatment system was designed around the extraction of WWW from an existing baffled concrete solid settling delta to a series of holding tanks which acted as additional settling tanks and rudimentary anaerobic digestors (Fig. 1). Each BSR was inoculated evenly at the top with 500 g of sand from an existing horizontal flow BSR used to treat winery effluent. The sand was then further acclimated by intermittent feeding with WWW 3 months before the start of the crush season in year 1 of the study. Due to technical problems with the control system during start-up, the BSRs were not feed as often as planned.

The BSRs were fed with the settled, pre-digested WWW and the final effluent flowed via gravity back into the settling delta from where the effluent was fed into a holding dam used for irrigation (Fig. 1). More specifically, the WWW was extracted from the delta via a submersible sludge pump into a 5000 L settling tank (ST1) via 4 upward facing inlets when the liquid level in ST1 dropped to 50%. To reduce disturbance of solids, the inlets were located 1 m meter from the bottom of the tank. After a settling period of 120 min, the settled WWW was fed by gravity via a floating outlet pipe controlled by a 1″ full bore electronic ball valve into a second 5000 L settling tank (ST2). The outlet was located 10 cm below the liquid surface level of ST2 to prevent extraction of floating biomass or foam. After a settling period of 120 min, the WWW was extracted via a centrifugal pump to two 2500 L holding tanks (T1, T2) elevated on a steel tank stand. T1 and T2 were fed and emptied alternatively, the flows to T1 and T2 being controlled by two solenoid valves (V1 and V2). The two BSRs (BSR1, BSR2) were fed from T1 outlet controlled via BV1 or T2 outlet controlled via BV2 which were connected via a manifold. BSR1 inlet was controlled via BV3 and BSR2 controlled via BV4 while BSR1 outlet was controlled by BV5 and BSR2 by BV6. The system was set up so that BSR1 and BSR2 could be operated in continuous/pulse or continuous mode of operation. The entire systems' logic was controlled via a RievTech micro PLC (PR-14 DC-DA-R) and two expansion units (PR-E-16 DC-DA-R) and a series of analogue and digital inputs and relay outputs together with basic remote control via an Accentronix Infinity Cellswitch.

2.2. Sampling and characterisation of influent and effluent

Grab samples were extracted from sampling ports in ST1. Influent and effluent samples were taken from the influent lines to and outlet points to/from BSR1 and BSR2, respectively using the schedule provided in Table 1. Intense sampling (Table 1) and monitoring was conducted during the crush season only (Day 1 to 72 year 1 and day 1 to 58 year 2) because (i) approximately 61% of winery effluent is generated during the crush season at this winery (ii) the organic load is highest during the crush season (Welz et al., 2016), and (iii) it has already been shown that performance of BSRs is only under stress during the crush season (Holtman et al., 2018). Start-up commenced on 13th February in year 1 and sampling began on the 20th of February (day 1). BSR1 was operated in pulse mode and BSR2 in continuous mode from day 1 to day 43, after which the order was reversed until day 72. Similarly, in year 2 BSR1 was



1. Existing concrete settling delta
2. Submersible sludge pump [P1] extracts wastewater from the existing delta to [ST1].
3. [ST1] fills via 4 upward facing inlets 1 m above the bottom. Solids settling time = 120 min.
4. A 1" ball valve [BV7] controls the gravity flow from 5000 L [ST1] to identical [ST2].
5. [ST2] is filled after the rest period of [ST1]. Both [ST1] & [ST2] have floating outlet pipes 100 mm below the surface.
6. Centrifugal pump [P2] extracts the settled supernatant from [ST2] and fills two 2500 L tanks [T1] & [T2] on tank stand. The flow direction is controlled by two solenoid valves [V1] & [V2].
7. Water flows alternatively via gravity from [T1] and [T2]. Flow and mode of operation is controlled by ball valves [BV1] and [BV2].
8. The Biosand reactors [BSR1] and [BSR2] can be operated in continuous or pulse fed mode of operation.
9. Treated wastewater is fed back into the settling delta

Fig. 1. Design layout schematic of the treatment system.

Table 1
Sampling schedule for the study period.

Influent and effluent to BSR1 and BSR2: COD, VOA, Total phenolics, pH, Electrical conductivity, TP, TN, Alkalinity, Na, Ca, Mg, K, P, Al	Influent and effluent to BSR1 and BSR2: COD, VOA, Total phenolics, pH, Electrical conductivity, Alkalinity	Settling tank: COD, VOA, Total phenolics, pH, Electrical conductivity, TP, TN, Alkalinity, Na, Ca, Mg, K, P, Al
Year 1 crush BSR1 pulse BSR2 in continuous mode		
Days; 1,10,14,16,22,29,34,37,43	Days: 6,24,27,31,38,41,45	Days: 16,37,43
Year 1 crush BSR1 continuous BSR2 in pulse mode		
Days: 50,57,64,72	Days: 48,55,59	Days: 50,57,64,72
Year 2 crush BSR1 pulse BSR2 in continuous mode		
Days: 1,8,22,36	Days: 3,6,8,13,17,20,24,27,31,34	Days: 1,8,22,36
Year 2 crush BSR1 continuous BSR2 in pulse mode		
Days: 43,52,57	Days: 38,41,45,55,58	Days: 43,57

BSR = biosand reactor, COD = chemical oxygen demand, VOA = volatile organic acid, TP = total phosphorous, TN = total nitrogen, ALK = total alkalinity, SALTS = Calcium, magnesium, potassium, sodium, aluminium and phosphorous.

operated pulse mode and BSR2 in continuous mode from day 1 to day 36, after which the order was reversed. Sampling and monitoring were terminated early in year 2 (day 58) because winery operations were interrupted due to an enforced hard lockdown as a result of the COVID-19 pandemic. Nevertheless, sufficient results were obtained in order to assess system performance. The system continues to operate effectively to date (year 4).

2.2.1. Analytical procedures

The concentrations of COD, total phosphorus (TP), total nitrogen (TN), alkalinity and volatile organic acids (VOA), were determined using a Merck (Merck®, Whitehouse Station, USA) Spectroquant® Pharo instrument and Merck Spectroquant® cell tests or kits according to manufacturer's instructions as previously described (Holtman et al., 2018). The total phenolic concentrations were determined using the Folin Ciocalteau method as previously described (Holtman et al., 2018).

2.2.2. Determination of pH, temperature and electrical conductivity

Probes were placed inside ST1 and ST2 to monitor electrical conductivity (EC, B&C C7335, K = 1.0, Carnate), temperature (PT100), pH (van London co. P822, Houston) and connected to an analog converter (Acdc Dynamics TRT-PT100). Data was recorded to an SD card connected to a micro PLC (RievTech PR-14 DC-DA-R and PR-E-16 DC-DA-R, Nanjing).

The pH of the lab samples was determined according to the manufacturer's instructions using a CyberScan pH 300 meter and appropriately calibrated pH probe PHWP300/02 K (Eutech instruments, Singapore).

2.3. Calculation of operational parameters

2.3.1. Flow rates, volume of wastewater treated and electricity consumption

The flow rate data was logged via two Kamstrup Multical 21 (02146VO1N94) 20 mm ultrasonic flow meters. This data was recorded by a Kamstrup Omnipower single phase electricity meter which also monitored the system's electricity consumption.

2.3.2. Hydraulic conductivity, hydraulic loading rate, organic loading rate

To counter biases from flow variability, the flow rate (Section 2.3.1) was converted to a 3-day lagging average for calculation of the HLR and OLR.

In CWs, the HLR or surface loading rate (SLR) and OLR are typically only calculated using the surface area of a wetland. In this study, the HLR and OLR were also determined using the entire volume of the reactor as previously described (Holtman et al., 2018; Mader et al., 2021). The ORR was determined by multiplying the OLR by the removal efficiency in terms of the COD.

3. Results and discussion

3.1. Performance of biosand reactors

The BSRs were evaluated in terms of (i) organic removal efficiency (COD, VOA, total polyphenolics), discussed in Section 3.1.1, (ii) inorganics and pH changes (pH, EC, alkalinity), discussed in Section 3.1.2, and, the (iii) hydraulic performance, discussed in Section 3.1.3. This is followed by (iv) a critical evaluation of the OLR, HLR and ORR that were achieved in the BSRs when operated in continuous and pulse modes, and comparison of the results to other passive systems treating WWW, discussed in Section 3.1.4.

3.1.1. Organic removal performance

The COD of samples for year 1 and year 2 are shown in Fig. 2A. Since an ideal pre-crush start-up/acclimation period could not be achieved in year 1 (Section 2.1), the COD removal efficiency was low (<50% for the first 3 weeks of operation). However, performance increased significantly over time. It can be seen in Fig. 2A that the COD concentration in the final effluent decreased with length of operation of the system during year 1. Overall, there was an average 88% (54–97%) COD removal efficiency in year 1 and 73% (33–93%) in year 2. The lower efficiency in year 2 may be attributed to differences in WWW characteristics and sampling periods related to variances in year-on-year cellar activities. The original vertical flow on-site pilot BSR system (Holtman et al., 2018) operated with a notably lower influent COD than the system described in this study over the two years of operation, however, COD removal rates were similar (70% vs. 79%), indicating notably superior COD removal performance with the new system. In terms of VOAs, in some instances they were formed within the BSRs as metabolic products, as previously demonstrated with WWW at laboratory scale and within settling basins (Welz et al., 2016; Welz et al., 2014).

Polyphenolics in WWW need to be reduced before discharge of the effluent because they may be toxic to microbes and plants (Arienzo et al., 2009; Mosse et al., 2011). In this study, the average removal efficiency of total polyphenolics during the monitoring period was 75%. The results compared favourably with the 77% (influent 18.4 mgGAE. L^{-1}) achieved with original horizontal flow system (Holtman et al., 2018) but at higher flow rates (Section 3.1.3). These results confirmed the ability of BSRs to effectively reduce polyphenolics in WWW.

3.1.2. Assessment of pH and inorganic changes

The pH of the WWW depends on the on the seasonal activities taking place in the cellar (Welz et al., 2016), but it is typically acidic (Vlyssides et al., 2005; Bories and Sire, 2010). In this study, the pH in ST1 ranged from 4.5 to 5.1 and 4.8 to 8.4 in year 1 and year 2, respectively (Fig. 3A). Overall, the BSRs increased the pH of the acidic effluent to >6 throughout the sampling periods. Similar results were found previously in a system containing sand from the same quarry site (Holtman et al., 2018).

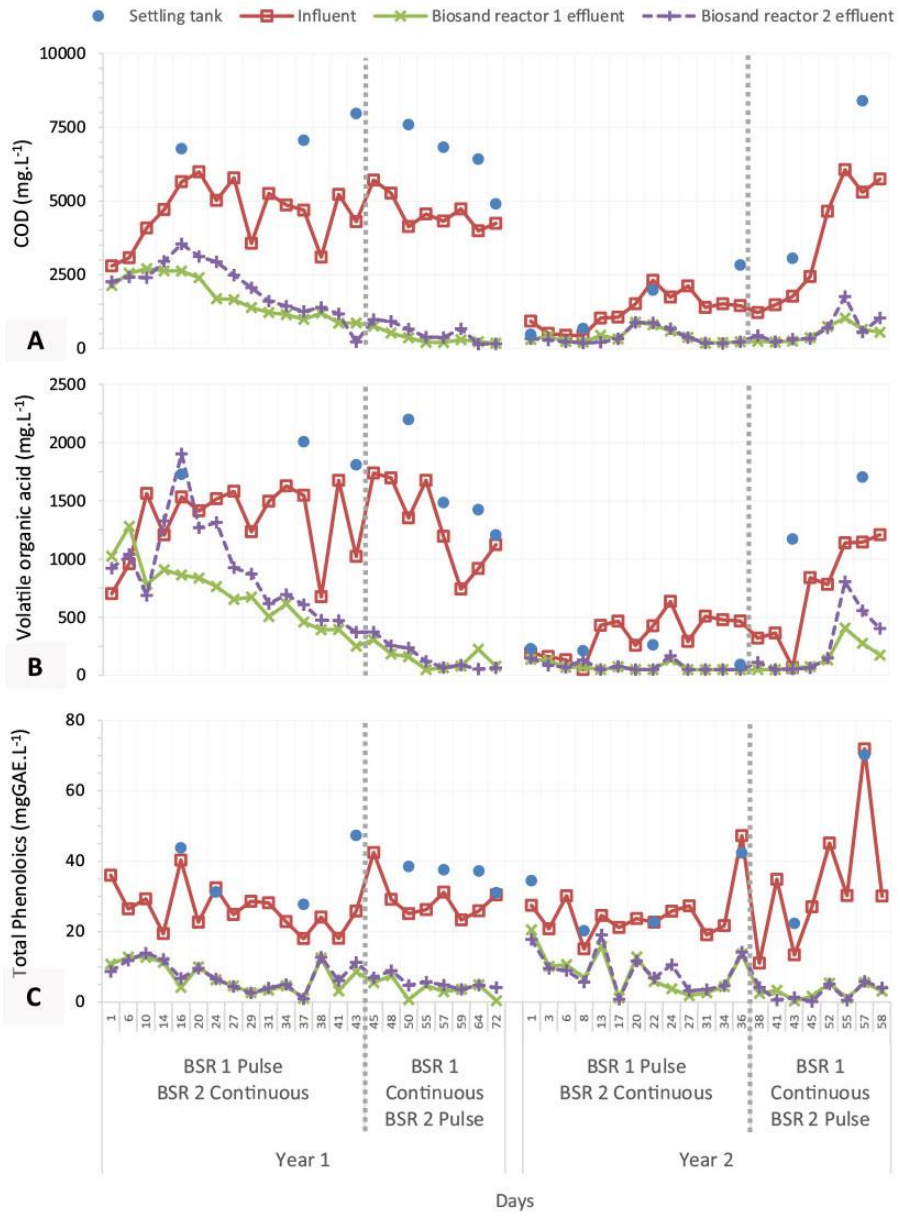


Fig. 2. The A) chemical oxygen demand, B) volatile organic acids, C) total phenolics samples taken from the biosand reactor treatment system during the pre-crush and crush period for year 1 and year 2.

The average electrical conductivity (EC) from ST1 for both periods was 1735 us.M^{-1} with respective averages for year 1 and 2 of 1690 us.M^{-1} and 1779 us.M^{-1} (Fig. 3C). Over the 2-year sampling period, the average EC increased by 75% from influent to effluent mainly due to the dissolution of calcite in the sand which was also responsible for pH buffering as previously described (Holtman et al., 2018; Welz et al., 2018a). The dissolution of calcite was also largely responsible for the increase in alkalinity from BSR inlet to outlet in year 1 and year 2 (Fig. 3C), also as previously described (Holtman et al., 2018).

The respective average influent TN and TP concentrations for year 1 were 1.4 mg.L^{-1} and 10.1 mg.L^{-1} and 31.8 mg.L^{-1} and 16.1 mg.L^{-1} in year 2 (data not shown). The average effluent concentrations from the BSRs in year 1 were 9.6 mg.L^{-1} TN and 2.4 mg.L^{-1} TP (ranges: 0 to 52 mg.L^{-1} and 0.2 to 24.4 mg.L^{-1} for TN and TP, respectively). In year 2, the average effluent TN from was 149.6 mg.L^{-1} (7.3–710 mg.L^{-1}) and average TP was 29.5 mg.L^{-1} (range: 4.8–67.5 mg.L^{-1}) (data not shown). The intermittent negative removal rates of TN may be attributed to microbial atmospheric N_2 fixation as previously described in BSRs (Welz et al., 2018b). From a practical perspective, the high effluent TP, and to a lesser extent high TN results may be seen as problematic in cases where

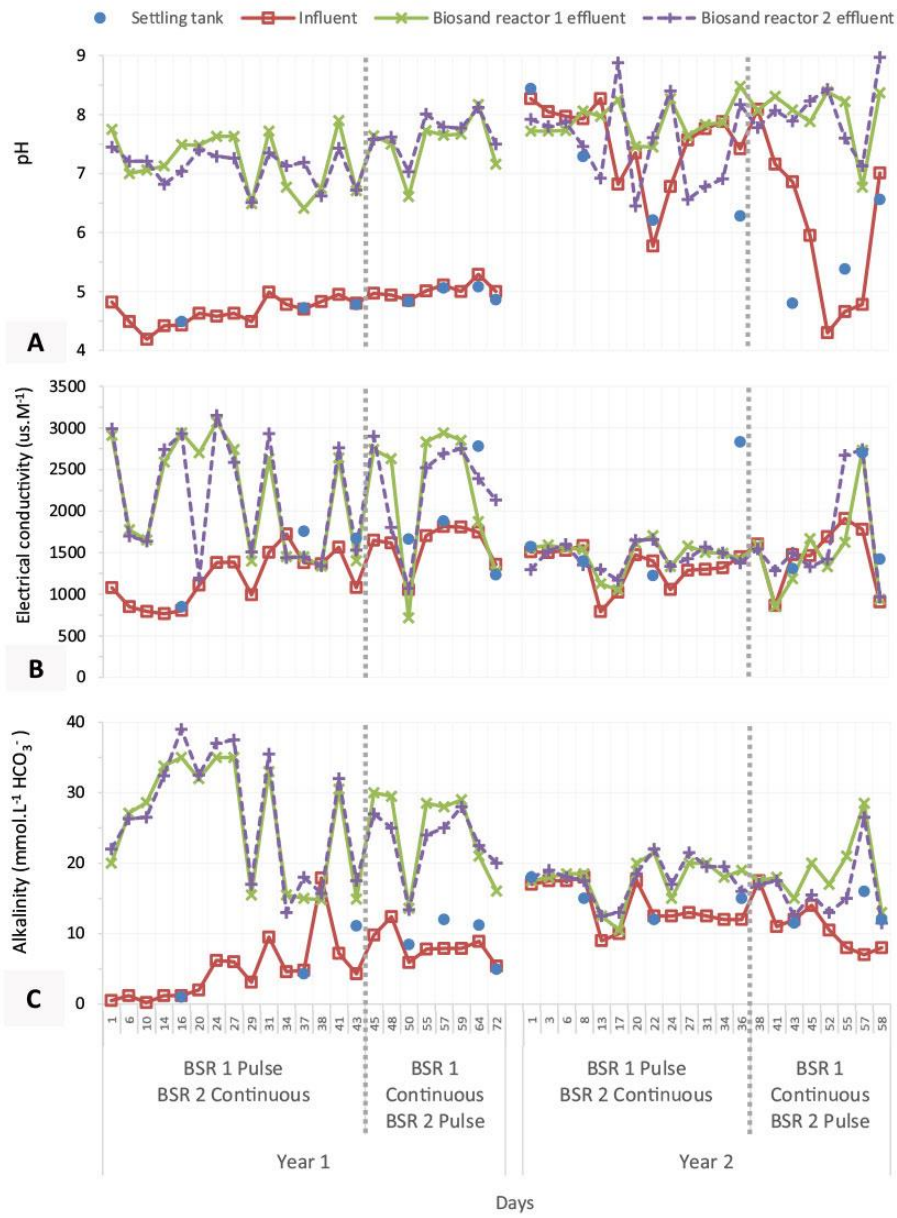
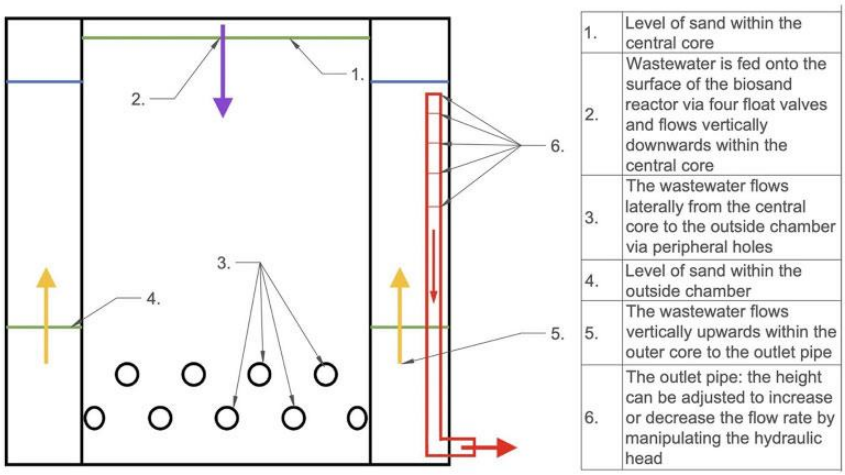


Fig. 3. The A) pH, B) electrical conductivity, C) alkalinity of samples taken from the biosand reactor treatment system during the pre-crush and crush period for year 1 and year 2.

the treated effluent is disposed directly to aquatic environments because of the risk of eutrophication. However, if the WWW is being used for irrigation purposes, as intended with the BSR systems, the presence of N and P is seen as beneficial as they are essential plant nutrients (Mader et al., 2021). Therefore, unlike many secondary wastewater treatment systems, removal of N and P is not a treatment objective.

3.1.3. *Hydraulic capacity and performance*

The BSRs used a novel design to increase the hydraulic performance while maintaining good pH neutralisation and organic removal performance, which was the primary objective of this study. It is envisaged that under normal operational circumstances, the fall across the BSRs (from the inside to the outside chamber) will be adjusted according to operational needs to increase or decrease flow rates, making the systems hydraulically versatile. In the study systems, the fall can be increased to up to 1700 mm during general operations to increase flow rates during high biomass and/or organic matter build-up (Fig. 4). However, for the purposes of the study, changes made to the fall during either of the two monitoring periods (year 1 and year 2 crush seasons) would have introduced an experimental variable, so no adjustments



1.	Level of sand within the central core
2.	Wastewater is fed onto the surface of the biosand reactor via four float valves and flows vertically downwards within the central core
3.	The wastewater flows laterally from the central core to the outside chamber via peripheral holes
4.	Level of sand within the outside chamber
5.	The wastewater flows vertically upwards within the outer core to the outlet pipe
6.	The outlet pipe: the height can be adjusted to increase or decrease the flow rate by manipulating the hydraulic head

Fig. 4. Section view of the novel design of the biosand reactors.

were made during either year. Nevertheless, no permanent clogging occurred in the BSRs and the temporal reduction in hydraulic conductivity (HC) was attributed to accumulation of functional biomass (microbial growth). The novel design and unrestricted flow from the inner to outer core ensured no clogging within or at the outlets of the BSRs.

In year 1, the fall was maintained at 200 mm, with a sand height of 1500 mm. Over the year 1 crush period, the BSRs used on average 0.97 $Kw.day^{-1}$ of electricity or 437 $L.Kw^{-1}$, to treat 30.2 m^3 of WWW at a flow rate of 425.3 L of WWW per day (205.5 $L.day^{-1}$ and 219.7 $L.day^{-1}$ in BSR1 and BSR2, respectively), with hydraulic flows of 659 $L.day^{-1}$ and 483 $L.day^{-1}$, respectively over the first two weeks (results not shown). This reduced to a flow of <100 $L.day^{-1}$ at the end of the crush season. It was theorised that the low flow rates were due to either (i) inorganic solids that may have entered the BSRs before the settling tanks were installed, and/or (ii) a lack in fall across the BSRs. Due to the unique design of the BSRs (Fig. 4), assumptions were made during the calculation of the fall that were not validated at a practical level. A new model was therefore applied that included a reduction in height of the sand in the outer chambers and reduction of the outlet height of the BSRs to increase the overall fall. It was calculated that this would result in an increase in treatment capacity to 1000 $L.day^{-1}$ for each BSR.

The operation of the system was suspended at the end of the year 1 monitoring period. At this point, the flow of incoming WWW into the delta had reduced to an impractical rate, and the surface of the delta was covered with organic sludge that required removal. Some adjustments were made, and the fall was increased to 800 mm in year 2 with a sand height of 300 mm in the outside chamber. Over the year 2 crush period, the combined BSRs treated an average of 1017 L $WWW.day^{-1}$, and a total volume of 66.0 m^3 WWW and used on average 1.44 $Kw.day^{-1}$ or 705 $L.Kw^{-1}$. The initial two-week flow rates were 790 $L.day^{-1}$ and 1057 $L.day^{-1}$ for BSR1 and BSR2, respectively. However, there was a reduction in flow rate over the treatment period to 500 $L.day^{-1}$. A build-up of organic matter was noted on the surface which was reducing the ingress of WWW into the top layer of sand, thereby reducing the overall HC of the BSRs. As with previous studies, the organic matter (including some microbial biomass) in the BSRs degraded during the off-crush period, restoring good flow rates (Holtman et al., 2018). This, however, takes time and does not rectify the problem experienced during the crush period itself. It is therefore suggested, as a simple remedy, that the organic layer is scarified in the affected areas on a weekly basis during the crush season to mitigate flow reduction in BSRs.

In the off-crush (non-monitoring) period of year 2, the system was re-started with both BSRs operated in continuous mode. Over the first week, the average flow rate was 3854 $L.day^{-1}$ for both BSRs combined, showing the effect of increased fall on the initial rates and the potential recovery of HC due to biomass degradation. The rates then dropped to 871 $L.day^{-1}$ and 426 $L.day^{-1}$ for BSR1 and 2, respectively by the third week and reduced further thereafter. It was hypothesized that this was an artefact attributable to a partial blockage in the influent line entering the ultrasonic flow meters - for experimental purposes, the influent WWW flow rates were monitored by ultrasonic meters which have small pre-filters. The hypothesis was validated by the fact that electricity was still consumed by the pump and the PLC logged events during periods when the ultrasonic meter recorded no flow.

3.1.4. Hydraulic and organic loading rates

The treatment capacity of CWs, and by inference BSRs, is critical. Low HLRs translate into large spatial footprints with a larger area to source, operate and maintain. In CWs, the HLR/SLR and OLR are typically calculated using the surface area of the CW because they are limited by O_2 transfer to the root systems of the wetland plants. BSRs have no such limitations, and the loading rate calculations can be based on the cross-sectional area in the direction of flow, the functional surface area (FSA) (HLR_{FSA} and OLR_{FSA}) or the entire volume of the reactor (HLR_{Vol} and OLR_{VOL}). The latter are more accurate efficiency indicators, especially when comparing the efficiencies obtained with different types of media (Holtman et al., 2018; Welz et al., 2018a).

The performance of the BSR system was compared with previously published data on other passive systems (CW/TWs) treating WWW specifically focusing on systems with reported porosities, which ranged from 25 to 40% and in the different studies and this study 29% (Table 2). If provided, the OLR and HLR in these studies were reported as HLR_{FSA} and OLR_{FSA} in the cited manuscripts. Wherever possible, the HLR_{Vol} and OLR_{Vol} were also calculated from available data and included in Table 2. Loading rates in terms of the volume of reactors allow a more accurate comparison of the treatment performance of different media and operation modes (Holtman et al., 2018; Mader et al., 2021). However, this still includes the volume taken up by the inert media and actual biodegradation of the WWW takes place within the pore spaces. In order to more accurately compare the actual biodegradation rates (i.e. microbial activity) from system to system, this was calculated using the porosity of the media to calculate the volume of voids $V_{Voids} = V_{VLF} = V \times Porosity$ (Eq. 1) and this value to calculate the OLR in the void liquid fraction (OLR_{VLF}) $OLR_{VLP} = OLR_{Vol}/V_{VLF}$ (Eq. 2) (Table 2). The same equation (Eq. 2) was used to determine the HLR_{VLF} and ORR_{VLF} for comparison of the different systems.

Table 2
Comparison of operational and design parameters and performance of wetlands treating winery wastewater.

Area	Functional volume			Void liquid fraction			Reactor	Refs.
HLR	HLR_{Vol}	OLR_{Vol}	ORR_{Vol}	HLR_{VLF}	OLR_{VLF}	ORR_{VLF}	HTC	
mm. d^{-1}	$L.m^{-3}.d^{-1}$	g $COD.m^3.d^{-1}$	g $COD.m^3.d^{-1}$	$L.m^{-3}.d^{-1}$	g $COD.m^3.d^{-1}$	g $COD.m^3.d^{-1}$	$m^2.m^{-3}$ $WW.d^{-1}$	
14.6*	41.7*	32.9 to 124.5	26.6 to 114.5*	131.7*	103.9 to 393.2*	84.2 to 361.7*	50.0	(Akratos et al., 2020)
7.3*	20.8*	16.4 to 62.1	13.3 to 57.1*	66.4*	52.3 to 198.2*	42.4 to 182.3*	100	
14	14 (VF)	152* (VF)	NG	UTC	UTC	UTC	9.6	(De La Varga et al., 2013a; De la Varga et al., 2013b)
24.8	43–82 (HF_1)	54* (HF_1)	38.3*	107.5–205* (HF_1)	135* (HF_1)	95.9* (HF_1)	57.7	
36.3	22–41 ($HF_{2,3}$)	27* ($HF_{2,3}$)	19.1	55–102.5* ($HF_{2,3}$)	67.5* ($HF_{2,3}$)	47.9* ($HF_{2,3}$)	57.7	
77–215	55–154* (VF)	31–333* (VF)	NG	UTC	UTC	UTC	9.6	(Serrano et al., 2011)
13–36	43–120* (HF_1)	12–183* (HF_1)	3.5 to 128.1*	107.5–300* (HF_1)	107.5 to 457.5* (HF_1)	29–320.3* (HF_1)	57.7	
13–36	22–60* ($HF_{2,3}$)	6.0–92* ($HF_{2,3}$)	1.4 to 64.4*	55–150* ($HF_{2,3}$)	50.0–230* ($HF2_{,3}$)	3.5–161.0* ($HF_{2,3}$)	57.7	
34	28*	37–176*	35.9 to 174.2*	77.8*	102.8 to 488.9*	99.7 to 484*	29.8	(Shepherd et al., 2001a; Grismer et al., 2001; Shepherd et al., 2001b)
333*	150	152	120.1*	517.2*	524.1*	414*	17.8	(Holtman et al., 2018; Welz et al., 2018a)
23	1) 25*	1)350*	19.3 to 22*	65.7*	71.4 to 71.4*	55 to 62.8*	43.9	(Mulidzi and Africa, 2007; Mulidzi, 2010)
45	2) 50*	2) NG	UTC	142.9*	UTC	UTC	22.2	
78.3	57.0	260.4	177.0	196.6	897.9	610.3	13.3	This study Year 1
206.5	150.3	322.9	232.3	518.4	1113.4	801.1	5.6	This study Year 2

HF = horizontal (subsurface) flow COD = chemical oxygen demand VF = vertical (subsurface) flow, VLF = void liquid fraction. UTC = Unable to calculate, * = Calculated HLR_{Vol} = volumetric hydraulic loading rate, OLR_{Vol} = volumetric organic loading rate, ORR_{Vol} = volumetric organic removal rate HLR_{VLF} = void liquid fraction hydraulic loading rate, OLR_{VLF} = void liquid fraction organic loading rate, ORR_{VLF} = void liquid fraction organic removal rate, HTC = hydraulic treatment capacity, HLR = Surface loading rate.

In terms of the $HLR_{Vol,}$ results obtained during year 1 compared favourably with most other studies, while in year 2, the average HLR_{Vol} was higher than the other passive systems with the exception of other BSRs (Holtman et al., 2018; Welz et al., 2018a) and a gravel-filled horizontal flow CW (Serrano et al., 2011). The highest OLR_{Vol} applied was in a horizontal flow gravel-filled CWs at a large winery/distillery in South Africa (Mulidzi and Africa, 2007; Mulidzi, 2010). The primary reason for the high OLR_{Vol} was that the average influent COD was highest at this site (14,000 $mgCOD.L^{-1}$). Due to the average influent COD to the study BSRs being either higher (year 1: 4568 $mgCOD.L^{-1}$) or comparable (year 2: 2148 $mgCOD.L^{-1}$) to the remaining systems that were included in the comparison (535–4720 $mgCOD.L^{-1}$), and the relatively high HLR_{Vol}, comparatively high OLR_{Vol} were applied in the BSRs.

Most notably, in terms of the average $ORR_{Vol,}$ the BSRs substantially outperformed all of the other passive systems included in the comparison over the 2-year study period (Table 2). When the ORR considered only the void fraction of the different media (ORR_{VLF}), the difference in magnitude of the results obtained in the BSRs and the other systems increased even further (Table 2). These results strongly suggest that the sand used in the BSRs provides a superior matrix for attachment and activity of the functional microbes responsible for biodegradation of organics in WWW, a significant finding. The HLR, OLR and ORR of the BSRs during different modes of operation are provided in Table 3.

The BSRs were also compared with other passive treatment systems terms of the hydraulic treatment capacity (HTC) (Table 2), as systems that occupy large tracts of valuable land that can potentially be used for viticulture are not desirable (Bolzonella et al., 2019). The HTC was calculated as the area of land required to treat 1 m^2 of wastewater d^{-1} *HTC = Area/Flow* (Eq. 3). The spatial footprints of the BSRs were notably lower than all the other systems. Some of these systems consisted of more than one TW in series (Hirzel et al., 2017; Ramond et al., 2013; Welz and le Roes-Hill, 2014; Shepherd et al., 2001a), each having their own spatial footprint. The spatial footprint of the study BSRs of 13.3 $m^2.m^{-3}$ $WW.day^{-1}$ and 5.6 $m^2.m^{-3}$ $WW.day^{-1}$ for year 1 and 2 respectively was notably lower that for the other systems (22.2 to 150 $m^2.m^{-3}$ $WW.day^{-1}$).

Table 3
Comparison of parameters in different modes of operation: averages and (ranges).

Year	Filter	Mode	Period	Influent COD	RE	HLR_{Vol}	OLR_{Vol}	ORR_{Vol}
				$mg.L^{-1}$	%	$L.m^{-3}$ $sand.day^{-1}$	$gCOD.m^{-3}$ $sand.day^{-1}$	$gCOD.m^{-3}$ $sand.day^{-1}$
1	BSF1	Pulse	First	4541 (2800–5990)	59% (17–84)	74 (24–44)	288 (82–1236)	133 (33–294)
	BSF2	Continuous			52% (19–95)	91 (0–526)	433 (0–3151)	180 (0–1510)
1	BSF1	Continuous	Second	4619 (3990–5710)	93% (87–96)	13 (3–22)	59 (15–124)	54 (14–108)
	BSF2	Pulse			89% (83–96)	7 (0.1–15)	32 (0.4–67)	28 (0.3–62)
2	BSF1	Pulse	First	1265 (441–2310)	63% (20–87)	177 (52–540)	190 (42–394)	114 (22–257)
	BSF2	Continuous			67% (42–89)	208 (3–717)	216 (4–985)	130 (4–310)
2	BSF1	Continuous	Second	3583 (1217–6060)	85% (80–90)	102 (29–207)	311 (101–638)	266 (87–536)
	BSF2	Pulse			81% (64–89)	61 (0.5–176)	176 (3–591)	149 (2–502)

BSR = biosand reactor, RE = removal efficiency, HLR_{Vol} = volumetric hydraulic loading rate, OLR_{Vol} = volumetric organic loading rate, ORR_{Vol} = volumetric organic removal rate.

3.2. Comparison of pulse and continuous mode of operation

The BSR systems were designed to allow different modes of operation. While continuous operation is simple, some researchers have shown that increased ORR may be achieved with intermittent or pulse operation modes. In order to definitively establish the preferential mode of operation, each BSR was operated alternatively in continuous and pulse mode each year during the crush periods (Table 1) and results were compared (Table 3).

3.2.1. Hydraulic and organic loading rates

On average continuous mode had greater HLR, OLR and ORR, outperforming pulse mode by average magnitudes of 21%, 31% and 30% respectively over the 2-year experimental period (Table 3). The ORR_{Vol} of VOA was 28% higher in continuous mode, and there was no difference in total phenolic removal efficiencies between the two modes (results not shown).

While the organic degradation of WWW relies on the microbial populations in BSRs and other similar systems, the HC decreases, and the HRT increases as a consequence of the functional microbial biomass which attaches to the inert medium and reduces the porosity. This retards the flow, but the increased HRT typically results in higher ORR (Welz et al., 2018a). There is a critical balance between HLR (and HRT) and system operation and performance. Systems operated with low HLR require large treatment plants with associated capital and operational costs and large spatial footprints, while the performance of those operated with high HLR (and low HRT) may be inefficient (Holtman et al., 2018; Juwarkar et al., 2010; Semple et al., 2007). In BSRs, higher HLR can be achieved by manipulating the hydraulics within the system by: (i) increasing the fall, (ii) adjusting the loading rates, and/or (iii) intermittent operation (Juwarkar et al., 2010; Semple et al., 2007; Achak et al., 2009; Knowles et al., 2011). Generally, the top layer of media or area around the inlet of TWs/CWs has the greatest accumulation of biomass. Several operational methods have been used to negate excessive biomass build-up, namely: (i) back washing, (ii) scarifying the surface layer, (iii) applying higher HLR to flush the solids out, and (iv) changing the direction of flow or areas of ingress to allow accumulation of biomass to dissipate during the rest period (Nivala et al., 2012). Then there are methods which involve the total or partial removal and replacement or offsite cleaning of the media which does have significant financial constraints (Nivala et al., 2012). Some of these methods involve the degradation of the biomass within the systems themselves. In this study, it was envisaged that the intermittent aerobic/anoxic conditions created by fill and drain cycles during pulse mode operation would result in reduced biomass and concomitant increased HC within the BSRs as described by Nivala et al. (2012). In addition, the drain cycle introduces air into the media which exposes the biomass to O_2. The O_2 is used as a terminal electron acceptor for aerobic heterotrophic metabolic processes, which are energetically more favourable than anoxic or anaerobic metabolic processes (Nivala et al., 2012; Maier et al., 2009; Torrens et al., 2009). However, when the BSRs were operated in pulse mode, the sand remained saturated after the drain cycle. A significant increase in drain cycle time was required to achieve a meaningful aerobic period. It was decided that this was not warranted as the hydraulic treatment capacity would be severely impacted. In addition, it has previously been shown that, contrary to expectations, higher degradation of ethanol and phenolics in synthetic WWW takes place in lower redox environments in sand-filled treatment systems, albeit with accumulation of VOAs which are formed as metabolic by-products (Welz and le Roes-Hill, 2014; Welz et al., 2016; Welz et al., 2014). The higher accumulation of VOAs in continuous mode of operation supports these previous findings.

Overall, it is recommended that due to the comparative operational simplicity, greater volumetric hydraulic loading, organic loading, and organic removal rates, that the systems are operated in continuous mode.

4. Conclusion

This study showed that continuous mode of operation of BSRs outperforms pulse mode of operation in terms of HLR, OLR, ORR by 21%, 31% and 30% respectively. The novel BSR systems provide a small spatial footprint compared to similar passive treatment systems with a reduction in the reactors special footprint ranging from a 40% to 96%. The systems provide a conducive environment for functional microbial growth and activity, allowing treatment of WWW at high ORL_{VLF} with no evidence of permanent clogging. These systems require minimal outside interference and do not require skilled labour for operation. Future designs should have outlets at different heights spaced 200 mm apart to allow more simple manipulation of flow rates.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix 4 Journal article 3 offprint

water

MDPI

Article

Calcite Dissolution and Bioneutralization of Acidic Wastewater in Biosand Reactors

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Abstract: Acidic wastewaters such as winery wastewater require treatment to increase the pH before discharge into the environment. Biosand filters have been shown to reduce the organic load while simultaneously providing a buffering function. Previous research has shown increases in pH which was assumed to mainly take place via dissolution of calcite from the sand particles. This study investigated the possible role of biotic mechanisms for pH adjustment in sand column experiments by comparing results obtained from irradiated (biotic) and non-irradiated (biotic and abiotic) sand columns extracted from biosand filters used to treat winery wastewater. The columns were fed with either synthetic winery wastewater or filtered water (control). It was shown that the specific hydroxide concentrations in the eluant from the non-irradiated columns was significantly ($p < 0.05$) higher than in the eluant from the irradiated columns (1.1×10^{-5} vs. 4.0×10^{-6} M/kgsand^{-1}), indicating the presence of both biotic (average $4.5 \pm 0.13\%$) and abiotic (average $95.5 \pm 0.16\%$) pH increases. Using multivariate statistical tools to analyze a combination of parameters linked with biotic and abiotic pH adjustment, significant differences (ANOVA, $p < 0.05$) were found between the four treatment groups (irradiated/non-irradiated SWW and control) and the groups showed good clustering in cluster plots (group average) linkages, and principal component analysis plots.

Keywords: abiotic; biotic; carbonate; dissolution; microbial; sand; acid mine drainage

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1. Introduction

The process of making wine results in the generation of 0.2 to 14 L of typically organic rich, acidic, and sometimes saline winery wastewater (WW) for each litre of wine produced [1–3]. It has been shown that biosand reactors (BSRs) containing locally available dune sand have higher organic removal rates (ORR) and spatial footprints than other passive systems treating WW [4]. These systems are inexpensive to install and maintain and are well suited for remediation of WW for irrigation purposes because they are able to reduce the organic load while simultaneously increasing the pH and sodium adsorption ratio (SAR) of acidic WW [1,4,5].

Calcite and aragonite are mineralised forms of calcium carbonate ($CaCO_3$) that make up limestone. In a previous study conducted with BSRs, it was assumed that dissolution of calcite was the primary abiotic WW buffering mechanism [1] because the sand particles consisted of quartz (81%) and calcite (18%) as the dominant minerals [5,6]. Apart from buffering acidic WW, irrigation with WW containing calcium (Ca^{2+}) from $CaCO_3$ dissolution can potentially improve the quality of sodic soils by increasing the SAR [7,8].

Calcite dissolution reactions (Equations (1)–(3)) are reversible and take place at the solid-liquid interface [9]. The reactions are mediated by the pH and the partial pressure of carbon dioxide (CO_2). The first reaction (Equation (1)) involves protonation of $CaCO_3$ to Ca^{2+} and

bicarbonate (HCO_3^-). In the other reactions, carbonic acid (H_2CO_3) and water (H_2O) are the reactants responsible for $CaCO_3$ dissolution (Equations (2) and (3), respectively).

$$CaCO_3 + H^+ \leftrightarrow Ca^{2+} + HCO_3^- \quad (1)$$

$$CaCO_3 + H_2CO_3 \leftrightarrow Ca^{2+} + 2HCO_3^- \quad (2)$$

$$CaCO_3 + H_2O \leftrightarrow Ca^{2+} + HCO_3^- + OH^- \quad (3)$$

More traditionally, the addition of limestone, calcite, or other $CaCO_3$-rich residues such as eggshells, seashells or concrete aggregates have been applied for passive remediation of acid mine drainage (AMD) [10,11], with various buffering and metal precipitation reactions taking place [12,13]. Calcite dissolution is also used for the mineralisation of desalinated potable water [9]. This is usually achieved by the addition of sulfuric acid (H_2SO_4) or by flushing with CO_2 to form H_2CO_3, but it has recently been shown that dissolution using acetate (CH_3COO^-) results in superior potable water quality [9]. The excellent dissolution kinetics achieved with CH_3COO^- [9] suggest that WW may be an ideal calcite dissolution agent because (i) volatile fatty acids (VFAs) constitute up to 60% of the organic fraction of WW [2] and (ii) VFAs are formed during organic biodegradation in BSRs, with CH_3COO^- being the major contributor to the chemical oxygen demand (COD) in the final effluent [14].

The rate of $CaCO_3$ dissolution is also correlated with particle size as smaller particles provide larger overall reaction surface areas [5,11]. In the case of AMD, the Ca^{2+} can react with sulfate (SO_4^{2-}) to form gypsum ($CaSO_4$) on the particle surfaces, slowing dissolution reaction kinetics [13]. In addition, efficiency of passive calcite-based systems for treatment of iron (Fe)-rich AMD can be restricted by coating of the particles with Fe oxides [11]. These reactions should theoretically be limited in the case of WW because Fe and SO_4^{2-} concentrations are lower and acidity is more likely associated with the presence of organic acids [2].

Changes in pH can also be microbially mediated (bioneutralization). For example, generation of alkalinity (Alk.) and increased pH has been associated with consumption of H^+ by microbial denitrification, SO_4^{2-} reduction, and reduction of metals stimulated by the addition of organic carbon (OC) as an electron donor [15]. Concurrent biotic and abiotic neutralisation of acidic saline waters has been demonstrated in bioreactors containing either compost or municipal organic waste and limestone, where limestone dissolution accounted for 78–91% of Alk., and bacterial SO_4^{2-} reduction for 9–22% [16]. In alkaline leachates and other higher pH waters, reverse reactions (Equations (1)–(3)) can lead to $CaCO_3$ precipitation, with concomitant pH decreases [17]. The reaction rates can be increased by aeration and microbial release of CO_2 from organic substrates *viz.* concurrent biotic and abiotic mechanisms [17]. Haloalkalophilic bacterial fermentative generation of organic acids has also been associated with bioneutralization of alkaline bauxite residues [18,19].

Passive biochemical reactors (PBRs) for remediation of AMD typically contain organic microbial electron donors such as wood chips, chicken manure, leaf compost, and lignocellulosic waste and inorganic buffering agents such as calcite [20]. Such systems operate best at pH values between 5 and 8 [20]. If some cases, Fe reducing bacteria may compete for substrate with the SO_4^{2-} reducing bacteria, retarding SO_4^{2-} reduction rates [20]. Similar principles have been applied for the rehabilitation of degraded soils with high metal concentrations by re-saturating dried sulfuric soils [21]. The reduced conditions lead to the formation of metal sulfides, which is enhanced by the action of SO_4^{2-} reducing bacteria in the presence of electron donors from lignocellulosic organic matter [21].

In lab-scale BSRs containing sand with no detectable calcite, the pH of acidic WW increased from inlet to outlet, strongly suggesting that biotic neutralization mechanisms exist in BSRs [22]. It is plausible that electron donors for reduction reactions are supplied by the major organics ethanol (C_2H_5OH), VFAs, sugars, (poly)phenolics and other minor organics, the quantities of which vary on a seasonal basis and from winery to winery [2].

In order to verify the hypothesis that both biotic and abiotic buffering of WW (and other acidic organic wastewaters) occur in BSRs, sand columns were extracted from pilot

scale BSRs that had been operational for >2 years and contained functionally adapted microbial communities. Half of the columns were irradiated, and both irradiated and non-irradiated columns were fed with either (i) filtered water (controls), or (ii) synthetic WW (SWW). Buffering in irradiated columns was assumed to be completely abiotic, while biotic buffering was determined by comparing the results obtained from the non-irradiated with those obtained from the irradiated columns.

2. Materials and Methods

2.1. Column Experiments: Set-Up

Twelve core sand samples were extracted from pilot vertical flow BSRs that had been operational at a winery in the Western Cape, South Africa for over 2 years [5] (Figure 1). The composition of the dune sand from the quarry site (approximately 81% quartz and 18% calcite) has been described previously in detail [5,6]. Cores were extracted from the surface of the BSRs using acrylic pipes (40 mm OD and 30 mm ID) which were sharpened on one end to promote penetration into the sand. The pipes were pushed carefully into the sand with as little disruption to the structure of the material as possible. After extraction of approximately 350 mm sand, the cores were capped. Nine of the 12 cores were sterilized by irradiation at 30 kGy at a commercial facility as previously described [23].

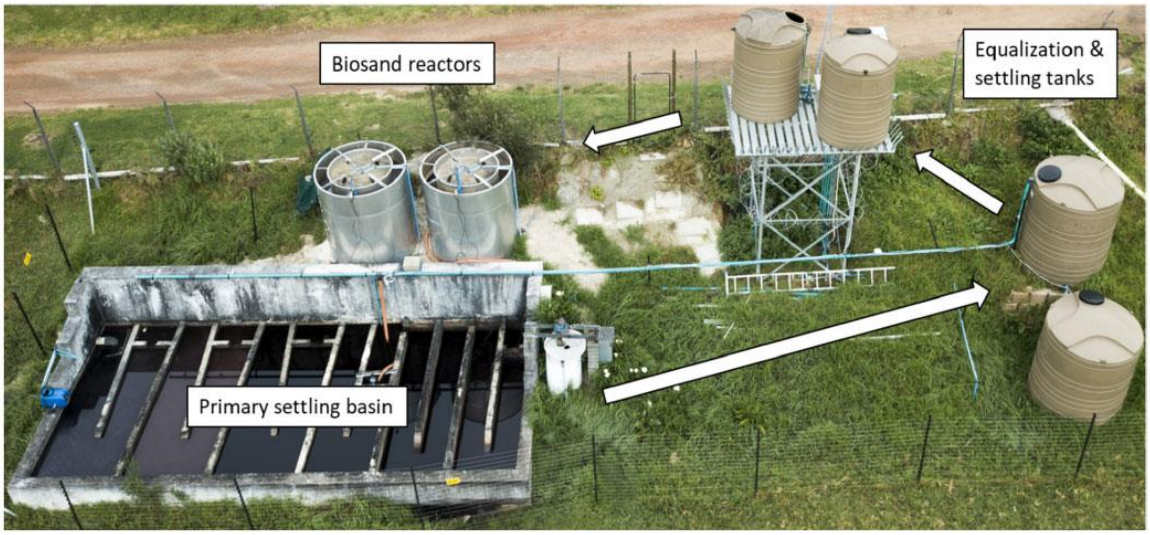

Figure 1. Set up of pilot biosand reactor system treating winery wastewater.

The cores were then set-up and used in flow-through column experiments (see graphical abstract for set-up). They were fitted with end pieces that retained the sand but did not impede the flow of liquid through the columns. Influent was pumped using individual infusing pumps via tubing ending in T-pieces onto the top of the sand and allowed to flow passively through the sand via gravity.

2.2. Operation of Column Experiments

In order to determine whether buffering (neutralization) of WW and possibly other acid wastewaters in BSRs is due to abiotic (notably via calcite dissolution), biotic (microbial) or combined biotic/abiotic factors, the irradiated and non-irradiated column replicates were fed each 24 h for 48 h with 400 mL of either: (i) filtered water (control), or (ii) synthetic WW with a pH 3.07. In addition, secondary negative controls consisting of fresh sand were fed with SWW (as per ii). The SWW components were added to give total COD (COD) concentrations of 1000 mg/L, made up of 500 mgCOD/L C_2H_5OH, 400 mgCOD/L acetic acid and 50 mgCOD/L gallic acid, 50 mgCOD/L vanillin, as previously described [23,24].

Each experiment was conducted in triplicate. In order to ensure robust microbial activity, the non-irradiated column experiments commenced within 4 h of coring. Each set of experimental replicates (n = 3) was fed with autoclaved influent from the same receptacle to ensure influent consistency. In order to reduce downstream microbial activity, the eluant was collected into separate autoclaved sealed beakers held within cooler boxes containing dry ice. The design operational parameters are provided in Table 1. The flow rate was set to mimic the hydraulic loading rate (HLR) of the BSRs from which they were extracted, which allowed full saturation of the sand in the columns without excessive pooling. The design HLR was calculated as previously described [1].

Table 1. Percentage calcium in sand from cores taken from biosand reactors treating winery wastewater.

Influent	Flow Rate (mL/h)	HLR ($L/m^3_{sand}.day^{-1}$)	OLR ($gCOD/m^3_{sand}.day^{-1}$)	HRT (h)
Control	8.3	808	NA	8.7
SWW	8.3	808	NA	8.7

Note: SWW = synthetic winery wastewater HLR = hydraulic loading rate OLR = organic loading rate HRT = hydraulic retention time NA = not applicable.

2.3. Eluant Sampling and Analytical Procedures

Composite eluant samples were taken after 24 h (0–24 h) and after 48 h (24–48 h). The pH was determined according to the manufacturer's instructions using a CyberScan pH300 meter (Eutech Instruments, Singapore) and appropriately calibrated pH probe PHWP300/02K (Eutech Instruments, Singapore). The pH was converted into OH^- concentrations before calculating the means and standard deviations from the mean. The electrical conductivity (EC) was determined using a hand-held Oakton ECTestr 11+ multi-range, cup-style pocket conductivity meter (Eutech Instruments, Singapore Cat No: 35665-35). This instrument is capable of reading conductivity with a range of 0 µS/m to 20.00 mS/m. The COD concentrations were determined on the same day of the sampling using a Merck (Merck®, Whitehouse Station, New Jersey, USA) Spectroquant® Pharo instrument and Merck Spectroquant® cell tests (cat. no. 1.14895.0001). Total Alk. was measured using the Merck titrimetric method with titration pipette MQuant catalogue number (1.111109.0001), according to the manufacturer's instructions. The organic composition of the eluants was determined using high pressure liquid chromatography (HPLC) as previously described [23,24]. At the end of the experiments, the sand in each column was allowed to drain for 24 h, dried and weighed (range 310–404 g) and the physicochemical results were specifically adjusted to account for the unavoidable variability in the amount of sand within each column to give specific values (per kg of sand).

2.4. Statistical Analysis

T-tests were performed using Microsoft Excel (Microsoft, Redmond, Washington, USA). Paired 2 sample for means T-tests were used to determine (i) significant differences between irradiated and non-irradiated experimental results, and (ii) correlations between physicochemical parameters for each column replicate. Two sample T-tests assuming unequal variances were used to determine significant differences between treatments (control and SWW). Differences were deemed significant if t-crit < t-stat and $p < 0.05$. Standard deviations (SD) were calculated as SD from the mean. Multivariate statistical analyses (principal component analyses (PCA), analysis of similarity (ANOSIM), and cluster analyses (group average linkage) on normalised physicochemical data using Primer 7®software (Primer-e, Auckland, New Zealand).

All statistical differences were deemed significant if $p < 0.05$ and $p \geq 0.001$ and highly significant if $p < 0.001$. These criteria are applied throughout the manuscript when referring to "significant" or "highly significant".

3. Results and Discussion

3.1. Operational Parameters

In order to closely mimic the physicochemical and microbial structures within the BSRs in the column experiments, the content of each column was kept intact from coring through experimentation. Although every effort was made to retrieve similar amounts of sand in each column (height approximately 350 mm), some variability in the weight of sand in each column was unavoidable (Table 2). This translated into some differences in the HLR and HRT in the columns, albeit in a relatively narrow range (Table 2). The measured HLR and HRT (Table 2) that were achieved were close to the design values (Table 1) based on the actual flow rates in the BSR from which the cores were extracted. The cores therefore provided a good approximation of the "real world" situation.

Table 2. Measured operational parameters (average ± SD and range, n = 3).

	Sand Height (mm)	Sand Weight (g)	HLR ($L/m^3_{sand}.day^{-1}$)	HRT (h)
Control	356 ± 8.4 (346–361)	383 ± 9.7 (375–394)	796 ± 19 (784–818)	8.81 ± 0.21 (8.57–8.94)
Control IR	352 ± 6.8 (344–357)	380 ± 8.7 (370–386)	805 ± 16 (793–822)	8.71 ± 0.17 (8.52–8.84)
SWW	358 ± 22 (337–380)	373 ± 30 (344–404)	792 ± 48 (745–840)	8.87 ± 0.53 (8.35–9.41)
SWW IR	363 ± 4 (359–367)	396 ± 4.5 (391–400)	779 ± 8.6 (771–788)	8.99 ± 0.10 (8.89–9.09)
SWW cont.	316 ± 5 (311–321)	376 ± 0 (376–376)	896 ± 14.2 (881–910)	7.83 ± 0.15 (7.70–7.95)

Note: HLR = hydraulic loading rate; HRT = hydraulic loading rate; IR = irradiated; SWW = synthetic winery wastewater; SWW cont. = control with new, unused sand.

3.2. Organic Biodegradation in Irradiated and Non-Irradiated Columns

Residual organics and inorganics were unavoidably present in the cores extracted from the working BSRs. Consequently, although no organics were added to the columns, residual COD leached into the column eluants (Figure 2a), adding to the experimental complexity. This can be seen by: (i) the high average specific COD concentrations (sp-COD) measured in the eluants from the control columns collected in the first 24 h, and (ii) the fact that the average spCOD was higher in the eluants collected over first 24 h (2527 $mgCOD/L.kg_{sand}^{-1}$) than provided in the influent (2831 $mgCOD/L.kg_{sand}^{-1}$) over the same period in the columns fed with SWW (Figure 2a).

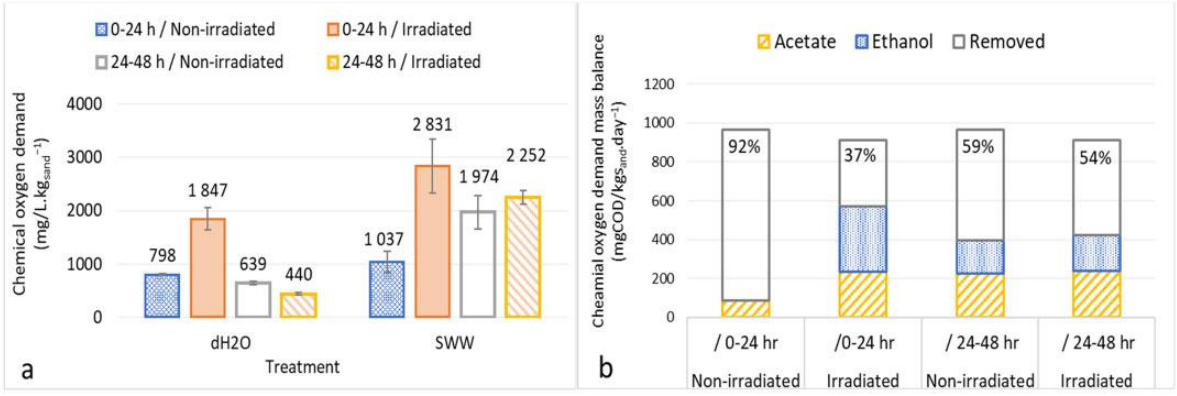

Figure 2. Specific chemical oxygen demand measurements for all the column replicates (**a**) and mass balance for ethanol and acetate added to the columns feed with synthetic winery wastewater (**b**).

The large and highly significant differences in average spCOD in the eluant collected from the irradiated columns of 1049 and 1804 mgCOD/L.kg_{sand}^{-1}, from the control columns and those fed with SWW, respectively, proved that good biodegradation rates were achieved in all the non-irradiated columns. However, no significant differences were found in the eluants that were collected after the first 24 h (24–48 h). It was therefore assumed that there was a temporal loss of sterility in the irradiated columns. Radiation is the preferred method for sterilizing sand, soil and sediment as it has minimal effects on the physicochemical properties of the substrate compared with other methods such as autoclaving [23,25,26]. Although sterile procedures were used and the radiation levels (30 kGy) were theoretically sufficient, factors such as shading and the presence of radiation resistant bacteria or spores can result in renewed microbial growth over time in substrates such as sand [23,25,26]. In summary, the highly significant differences in the spCOD measurements in the eluants from all the irradiated and non-irradiated columns over the first 24 h confirmed biotic activity and the relevance of the biotic/abiotic experiment, but indicated that interpretation of the physicochemical results are less relevant after 24 h.

For the columns fed with SWW, the OLR for the irradiated and non-irradiated columns were 772 ± 46 (range 726–819) gCOD/L.kg_{sand}.day^{-1} and 760 ± 8.4 (range 752–768) gCOD/L.kgs_{and}.day^{-1}, respectively, providing a reasonable approximation of the design values (831 gCOD/L.kg_{sand}.day^{-1}) calculated for the operational BSR. Selected organics were measured in the eluant samples to substantiate organic biodegradation. Samples were screened for sugars, glycerol, C_2H_5OH, VFAs (CH_3COO^-, propionate, butyrate), and selected phenolics (vanillin, gallic acid, vanillic acid, catechol). If present, the concentrations were measured. In the eluant from the control columns, random and negligible amounts of fructose and glycerol were detected (<8 mg/L average per experimental triplicate and CH_3COO^- was also detected (0–32 mg/L), but only in the eluants collected during the first 24 h. No particular trends were discernible between replicates. This was not unexpected as the columns had been extracted from different spatial locations within the BSRs and some variability was inevitable.

For the columns fed with SWW, mass balances were determined for the amounts of C_2H_5OH and CH_3COO^- that were added in the influent and collected in the eluants. Over the first 24 h, all of the C_2H_5OH was removed in the non-irradiated columns, with 92% spCOD removal, while only 26% of the C_2H_5OH was removed in the irradiated columns, with only 37% spCOD removal (Figure 2b). These removal rates are likely over-estimated as existing WW from the BSR system would have eluted out first from the columns. The C_2H_5OH and CH_3COO^- biodegradation rates were similar in the eluants collected from the irradiated and non-irradiated columns between 24 and 48 h (overall 54% and 59%, respectively). By way of comparison, when operated in continuous mode, the COD removal rates achieved during the crush periods in the vertical flow BSR system that the cores were extracted from ranged from 37% to 95% in year 1 of operation (including the start-up period), and 42% to 90% in year 2 of operation (Figure 3a).

The C_2H_5OH/CH_3COO^- mass balances therefore proved unequivocally that biodegradation was taking place preferentially within the non-irradiated columns during the first 24 h and that there was a temporal loss of sterility in the irradiated columns.

It was not possible to identify the exact biotic neutralization mechanisms that took place within the cores due to: (i) the complexity and variability of the substrate (WW residuals) already existing within the pore spaces of the extracted cores, (ii) the possible presence of multiple biotic neutralization mechanisms, and (iii) the fact that elucidation of these mechanisms would require addition and monitoring of a range of different chemicals such as sulfates and nitrates which was not feasible in the context of this study.

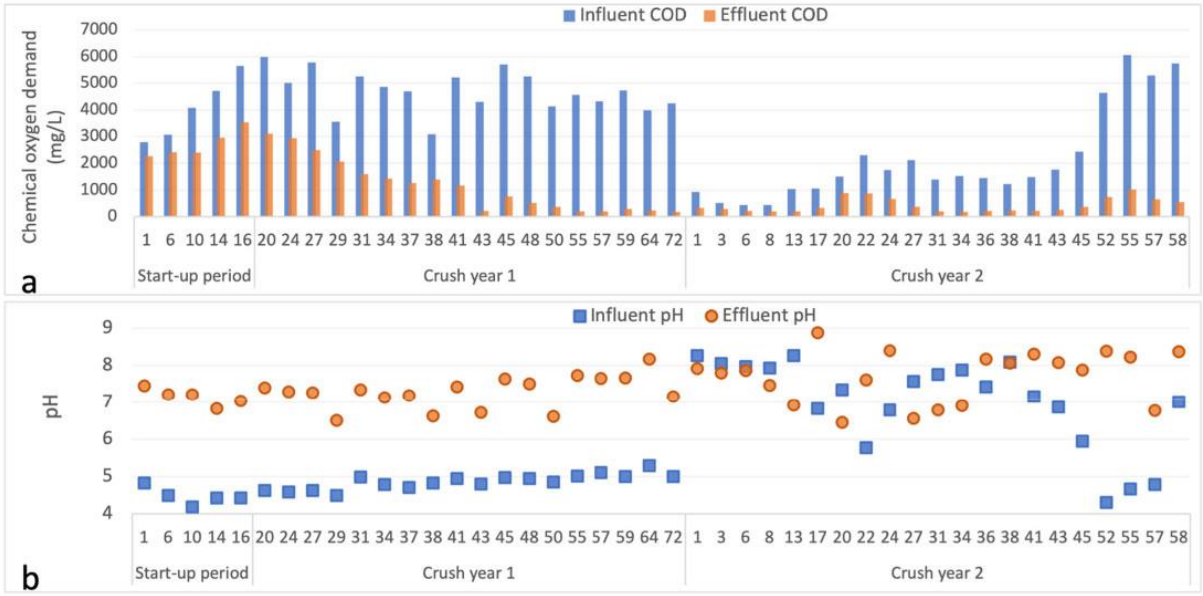

Figure 3. Chemical oxygen demand (**a**) and pH (**b**) measurements from the pilot biological sand reactor system (adapted from [4]).

3.3. Analysis of Eluant Hydroxide Ion, Alkalinity and Calcium Concentrations and Electrical Conductivity

In the pilot BSR system, the influent pH ranged from 4.2 to 8.3, but the eluant pH was maintained in the range of 6.5 to 8.9 (Figure 3b). In comparison, the eluant pH values from the columns ranged from 7.9 to 8.8 with influent pH values of 6.7 and 3.07 for the control (filtered water) and SWW, respectively, demonstrating similar functionalities between the BSR and columns.

Due to the loss of sterility in the columns after 24 h and the fact that the focus of the study was on determining the presence of biotic pH adjustment mechanisms, the discussion on the physicochemical analyses is mainly concentrated on the results obtained within the first 24 h. Details on the primary abiotic neutralization mechanism, namely, calcite dissolution, as well as the changes in the sizes and shapes of the calcite particles has previously been described in detail [5].

The specific OH^- concentrations ($spOH^-$) (Figure 4a) were significantly higher in the eluants collected from the non-irradiated than the irradiated columns for the control columns as well as those fed with SWW during the first 24 h of the experiment, clearly demonstrating a biotic component to increased pH. This was substantiated by the fact that there was no significant difference in the $spOH^-$ in the eluants from the irradiated and non-irradiated samples collected between 24 and 48 h. It was hypothesized that the temporal loss of sterility in the irradiated columns allowed both biotic and abiotic pH adjustments to take place simultaneously in all columns after 24 h (in contrast to only abiotic mechanisms in the irradiated columns initially). The average biotic and abiotic contributions to $spOH^-$ increases were calculated as 4.5 ± 0.13% and 95.5 ± 0.16%, respectively. This is likely an over-estimate as some microbial growth in the irradiated columns may have commenced before 24 h.

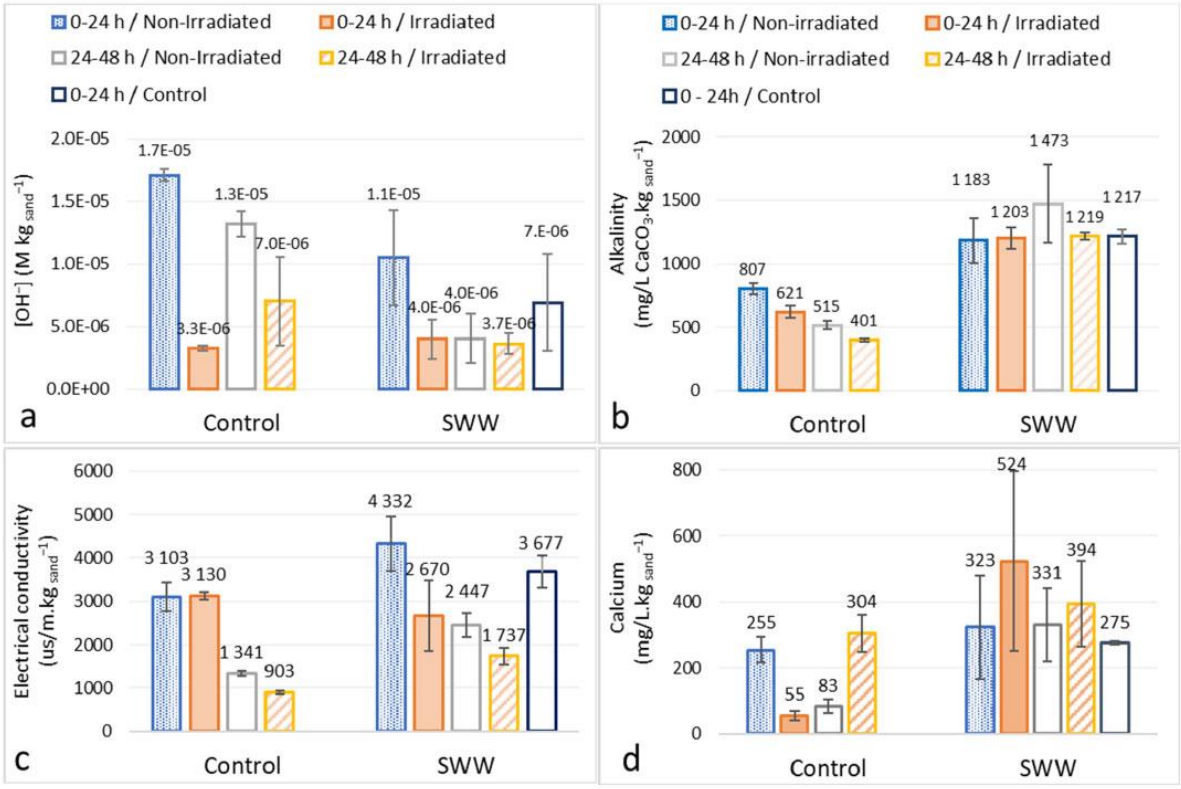

Figure 4. Average specific concentrations of hydroxide ions (**a**), alkalinity (**b**), electrical conductivity (**c**) and calcium (**d**) in column eluent (n = 3 replicates, error bars represent standard deviation from the mean).

With the exception of spCa and spAlk. (Pearsons' = 0.821), there were no significant correlations between the inter-related spOH$^-$, spAlk. spEC and specific Ca (spCa), the parameters most likely to reflect pH adjustment via calcite dissolution and/or biotic factors. There were no significant differences in the specific alkalinity (spAlk.), but highly significant differences in the specific EC (spEC) measurements in the 0–24 h eluants from the irradiated and non-irradiated columns fed with SWW (Figure 4b,c). In the case of spAlk. and specific SpCa, the measurements were significantly higher in the eluants from columns fed with SWW than from the control columns, indicating good solubilisation of calcite by the SWW as previously shown with CH_3COO^- [9].

It has previously been shown that with fresh sand, abiotic calcite dissolution kinetics and mass balances can be accurately calculated using the Ca concentrations in the eluant from sand columns as a proxy for $CaCO_3$ dissolution [5]. Using the calculated mass-balances from the column experiments in conjunction with historical calcium loss data obtained from the sand in a pilot BSF system in-situ at a winery, it was also shown the calcite would not be expended within the feasible lifespans of such BSF systems treating WW [5]. However, in this study, the eluant spCa concentrations were highly variable, and did not exhibit a distinct pattern (Figure 4d), indicating the complex nature of this 'real world' study using sand extracted from pilot BSRs, necessitated by the need to obtain functional microbial communities acclimated to WW.

It has also been previously been shown that laboratory-scale BSRs containing river sand with minimal calcite (<0.3% Ca) were able to increase the pH of diluted WW from 4.2 to 7.7 [6,22]. The lack of calcite in this system suggested that biotic pH adjustment was the primary WW neutralization mechanism, but this was not substantiated and only a

limited number of samples were taken. This study has shown unequivocally that biotic pH adjustment can occur in BSRs treating WW. This is noteworthy because increases in the pH of acidic WW (and possibly other acidic organic effluents) may continue once calcite has been expended or can occur in instances where calcite-poor sands are employed. However, the use of calcite-containing sand is still recommended because the addition of Ca to the final effluent reduces the SAR and protects the receiving environment from becoming sodic if the effluent is used for irrigation purposes [1]. In such systems, the SAR and effluent pH should be monitored to the ensure that the receiving soils are protected, as previously described [5].

Not only were residual organics present in the pore water of the columns, but also other dissolved solids, most notably Na and K, which are commonly found in high concentrations in WW. By examining the Ca, Na and K concentrations measured in the eluant of all the replicates for the first (Figure 5a) and second (Figure 5b) 24-h period, it is clear that there was considerable variation in the character of the interpore WW, and that complex biotic and abiotic interactions were responsible for leaching of inorganics from the columns. As alluded to previously, univariate statistical analyses only showed a significant correlation between spCa and spAlk, demonstrating the effect of confounding variables on the study results.

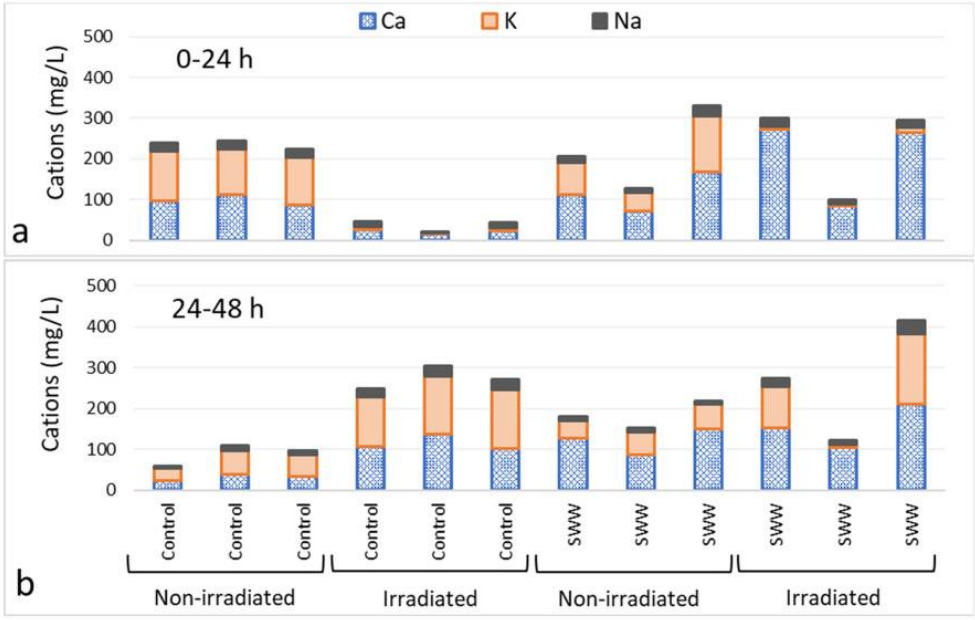

Figure 5. Major cations measured in the eluant from each column replicate for the first (**a**) and second (**b**) day of the experiment. Figure (**a**) is vertically aligned with (**b**).

In summary, the trends towards: (i) higher values of indicator parameters in the eluant from columns fed with SWW in comparison to those fed with filtered water and (ii) differences in values of indicator parameters in eluants from irradiated and non-irradiated columns, showed that: (i) SWW increased the solubilisation and leaching of Ca and other dissolved salts from the columns, (ii) solubilisation and/or leaching was increased by both biotic and abiotic interactions (iii) both biotic and abiotic mechanisms were responsible for pH increases in the eluant from inlet to outlet in non-irradiated columns.

However, due to the presence of confounding variables as a consequence of the organic and inorganic residuals within the columns, results were not consistent for the indicator parameters used to demonstrate biotic and abiotic pH adjustment mechanisms.

Multivariate statistical analyses were therefore used to assess the indicator parameters spAlk., spEC and spOH$^-$ simultaneously (Figure 6).

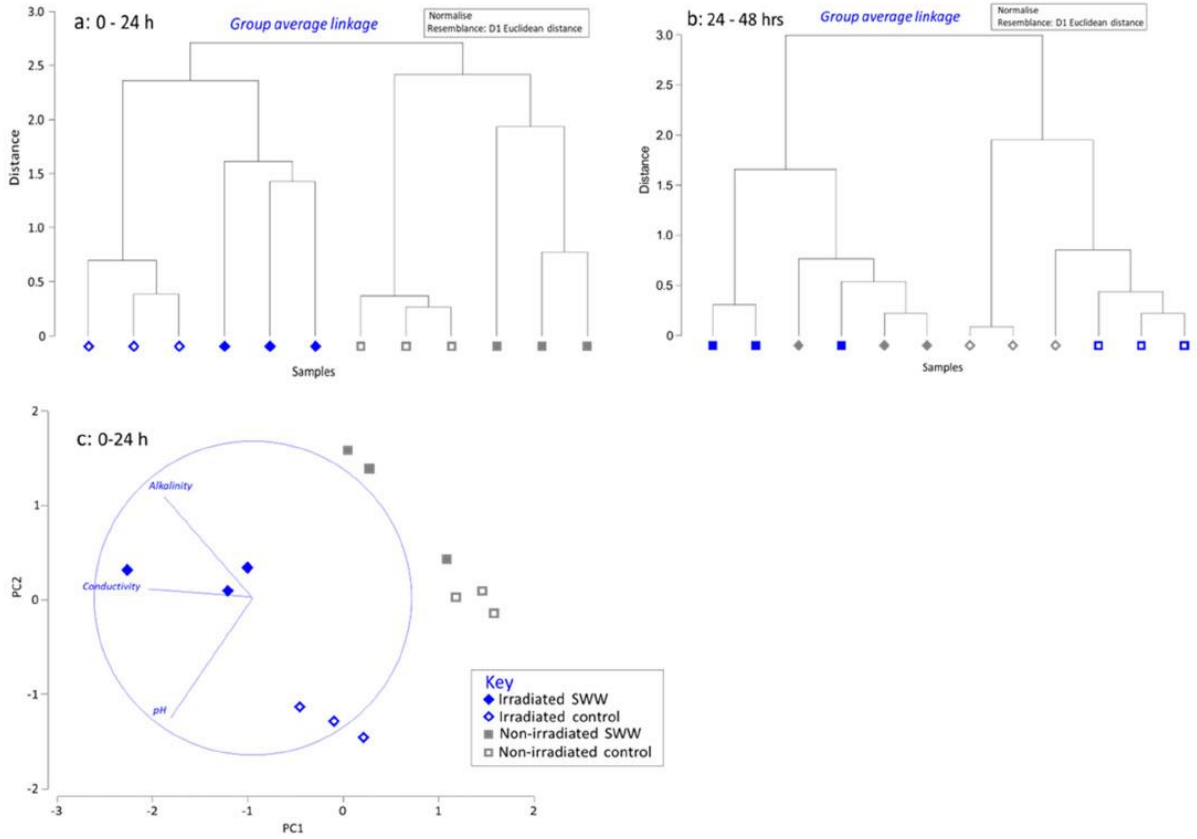

Figure 6. Cluster plots of selected physicochemical parameters showing the different treatment groups in eluant samples taken between 0–24 h (**a**) and 24–48 h (**b**), and principal component analyses of the same data from eluant samples taken between 0–24 h (**c**).

For combined spOH$^-$, spAlk and spEC eluant measurements, there were significant (ANOVA) inter-group differences and intra-group similarities (irradiated SWW, non-irradiated SWW, irradiated control, non-irradiated control). In samples taken during the first 24 h of the experiment, the replicates fell into four distinct clusters (Figure 6a). In samples taken between 24 and 48 h, the irradiated and non-irradiated groups formed two initial clusters, but there was less distinction between the different treatments (SWW or control) (Figure 6b). Despite the loss of sterility, it was expected that the irradiated and non-irradiated column eluants would have different characteristics over the course of the experiment because of the higher biotically induced leaching of residuals in the first 24 h from the non-irradiated columns (Figure 5). Overall, the multivariate analyses, including the PCA (Figure 6c), validated the assumptions made using the univariate analyses results.

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Conflicts of Interest: The authors declare no conflict of interest.

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Appendix 5 Journal article 4 offprint

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Effect of Particle Character and Calcite Dissolution on the Hydraulic Conductivity and Longevity of Biosand Filters Treating Winery and Other Acidic Effluents

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Abstract: Acidic effluent such as winery wastewater is challenging to remediate. Biological sand reactors can simultaneously remove organics and neutralize winery wastewater via biotic and abiotic mechanisms. The systems have been shown to be suitable for treating the intermittent flow of wastewater at small wineries. It has been shown that dissolution of calcite is the most important abiotic mechanism for increasing the pH of the influent. In this study, sand column experiments were used to determine the effects of (i) sand particle size distribution on calcite dissolution kinetics, and (ii) the effects of calcite particle dissolution on the hydraulic conductivity. The results were then used to calculate the theoretical temporal abiotic neutralization capacity of biological sand reactors with differently sized sand fractions, including unfractionated (raw) sand. The results were compared with those determined from a pilot system treating winery wastewater over a period of 3 years. Sand fractions with larger particles contained lower amounts of calcite (using Ca as a proxy), but exhibited higher hydraulic conductivities (3.0 ± 0.05 %Ca and 2.57 to 2.75 mm·s^{-1}, respectively) than those containing smaller particles and/or raw sand (4.8 ± 0.04 to 6.8 ± 0.03 %Ca and 0.19 to 1.25 mm·s^{-1}, respectively). The theoretical abiotic neutralization capacity of biological sand reactors was compared with a pilot system with the same flow rates, and a temporal abiotic neutralization capacity of 37 years was calculated for biological sand reactors, which compared favorably with the theoretical results obtained for wastewater with pH values between 2 (8.2 years) and 3 (82 years). It was concluded that biological sand filters with around 10% calcite will be able to abiotically neutralize winery wastewater and other wastewaters with similar acidities for the projected life span of the system. Future work should focus on determining the effect of sand grain size on the bioremediation capacity, as well as the use of biological sand reactors for treating other acidic organic wastewaters such as fruit processing, food production and distillery wastewater.

Keywords: mineralogy; neutralization; remediation; sand particles; wastewater; winery wastewater; water treatment; biological remediation

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1. Introduction

In the cycle of production, development and processing of raw material to a final product, waste and/or wastewater (WW) is generated. The global shortage of clean water is aggravated by the rapid expansion of industries and the subsequent increase in the overall volume of WW, including acidic WW, which has become a global environmental challenge [1–3]. If the acid WW is not sufficiently remediated, there is potential for adverse impacts on the environment and on human health [2,4]. If the WW is treated before discharge, the energy and cost requirements as well the quality and quantity of the acid WW inform the choice of treatment method. Physicochemical methods include adsorption, extraction, distillation, and membrane filtration [5–7]. However, these treatment systems

require large capital outlays and skilled personnel which may not be economically feasible for some industries, especially those that do not generate large quantities of WW. Wineries typically generate acidic WW that is highly seasonal in quantity and quality, but most smaller wineries cannot afford to install, maintain and operate sophisticated WW treatment systems.

Biosand reactors (BSRs), otherwise known as biological sand filters (BSF) or unplanted constructed/treatment wetlands are cost-efficient, low maintenance treatment systems that are able to effectively biodegrade the organic fraction of winery wastewater (WWW). Operational results from a pilot horizontal flow system as well as a more advanced vertical flow system with a novel hydraulic design have shown that the systems are also effective in neutralizing acidic WWW while increasing the sodium adsorption ratio (SAR) of the effluent [8,9]. The neutralization mechanism has been attributed to abiotic dissolution of calcite present in the sand [8,9] and may be applicable to other forms of acidic WW, similar to passive systems for treatment of acid mine drainage [10,11].

While there are in depth reports in the literature on determining and modelling particle shapes in sand [12–14], and it has been shown that stress pressure is the major factor influencing the size and shape of sand particles, including calcareous particles [15], there is little information in the literature comparing the shape and size of particles in sand with mixed mineralogy. Uing QEMSCAN® analyses [16,17], demonstrated differences in particle shape related to minerology in dune sand containing approximately 18% calcite and 81% quartz. In this case, it was found that the calcite particles were more angular and less round than the quartz particles. As shape plays a major role in particle packing, the hydraulic conductivity (HC) of the sand could not be predicted accurately using existing models which assume particle sphericity [17].

The dissolution kinetics of $CaCO_3$ from the calcite is affected by the pH and partial pressure of CO_2. Three heterogenous reactions usually take place simultaneously at the solid–liquid interface, namely: solid surface protonation where the H^+ ions in solution diffuse to the solid surface (Equation (1)), surface interaction with carbonic acid (H_2CO_3) where it adsorbs to the surface of the calcite (Equation (2)), and surface hydration where the H_2O migrates to an active site (Equation (3)) [18]. The exothermic dissolution reaction (Equation (4)) results in an increase in temperature of the solution and a decrease in calcite solubility [19]. Once solubilised, the products desorb into solution and migrate away from the reaction sites into the bulk solution [18]. Solubilisation is driven at lower pH values, while reprecipitation can take place when the pH of the WWW increases. While BSRs are effective at neutralizing acidic WWW, the longevity of the abiotic calcite dissolution process is unknown and cannot be modelled using kinetics due to the seasonal variability in the quality and quantity of this effluent, both inter- and intra-winery [8,9,20]. In addition, flow rates are confounded by the attachment of functional microbial biomass to the sand particles creating ever-changing porosities in the sand matrix [17]. The pH of the liquid is the most important parameter affecting calcite dissolution. Although WWW generally has a low pH and high sodium (Na) concentration, the pH varies from acidic to alkaline depending on the seasonal cellar practices taking place at the time of WWW generation [21,22].

$$CaCO_3 + H^+ \leftrightarrow Ca^{2+} + HCO_3^- \quad (1)$$

$$CaCO_3 + H_2CO_3 \leftrightarrow Ca^{2+} + 2HCO_3^- \quad (2)$$

$$CaCO_3 + H_2O \leftrightarrow Ca^{2+} + HCO_3^- + OH^- \quad (3)$$

$$CaCO_3 \leftrightarrow Ca^{2+} + CO_3^{2-} \quad (4)$$

This study was conducted in order to ascertain: (i) How BSR systems may function over time in terms of calcite removal *viz* how long before the abiotic neutralizing capacity is expended, (ii) Whether calcite dissolution kinetics can be improved by using different sand size fractions in BSRs, (ii) Whether calcite dissolution positively or negatively affects

the HC of fractionated and/or raw sand, (iii) How results obtained from *ex-situ* column experiments may relate to field data from operational BSRs.

2. Materials and Methods

2.1. Column Experiments: Set-Up and Operation

The experimental set-up (Figure 1) consisted of a series of identical clear acrylic columns with internal diameters of 30 mm and lengths of 500 mm. The particle size distribution of the raw sand was determined (Section 2.4.2), and the sand was then partitioned into six different size fractions. Each column was filled with 100 g of Dune quarry sand from Philippi, Cape Town, South Africa with either raw (unfractionated) sand or sand with different size fractions (Table 1). The bottom caps of the columns consisted of stainless-steel screens and open cell polyurethane that retained the sand but did not impede the flow rate. The sand was saturated and allowed to settle for 24 h and the HC was determined by the falling head method using tap water as described in Section Calculation of Hydraulic Conductivity by the Falling Head Method. The columns were then placed in a 37 °C constant environment room overnight to allow the sand to dry.

Each column was dosed continuously with 2.5 L of either 0.1 M HCl (tests) or distilled water (dH_2O) (negative control) using IVAC volumetric pumps (Model 597) at a flow rate of 10 $mL \cdot h^{-1}$ for a total of 10.4 days at a hydraulic retention time (HRT) of approximately 1.8 h and a hydraulic loading rate (HLR) of ±3850 $L \cdot m^{-3}$ of sand·day^{-1}. The volume of influent was theoretically calculated according to Equation (1) to ensure complete dissolution of calcite in the original fraction of sand. The effluent was collected in enclosed Erlenmeyer flasks which were emptied daily, and the contents from each column was pooled and stored at 3 °C until the end of each respective column experiment. After the dosing period, the final HC was measured and the sand was allowed to dry at 37 °C and then weighed. Each experiment was conducted in triplicate.

Figure 1. Experimental set-up.

Table 1. Sand particle size fractions in columns (100 g per column).

Column (n = 3)	Particle Size (mm)	Amount (% wt.wt)	Ca in Sand (% wt.wt)	Comments
1 & 2	>1.00–2.00	10.0	5.4 ± 0.01	Raw (unfractionated) sand
	>0.600–1.00	24.3		
	>0.425–0.600	18.2		
	>0.300–0.425	19.7		
	>0.150–0.300	25.3		
	>0.075–0.150	2.1		
	<0.075	0.4		
3	>1.00–2.00	29.2	3.0 ± 0.05	Homogenized fractions
	>0.600–1.00	70.8		
4	0.425–0.600	48.0	6.7 ± 0.07	Homogenized fractions
	>0.300–0.425	52.0		
5	0.150–0.300	91.0	6.8 ± 0.03	Homogenized fractions
	>0.075–0.150	7.6		
	<0.075	1.4		
6	1.00–2.00	13.9	4.8 ± 0.04	Homogenized fractions
	>0.600–1.00	13.7		
	>0.425–0.600	25.2		
	>0.300–0.425	27.3		
7	1.00–2.00	10.3	5.6 ± 0.019	Homogenized fractions
	>0.600–1.00	24.9		
	>0.425–0.600	18.7		
	>0.300–0.425	20.2		
	>0.150–0.300	25.9		

2.1.1. Calculation of Operational Parameters

Calculation of Hydraulic Conductivity by the Falling Head Method

The HC or co-efficient of permeability (k) of a porous media is the ease of which water passes through it and is defined by Darcy's Law. This can be rewritten in terms of the falling head conditions as in Equation (5):

$$k = (2.303aL/At)\cdot Log_{10}(h_1/h_2) \quad (5)$$

where a is the cross-sectional area of the standpipe, L is the length of the porous media, A is the cross-sectional area of the porous media, t is the time the liquid takes to drop from h_1 to h_2, and h_1 and h_2 are the start and stop levels above the outlet. In this study, in the HC experiment, h_1 and h_2 were 470 mm and 145 mm above the stainless-steel sieve, respectively. The times taken for the water to drop from h_1 to h_2 were recorded with a stopwatch. Each measurement was conducted in triplicate.

2.1.2. Hydraulic Loading Rate

The HLR was calculated in terms of the volume of sand within the column as previously described [8] and was on average 2244 $L{\cdot}m^{-3}$ of $sand{\cdot}day^{-1}$.

2.2. Biosand Filters: Set Up and Operation

A novel pilot scale BSR/BSF was installed and operated at a small cellar within the Western Cape, South Africa. The system consisted of four units (1.73 × 1.05 × 0.42 m) filled with a total volume of 7.26 m^3 sand which were gravity fed and operated in horizontal subsurface flow mode (Figure 2). The system treated approximately 402 $L{\cdot}day^{-1}$ or 137 $L{\cdot}m^{-3}$ of $sand{\cdot}day^{-1}$, and the performance results have been published elsewhere [8].

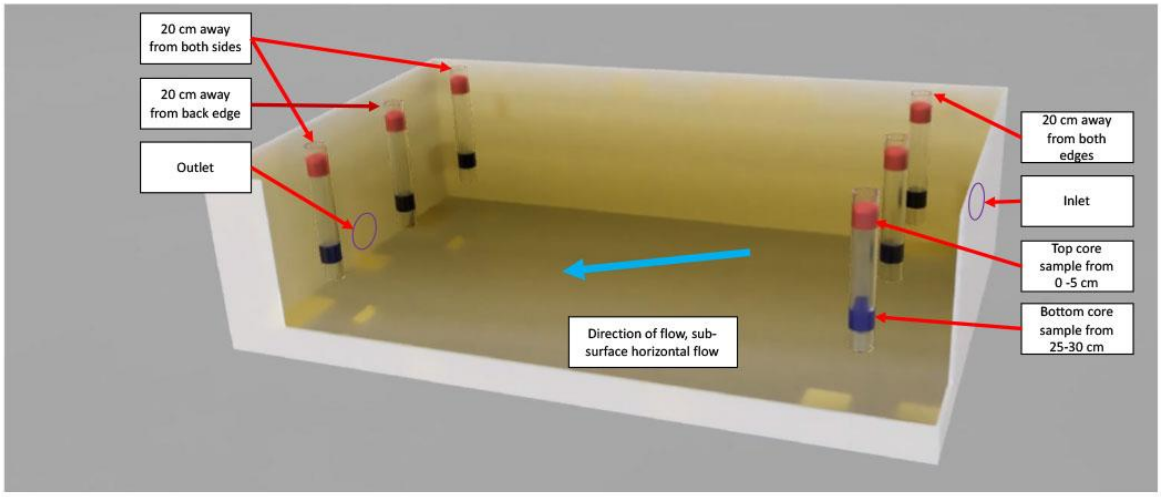


Figure 2. Sample locations of onsite treatment system.

2.3. Sampling

For the column experiments, the effluent from each column was collected and analysed daily, then pooled and stored at 3 °C until the end of each experimental run. Samples of homogenised fractions and raw unfractionated sands (Table 1) were set aside before the start of the experiments. At the end of the experimental period, the contents of each column were homogenised and sampled.

In order to compare the *ex-situ* experimental data obtained with the columns with *in-situ* operational data, core samples were extracted from each of the 4 BSR modules each year for three years. At each sampling instance, six sand cores were extracted from each module (three from the inlet and three from the outlet, as shown in Figure 2). The contents of each core were partitioned into samples from the top (0–5 cm below the surface) and bottom (25–30 cm below the surface) of the BSRs. A total of 144 samples were collected (48 each year). All the sand samples were dried thoroughly before further analyses.

An additional set of samples were taken at 3.2 years at the inlet, middle and outlet from one filter at (0–5 cm below the surface) and middle (25–30 cm below the surface) bottom (45–50 cm below the surface) of the BSRs.

2.4. Analytical Procedures

2.4.1. Effluent

The pH of the daily and pooled effluent was determined according to the manufacturer's instructions using a CyberScan pH300 meter and appropriately calibrated pH probe PHWP300/02K (Eutech instruments, Singapore). Concentrations of Ca in the pooled effluent samples were determined using a Thermo ICap 6200 ICP-AES plasma optical emission spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) at the Central Analytical Facility at Stellenbosch university (Stellenbosch, South Africa) according to the manufacturers' instructions.

2.4.2. Sand

The particle size distribution of the sand was performed according to SANS 3001 (Method AG1, PR5, AG21) by Cetlab, South Africa.

The sand samples were ground to a fine powder by swing milling and the elemental composition was determined by the Central Analytical Facility by X-ray fluorescence (XRF) spectrometry on a PANalytical (Almelo, The Netherlands) wavelength dispersive spectrometer according to the manufacturer's instructions.

Fractionated samples of sand were analysed by automated scanning electron microscopy using a FEI QEMSCAN® (Quantitive Evaluation of Minerals by SCANning Electron Microscopy) instrument as previously described [23] (Thermo Fisher Scientific, Waltham, MA, USA) and iDiscover™ software. The roundness and aspect ratios of the different mineral fractions determined using the iDiscover™ software were used to compare the shapes and sizes of particles with different mineral compositions according to the method described by [16].

3. Results and Discussion

3.1. Calcite Dissolution in Raw Sand and Fractionated Sand: Column Experiments

The concentration of Ca was measured as an equimolar proxy for dissolution of $CaCO_3$ from the calcite particles (Equations (1)–(3)). In order to cross check $CaCO_3$ solubilisation, the total amount of Ca captured in the column effluent was compared with the total amount of Ca lost from the sand in the columns (Figure 3A). Results were generally in good agreement with one another, with no significant differences between the amount of Ca lost and Ca captured ($p > 0.05$, paired T-test).

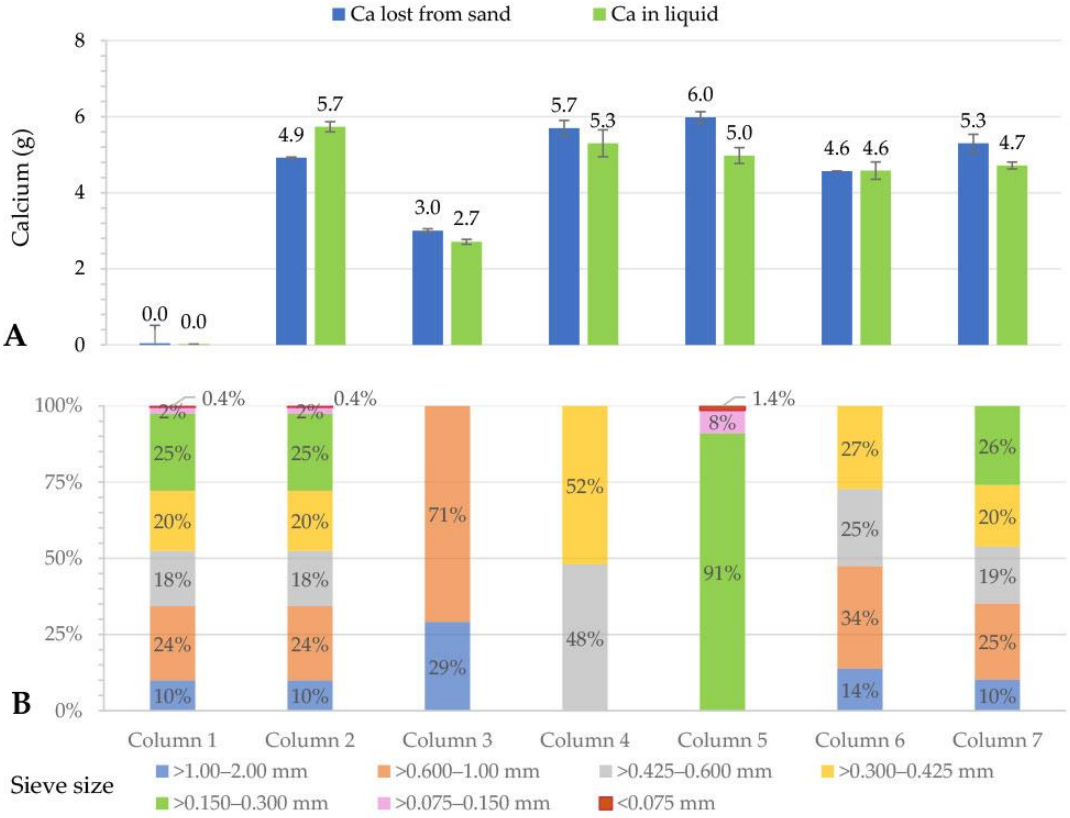


Figure 3. Mass balance of the calcium captured in the effluent and the calcium lost from the sand (**A**), and the distribution of grain size fractions in the columns (**B**). The bars from plot A are aligned vertically with those from plot B, both representing columns 1 to 7.

Only negligible amounts (<1%) of $CaCO_3$ were solubilised from the negative control columns containing raw unfractionated sand treated with dH_2O (column 1, Figure 3). In the case of the test columns, less $CaCO_3$ was mineralised in the columns containing the larger sand particles (column 3) than in the other columns. This was an anomaly unique to

column 3 because: (i) the initial Ca concentration in the sand (3% wt.wt) was lower than in the other columns (4.8–6.8% wt.wt, Table 1), (ii) 99% of the Ca was removed during the experimental period, and (iii) the neutralization endpoint was achieved between day 6 and day 7 of the experiment (Figure 4). In comparison, neutralization endpoints were achieved after 10 days in the columns containing raw sand (column 2), and those with medium/large particles sizes only (columns 6 and 7), with 91%, 96% and 95%, respectively, of the Ca being solubilised. In contrast, only 84–85% of the Ca was solubilised in the columns that contained only small sand particles (columns 4 and 5), and no neutralization endpoint was achieved after 11 days. However, these columns contained the highest Ca concentrations (≥6.7 g), and the amount of HCl solution added was theoretically calculated to solubilise 5.00 g $CaCO_3$.

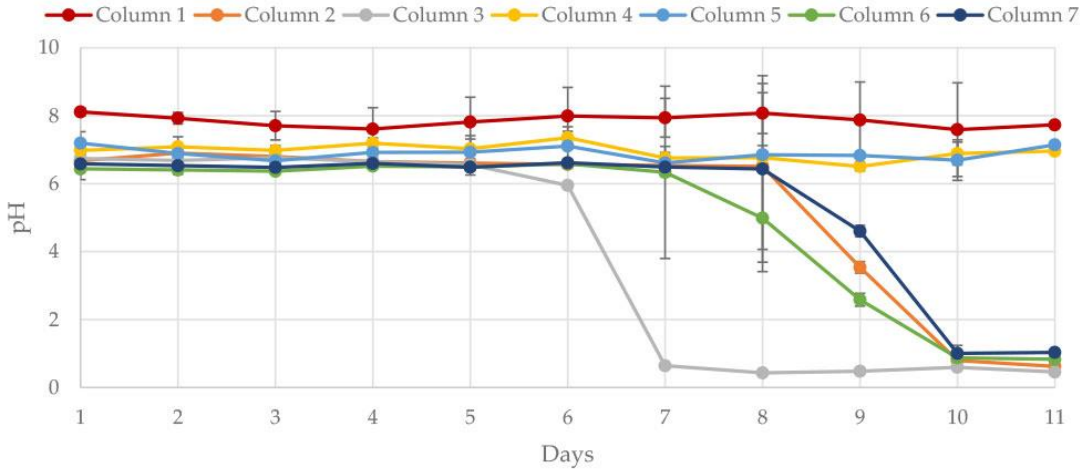


Figure 4. Daily effluent pH measurements used to compare neutralization efficiencies and endpoints.

Overall, as shown in Figure 4 there were no significant differences in the average pH values measured in the effluent from the test columns (2–7) over the first 5 days of the experimental period (ANOVA: F crit > F, $p > 0.05$), indicating that $CaCO_3$ solubilisation may not be significantly affected by particle size provided sufficient calcite is available. The results clearly demonstrate that from a long-term neutralization perspective, it is important to include sand particles with higher calcite concentrations in BSRs. In this study, more of the smaller sand particles were composed of calcite, as discussed in more detail in Section 3.3. This may also be beneficial in terms of dissolution kinetics because the overall reaction surface area is larger with smaller particles [10].

3.2. Hydraulic Conductivities of Raw and Fractionated Sand: Column Experiments

The column $CaCO_3$ dissolution experiments (Section 3.1) provided insight into which fractions of sand and combinations thereof can maximise the capacity of BSRs to neutralize acidic WW such as WWW. However, the HC of BSRs needs to be maintained at rates that will allow sufficient HRT for treatment but not impede the flow to the extent where the treatment capacity becomes limited [9,24]. For spherical sand particles, the HC increases as the particles increase in size and/or become less uniform in size [25,26]. More holistically, the particle size, particle size distribution (PSD) and particle morphology all influence the manner in which the particles physically pack together [27–29]. The porosity as well as the intrapore distribution space are key parameters influencing the HC, and these are dependent on particle packing [27–29].

To determine whether particular fractions of sand particles, or combinations thereof could offer both good flow properties as well as neutralization efficiencies, the HCs of the sand-containing columns were experimentally determined (Figure 5). There were no

significant differences ($p > 0.05$, paired T-test) in the HC measurements taken before and after $CaCO_3$ solubilisation, indicating that the loss of the calcite particles did not have a negative effect on particle packing. The HC measurements in column 3 containing the largest particles (>0.6 to 2 mm) were more than 10-fold higher than those in the columns 1,2 and 5 containing the smallest particles (≤0.150 mm). In all the columns containing fractionated sand mixes without particles ≤0.150 mm (columns 3, 4, and 6), the HC was higher than that in the columns containing raw (unfractionated) sand. The second highest HC was measured in the columns containing all fractions of sand >0.150 mm (column 6); however, this was only half the HCs measured in the columns containing the larger particles (column 3) but double the HC of the raw sand (columns 1 and 2).

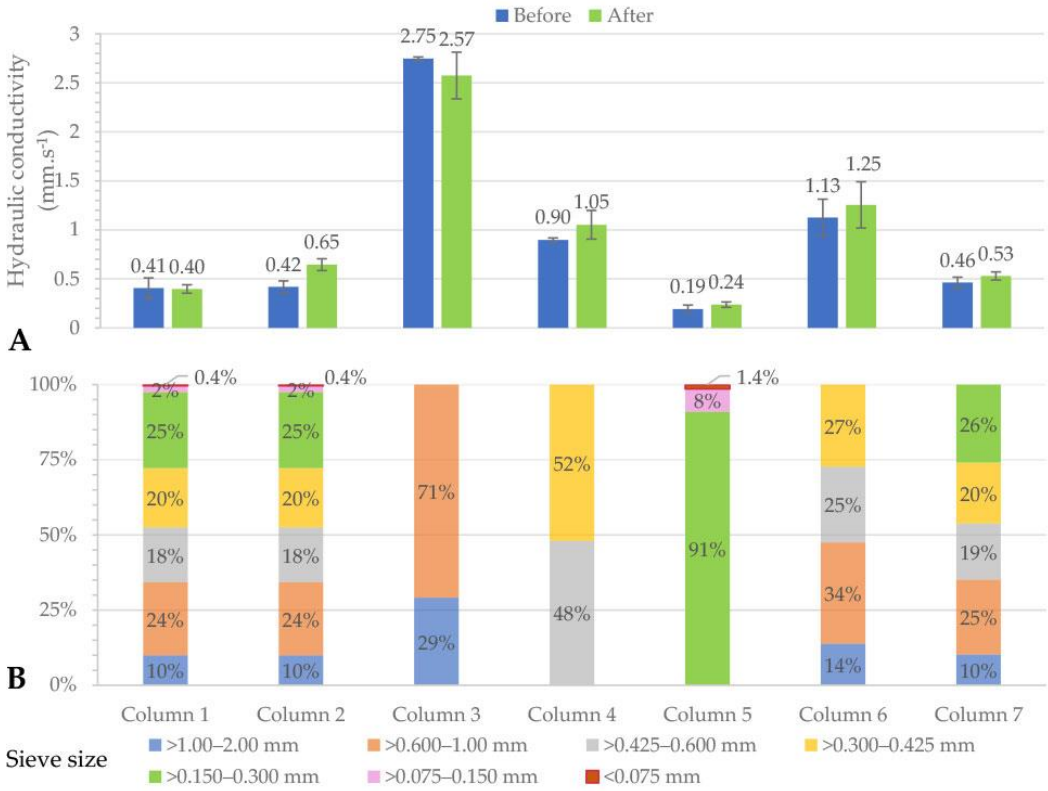


Figure 5. Measured hydraulic conductivity in sand columns before and after calcite dissolution (**A**), and the distribution of grain size fractions in the columns (**B**). The bars from plot A are aligned vertically with those from plot B, both representing columns 1 to 7.

In summary, the column experiments showed that prudent fractionation of sand particles can increase the HC of sand-based treatment systems significantly. As expected, the columns with the most and least efficient HCs were those containing the largest and smallest particles, respectively. The converse was true when considering the neutralization capacity (Section 3.1). When designing BSR systems, if the HC of raw sand is too low to achieve design HLRs, it may justify the cost of fractionating the sand. Following this, it should be considered which fractions of sand afford the best treatment performance. In this study, there was a trade-off between achievable flow rates and neutralization capacity. It must also be noted that while neutralization of acidic WW was emphasised in this study, other important factors such as the bioremediation capacity may also differ between sand fractions.

3.3. Temporal Abiotic Neutralization Capacity (TANC) of Biological Sand Reactors

3.3.1. Theoretical Values Based on Data Obtained from Column Experiments

To date, operational BSR systems have been filled with raw (unfractionated sand) [8,9]. In the column replicates containing raw sand (5.4 ± 0.01 g), the neutralization endpoint was reached after 10 days, and 91% of the Ca in the columns was solubilised and washed out after 11 days. These results indicated that 9% of the calcite in the raw sand was recalcitrant to solubilisation and/or that preferential flow paths existed within the column intra-pores so that some calcite particles were not exposed to the acidic influent. The latter is more likely, as smaller particles can fill the void spaces afforded by larger particles, hampering flow on a spatial level, as evidenced by low HC values previously measured using sand with mixed grain sizes [17].

The longevity of BSRs in terms of the temporal abiotic neutralization capacity (TANC) is theoretically affected by three main factors: the available calcite, the HC (or flow rate), and the influent WW composition. The pH is the most important WW parameter affecting calcite dissolution kinetics. Although the calcite in a particular batch of sand is finite, it is anticipated that the neutralization capacity may be supplemented in a dedicated upstream or downstream process similar to a permeable reactive barrier (PRB) once the calcite within BSRs is expended [30].

Simplified reaction kinetics (Equation (6)) using the major overall chemical reaction were used to determine the TANC of BSR systems containing the raw sand and the different sand fractions used in the column experiments. Based on Equation (4), Equation (7) was formulated to calculate the longevity of BSRs per cubic meter of sand by using the mass of available calcium concentration with the sand and dividing it by the amount of H^+ applied to the sand due to the influent pH values (Ca as a proxy for $CaCO_3$).

$$CaCO_3 + 2HCl \leftrightarrow CaCl_2 + CO_2 + H_2O \quad (6)$$

$$T_{years} = \%Ca \times \rho \div (731.4235 \times Q \times (10^{-pH} - 10^{-7})) \quad (7)$$

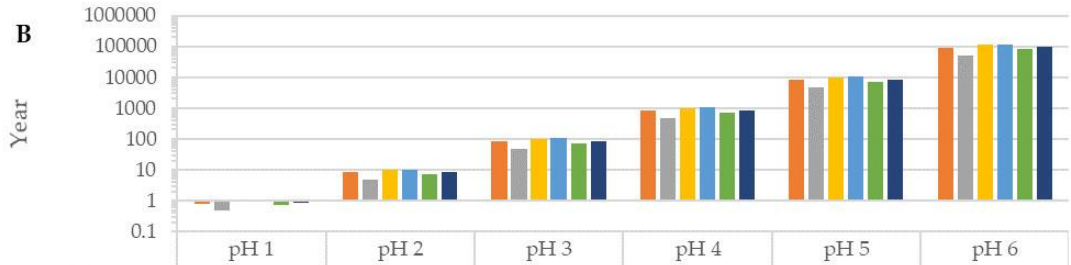
In Equation (7) the %Ca is the percentage calcium available in the sand, ρ is the density of the sand, Q is the flow rate of a reactor expressed in $L{\cdot}m^{-3}$ of $sand{\cdot}day^{-1}$, and 731.4235 is a constant for converting the molar masses of Ca to H^+ required per annum at a given Q. The pH is converted into the concentration of H^+, and effluent pH values > 7 are ignored.

The theoretical TANCs based on a pilot BSR system containing raw sand from the same quarry site and operated in horizontal subsurface flow mode [8] were calculated for the raw sand and different sand fractions used in the column experiments. The experimental HCs measured in the column experiments (Figure 5A) were notably higher than the actual HC (0.040 $mm{\cdot}s^{-1}$) calculated from the *in-situ* flow rates (150 $L{\cdot}m^{-3}$ of $sand.d^{-1}$) in the pilot BSR system [8].

When flow rates were extrapolated from the experimental HCs determined in the column experiments to the scale (0.735 m^3) of the pilot BSR system, the TANC ranged from around 2 months to over 15.8 centuries for influent with pH values ranging from 2 to 6 (Figure 6A). The HC values for the columns containing raw sand (column 2 replicates) and the columns with the highest HC and least amount of Ca (column 3 replicates) would translate into theoretical flow rates of 2400 $L{\cdot}m^3$ of $sand{\cdot}day^{-1}$ and 15,600 $L{\cdot}m^3$ of $sand{\cdot}day^{-1}$, respectively, in the BSR system. Although such high flow rates can increase treatment capacity, the RTs of 10.4 and 1.5 h, respectively, would be insufficient for effective WW bioremediation. In reality, BSRs can be designed with mechanisms to retard flow rates/RTs and, to a lesser extent, to increase flow rates [9].

The HC values obtained in the column experiments did not account for the accumulation of functional biomass, which was shown to reduce the HC by up to 80% without problematic clogging [8]. The microbial biomass in sand-based systems promotes not only biodegradation, but also flocculation, adsorption and interception [31].

	pH 1	pH 2	pH 3	pH 4	pH 5	pH 6
Column 2	0.05	0.5	5.1	51	520	5716
Column 3	0.00	0.0	0.4	4.5	45	495
Column 4	0.03	0.3	3.0	30	302	3318
Column 5	0.14	1.4	14	143	1439	15834
Column 6	0.02	0.2	1.7	17	172	1894
Column 7	0.05	0.5	4.9	49	493	5428



	pH 1	pH 2	pH 3	pH 4	pH 5	pH 6
Column 2	0.8	8.2	82	818	8251	90762
Column 3	0.5	4.6	46	464	4678	51460
Column 4	1.0	10.1	101	1015	10239	112629
Column 5	1.0	10.4	104	1039	10487	115358
Column 6	0.7	7.3	73	728	7343	80771
Column 7	0.9	8.6	86	857	8645	95095

Figure 6. Log graphs and tables showing the theoretical abiotic neutralization capacity values of a horizontal flow gravity-fed biological sand reactor system with flow rates based on: (**A**) Column experiments (this study), and (**B**) *in-situ* flow rates in a pilot biological sand reactor system as previosuly described [8]).

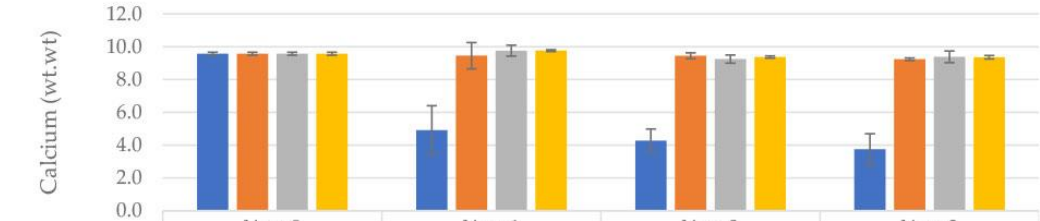
Taking all these factors into account, the TANCs were re-calculated using the actual HC achieved in the pilot BSR system (Figure 6B). In each case, the values increased. For example, the theoretical TANC of a BSR containing the same sand as column 2 increased from around 6 months to 8 years and from 5 to 90 centuries at influent pH values from 2 to 6, respectively.

3.3.2. Validation of Theoretical Results with Data Obtained from Operational Biological Sand Reactors

In order to validate the TANC results obtained using Equation (7) (Figure 6), a three-part approach was adopted using the results from the on-site BSR system and the measured flow rates of that system. Three chemical parameters were assessed: (i) the Ca concentrations in sand cores taken annually over a three-year period, (ii) The difference in Ca concentrations between influent and effluent (Ca solubilised), and (iii) the average H^+ of the influent calculated from the pH values [8].

Results Based on the Calcium Concentrations in Core Samples

The Ca was preferentially removed from the top inlet of the BSR modules, with negligible removal in the other core samples (Figure 7A). The different spatial results were anticipated as the WWW is expected to become gradually less acidic as it passes through the BSR modules, perhaps even precipitate again as $CaCO_3$ in some instances towards the outlet. Despite the spatial variation, it was estimated from averaged inlet, middle and outlet core results taken after 3.2 years of operation that 8.6% of the calcite or a 5.3 kg Ca·m^{-3} of sand·year^{-1} had been solubilised (Table 2), translating into a TANC of approximately 37 years with an influent of the same strength and composition.



	Year 0	Year 1	Year 2	Year 3
Inlet top	9.6	4.9	4.3	3.8
Inlet bottom	9.6	9.5	9.4	9.2
Outlet top	9.6	9.8	9.2	9.4
Outlet bottom	9.6	9.8	9.4	9.4

Figure 7. Percentage calcium in sand from cores taken from biosand reactors treating winery wastewater for three years.

Table 2. Percentage calcium in sand from cores taken from biosand reactors treating winery wastewater.

Position	Inlet (ca wt.wt)	Middle (ca wt.wt)	Outlet (ca wt.wt)
Top (0–5 cm)	4.5	9.0	9.2
Middle (25–30 cm)	9.2	9.3	9.4
Bottom (45–50 cm)	9.4	9.2	9.5

Results Based on Influent and Effluent Calcium Concentrations

The Ca concentration increased by an average of 24 ± 32 mg·L^{-1} from influent to effluent, which translates into a removal of 1.3 kg ± Ca·m^{-3} of sand·year^{-1} as previously published [8]. The initial Ca concertation in the sand was 159 kgCa·m^{-3} of sand (9.6% wt.wt), which would result in a TANC of 132 years.

Results Based on Influent pH Values

The pH measurements in the WWW influent ranged from 4.55 to 7.95 (n = 33) as previously published by [8]. The amount of Ca that could theoretically be solubilised for the amount of H^+ added was 0.003 mol·year^{-1}, indicating a TANC of 17,652 years using Equation (7).

In summary, the three approaches provided notably different results ranging from 37 years to 17,652 years. Amongst other factors, the accuracy of the results based on the influent and effluent values was compromised by the variability of WWW and temporal sampling, while those based on Ca removal from the BSRs were compromised to a lesser extent by the spatial variation in calcite dissolution. Nonetheless, the preservation of calcite within the middle and outlet of the BSRs after 3.2 years provided unequivocal evidence of system longevity. The TANC of 37 years based on temporal changes in the %Ca in the extracted cores compared favourably with the theoretical TANC based on the results obtained from column 2 containing raw sand, with the actual BSR flow rates for influent with pH between 2 (8.2 years) and 3 (82 years).

3.4. Changes in the Character of the Sand Particles before and after Calcite Dissolution in Columns and Biological Sand Reactors

The changes in the character of the sand particles after calcite dissolution were assessed using two batches of raw sand from the same quarry site, namely, the sand used in the column experiments, and the sand used in the BSR system. Only sand taken from the top inlet area of the BSRs was used to assess changes in the BSR system sand as negligible calcite dissolution was observed in the other spatial niches (Table 2). In the case of the columns, the calcite was dissolved artificially using HCl, while in the BSRs, real WWW was responsible for any dissolution that occurred.

3.4.1. Chemical Composition of Sand

The major minerals and accompanying elements in both batches of sand were calcite and Ca, and quartz and Si, respectively. In both batches of sand, decreases in Ca and calcite were accompanied by relative increases in Si and quartz (Tables 3 and 4). Other elements and minerals were only present in very low concentrations, and only negligible relative or actual increases or decreases occurred (Tables 3 and 4).

Table 3. Average major elemental composition (% wt.wt) of the raw sand determined using X ray diffraction (n = 4).

	Al	Ca	Cr	Fe	K	Mg	Mn	Na	P	Si	Ti
Column before	0.15	5.39	bdl	0.05	0.11	0.01	bdl	0.06	0.01	43.0	0.03
Column after	0.16	0.54	bdl	0.04	0.12	bdl	0.01	0.02	0.00	49.0	0.02
BSR year 0	0.18	9.57	bdl	0.06	0.13	0.06	bdl	0.10	0.01	37.7	0.03
BSR year 3	0.24	4.26	bdl	0.09	0.17	0.06	0.01	0.04	0.02	43.9	0.03

Note(s): bdl = below detectable limit.

Table 4. Mineral composition of the raw sand determined using QEMSCAN®.

	Column before				Column after			
Size fraction (µm)	Combined	1180>	>425	<425	Combined	600>	>300	<300
Quartz	81.3	87.8	80.0	82.5	87.49	97.30	99.36	97.91
Feldspar	0.8	0.1	0.9	0.8	1.25	2.46	0.50	1.56
Mica	ND	ND	ND	ND	0.07	0.14	0.02	0.09
Other silicates	ND	ND	ND	ND	0.06	0.03	0.04	0.13
Calcite	17.6	11.9	19.0	16.2	0.02	0.01	0.02	0.06
Fe-oxides/hydroxides	0.1	0.1	0.1	0.1	0.08	0.05	0.05	0.18
Others *	0.1	0.0	0.1	0.2	0.03	0.01	0.01	0.08

Note(s): ND = not detected, * = includes accessory minerals e.g., fluorite and apatite.

In the columns, the %Ca and calcite were reduced by 91%, and 99.9%, respectively (Tables 3 and 4), indicating almost complete dissolution of calcite, but there was some residual Ca, either alone or complexed with other anions in the sand. In contrast, in the BSRs, the %Ca and calcite were reduced by 57% and 29.7%, respectively, after 3 years of operation. The absence and presence of calcite in the column and BSR sands at the end of the respective experimental periods were determined by QEMSCAN® using thousands of automated digital images, an example of which is shown in Figure 8 The anomalous result between the %calcite and Ca reduction in the BSFs can be explained by the addition of Ca from the influent WWW which contained an average of 36.2 $mg \cdot L^{-1}$ Ca (range 6.6 to 104.0 $mg \cdot L^{-1}$) [8].

3.4.2. Size and Shape of Sand Particles

After respective dissolution with HCl and WWW, the sand particle sizes between 0.1 and 1 mm in both the columns and the BSR sands decreased, but the differences were negligible in the BSR sand (Figure 9). This could be related to some extent to the lower

calcite dissolution in the BSR and/or physical effects of the HCl on the non-calcite particles in the column sand.

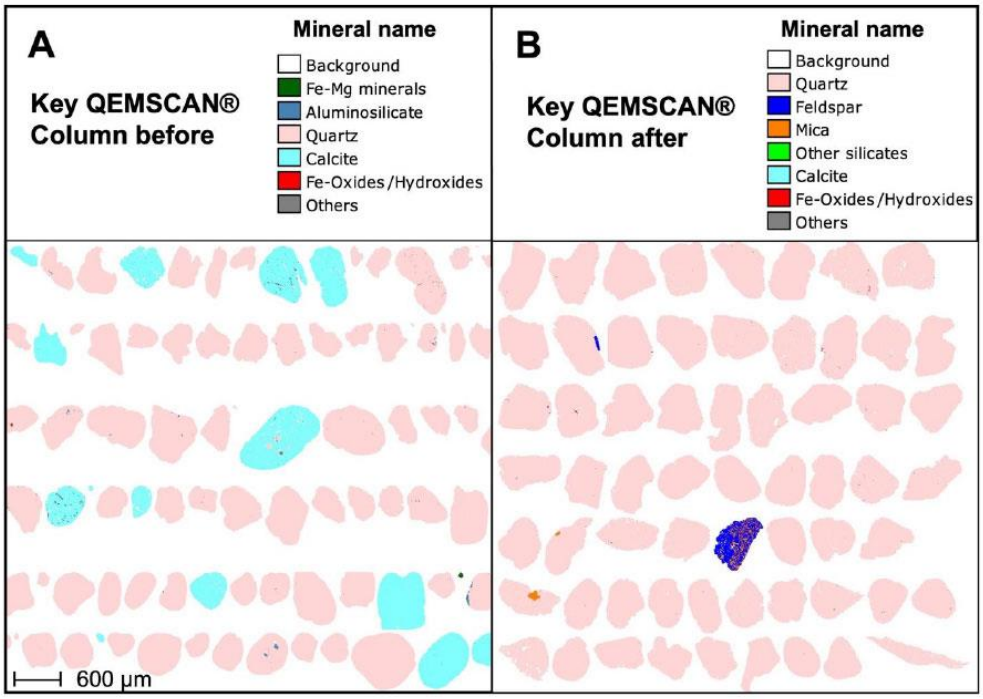


Figure 8. Particle image of column sand before (**A**) and after (**B**) calcite dissolution.

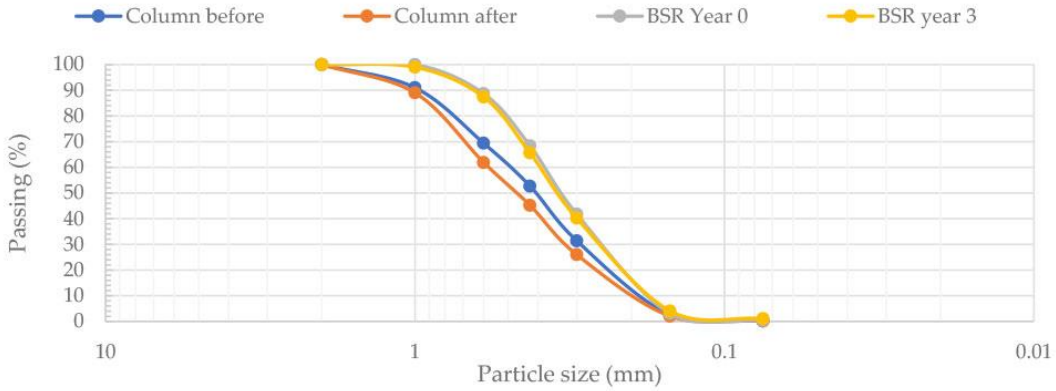


Figure 9. Particle size distribution curves for the batches of sand used in the column 2 experiments and the biological sand reactors at the beginning and end of the respective experimental periods.

The shape of particles in terms of roundness and elongation can be described using roundness and aspect ratios [16]. Roundness is the ratio of the area of the particle to the smallest perfect circle able to fit around the particle outline, with rounder particles having higher roundness ratios. The aspect ratio is a ratio of the lengths of the long and short particle axes, so that higher ratios equate to more elongated particles [16]. In this study, the mineral composition of the grains was relatively pure and the quartz particles of both batches of raw sand before dissolution were generally rounder and more elongated than the calcite quartz particles (Table 5, Figures 8 and 10).

Table 5. Relationship of mass % distribution of quartz and calcite particles in terms of aspect ratio and roundness.

				Column					
Aspect ratio	<0.2	<0.3	<0.4	<0.5	<0.6	<0.7	<0.8	<0.9	<1.0
%Quartz before (n = 2572)	0.00	0.02	0.15	2.19	8.24	18.19	28.93	26.88	15.40
%Quartz after (n = 5191)	0.00	0.18	0.96	4.40	12.48	23.30	27.44	23.03	8.15
%Calcite before(n = 253)	0.53	1.81	4.60	15.85	13.77	21.58	18.48	15.10	8.28
%Calcite after(n = 478) *	0.00	0.00	0.79	2.33	33.39	16.38	1.76	3.28	42.07
Roundness	<0.2	<0.3	<0.4	<0.5	<0.6	<0.7	<0.8	<0.9	<1.0
%Quartz before (n = 2572)	0.00	0.10	1.17	8.13	18.86	33.26	31.47	7.00	0.00
%Quartz after = 5191)	0.10	0.66	3.99	14.72	29.13	32.06	16.69	2.66	0.01
%Calcite before (n = 253)	0.53	1.81	4.60	15.85	13.77	21.58	18.48	15.10	8.28
%Calcite after(n = 478) *	0.00	4.78	6.95	9.26	32.95	5.38	0.00	1.17	39.50
				BSR					
Aspect ratio	<0.2	<0.3	<0.4	<0.5	<0.6	<0.7	<0.8	<0.9	<1.0
%Quartz year 0 (n = 5267)	0.00	0.08	1.17	5.35	14.22	21.47	29.24	20.33	8.13
%Quartz year 3 (n = 5472)	0.00	0.12	1.12	5.34	13.90	23.78	25.59	20.11	10.04
%Calcite year 0 (n = 4768)	0.96	4.20	16.69	18.99	22.18	14.48	11.20	7.87	3.42
%Calcite year 3 (n = 2999)	0.52	5.88	17.11	16.90	17.31	19.77	11.89	7.53	3.07
Roundness	<0.2	<0.3	<0.4	<0.5	<0.6	<0.7	<0.8	<0.9	<1.0
%Quartz year 0 (n = 5267)	0.06	0.89	5.12	17.78	30.59	31.48	12.00	2.03	0.06
%Quartz year 3(n = 5472)	0.02	0.92	5.86	16.82	32.08	26.09	15.51	2.66	0.05
%Calcite year 0 (n = 4768)	1.67	11.11	24.11	27.00	18.75	11.48	4.50	1.16	0.22
%Calcite year 3 (n = 2999)	1.50	11.01	25.34	25.14	19.25	13.40	3.46	0.70	0.18

Note(s): * = calcite in too low quantities for reliable shape data.

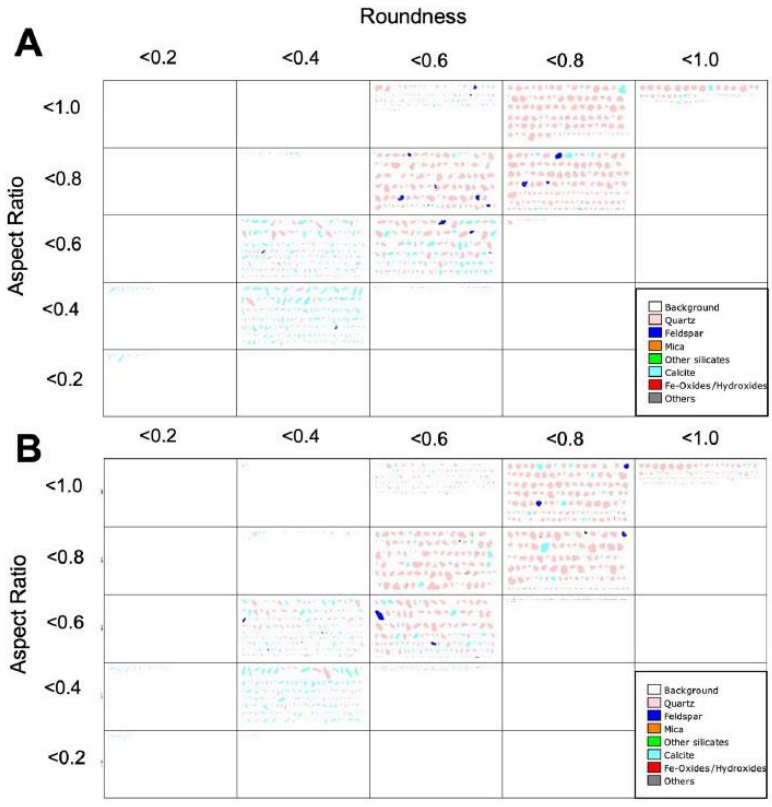


Figure 10. Image showing the mineralogy and relative roundness and aspect ratios of the different sizes of sand particles from the biological sand reactors of core samples taken at year 0 (**A**) and year 3 (**B**).

In the columns, only 9% of the original calcite particles remained after dissolution. Although the roundness and aspect ratio mass balances of these particles showed marked temporal differences, no trends were noted (Table 5). The quartz particles, however, became less round as well as less elongated, possibly due to the action of the HCl on the particle surfaces.

In the BSRs, there were no significant temporal changes in the roundness or aspect ratios of either the calcite or quartz particles ($p > 0.05$, paired t-test). It was hypothesized that the less acidic WWW allowed the particles to maintain their structure in comparison with the particles subjected to HCl in the columns. In addition, less calcite was solubilised in the BSRs.

4. Conclusions

Biosand reactors containing unfractionated sand have a TANC longer than the projected lifespan of the related infrastructure. The hydraulic conductivity is unaffected by dissolution of calcite particles but can be increased by removing smaller sand particles. In real world situations, it is suggested that the sand is replaced every 10 to 15 years or when the effluent pH is no longer increased though the filters. Based on the results of this study, future research should focus on the bioremediation performance of BSRs containing selected fractions of sand, as well as changes in HC that occur due to build-up of functional biomass in these systems. It is suggested that future BSRs should operate in vertical flow conditions which allows for sufficient head across the filter together with the ability to manipulate flow rates, and the systems should be operated with an HRT of no less than 24 h to allow sufficient microbial interaction with the wastewater. It is also suggested that the optimal grading would be sand particles > than 0.450 mm which would be a combination of the sand used in column 3 and column 4 in this study. For the sand used in this study, this would constitute 54% of the entire grading and would provide the 46% fraction with smaller particles for use in concrete mixes where smaller particles are more desirable. The projected TANC of such a system would be 68 years. In addition, the use of BSRs for the remediation of other acidic organic WW should be investigated.

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Appendix 6 Journal article 5 offprint

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ORIGINAL ARTICLE

Anaerobic digestion of primary winery wastewater sludge and evaluation of the character of the digestate as a potential fertilizer

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Abstract
Sludge is generated from settling of winery wastewater from seasonal cellar activities. Most primary winery wastewater sludge is generated during the crush season and is often disposed to landfill. This practice is an economic and environmental burden and ignores the potential for valorization. In this study, a series of biochemical methane potential tests were conducted using seasonal batches of sludge with and without the addition of selected micronutrients (Co, Cu, Ni) at different inoculum to substrate ratios and temperatures (ambient vs 37 °C). The highest specific CH_4 yields were obtained at an inoculum to substrate ratio of 4 with the addition of micronutrients from sludge collected during (206 ± 2.7 $mLCH_4/gVS_{added}$) and after (177 ± 1.4 $mLCH_4/gVS_{added}$) the crush season at 37 °C and ambient temperatures (15.3 to 27.1 °C), respectively. In some instances, digestion at 37 °C appeared to promote inhibited steady-state conditions leading to lower CH_4 yields than at ambient temperatures. This suggested that the sludge could be easily digested without heating, particularly during the warmer months of grape harvesting and crushing. The composition of the digestate indicated that it may be suitable as an agricultural fertilizer, with high concentrations of N (21.5 to 27.7 g/kg dry weight) and C (229 to 277 g/kg dry weight), as well as the presence of all essential micronutrients.

Keywords Anaerobic digestion · Fertilizer · Irrigation · Reuse · Valorization · Waste

1 Introduction

Solid and liquid wastes are generated by the wine industry through winemaking processes. Solid wastes from winemaking include lees, fining agents, filtration aids, and agricultural waste known as grape marc or pomace (skins, seeds, and stems). Winery wastewater (WWW) is generated when cellar equipment and floors are washed and/or sterilized and contains solid grape residuals, chemicals, and filter aids in variable quantities [1]. Over the last three decades, a number of industrial biorefineries have been set up to extract value-added products from grape pomace and lees including bioactive compounds such as phenolics, dietary fiber, cellulose, grapeseed oil, and tartaric acid [2–8]. Valorization approaches that have been explored on a more theoretical level include fermentation of pomace for bioethanol production, energy production from gasification or pyrolysis of pomace, and extraction of condensed tannins for use as wood adhesives [9–13]. Grape pomace has also been composted, vermi-composted, or co-composted with substrates such as green waste, municipal waste activated sludge (WAS), olive mill waste, and spent winery filter material as a lower value valorization option [14–18]. Co-composting may enhance the quality of the compost [14, 16]. Application of such compost to agricultural land may increase plant yield, as in the case of fertilizer obtained from co-composting of pomace, WWW, olive mill sludge, olive mill wastewater, and green waste, which was shown to increase radish yield [17].

Primary winery wastewater sludge (PWWS) and secondary winery wastewater sludge (SWWS) are indirect solid wastes that are generated by the wine industry. Unlike direct forms of solid winery wastes, WWS is not suitable for higher value valorization. Indeed, some wineries contract commercial companies to remove WWS and

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dispose it to landfill, which is (i) an economic burden on the industry, (ii) a burden on landfill sites, and (iii) ignores the potential for valorization of the organic-rich waste.

In water-stressed countries, including South Africa, Australia, and some areas of the USA, cellar effluent is often used for beneficial irrigation after primary settling and pH adjustment if required. This results in the generation of copious amounts of PWWS. Conversely, in many wineries, especially those in Europe, WWW is either discharged into municipal sewers for concurrent treatment with domestic wastewater or it undergoes secondary treatment in situ generating SWWS that differs in character from PWWS [19, 20]. Although SWWS and PWWS differ in nature, both are malodorous and potentially toxic to the environment, mainly due to high concentrations of (poly) phenolics and volatile fatty acids (VFA). It has been shown that stabilized SWWS from constructed wetlands can be used directly as a soil conditioner [19] and that SWWS from a continuous activated sludge (CAS) process is a suitable substrate for anaerobic digestion (AD) [21].

From economies of scale, composting and AD are generally preferred for decentralized and centralized biodegradation of organic matter, respectively [22]. However, composting can be labor-intensive because compost piles need regular turning and stalks may need to be ground [14]. In addition, a large spatial footprint is required as the process is slow. Anaerobic digestion occurs at a faster rate, requires a smaller spatial footprint, and emits less greenhouse gases than composting, provided the biogas from AD is utilized [22]. Promising results have been obtained from AD of grape pomace, lees, and grape marc [21, 23–26]. Arguably, in contrast to WWS, these are already being used for extraction of more valuable products than biogas. Nonetheless, these studies indicate that valorization of WWS via AD may be a viable remediation approach. Previous studies of AD of primary and secondary WWS were ineffective (< 30 mLCH_4/gVS) in relation to other winery wastes like grape marc [27]. To the best of our knowledge, no other studies have been conducted on the AD of PWWS, including the addition of micronutrients and the use of digestate as a fertilizer.

The aims of this study were therefore to determine the biochemical methane potential (BMP) of PWWS from a winery during and after the crush season at different inoculum to substrate (ISR) ratios, with and without the addition of micronutrients at different temperatures (ambient and 37 °C). Furthermore, the digestates were characterized and compared with commercial organic fertilizers in terms of their macro- and micronutrient compositions.

2 Materials and methods

2.1 Biochemical methane potential experiments

The inoculum was prepared by randomly feeding the digestate obtained from previous AD experiments [28] with PWWS for > 6 months before the start of the first experiments. The composition of the biogas produced by the inoculum was monitored on a weekly basis. The inoculum was deemed ready for use once the CH_4% of the biogas was > 60%.

Approximately 100 L of PWWS was collected from during and after the crush season. The PWWS was transported to the laboratory immediately and thoroughly homogenized using a blender. The PWSS was fully characterized together with the inoculum. As the microbial communities are naturally dynamic in nature, the inoculum for each of the four experiments was characterized separately.

Two experiments were conducted with both the crush and the post-crush PWSS, giving a total of four sets of experiments: (i) crush experiment 1, (ii) crush experiment 2, (iii) post-crush experiment 1, and (iv) post-crush experiment 2. Experiment 1 for both PWWS substrates was conducted at ambient temperature (20.7 °C ± 2.0 °C) using a combination of two variables inoculum to substrate ratio (ISR) (2:1, 3:1, 4:1) and nutrient addition (nutrients/no nutrients) (Table 1). The character of the PWSS taken during the crush

Table 1 Parameters used for experiments 1 and 2

	Crush season PWWS			Post-crush season PWWS		
	Experiment 1	Experiment 2		Experiment 1	Experiment 2	
Temperature	Ambient	Ambient	37 °C	Ambient	Ambient	37 °C
ISR 2	✓	✗	✗	✓	✗	✗
ISR 2 + N	✓	✗	✗	✓	✗	✗
ISR 3	✓	✗	✗	✓	✗	✗
ISR 3 + N	✓	✗	✗	✓	✗	✗
ISR 4	✓	✓	✓	✓	✓	✓
ISR 4 + N	✓	✓	✓	✓	✓	✓

PWWS, primary winery wastewater sludge; ***ISR***, inoculum to substrate ratio; ***N***, nutrients (5 mg/L Cu^{2+}, 20 mg/L Ni^{2+}, 20 mg/L Co^{2+})

season informed the choice of (micro)nutrients (5 mg/L copper (Cu^{2+}), 20 mg/L nickel (Ni^{2+}), 20 mg/L cobalt (Co^{2+})). These metabolic co-factors were deficient in both batches of PWWS [29, 30]. Three controls were included in each experiment: inoculum only, substrate only, and inoculum + acetate, all with and without nutrients. All BMP experiments, including controls, were conducted in duplicate.

The experimental set-ups for the variable combination resulting in the most efficient performance in terms of specific biogas and CH_4 generation in experiment 1 were repeated at ambient (19.4 °C ± 1.77 °C) and mesophilic (37 °C) temperatures simultaneously to determine the effect of these temperatures on AD efficiency (experiment 2). The sets of experiments were first performed during the crush season using the crush season sludge, followed by the post-crush season sludge.

For reference, the results for characterization of all of the reactor contents at the beginning and end of the experiments are provided in the Supplementary material (Supplementary Tables 1 and 2).

2.2 Biogas measurements

Biogas was collected directly from the reactors into 2-L Supelco (Bellefonte, USA) foil gas sampling bags. The volume of biogas was determined three times per week on designated days by extracting the gas from each bag into graduated gas tight syringes. Volumes were measured at 20.7 °C ± 2.0 °C and pressure of 1008.7 ± 2.7 hpa. The composition of the biogas was determined using a Geotech (Warwickshire, UK) Biogas 5000 instrument. The performance of AD was assessed using the specific total biogas and CH_4 yields based on volatile solids added (mL/gVS).

2.3 Physicochemical analyses

Total solid (TS) and total volatile solid (TVS) concentrations were determined by standard methods (weight loss on ignition at 105 °C and 550 °C), respectively. A Merck (Darmstadt, Germany) Spectroquant Prove® 600 spectrophotometer together with Merck cell tests or kits was used to determine chemical oxygen demand (COD) (cat no: 14555), volatile fatty acids (VFA) measured as total volatile organic acids (VOAt) (cat no: 01763), total organic carbon (TOC) (cat no: 14879), total nitrogen (TN) (cat no: 14537), total alkalinity (TAlk) (cat no: 101758), total phosphorous (TP) (cat no: 14729), ammonia as NH_4^+ (cat no: 00683), sulfate SO_4^{2-} (cat no: 14791), sulfide (S^{2-}) (cat no: 14779), and chloride (Cl^-) (cat no: 14789) concentrations, according to manufacturers' instructions. Quantification of carbon, hydrogen, nitrogen, and sulfur (CHNS) concentrations (%wt.wt) was determined at the Central Analytical Facility of Stellenbosh University using an Elementar (Hamburg, Germany) Vario EL cube Elemental analyzer according to the manufacturers' instructions. Major and minor elements were quantified at the same facility using a Thermo ICap 6200 ICP-AES instrument for trace analyses, while ultra-trace analyses were performed on an Agilent (Santa Clara, USA) 7900 ICP-MS instrument, according to manufacturers' instructions.

3 Results and discussion

3.1 Characterization of wastewater, primary winery wastewater sludge, and inoculum and theoretical suitability of sludge for anaerobic digestion

3.1.1 Organic fractions, ammonia, and sulfides

Due to the seasonal nature of winery operations, there is a considerable intra- and inter-site variation in the characteristics of WWW and by inference in PWSS [1]. Samples of WWW taken from the same winery over the crush season exhibited a high COD:N ratio of 226 (Table 2). High COD:N ratios are typical of WWW, and a source of N is often added to secondary wastewater treatment systems to assist bioremediation [1]. Notably lower COD:N ratios were measured in the PWWS (12.9 and 24.1 for the crush and post-crush season PWWS, respectively). It was therefore noted that N partitioned preferentially into the PWWS and indicated that the character of the PWWS cannot be extrapolated directly from that of the WWW.

In this study, the C content from the (CHNS) elemental analysis was notably higher in the post-crush than the crush season PWWS, but the converse was true for the TOC. The post-crush PWWS exhibited significantly higher (ANOVA, $p > 0.05$) concentrations of TS, TVS, and COD than the crush PWWS (Table 2). The higher TOC in the crush season PWWS was due to the presence of fresh pomace residues from washing equipment and floors during the crushing. The post-crush PWWS still contained some pomace residues and solubilized degradation products because the settling delta at the winery had not been completely de-sludged after the crush period. Overall, the organic character of the PWSS adequately reflected seasonal operational variabilities.

Theoretically, the C:N ratio of the crush season PWWS (11.6) and post-crush season PWWS (7.1) were both below optimal for AD of carbonaceous substrates, which has been widely reported as 20–30 [31]. The AD process for carbonaceous substrates with lower C:N ratios may be susceptible to instability due to the potential accumulation of NH_4^+ and VFAs. High concentrations of VFAs can inhibit methanogenesis leading to bioreactor instability or failure [32]. In this study, The VFAs contributed 21% and 32% to the crush

Table 2 Characteristics of primary winery wastewater sludge and inoculum used in the biochemical methane potential experiments as well as parameters measured in the wastewater over the same period (average and standard deviation from the mean, $n=3$)

Parameter ($n=3$)	Crush PWWS	Crush inoculum 1	Crush inoculum 2	Post-crush PWWS	Post-crush inoculum 1	Post-crush inoculum 2	Winery effluent
Elemental CHNS analysis (g/kg dry weight)							
C	29.4 ± 2.45	32.5 ± 2.2	25.2 ± 2.9	40.1 ± 1.3	26.5 ± 0.3	20.4 ± 3.9	NA
H	0.55 ± 0.05	4.86 ± 0.55	4.85 ± 0.42	6.61 ± 0.43	3.62 ± 0.52	2.28 ± 0.64	NA
N	2.54 ± 0.04	2.86 ± 0.16	1.89 ± 0.25	5.67 ± 0.14	2.25 ± 0.20	1.78 ± 0.39	NA
S	0.38 ± 0.04	0.89 ± 0.05	0.57 ± 0.15	0.34 ± 0.03	0.38 ± 0.07	0.38 ± 0.08	NA
C:N	11.6	11.6	13.3	7.1	11.8	11.5	NA
Characterization (g/L wet weight homogenized sludge, g/L wastewater)							
COD	149 ± 5	35.5 ± 1.0	206 ± 14	346 ± 26	95.2 ± 8.8	71.7 ± 5.6	6.79 ± 1.20
TP	0.70 ± 0.01	0.17 ± 0.01	0.82 ± 0.20	1.65 ± 0.13	0.49 ± 0.09	0.44 ± 0.06	0.20 ± 0.07
TN	11.5 ± 0.5	2.3 ± 0.4	2.5 ± 1.07	14.3 ± 0.5	2.4 ± 0.8	4.5 ± 0.1	0.03 ± 0.02
VFA	28.5 ± 0.7	8.98 ± 0.35	44.6 ± 10.4	104 ± 5	26.1 ± 1.1	23.3 ± 2.36	1.37 ± 0.15
HS^-	0.08 ± 0.01	0.06 ± 0.01	0.08 ± 0.01	0.75 ± 0.02	0.11 ± 0.01	0.13 ± 0.02	ND
NH_4^+-N	1.89 ± 0.04	1.3 ± 0.17	5.39 ± 0.06	1.18 ± 0.35	1.02 ± 0.21	1.16 ± 0.13	ND
TS	173 ± 10	257 ± 7	62.3 ± 4.1	301 ± 14	145 ± 2	117 ± 1	ND
VS	91.9 ± 11	97.8 ± 8.7	32.8 ± 22	247 ± 9	67.9 ± 1.5	85.3 ± 1.4	ND
COD:VS	1.62	0.36	6.28	1.40	1.40	0.84	ND
VS:TS	0.53	0.38	0.53	0.82	0.49	0.72	ND

PWWS, primary winery wastewater sludge; ***Inoculum 1***, inoculum for ambient experiments (experiment 1); ***NA***, not applicable; ***ND***, not determined; ***Inoculum 2***, inoculum used for ambient vs mesosphilic experiments (experiment 2)

and post-crush season PWWS CODs, respectively, based on the theoretical COD yield of acetic acid [33]. Similarly, the stoichiometric contribution of VFAs to the TOC for the crush and post-crush PWWS was 40% and 62%, respectively. In this case, the total VFA concentrations of the PWWS and inocula (Table 2) were above previously reported inhibitory thresholds of 5.80–6.90 g/L [34].

Ammonia in WWW emanates from cellar cleaning products and/or from hydrolysis of proteins from lees and grapes. The pH, temperature, C:N ratio, and elemental concentrations influence the degree of toxicity of NH_4^+ on the sensitive methanogenic archaea during AD [35]. Hence, a range of inhibitory concentrations (IC) has been reported in literature, from 2 to 14 g/L as total NH_3-N and 0.053 to 1.45 g/L as NH_3 [29]. In this study, the NH_4^+ concentrations in the PWWS fell within these previously reported ranges for inhibition of AD (Table 2).

The S^{2-} concentration measured in the post-crush PWWS was 89% higher than the concentration measured in the crush season PWWS (0.75 g/L and 0.08 g/L, respectively). For hydrogenotrophic and aceticlastic methanogens, IC_{50} ranges reported in literature for H_2S are 0.043–0.125 g/L and 0.014–0.060 g/L, respectively [36]. In this study, the measured equivalent H_2S concentrations for the PWWS substrates were within the inhibitory range, particularly for aceticlastic methanogens. However, HS^- is likely to ppt. as metal sulfides during AD. This can alleviate toxicity by reducing the HS^- concentration, and in some instances, reducing the bioavailability of concentrations of toxic cations by co-ppt. Conversely, AD can be retarded if the bioavailabilities of essential metabolic co-factors are reduced to below functional microbial requirements.

Although the measured VFA, NH_4^+, and HS^- concentrations were above inhibitory values reported in literature, this does not necessarily translate into poor performance as each substrate and inoculum is unique. Indeed, it has been shown that substrates that appear unsuitable for AD may yield good AD results after long-term acclimation of the functional microbial communities to the particular physico-chemical milieu provided by the substrate [37].

3.1.2 Elemental composition of primary winery wastewater sludge and inoculum

The elemental composition of grapes varies according to the soil geochemistry, the agricultural practices, and the grape varietal and rootstock. Potassium (K^+), the element found in the highest concentrations in the crush season PWWS, is found in high concentrations in grapes and can also be present in cellar cleaning products. Sodium is also typically present in high concentrations in WWW because caustic soda (NaOH) is the main cleaning and sterilizing agent used in most cellars [1]. However, Na^+ tends to remain in the soluble fraction of WWW [38], while much of the K remains in the PWWS because it is a constituent of the pomace. This is reflected by the relatively low concentrations of

Na compared with K in the PWWS, particularly in the crush season PWWS (Table 3).

In the case of metals, a large range of concentrations expected to inhibit AD has been reported in literature. This is due to variabilities in substrates, the degree of functional microbial acclimation to the intended substrates, substance synergism, and antagonism and operational differences [39]. Acclimated microbial communities may adapt to prevent metal toxicity or deficiency by altering their rate limiting flux and/or protecting key enzymes from reactive metals [40]. Soluble microbial products (SMPs) and extracellular polymeric substances (EPS) also play significant roles in chelating metals, thereby reducing their bioavailability to microbes in anaerobic digesters [40]. Metals are not only inhibitory—many are also essential metabolic co-factors, and it is common practice to supplement AD reactors with a co-factor cocktail to enhance AD. In this study, the metabolic co-factors Ni, Zn, Co, and Cu were either within or below the optimal range for AD [29], hence the addition of Ni, Co, and Zn as micronutrients in the experiments.

3.2 Biochemical methane potential tests

The quality and quantity of CH_4 generated from AD are fundamentally dependent on the oxidation state of the organic C. Higher CH_4 yields are associated with more reduced substrates [41]. Sludge characteristics are often used to infer CH_4 generation potential [42]. Buswell's equation has been used for decades to estimate the theoretical BMP of organic substrates assuming 100% conversion into carbon dioxide (CO_2) and CH_4 [43]. However, the theoretical yield is usually overestimated as the methanogenic archaea are highly susceptible to inhibition [29, 35]. The yields were therefore determined experimentally in this study.

3.2.1 Anaerobic digestion of primary winery wastewater sludge from the crush season

The first set of BMP experiments was conducted using PWWS taken during the crush season. Although it is well known that stable mesophilic (30–38 °C) and thermophilic (40–60 °C) temperatures typically increase the rate of AD, digestion still occurs under ambient conditions in some temperate and tropical climates [44]. In order to ascertain whether AD of PWWS could feasibly be conducted without having to install and operate sophisticated heating equipment, the BMP experiments were first conducted in the laboratory under ambient conditions in a non-temperature-controlled environment. Most WWW (and hence PWWS) is generated during the crush period, which falls within the

Table 3 Elemental cation concentrations in primary winery wastewater sludge and inoculum used in the biochemical methane potential experiments

	Crush PWWS	Crush inoculum 1	Crush inoculum 2	Post-crush PWWS	Post-crush inoculum 1	Post-crush inoculum 2
Major elemental parameters (g/kg dry weight)						
Fe	3.83 ± 0.13	3.59 ± 0.11	4.23 ± 0.95	2.96 ± 0.12	4.85 ± 0.85	5.02 ± 0.36
Al	11.1 ± 0.14	7.18 ± 0.24	10.2 ± 2.23	10.7 ± 0.22	13.0 ± 2.03	10.0 ± 1.16
Ca	7.37 ± 0.45	22.1 ± 0.19	10.4 ± 2.07	0.91 ± 0.12	10.3 ± 1.15	14.4 ± 3.03
K	51.8 ± 4.70	43.2 ± 1.39	29.5 ± 4.17	3.73 ± 0.12	61.5 ± 24.7	39.8 ± 15.7
Mg	1.60 ± 0.23	3.74 ± 0.19	2.32 ± 0.44	1.21 ± 0.41	2.19 ± 0.21	1.99 ± 0.13
Na	1.03 ± 0.16	30.9 ± 0.99	3.40 ± 0.09	0.18 ± 0.05	4.72 ± 1.61	3.75 ± 1.69
P	2.85 ± 0.12	5.24 ± 0.15	4.14 ± 0.93	5.40 ± 0.11	2.91 ± 0.05	3.80 ± 0.31
Si	0.91 ± 0.01	1.39 ± 0.12	0.65 ± 0.11	2.52 ± 0.53	1.57 ± 0.27	1.78 ± 0.35
Minor elemental parameters (mg/kg dry weight)						
B	68.1 ± 48.3	54.6 ± 5.60	40.5 ± 7.58	BDL	45.3 ± 2.00	46.4 ± 2.50
Mn	39.7 ± 1.60	333 ± 3.80	93.0 ± 17,0	23.4 ± 2.20	74.5 ± 13.5	80.3 ± 11.3
Co	1.30 ± 0.50	16.9 ± 0.83	26.3 ± 5.84	BDL	20.4 ± 8.90	BDL
Ni	36.9 ± 4.9	11.6 ± 0.70	52.9 ± 12.1	12.9 ± 3.10	49.5 ± 5.40	36.0 ± 6.30
Cu	147 ± 12.7	168 ± 8.10	157 ± 34.5	120 ± 3.00	208 ± 30.3	197 ± 79.4
Zn	300 ± 15.0	1443 ± 71	449 ± 100	354 ± 2.4	511 ± 82.6	385 ± 35.2
Sr	21.6 ± 1.70	90.4 ± 0.70	51.14 ± 9.67	67.1 ± 11.9	39.1 ± 3.00	67.1 ± 11.9
Mo	3.01 ± 1.30	5.0 ± 0.2	1.70 ± 0.47	ND	ND	ND
Pb	34.2 ± 6.40	19.1 ± 6.70	24.3 ± 7.30	34.7 ± 8.60	29.7 ± 6.30	35.1 ± 10.5
Ba	133 ± 8.60	42.8 ± 4.90	72.9 ± 19.2	69.8 ± 2.50	114 ± 29.6	114 ± 13.1

BDL, below detection limit; *PWWS*, primary winery wastewater sludge; *Inoculum 1*, inoculum for ambient experiments (experiment 1); *Inoculum 2*, inoculum used for ambient vs mesosphilic experiments (experiment 2); *ND*, not determined

warm summer/autumn months in wine-producing countries. As a cost-effective option, it is feasible that PWWS could be pumped from existing settling tanks/deltas into simple digesters. These could be off-the-shelf polyethylene tanks, for example. Black containers exposed to sunlight could absorb heat and assist with increasing the temperatures of the reactor contents. Alternatively, specialized insulated reactors used in combination with solar heating could be employed.

Promising results were obtained at ambient temperatures (experiment 1). Good specific biogas (Fig. 1A) and CH_4 generation (Fig. 1B) were achieved within 40 days (Fig. 2). In terms of ISR and nutrient addition, both biogas and CH_4 generation increased with increased ISR and nutrient addition. In practice, reactor capacity is reduced at lower ISRs as less digestate needs to be retained between batches (for batch reactors), and more solids can be wasted in the case of continuous reactors. In this study, the comparatively low efficiency at ISR2 obtained during (ambient) experiment 1 would not support the use of this ISR for piloting a system to treat PWWS. Although reasonable absolute cumulative CH_4 generation was achieved at ISR2, the quantities were masked when corrected for the contribution by the inoculum control as prescribed in the standard method applied [45]. This led to some anomalous negative generation rates at ISR2. Although the inoculum had been pre-acclimated to a different batch of PWWS, it was hypothesized that the sensitive microbial populations needed to adjust to the slight change in substrate, especially when present in lower concentrations (ISR2). Many researchers do not pre-acclimate inocula to the intended substrate, which can reduce AD efficiency as acclimation then needs to take place during the experimental period [39]. This may lead to long lag phases, and in some cases, the microbial consortia may completely fail to adapt to the new substrate. In an attempt to standardize testing to allow inter- and intra-study comparisons, some standardized methodologies prescribe the use of inocula from municipal wastewater sludge digesters for all AD testing [45]. While

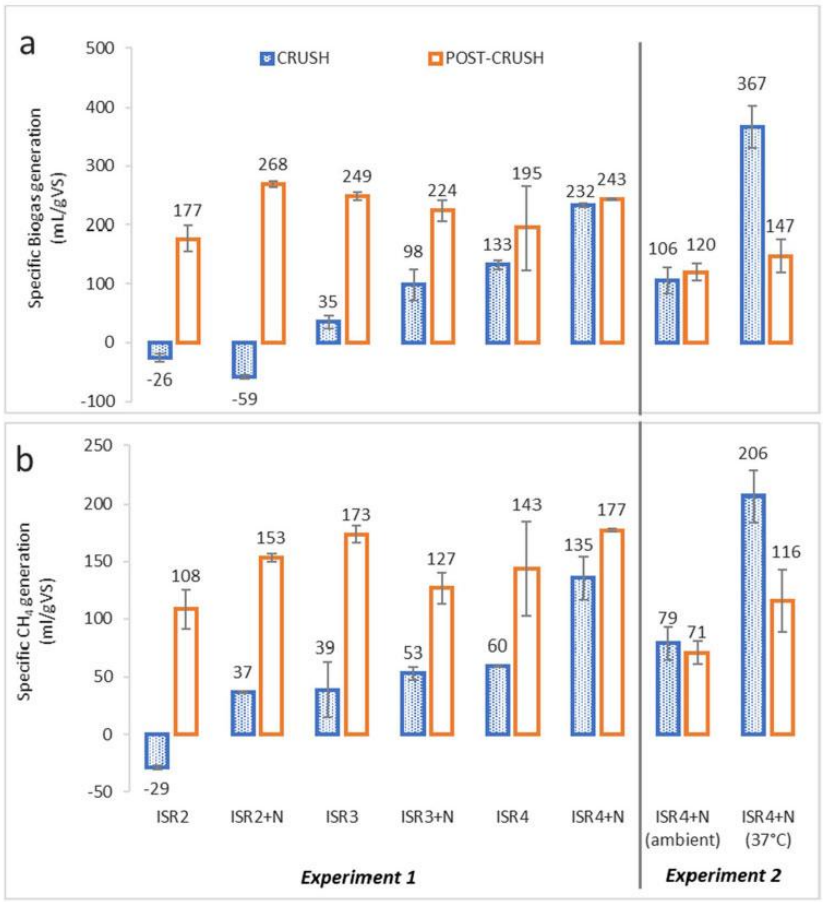

Fig. 1 Corrected specific biogas (**A**) and methane (**B**) measured in reactors. ISR, inoculum to substrate ratio; N, (micro) nutrients (Co, Cu, Ni). Experiment 1 at ambient temperature and experiment 2 at ambient and 37 °C

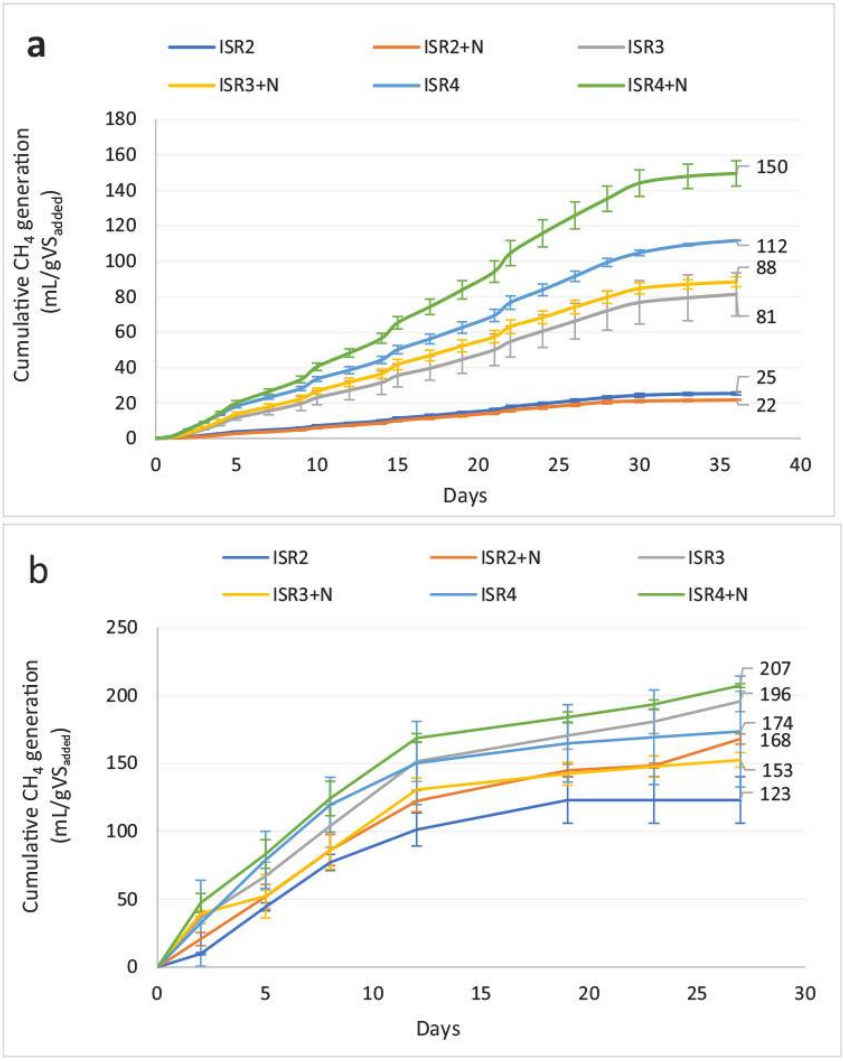

Fig. 2 Cumulative methane generation with crush season sludge (**A**) and post-crush season (**B**) primary winery wastewater sludge at ambient temperatures

this may be acceptable if a similar substrate is being tested, the sudden exposure of sensitive functional microbial species (such as methanogens) to completely different physicochemical environments may not be prudent. For example, it has been shown that methanogenesis was completely inhibited using a non-acclimated inoculum from digesters treating abattoir effluent [46], while good methanogenesis was obtained using a specifically pre-acclimated inoculum to digest the same tannery effluent [47].

In this study, the advantage of using acclimated inocula was jeopardized by the presence of residual substrate in the inoculum at the start of the experiments. Ideally, the substrate should be depleted in inocula used for BMP experiments [45]. The residual substrate was due to the nature of the PWWS that contained slowly biodegradable lignocellulosic material from the grape pomace, making substrate exhaustion impossible without compromising on the viability of the sensitive methanogenic populations via starvation. The presence of residual substrate resulted in biogas and CH_4 generation in the inoculum control. However, the origin of the substrate in the inoculum was the same as the experimental substrate, and the specific yields were corrected to include the biogas contribution from the inoculum in the results (Fig. 1). Notwithstanding the biogas contribution from the inoculum substrate, around 1.5 L CH_4 was generated from 1 L reactor contents at ISR2 during experiment 1 (Fig. 2A).

To assess the degree to which AD could be enhanced at under stable mesophilic conditions, the optimal CH_4 generation conditions (ISR4 with nutrient addition) were applied

under both ambient and controlled mesophilic (37 °C) conditions simultaneously (experiment 2, Fig. 1). Under ambient conditions, the biogas and CH_4 yields were lower than those attained during experiment 1, once again demonstrating the variability in biogas yields that can be obtained when even slightly different inocula are used. In this case, all of the other experimental factors remained consistent between experiment 1 and experiment 2. The same inoculum had been maintained by feeding with the same PWWS. The results suggest that it is impossible to avoid temporal variations in the microbial community structure and function when maintaining inocula. However, the 2.6-fold higher specific CH_4 generation obtained at 37 °C clearly demonstrated that, as expected, performance efficiency could be increased significantly by increasing and controlling the temperature within an optimal range.

In comparison to the theoretical yield (530 $mLCH_4/gVS_{added}$ and 58% CH_4) calculated from the COD:VS ratio [41], the maximum yield (206 ± 2.7 $mLCH_4/gVS_{added}$) of the crush season PWWS was only 39% of the theoretical, but the CH_4 composition of the biogas (56%) was close to the theoretical value.

3.2.2 Anaerobic digestion of primary winery wastewater sludge from the post-crush season

In contrast to results obtained with crush season PWWS, no clear trend on the effect of ISR and nutrient addition on AD efficiency of post-crush PWWS was demonstrated (Fig. 1). However, in terms of absolute cumulative CH_4 generation (Fig. 2B), the highest yield was obtained at ISR2 with nutrient addition, and the lowest at ISR4, strongly suggesting that there were sufficient active functional microbes present at ISR2 for efficient methanogenesis. At ambient temperature (experiment 1), the yields attained were all higher with the post-crush season PWWS than with the crush season PWWS. This may have been related to the inoculum, and/or the presence of higher concentrations of readily biodegradable organics, including VFAs in the post-crush PWWS (COD 346 g/L, VFA 104 g/L) than in the crush PWWS (COD 149 g/L, VFA 29 g/L) and/or the use of a more robust inoculum.

The results of experiment 2 (ambient vs 37 °C) showed a similar trend to those obtained during AD of crush season PWWS in that substantially lower specific biogas and CH_4 yields were obtained at ambient temperature (ISR4 with nutrients) during experiment 2 than during experiment 1. In contrast to the results obtained with crush season PWWS, the amount of biogas was only negligibly higher (27 mL/gVS_{added}) at 37 °C than under ambient conditions during experiment 2. However, due to a promisingly high CH_4 contribution at 37 °C (79%) when compared to ambient conditions (59%), there was a greater difference in the specific CH_4 yield (45 mL/gVS_{added}). In comparison to the theoretical yield (496 $mLCH_4/gVS_{added}$) calculated from the COD:VS ratio [41], the maximum yield (177 ± 1.4 $mLCH_4/gVS_{added}$) was only 36% of the theoretical maximum, but the CH_4 composition of the biogas fell within the theoretical range (50–71%) under ambient conditions and was notably higher than the theoretical yield at 37 °C.

The differences in yields in experiments 1 and 2 with the same substrate at ambient temperatures could be explained by the fact that the average ambient temperatures during experiment 1 (20.7 ± 2.0 °C) were higher than those during experiment 2 (19.8 ± 1.87). However, greater temperature variations (range of temperatures) occurred during experiment 1 (16.8 to 27.1 °C) in comparison with experiment 2 (15.3 to 22.8 °C), which could theoretically lead to greater methanogenic community instability and reduced AD efficiency [48]. Furthermore, in comparison to lower temperatures, mesophilic and thermophilic temperatures typically improve reaction kinetics and promote methanogenesis [48–50]. Therefore, temperature differences and fluctuations do not appear to explain why the yields were also less at 37 °C than at ambient temperatures during experiment 2, even though higher temperatures can promote some reactor instability due to an increased rate of dissociation/solubilization of inhibitors as previously described [28].

Apart from the complexity of the physicochemical and biochemical reactions, these results appear to confirm that that the inoculum (as the only other variable) was less active during experiment 2, once again highlighting (i) the importance of having a suitable functional microbial consortium for BMP experiments and for larger scale reactor start-up and (ii) the inherent difficulty of comparing intra- and inter-study results. Typically, although BMP studies often include multiple variables, they do not account for temporal microbial variations as they are conducted as singular experiments.

Notwithstanding the inherent difficulties already alluded to regarding inter- and intra-study comparisons, results were compared with those reported in literature for AD of winery solid wastes under mesophilic conditions. All but one study used sludge from municipal wastewater treatment plants as the inoculum. Higher specific CH_4 yields (365 ± 20 $mLCH_4/gVS$) were obtained digesting pomace in 250-mL semi-continuous reactors [25]. However, the hydraulic retention time of 104 days was considerably longer than with PWWS where most biogas was generated within 30 days (Fig. 2). The highest specific CH_4 yields were obtained for batch AD of lees (876 ± 45 $mLCH_4/gVS$), grape must (838 $mLCH_4/gVS$), and SWWS (690 ± 25 $mLCH_4/gVS$) in 500 mL digesters with 57 days RT [21, 27]. These substrates have also been successfully co-AD at pilot scale in 230-L continuously stirred tank reactors, but only the overall biogas measurements were reported in this case (average 0.450 $m.^3/kg\ COD_{fed}$) [23]. It

has been calculated that sufficient biogas could be generated from co-AD of these substrates to support winery operations [21]. The lowest yields (6.45 $mLCH_4/gVS$) were obtained with co-AD of dried spent grape pomace and cheese whey (100-mL digesters, 58 days), despite using a well-acclimated inoculum from a laboratory digester [24]. Similarly, the AD of stems, PWWS, and secondary WWS were stunted (< 30 $mLCH_4/gVS$) likely due to poor acclimation of inoculum to substrate [27]

Overall, this study demonstrated that PWWS is a suitable substrate for AD, with no lag phase and complete digestion taking place within 30–40 days even under ambient conditions. In general, nutrient addition and controlled higher temperatures (37 °C) enhanced biogas and CH_4 generation at higher ISRs, indicating the increased metabolic co-factor demand associated with higher concentrations and activities of the functional microbial communities (higher ISR).

3.2.3 Evaluation of methane generation kinetic models

To evaluate the effectiveness of kinetic models for predicting the AD of PWWS, commonly applied models (cone, logistic, modified Gompertz, and first order) were fitted to the cumulative CH_4 production from experiment 1 for both crush and post-crush PWWS. In both cases, the cone, logistic, and first-order models fitted the experimental data with high precision (adjusted $R^2 > 0.958$). For optimal reactors (ISR4 + N), the models predicted the experimental data in the order: Logistic > Cone > First order > modified Gompertz according to AIC (Akaike information criterion) for the crush season PWWS, and predicted maximum CH_4 yields of 151.5, 142.0, 144.1, and 121.2 $mlCH_4/gVS_{added}$, respectively. For the post-crush PWWS, the model fit order was Cone > First order > Logistic > modified Gompertz, with predicted maximum methane yields of 199.4, 203.8, 195.2, and 155.7 $mlCH_4/gVS_{added}$, respectively. For reference, the complete set of calculated kinetic parameters has been provided in the Supplementary Material (Supplementary Tables 2 and 3).

3.3 Evaluation of digestate of crush and post-crush primary winery wastewater sludge as a soil conditioner/fertilizer

In-depth theoretical analyses of fertilizers need to account for a variety of different soil types and plants, ideally including pot and/or field experiments. Such analyses were beyond the scope of this study which was instead limited to a brief evaluation of the PWWS as a "proof of concept" to determine whether it may be suitable as a fertilizer. This was achieved by (i) comparing the macronutrient and micronutrient composition of the digestates with typical organic fertilizers and (ii) evaluating whether any elements were present in quantities that may be detrimental to plant and/or soil health.

The PWWS digestate composition was compared with a range of four commercial agricultural organic fertilizers based on composted chicken manure. Two of these were enriched, one with kelp and fishmeal, and the other with Ca and P. Both inorganic and organic forms of C are important as much C is being lost from the soil due to agriculture, other anthropogenic land use practices, and climate change across the globe [51, 52]. The total C concentrations were 2.0- to 2.6-fold higher in the PWWS than in the commercial fertilizers, indicating that would be a valuable source of C as a fertilizer. In terms of organic C, decayed organic material (humus) increases the water retention capacity and the cation exchange capacity of soils, assists with soil aeration, and provides a reservoir for gradual release of plant nutrients [53, 54]. All the PWWS digestates contained notable amounts of organic carbon (measured as TOC), and the amount was higher in the crush season PWWS digestate (average 261 g/kg) than in the post-crush season PWWS digestate (average 104 g/kg) (Table 3). Given the worldwide average soil organic carbon (SOC) concentration of 15.5 g/kg, even the post-crush PWWS could be a valuable contributor of humic material, especially in the case of sandy soils [55].

Different plants require macronutrients in different quantities, and some soils themselves contain sufficient elements. For example, as the name suggests, calcareous soils have high Ca concentrations, but have limited P and Zn availability [56]. To cater for different requirements, fertilizers contain variable ratios of the macronutrients N, P, and K, among other elements. The PWWS digestates contained similar amounts of N and K to the commercial fertilizers but were deficient in P, Ca, and S (Table 4). Depending on the soil type and crop, the PWWS may require supplementation with a source of these elements. Waste gypsum is an example of a sustainable source of Ca and S. In terms of micronutrients, both batches of PWWS digestates contained reasonable concentrations in comparison to the commercial organic fertilizers (Table 4), but both the commercial fertilizers and the PWWS contained high concentrations of Fe. Although Fe deficiency can be detrimental to plant growth, it can be toxic to plants in highly acidic or hypoxic soils (usually formed via waterlogging) as Fe^{3+} is reduced to Fe^{2+} which is more bioavailable than the oxidized form [26]. In well-aerated less acidic to alkaline soils, relatively high Fe concentrations are generally not problematic [26].

Unlike K, Na is not a plant nutrient and can negatively affect the soil structure by binding with negatively charged soil particles. This can be offset to some extent by the presence of divalent cations (Ca^{2+} and Mg^{2+}) that have more than one binding site and replace Na^+ [57]. Although the Na

Table 4 Concentrations of essential plant nutrients, sodium, and sodium adsorption ratio in the digestates from optimal anaerobic digestion at 37 °C and ambient temperatures

	Crush PWWS		Post-crush PWWS		Commercial*
	37 °C	Ambient	37 °C	Ambient	
Macronutrients (g/kg dry weight)					
Elemental CHNS analysis					
C	256.9 ± 8.1	277.8 ± 1.5	271.7 ± .9.1	229.2 ± 4.9	106–115
H	40.9 ± 3.7	43.8 ± 2.6	30.7 ± 1.1	51.0 ± 1.9	-
N	25.5 ± 1.4	25.6 ± 0.8	27.7 ± 0.1	21. ± 0.5	26–34
S	3.0 ± 0.2	3.0 ± 0.02	5.1 ± 0.5	3.6 ± 1.1	9–10
XRF analyses					
Ca	10.5 ± 0.24	9.8 ± 1.57	10.36 ± 0.83	9.69 ± 1.66	35–60
K	38.3 ± 2.11	37.9 ± 3.47	38.42 ± 1.91	27.67 ± 6.63	30–33
Mg	2.20 ± 0.07	2.00 ± 0.01	2.93 ± 0.09	2.38 ± 0.55	6–7
P	3.4 ± 0.01	3.3 ± 0.51	5.15 ± 0.43	4.47 ± 0.69	17–27
Micronutrients (mg/kg dry weight, **g/kg dry weight)					
XRF analyses					
Fe**	5.1 ± 0.01	5.0 ± 0.02	5.72 ± 0.04	4.76 ± 1.57	5
B	62 ± 11	57 ± 1.4	50 ± 0.1	40 ± 11	50
Mn	80 ± 1.1	70 ± 1.6	90 ± 1.0	80 ± 22	570–610
Cu	25 ± 2.2	23 ± 1.1	250 ± 17	180 ± 56	60
Zn	57 ± 2.8	49 ± 1.8	620 ± 7.9	450 ± 133	500–540
Mo	2.0 ± 0.1	4.0 ± 0.0	ND	ND	5
Other (g/kg dry weight)					
TOC	282 ± 13.0	241 ± 17.1	98.7 ± 10.6	110 ± 2.82	ND
Na	3.20 ± 0.17	3.10 ± 0.19	3.26 ± 0.08	2.28 ± 0.57	ND
Ratios					
C:N	10.08	10.87	9.82	10.66	3.73
SAR	1.3	1.3	1.2	0.9	ND

*Range of organic fertilizers based on composed chicken manure (Bio ganic® Bio ganic crumble®), enriched with kelp and fishmeal (Bio ocean®), enriched with calcium and phosphate (Bio rock®). *TOC*, total organic carbon; *SAR*, sodium adsorption ratio; *ND*, not determined

concentrations in the digestates were high (Table 4), the concentrations in the PWSS before AD were relatively low in comparison to the inocula (Table 4), clearly demonstrating that most of the Na in the digestates emanated from the inocula. The original starter culture was taken from AD of highly saline tannery effluent, and thereafter, the inoculum was fed constantly with WWW which also resulted in accumulation of Na over time. In reality, Na remains soluble in WWW, and is not expected to accumulate to high concentrations in PWWS [1, 38] as evidenced by the results in Table 3. Even with the anomalously high concentrations measured in the PWWS digestates, any sodicity risk was offset by high concentrations of Ca and Mg that reduced the sodium adsorption ratio (SAR) to ≤ 1.3. To put this in perspective, soils with and SAR > 13 are considered sodic [57, 58] and those with SAR < 5 pose a low risk [59]. Nevertheless, the Na levels will be closely monitored in future studies.

For reference, the character of the digestates from all of the reactor contents is included in the Supplementary Material (Supplementary Table 5).

Overall, the composition of the PWWS showed excellent potential for addition of C, N, and micronutrients to soils. Further work will be conducted using pot experiments to determine the effects of PWSS digestates on crop growth in different soils.

4 Conclusions

Concurrent AD of PWWS for bioenergy and biofertilizer supports a circular economy. During the warmer post-crush months, good biogas and CH_4 yields from AD may be feasible without the need for heating, making it economically

viable for smaller wineries. The digestate is a promising agricultural fertilizer, especially for addition of C, N, and micronutrients to soils. To prevent potential Fe toxicity, it is recommended that it should not be used in highly acidic or waterlogged soils. Future studies will focus on conducting pot experiments with different batches of digestates and comparing the results.

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Declarations

Competing interests The authors declare no competing interests.

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Appendix 7 Journal Article 5 – Supplementary data

Supplementary Table A7-1 Initial characterisation of reactor contents (Experiment 1)

	COD (g/L)	VOA (g/L)	TN (mg/L)	TP (mg/L)	NH_4^+(mg/L)	S^{2-} (mg/L)
Crush						
ISR 2	78,4	22,1	2397,3	395,0	647,7	117,1
ISR 3	69,7	19,5	2040,7	353,7	618,2	98,4
ISR 4	65,2	18,2	1855,2	332,2	602,8	88,7
Inoculum	52,4	14,3	1327,3	271,2	559,2	61,1
PWWS	121,2	36,4	4993,3	577,7	413,0	261,3
Post-crush						
ISR 2	147,5	31,2	218,1	712,7	3070,7	117,1
ISR 3	110,2	24,1	160,7	535,6	2599,0	95,6
ISR 4	93,8	20,9	135,5	457,6	2391,5	86,2
Inoculum	43,1	11,2	57,5	216,7	1750,0	57,1
PWWS	104,4	20,0	160,6	496,1	1320,7	60,0

Supplementary Table A7-2 Final characterisation of reactor contents (Experiment 1)

	COD (g/L)	VOA(g/L)	TN(mg/L)	TP(mg/L)	NH4+(mg/L)	S2-(mg/L)
Crush						
ISR2	44,4 ± 4,1	18,9 ± 7,5	3075 ± 12,2	334,0 ± 43,8	1457,5 ± 10,6	75,0 ± 13,4
ISR2+N	46,4 ± 2,7	13,9 ±0,8	3680 ± 226,3	319,5 ± 9,2	2017,5 ± 456,1	61,8 ± 0,4
ISR3	37,4 ± 13,2	13,1 ± 2,6	3040 ± 0,0	257,5 ± 26,2	2000,0 ± 1131,4	64,5 ± 21,2
ISR3+N	33,5 ± 14,7	10,9 ± 1,4	2860 ± 1045,5	257,0 ± 48,1	1565,0 ± 0,0	52,3 ± 11,0
ISR4	39,5 ± 7,1	17,0 ± 1,1	2830 ± 353,6	272,5 ± 12,0	1402,5 ± 67,2	69,8 ± 20,2
ISR4+N	27,3 ± 0,9	10,5 ± 0,2	3000 ± 622,3	280 ± 17,0	1880,0 ± 636,4	56,8 ± 15,2
Post-crush						
ISR2	170,1 ± 28,1	33,9 ± 2,6	1110 ± 1145,5	432,8 ± 52,0	2365,0 ± 247,5	60,0 ± 0,0
ISR2+N	180,3 ± 10,8	34,8 ± 1,9	2350 ±551,5	416,0 ± 95,5	2420 ± 198,0	78,8 ± 6,7
ISR3	153,6 ± 22,9	16,4 ± 2,0	2040 ± 84,9	264,3 ± 56,9	1705 ± 148,5	80,3 ± 0,4
ISR3+N	158,1 ± 12,8	23,9 ± 1,8	2165 ± 586,9	264,0 ± 38,2	2320 ± 84,9	59,0 ± 0,7
ISR4	131,7 ± 8,6	19,2 ± 4,1	2100 ± 452,5	268,8 ± 45,6	1890 ± 28,3	63,3 ± 1,1
ISR4+N	120,8 ± 0,6	15,6 ± 4,3	1840 ± 736,7	228,0 ± 58,0	1905 ± 77,8	61,8 ± 0,4

Supplementary Table A7-3 Kinetic parameters for AD of crush season PWWS

	Model	Kinetic parameters					Adj R^2	AIC	RMSE
		A ($mLCH_4/gVS$)	μ_m ($mLCH_4$ /gVSd)	λ (d)	K	n			
ISR 2	Logistic	27,1	1,08	5,1			0,993	67,2	0,55
	Cone	54,7			0,027	1,47	0,993	62,1	0,51
	Gompertz	7,4	0,43	8,61			0,655	207	3,83
	1st order	2490,3			3E-04		0,989	84,7	0,70
ISR 2+N	Logistic	22,7	1,00	5,53			0,993	53,4	0,45
	Cone	34,4			0,041	1,78	0,994	51,9	0,45
	Gompertz	9,4	0,34	14,3			0,996	38,4	0,37
	First order	120,0			0,006		0,981	95,3	0,81
ISR 3	Logistic	86,9	3,32	5,02			0,991	157	1,92
	Cone	211,1			0,021	1,36	0,995	135	1,42
	Gompertz	37,4	1,11	15,8			0,996	129	1,29
	First order	39035,9			6E-5		0,997	115	1,07
ISR 3+N	Logistic	92,4	3,71	4,44			0,991	163	2,08
	Cone	184,3			0,028	1,41	0,998	102	0,90
	Gompertz	38,7	1,25	30,7			0,997	131	1,33
	First order	633,3			0,005		0,997	125	1,23
ISR 4	Logistic	119,5	4,44	4,34			0,991	180	2,64
	Cone	351,1			0,016	1,24	0,994	160	2,00
	Gompertz	51,4	1,49	15,4			0,994	162	2,06
	First order	1588,2			0.002		0,998	121	1,16
ISR 4+N	Logistic	159,3	6,49	5,54			0,993	192	3,11
	Cone	286			0,028	1,45	0,963	252	7,15
	Gompertz	45	2,63568765	10			0,655	335	22,7
	First order	1199,5			0,004		0,958	261	8,09

RMSE = Root mean square error

AIC = Akaike information criterion

Supplementary Table A7-4 Kinetic parameters for Post-crush season PWWS

	Model	Kinetic parameters					Adj R^2	AIC	RMSE
		A ($mLCH_4/gVS$)	μ_m ($mLCH_4$ /gVSd)	ʎ (d)	K	n			
ISR 2	Logistic	121,7	12,51	1,97			0,990	134	2,50
	Cone	131,8			0,147	2,18	0,998	81,5	0,94
	Gompertz	34,4	7,27	1,5			0,325	248	20,62
	First order	141,6			0,091		0,977	157	3,85
ISR 2+N	Logistic	154,8	12,84	1,49			0,975	160	4,05
	Cone	194,0			0,109	1,52	0,992	128	2,25
	Gompertz	58,8	4,42	5,69			0,991	142	2,87
	First order	120,0			0,174		0,812	226	13,66
ISR 3	Logistic	184,0	14,79	0,9			0,982	168	4,71
	Cone	246,8			0,103	1,27	0,986	148	3,24
	Gompertz	69,8	5,14	5,22			0,989	155	3,66
	First order	214,4			0.087		0,992	148	3,23
ISR 3+N	Logistic	149,3	12,38	0,74			0,979	161	4,12
	Cone	197,2			0,112	1,21	0,954	167	4,63
	Gompertz	56,3	4,30	30,7			0,98	160	4,07
	First order	166,4			0,100		0,977	164	4,36
ISR 4	Logistic	167,5	17,61	0,9			0,988	153	3,58
	Cone	187,9			0,175	1,65	0,996	109	1,57
	Gompertz	62,6	6,30	4,11			0,997	120	1,92
	First order	181,6			0,130		0,993	139	2,74
ISR 4+N	Logistic	195,3	16,71	0,38			0,964	176	5,47
	Cone	264			0,076	1,56	0,995	121	1,98
	Gompertz	57	9,54	2			0,149	275	34,2
	First order	214,6			0,111		0,993	146	3,09

RMSE = Root mean square error

AIC = Akaike information criterion

Supplementary Table A7-5 Characteristics of digestate from all reactor

	Crush						Post-Crush					
	ISR2	ISR2+N	ISR3	ISR3+N	ISR4	ISR4+N	ISR2	ISR2+N	ISR3	ISR3+N	ISR4	ISR4+N
Elemental CHNS analysis (%wt.wt. dry weight); TOC & NH_4^+ (g/L wet weight homogenised digestate)												
C	29.92±2.46	29.6±0.64	30.44±0.15	28.97±2.35	26.93±0.85	28.03±1.8	27.70	26.26±1.26	25.16±1.17	24.90±0.19	23.93±1.24	21.02±1.22
H	5.1±0.71	5.85±1.7	4.42±0.47	6.56±0.57	4.7±0.01	6.42±0.39	4.46	3.32±0.41	3.15±0.17	2.93±0.01	2.95±025	3.51±1.44
N	2.4±0.07	2.4±0.21	2.6±0.31	2.36±0.15	2.43±0.09	2.65±0.08	3.35	2.35±0.09	2.17±0.11	2.23±0.10	2.23±0.13	2.0 ±0.15
S	0.43±0.05	4.92±6.6	0.42±0.06	3.25±3.4	0.46±0.05	2.87±1.55	0.49	0.45±0.06	0.48±0.05	0.36 ±0.09	0.46±0.04	0.37±0.05
C:N	12.47	12.33	11.71	12.28	11.08	10.58	8.27	11.20	11.62	11.16	10.73	10.28
TOC	13.97±3.29	16.41±0.30	15.60±8.59	9.45±2.58	11.98±1.45	10.54±7.51	4.67±1.00	7.76±0.51	7.39±2.36	6.27±0.52	7.26±1.60	8.10±3.76
NH_4^+	2.37±0.25	2.42±0.20	1.71±0.15	2.32±0.08	1.89±0.03	1.91±0.08	1.46±0.01	2.02±0.46	2.00±1.13	1.57±0.00	1.40±0.07	1.88±0.64
Major elemental parameters (g/kg dry weight)												
Fe	4.73±0.45	4.46±0.46	4.42±0.28	5.28±0.2	4.79±0.53	4.44±0.34	5.28	5.36±0.15	4.97±0.30	4.75±0.58	4.82±0.90	5.17±0.09
Al	11.2±1.41	10.31±1.1	10.25±0.47	12.22±0.68	11.65±1.1	10.64±1.3	15.98	14.80±0.45	14.19±0.16	13.36±1.21	13.75±1.67	14.32±0.72
Ca	15.82±1.15	14.84±0.45	17.66±1.94	17.88±1.00	19.40±1.8	18.96±2.9	5.73	8.18±0.15	7.76±0.70	8.25±1.42	7.21±2.94	8.57±0.94
K	61.55±8.86	55.59±7.85	61.31±4.97	65.99±11.63	68.28±27.31	80.43±18.54	31.19	63.58±10.89	73.64±21.55	93.86±27.01	47.09±21.34	81.82±15.65
Mg	2.52±0.12	2.36±0.42	2.48±0.45	2.75±0.45	2.61±0.09	2.52±0.21	2.47	2.47±0.04	2.33±0.03	2.31±0.14	2.21±0.39	2.47±0.02
Na	11.69±1.11	11.17±0.03	15.07±0.79	13.33±0.62	18.70±7.12	19.76±7.05	2.66	4.81±0.90	5.39±1.48	6.52±1.94	3.79±1.48	5.90±0.99
P	4.14±0.12	3.91±0.70	4.19±0.62	4.75±1.02	4.10±0.05	4.25±0.03	4.64	4.75±0.53	4.61±0.40	5.48±0.76	4.31±0.24	4.83±0.47
Si	2.16±0.36	2.13±0.62	2.11±0.33	2.59±0.21	1.52±0.51	2.44±0.29	2.66	2.73±0.03	2.33±0.07	2.34±0.08	2.44±0.34	1.90±0.08
Selected minor elemental parameters (g/kg dry weight)												
B	0.06±0.00	0.06±0.00	0.07±0.00	0.07±0.01	0.05±0.00	0.07±0.01	0.05	0.05±0.00	0.05±0.00	0.05±0.00	0.05±0.01	0.05±0.00
Cr	0.55±0.07	0.55±0.01	0.71±0.11	0.64 ±0.04	0.81± 0.08	0.76±0.21	0.12	0.15±0.01	0.13±0.00	0.13±0.02	0.12±0.04	0.14±0.01
Mn	0.14±0.01	0.14±0.00	0.18±0.04	0.19 ±0.03	0.18±0.00	0.17±0.03	0.06	0.06±0.00	0.06 ±0.00	0.06±0.01	0.06±0.02	0.07±0.00
Co	0.01±0.00	0.01±0.00	0.01±0.01	0.01 ±0.00	0.01±0.00	0.02±0.01	0.05	0.15±0.00	BDL	0.11±0.00	0.02±0.03	0.09±0.00
Ni	0.01±0.00	0.04±0.00	0.03±0.02	0.04 ±0.01	0.01±0.00	0.04±0.01	0.09	0.21±0.01	0.02±0.00	0.16±0.01	0.05±0.03	0.17±0.01
Cu	0.19±0.01	0.23±0.01	0.22±0.07	0.24 ±0.01	0.19±0.03	0.25±0.03	0.25	0.25±0.02	0.20±0.00	0.29±0.03	0.21±0.01	0.30±0.00
Zn	0.99±0.1	0.99±0.06	1.06±0.17	1.07±0.01	1.16±0.17	1.02±0.09	0.59	0.55±0.01	0.56±0.04	0.54±0.10	0.52±0.04	0.55±0.03
Sr	0.06±0.00	0.06±0.00	0.07±0.01	0.07±0.01	0.07±0.01	0.07±0.01	0.03	0.04±0.00	0.04±0.00	0.04±0.01	0.04±0.01	0.04±0.01
Mo	0.003±0.00	0.003±0.00	0.003±0.00	0.004±0.00	0.003±0.00	0.003±0.00	ND	ND	ND	ND	ND	ND
Pb	0.03±0.00	0.03±0.00	0.02±0.00	0.03±0.01	0.02±0.01	0.02±0.00	0.06	0.04±0.00	0.04±0.00	0.04±0.01	0.09±0.06	0.05±0.02
Ba	0.1±0.04	0.09 ±0.01	0.08 ±0.01	0.11±0.03	0.09±0.03	0.07±0.01	0.10	0.12±0.00	0.12±0.03	0.09±0.01	0.10±0.02	0.12±0.01

Appendix 8 Journal article 6 offprint

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Biosand reactors for remediation of winery effluent in support of a circular economy and the positive effect of sand fractionation on hydraulic and operational performance

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ABSTRACT

There is an extensive body of knowledge pertaining to the treatment of winery wastewater and other acidic effluents in biosand reactors. This manuscript compares the performance of biosand reactors containing raw sand and fractionated sand. Fractionation of sand (particles <0.425 mm removed) increased the hydraulic conductivity of the sand matrix 9-fold when compared to raw sand: from 0.285 mm·s^{-1} to 2.50 mm·s^{-1} and 0.129 mm·s^{-1} to 1.11 mm mm·s^{-1} before and after start-up, respectively. Similar results for both sands were obtained in terms of organic removal performance (94 % and 95 %, respectively) and neutralization of acidic (pH 4.9) winery wastewater. Results indicate that one 5.6 m^3 biosand reactor module containing 2.9 m^3 of fractionated sand can theoretically treat 8102 L·d^{-1}, which is the approximate volume generated from wineries crushing 329 to 547 t of grapes per annum. This is notably higher than the design flow rate of 1000 L·d^{-1} in used in a pilot system containing raw sand. Furthermore, a zero-waste biosand reactor model is presented for treatment of winery wastewater. The strategy includes reuse of treated effluent for irrigation, anaerobic digestion of primary winery wastewater sludge and use of the digestate as an agricultural fertilizer, and re-purposing of the residual sand.

1. Introduction

Fermentation of grape juice to wine relies on the microbially mediated conversion of grape sugars to ethanol with the concurrent formation of other metabolites which add to the complexity of the final product [1–4]. This winemaking process and ancillary operations generate solid wastes, by-products, and winery wastewater (WW). Many solid waste streams, including lees and marc, are valorised on-site or in dedicated biorefineries [5], but due to its nature, WW has little beneficial use. Winery wastewater fluctuates in physiochemical quality, with the highest organic and volumetric loads occurring during the crush/harvest period [6–8]. After primary settling and pH adjustment, 'beneficial' irrigation is often used as a means of WW disposal, particularly in water stressed countries [9]. Depending on cellar activities and practices, the WW can contain high concentrations of organic molecules and inorganics. For example, high sodium (Na) loads can emanate from the ubiquitous use of the relatively inexpensive and effective cleaning product, sodium carbonate (Na_2CO_3) [6]. Polyphenolics in WW can be phytotoxic, and highly saline WW may cause some soils to become sodic [6,10–12]. Irrigation with untreated WW can therefore pose a threat to the receiving environment. It is therefore prudent to remediate WW before discharge on land or to aquatic environments.

There are several WW treatment options available which vary in cost, complexity, and efficiency. The challenge for designers of WW treatment systems is the seasonal variability in the quality and quantity of WW [13–15]. Depending on the local legislation, the amount of WW generated, and the location of the cellar, different levels of remediation may be required. While complex treatment systems may be effective, they are generally not applicable for smaller wineries due to cost and staff constraints [16]. Constructed/treatment wetlands have been extensively used as passive WW treatment systems, but typically have large spatial footprints and the WW may be phytotoxic to the plants [17]. Biosand reactors (BSRs), alternatively known as biological sand filters or unplanted constructed/treatment wetlands, are cost effective, require minimal maintenance and provide an environment for biodegradation of the organic fraction of WW and neutralization of acidic WW.

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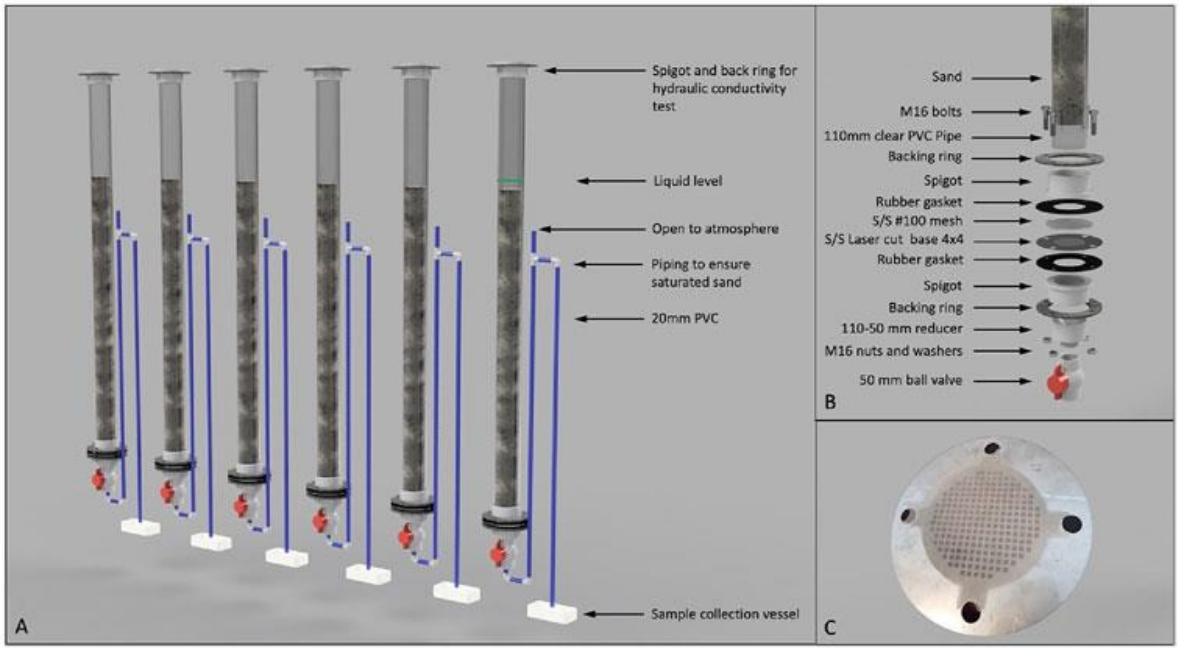

Fig. 1. Layout of experimental set-up of column experiment together with outlet piping (A), exploded view of outlet filter of column (B), stainless steel number 100 mesh and the stainless-steel laser cut base with 4 × 4 mm square openings (C).

After start-up, the hydraulic conductivity (HC) of the BSRs decreases due to the formation of functional biomass. This is inversely correlated with organic loading and therefore reversible, so that the systems do not require backwashing after the crush season even after long-term operation [18–21]. When operated in vertical mode, BSRs have smaller spatial footprints and higher specific organic removal rates (ORR) than other passive treatment systems, and can be retrofitted to existing treatment systems [21].

One of the challenges that has previously been associated with pilot BSRs is the large (up to 95 %) reduction in the HC of the sand due to build-up of biomass during WW treatment [21]. The biomass is a necessary functional component of BSRs and is most abundant during periods of high organic loading. During this time, it retards the flow of WW, causing a transient reduction in the hydraulic capacity of BSRs [18,21,22]. The size of sand particles is a critical factor governing the HC of soil and sand environments, with liquid flow rates increasing as particles increase in size [22,23]. It was therefore hypothesized that: (i) the hydraulic performance of BSRs during the crush period could be improved by fractionating the sand and using only the larger sand particles in the systems, and (ii) the smaller sand particles could be used for another purpose. For example, smaller sand particles are more desirable in self-compacting concrete (SCC) mixes [24]. The proposed dual usage of the sand feeds into the principles of a circular economy.

In passive treatment systems, there is theoretically a balance between hydraulic retention time (HRT) and remediation efficiency [25,26] and functional microbial communities are influenced by the character of sand particles in BSRs [27]. It was therefore recognized that the fractionation strategy could have a negative impact on BSR performance. Column replicates containing raw or fractionated sand (particles >0.425 mm) were therefore used to compare (i) The HC before and after build-up of biomass consequent to feeding with WW, (ii) organic removal rates, and (iii) WW neutralization rates. This study is the final in a series of studies pertaining to remediation of WW in BSRs. The manuscript therefore contains a section exploring the holistic use of BSRs and proposed ancillary methods for treating WW and WW sludge in the context of resource utilization and reuse for a bio-circular economy.

2. Materials and methods

2.1. Experimental set-up and operation

The experimental set-up consisted of six identical clear 2000 mm tall polyvinyl chloride (PVC) columns with internal diameters of 105 mm (Fig. 1A). Each column contained approximately 20 kg of sand to a height of 1450 mm (Table 1). The columns were operated in triplicate with one set containing raw (unfractionated) sand and the second containing sand with all particles <0.425 mm removed (fractionated sand) (Fig. 2). The raw sand has an effective size, uniformity coefficient and fineness modulus of D_{10} = 0.36, 2.3 and 1.67 respectively and D_{10} = 0.36, 2.0 and 2.78 respectively for the fractionated sand. To prevent

Table 1
Operational parameters of sand columns.

Sand type	Sand height (mm)	Sand weight (g)	HRT (h)	HLR ($L \cdot m^3$ sand-day^{-1})
Raw	1450 ± 10.8 [1435–1460]	20,000 ± 0 [20000–20,000]	36.7 ± 0.3 [36.3–36.9]	191.2 ± 1.4 [189.9–193.1]
Fractionated	1444 ± 7.4 [1435–1453]	21,000 ± 408 [20500–21,500]	37.6 ± 0.3 [37.4–37.9]	191.9 ± 1.0 [190.8–193.1]

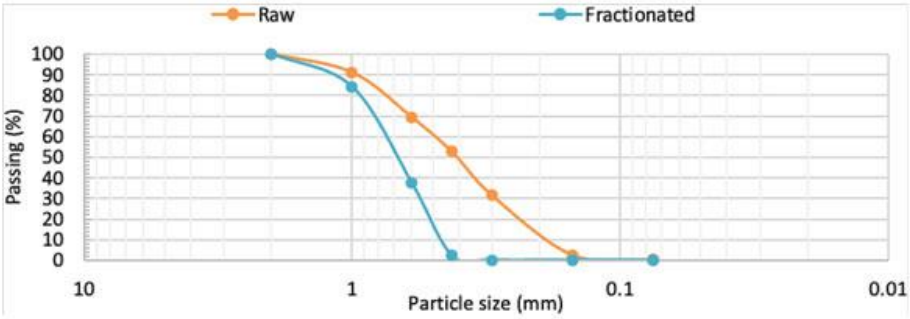

Fig. 2. Particle size distribution curves for raw and fractionated sand.

Table 2
Feeding schedule and physicochemical analysis of influent winery wastewater.

Period (day)	Volume (L)	COD ($mg \cdot L^{-1}$)	TN ($mg \cdot L^{-1}$)	TP ($mg \cdot L^{-1}$)	TPP ($mg\ GAE \cdot L^{-1}$)	pH (pH)	Alk ($mg\ HCO^{-} \cdot L^{-1}$)	Con ($\mu S \cdot m^{-1}$)
Days 0–21[1]	4*	101	0.0	0.0	3.6	6.8	92	161
Days 21–42[1]	4*	206	0.0	0.0	2.4	6.8	67	153
Days 42–63[1]	8*	492	0.5	1.6	10.3	6.8	116	230
Days 63–84[1]	8*	961	2.6	5.8	16.4	6.6	134	438
Days 84–105[1]	13	1013	20.8	21.1	17.7	6.8	183	470
Days 105–126[2]	21	2538	73.1	9.2	55.3	5.6	409	1286
Days 126–130[3]	8	4681	89.3	29.6	38.2	4.9	311	1551

COD = chemical oxygen demand, TN = total nitrogen, TP = total phosphate, TPP = total poly-phenolics, GAE = gallic acid equivalents, Alk = Alkalinity, HCO = bicarbonate, Con = electrical conductivity, S = siemens, [1,2,3] = batch number of the winery wastewater.
* Wastewater diluted with filtered water.

sand washout and to ensure permanent saturation but unimpeded flow when required during operation, a complex sealing system consisting of gaskets, mesh screens, spigots, backing rings and ball valves were installed at the bottom outlet of each column. To increase the column lengths for sand HC measurements, spigots and backing rings were also installed at the top of the columns (Fig. 1B, C).

For the HC tests, the outlet piping was removed from the columns so that the flow was only restricted by the 50 mm ball valves. To increase the hydraulic head, temporary 2000 mm column lengths were joined to the top of the columns above the mesh and a stopwatch was used to measure the time it took for the water level to drop each 200 mm, with the final measurement being taken at a hydraulic head of 1520 mm. HC measurements for each column were conducted in triplicate.

For the performance evaluation experiments, each column was fed with WW. The hydraulic loading rate (HLR) was calculated in terms of the volume of sand within the column as previously described [18] (Table 1). The columns were fed via IVAC volumetric pumps (Model 597) at a rate of 2.4 $L \cdot day^{-1}$ with operational parameters as shown in Table 1. All the pumps were fed from the same holding vessel to ensure influent consistency.

Approximately 3 kg of sand was extracted from an operational BSR system containing functional biomass [21]. Distilled water was added to the extracted sand and sonicated. After the initial HC test, the columns were allowed to drain for 48 h then inoculated with 1 L of the supernatant fluid from the sonicated sand to the top of each column, which were then allowed to acclimate for 14 days.

After inoculation, starting at days 0, 21, 42, 63, 84, 105 and 126, the columns were fed with different batches of WW at 100 $mL \cdot h^{-1}$ for the allotted volume, sampled and outlet was closed till the next dosing (Table 2). During the start-up period (days 0 to 83), the WW was diluted with filtered water and the columns were fed with WW in increasing concentrations to allow the functional microbial communities to gradually acclimate to the WW. This 'incremental priming' has been shown to increase system performance in BSRs [28]. The systems were considered operational from day 84 (Table 2).

[] = Range, HRT = hydraulic retention time, HLR = hydraulic loading rate.

2.2. Sampling and characterisation of influent and effluent

From the third feeding period (days 42–63), effluent samples were taken over the last hour of each feeding period and analysed immediately. The pH was determined according to the manufacturer's instructions using a CyberScan pH 300 meter and appropriately calibrated pH probe PHWP300/02K (Eutech instruments, Singapore). The electrical conductivity (EC) was determined using a hand-held Oakton ECTestr 11+ multirange, cup-style pocket conductivity meter (Eutech Instruments, Singapore Cat No: 35665-35) with a range of 0 µS/m to 20 mS/m. The COD concentrations were determined using a Merck (Merck®, Whitehouse Station, USA) Spectroquant® Pharo instrument and Merck Spectroquant® cell tests (cat. no. 1.14895.0001, 1.14541.0001 and 1.14691.0001) according to manufactures instructions. The total phenolic concentrations were determined using the Folin-Ciocalteu micro method [29] using a Merck®Folin-Ciocalteu reagent (Cat No: 1.09001.0500). Total Alkalinity was measured using the Merck titrimetric method with titration pipette MQuant catalogue number (1.111109.0001), according to the manufacturer's instructions.

2.3. Characterisation of sand

The particle size distribution was performed using the SANS 3001 (Method AG1, PR5, AG21) by Cetlab, South Africa.

Fractionated samples of sand analysed by automated scanning electron microscopy using a FEI QEMSCAN® (Quantitive Evaluation of Minerals by SCANning Electron Microscopy) instrument (Thermo Fisher Scientific, Waltham, USA) and iDiscover™ software. The roundness and aspect ratios of the different mineral fractions determined using the iDiscover™ software were used to compare the shapes and sizes of

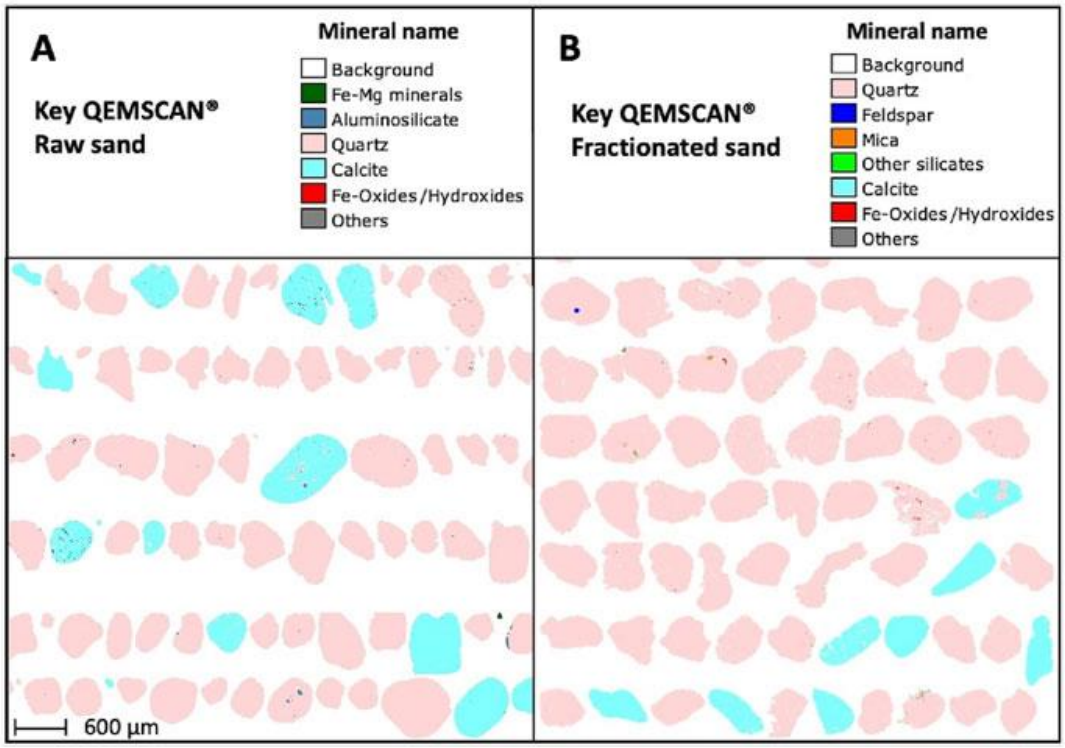

Fig. 3. Computer generated (QEMSCAN®) images of the raw (A) and fractionated (B) sand particles.

particles with different mineral composition as previously described [23,30].

3. Results and discussion

3.1. Hydraulic performance

The saturated HC is the intrinsic value which describes the rate at which fluid moves though a saturated porous media and is affected by the porosity of the medium, particle packing, particle size [23] and uniformity of the particles [22]. In this study, indices obtained via QEMSCAN® were used to compare the morphologies of the raw and fractionated sand [23,30]. For the raw sand, the quartz and calcite contributions were 81.3 % (wt.wt.) and 17.6 % (wt.wt.), while for the fractionated sand, they were 74.0 % (wt.wt.) and 9.6 % (wt.wt.) respectively. Computer generated images of the morphology and mineralogy of the raw and fractionated sand obtained using via QEMSCAN are shown in Fig. 3. Additional information of the character of the raw sand can be found in literature [22,23].

It was previously established that the HC of the sand with the smaller fractions (>0.6 mm) removed exhibited a significantly higher ($p < 0.05$) HC (2.75 ± 0.02 mm·s^{-1}) than the raw sand (0.41 ± 0.10 mm·s^{-1}), but the relative losses in HC due to the formation of functional biomass were not determined [19,22]. In this study, the HC results before and after biomass formation were very similar to those obtained in an earlier experiment conducted using columns containing a batch of sand from the same quarry site fed with synthetic WW for 14 weeks [22]. The initial comparative HC values of the raw sand batches were: 0.28 ± 0.05 mm·s^{-1} (this study) and 0.284 mm·s^{-1} [22], with respective reductions in HC due to the growth of functional biomass of 52.3 ± 6 % and 52 %.

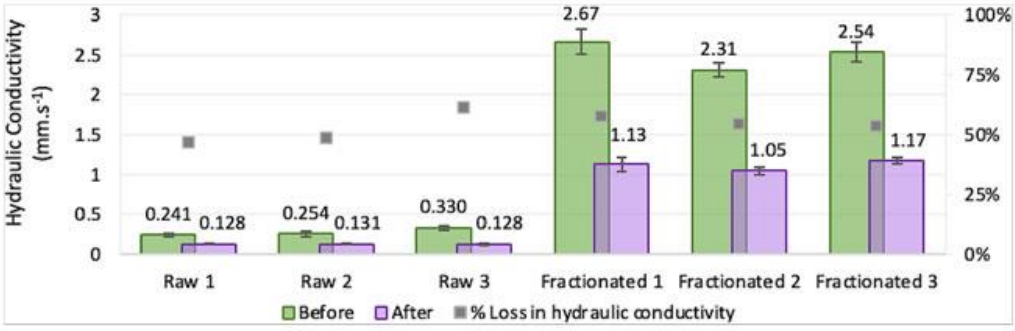

Fig. 4. The hydraulic conductivity of raw and fractionated sand measured in experimental columns before and after feeding with winery wastewater.

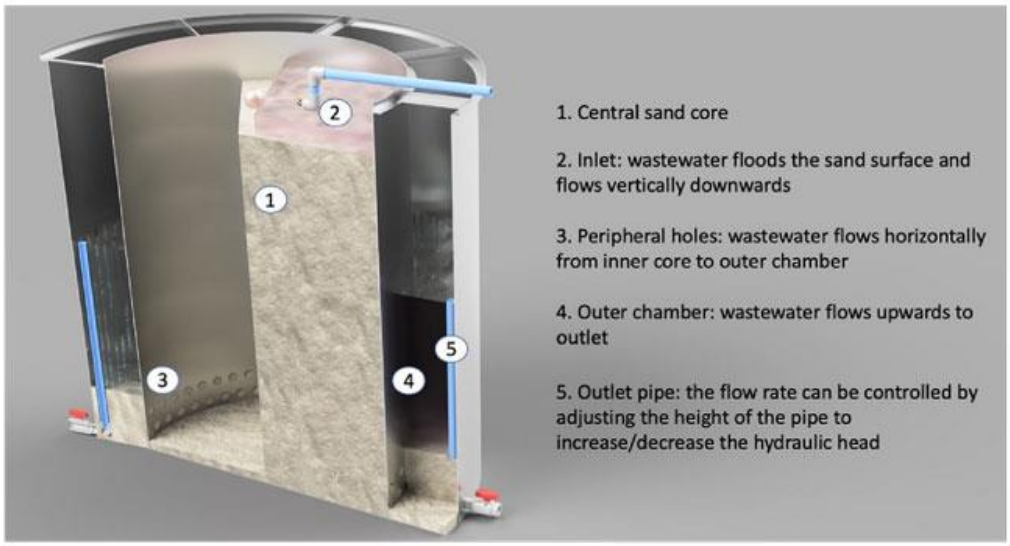

Fig. 5. Cross-section of a novel biosand filter module. (Adapted from Holtman et al. [21]).

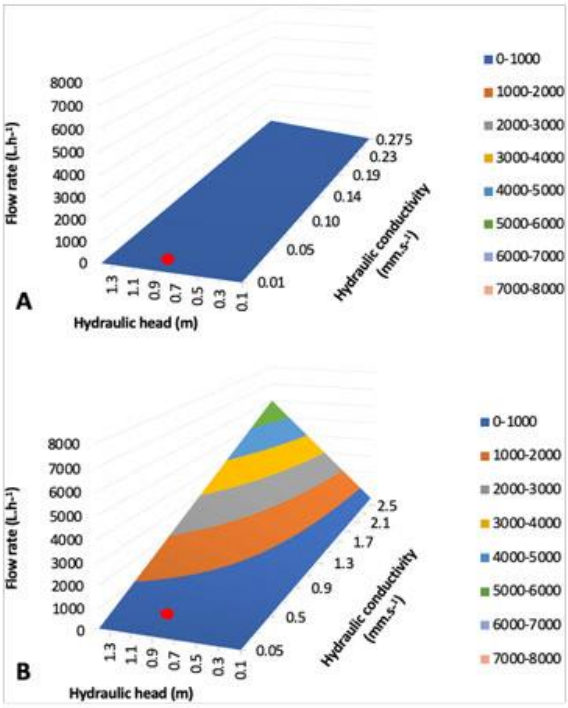

Fig. 6. Potential ranges of flow rates that can be achieved in biosand reactors containing raw (A) and fractionated (B) sand before and after biomass accumulation. The theoretical flow rates, used in Table 3, after biomass accumulation are denoted with a red circle. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

For the fractionated sand, the initial HC of 2.50 ± 0.20 mm·s^{-1} reduced by 55.4 ± 2 % after feeding with WW for 19 weeks, but the HC (average 1.15 ± 0.081 mm·s^{-1}) was still >4 fold higher than the initial HC of the raw sand (Fig. 4).

The HC results were applied to a pilot BSR system with a novel design [21] shown in Fig. 5. In the system, pre-settled WW is applied to the surface of the BSR modules and flows passively through the system as indicated in the diagram. The adjustable outlets allow the HLR to be manipulated by changing the hydraulic head to the maximum rate dictated by the HC. It was found that the range of possible flow rates was considerably greater when using fractionated sand in comparison to raw sand in the BSR modules (Fig. 6).

When the pilot system was operated with raw sand and a hydraulic head of 0.8 m, the maximum achievable flow rate during the crush season was <250 L·d^{-1} per module [21]. By applying the HC results obtained in the column experiments containing fractionated sand, it was calculated that this rate could theoretically be increased to 114,681 L·d^{-1} (before) and 5455 L·d^{-1} (after) biomass accumulation during the crush season. This would reduce the HRT for the reactor module to 17.8 h during the crush season (Fig. 6). In reality, the maximum operational flow, with a hydraulic head of 0.8 m, would be limited to 8102 L·d^{-1} which equates to a HLR of 3212 L·m^{-3}of sand.d^{-1} in order to provide sufficient HRT (12h) for bioremediation of the WW. In other words, when calculating the WW volume generated/tonne of grapes crushed previously formulated [31], one BSR module containing fractionated sand would be required for wineries that crush 547 t or 329 t of grapes, respectively, producing 50 % or 80 % of their WW during the crush season. In comparison, a single BSR module would be capable of treating the WW from wineries crushing approximately three times as many grapes when applying the WW generation volume of 3.05 m^3 WW/tonne of grapes crushed formulated by another group of researchers [32].

3.2. Remediation performance of biosand filters with fractionated and raw sand

3.2.1. Organic removal rates

Due to the organic nature of WW, the organic fraction was measured using COD as a proxy [6]. It has been conclusively established that BSRs containing raw sand are capable of significantly reducing the COD of

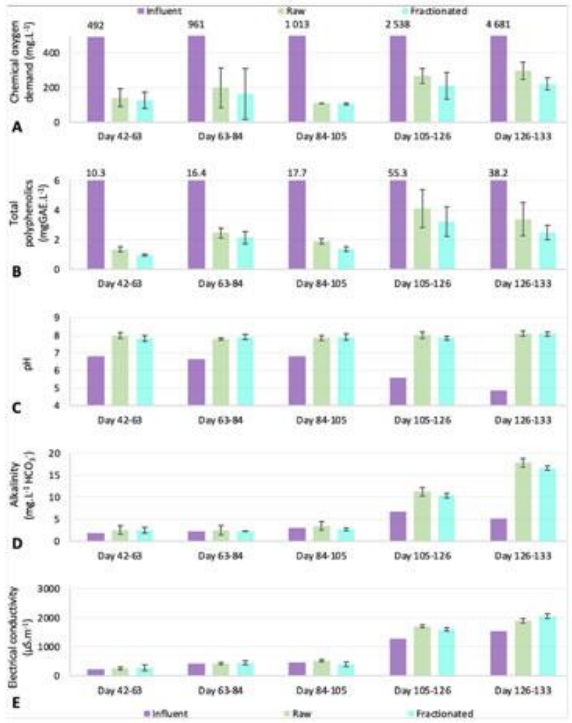

Fig. 7. Results of A) chemical oxygen demand, B) total polyphenolics, C) pH, D) alkalinity and E) electrical conductivity of influent and samples taken from experimental columns containing raw and fractionated sand.

WW, including the concentrations of polyphenolics [18,21,33,34]. A pilot system containing raw sand was able to treat WW at higher organic loading rates (279 g COD·m^{-3} of sand·day^{-1}) with a smaller spatial footprint than reported for other passive systems (constructed/treatment wetlands) [21].

In this study, the use of fractionated sand had a positive influence, not only on hydraulic performance, but on COD removal efficiency (Fig. 7A). The COD removal efficiencies in the columns containing raw and fractionated sand ranged from 71 % to 94 %, and 74 % to 95 %, respectively. For the final sampling instance (with the most concentrated WW), the organic removal efficiency in the columns containing fractionated sand was 95 % (±1 %) with influent and effluent concentrations of 4681 mg·L^{-1} and 222 mg·L^{-1} (±43), respectively. This was comparable with the 94 % (±1 %) organic removal efficiency and effluent concentration of 298 mg·L^{-1} (±61) obtained with the raw sand (Fig. 7A). The trend continued with the removal of total polyphenolics with the fractionated sand columns having slightly higher removal efficiencies (87 % to 94 %) than the raw sand columns (87 % to 93 %) with influent values ranging from 10.4 to 55.3 mg GAE·L^{-1} (Fig. 7B).

3.2.2. Neutralization of acidic winery wastewater

Winery wastewater is typically acidic, which may have a negative impact on the receiving environment. It has been established that BSRs are capable of neutralizing acidic WW and increasing the sodium adsorption ratio (SAR) [18]. The main pH adjustment mechanism in BSRs is via abiotic dissolution of calcite [19,23], but biotic mechanisms are also involved [19]. It is possible that some calcite solubilises and then re-precipitates when the WW flows through BSRs and becomes less acidic. However, QEMSCAN results have shown that no other Ca-based minerals such as anhydrite or gypsum are formed [19,23]. The longevity of BSR systems in terms of calcite dissolution capacity is beyond the projected life of the systems, with a lifespan of 37 years being calculated using data from column and in-situ experiments for systems containing approximately 10 % calcite. Furthermore, it has been shown that loss of calcite particles does not have a deleterious effect ($p > 0.05$) on the HC [23].

In this study, the pH in the final effluent from the columns containing both raw and fractionated sand was slightly alkaline, with no significant ($p > 0.5$) differences between effluent values. In the final sampling instance, the pH of the acidic WW increased from 4.86 to 8.13 (±0.17) and 8.10 (±0.14) after treatment in the columns containing raw and fractionated sand, respectively (Fig. 7C). There were temporal changes over the sampling period for both total alkalinity and EC of the effluent WW (Fig. 7D, E). Both of these parameters increased when the columns were fed with more concentrated WW with lower pH values, supporting previous results that showed that calcite dissolution increases with decreases in acidity [19,23]. Overall, the result showed that fractionation of sand did not have a negative impact on the WW pH neutralization mechanisms.

Table 3
Comparison of hydraulic and organic loading rates of biological sand reactors and other passive systems treating winery wastewater.

HLR mm·d^{-1}	HLR_{Vol} L·m^{-3}·d^{-1}	OLR_{Vol} g COD·m^{3}·d^{-1}	Ref
14.6*	41.7*	32.9 to 124.5	[35]
7.3*	20.8*	16.4 to 62.1	
14	14 (VF)	152* (VF)	[36,37]
24.8	43–82 (HF_1)	54* (HF_1)	
36.3	22–41 ($HF_{2,3}$)	27* ($HF_{2,3}$)	
77–215	55–154* (VF)	31–333* (VF)	[25]
13–36	43–120* (HF_1)	12–183* (HF_1)	
13–36	22–60* ($HF_{2,3}$)	6.0–92* ($HF_{2,3}$)	
34	28*	37–176*	[38–40]
333*	150	152	[18,22]
23	1) 25*	1) 350*	[41,42]
45	2) 50*	2) NG	
78.3	57.0	260.4	[21] Year 1
206.5	150.3	322.9	[21] Year 2
425	223	447#	This study: raw sand after biomass accumulation during the crush season
4110	2163	4326#	This study: fractionated sand after biomass accumulation during the crush season

HLR = hydraulic (surface) loading rate, HF = horizontal (subsurface) flow, VF = vertical (subsurface) flow.
* Calculated, HLR_{Vol} = volumetric hydraulic loading rate, OLR_{Vol} = volumetric organic loading rate.
# Theoretical value with assumed influent of 2000 mg (COD).L^{-1}
(Adapted from [21]).

3.2.3. Comparison of hydraulic and organic loading of biosand reactors with other passive treatment systems treating winery wastewater

The theoretical organic loading rates (OLR) and HLRs loading rates measured in the pilot BSR system containing raw sand were notably higher than in other passive systems treating WW, translating into a comparatively lower spatial footprint [21]. Based on the results obtained from this study when using fractionated sand and applying a hydraulic head of 0.8 m (Fig. 5), the theoretical OLRs and HLRs after biomass accumulation during the crush season were typically an order of magnitude higher than in other systems, including the pilot BSR system containing raw sand (Table 3).

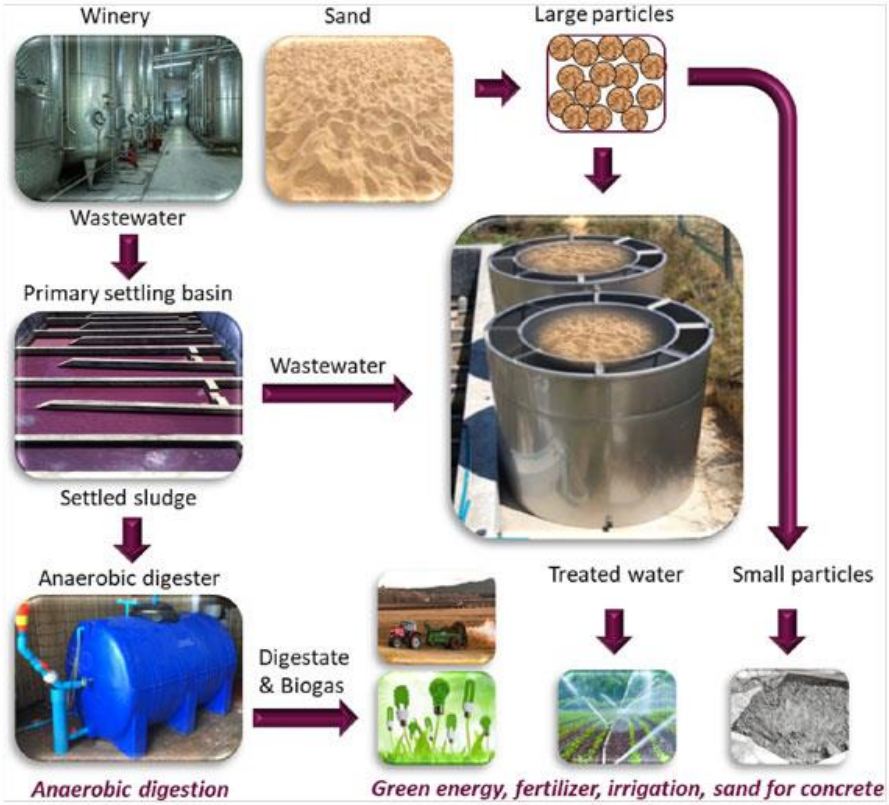

Fig. 8. Schematic of proposed 'zero waste' biosand reactor-based system for valorisation of winery wastewater.

Table 4
Design criteria for biosand reactor systems treating winery wastewater and/or digesting primary winery wastewater sludge.

Parameter	Units	Tonnes of grapes crushed							
		10	100	500	1000	2000	5000	10,000	20,000
Calculated volume of wastewater produced yearly*	m^3·year^{-1}	37	313	1387	2633	4996	11,654	22,116	41,971
Produced 80 % of wastewater in crush#	m^3·day^{-1}	0.3	2.7	12	23	43	100	190	361
Produced 50 % of wastewater in crush#	m^3·day^{-1}	0.2	1.7	7.5	14	27	63	119	226
Number of 5.6 m^3 BSR modules required for 80 % wastewater in crush#	Each	1	1	2	3	6	13	24	45
Number of 5.6 m^3 BSR modules required for 50 % wastewater in crush#	Each	1	1	1	2	4	8	15	28
Calculated volume of sludge produced per year	kg·year^{-1}	84	709	3137	5953	11,297	26,349	50,004	94,896
Produced 80 % of sludge in crush#	kg·day^{-1}	0.7	6.1	27	51	97	227	430	816
Produced 50 % of sludge in crush#	kg·day^{-1}	0.5	3.8	17	32	61	142	269	510
Calculated volume of methane produced per year	ML CH_4	11	92	405	769	1460	3406	6464	12,266
Produced 80 % of methane in crush#	ML CH_4·day^{-1}	0.1	0.8	3.5	6.6	13	29	56	106
Produced 50 % of methane in crush#	ML CH_4·day^{-1}	0.1	0.5	2.2	4.1	7.9	18	35	66
Potential power produced per year	kW	23	192	851	1616	3066	7152	13,574	25,760
Produced 80 % of electricity in crush#	kW·day^{-1}	0.2	1.7	7.3	14	26	62	117	222
Produced 50 % of electricity in crush#	kW·day^{-2}	0.1	1.0	4.6	8.7	16	38	73	138

* Calculated using Sheridan 2003 [31]
\# Converted to a daily flow rate for a winery producing ether 50 % or 80 % of their wastewater during a 3 month crush season, ML = Mega litres.

3.3. Sustainable and circular economy approach for valorisation of winery wastewater: towards zero waste for wineries

Circular economy principles have been envisaged, evaluated, and/or applied in the wine industry for a range of products, services, and solid wastes/by-products. These include wine packaging [43], multiple external and internal stakeholder engagements and activities [44], biorefineries for the extraction of tartrates, antioxidants and other value-added products from grape pomace and lees [7,8], and the use of solids wastes for animal feed and composting.

Passive systems such as constructed/treatment wetlands and BSRs are sustainable options for remediation of WW [17,18,20,21,45]. Building on the results obtained from previous studies, a circular economy and zero waste model for WW remediation and valorisation based on the use of BSRs is proposed (Fig. 8). The intention is to promote the design and implementation of integrated sustainable systems for

reuse and recycling of WW and primary winery wastewater sludge (PWWS) in order to contribute to minimizing waste formation and maximizing resource recovery in wineries [46].

In comparison to other passive treatment systems, BSRs containing fractionated sand (particles >0.425 mm) are envisaged to have even smaller spatial footprints and higher OLRs than previous systems containing raw sand (Section 3.2.2. [19]). As alluded to in Section 3.1, it is feasible that these 'new' BSR systems can be implemented at wineries crushing around 547 t of grapes and generating 1507 m^3 of WW or less per annum, taking into account 50 % peak flows during the crush period. This amount of WW can theoretically be treated using just one 5.6 m^3 BSR module containing 2.9 m^3 of sand. The WW treatment capacity can be increased by adding additional modules where practically possible (Table 4). Alternatively, the size of the modules may be increased. While sand containing larger particles is more desirable for use in BSRs, the cost of fractionating the sand can be offset by using the finer sand fractions, for example, SCC in the building industry [24].

Remediation of WW using BSRs renders it suitable for irrigation purposes, reducing water consumption which is important in water-stressed wine-producing areas such as South Africa, Australia and California, USA [47,48].

Before WW is remediated in BSRs, the solids are removed using primary settling. In a survey conducted in South Africa, 25 % of wineries indicated that they use commercial companies to remove PWSS and dump it in landfills. This is an economic and environmental burden on the industry and is more likely to take place in medium and larger wineries than smaller wineries. In addition, medium-large wineries tend to dispose of PWWS more frequently than smaller wineries [49,50]. In another study, it was found that the Na in WW does not partition into the PWWS, and that it is amendable to anaerobic digestion (AD), generating up to 206 ± 2.7 mL CH_4/gVS_{added} [5]. These results suggest that it may be feasible for biogas generated from co-digestion of PWWS and other available organic substrates to be used as part of a renewable energy mix in wineries [5,51]. In addition, it was shown that the digestate from AD of PWWS has potential as an organic agricultural fertilizer as it contains micronutrients and high concentrations of N (21.5 to 27.7 g/kg dry weight) and C (229 to 277 g/kg dry weight) [5].

In summary, based on the results of this research and previous findings, the a zero waste model is proposed which consists of the following objectives: (i) to fractionate sand and use the fraction with larger particles (>0.425 mm) in BSRs and the smaller fraction in high performance concrete i.e. SCC, (ii) to remediate WW in BSRs and use the treated effluent for irrigation, (iii) to digest PWWS and use the biogas for energy and the digestate as an agricultural fertilizer (Fig. 8). Data for different size categories of wineries generating different volumes of WW are provided in (Table 4).

The volume and character of WW is incumbent on cellar practices and differs from winery to winery [6,32,52]. In most cases, the highest volume of WW (up to 80 %) is usually generated during the standard three-month crush period, but this figure can vary quite widely. For example, some wineries do not crush while other do not bottle on site and a number of wineries have bottling facilities which will result in greatly different flow rates with the latter generating considerable quantities outside the crush season [17,53,54] Table 4 provides data that can assist practitioners to capacitate integrated BSR systems for different sized wineries based on the amount of grapes crushed per annum and maximum peak flows (50–80 % during crush period). The amount of methane generated can be estimated using the volume of total sludge (TS) 2261 mg $TS{\cdot}L^{-1}$ of wastewater [49], in press, converting the TS to volatile solids (VS) TS:VS ratio of 0.675 and VF to methane 191.5 $mLCH_4{\cdot}gVS^{-1}$ [5]. In addition the average calorific value of 6 $kWh{\cdot}m^3$ and a conversion efficiency of 35 % was used to calculate the electricity generation [56]. For example, a winery crushing 1000 t of grapes would require 3 BSR modules with fractionated sand, if 80 % of the WW is produced in the crush. The sludge would potentially yield 1266 kWh of electricity from the methane.

4. Conclusion

To conclude, a circular economy approach for the treatment and valorisation of waste gives a purpose and a value to waste. The BSR system containing raw sand has a proven track record in terms of treatment of WW. Previously, a drawback was associated with reduction in HC due to the accumulation of functional biomass during the crush season that reduced the BSR treatment capacity to below the design limit. This study showed that the HC and flow rate can be increase 9-fold by using fractionated sand with the smaller particles removed. With the experimental test setup, BSR columns containing fractionated sand also showed excellent organic removal efficiencies and increased the pH of acidic WW. With a zero-waste model, it is suggested that the larger fraction of sand (>0.425 mm) is used in BSRs and the smaller fraction in high performance concretes. The solid fraction of WW can be anaerobically digested producing biogas which may be used for energy and digestate used as an agricultural fertilizer. The quality of the remediated WW is acceptable for irrigation purposes.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Pamela J Welz reports financial support was provided by Wine Industry Network for Expertise and Technology.

Data availability

Data will be made available on request.

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