ANAEROBIC TREATMENT OF TANNERY EFFLUENTS FOR RESOURCE RECOVERY IN A CIRCULAR BIOECONOMY

by

Ashton Busani Mpofu

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SUPERVISORS

Dr. Pamela Jean Welz

Senior researcher

Applied Microbial and Health Biotechnology Institute

Cape Peninsula University of Technology

Bellville campus

A.Prof. Oluwaseun Oyekanmi Oyekola

Associate professor: Chemical Engineering

Faculty of Engineering and the Built Environment

Cape Peninsula University of Technology,

Bellville campus

DECLARATION

I, **Ashton Busani Mpofu** declare that the work contained in this thesis has not been previously submitted to meet requirements for an award at this or any other higher education institution. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made.

Signature:

Date: 30 November 2022

ABSTRACT

The current economy of tanneries in developing countries is mainly linear, with certain elements of circularity as it promotes the recycling of skin/hides and indirect water reuse. In view of the global efforts to achieve sustainable development, the concept of a circular bioeconomy through the application of anaerobic digestion (AD) has gained significant traction in solving the tannery industry's economic and waste management challenges. However, its application is susceptible to inhibition by recoverable toxicants particularly sulfur species, metals and ammonia (NH_3). This study investigated the AD raw slaughterhouse–ostrich (SOTE) and bovine–ovine (BOTE) tannery effluent for resource recovery. It further investigated the use of a novel hybrid linear flow channel reactor (HLFCR) for the pre-treatment of TWW to remove inhibitory sulfur species and recover it as S^0, while making TWW more amenable for methane (CH_4) recovery in anaerobic sequential batch reactors (AnSBR).

Standardised biochemical methane potential (BMP) tests were conducted on raw SOTE and BOTE to determine the effects of inoculum to substrate ratio (ISR), sulfate concentration [SO_4^{2-}], and/or substrate to substrate ratio (SSR) on the anaerobic biodegradability (Bₒ), CH_4 yields and process kinetics. The effect of these factors and process optimisation was assessed using response surface methodology (RSM). The optimal conditions were then applied in AnSBR with/out pre-treatment with HLFCR.

The BMP tests on SOTE demonstrated the inhibition effect of high [SO_4^{2-}] and/or lower ISR on CH_4 yields while tests on BOTE showed that beamhouse effluent (BHE) was more amenable for AD than tanyard effluent (TYE). Bioreactors with higher TYE compositions (v/v) and operating at very high/low ISR (3<ISR≤2) suffered severe methanogenesis inhibition. However, it was established that all reactors were active regardless of inhibition. It was also apparent that at least a fraction of the HS^- formed from sulfidogenesis was oxidised into S^0 and a white-yellowish layer formed at the interface of the bulk liquid and head space for both BOTE and SOTE. As expected, higher CH_4 yields (93–130 mLCH$_4$/gVS) were achieved at [SO_4^{2-}] within the range (646±417 mg/L) measured in SOTE batches. This meant there was no need for the pre-treatment of SOTE to remove sulfur species. The optimum theoretical operating conditions for maximal CH_4 yield and biodegradability (Bₒ) for SOTE were [SO_4^{2-}] = 922 mg/L and ISR = 3.7, while for BOTE they were determined as ISR = 2.5 and 100% BHE. The AD of SOTE with [SO_4^{2-}] ≈ 680 mg/L in AnSBRs at ISR = 4 while mixing at 50–300 rpm using a pitched four-blade marine impeller achieved 31–208 mLCH$_4$/gVS. The optimal mixing conditions at 200 rpm (continuous) achieved 208 mLCH$_4$/gVS and 49% total solids reduction.

The feasibility of using a novel integrated biological system (IBS) that comprised of a novel HLFCR and an AnSBR connected in series was investigated in treating 100%BHE. The IBS's optimum operating configuration and conditions were single-stage (8 days HRT) or two-stage (4 days HRT) HLFCR and 50 rpm continuous mixing in an AnSBR. These operating conditions achieved the removal of 80–91% total COD, 78–98% TOC, 89–91% SO_4^{2-}, 92–93% S^{2-}, 50–73 PO_4^{2-}, and 48–60% total nitrogen while recovering 241±4 $mLCH_4/gCOD_{added}$ after 20–24 days. Single-stage HLFCR (8 days HRT, pH=7.0–7.8, ambient temperature and −366<ORP<−322) recovered about 16–25% of the inlet sulfur as S^0. The composition range of the harvested floating sulfur biofilm (FSB) was 10.8–14% C, 1.5–1.9% N, 1.9–4.4% H, and 26–39.6% S. Mixing continuously at 50 rpm significantly improved (F test, p>0.05) the cumulative CH_4 yield by 82–98%, 33– 65% and 63–71% compared to raw BHE, unmixed and intermittently mixed AnSBRs treating HLFCR pre-treated BHE, respectively. The AD of settled solids from HLFCR in an unmixed reactor operating at ISR = 4 produced 88±2 $mLCH_4/gVS_{added}$.

The final treated effluent of SOTE, BHE and that of IBS met many of the irrigation standards for most developing countries leading in leather production except for Na, Cl, and NH_3. In the South African context, the treated SOTE and BHE met the standards for irrigating up to 500 m³/day for BMP tests and 50 m³/day for BHE in the IBS. Similarly, metal concentrations in the SOTE and BHE digestate were within the recommended limits for class A1a/b sludge suitable for agricultural use in South Africa (SA) and other developing countries, except for Cr. Chrome (III) is insoluble in water and hence it attaches and settles with solids. Nonetheless, the treated BHE from IBS can be further treated for the recovery of VOA (2284– 2465 mg/L), and NH_4 (232–444 mg/L) that were still present in high concentrations due to hydrolysis and/or diluted to meet irrigating up to 2000 m³/day. The full-scale application of the IBS at a local medium-sized tannery treating 2258 m³/day of BHE would produce a floating sulfur biofilm with 33% S^0, 3420 m³ of CH_4, and 31 tonnes of biofertiliser. The sale of recovered resources and potential savings from 72% reduction in electricity demand and 62% in sludge disposal had a potential revenue of US$5559. The integrated system has a payback period of about 5 years and an internal rate of return of about 18%.
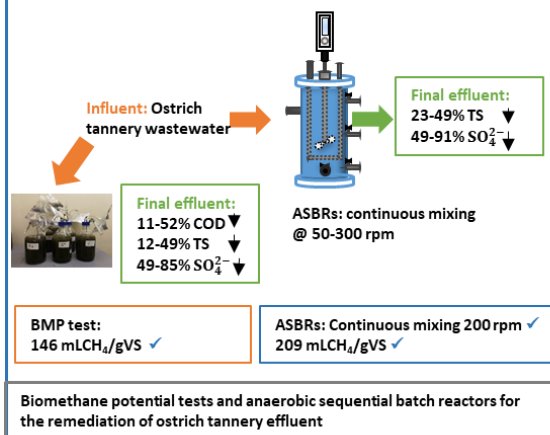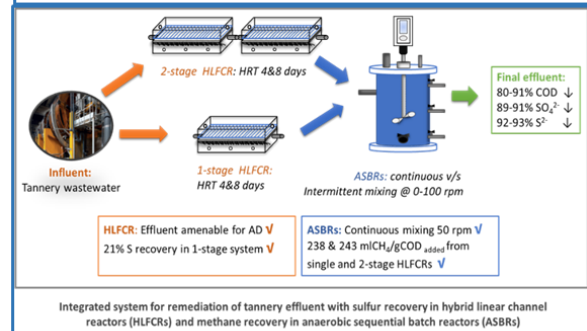
PREFACE

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Influent: Ostrich tannery wastewater

Final effluent:
23-49% TS ▼
49-91% SO_4^{2-} ▼

Final effluent:
11-52% COD ▼
12-49% TS ▼
49-85% SO_4^{2-} ▼

ASBRs: continuous mixing @ 50-300 rpm

BMP test:
146 mLCH$_4$/gVS ✓

ASBRs: Continuous mixing 200 rpm ✓
209 mLCH$_4$/gVS ✓

Biomethane potential tests and anaerobic sequential batch reactors for the remediation of ostrich tannery effluent

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2-stage HLFCR: HRT 4&8 days

Influent: Tannery wastewater

1-stage HLFCR: HRT 4&8 days

ASBRs: continuous v/s Intermittent mixing @ 0-100 rpm

Final effluent:
80-91% COD ↓
89-91% SO_4^{2-} ↓
92-93% S^{2-} ↓

HLFCR: Effluent amenable for AD √
21% S recovery in 1-stage system √

ASBRs: Continuous mixing 50 rpm √
238 & 243 mlCH$_4$/gCOD $_{added}$ from single and 2-stage HLFCRs √

Integrated system for remediation of tannery effluent with sulfur recovery in hybrid linear channel reactors (HLFCRs) and methane recovery in anaerobic sequential batch reactors (ASBRs)

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DEDICATION

Dedicated to my family the "Phangwanas"

To my late grandparents Jarvis and Angeline, my parents Busani and Siphephile, and my lovely sister Ashley for being my bolsters, critics and catapults.

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LIST OF OUTPUTS

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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:

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Mpofu, A.B., Kaira, M.A., Welz, P.J. & Oyekola, O.O. 2022. Anaerobic co-digestion of tannery effluents: Process optimisation for resource recovery, recycling and reuse in a biocircular economy. Journal of Process Safety and Environmental Protection. https://doi:10.1016/j.psep.2021.12.027

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Kibangou, V.A., Lilly, M., **Mpofu, A.B**., de Jonge, N., Welz, P.J. & Oyekola, O.O. 2021. Sulfate-reducing and methanogenic microbial community responses during anaerobic digestion of tannery effluent. Bioresource Technology Journal. https://doi.org/10.1016/j.biortech.2021.126308

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Mpofu A.B., Kaira, M.A., Welz, P.J, Oyekola, O.O. 2022. 'Anaerobic co-digestion of tannery effluents: Process optimisation for resource recovery, recycling and reuse in a biocircular economy'. *World Sustainable Energy Days 2022*: Linz, Austria, 4–8 April 2022.

Mpofu, A.B., Welz, P.J. & Oyekola, O.O. 2021. 'Anaerobic treatment of tannery effluents in the context of a biocircular economy for developing countries'. *8th International Conference on Engineering for Waste and Biomass Valorisation*: Guelph, Canada/online, 31 May – 4June 2021.

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Mpofu, A.B., Welz, P.J. & Oyekola, O.O. 2020. Co-digestion of tannery waste-activated sludge with slaughterhouse sludge to improve organic biodegradability and biomethane generation. Journal of Process Safety and Environmental Protection. https://doi.org/10.1016/j.psep.2019.09.018

Published book chapter:

Godfrey, L., Roman, H., Smout, S., Maserumule, R., **Mpofu, A**., Ryan, G., and Mokoena, K., 2021. Circular Economy: Recent Trends in Global Perspective. Unlocking the opportunities of a circular economy in South Africa. ISBN: 978-981-16-0913-8 (e-book). https://doi.org/10.1007/978-981-16-0913-8

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ABBREVIATIONS AND ACRONYMS

Abbreviation	Description
AAE	Acetic acid equivalent
AD/AcoD	Anaerobic digestion/codigestion
Add	Added
AES	Atomic emission spectroscopy
AIC	Akaike's information criterion
AFFB	Anaerobic fixed film bed reactor
Alk	Total alkalinity
AMs	Aceticlastic methanogens
AnMBR	Anaerobic membrane bioreactors
ANAMMOX	Anaerobic ammonia oxidation
ANOVA	Analysis of variance
AnSBR	Anaerobic sequencing batch reactor
AS	Acclimated sludge
ASTM	American Society for Testing and Materials
BET	Brunauer–Emmett–Teller
BHE	Beamhouse effluent
BMP	Biochemical methane potential
BOD	Biological oxygen demand
BWW	Bovine wastewater
CCD	Central composite design
C/N	Carbon to nitrogen ratio
CODs	Soluble COD
CODt	Total chemical oxygen demand
Cr-liq	Chrome liquor
D/AnS	Domestic/anaerobic sludge
FSB	Floating sulfur biofilm
GTWW	General tannery wastewater (BHE+ tanyard)
HAc	Acetic acid
HBu	Butyric acid
HLFCR	Hybrid linear flow channel reactor
H/SRT	Hydraulic/solid retention time
HMs	Hydrogenotrophic methanogens
HPr	Propionic acid
ICP	inductively coupled plasma
ISR	Inoculum to substrate ratio
Lab	Laboratory
LCFA	Long chain fatty acids
LS	Leather shavings

MAD	Mesophilic anaerobic digestion
mcrA	Methyl coenzyme M reductase
ML	Mega litres
MS	Mass spectrometry
OLR	Organic loading rate
ORP	Oxygen reduction potential
PVDF$_{mem}$	Polyvinylidene fluoride membrane
R	Reactor
R$_V$	Reactor volume
Rem	Removed
RMSE	Root mean square error
Rpm	Revolutions per minute
RSM	Response surface methodology
S-Cont	Semi-continuous
SHS	Slaughterhouse sludge
SL	Soak liquor
SMA	Specific methanogenic activity
SOTE	Slaughterhouse-ostrich tannery effluent blend
SRB	Sulfate reducing bacteria
SS	Suspended solids
Strip	Stripping
SWW	Slaughterhouse wastewater
TDS	Total dissolved solids
TAD	Thermophilic anaerobic digestion
TKN	Total Kjeldahl Nitrogen
TL	Tanning liquor
TN	Total nitrogen
TOC	Total organic carbon
TP	Total phosphate
TS	Total solids
TSS	Total suspended solids
TSW	Tannery solid waste
TVS	Total volatile solids
TWAS	Tannery waste activated sludge
TWW	Tannery wastewater
TWWTP	Tannery wastewater treatment plant
TY	Tanyard
UACF	Upflow anaerobic contact filter
UASB	Upflow anaerobic sludge blanket
UN-FAO	United Nations Food and Agriculture organisation
VOAs	Volatile organic acids

LIST OF SYMBOLS

Symbol	Description	Unit
A	Ultimate methane yield	$mLCH_4/gVS$
A^0	Brunauer–Emmett–Teller	m^2/g
B$_o$	Anaerobic biodegradability	%
K	Rate constant	d^{-1}
p	*p*robability	
R	Pearson's correlation	
μ$_m$	Maximum specific methane generation rate	$mLCH_4/gVSd^{-1}$
R^2	Coefficient of determination	
Λ	Lag phase	days

CHEMICAL FORMULAE

Element/compound	Description
$Al_2(SO_4)_3$	Aluminium sulfate
B	Boron
Ba	Barium
Ca	Calcium
$(Ca(OH)_2)$	Calcium hydroxide/slaked lime
CH_4	Methane
CH_3COOH	Acetic acid
Cd	Cadmium
Cr_3SO_4	Chrome sulfate
Cl	Chloride
CO_2	Carbon dioxide
Cu	Copper
Fe	Iron
$FeCl_3$	Ferric chloride
HS	Hydrogen sulfide
H_2	Hydrogen
HNO_3	Nitric acid
K_2SO_4	Potassium sulfate
$MgSO_4$	Magnesium sulfate
Mg	Magnesium
$MgNH_4PO_4 \cdot 6H_2O$	Struvite
Mn	Manganese
Mo	Molybdenum
Na	Sodium
NO_3^-/NO_2^-	Nitrate/Nitrite
NH_3/NH_4	Ammonia/um
NH_3N	Ammonia nitrogen
Ni	Nickel
O_2	Oxygen
PO_4	Phosphate
Pb	Lead
$[Al_2(OH)_nCl_{6-n}]_m$	poly aluminium ferric chloride (PAFC)
SO4	Sulfate
S^{2-}	Sulfide
S^0	Elemental sulfur
St	Strontium
Zn	Zinc

GLOSSARY

Term	Description
Anoxic	An environment in which bacteria use nitrate and/or nitrite ions
Biogas	A gaseous mixture of methane, carbon dioxide and hydrogen sulfide derived from bacterial decomposition of organic matter.
Biochemical oxygen demand (BOD)	The quantity of oxygen required by microorganisms to degrade organic materials and oxidisable inorganic matter in the substrate at standard conditions (Buljan & Král, 2011). The reaction time is typically limited to five days.
Characteristics	General physical, biochemical and biological classes of wastewater elements.
Chemical oxygen demand (COD)	A measure of oxygen intake during the breakdown of organic matter and the oxidation of inorganic substances such as sulfur, ammonia, and chrome. Thus, it is a measurement of the level of the contamination level (how much organic and inorganic compounds are present).
Liming	The addition of a liming agent such as calcium hydroxide $[Ca(OH)_2]$ to induce opening of the collagen structure of the hide by removing interstitial material.
Mixing	The process of increasing the homogeneity of a medium by using physical methods.
Pickling	The addition of chemical acids and brine to lower the pH and prevent swelling of hides so as to facilitate the penetration of tanning agents in the tanning stage.
Sludge	Semi-solid slurry containing organic matter produced from wastewater gravity treatment processes such as clarifiers and sedimentation tanks.
Tanning	The process of converting animal hide/skin into leather by the use of chemicals.
Unhairing	A process by which the hair of the hide/skin is converted into a pulp by soaking the hide/skin into a liquid containing sodium sulfide (Na_2S) or sodium hydrosulfide (NaHS).
Wet blue	Chrome-tanned skin/hide that has been sammyed to remove surplus moisture. Further processing is required to produce leather

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CHAPTER 1

INTRODUCTION

[The chapter provides an introduction, background, and motivation to the study. Additionally, the hypothesis and novelty of the study is presented].

1.1 Introduction and background

Leather is one of the world's most widely traded commodities, with every country having a leather products industry and a market for finished leather goods in one form or another. The leather tanning and leather products industries play prominent roles in the developing world's economy, with an estimated global trade value of approximately US$100 billion per year. The industries prevent landfilling of over 95% of the skins/hides from the meat and dairy industries (UN-FAO, 2013). The developing world dominates the industry and supplies >60% of the world's skins/hides (Buljan & Král, 2015; Swartz et al., 2017). This may be attributed to the availability of a large low-cost labour force, less stringent environmental regulations and cheaper TWW (tannery wastewater) treatment and disposal cost (Swartz et al., 2017). In line with global efforts for cleaner production, tanners are endeavouring to lower their water consumption, promote improved uptake of chemicals, re-use and/or recycle process liquor, process water and/or solid waste, reduce content and/or eliminate the wide use of specific pollutants such as chrome sulfate (Cr_3SO_4). However, the implementation of these techniques has been challenging for most tanneries in the developing world due to the related additional or capital costs, and perception on the possibility of jeopardising leather quality (Mpofu, 2018).

There has been an increasing environmental concern regarding pollution of the environment by some tanneries in the developing world that dispose of their untreated or partially treated TWW and/or tannery sludge to nearby water resources and/or agricultural land (Haque et al., 2019; Mpofu, 2018; Saxena et al., 2015). Sludge management costs account for nearly 35% of the overall budget or 50-55% of the process and maintenance costs of tannery wastewater treatment plants (TWWTPs), while energy costs may account for up to 60% of the total costs in TWWTPs (Akyol et al., 2014; Divyalakshmi et al., 2015; Sodhi et al., 2018). Moreover, TWWTPs have higher carbon footprints than conventional biological municipal wastewater treatment works (Giaccherini et al., 2017).

In light of the global adoption of circular bioeconomy principles of recovering, reusing and recycling of resources to promote sustainable economic growth, the TWWTPs are increasingly being regarded as potential biorefineries. Prospective products that can be derived from TWW are: first-level products (organic acids, metals and industrial enzymes), second-level products (biofuels and bioenergy, such as biogas, hydrogen, lipids for biodiesel and biomass for combustion, gasification or pyrolysis), third-level products (processed biomass, such as fertiliser, animal feed, fibre and compost); and fourth-level products (acceptable quality water that can be reused on a 'fit for purpose' basis). Previous studies on the AD of TWW mostly reported on biogas recovery for onsite use in electricity production and/or for heating

purposes. However, the availability of microbial toxicants in the TWW prompted more studies on pre-treatment, particularly coagulation and co-digestion (AcoD) in order to improve process efficiency. Successes in these pre-treatment and AcoD studies have led to the realisation that these 'toxicants' can potentially be recovered as value-added products, and have spurred this research.

1.2 Research problem statement

The current economy of tanneries, particularly in the developing world that dominate leather production, is mainly linear and lacks economic and environmental sustainability. Tanneries consume significant volumes of water and chemicals. They generate large amounts of complex tannery wastewater (TWW) and wet sludge laden with pollutants. Tanneries in developing countries are increasingly reported as environmental polluters due to the inadequate or partial treatment of TWW and/or sludge and their improper disposal on the environment. The widely used physicochemical treatment methods and/or conventional activated sludge process for remediation of TWW produce excess sludge, have high carbon emissions, are energy intensive and are generally costly. Although, the application of AD looks attractive in promoting a circular bioeconomy through the recovery of reusable, recyclable and value-added products, pollutants ($H_2S/HS^-/SO_4^{2-}/NH_3/NH_4^+$/metals), dosed as feed chemicals during processing make TWW and sludge unsuitable for anaerobic treatment.

1.3 Research rationale and novelty

The AD process has been successfully employed for the treatment of TWW to meet the stipulated discharge standards and/or for the production of biogas. The previous studies did not focus on recovery of other value-added products except for biogas (methane). However, the process seldom proceeds without inhibition, particularly by NH_3, SO_4^{2-}, H_2S, Cr, lipids, VOAs and O_2 (aq) contained in TWW and/or released as by-products during processing. This initially prompted studies on abiotic pre-treatment methods such as coagulation for the removal of sulfide and Cr. Additional studies focused on anaerobic co-digestion (AcoD) of tannery effluents with tannery solid wastes (leather shavings, sludge and/or fleshings), cow dung, and/or fresh wheat straw (*Triticum aestivum*) for biogas (methane) recovery. Another study investigated the AcoD of pre-treated (coagulated) beamhouse (BHE) and tanyard effluent (TYE) at 50% volumetric composition *(%v/v)*. Other studies demonstrated the feasibility of recovering S^0 from pre-treated TWW through two-stage biological systems comprising of biological sulfate reducing (BSR) reactors promoting sulfidogenesis and downstream bioreactors promoting sulfide oxidation (SO) by injecting air. A follow up study integrated BSR and SO in an up-flow hybrid anoxic bioreactor while injecting air from the bottom. However, the studies focused on the treatment of primary treated TWW and

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synthetic wastewater, respectively, for pollutants (NH_4^+, SO_4^{2-} and COD) removal in order to meet discharge standards.

To the authors' knowledge, this was the first study that (i) exclusively reported on the characteristics of bovine-ovine TYE and BHE, and a blend of effluents from ostrich slaughterhouse and tannery (SOTE), (ii) anaerobically digested SOTE, (iii) investigated the AcoD of BHE and TYE at different volumetric compositions *(%v/v)*, (iv) investigated the impact of intermittent and continuous mechanical mixing using a four blade marine impeller at different speeds in AnSBRs during the AD of raw and biologically pre-treated TWW, (v) optimised and applied an integrated biological system comprising of a novel HLFCR coupled with an AnSBR for the recovery of resources from TWW, and (vi) investigated the microbial characteristics (ecology and composition) and AD process kinetics of SOTE during different mixing regimes.

1.4   Hypotheses

It was hypothesised that AcoD of bovine-ovine BHE and TYE at different compositions (*v/v*) could be instrumental in abating inhibition and augmenting deficient nutrients for AD. Secondly, it was hypothesised that upstream removal of inhibitors (SO_4^{2-}, H_2S/HS^-/NH_3/NH_4^+ and metals) in a passive BSR and SO system could improve AD of SOTE, BHE and/or TYE. Furthermore, the removed sulfur species could be recovered as S^0, nitrogen species as N_2 in biogas and metal precipitates in sludge, and the treated final effluent could be used in a fit-for-purpose basis.

1.5   Significance of research

The successful anaerobic treatment of tannery effluent will recover reusable, recyclable and value-added products such as CH_4, H_2S/S^0, NH_4^+ rich centrate [for struvite ($MgNH_4PO_4 \cdot 6H_2O$) recovery], biofertiliser/compost or activated carbon (if metal levels are acceptable), concrete/clay aggregate, and reusable water. This will provide an economic benefit to the tanneries through selling recovered products and/or their reuse, reducing dependence on new resources. This will significantly address the current shortage of landfill sites, and the potential negative impact of landfills, tannery effluents and sludge on the environment. Maximising CH_4 yield will provide a clean, affordable and a renewable energy source for onsite electricity generation. This will reduce the dependence of tanneries on external electricity supply, which is currently under strain in developing countries, including South Africa. The high costs of electricity, waste management (effluent/sludge treatment and/or disposal), and other associated costs can be significantly reduced. Furthermore, this research will benefit tannery industries by alleviating stringent fines often incurred due to non-compliance of tannery effluents discharged to municipal sewers

or environment. This will present TWWTPs as economical biorefineries and bioremediation centres that promote sustainable economic growth and development, particularly of developing countries.

1.6   Research aims and objectives

This study sought to promote a circular bioeconomy and net positive tanneries in developing countries by developing an integrated biological treatment system (IBS) for the recovery of reusable/recyclable methane, sulfur, biofertiliser, and process/irrigation water from SOTE and bovine/ovine BHE and/or TYE using a novel hybrid linear flow channel reactor (HLFCR) connected to a mechanically mixed anaerobic sequential batch reactor (AnSBR).

1.6.1   Research objectives:

- To determine the physicochemical characteristics of different tannery effluent streams: bovine-ovine BHE and TYE and SOTE
- To prepare robust microbial inocula well acclimated to the tannery effluents
- To determine the characteristics (ecology and composition) of the prepared inocula initially and during AD at different operating conditions
- To investigate the AD of different streams (co-digestion) and optimisation of the AD process using response surface methodology (RSM) based on a full factorial central composite experimental design (CCD) while varying the inoculum to substrate ratio (ISR), retention time, substrate to substrate ratio (SSR), continuous and intermittent mixing and/or sulfate concentration
- To determine the feasibility of recovering elemental sulfur (S^0), biogas/CH_4, reusable water and biofertiliser using standardised biomethane potential experiments (BMP) and an integrated biological system (IBS) comprising of a hybrid linear flow channel reactor (HLFCR) and an anaerobic sequential batch reactor (AnSBR)
- To perform mass balances in order to quantify resource recovery rates
- To determine the suitability of the recovered resources for recycling and/or reuse in the tannery operations and/or in agricultural application
- To determine the kinetics of the AD process during the recovery of resources using nonlinear regression methods
- Conduct an economic feasibility study on the application of the proposed IBS at the study site

1.7   Research questions

- What are the characteristics of the different streams produced in a tannery?
- Based on TWW characteristics, is it suitable for AD?

- What is/are the best product/s that can be recovered from TWW?
- Do the prepared inocula contain the desired microbial species for recovering targeted resources?
- Can co-digestion of different tannery effluent streams improve process efficiency?
- What are the optimal operating conditions for maximum recovery of reusable, recyclable and/or value-added products?
- What are the process kinetics?
- Are the recovered products suitable for recycling and/or reuse?
- Is the process economically feasible?

1.8   Delineation

This study was limited to investigating the feasibility of recovering reusable, recyclable and value-added products from TWW obtained from chosen tanneries in South Africa. The further processing or purification of the recovered resources was not investigated.

1.9   Thesis outline

This thesis reports on a number of inter-related studies focussed on the optimisation of the anaerobic treatment of ostrich and bovine TWW for resource recovery to promote a circular bioeconomy and net positive tannery operations. The studies further investigate the characteristics of different tannery effluent streams, their anaerobic mono and co-digestion, process kinetics and suitability of the recovered resources for recycling and/or reuse.

Chapter 1 introduces the research, provides background to the research problems, aims and objectives and rationale of the studies.

Chapter 2 provides a review of the anaerobic digestion (AD) of TWW, including operational factors and process inhibitors affecting AD. Subsequently, the leather tanning process and the characteristics of the tannery effluents is covered as it is a source of toxicants that inhibit AD. The chapter further provides an overview of the pre-treatment and co-digestion of TWW to abate inhibition. Lastly, the chapter describes the potential of recovering resources for recycling and reuse from TWW using AD. This objective is satisfied in the subsequent chapters.

Chapter 3 is the first chapter that provides Results and Discussion of the study. The chapter evaluates the AD of raw ostrich TWW at different sulfate concentrations for resource recovery using the bio-methane potential (BMP) protocol. The content includes a descriptions and discussions on the characteristics of ostrich TWW, optimisation of the AD using the response surface methodology (RSM) and the recovery of

resources for recycling and reuse. Additionally, the chapter reports on the kinetics of the cumulative methane production determined using nonlinear regression methods.

Chapter 4 reports on the characteristics of beamhouse (BHE) and tanyard (TYE) effluent batches. The chapter describes the optimisation of the anaerobic mono and co-digestion of both effluents by varying the volumetric blend (% vol/vol) and inoculum to substrate ratio (ISR) using the BMP protocol and RSM. It further evaluates the recovery of resources and the kinetics of the cumulative methane production using nonlinear regression methods. The chapter also evaluates the economic feasibility of the process and the suitability of the recovered resources for reuse in agriculture. The results from this chapter were further explored in chapter 5.

Chapter 5 introduces the use of hybrid linear flow channel reactor (HLFCR) for the pre-treatment of beamhouse effluent (BHE) to recover elemental sulfur and make the treated effluent more amenable for AD in anaerobic sequential batch reactors (AnSBR). The chapter describes the optimisation of mechanical mixing in AnSBR by varying mixing rate, time and regime (continuous and intermittent). Additionally, it evaluates the recovery of resources and their suitability for recycling and reuse within the tannery and/or in agriculture. The chapter also evaluates and report on the kinetics of the cumulative methane production and the economic feasibility of the optimised process.

Chapter 6 The chapter includes a synopsis on how the aims and objectives of the study were addressed, and provides a conclusion and recommendations for further research.

CHAPTER 2

ANAEROBIC TREATMENT OF TANNERY WASTEWATER IN THE CONTEXT OF A CIRCULAR BIOECONOMY FOR DEVELOPING COUNTRIES

This chapter was wholly published as:

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[The chapter provides a review of the anaerobic treatment of tannery effluents for the purposes of recovering resources other than biogas. Furthermore, the chapter identifies research gaps in the application of AD to promote a circular bioeconomy].

2.1 Introduction

Leather is one of the world's most widely traded commodities, with every country having a leather products industry and a market for finished leather goods in one form or another. The leather tanning and leather products industry plays a prominent role in the developing world's economy, with an estimated global trade value of approximately US$$100 \times 10^9$/year. The industry recycles and prevents landfilling of over 95% of the skins/hides from the meat and dairy industries (UN-FAO, 2013). The developing world dominates the industry and supplies >60% of the world's skins/hides (Buljan & Král, 2015). This may be attributed to the availability of a large low-cost labour force, less stringent environmental regulations, cheaper treatment and disposal costs of tannery wastewater (TWW) (Swartz et al., 2017). In line with global efforts for cleaner production, tanners are endeavouring to lower their water consumption, promote improved uptake of chemicals, re-use and/or recycle process liquor, process water and/or solid waste, reduce content and/or eliminate the wide use of specific pollutants such as chrome sulfate (Cr_3SO_4). However, the implementation of these techniques has been challenging for most tanneries in the developing world due to the related additional or capital costs, and perception on the possibility of jeopardising leather quality (Mpofu, 2018).

There has been an increasing environmental concern regarding pollution of the environment by some tanneries in the developing world that dispose of their untreated or partially treated TWW and/or tannery sludge to nearby water resources and/or agricultural land (Mpofu, 2018). Sludge management costs account for nearly 30-40% of the overall budget or 50-55% of the process and maintenance costs of tannery wastewater treatment plants (WWTPs) (Akyol et al., 2014), while energy costs may account for up to 60% of the total costs in tannery WWTPs (Buljan, 2005). Moreover, tannery WWTPs have higher carbon footprints than conventional biological municipal wastewater treatment works (Giaccherini et al., 2017).

In light of the global adoption of circular bioeconomy principles of recovering, reusing and recycling of resources to promote sustainable economic growth, tannery WWTPs are increasingly being regarded as potential biorefineries. Potential products that can be derived from TWW are: first-level products (organic acids, metals and industrial enzymes), second-level products (biofuels and bioenergy, such as biogas, hydrogen, lipids for biodiesel and biomass for combustion, gasification or pyrolysis), third-level products (processed biomass, such as fertiliser, fibre and compost); and fourth-level products (acceptable quality water that can be reused on a 'fit for purpose' basis). Previous studies on the anaerobic digestion (AD) of TWW mostly reported on biogas recovery for onsite use in electricity production and/or for heating

purposes. However, the availability of microbial toxicants in the TWW prompted more studies on co-digestion (AcoD) and pre-treatment, particularly coagulation in order to improve process efficiency. Successes in these pre-treatment and AcoD studies have led to the realisation that these 'toxicants' can potentially be recovered as value-added products, and have spurred further research.

Reviews on AD of TWW and recovery of resources other than biogas are limited. Saxena et al. (2015)and Zhao & Chen, (2019) reviewed the toxicity of TWW and the various physical, chemical and biological methods used either alone or in combination to remove pollutants from TWW. The reviews did not focus on recovery, instead they focused on the treatment of TWW to meet the stipulated discharge standards. Reviews by Durai & Rajasimman, (2011); Midha & Dey, (2008); Sabumon, (2016); Sanjay & Vaishnav Raj, (2014) and a book chapter by Doble & Kumar, (2005) reviewed the biological treatment of TWW and highlighted the use of microorganisms for the removal of the main pollutants in TWW; chromium (Cr) and sulfide (H_2S). Additionally, Sabumon et al. (2016) extensively reported on biological nitrogen removal and competition between sulfate reducing bacteria (SRB), other bacteria and/or achaea. A review by Sawalha et al. (2020) gave an overview of the various treatment methods excluding AD for removing, recycling or replacing tanning chemicals, especially $Cr_3SO_4$and sodium sulfide (Na_2S). A book chapter by Mannucci & Lubello, (2020) focused on the use of membrane bioreactors for the treatment and recovery of reusable water from TWW. The only exclusive review on AD was done by Mannucci et al., (2010). However, the review did not include a discussion on resource recovery, and focused on effluent from vegetable tanning. While effluent from vegetable tanning is notably less toxic than TWW from chrome tanning, it is currently not widely applied by the tanning industry.

Therefore, this chapter seeks to (i) critically review the AD of both chrome and vegetable TWW in the context of a circular bioeconomy for developing countries, (ii) provide an update on the research developments in the AD of TWW (iii) identify existing research gaps, (iv) discuss ambiguities and inconsistencies, especially those pertaining to the biomethane potential (BMP) protocol and (v) provide future research perspectives to spur further research and development for this study.

2.2 Methodology

Relevant international informational databases and bibliometric resources; Science Direct, Springer, MDPI, and Wiley were used to retrieve over 200 articles, reports and book chapters in the period from 1979 to 2020. The following key words were used in different combinations to retrieve literature: anaerobic digestion; treatment; tannery wastewater; tannery effluent; inhibition; circular economy; resource recovery; biogas; and co-digestion. The analysis of the selected data and the structure of this

review is presented with due consideration being given to the leather tanning processes that generate TWW, the characteristics of the TWW emanating from the different and the recovery of resources.

2.3   Leather tanning process and tannery wastewater generation

Conventionally, tanning consumes about 20-80 m^3/t of raw hide/skin and yields approximately 200 kg of leather, 15-45 m^3 of TWW containing pollutants dosed as chemicals and 500 kg wet sludge (Buljan & Král, 2015). The process consumes over 130 different types of chemicals used in tanning, mainly in the form of metal salts, hydro/oxides and acids. Chrome tanning remains the principal method accounting for 80-90% of the leather produced worldwide (Swartz et al., 2017). The main wet blue or fellmongery process consists of beam-house (soaking - deliming and bating or pickling) and tanning processes (tanning - sammying), while the dye house or leather finishing process consists of optional retanning, dyeing, fat liquoring and dry finishing (Figure 2-1).

Beam-house processes are the most polluting processes contributing more than 60% of salinity, 80% of the organic pollution load or biological oxygen demand (BOD) and 40-70% of ammonia nitrogen (NH$_3$N) in TWW mainly emanating from soaking, un-hairing/liming, and deliming/bating processes, respectively (Buljan & Král, 2019; Buljan & Král, 2015). The tanning process contributes over 90% chrome (Cr) and 60% sulfate (SO_4^{2-}) as the main pollutants in TWW (Bosnic et al., 2003). Enormous amounts of liming chemicals and salinity in TWW can be reduced by fleshing before liming (green fleshing) and tanning fresh (green) or chilled skin/hides, respectively. Therefore, the vertical integration of the value chain for feedlot businesses such as slaughterhouses that can supply fresh skin/hides can be promoted by amalgamating them with tanneries (Mpofu et al., 2020b).
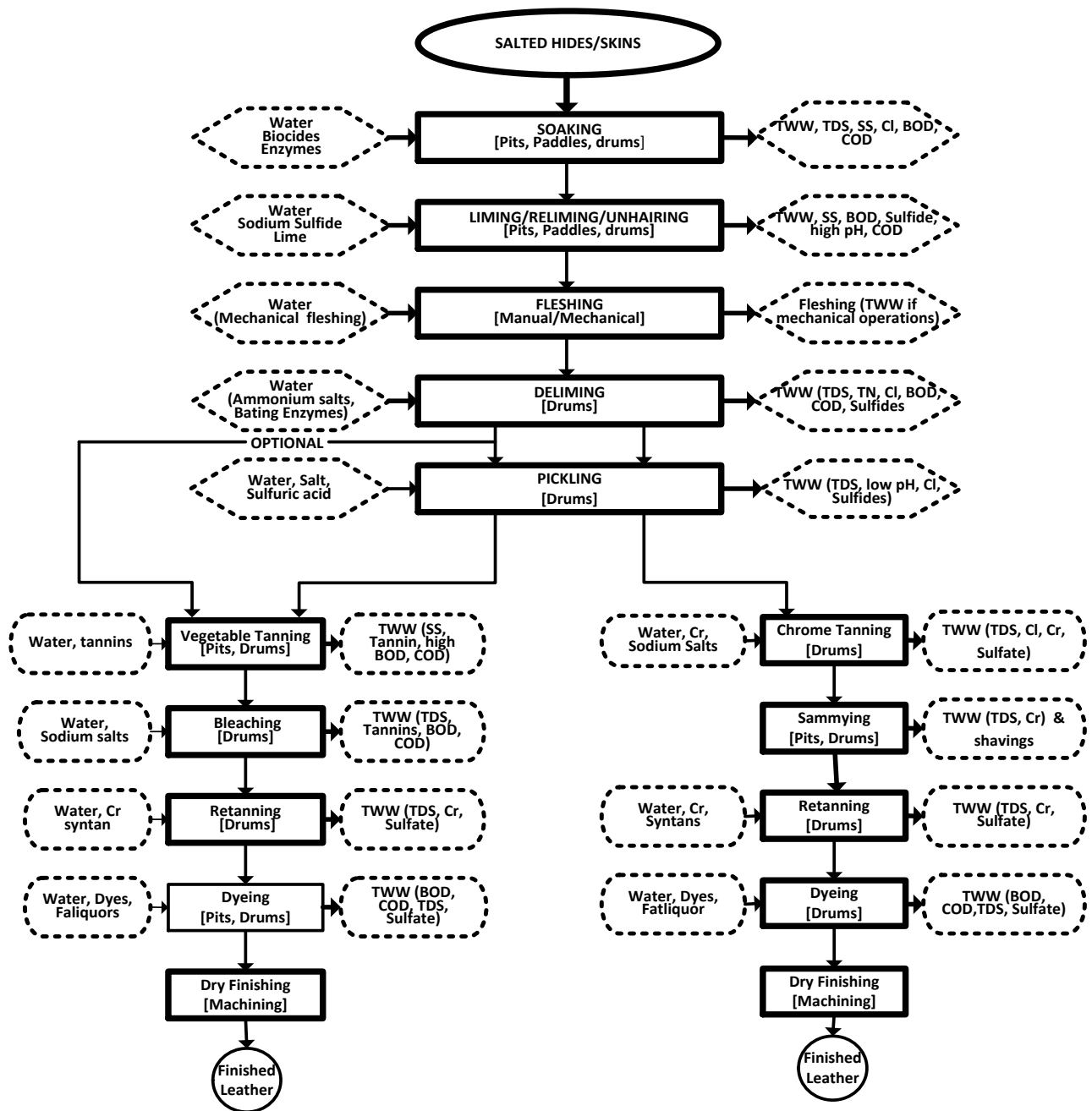
Figure 2-1: Leather-processing flow sheet showing inputs and wastewater outputs for each process

(adapted from Buljan & Král, 2019)

2.4 Tannery wastewater treatment

The integrated Tannery WWTPs comprise of preliminary, primary, secondary (biological), and tertiary/advanced treatment processes and are site specific. Tanneries only involved in leather finishing (Figure 2-1) generally do not employ secondary treatment due to low BOD concentrations of the TWW. Sometimes tanneries segregate and treat the soaking, beam-house and tan yard effluents separately in order to avoid pollutant overload. The partially treated effluents are typically mixed together later in a balancing tank and subsequently treated in a conventional activated sludge process (ASP), which remains the principal method for TWW treatment worldwide (Swartz et al., 2017). However, ASP has a high carbon footprint related to high energy requirements for aeration, consumes enormous amounts of energy and generates a large amount of excess sludge (Giaccherini et al., 2017). The total energy intensity (kWh/kgCOD$_{removed}$) and off site CO_2 equivalent emission (kg of COD$_{removed}$) from Tannery WWTPs is about 260% and 400% higher than municipal wastewater treatment processes, respectively (Giaccherini et al., 2017). Similarly, emerging and widely researched physicochemical treatment technologies such as advanced oxidative processes (AOPs), adsorption, electrocoagulation, ion exchange, membrane filtration and membrane bioreactors are characterised by high capital, operating and maintenance costs, consume chemicals and/or produce harmful by products.

In the context of a bio-circular economy in the developing world, AD is more favourable for treating TWW. However, its adoption is limited by process instability, slow reaction rates and low biogas recovery due to the presence of recoverable microbial toxicants in the TWW such as H_2S, NH_3, VOAs and metals (Table 2-1). However, there is limited information on metal concentrations in TWW except for Cr. A study by Kim et al. (2014) reported on [Ni] = 0.02 mg/L and [Pb] = 0.08 mg/L, while Mekonnen et al. (2017) reported on [Cu] = 0.83 mg/L. These were the only studies in this review that reported on [Ni], [Pb] and [Cu] concentrations in TWW.

Table 2-1: Tannery effluent characteristics reported in literature

pH	TDS	TSS (TS)	BOD$_5$	COD	HS	SO$_4$ (S)	TKN (TN)	NH$_4$	TP	TOC	Cl	Na	Ca	Fe	Cr	Mg	Reference
	g/L	g/L	g/L	g/L	mg/L	g/L	mg/L	mg/L	mg/L	g/L	g/L	g/L	g/L	mg/L	mg/L	mg/L	
6.0	24.2	1.22	0.14								4.05				4166		Ahsan et al., 2019
8.7	7.04	2.21		7.27	269	0.48	(112)	262	12.4						28.0		Alemu et al., 2016
4.1		0.91		2.60				180			1.69				570		Deghles and Kurt, 2016
3.8				5.80		1.30					23.3				7000		Elabbas et al., 2015
4.0	6.60	2.25	2.04	4.10				52	63						840		Elmagd & Mahmoud, 2014
7.1	13.3	2.82	0.63	4.80	228		225	128	-						11.2		Sanjay & Vaishnav Raj, 2014
				2.53	10	1.81	(190)	126	14								Mannucci et al., 2014
8.5	8.50	(10.1)	0.76	5.68	185	(2.68)	2.87	-	165	0.51	6.58	17.4	2.98		521		Bhattacharya et al., 2013
8.7		3.41		11.6											1.80		Tamilchelvan & Mohan, 2013
		0.99		55.0	0.1			74	2.6						198		Stoller et al., 2013
6.6		2.87	2.70	6.86		0.75		70.5			2.84				140		Lofrano et al., 2013
9.2	17.7	16.9		5.78							4.70	3.08			9.86		Sharma & Malaviya, 2013
7.2	0.32		0.15	0.93											3.90		Asfaw et al., 2012
12	6.10	7.97	0.25	0.51							0.40				-		Subramani & Haribalaji, 2012
7.2		(14.5)		16.5	287						1.60				6.00		Rajesh Banu & Kaliappan, 2007
7.8	12.9	1.15	1.74	6.24	232		168	115	21						13.3		Durai et al., 2010
6.9		0.98		3.69	27		(306)	222			5.76						Munz et al., 2008
6.2			0.10	1.06	65	1.06	162		10		1.07						Aboulhassan et al., 2008
7.1		2.82		4.80			225	128							95.0		Ganesh et al., 2006
7.7	36.8	5.30		2.20			(270)	150	21								Lefebvre et al., 2006
9.3	7.20			4.38	115	0.46	(480)	251	20.8		9.11	2.69	0.34	7.60	6.20	48.0	Mekonnen et al., 2017
8.4		(2.48)		4.47	196	0.45	(915)	282				0.80	58.0	-	0.26	13.4	Berhe & Leta, 2018
6.8		2.40		6.50		3.90	0.22	90.0	11.5								Guerrero et al., 2013
-		3.07	4.33	7.26		1.20											El-Sheikh et al., 2011
5.6		(31.5)		23.6	52	2.71	(0.18)	132	50	10.2	5.73	4.60					Daryapurkar et al., 2001
10		2.26	2.10	6.10			(505)	320	5.83			0.14		0.28	7.60	3.19	*Kim et al., 2014

No data   *average and/or median values were given for studies that reported concentrations as a range

2.5 Anaerobic treatment of tannery wastewater

Like all the biological processes, optimum operating conditions are vital for the efficiency of AD, which is mainly measured in terms of cumulative gas (biogas or CH_4) production and/or yield, substrate conversion and/or removal efficiency [biodegradability (B_0)]. Process efficiency is affected by the characteristics of TWW and inoculum, which depend on the operations at source. Given these variations, it is vital that the experimentation procedure is standardised and a comprehensive characterisation of the substrate/s, inoculum/inocula, biogas and at least the reactor (digester) contents is conducted. This will aid in understanding process inhibition, synergistic and/or antagonistic effects when co-digesting, microbial activity, bioavailability of metals, and in explaining the results. However, the widely used BMP assays for testing B_0 and CH_4 potentials vary from one study to another.

2.5.1 Biomethane potential experiments

The wide variability in BMP protocols used in studies published between 2007 and 2018 has been comprehensively reviewed by Ohemeng-Ntiamoah and Datta, (2019). The latest attempts by Holliger et al. (2016) and Steinmetz et al. (2016) to standardise the BMP protocol have not been fully adopted and none of the considered studies (Table 2-2) used these protocols. Some studies continue to use the German standard (VDI 4630, 2006), method by Angelidaki et al. (2009) and Owen et al. (1979). Laboratory scale semi/continuous operations that are sometimes applied also vary widely, manipulate different variables compared to BMP assays and cannot be easily standardised. There is significant variability in the results presented in this review due to the differences in reporting and in the protocols such as reactor type, mixing and size, inoculum source and operating quantity, temperature, controls and addition of nutrients and/or water (Table 2-2). Therefore, this review does not focus on comparing BMP protocols, process efficiencies and kinetics of the different studies, but rather the feasibility of recovering resources from TWW using AD.

2.6 Influence of operational parameters on anaerobic digestion

The investigated operational parameters for AD of TWW are mainly the feed rate, the organic loading rate (OLR), the influent characteristics (particularly COD, SO_4^{2-}, Cr, tannin, Cl, H_2S and NH_3 concentrations), hydraulic or solids retention time (HRT or SRT), pH and mode of operation (batch, semi/continuous). Some studies often investigate the influence of oxygen reduction potential (ORP), inoculum source and amount applied. In order to control the feed concentrations of toxicants, co-digestion (Section 3.4 and 4.4) and pre-treatment (5.5.1) experiments have been conducted.

2.6.1 Inoculum

The majority of the studies dealing with the AD of TWW used either activated sludge, domestic anaerobic sludge, sewage sludge and/or manure and did not report on the microbial and physicochemical characteristics of the inoculum (Table 2-2). The inocula in some studies were not acclimatised and/or preincubated, and worse reactors were operated at low retention times (RT) and without controls. Preincubation of inocula for 2 to 5 days to reduce endogenous biogas production is recommended and a control reactor must be set up to measure the residual endogenous biogas (Holliger et al., 2016). Generally, without regarding the operating conditions, studies using sewage sludge inoculum reported lower BMPs while those using domestic/anaerobic sludge or manure reported higher BMPs (Table 2-2). This is likely due to the presence of active, diverse and robust microbial consortium in the latter inoculum.

2.6.2 Reactor design

Single-stage, high rate anaerobic reactors such as the up-flow anaerobic sludge blanket (UASB), up-flow anaerobic filters (UAFF/UAFFFB/UACF/UAFBR) and anaerobic sequential batch reactors (AnSBR) are the most widely investigated and applied reactors for TWW treatment (Table 2-2). Batch systems particularly AnSBR, represent the most widely employed (Table 2-2), simplest and cheapest technology, and are therefore suitable for tanneries in developing countries (Mekonnen et al., 2017). The UASB and the different anaerobic filters reactors become less suitable for treating raw TWW, particularly beamhouse and combined tannery effluents with high suspended solids (SS) and salinity which affects bio-granulation (Lefebvre et al., 2006). Moreover, anaerobic membrane bioreactors (AnMBR) also offer similar treatment advantages to AnSBR and their feasibility in treating TWW was demonstrated by Umaiyakunjaram & Shanmugam, (2016) using a submerged AnMBR. Though the application of AnMBRs is promising; fouling, inhibition (particularly by Cr and H_2S) and their lifecycle costs are prohibitive (Mannucci & Lubello, 2020). To date, there are very few studies that have been conducted in this field and there are limited full-scale application of AnMBRs in tannery WWTPs.

The benefits of two-stage systems during treatment of TWW are sometimes insignificant compared to single-stage systems, in terms of CH_4/biogas production and B_o (Berhe & Leta, 2017). However, there are no studies that have been specifically performed to compare single-stage and two-stage configurations during the AD of TWW. There is also a lack of studies investigating the impact of reactor size, headspace volume and reactor configurations on BMP. A wide range of reactor sizes is used in BMP studies (Table 2-2).

The AnSBR may require some minimal agitation using an impeller, recycled biogas or TWW to improve mass transfer and contact with suspended biomass. The electricity demand of a full-scale AD plant can be in the range of 8-24% of the produced biogas (Berglund & Börjesson, 2006), with 14-54% of a WWTPs' total energy demand removed by mechanical mixing (Dachs & Rehm, 2006). Thus, the need for implementation of energy efficient measures such as reducing mixing time, intensity (shear rate), biogas or effluent recycling speed (pump power size), and/or ultimate elimination of mixing systems. Studies presented in Table 2-2 were conducted without mixing, except for those by Bonoli et al. (2014); Mekonnen et al. (2017); and Umaiyakunjaram & Shanmugam, (2016). Nonetheless, these few studies did not investigate the effect of mixing. There is a lack of studies investigating the different mixing modes (continuous or intermittent mixing), intensity and set ups during the AD of TWW.

2.6.3 Hydraulic and solids retention time (HRT/SRT)

The HRT and SRT (H/SRT) are the principal drivers of process efficiency, the selection of predominant microbial species, the composition of fermentative products, and the concentrations of inhibitors. The optimum operational H/SRT during the AD of TWW at ±35°C is about 0.5–5.0 days for continuous reactors and ≥10 days for semi continuous and batch reactors (Table 2-2). According to Rittmann & McCarty, (2001), the limiting SRT for aceticlastic methanogens (AMs) and HMs at 35°C are 4 and 0.76 days, respectively. Ideally, for perfectly mixed reactors, S/HRT is inversely proportional to OLR, net specific growth rate and inoculum to substrate ratio (ISR) or feed to microorganism ratio (F:M).

Generally, when treating complex effluents such as TWW, increasing S/HRT (decreasing OLR) leads to increased process efficiency due to reduced concentration of inhibitory substances. Low HRT (<24 hr) in continuous reactors can lead to biomass washout (Daryapurkar et al., 2001). Batch reactors, particularly those co-digesting TWW with solid substrates and/or using unacclimatised inoculum require longer H/SRT to overcome inhibition (Table 2-2). The optimum operating HRT or OLR often differs for CH_4 yield and B_o (El-Sheikh et al., 2011). Mpofu et al. (2020a) observed reversible inhibition with long lag phases of more than the 3 days using long term acclimated inoculum formed by mixing various sludges as proposed by Steinmetz et al. (2016). The standard BMP protocol recommend 3 days as the ultimate process termination time.

2.6.4   Organic loading rate and inoculum to substrate ratio

The OLR [mass of COD or volatile solids (VS) fed per unit time) dictates the amount of toxicants fed into the reactor and can be controlled by manipulating S/HRT and ISR (gVS/gVS). In batch reactors, enough supply of inoculum (functional microorganisms) must be supplied per TWW organic load (ISR) to start the AD process. However, apart from studies conducted by Achouri et al. (2017) and Vazifehkhoran et al. (2018), other batch studies (Table 2-2) did not report on the operational ISR.

Generally, during AD of TWW, OLR and ISR are respectively positively and negatively correlated with biogas yields. However, biogas yields may eventually decline when the bioavailability of toxicants become inhibitory to microorganisms. This causes reductions in B_o (COD, TS, VS and SO_4^{2-}), CH_4 yield and quality due to overloading and inhibition, particularly of the sensitive methanogens (Berhe & Leta, 2018). High OLR and an imbalance of acidogenic, acetogenic and methanogenic degradation rates may lead to the accumulation of VOAs and subsequently low reactor pH (Mekonnen et al., 2017). Conversely, B_o may momentarily increase with increasing OLR until a toxic level is reached depending on the initial OLR (Torkian et al., 2003). Operating at very low OLRs and high ISR, affects the economic feasibility of the process.

2.6.5   Temperature

Temperature has a direct effect on the microbial metabolic activities, process factors such as mass transfer rates, solubility, viscosity, toxicity and settling characteristics of biosolids and indirectly on biogas quality, and yield (Appels et al., 2008). Conventionally, mesophilic AD (MAD) at 35–40°C is preferable to thermophilic AD (TAD) when treating TWW. TAD may exacerbate inhibition instead of improving reaction kinetics and process efficiency.

Mpofu et al. (2020a) demonstrated the positive effect of temperature increase on ultimate CH_4 yield (A) which coincided with a decrease in the methanogenesis rate (k) while treating TWW sludge. The results defied the Arrhenius relationship, as a 10 °C increase in temperature resulted in decreased reaction rates of at least 15%. Apart from NH_3, the solubility (pKa) of most inhibiting unionised substances such as H_2S, CO_2, H_2, and metal salts follow Henry's law, and decrease with increasing temperature.

Several studies have reported that lower temperatures may achieve comparable results to what is conventionally considered optimal for MAD when treating ammonia rich substrates such as of TWW (Mpofu et al., 2020a). Song et al. (2003) reported a 22% decrease in COD removal efficiency due to a 17°C shock temperature decreases during operation. Nonetheless, the feasibility of treating TWW in an

unmixed ambient anaerobic reactor, suitable for tanneries in the developing world with temperate climates, has been demonstrated (Table 2-2). There is, however, a lack of investigations on reactor heating technologies such as underground reactors, passive solar heating, coating reactors with material that adsorb heat and/or insulating material, suitable for low income areas.

2.6.6   Reactor pH, volatile organic acids and alkalinity ratio

Generally, a neutral pH (7.0±0.5) is considered optimal during the AD of TWW as it supports the fair distribution of soluble and insoluble concentrations of compounds, and promotes growth and activity of functional microbial species. The optimum pH range for methanogens is 6.5 – 8.0, acetogens is 5.8 – 8.5 (Amani et al., 2010), SRB is 7.5 – 7.9 (Genschow et al., 1996), acidogens and hydrolytic bacteria is 5.5 – 6.5 (Berhe & Leta, 2017).

TWW generally has a good buffering capacity ($NH_3 - NH_4^+$ and $CO_2 - HCO_3^- - CO_3^{2-}$) (Table 2-1) which may help to retain suitable pH levels (Berhe & Leta, 2018). The naturally high VOAs concentrations in TWW itself, coupled with high OLR, particularly during start-up may cause reactor acidification (souring), pH and alkalinity drop, and inhibition of functional microbes. Likewise, the high concentration of alkalinity ($CaCO_3$ or OH^-) and low C/N ratio of TWW may lead to ammonification (Berhe & Leta, 2018), pH increase (Agustini et al., 2019) and subsequently reversible inhibition of methanogenesis or ultimate reactor failure. The degradation of VOAs by SRB (acetogenesis and/or sulfidogenesis) increases pH. Reactor VOA:alkalinity is an indicator of reactor stability (Gao et al., 2015) with VOA:alkalinity <0.3–0.4 indicating a stable reactor, whereas ratios >0.8 indicate significant reactor instability. It has been reported that the AD process is steady at alkalinity concentrations in the range of 1000 – 4000 mg $CaCO_3$/L (Banu & Kaliappan, 2007; Berhe & Leta, 2018). However, the use of chemicals for pH correction exacerbate the operating costs and may render the process uneconomical.

2.6.7   Oxidation-Reduction potential (ORP)

Thermodynamically, O_2 is the most favourable electron acceptor and under anoxic conditions, NO_3^-, NO_2^-, Fe^{2+}/Fe^{3+}, SO_4^{2-} or CO_2 act as electron acceptors (Song et al., 2003a). However, high concentrations of oxidising agents should be avoided as ORP between -300 and -330 mV is necessary for optimal growth and activity of methanogens (Zupančič et al., 2012). Song et al. (2003) reported optimal conditions for methanogesisis at an ORP range of -500 to -550 mV. Sabumon, (2008a) successfully promoted sulfidogenesis and anaerobic ammonia oxidation (ANAMMOX) at an ORP of -320 ± 25 mV for the removal of SO_4^{2-} and NH_4^+ from TWW.

Facultative bacteria (acidogens) can coincidentally use the available O_2 as an electron donor, thereby protecting methanogens from O_2 toxicity (Khanal et al., 2003) and promoting the development of genera such as: *Methanobrevibacter thermoautotrophicum, Methanobrevibacter arboriphilus, Methanosarcina barkeri and Methanosaeta* that can tolerate intrinsic oxygen (Huang & Khanal, 2004). Huang & Khanal. (2004) recorded a 13.5% increase in total COD removal due to increased activity of facultative heterotrophs enhanced by O_2 availability. Furthermore, the authors demonstrated the feasibility of controlling ORP by injecting O_2 to effect a 165% HS^- reduction with concomitant 46% improvement in CH_4 yield at feed SO_4^{2-} concentrations as high as 6000 mg/L. There is, however, a lack of studies investigating controlled injection of O_2 to alleviate HS^- inhibition and/or to produce S^0.

2.6.8   Carbon:nitrogen ratio and co-digestion

The low C:N ratios of TWW (Table 2-2) are considered a key indicator of potential AD instability due to accumulation of potential inhibitors, particularly NH_3 and VOAs. Optimal C:N ratios for TWW are in the range of 6–9 (Berhe & Leta, 2018). AcoD of TWW with substrates with high C:N ratios such as dairy wastewater, wheat straw and tannery solid waste (TSW) has proven effective for improving AD (Table 2-2). However, when choosing a co-substrate, it is important to consider that some substrates may lead to synergetic co-inhibition. For example, AcoD of nitrogenous and lipid rich-waste can lead to $NH_3 - LCFA$ co-inhibition (Mpofu et al., 2020b). In addition, the origin of the co-substrate must be near the tannery concerned in order to make the process cost-effective.

Berhe & Leta. (2017) co-digested TWW and dairy wastewater (DWW) at an optimal 50:50 (v/v) mix ratio in a hydrolytic-acidogenic reactor and achieved 55.5% acidification. The authors later investigated the AcoD of TWW with TSW and reported optimum VOAs production at 50:50 (v/v). The methanogenic reactor displayed process stability and recorded a 34.7% increase in biogas production (Berhe & Leta, 2018). Similarly, Agustini et al. (2019) demonstrated optimal performance while co-digesting TSW with TWW at 80:20 (v/v). A 66% reduction in TOC was achieved, like those achieved using an engineered nutrient solution. The study showed a reduction of 8, 18 and 23% of electricity, TWW treatment and TSW disposal costs, respectively. The studies concluded that AcoD of TWW improved the C:N ratio, balanced nutrients, provided a stable buffering capacity, and abated NH_3 inhibition.

2.7   Process inhibitors

The success of implementing AD in a tannery WWTPs depends on the characteristics and the discharge volumes of TWW. These are influenced by the tannery and WWTPs operations (Figure 2-1) which are dependent on the type of raw materials and the required quality of the finished leather. There are structural differences in b/ovine hides and exotic skins in terms of fat content, thickness, hair, size, among others which influence the tanning process and TWW characteristics. Consequently, the characteristics of TWW vary both inter- and inter-site, as evidenced by the variability in the characteristics shown in Table 2-2.

Inhibitory substances such as NH_3, SO_4^{2-}, H_2S, metals, lipids, VOAs and O_2 may be contained in TWW and/or are released as by-products. Some of these substances are essential in small concentrations for microbial growth, but inhibit AD at higher concentrations (Chen et al., 2008). A wide range of inhibitory concentrations have been reported due to the varying bacteriological mechanisms (acclimation, synergism and antagonism) on different reactor operating conditions. However, many manuscripts do not provide comprehensive results of TWW character, nor the process stream source of TWW.

2.7.1   Pre-treatment

A number of studies have investigated the pre-treatment of TWW using coagulation prior to AD in order to remove potential toxicants, particularly sulfide and Cr. Conventional alkaline coagulation is achieved by the application of slaked lime ($Ca(OH)_2$), aluminium sulfate [Alum $-$ $Al_2(SO_4)_3$] and anionic polyelectrolytes (Buljan & Ivan Král, 2011). Ferric chloride (FeCl$_3$) is commonly used by the tanning industry and is the subject of most coagulant experiments. Song and Williams, (2004) and Song et al. (2001) have demonstrated FeCl$_3$ as more efficient than $Al_2(SO_4)_3$ in pollutant removal, particularly Cr and sulfide, respectively. In contrast, Aboulhassan et al. (2008) reported that $Al_2(SO_4)_3$ and $Ca(OH)_2$ were more efficient than FeCl$_3$ in the improvement of B$_0$ and in the removal of Cr, COD and colour. In a lab scale study, poly aluminium ferric chloride (PAFC$-$ $[Al_2(OH)_nCl_{6-n}]_m$) coagulant achieved >75% COD and >95% SS removal and was demonstrated to be more efficient than FeCl$_3$, ferrous chloride (FeCl$_2$), and $Al_2(SO_4)_3$ (Lofrano et al., 2006).

Studies by Song & Williams. (2004) and Song et al. (2001) reported improved B$_o$ of TWW after the successful removal of 30–37% COD, 38–69% SS, 74–99% Cr, 80–100% total sulfide and 85–86% colour using pre-treatment coagulation with 800 mg/L of FeCl$_3$ and $Al_2(SO_4)_3$. Aboulhassan et al. (2008) later reported comparable results (p>0.05) for colour (76–92%), and Cr (79–97%) removal while COD (100%) removal varied significantly. However, FeCl$_3$ and $Al_2(SO_4)_3$ were dosed at higher concentrations of 400

and 600 mg/L, respectively. Achouri et al. (2017) recently reported the lower removal efficiencies of 0.44–29% for total Cr, 2.4–19% for total sulfide, 0.9–29% for COD, and 0.3–56% for TS depending on the $FeCl_3$ dosage. The highest pollutant removal efficiencies at 1000 mg $FeCl_3$/L corresponded with 25% reduction in RT, 10% increase in biogas production and 43% increase in COD removal (Achouri et al., 2017). The differences in removal efficiencies were partly due to varying initial concentrations, coagulant dosages and operating pH. Achouri et al. (2017) had fed TWW with high Cr and sulfide concentrations of 150 and 380 mg/L, while Aboulhassan et al. (2008) fed 12 and 3.3 mg/L and Song et al. (2001) fed at 20 and 100 mg/L, respectively.

Coagulation may lead to a decrease in biogas/CH_4 yield and B_0 due to increased concentrations of cations (Fe, Ca and Al) or anions (SO_4^{2-}) and/or precipitation of organic matter, producing excess sludge (Genschow et al., 1997). To mitigate this, Wiemann et al. (1998) employed a H_2S stripping system and achieved 70% H_2S removal in the influent TWW and 15% improvement in B_0. The stripped H_2S was precipitated with $FeCl_3$ and could be recovered. Most of all, pre-treatment increases the treatment and sludge disposal costs. Pre-treatment using AD by promoting sulfidogenesis may be an alternative. Liu et al. (2016) successfully utilised a UASB reactor as a hydrolysis/acidogenesis pretreatement process for the removal of a considerable proportion of readily biodegradable organics and improved B_0 of TWW. However, the process must be optimised in order to ensure the effluent has enough nutrients and lower sulfide (H_2S/HS^-/S_2^-) concentrations suitable for methanogenesis.

2.7.2 Solids

Generally, a VS:TS ratio >0.8 is required for efficient reactor performance. Higher TS and/or SS content in TWW reduce mass transfer, settling rates (Song et al., 2000), and may alter other rheological properties and consequently affect the microbial communities, their activity, and metabolic pathways.

Akyol et al. (2014) observed VOA accumulation while operating at 5% with no significant difference in CH_4 yields. Lefebvre et al. (2006) reported reactor inefficiency while operating at 6.1 g SS/L. The optimal %TS depends on reactor design, TWW characteristics and OLR. Therefore, in order to promote process efficiency, several studies have investigated the use of physicochemical pre-treatment processes to remove excess SS. Inevitably, AD of TWW produces excess sludge (digestate) that is composed of recalcitrant material, particularly toxic metal salts. Depending on the ultimate composition of the digestate, it may be recycled for example, as a biofertiliser, concrete aggregate, and/or source of biomass for downstream applications.

Table 2-2: Tannery effluent anaerobic co/digestion at different operating conditions

Reactors	Batch Digester	UASB	2-stage AnSBR	Batch AFFFB	Batch UAFFB	Batch digester	1-stage UAFBR	Batch digester	1-stage UACF	Batch digester
Scale	Lab-scale	Lab-scale	Full-scale	Lab-scale	Pilot-scale	Lab-scale	Lab-scale	Lab-scale	Pilot-scale	Lab-scale
Reactor volume (L)	0.15	94	850	17.5	-	1	2	1	1930	-
Pre-treatment	-	Physchemical	Hydrolysis	Physchemical	Coagulation	Coagulation	Coagulation	Coagulation	Coagulation/-	Strip/ Coagulation
Substrate	GTWW	GTWW	GTWW	GTWW+ Cr liq	GTWW	GTWW	$GTWW_v$	GTWW	$GTWW_v$/ $GTWW_{Cr}$	BWW
Co-substrate (type)		-	-	-	-	_	-	Wheat straw	-	-
Co-substrate (%vol)	-	-	-	-	-	_	-	10	-	-
Operation mode	Batch	Continuous	S-continuous	Batch	Batch	S-continuous	Batch	Batch	Continuous	Batch
Inoculum	Swine	-	TWW	Manure+sewage	-	DAnS	DAnS	Manure	Manure+sewage	AnS
Nutrients added	-	-	No	No	No	-	No	Yes	No	No
Topped with water	-	-	No	No	No	-	No	No	No	Yes
Acclimation (days)	-	60	35	25	-	-	20	-	175	-
Controls	-	No	-	No	No	-	No	-	No	
ISR (TVS/TVS)	-	-	-	-	-	-	-	3-4	-	-
H/SRT (days)	58	0.5	40	0.5-5.0	3.5	10	16	30-60	2.5	1.9/2.4
Temp (°C)	37	Ambient	40	35	34	37	37	37	Ambient	35
pH	6.8	7.0	7.6-.8.0	7.0	5.0 - 7.0	-	5.0 -7.0	-	7.0-8.3	7.4
C/N	-	-	3.9	2.6-18	-	-	-	-	-	-
OLR (gCOD/L.day^{-1})	-	-	2.0-2.5	-	1.0-2.2	0.33	-	0.6	-	-
Influent COD_t (g/L)	6.5	7.26	-	0.9-7.2	5.71	2.2	-	-	-/19.7-20.0	2570/2130
COD_t removal (%)	88.5	82.4	-	45	70	64	75	-	95/86-89	90/65
Influent COD_s (g/L)	-	-	13.5	-	-	-	-	-	-	-
COD_s removal (%)	-	-	88	-	-	-	-	-	-	-
Biogas (mL/L_{RV}.day^{-1})	-	5681	-	7.3-2276	-	-	-	-	2.45/2.42-2.49	-
Biogas (mL/gVS)	-	137	-	-	-	-	-	-	-	-
Biogas (mL/gCOD$_{Add}$)	-	-	312	-	75	-	-	-	-	400/420
CH$_4$ yield (mL/gVS)	-	-	-	-	-	-	-	314	-	-
CH$_4$ yield (mL/gCOD$_{add}$)	54	-	-	-	-	0.21	270	-	-	-
CH$_4$ yield (mL/gCOD$_{rem}$)	-	-	-	-	-	-	360	-	-	-/300
Average CH$_4$ (%)	-	-	66	59	-	-	65-73	-	-	-/69
References	(Guerrero et al., 2013)	(El-Sheikh et al., 2011)	(Bonoli et al., 2014)	(Daryapurkar et al., 2001)	(Genschow et al., 1997)	(Song et al., 2001)	(Song et al., 2003a)	(Vazifehkhoran et al., 2018)	(Vijayaraghavan & Murthy, 1997)	(Schenk et al., 1999)

AS = acclimated sludge   BMP = biochemical methane potential   CH$_4$ = methane   C/N = carbon to nitrogen ratio   D/AnS = domestic/anaerobic sludge

COD_t = total COD   ISR = inoculum to substrate ratio   Add = added   H/SRT, hydraulic/solid retention time   AnSBR = anaerobic sequencing batch reactor

LS = leather shavings   GTWWv, general chrome TWW   Gran =granule   OLR = organic loading rate   UACF = upflow anaerobic contact filter

Reactors	Batch digester	2- stage AnSBR	Batch BMP	2- stage AnSBR	Hybrid UASB	UASB	Submerged Pit	SAnMBR PVDF mem	1 - stage AnSBR	Batch BMP	1st -stage UASB
Scale	Lab-scale	Lab-scale	Lab-scale	Full-scale	Lab-scale	Lab-scale	Pilot-scale	Pilot scale	Pilot-scale	Lab-scale	Lab-scale
Reactor volume (L)	2	0.6	1	113 000*	5	5	4 000	80	100 000	0.3	4.2
Pre-treatment	-/ Cavitation	Hydrolysis	-/ Coagulation	Hydrolysis	-	-	-	-	-	-	-
Substrate	GTWW	GTWW	GTWW	GTWW	$GTWW_V$	SL	GTWW	GTWW	GTWW	LS	GTWW
Co-substrate (type)	-	-/TSW	-	-	-	-	-	-	-	BHE	-
Co-substrate (%vol)	-	-/50	-	-	-	-	-	-	-	0-100%	-
Operation mode	Batch	S-cont.	Batch	S-cont.	Cont.	Cont.	Cont.	Cont.	Batch	Batch	Continuous
Inoculum	Sewage	Manure	DAS	-	DAnS	$UASB_{sludge}$	Sewage	AnS	AS	AS	UASB gran
Nutrients added	-	-	Yes	No	-	No	No	No	No	Yes	Yes
Topped with water	-	-	Yes	No		No	No	No	No	Yes	No
Acclimation (days)	36	30	0	-	-	LT	30	60	-	LT	50
Controls	-	No	Yes	No	No	No	No	No	-	No	No
ISR (TVS/TVS)	-	-	1.5	-	-	-	-	-	-	-	-
H/SRT (days)	35	20	37	-	2.5	5	8	1.7	4	150	30
Temp (°C)	37±0.2	38±2	35	-	33-42	Ambient	20-30	-	31	35	-
pH	-	-/6.80	7.00	7.1		7.5-7.8		7.1-8.7	-	7.33	-
C/N	-	-	-	-		-			-	-	-
OLR (gCOD/L.day^{-1})	-	0.21/0.28	-	1.0-2.2	3.1	0.5	0.6	6	-	-	2.23
Influent COD_t (g/L)	6.5/8.2	4.2/5.5	23.7/17.3	-	14.0	2.6	-	11.2	0.65	-	8.9
COD_t removal (%)	43	56/75	45/88	-	88	78	80	90	85	-	63.4
Influent COD_s (g/L)	-	2.2/3.3	-/20.6	-	-	-	-	7.6	-	-	-
COD_s removal (%)	-	68/75	-	-	-	-	-	-	-	-	-
Biogas (mL/L_{RV}.day^{-1})	-	-	-	230-319*	628*	-	9.8*	-	298	-	-
Biogas (mL/gVS)	-/137	-	≈653/737	-	-	-	-	-	-	21	-
Biogas (mL/gCOD$_{rem}$)	-	-	-	-	-	470	-	-	-	-	-
CH$_4$ yield (mL/gVS)	7.6/26.5	-	437/703	-	-	-	-	-	-	9	-
CH$_4$ yield (mL/gCOD$_{add}$)	54/-	656/896	-	-	-	-	-	-	300	-	-
CH$_4$ yield (mL/gCOD$_{rem}$)	-	-	-	-	-	-	-	160	70	52	160
Average CH$_4$ (%)	11/19	45/61	58/84	61	-	-	-	86	-	-	-
References	(Saxena et al., 2019)	(Berhe & Leta, 2018)	(Achouri et al., 2017)	(Alemu et al., 2016)	(Rajesh Banu & Kaliappan, 2007)	(Lefebvre et al., 2006)	(Tadesse et al., 2003)	(Umaiyakunjar am & Shanmugam, 2016)	(Mekonnen et al., 2017)	(Agustini et al., 2019)	(Liu et al., 2016)

AFFB = anaerobic fixed film bed reactor   BHE = beamhouse effluent   BWW = bovine wastewater   Lab = laboratory   UASB = upflow anaerobic sludge blanket

Strip = stripping   TSW = tannery solid waste   RV = reactor volume   S-Cont = semi-continuous   CODs = soluble COD

GTWWv = general vegetable TWW   TVS = total volatile solids   Vol = volume   Cont = continous

2.7.3 Lipids

TWW contains significant amounts of biodegradable aliphatic organic compounds that can cause direct inhibition of AD, or indirect inhibition through their metabolites [long chain fatty acids and volatile organic acids metabolites (LCFAs and VOAs)] during AD (Reemtsma & Jekel, 1997). Lipids have a higher BMP and B_o, though with a lower degradation rate (Appels et al., 2011). The widely reported hypothesis for lipid inhibition is the adsorption of the hydrophobic LCFAs onto the cell wall and membrane of microorganisms. This blocks mass transfer of metabolites, enzyme accessibility and causes the flotation and/or washout of active biomass (Appels et al., 2011). Sludge flotation is also exacerbated by the high density of TWW due to hyper salinity which hampers gravity settling. Furthermore, the measurement of COD is affected by the presence of VOAs and aromatic compounds that are not fully oxidised (Angelidaki et al., 2009).

Mitigation measures such as addition of Ca, adsorbents, increasing ISR, and co-digestion with less biodegradable substrates have proven successful (Chen et al., 2014). According to Berhe & Leta. (2018), high concentration of Ca during the AcoD of TWW and TSW reacted with LCFAs and precipitated as insoluble salts (6.50 – 37.4 mg/g fat) leading to an increase in biogas production and floating sludge. Other biotoxic organic compounds introduced during leather processing include chlorophenols, halogenated aliphatics, alcohols, aldehydes, surfactants, ethers, carboxylic acids, and amines (Chen et al., 2014). Although several studies have reported VOA inhibition during the AD of TWW, there are limited studies that exclusively investigate inhibition by organic compounds, particularly LCFAs and VOAs.

2.7.4 Volatile organic acids

Free VOAs can easily permeate the bacterial cell membrane and cause pH reduction, thereby disrupting cell homoeostasis which may cause reactor foaming (Appels et al., 2008). The inhibition threshold ranges for different VOAs during AD reported in literature vary widely depending on the operating conditions: 4000–6900 mg/L for total VOA (VOA_t), 800–4000 mg/L for HAc, and 900 – 2000 mg/L for HPr (Mpofu, 2018). The IC_{50} of VOAs on specific methanogenic activity (SMA) have been reported as 13000, 15000 and 3500 mg/L for HAc, HBu and HPr, respectively in granular

UASB sludge (Dogan et al., 2005). In contrast, some researchers have established that VOA_t, HAc and/or HPr at concentrations up to 10000 mg/L may have no significant effect on CH_4 production (Demirel & Yenigün, 2002). However, most of these studies used organic acids as feed, and there are limited studies that exclusively investigate the effect of VOAs during AD of TWW.

Berhe & Leta. (2018) and Berhe & Leta. (2017) investigated two phase AcoD of TWW with TSW and dairy effluent and reported on the accumulation of VOA_t up to 8300 and 4980 mg CH_3COOH/L, respectively. The authors reported that high OLRs inhibited acidogenes but did not investigate direct inhibition by VOAs. Generally, VOAs inhibition during AD of TWW is superseded by the effect of operational parameters such as temperature, pH, OLR, S/HRT and other inhibitors such as NH_3, H_2S, metals and lipids. Mpofu et al. (2020b) observed an accumulation of VOAs (HAc, HPr and HBu) in the range 2360–5960 mg/L, presumably due to inhibition of methanogens and/or acetogens by LCFAs and/or NH_3 and/or H_2S. However, most of the studies investigating the effect VOAs on AD used organic acids as feed, and there are limited studies that exclusively investigate the effect of VOAs during AD of TWW.

2.7.5 Metals

High levels of inorganic salts added during leather processing (Figure 2-1) and TWW treatment can contribute significant concentrations of non-biodegradable metal ions (cations) in the reactor that may easily accumulate and exceed the optimal requirement for bacterial growth and metabolism, leading to metal inhibition/toxicity. BMP protocols recommend the addition of nutrient/basic medium (inorganic salts) when they are deficient in the inoculum and/or substrate and/or addition of tap, which is obviously a source of micronutrients. The recommended nutrient medium/solution varies by author. Work by Agustini et al. (2019) proved that the addition of a nutrient solution containing organics reduced the bioavailability of Cr and enhanced process efficiency. The authors also demonstrated that the addition of water (deionised) increased the bioavailability of Cr and led to process inhibition.

Metal inhibition by common cations such as K, Na, Ca, Mg, Cu, Fe and Cr is one of the major causes of reactor upset or failure (Chen et al., 2008). The differences in inhibiting concentrations of similar

metals dosed with different anions (Cl^-, SO_4^{2-}, S_2^-, NO_3^-, or CO_3^{2-}) proved that metal inhibition strongly depends on metal species as free ions, complex bound and/or precipitates and their physico-chemical properties. Concentrations of 5 – 10 g Cl^-/L were reported as inhibitory to methanogens while SRB require 0.5 – 20 g/L of NaCl (Vazifehkhoran et al., 2018). Generally, methanogens are reported as the most sensitive to metal inhibition. Some studies reported acetogens, particularly those degrading HPr and HBu and hydrolytic bacteria as more sensitive to some metals than methanogens (Feijoo et al., 1995). The SRB are most resilient and are helpful in the precipitation of metal sulfides during AD (Vazifehkhoran et al., 2018). However, this can also lead to lower CH_4 yields due to precipitation of organic matter and reduced bioavailability of metals. The hypersalinity of TWW can promote the floating of matter leading to effluents with high SS and this can be mistakenly attributed to metal inhibition. Successful precipitation of metal is undesirable for digestate destined for use as a biofertiliser.

The presence of metal mixtures in TWW may exhibit antagonistic and synergistic effects to inhibition (Feijoo et al., 1995). Therefore, some reactors displayed steady-state operations at metal concentrations >10 g/L for Na (Mekonnen et al., 2017) and ≤140 mg/L for Cr (Vijayaraghavan & Murthy, 1997). This is likely to have been the case that led to the reporting of higher inhibitory concentrations (IC) for Fe and Ca by Jackson-Moss and Duncan, (1990, 1989) and Cr by Jackson-Moss et al. (1989), respectively. The general trend of mean IC on VOA degradation and methanogenesis using suspended and granular sludge is Cd>Cu>Cr>Zn>Pb>Ni, Zn>Cr>Cu>Cd>Ni>Pb and Zn>Ni>Cu>Cr>Cd, respectively (Lin, 1992). Table 2-3 shows the optimal and IC range of metals during AD. The differences in the IC in literature are due to the complexity of the biochemical reactions, microbial species and their adaptation, and operating conditions. Additionally, most studies used VOAs and/or carbohydrates (e.g. starch) as feed and there is a lack of studies investigating the effect of metals during the AD of TWW.

Table 2-3: Optimum and inhibitory concentrations of metals on anaerobic digestion

Metal	Potassium	Sodium	Calcium	Magnesium	Copper	Chrome	Iron
Optimum (mg/L)	-	(230–350)[2]	100[1]-352[5]	(41-53)[5]	-	-	-
Inhibitory (mg/L)	(2500-4500)[5]	(3000-24000)[7]	2500[6]-7000[1]	(1000-1500)[6]	100[6]	60[8]-5000[4]	(20-5650)[3]

Appels et al., 2011[1]   Feijoo et al., 1995[2]   Jackson-Moss & Duncan, 1990[3]   Jackson-Moss et al., 1989[4]
Mekonnen et al., 2017[5]   Tchobanoglous et al., 2003[6]   Rinzema et al., 1988[7]   Vijayaraghavan & Murthy, 1997[8]

2.7.6 Sulfate and sulfide

Sulfate is relatively non-inhibitory directly, but it promotes the growth of SRB that compete with acetogens and methanogens for substrates. This is assumed as the primary methanogenesis inhibitor ahead of unionised sulfide (H_2S) produced from SO_4^{2-} reduction by SRB (Tadesse et al., 2003). Sulfides reduce the bioavailability of essential stimulatory/toxic non-alkali metals needed by bacteria by causing their precipitation. Precipitates can reversibly inhibit microbes by blocking their access to substrates (Utgikar et al., 2002). The H_2S solubility is directly proportional to pH and inversely proportional to temperature. Methanogens are more sensitive to temperature and pH variations than H_2S or SO_4^{2-} inhibition (Shin et al., 1997). According to Vazifehkhoran et al. (2018), sulfide speciation with pH dictates that inhibition at pH > 7.2 is mainly due to the concentration of total sulfide ($H_2S + HS^-$).

The reported H_2S inhibitory concentration range for methanogenesis in this study was 50–280 mg/L. Conversely, Vijayaraghavan & Murthy, (1997) showed that a UACF treating TWW and operating at pH $7.0 - 8.3$ did not suffer H_2S inhibition at concentrations as high as 180 mg/L. Operating anaerobic reactors at pH 7.0–8.0 offers optimal pH levels for methanogens and reduces the concentration of H_2S (Huang & Khanal, 2004). In general, SRB are known to be more resilient to sulfide inhibition compared to methanogens, and their inhibitory range in literature was reported as $50 - 550$ mg/L (Mpofu, 2018). The syntrophic degradation of VOAs by SRB may play a major role in improving B_o without any decrease in CH_4 yield. According to Guerrero et al. (2013), the COD:SO_4^{2-} ratio takes precedence over pH in inhibiting methanogenic activity at high SO_4^{2-} concentrations. The critical pro-methanogenesis COD:SO_4^{2-} ratios were reported as low as 0.5–3.0 under different operating conditions (Chou et al., 2008). Huang & Khanal, (2004) reported SO_4^{2-} levels >6000 mg/L as inhibitory to methanogenesis during the AD of TWW whereas Guerrero et al. (2013) observed methanogenesis inhibition at SO_4^{2-} levels >8450 mg/L. Available Information on $H_2S/HS^-/S^{2-}$ toxicity during the AD

of TWW is inconclusive as most studies, particularly BMP, do not take into account the instantaneous reactor pH, concentration of other substances and bacterial adaptation. Furthermore, most studies explore inhibition of biogas production without investigating the effect of $H_2S/HS^-/S^{2-}$ on other bacterial groups. There is no economically feasible and sustainable technique that has been developed for the selective inhibition of SRB to promote CH_4 recovery from TWW.

2.7.7 Ammonia

The AD of TWW is restricted by high concentrations of NH_3 resulting from protein fermentation and/or NH_4^+ salts applied during deliming and bating. Ammonia or ammonium (NH_3/NH_4^+) is an essential nitrogen (N) nutrient for bacterial growth and a buffer (Berhe & Leta, 2017). However, similar to H_2S, the presence of NH_3 presents a more pronounced inhibition effect compared to NH_4^+. The reactor operating conditions such as pH, temperature, bacterial acclimation, C:N ratio, and concentration of other toxicants, influence the IC of NH_3 (Appels et al., 2008). Hence, the different IC reported in consulted literature for this study, range from 2000–14000 mg/L as total NH_3N ($NH_3+NH_4^+$) and 53–1450 mg/L as NH_3. The widely reported threshold limit of NH_3 on acclimated and unacclimated AMs is 700 mg/L and 100–200 mg/L, respectively. However, a steady operation was reported at 703 mg NH_3/L without inhibition using acclimated methanogens (Umaiyakunjaram & Shanmugam, 2016).

Generally, under elevated NH_3 levels (140–280 mg/L at 37–38°C and pH 7.5–8.0), CH_4 formation shifts to and/or is dominated by the SAO+HM pathways (Westerholm et al., 2016). This may be attributed to *Methanosarcina* spp. that have been reported to survive with SAOB at high NH_3 and H_2S levels as they are good H_2 scavengers (De Vrieze et al., 2012). Other TWW constituents and/or operating conditions may have exerted antagonistic effects on NH_3 inhibition. The inhibition of HMs leads to high H_2 partial pressures, which in turn inhibits HPr degrading acetogens and promotes HPr accumulation (Ariunbaatar et al., 2015) whereas inhibition of AMs promotes accumulation of HAc. Methanogens have a low conversion rate for HPr and it is regarded as the most toxic VOA.

2.8 Recoverable resources

2.8.1 Valorisation of tannery wastewater and digestate

2.8.1.1 *Nitrogen recovery*

Although N recovery from TWW is possible using ANAMMOX and denitrification, there are no economically feasible and sustainable techniques that have been developed to date. Instead, ANAMMOX and denitrification processes are employed to decrease the aeration rates, improve the energy efficiency of Tannery WWTPs and minimise the production of excess sludge (Gherghel et al., 2019). However, more focused studies are required for the development of an anoxic mixed culture for N recovery from TWW.

2.8.1.2 *Ammonia recovery*

The hydrolysis of TWW during AD leads to the accumulation of NH_3/NH_4^+ that becomes inhibitory at certain concentrations. Previous studies have indicated that anaerobically treated TWW contains concentrations of NH_4^+ as high as 9000 mg/L (Bonoli et al., 2014). This presents an opportunity for the treatment of reactor centrate to recover NH_4^+. Struvite ($MgNH_4PO_4 \cdot 6H_2O$) precipitation and NH_3 stripping are the most plausible methods for NH_4^+ recovery suitable for third world countries.

Struvite formation involves the addition of deficient Mg and P to the NH_4^+ rich TWW centrate in order to precipitate $MgNH_4PO_4 \cdot 6H_2O$ as a slow release fertiliser. However, this affects the economic feasibility of the process. Similarly, NH_3 stripping requires heating and use of pH adjusters. The stripped NH_3 can then be recovered or adsorbed by acid solutions such as H_2SO_4 to form $(NH_4)_2SO_4$. There is a need to investigate the feasibility of recovering fertilisers, $MgNH_4PO_4 \cdot 6H_2O$ and $(NH_4)_2SO_4$ from TWW centrates for agricultural application in developing countries.

2.8.1.3 *Sulfur and sulfide recovery*

Recent research developments have shown the possibility of recovering H_2S and S^0 from SO_4^{2-} rich effluents such as TWW in a cost-effective way suitable for developing countries. Boshoff et al. (2004) demonstrated the feasibility of using TWW as a source of organic carbon in producing HS^-/S^{2-} which can precipitate metals. Partial oxidation of $H_2S/HS^-/S^{2-}$ produced by AD using O_2 or an alternative electron acceptor such as NO_3^- or NO_2^- already present in TWW can produce S^0. Khanal et al. (2003)

and Sabumon, (2008b) demonstrated the feasibility of abating $H_2S/HS^-/S^{2-}$ inhibition by injecting controlled amounts of O_2 (-290mV≤ORP≤-270mV) to produce to S^0 with a simultaneous 46% increase in CH_4 yield and B_o, respectively. On the other hand, the authors reported autotrophic and heterotrophic denitrification having successfully oxidised sulfide to S^0 using NO_3^- and/or NO_2^- present in TWW. It was found that 63–66% of input sulfur (SO_4^{2-} and S^{2-}) can be recovered as S^0. To date, there are limited studies reporting the recovery of H_2S and S^0 from TWW.

For the recovery of S^0, inversed fluidized bed reactors represent a suitable option as it can easily be separated from denitrifying biomass due to differences in densities (Lin et al., 2018). The produced S^0 can be used as a raw material for fertiliser, H_2SO_4, insecticide, or pesticide manufacturing.

2.8.1.4 *Metal recovery*

Metals present in TWW can be precipitated during AD and recovered from sludge. The sludge can be thermally treated via incineration, pyrolysis, gasification, ultrasonication and microwaving to recover metals (Gherghel et al., 2019). Acid or bio leaching can be employed to recover metals from the ash residue or directly from the tannery sludge. Kokkinos et al. (2019) and Zeng et al. (2016) demonstrated the feasibility recovering 65–93% and 80–97% of Cr (III) using acid and bioleaching, respectively. The concentration of metals in ash after recovering energy and their leaching provides a relatively cheaper option for developing countries compared to landfilling.

2.8.1.5 *Volatile organic acid and hydrogen recovery*

The production of bio-hydrogen (H_2) from TWW can be promoted by optimising the AD process and splitting it into two phases: hydrolysis – acidogenesis (dark fermentation), and acetogenesis - methanogenesis. The first reactor, namely, dark fermentation produces H_2 rich biogas and VOAs. Berhe & Leta, 2018; (2017) reported the accumulation of VOA_t up to 8300 and 4980 mg CH_3COOH/L in the first and second reactors, respectively, while using two phase AD of TWW. However, studies have not fully explored this avenue for H_2 and/or VOAs recovery.

2.8.1.6 *Energy-Incineration*

Tannery sludge/digestate thermal stabilisation using processes such as incineration, pyrolysis and gasification can be applied for the recovery of energy and a stabilised product in the form of ash or

activated carbon. However, there is limited information on the latter processes. Kavouras et al. (2015) reported the beneficial anoxic over oxic incineration of tannery sludge at 500 °C to recover Cr (III) and conditioning of tannery ash for further beneficiation via vitrification. Nonetheless, oxic incineration of tannery sludge has been successfully applied by Abreu and Toffoli, (2009) after pre-treatment to produce raw material for (vitrification) ceramics. Thermal treatment offsets volatile heavy metals particularly Cr (VI) which is highly mobile, carcinogenic and toxic (Abreu & Toffoli, 2009). Conversely, Basegio et al. (2002) reported the immobilisation of metals, including Cr, and emission of S^0 and Cl^- compounds during incineration.

2.8.1.7 *Activated Carbon*

The use of tannery sludge or digestate as an adsorbent for wastewater treatment offers a simple, low cost and sustainable alternative suitable for developing countries. Tannery digestate contains a substantial amount of residual carbon that can be physically and/or chemically activated (Geethakarthi & Phanikumar, 2012). Geethakarthi & Phanikumar, (2012) developed an effective activated carbon adsorbent from tannery sludge which possessed a higher Brunauer–Emmett–Teller (BET) surface area of 188 m^2/g and a micropore size distribution of <20 A°. The prepared activated carbon (1.5 g/L) achieved up to 95% efficiency in removing anionic azo reactive dye RR31 fed at a concentration of 40 mg/L. Mella et al. (2019) achieved removal efficiencies of up to 71% and 73% of Acid Brown 414 and Acid Orange 142 azo dyes from TWW, respectively.

2.8.1.8 *Concrete and clay aggregate*

Generally, ceramic and glass processes are considered versatile in managing hazardous waste such as tannery sludge or digestate. Incinerated tannery sludge or digestate has been successfully used to immobilise heavy metals in a stable matrix during the manufacturing of ceramic products and/or bricks. Abreu & Toffoli, (2009) reported a gain in aesthetics (colour tone, texture, and brightness) of the ceramic tile glaze after the incorporation of tannery sludge. Incorporation of TWW sediment, clay, and alkaline slag produced a ceramic with good thermal and sound insulating properties that made it suitable for use in constructing inside walls (Belyakov et al., 1998). However, the amount of TWW sediment needed for maximum insulation properties was not investigated. Basegio et al. (2002)

optimised and reported the addition of 10% tannery sludge to be environmentally safe to produce ceramic bricks whose technological properties were compatible with conventional clay bricks. Likewise, Juel et al. (2017) also sustainably stabilised 10–40% of tannery sludge in clay bricks. This provided an energy saving of 15–47% during incineration and the amended clay bricks met the Bangladesh and American Society for Testing and Materials (ASTM) standards.

Overall, co-composting of tannery digestate or sludge with other organic wastes and its integration in clay or ceramic products offers a simple, cheaper and sustainable disposal alternative suitable for developing countries. However, the tannery digestate/sludge must be strategically co-composted or incorporated in order to optimise the concentration of nutrients suitable for a particular soil, plant, brick or clay product type. Therefore, long-term studies are needed to ascertain the environmental safety of these practices.

2.8.1.9 *Biofertiliser and compost*

Tannery digestate is bio-stabilised and contains the essential macro-nutrients (N, P, K, Ca, Mg, S) and micro-nutrients (Cu, Mn, Mo, Ni, Fe, B, Zn and Cl) needed by plants for growth (Feyisa et al., 2019). However, the agricultural application of tannery digestate faces many barriers due to the presence of these nutrients in very high concentrations that are toxic to the plants and the environment (Sodhi et al., 2018). Heavy metals (Na, Mg, Mn, Pb, Cr, Cu, Fe and Cd) may accumulate in plants and subsequently, enter the food chain and cause serious health problems. This is one of the major reasons why unstabilised tannery sludge is not suitable for agricultural application and is rather referred to as hazardous. Feyisa et al. (2019) produced a digestate from the co-digestion of TWW with TSW which was highly toxic to the germination of *Glycine max* (soybean) seeds. Gupta et al. (2010) observed the accumulation of Pb, Mn, Cd, Ni, and Fe in the shoots of *Brassica campestris* after applying a mixture of digestate and fly ash (1:4) while Nunes et al. (2018) observed Cr accumulation in sweet peppers after the application of tannery sludge based vermicompost in the order: fruits>stem/stalks>leaves=root. Conversely, tannery digestate or sludge has been successfully applied as an organic fertiliser and in some instances after co-composting with other organic waste such as livestock manure in order to optimise the concentration of nutrients.

Zemleduch-Barylska & Lorenc-Plucińska, (2016) and Patel et al. (2015) proved the successful application of tannery sludge without significant metal accumulation in *Populus canescens* and *Ocimum basilicum,* respectively. Nakatani et al. (2011) demonstrated an improvement in the soil enzymatic activities related to N cycling (*asparaginase and urease)* after the application of a mixture of beamhouse and primary tannery sludge (1:1) that increased inorganic N and soil pH. The authors further demonstrated the positive effect of tannery sludge application on chemical properties, increasing pH, electrical conductivity and the bioavailability of P and N in the soil (Nakatani et al., 2012). The application of tannery digestate and fly ash mixture (1:4) for the cultivation of *Brassica campestris* improved the yield and oil content (Gupta et al., 2010). Similarly, Patel et al. (2015) observed the same benefits after applying tannery sludge at 1:1 ratio with the soil while cultivating *Ocimum basilicum*. The results proved that tannery sludge is a slow releasing fertiliser.

Vig et al. (2011) reported nutrient enrichment of tannery sludge after vermicomposting with cattle manure while vermicomposting of tannery sludge, wet blue offcuts, sawdust and cattle manure achieved a 300% higher yield compared to using an NPK chemical fertiliser (Nunes et al., 2018). A class 1 type compost (European standard) was produced after co-composting the tannery digestate with sawdust and cow dung (Feyisa et al., 2019). On the hand, products from co-composting of tannery sludge with sawdust, chicken manure, molasses, and rice bran (Haroun et al., 2007); human hair, cattle manure, municipal solid waste and roadside pond sediment (Karak et al., 2017); sugarcane straw and cattle manure (Santos et al., 2011) had permissible metal concentrations for agricultural use and their application enhanced microbial biomass and/or activity in the soil. These low metal concentrations in the compost were attributed to leaching during composting and their potential bioavailability was less than 2% (Haroun et al., 2009). Wickliff et al. (1984) had earlier reported higher yields of tall fescue, bush bean, and maize in the long term after applying tannery sludge when compared to using a commercial N fertiliser. A ten-year study on the soil application (yearly) of composted tannery sludge reported on the improvement of chemical properties (increase in macro and micro elements) of the soil while the microbial biomass and enzymes activity decreased due to Cr accumulation (Araujo et al., 2020). The authors recommended further studies in reducing or eliminating Cr concentrations from

the tannery sludge or its bioavailability prior to composting. Co-composting with a variety of organic wastes that are readily accessible by a tannery should be also investigated.

Nonetheless, the successful use of tannery sludge as a biofertiliser and/or compost will depend on the metal concentrations particularly Cr, and the country's wastewater sludge, organic waste, organic fertiliser and/or tannery waste management regulations. Some countries classify tannery sludge (digestate) as hazardous material that is only disposable in hazardous landfills (Mpofu, et al., 2020b). Therefore, TWW from vegetable tanning is most ideal for the recovery of compost and/or biofertiliser as it contains negligible or minimal amounts of Cr (Table 2-1).

2.8.2 Water reuse

Tanneries in the developing world are increasingly adopting TWW recycling techniques such as single process recycling, floor washing and/or permitted irrigation. The use of membrane technologies particularly membrane bioreactors can treat TWW to standards that promote reuse (Mannucci & Lubello, 2020). Generally, disposal of TWW onto neighbouring agricultural land is a common practice provided given it meets local discharge criteria. Tannery wastewater contains nutrients that are essential for plant growth. However, excessive application may lead to the accumulation of heavy metals that may alter the physical and chemical characteristics of the soil and may enter the food chain. To mitigate this, Maqbool et al. (2018) concluded that irrigating with 50:50 (v/v) tap water and TWW might be a sustainable alternative for increasing vegetable growth.

Moreover, tanning and pickling wastewater can be treated via electro-oxidation and successfully recycled into the tanning process (Sundarapandiyan et al., 2010). Haque et al. (2019) also demonstrated the successful use of TWW as mixing liquid in the tiling mortar for walls and floors. However, the moulded mortar blocks had a 6–14% lower compressive strength compared to blocks manufactured with potable water. Therefore, further studies are required to investigate the feasibility of using anaerobically treated TWW for irrigation, recycling and/or concrete mixing.

2.9 Summary

This chapter has demonstrated that the wide scale application of anaerobic digestion in tannery wastewater treatment plants of developing countries presents an opportunity for establishing bioremediation centres and economical biorefineries that will recover resources, and promote sustainable economic development. However, each tannery must conduct techno-economic feasibility studies and market research in order to ascertain the most economically feasible recoverable resource/s. There is also a vital need for the upskilling of process controllers, provision of funding or incentivised innovation and progressive policies and regulations that support a full transition of tanneries to a circular bioeconomy.

This review also identified research gaps in (i) studies providing an exclusive characterisation of different TE streams, (ii) studies focusing on AcoD of different TE streams, (iii) studies reporting on the microbiology and process kinetics of bioreactors during the AD/AcoD of TEs, (iv) studies investigating the impact of mixing regime (speed and time) during the AD/AcoD of TEs, (v) studies investigating the application of a biological system for the recovery of value added products besides biogas from TEs, and (vi) studies reporting on the techno-economic feasibility of recovering resources from TEs using a biological method

CHAPTER 3

ANAEROBIC CO-DIGESTION OF TANNERY AND SLAUGHTERHOUSE WASTEWATER FOR SOLIDS REDUCTION AND RESOURCE RECOVERY: EFFECT OF SULFATE CONCENTRATION AND INOCULUM TO SUBSTRATE RATIO

[This chapter investigated the effect of sulfate concentrations and mechanical mixing (speed and time) during the mono AD of a blend of slaughterhouse and ostrich tannery effluent using BMP tests and anaerobic sequential batch reactors for resource recovery].

3.1 Introduction

The leather tanning and products industries play prominent roles in the world's economy, particularly for developing countries. South Africa is a net exporter of hides/skins, and is a renowned producer of exotic ostrich leather. The industry sustainably recycles skins which are by-products of the meat industry and prevents their disposal on to the environment. However, tanneries solve one problem and create another as they produce large amounts of tannery wastewater (TWW) loaded with toxic metal salts, and in/organic substances. Conventionally, a tonne of raw hide/skin yields approximately 200 kg of leather, 500 kg wet sludge, and 50–15000 m^3 of liquid effluent containing residual processing chemicals (Buljan & Král, 2015). Typically, TWW contains high loads of chromium (Cr), sodium (Na), chlorides (Cl), nitrogen (TN), sulfate (SO_4^{2-}), sulfide (S^{2-}/H_2S/HS^-), and suspended solids (SS) (Buljan & I Král, 2011). In addition, the process generates in-plant solid wastes such as untanned raw trimmings, fleshings, tanned waste blue splits, trimmings, and shavings. Most of the solids emanate from wet-blue processing and 80% is generated by the beamhouse processes (Kanagaraj et al., 2006). Tanneries are therefore regarded as one of the most polluting industries particularly in developing countries that dominate the industry and supply >60% of the world's skins/hides (Buljan & Král, 2015).

The adequate management of TWW and tannery solid waste, particularly sludge is onerous and expensive and impacts on the profitability of the tanneries. Sludge management costs account for nearly 40% of the overall budget and 55% of the process and maintenance costs of tannery wastewater treatment plants (TWWTPs) (Akyol et al., 2014), while the costs of energy may be up to 60% of the total costs incurred in TWWTPs (Buljan, 2005). The amalgamation of slaughterhouses and tanneries presents an opportunity to integrate the value chain, promote the processing of fresh skins and co-treatment of TWW with slaughterhouse wastewater (SWW) (Mpofu, 2020b). The authors demonstrated the synergistic effect of co-digesting (AcoD) tannery waste-activated sludge (TWAS) with slaughterhouse sludge (SHS). There is a lack of studies focusing on the AcoD of TWW with slaughterhouse wastewater (SWW) while evaluating process kinetics and recovering valuable resources. This is innovative approach will integrate the value chain and promote cleaner production

through the processing of fresh or chilled skin/hides. This will eliminate sodium chloride (NaCl) preservation, the soaking stage, and NaCl availability in TWW.

However, the successful application of anaerobic digestion (AD) in treating TWW and SWW is hindered by microbial inhibitors, notably inorganic sulfur (S) species, ammonia (NH_3), volatile organic acids (VOA), and heavy metals. The presence of SO_4^{2-} favours the growth of sulfate-reducing bacteria (SRB) which compete with methanogens for available carbon sources (acetate (CH_3COOH), and carbon dioxide/hydrogen (CO_2/H_2). The availability of microbial inhibitors in TWW had prompted investigations on AcoD and pre-treatment, particularly coagulation in order to improve process efficiency. Successes in pre-treatment studies have led to the realization that these 'toxicants' can potentially be recovered as value-added products. In light of the global adoption of circular bioeconomy principles, TWWTPs are increasingly being regarded as potential biorefineries. A review by Mpofu et al. (2021a) reported on the feasibility of using AD to recover sulfur species (H_2S and S^0), H_2, and VOA rich biogas, biofertilizer/compost, metals, activated carbon, and/or reusable water. Therefore, the aim of this chapter was to determine the AD process efficiency and kinetics during the AcoD of ostrich TWW and SWW at different SO_4^{2-} concentrations using an acclimated inoculum. The study also seeks to ascertain whether the adoption of AD can: (i) improve the quality of treated TWW to a standard that promotes reuse (ii) and/or recover elemental sulfur (S^0), (iii) and/or recover renewable energy as biogas, and/or (iv) reduce the volume of sludge from TWWTPs, thereby improving the overall environmental and economic performance of tanneries.

3.2   Materials and Methods

3.2.1   Sampling

Samples used in this study were collected from an ostrich tannery that is integrated with a slaughterhouse (IOT). The tannery processes mainly fresh ostrich skins via wet-blue tanning. The onsite wastewater treatment plant treats a blend of TWW from the tannery and SWW from slaughterhouse. The blended SOTE is treated using the activated sludge process (ASP). Six 50 L composite SOTE samples were obtained from the balancing tank every 2 weeks over the course of 5 months (March to September 2018), to allow for fluctuations in effluent quality.

3.2.2 Analytical Methods

The loss on ignition standard methods were used to determine the concentration of total solids (TS) at 105 °C in an oven and total volatile solids (VS) in furnace at 550 °C, respectively (American Public Health Association et al., 2017). Soluble fractions of the reactors were sampled before mixing and were analyzed to determine process efficiency. A Merck Spectroquant Pharo® Spectrophotometer (Darmstadt, Germany) together with Merck cell tests or kits were used to determine the concentration of substances (Table 3-1): chemical oxygen demand (COD) (cat no: 14555), 5-day biological oxygen demand (BOD$_5$) (cat no: 00687), total organic carbon (TOC) (cat no: 14879), total volatile organic acids (VOA$_t$) as acetic acid equivalents (AAE) (cat no: 01763), total sulfate (SO$_4{}^{2-}$) (cat no: 118389), total (S^{2-}) as (HS$^-$) (cat no: 14779), total nitrogen (TN) (cat no: 14537), nitrate (NO$_3{}^-$) (cat no: 114776), nitrite (NO$_2{}^-$) (cat no: 114776), total phosphate (TP) as phosphorous (PO$_4{}^{2-}-$P) (cat no: 14729), total ammonia nitrogen (TAN) (NH$_3-$N) (cat no: 00683), and total alkalinity (Alk) as calcium carbonate (CaCO$_3$) (cat no: 101758), following the prescribed procedures.

The concentrations of metals (Al, Cd, Cr, Co, Cu, Fe, Ni, Pb, and Zn) and other cations (Ca, Cl, K, Mg, and Na) were acidified with nitric acid (HNO$_3$) and were quantified by inductively coupled plasma (ICP) atomic emission spectroscopy (AES) using a Thermo ICap 6200 ICP-AES instrument, while ultra-trace analyses were performed by ICP-mass spectrometry (MS) using an Agilent (Santa Clara, USA) 7900 ICP-MS instrument.

3.2.3 Biomethane Potential Experiments

The biomethane potential (BMP) experimental protocol described by Holliger et al. (2016) was followed in this study. The constant inoculum amount added in all the reactors was 176 mL while the blended SOTE varied from 850 mL to 2120 mL. The reactors were 2.5 L (total volume) screw-capped borosilicate bottles with modified lids fitted with o-rings containing stainless steel inserts with gas-tight ports and tubing to allow sampling and biogas collection. All reactors were topped up to 2.3 L using deionized water. There was a high TVS ratio between inoculum and SOTE (~25:1), and the volume occupied by the inoculum was relatively low, even at high inoculum to substrate ratios (ISRs). An acclimated inoculum was prepared by feeding SOTE to digestate obtained from mesophilic batch

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reactors treating TWAS. The inoculum was kept at 37 °C and was fed with SOTE until biogas production and quality stabilized (Mpofu, 2018).

The effect of 2 numeric factors were investigated: SO_4^{2-} (665–2000 mg/L) and ISR (2–5), which were assessed using 2 responses: anaerobic biodegradability B_o (%COD, %TS, %TVS, and %TOC reduction)], and maximum CH_4 yield (mLCH_4/gVS), using response surface methodology (RSM). The RSM was based on a full factorial central composite experimental design (CCD) with 13 runs, 5 replicates (R4, R8-R11) and 5 levels for each factor. The experimental design matrix (Table 3-2) was generated using Design-Expert® Software Version 11 (Stat-Ease, Inc., Minneapolis, MN, USA). Two sets of each reactor and negative controls (inoculum and substrates only) reactors were set up. However, no positive controls were setup. Magnesium sulfate (MgSO_4) and potassium sulfate (K_2SO_4) were added to the SOTE of selected reactors to mimic the upper range of SO_4^{2-} expected in the effluent stream. Reactors were manually mixed once a day for two minutes.

3.2.4 Set-up and operation of anaerobic sequential batch bioreactors

Two 20 L gas-tight polyethylene anaerobic sequential batch bioreactors (AnSBRs) were set up (Figure 3-1B) and operated at an inoculum to substrate ratio (ISR) determined as optimum from the BMP experiments (B), 37±2°C, and pH 7±0.5 (Mpofu et al., 2022). The AnSBR contents were mixed using a Heidolph Instruments (Schwabach, Germany) Hei-torque 100 programmable overhead stirrer connected to a shaft and pitched four blade marine impeller (Figure 3-1B).

3.2.5 Biogas Sampling and Analysis

The gas ports from the bioreactors were connected to individual gas sampling bags. When sufficient biogas was produced (>200 mL), samples were analyzed qualitatively and quantitatively. The CH_4, CO_2, CO and oxygen (O_2) content (%vol), as well as the H_2S content (parts per million (ppm) of the gas were determined using a Geotech biogas 5000 analyzer (Warwickshire, England) according to the manufacturers' instructions. Biogas volume was determined using a gas syringe.

Figure 3-1: Set up of bioreactors for biochemical methane potential tests (A) and anaerobic sequential batch reactors, and related control systems (B))

3.3 Results and Discussion

3.3.1 Characteristics of Ostrich Tannery Effluent

It has been shown that there are significant differences in the TWW generated from the processing of either ostrich skins or bovine hides (Mpofu et al., 2021a). One contributing factor is the differences in the tanning and TWW treatment processes. In this study, the variations in the slaughterhouse and tannery operations was the main contributing factor.

3.3.1.1 *Chemical and Biological Oxygen Demand and Solids Concentrations in the Ostrich Tannery Effluent*

As expected, there was a batch-to-batch variation in the parameters that were measured in the SOTE (Table 3-1). The TS, TVS, and COD significantly varied (ANOVA, $p < 0.05$) and batch 4 (June-winter) was higher. Apart from routine differences in the daily industrial processes, it was hypothesized that (i) samples containing different proportions of SWW and/or (ii) ostrich skins containing a higher amount of fat during colder months may have played contributory roles (Mpofu, 2018). Indeed, the TS, TVS, and COD concentrations exhibited an inverse relationship with the monthly average temperature profile of the area. The BOD:COD ratio (=0.10) in batch 4 was the lowest of all the batches, but the BOD itself was within the range of the other batches. These results suggest that, if organic solids, notably fats, were responsible for the high TS, TVS, and COD concentrations in batch 4, then these were mainly recalcitrant in nature.

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Table 3-1: Characteristics of different batches of ostrich tannery effluent

Parameter	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Mean	SD
TOC (mg/L)	2467	3380	4530	9080	485	820	3460	3148
COD (mg/L)	7945	8143	7903	15690	4387	7235	8551	3768
BOD (mg/L)	3532	1472	1542	1515	1531	1552	1857	821
VOA_t (mg/L AAE)	3070	2800	2440	2480	2120	1800	2452	456
TN (mg/L)	440	235	180	260	220	530	311	140
TAN (mg/L NH_3-N)	18.8	13.5	16.2	41.0	13.2	9.60	18.7	11.3
NO_3 (mg/L)	143.8	39.0	28.6	18.7	11.5	54.7	49.4	48.7
TP (mg/L PO_4^{2-}-P)	6.65	5.10	5.05	17.8	5.00	4.90	7.41	5.11
SO_4^{2-} (mg/L)	1114	626	352	424	173	1186	646	417
HS^- (mg/L)	ND	2.38	5.70	2.20	0.00	0.12	2.08	2.31
Cl (mg/L)	2038	1547	1294	1022	911	2369	1530	576
TS (g/L)	7.85	8.07	8.38	19.4	5.53	4.69	8.98	5.30
TVS (g/L)	3.61	4.06	4.97	14.6	2.82	2.32	5.40	4.61
K (mg/L)	11.7	19.9	13.2	12.3	10.7	6.6	12.4	4.3
Na (mg/L)	1477	1315	1953	2789	964	754	1542	740
Fe (µg/L)	3272	3081	606	497	193	282	1322	1446
Ca (mg/L)	11.7	24.0	24.2	6.9	17.6	16.8	16.9	6.8
Mg (mg/L)	19.4	15.5	39.5	55.3	14.9	13.2	26.3	17.2
Zn (µg/L)	1568	674	439	401	229	198	585	511
Cu (µg/L)	304	136	16.6	65.9	12.7	12.1	91.2	115
Co (µg/L)	7.7	4.7	1.6	1.9	44.2	1.9	10.3	16.8
Cd (µg/L)	2.27	1.08	0.18	0.27	0.18	0.18	0.69	0.85
Ni (µg/L)	73.1	18.4	18.7	21.3	5.6	8.4	24.2	24.7
Cr (µg/L)	766	57	1094	350	584	136	498	395
Pb (µg/L)	8.4	2.3	4.8	6.8	5.2	4.1	5.3	2.1
Al (µg/L)	1798	2366	583	624	85	101	926	941
Alk (g/L $CaCO_3$)	245	236	330	264	297	308	280	37.0
EC (mS/cm)	8.22	8.27	8.81	11.87	4.04	3.61	7.47	3.13
pH	6.49	6.73	7.33	7.09	6.92	6.93	ND	ND
TVS:TS	0.46	0.50	0.59	0.76	0.51	0.49	ND	ND
BOD:COD	0.44	0.18	0.20	0.10	0.35	0.21	ND	ND
C:N	5.61	14.38	25.17	34.92	2.20	1.55	ND	ND
VFA:Alk	12.5	11.9	7.39	9.39	7.14	5.84	ND	ND
COD: SO_4^{2-}	7.13	13.0	22.4	37.0	25.3	6.10	ND	ND
COD:TVS	2.20	2.00	1.59	1.07	1.56	3.12	ND	ND

ND = no data given   SD = standard deviation.

The gCOD:gTVS ratios of batches 1–6 were 2.20, 2.00, 1.59, 1.07, 1.56, and 3.12, respectively. These results suggested that batches 1, 2, and 6 were more lipid-like in character (gCOD:gTVS = 2.9) due to the low TVS content, whilst the other batches were more protein-like (gCOD:gTVS = 1.42) (Angelidaki & Sanders, 2004). It was postulated that the insignificant differences (ANOVA, $p > 0.05$) in TVS:TS ratios and gCOD:gTVS were mainly due to the elucidated factors that caused differences in the TVS content of the SOTE. Assuming no inhibition, CH_4 yields of approximately 1000 mL/gTVS for lipid-like batches and 415–496 mL/gTVS for protein-like batches with 50%–71% CH_4 are expected (Angelidaki & Sanders, 2004). Despite the lack of TVS:TS ratios being reported in literature, it was envisaged that

batch 4 samples would be the most suitable for AD as TVS:TS ratios >0.8 are required for efficient reactor performance (Zhang et al., 2007).

Concentration of Nitrogen, Carbon and Volatile Organic Acids in the Ostrich Tannery Effluent

Total nitrogen concentrations (TN) in TWW are widely reported in literature, and are typically high (Mpofu et al., 2021a). The TN determined in this study for SOTE (Table 3-1) were in keeping with literature values. In contrast to TN, there is a lack of studies that report TOC and C:N ratios of TWW. Except for batch 5 (485 mg/L, end July 2018), the TOC (820–9080 mg/L, Table 3-1) in this study were higher than 510 mg/L reported by Bhattacharya et al. (2013). Although, the optimal C:N range for AD is 20–30 (Sri Bala Kameswari et al., 2014), the optimal range for TWW has been reported as 6–9 (Berhe & Leta, 2018). Anaerobic reactors operating at lower than optimal C:N ratios are likely to suffer NH_3 and VOA inhibition during AD. In this study, although the TN of batch 3 and 4 (May–June) was low, the TOC concentrations were high compared to other batches. Batch 3 exhibited an optimal C:N ratio (25.2 ± 0.9) for AD whilst batch 4 was above optimal (34.9 ± 1.6) and the rest were below optimal (1.55– 14.4).

Animal skins have a layer of fat that can increase in colder months and result in increased lipid concentrations in TWW. Fat floats were observed in the SOTE, particularly in batch 4. Generally, lipids have high CH_4 yields and B_o, and require long retention times due to low degradation rates (Appels et al., 2011). High lipid concentrations (long chain fatty acids) in SOTE may theoretically inhibit AD (Appels et al., 2011). In this study, the measured VOA_t concentrations (1.80–3.07 g/L) were mostly below the inhibitory thresholds (VOA_t = 5.80–6.90 g/L) (Buyukkamaci & Filibeli, 2004). The VOA:ALK ratios of SOTE (0.53–0.62) were >0.4, indicated the possibility of AD process instability and failure (Gao et al., 2015). However, the speciation of $NH_3 - NH_4$ during the AD of nitrogenous wastes serves as a buffer and plays a vital role in maintaining a relatively constant pH (Berhe & Leta, 2018; Mpofu et al., 2020a).

Inorganic Characteristics of Ostrich Tannery Effluents

The TN and NO_3^- were not significantly different ($p > 0.05$) in batches 2–5, and highest in batches 1 and 6. The NH_3 concentration in batch 4 was notably higher than in the other batches. The batch-to-

batch variation in N species was assumed to be due to process variations, environmental factors, effluent constituents and microbial activity (hydrolysis). The NH_3 concentrations were all below the inhibiting range of 53–1450 mg/L for AD (Chen et al., 2008). As expected in SOTE, high SO_4^{2-}, Na, Cl, Cr, Fe, and Ca concentrations were found. The concentration trends of Na, Cl, Fe, Cr, TS, and COD were similar (r = 0.64–0.86) from batch to batch.

Significant differences (ANOVA, $p < 0.05$) in SO_4^{2-} and HS$^-$ were assumed to emanate mainly from the differences in tanning operations rather than differences in the TWWTP performance and environmental factors. The high COD: SO_4^{2-} ratios (13–37) of batch 2–5 were above the reported ranges for favoring methanogenesis over sulfidogenesis (Guerrero et al., 2013). However, batch 1 and 6 were within the 1–7 range and capable of supporting either methanogenesis or sulfidogenesis. The macronutrient (C:N:P:S) ratios were in the range 2.1–64:1.1–3.8:0.01–0.3:1 (data not shown) and were not equivalent to the optimal ratio of 500–600:15:5:1 for AD (Deublein & Steinhauser, 2008). Therefore, acclimatization of the inoculum was most vital in ensuring effective AD.

3.3.1.4 *Metal Characteristics of Ostrich Tannery Wastewater*

The concentration of most metals (Na-Mg; Zn-Cu-Ni-Cd-Fe-Al; Ni-Pb; and Cr-Al) in the SOTE samples displayed a similar trend (r = 0.73–0.99), with the first 2 batches having the highest concentrations. The IC_{50} values for methanogens and acetogens have been reported as 11 g/L Na, 28 g/L K, 4.8 g/L Ca, 4–8 mg/L Cd, 100–400 mg/L Ni, 17–58 mg/L Zn, 67 mg/L Pb, 8.3–3000 mg/L Cr, and 0.7–5.65 g/L Fe (Lin, 1992; Zayed & Winter, 2000). The metal concentrations in the SOTE were below the reported IC_{50}. Some metals, such as Ni, Zn, Co, Cu, and Ca are also necessary as metabolic co-factors, and in this study, they were either within or below the optimal range for AD. However, inhibiting and optimal metal concentrations strongly depend on their availability as free ions, their physico-chemical properties, operating conditions, microbial species and their adaptation (Thanh et al., 2016). The presence of metal mixtures in TWW may exhibit antagonistic and synergistic effects to inhibition (Feijoo et al., 1995).

3.4 Biochemical Methane Potential Experiments

Methanogenesis is generally considered to be the slowest, most sensitive, and often rate-limiting reaction when processing tannery effluents as they are laden with soluble and/or unionised toxicants such as NH_3/NH_4^+, SO_4^{2-}, H_2S/HS^-, VOA, and metals (Mpofu et al., 2021a).

3.4.1 Cumulative Methane Generation

In this study, negligible biogas that could not be quantified was generated in the inoculum and substrate controls. In reactors with ISR ≥ 3, and SO_4^{2-} ≤ 710 mg/L lag phases between 5 and 23 days were experienced before CH_4 generation commenced (Figure 3-2), reflecting complete, but transient inhibition. In addition, in four of five replicates with ISR = 3, close to 50 days were required for CH_4 generation to reach completion. The SO_4^{2-} concentrations in these reactors was in the upper range expected in the SOTE from the tannery concerned (SO_4^{2-} = 352–1186 mg/L, n = 6, Table 3-1). The average cumulative CH_4 yield in these reactors ranged from 98 to 146 mL/gVS, higher than the CH_4 yield reported by Saxena et al. (Saxena et al., 2019), but lower than that reported by Achouri et al. (Achouri et al., 2017) for AD of TWW without pre-treatment (7.6 mL/gVS and 753 mL/gVS, respectively, after 35 and 37 days, respectively). Studies by Mpofu et al. (2020a; 2020b) also reported long lag phases of >60 days during mono-digestion of ostrich TWAS, and 20 days during co-digestion (AcoD) (50%/50% *v/v*) with ostrich SHS that led to retention times of 108 and 50 days, respectively.

In the reactors with SO_4^{2-} ≥ 1960 mg/L (i.e., above the range expected in the SOTE from the study site), methanogenesis was severely inhibited at all ISRs (Figure 3-2B). However, at ISR ≥ 3 and SO_4^{2-} ≤ 710 mg/L (mid concentration range from study site), no lag phase was experienced, and more than 92% of the cumulative CH_4 generation of 130–139 mL was obtained within 10 days of operation (Figure 3-2C). These results are very promising and strongly suggest that by optimizing the sludge recycle ratio and/or SRT, efficient AD can be achieved, provided the SO_4^{2-} concentration is kept below a particular (high) threshold. Further experiments need to be conducted to optimize these, and other factors (such as mixing), in continuous or semi-continuous systems.
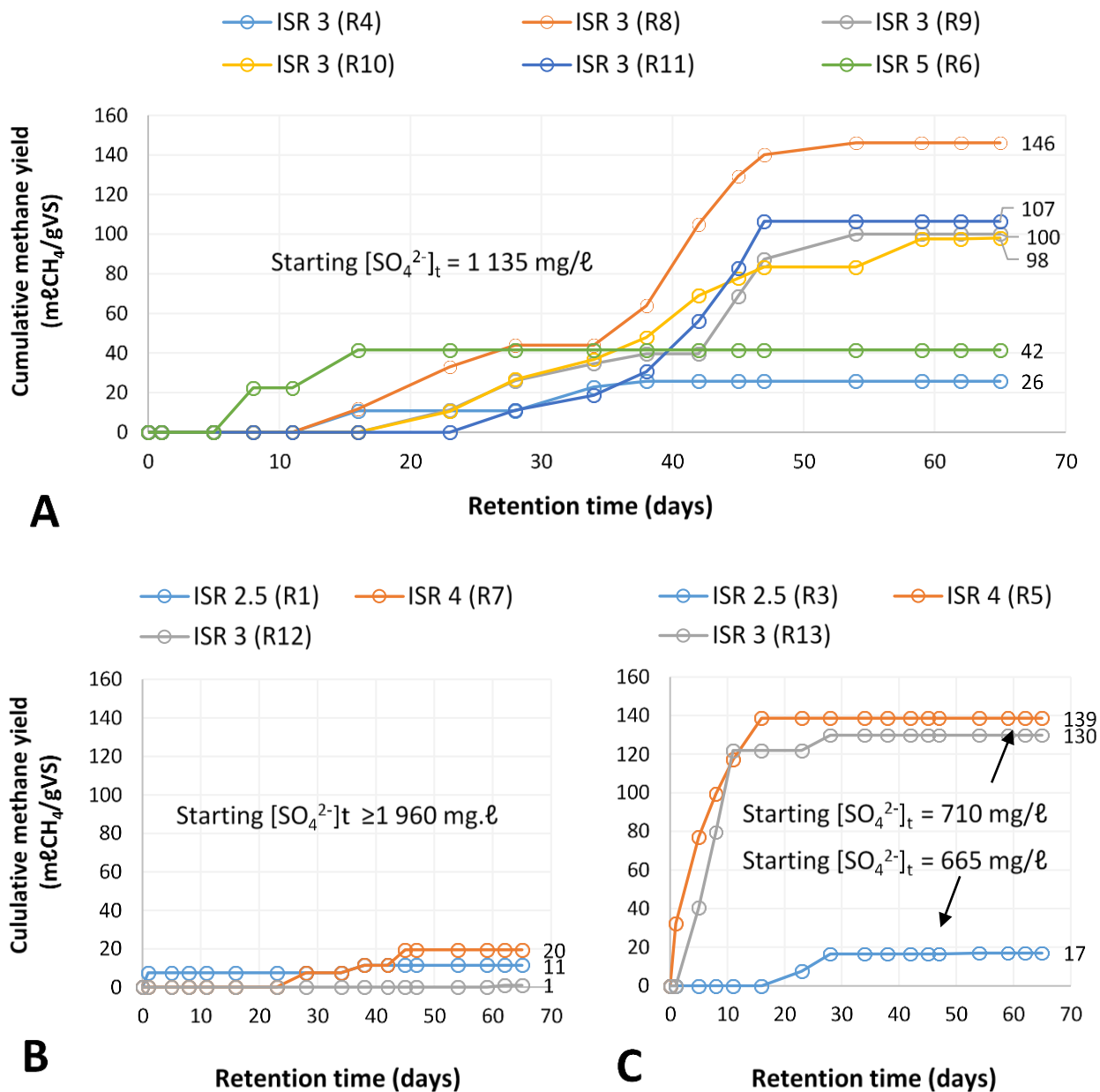
Figure 3-2: Cumulative methane yields of reactors operating at different sulfate concentrations and inoculum to substrate ratios, A:sulfate concentrations = 1135mg/L, B: sulfate concentration ≥ 1960 mg/L, C: sulfate concentrations = 665 and 710 mg/L.

3.4.2 Hydrolysis and pH Changes

The lack of CH_4 generation in some of the reactors in the first 15 days reflected poor or absent methanogenic activity, but not necessarily a lack of other metabolic processes. Analysis of selected physicochemical parameters of the reactor contents established that hydrolysis, acidogenesis and acetogenesis took place.

It was assumed that the primary mechanism for NH_3 release (76%–89% increase after 20 days), was protein hydrolysis. The overall increase in VOA in some reactors, and 38–80% reduction in FOG clearly indicated that hydrolysis of lipids also occurred. The NH_3 concentration in samples taken at day 0 and at day 20 in all reactors fell above the minimum inhibitory concentration (MIC) reported in literature for AD (53 mg/L; (Rajagopal et al., 2013)), but fell well below this MIC at the end of the study (day 62). Temporal NH_3 increases (25%–147%) between day 0 and day 20 were observed in reactors operating at higher ISRs (>2.5) and/or lower SO_4^{2-} ≤ 1335 mg/L, while decreases (5%–51%) were observed for reactors operating at lower ISRs ≤ 2.5 and/or higher SO_4^{2-} ≥ 1335 mg/L. The pH in the reactors remained largely within the optimal range for methanogens over the first 20 days. However, values measured in all reactors at the end of the study were slightly higher than the optimal range (6.5 – 8.0) (Amani et al., 2010). Buffering capacity provided by high NH_3/NH_4^+ concentration probably compensated to some extent for the initial high VFA:ALK of the reactor contents.

In terms of VFA:ALK, initial ratios (>0.4) suggested that potentially unstable operational conditions for methanogenesis existed during start-up, which stabilized after 20 days (<0.3–0.4) in all reactors with the exception of R3 and R12 which generated minimal CH_4. There was a notable increase in alkalinity, suggesting microbial utilisation of H^+ in the reactors, for example by oxidising homoacetogens, chemolithotrophic sulfur oxidising bacteria (SOB), and/or hydrogenotrophic methanogens (HMs).

3.4.3   Acidogenesis, Acetogenesis and Changes in Volatile Organic Acid Concentration

The initial and final VOA concentrations in R1 and R12 increased by 16% and 17%, respectively. In contrast, decreases ranging from 7% to 60% were noted in other reactors. Together with changes in the VOA, SO_4^{2-} was reduced to H_2S, suggesting that both acidogenesis and acetogenesis occurred during the lag phase. The accumulation of VOA in the two reactors (R1 and R12) operating at high SO_4^{2-} ≥ 1960 mg/L and ISR ≤ 3 suggested the involvement of SRB in the breakdown of complex substrates.

The VOA concentration at any point in time depends on the balance between the breakdown of the products of hydrolysis into VOA by acidogens, and utilization of the VOA by acetogens and/or aceticlastic methanogens. Increased VOA concentrations could therefore be attributed to inhibition

of the latter two metabolic groups. In contrast, decreased VOA concentrations could be attributed to either inhibition of acidogens (decreased formation) and/or efficient acetogenic/methanogenic activity (utilization). High H^+ partial pressures greater than 10^{-4} atmospheres are also known to inhibit propionate (HPr), butyrate (HBu), and ethanol degrading acetogens (Gerardi, 2003).

Lipid inhibition may have occurred in R1, R2 and R12, which may have led to decreased acidogenesis and/or acetogenesis and subsequent accumulation of VOA.

3.4.4 Sulfidogenesis, Sulfite Oxidation and Methanogenesis

Notable reductions in SO_4^{2-} occurred in all the reactors over the study period, with concomitant increases in H_2S over the first 20 days of operation. It was assumed that sulfidogenesis was largely responsible for the simultaneous increase in H_2S and reduction in SO_4^{2-}, and that SRB dominated not only HS^- generation, but also contributed to organic substrate utilization. Furthermore, at pH > 8, H_2S is solubilized to HS^-, such that as the pH increased in the reactors, some of the HS^- may have precipitated with metals. This can reduce direct HS^- toxicity on functional microbial species, but it can also reduce the bioavailability of essential methanogenic micronutrients. The HS^- concentrations measured at day 20 fell within the inhibitory range (IC$_{50}$ = 43–125 mg/L at pH 7–8) for methanogenesis (O'Flaherty et al., 1998).

Nonetheless, it was apparent that about 43%–96% of the formed S^{2-} from sulfidogenesis was oxidized into elemental sulfur (S^0) as a white layer was formed at the interface of the bulk liquid and head-space in the reactors. This phenomenon has been described by Sabumon, (2008a; 2008b), whom observed the formation of S^0 as the main intermediary product of HS^- and H_2S oxidation during treatment of TWW. Moraes et al. (2012) reported that SO_4^{2-} may be re-formed by oxidation of thiosulfate ($S_2O_4^{2-}$) and elemental S^0. This may have been the case with R1 and R2 where a 91% reduction in SO_4^{2-} by day 20 was followed by 99% increase by day 62. Chemolithotrophic SOB can simultaneously reduce oxidized N compounds (NO_2^- and NO_3^-) and oxidize S^{2-} under anoxic conditions (Equation 3-1 to Equation 3-4). Other inorganic reduced S compounds such as $S_2O_4^{2-}$ and S^0 can also be used as electron donors by SOB. In this study, the notable decrease in the NH_3 between day 20 and day 62 in the reactors supports denitrification having occurred, which would have made

NO_2^- and NO_3^- available as electron donors for SOB. Furthermore, consumption of H^+ by SOB could explain the anomalous increase in alkalinity in all reactors, except R5 and R8. However, due to the complexity of the physicochemical and biological processes, detailed mass balances for S were not determined.

$$5HS^- + NO_3^- + 3H^+ \rightarrow 5SO_4^{2-} + 4N_2 + 4H_2O \quad \Delta G - 3848kJ/mole \qquad \textbf{Equation 3-1}$$
$$3HS^- + 8NO_2^- + 5H^+ \rightarrow 3SO_4^{2-} + 4N_2 + 4H_2O \quad \Delta G - 2944kJ/mole \qquad \textbf{Equation 3-2}$$
$$5HS^- + 2NO_3^- + 7H^+ \rightarrow 5S_0 + N_2 + 6H_2O \quad \Delta G - 253kJ/mole \qquad \textbf{Equation 3-3}$$
$$3HS^- + 2NO_2^- + 5H^+ \rightarrow 3S_0 + N_2 + 4H_2O \quad \Delta G - 306kJ/mole \qquad \textbf{Equation 3-4}$$
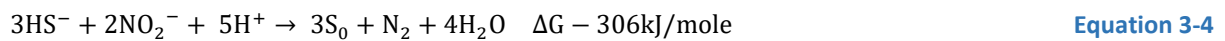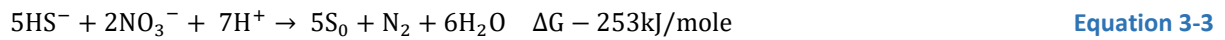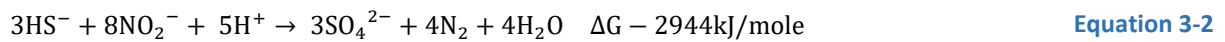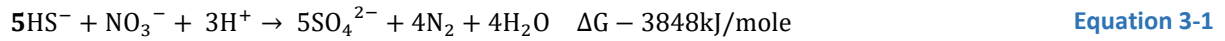
The COD: SO_4^{2-} ratios were consistently <10, suggesting that sulfidogenesis would be favored over methanogenesis. It was therefore hypothesized that (i) methanogenesis was favored in reactors R5 and R13 where no lag phase for CH_4 generation was experienced (ISR ≥ 3 and SO_4^{2-} ≤ 710 mg/L), (ii) sulfidogenesis initially dominated, followed by methanogenesis in the reactors that generated CH_4 after lag phases, and (iii) sulfidogenesis was favored, and methanogenesis was severely and continually inhibited in reactors with high SO_4^{2-} of ≥ 1960 mg/L (R1, R7, R12). This was supported by qualitative analysis of the biogas from R1 (230 mL biogas: 78 ppm H_2S, 0.1% O_2, 44.8% CH_4). It was hypothesised that the physicochemical and biological milieu in R1 and R12 inhibited aceticlastic methanogens (AMs) and/or acetogens, leading to an accumulation of VOA between day 20 and day 62, which exacerbated methanogenic inhibition.

The results strongly suggested that SRB played a key role in the syntrophic degradation of hydrolysis metabolites and VOAs in most of the reactors. This could in turn have led to the dominance of HMs after the lag phase, which was suggested by the $CH_4 : CO_2$ (>1) for the reversible inhibited reactors (Ariunbaatar et al., 2015). It was conclusively established that SO_4^{2-} ≥ 1960 mg/L caused almost complete methanogenic inhibition, while no inhibition occurred when reactors were operated at SO_4^{2-} ≤ 710 mg/L and ISR ≥ 3. Furthermore, both reactors that were operated at the lowest ISR (=2.5) were almost completely inhibited, suggesting that the initial methanogenic population density was too low to become established in that particular environment.

3.4.5 Metal Inhibition or Stimulation and Macronutrient Limitation

Depending on the speciation and concentration, bioavailable metals and other ions may either promote or inhibit methanogenesis. In this study, the concentrations of all the essential metals decreased, except for Ni in R1, R5, R7, R11, R12, and R13. More holistically, the concentrations of most soluble metals decreased in the reactors, except for R7 and/or R12. In contrast Ca increased concurrently with a decrease in VOA, NH_3, and H_2S except in R2, R7, and R12 that operated at high SO_4^{2-} concentrations and/or lower ISR. Interestingly, the same reactors exhibited a low CH_4 yield and experienced longer lag phases. This supports the observed flocs in the different reactors which may have formed in an effort to adapt and prevent metal toxicity or deficiency through excretion of extracellular polymeric substances and soluble microbial products (Thanh et al., 2016).

It is plausible that inhibition was initially caused by SO_4^{2-}, followed by NH_3, H_2S, and/or nutrient limitation. The C:N range was below optimal in all reactors at the beginning and end of the study. The initial C:N:P ratios ranged from 280:37:1 to 111:26:1, indicating both N and P were limiting macronutrients. However, although there was a notable reduction in concentration, bioavailable (soluble) P was still present at day 62. Furthermore, the HS^- that was generated may have reduced the bioavailability of inhibitory and/or stimulatory micronutrients via precipitation (Appels et al., 2011). The precipitates may have further reversibly inhibited functional microorganisms by blocking their access to substrates (Utgikar et al., 2002).

3.5 Optimisation of Cumulative Methane Yield and Anaerobic Biodegradability

The experimental gas yields (CH_4 and biogas) and B_0 (% reduction of TOC, TS, VS, COD) (
Table 3-2) were modelled using linear equations and quadratic polynomials with up to second degree interaction terms (Figure 3-3).

Table 3-2: Experimental design matrix showing methane yield and biodegradability results

Reactor	A:SO_4^{2-}	B:ISR	Biogas Yield	Methane Yield	Average CH_4	Biodegradability Indicators (% Reduction)				
						TOC	Sulfate	TS	VS	COD
	(mg/L)		(mL/gVS$_{added}$)	(mL/gVS$_{added}$)	%	(%)	(%)	(%)	(%)	(%)
R1	1960	2.5	129	12.0	9.30	87.6	59.7	11.7	15.2	16.6
R2	1335	2.0	4.97	1.71	34.4	93.1	63.4	11.5	19.2	13.8
R3	710	2.5	83.6	17.0	20.3	76.2	77.1	20.1	28.5	19.2
R4	1335	3.0	79.8	25.7	32.2	81.3	73.6	19.3	26.7	24.4
R5	710	4.0	361	93.3	25.9	82.5	68.0	29.1	40.9	52.0
R6	1335	5.0	180	41.5	23.1	88.3	81.1	26.0	55.0	10.8
R7	1960	4.0	110	19.5	17.7	77.1	80.4	28.3	33.3	14.9
R8	1335	3.0	337	146	43.4	82.7	80.4	26.1	30.5	43.6
R9	1335	3.0	260	100	38.5	69.0	85.3	49.2	51.7	24.6
R10	1335	3.0	265	102	38.5	61.3	79.6	37.0	40.8	43.0
R11	1335	3.0	280	103	36.9	75.3	75.0	35.8	37.7	24.3
R12	2000	3.0	9.13	1.02	11.2	23.2	49.3	19.0	27.5	39.3
R13	665	3.0	290	130	44.8	78.5	77.8	29.0	35.4	25.5

COD = chemical oxygen demand   Conc = concentration   ISR = inoculum to substrate ratio   R = reactor

TOC = total organic carbon   TS = total solids   VS = volatile solids.

The models (Equation 3-5–Equation 3-11) were significant (F test, $p < 0.05$) and there was only 0.12– 3.20% probability that this may have been caused by natural system variation (Table 3-3). The F test showed that ISR and SO_4^{2-} and their interaction ISR^2, (SO_4^{2-})^2 and ISR(SO_4^{2-}) were all significant ($p < 0.05$) model terms for biogas yield, while ISR and its interaction with SO_4^{2-} (ISR(SO_4^{2-})) were the only non-significant terms (F test, $p > 0.05$) on CH_4 yield. The interaction of both factors ISR(SO_4^{2-}) was the only significant factor (F test, $p < 0.05$) on sulfate reduction whilst (SO_4^{2-}) and its interaction (SO_4^{2-})^2 were the only significant factors (F test, $p < 0.05$) affecting COD reduction (Table 3-3). Nonetheless, both factors were significant in achieving the maximization of gas yields and B$_o$. The correlation coefficients (R^2) of the models (Table 3-3) indicated that only 13.3%, 23.2%, 29.1%, and 16.1% of the variability in biogas yield, CH_4 yield, % SO_4^{2-}, and %COD reduction was not explained by the models, respectively. The models' adj. R^2 values were 0.81, 0.61, 0.55, and 0.76, respectively, suggesting moderate to good predictability of the gas yields and B$_o$.

In contrast, the general quadratic polynomial and linear equations did not fit the %TOC, %VS, and %TS reduction data very well (adj R$^2 \leq 0.28$) and returned negative predicted R^2. This indicated that the overall mean better predicted B$_o$ (Stat-Ease, Inc., Minneapolis, MN, USA). This is in agreement with the observation that regardless of inhibition of CH_4 generation, other processes occurred. Therefore,

Equation 3-5–Equation 3-11 were used to simulate and optimize the B_o and cumulative gas yields as plotted in Figure 3-4.

Table 3-3: Summary of the statistical results of the fitted models

Models	Std Dev	Overall F Test p-Value	F Test (LOF) p Value	R²	Adj R²	Adeq Prec	AIC
Biogas-Quadratic	2.41	0.0012	13.8	0.87	0.81	15.4	71.9
CH₄-Quadratic	0.005	0.032	4.87	0.77	0.61	6.4	−83.6
TOC-Mean	ND	ND	ND	ND	ND	ND	112
Sulfate-Quadratic	6.87	0.027	4.72	0.71	0.55	7.70	99.3
TS-Mean	ND	ND	ND	ND	ND	ND	ND
VS-Mean	ND	ND	ND	ND	ND	ND	ND
COD-Quadratic	0.01	0.003	10.7	0.84	0.76	10.9	−68.7

Adeq Prec = adequate precision   Adj = adjusted   AIC = Akaike's information criterion   LOF = lack of fit

ND = no data   Pred = predicted R^2 = coefficient of determination   Std dev = standard deviation

TOC = total organic carbon   TS = total solids   VS = volatile solids.

$$\text{Biogas yield} = 40.4\text{ISR}^2 + 0.07[\text{SO}_4]\text{ISR} - 422\text{ISR} - 0.2[\text{SO}_4] - 834 \qquad \textbf{Equation 3-5}$$

$$\text{CH}_4 \text{ yield} = 0.12 + 1.6 \times 10^{-8}[\text{SO}_4]^2 + 6.0\text{ISR}^2 - 0.05\text{ISR} - 4.2 \times 10^{-5}[\text{SO}_4] \qquad \textbf{Equation 3-6}$$
$$+ 2.6 \times 10^{-6}[\text{SO}_4]\text{ISR}$$

$$\% \text{TOC}_{\text{reduction}} = 75.1 \qquad \textbf{Equation 3-7}$$

$$\% \text{VS}_{\text{reduction}} = 27.5 \qquad \textbf{Equation 3-8}$$

$$\% \text{TS}_{\text{reduction}} = 27.4 \qquad \textbf{Equation 3-9}$$

$$\% \text{COD}_{\text{reduction}} = \frac{1}{0.39 - 7 \times 10^{-5}[\text{SO}_4] - 0.19(\text{ISR})} \qquad \textbf{Equation 3-10}$$

$$\% \text{SO}_{4_{reduction}} = 0.02[SO_4]ISR - 0.011[SO_4] - 19.9ISR + 115 \qquad \textbf{Equation 3-11}$$

The cumulative CH_4 yields and the average $\%CH_4$ varied from 0 to 146 mL/gVS and 9.3% to 44.8%, respectively. The corresponding reduction efficiencies were SO_4^{2-} (49.3%–85.3%), TOC (23.2–93.1%), VS (15.2–55.0%), TS (11.5–49.2%), and COD (10.8–52.0%) (Table 3-2). The CH_4 yields were compared to the controls (inoculum only) and corrected CH_4 yield reported. Based on the interest to maximize CH_4 yield and B_o, the theoretical optimum operating conditions were found to be at SO_4^{2-} = 922 mg/L and ISR = 3.72 with a desirability of 0.65. These optimum conditions are expected to generate 361 mL biogas/gVS, 235 mLCH₄/gVS and reduction efficiencies of 27.5% VS, 27.4% TS, 75.1% TOC, 75.6% SO_4^{2-}, and 41.1% COD. This implies that the mass of sludge will be reduced by about 27% (dry mass) and SO_4^{2-} by 76%, with a fraction of it recovered as S^0.

The CH_4 yields obtained in this study (Table 3-2) were comparable to those obtained by Vazifehkhoran et al. (2018) and higher than those reported by Agustini et al. (2019) and Saxena et al. (2019). However, Achouri et al. (2017) reported higher gas yields and no lag phase, presumably due to the higher dilutions (34% and 53%) using tap water, uncorrected gas yields, addition of micronutrients and use of a blend of tanyard (TYE) and beamhouse (BHE) effluent. Interestingly, Mpofu et al., (2020b) operated 0.5 L batch reactors at SO_4^{2-} = 494–562mg/L, 37 ± 2 °C, pH = 7.0 ± 0.5 and ISR (=4) while codigesting TWAS and SHS (50% v/v). The authors reported biogas yields of 333 – 431 mL/gVS, CH_4 yield of 170–215 mLCH$_4$/gVS, 50–53% CH_4 (average), and Bo of 54.4%–68.5% VS, 45.5%–50.7% TS and 43.2%–48.2% COD. The %COD reduction achieved in this study were lower compared to other studies using AnSBR and other continuous reactors (UASB, UAFFB, UAFBR, SAnMBR, and UACF) (Mpofu et al., 2021a). They were however in the same range with 45%, 56%, 45%, and 43%, accomplished by Achouri et al. (2017), Berhe & Leta, (2018), Daryapurkar et al. (2001), and Saxena et al. (2019) respectively, using batch reactors.
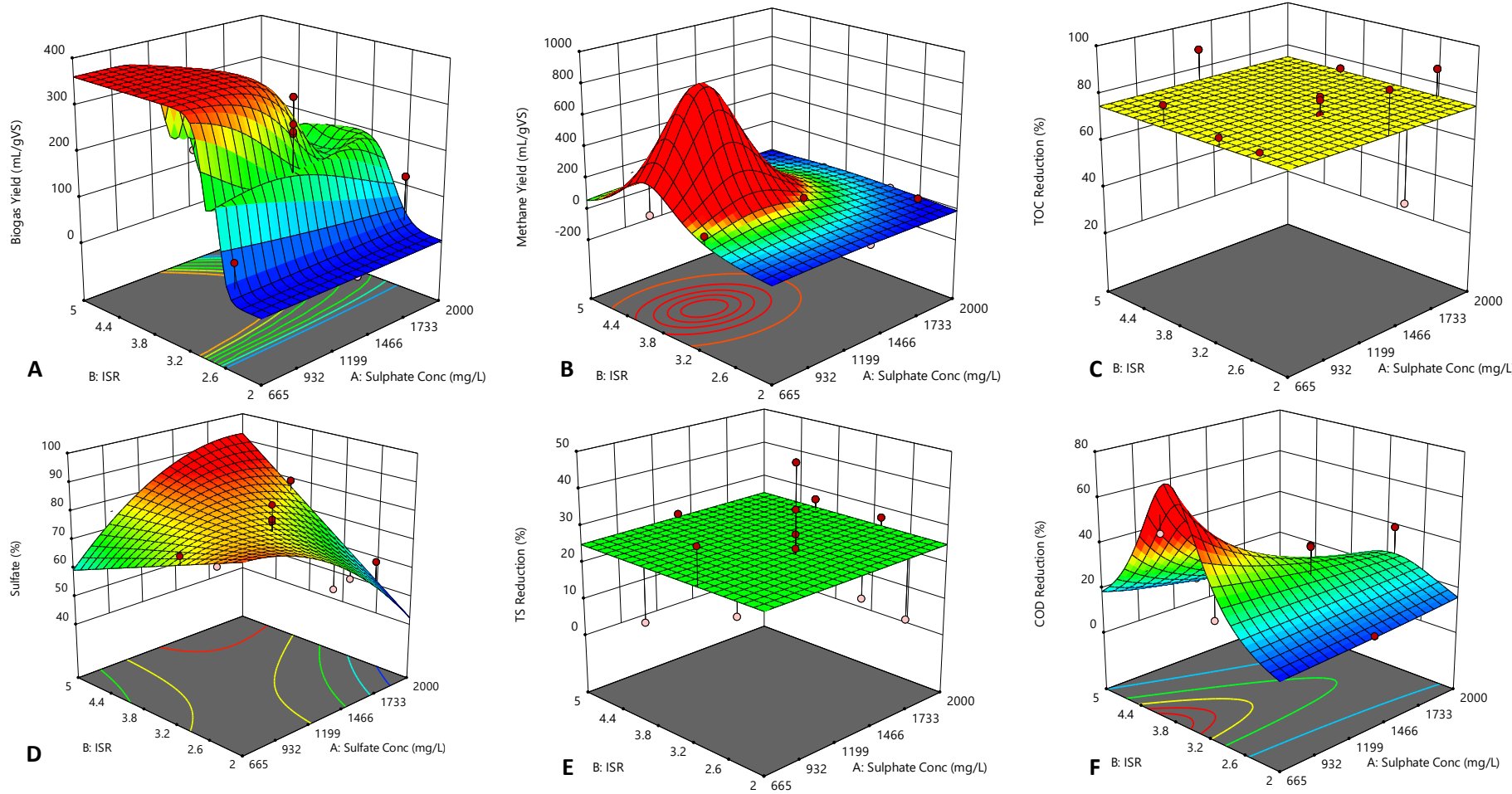
Figure 3-3: Effect of sulfate concentration and inoculum to substrate ration on: (A)—biogas yield; (B)—cumulative methane yield; (C)—total organic carbon reduction; (D)—total solids reduction; (E)—volatile solids reduction; and (F)—COD reduction during anaerobic digestion of ostrich slaughterhouse-tannery effluent

3.5.1 Water reuse

The resulting treated SOTE met the stipulated wastewater limit values applicable for the irrigation of land with up to 50 and 500 m^3/day. However, treated SOTE did not meet the limit values for irrigating with 2000 m^3/day in terms of SS, COD, NH_3, and Cl (Department of Water Affairs, 2013). This can be mitigated by mixing treated SOTE and tap water at an optimised volumetric ratio that meets the irrigation standard. Maqbool et al. (2018) concluded that irrigating with 50:50 (*v/v*) tap water and TWW might be a sustainable alternative for increasing vegetable growth.

3.5.2 Correlative analysis of variables on methane yield and anaerobic biodegradability

There was a weak linear insignificant relationship (r ≤ −0.29, (F test, $p > 0.05$)) between SO_4^{2-} and %solids reduction. Similarly, ISR did not linearly correlate with B_o (TS, COD, and TOC), average %CH_4 and CH_4 yield (−0.16 ≤ r ≤ 0.12, (F test, $p > 0.05$)). There was a strong significant positive linear correlation (r = 0.71, (F test, $p < 0.05$)) between ISR and %VS reduction, while weak insignificant negative linear relationships (−0.29 < r ≤ −0.21, (F test, $p > 0.05$)) existed between SO_4^{2-} with %COD and %TOC reduction and ISR with biogas yield and % SO_4^{2-} reduction (0.31 ≤ r ≤ 0.34, (F test, $p > 0.05$). Mpofu, (2018) also reported a lack of correlation (r < 0.19, (F test, $p > 0.05$)) between ISR and %solids reduction, strong positive correlation (r = 0.84, (F test, $p < 0.05$)) with gas yields and a moderate positive correlation (r = 0.46, (F test, $p > 0.05$)) with %COD reduction. A moderate negative linear relationship (−0.39 < r ≤ −0.53, (F test, $p > 0.05$)) existed between SO_4^{2-} with % SO_4^{2-} reduction, average %CH_4, biogas and CH_4 yield. These results confirm that (i) high SO_4^{2-} promoted sulfidogenesis over methanogenesis, which negatively affected gas yields, (ii) gas yields and SO_4^{2-} removal could be improved by decreasing the influent SO_4^{2-} to around 922 mg/L and increasing the ISR to 3.7, and (iii) the strong significant linear relationship between %SO_4^{2-} reduction with %TS (r = 066, (F test, $p < 0.05$)) and %VS (r = 0.64, (F test, $p < 0.05$)) reduction indicated the importance of SRB in degrading hydrolysis products. This may have created a better environment for the hydrolytic bacteria.

3.6 Kinetic study of cumulative methane production

There is a lack of studies that report on the AD kinetics while treating TWW and worse for SOTE. In order to evaluate the performance and the kinetics of the BMP experiment, the modified Gompertz, logistic, first-order, and cone models were fitted onto the cumulative CH_4 yield data (Table 3-3) using non-linear regression. The models displayed a perfect fit to the cumulative CH_4 yields with high precision in the order Logistic > Cone > modified Gompertz > first-order (Figure 3-4, Table 3-4).

According to the statistical parameters (Table 3-4), the first-order model was the worst predictor (Adj R^2 = 0.437–0.763) for most reactors that experienced a lag phase except for R5 (Adj R^2 = 0.984), R6 (Adj R^2 = 0.883) and R13 (Adj R^2 = 0.939) that experienced shorter lag phases of 3 to 6 days. The exponential–plateau curve displayed by the first-order model accurately fits data without or with short lag phases. The first-order model predicted the lowest kinetic values for maximum microbial specific growth rate (μ_m). In contrast, the rest of the models best fitted (0.827 ≤ Adj R^2 ≤ 0.999) reactors with long lag phases of 14 to 35 days as they display a sigmoidal shape with lag, exponential and stationary phases. However, the modified Gompertz model yielded the highest lag phases (λ) and significantly under predicted the ultimate CH_4 yields (A) by >63%. The cone (Adj R^2 = 0.960–0.999) and logistic (Adj R^2 = 0.956–0.985) models gave better approximations of the kinetic constants.

The range of the kinetic constants: A, μ_m, K and λ obtained in this study were 11.2 – 139 mLCH_4/gVS, 0.171 – 17.5 mLCH_4/gVSd^{-1}, 0.025–0.27 day^{-1} and 0–35 days, respectively. The highest K and μ_m were found in reactors R5 and R13 operating at lower SO_4^{2-} of 710 and 665 mg/L and ISR of 4 and 3, respectively. This confirms that from the range of parameters tested, higher ISR and low SO_4^{2-} provided the most ideal environment for proliferation of methanogens. The CH_4 production μ_m = 0.015 – 17.5 mLCH_4/gVSd^{-1} obtained in this study were comparable to μ_m = 2.04 – 5.48 mLCH_4/gVSd^{-1} reported by Sri Bala Kameswari et al. (2014), and μ_m = 0.08 – 5.49 mLCH_4/gVSd^{-1} reported by Mpofu et al. (2020a) for AD of tannery sludge. Furthermore, they were similar to μ_m = 6.0–18.1 mLCH_4/gVSd^{-1} reported by Mpofu et al. (2020b) while co-digesting TWAS and SHS. The K values in this study were higher than K = 0.0185 – 0.0239 d^{-1} reported by Thangamani et al. (2010; 2009) during the AcoD of tannery sludge and solid wastes and K = 0.008 – 0.14 day^{-1} reported by Mpofu et al. (2020a; 2020b) during the mono and AcoD of TWAS and SHS, respectively. Generally, low K values indicate efficient AD similar to natural systems that operate under slow but steady reaction rates (Li et al., 2018). This study proved the advantage of liquid (wet/low solids) over solid (dry/high solids) AD and the synergistic effect of AcoD of SOTE with SWW. Nonetheless, there is a need for more studies on the kinetics of AD of TWW.
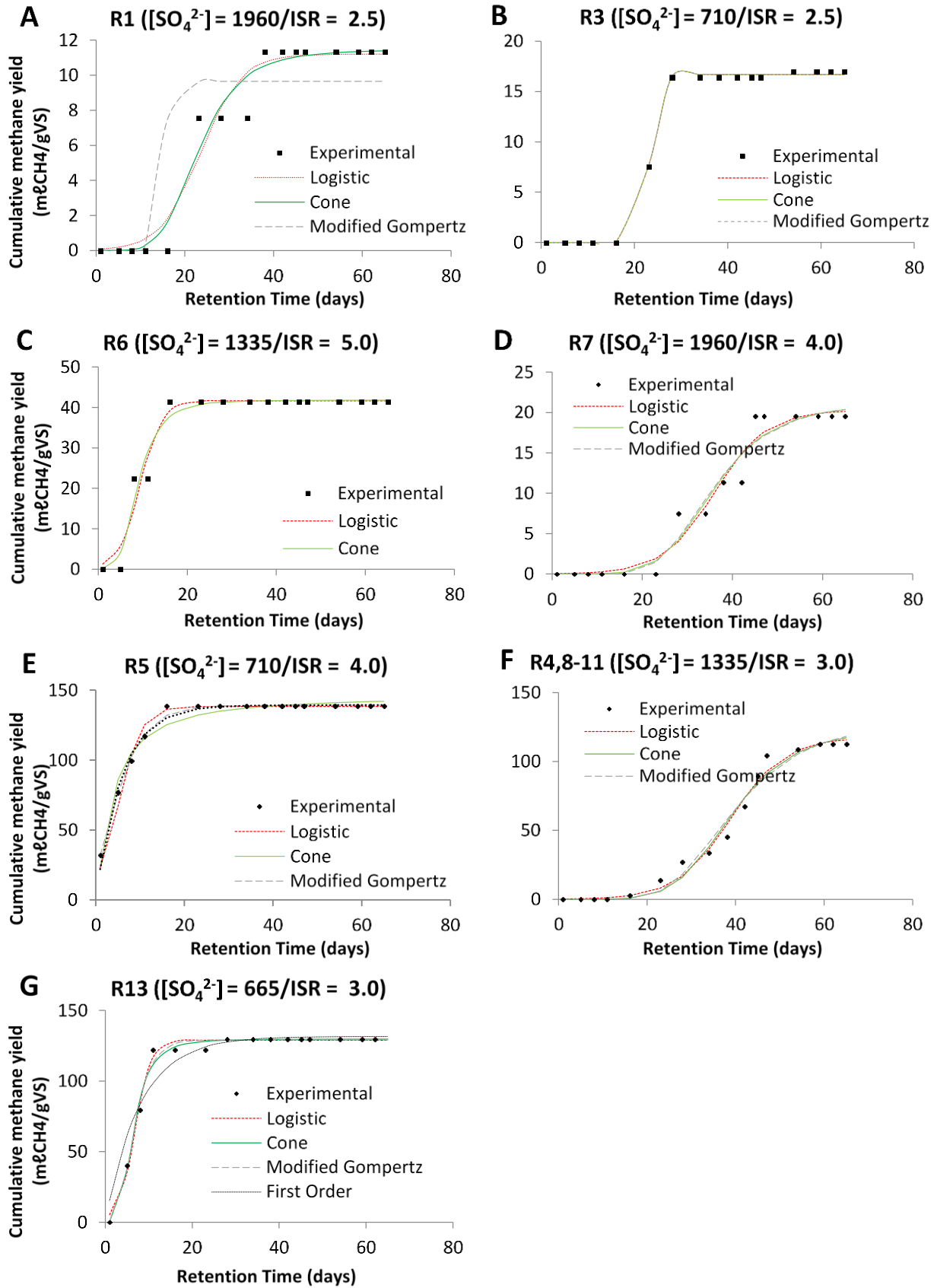
Figure 3-4: Graphs depicting the experimental and model curves for cumulative methane yields for reactors operating at different sulfate concentrations and ISR, respectively. A: 1960 mg/L and 2.5, B: 710 mg/L and 2.5, C: 1335 mg/L and 5, D: 1960 mg/L and 4, E: 710 mg/L and 4, F: 1335 mg/L and 3, G: 665 mg/L and 3 (note the different scales on the y axes).

Table 3-4: Kinetic parameters and goodness of fit of models fitted onto cumulative methane yields of biomethane potential bioreactors

Reactor	Model	Kinetic Parameters					Adj R^2	p Val. Prob > F	AIC	RMSE
(SO_4^{2-}/ISR)		A (mLCH$_4$/gVS)	μ_m (mLCH$_4$/gVSd^{-1})	λ (d)	K	n				
R1 (1960/2.5)	Cone	11.5	ND	ND	0.043	4.92	0.965	0.44	90.5	0.46
	Logistic	11.2	0.61	14.4	ND	ND	0.956	0.41	105	0.51
	First-order	13.0	0.03	ND	ND	ND	0.827	0.08	195	1.02
	Gompertz	3.55	0.69	13.5	ND	ND	0.676	0.23	235	1.38
R3 (710/2.5)	Logistic	16.7	3.63	20.9	ND	ND	0.999	0.50	99.0	0.11
	Cone	16.7	ND	ND	0.043	21.9	0.999	0.50	99.2	0.11
	Gompertz	6.14	1.76	22.7	ND	ND	0.999	0.50	99.2	0.11
	First-order	28.8	0.02	ND	ND	ND	0.831	0.18	251	1.57
R4 (1335/3.0)	Logistic	26.5	1.06	12.9	ND	ND	0.955	0.45	209	1.13
	Gompertz	9.99	0.36	20.6	ND	ND	0.951	0.45	213	1.18
	Cone	28.2	ND	ND	0.04	3.25	0.949	0.46	217	1.20
	First-order	47.3	0.015	ND	ND	ND	0.883	0.24	272	1.84
R5 (710/4.0)	Gompertz	51.2	4.20	2.75	ND	ND	0.996	0.50	183	0.93
	First-order	139	0.172	ND	ND	ND	0.984	0.43	270	1.82
	Logistic	138	13.40	0	ND	ND	0.979	0.38	286	2.06
	Cone	146	ND	ND	0.27	1.22	0.966	0.47	319	2.65
R6 (1335/5.0)	Gompertz	15.3	1.71	7.80	ND	ND	0.968	0.45	222	1.25
	Cone	41.9	ND	ND	0.112	3.78	0.966	0.44	225	1.29
	Logistic	41.6	4.32	4.59	ND	ND	0.961	0.42	234	1.37
	First-order	43.3	0.083	ND	ND	ND	0.883	0.23	306	2.40
R7 (1960/4.0)	Logistic	20.3	0.88	24.5	ND	ND	0.962	0.46	165	0.81
	Cone	21.1	ND	ND	0.03	5.60	0.960	0.47	168	0.83
	Gompertz	7.72	0.31	32.2	ND	ND	0.960	0.47	169	0.83
	First-order	25.0	0.02	ND	ND	ND	0.763	0.04	285	2.04
R8; R9; R10 & R11 (1335/3.0)	Logistic	117	4.82	26.7	ND	ND	0.987	0.50	314	2.55
	Cone	124	ND	ND	0.025	5.68	0.982	0.48	337	3.04
	Gompertz	46.6	1.55	35.3	ND	ND	0.979	0.49	347	3.29
	First-order	38.0	0.021	ND	ND	ND	0.437	0.03	648	4.81
R13 (665/3.0)	Logistic	129	17.5	3.12	ND	ND	0.991	0.47	265	1.75
	Gompertz	47.5	6.43	5.55	ND	ND	0.991	0.49	267	1.77
	Cone	130	ND	ND	0.15	3.44	0.990	0.50	271	1.83
	First-order	132	0.125	ND	ND	ND	0.939	0.29	389	4.54

A = ultimate CH$_4$ yield μ_m = maximum CH$_4$ production rate (specific microbial growth rate) λ = lag phase

K = specific rate constant   n = shape factor constant   ND = no data   AIC = Akaike's information criterion

RMSE = root mean square error   R = reactor   R^2 = coefficient of determination   RT = retention time.

In order to understand the influence of ISR and SO_4^{2-} on the process kinetics, reactors operating at the same ISR and same SO_4^{2-} were analysed. For reactors operating at constant ISR, an increase in SO_4^{2-} led to a significant (ANOVA, $p < 0.05$) decrease in A, μ_m and K, and a significant (ANOVA, $p < 0.05$) increase λ due to the inhibitory effect of S species on methanogenesis. In contrast, an increase in SO_4^{2-} led to a 34% decrease in λ and a constant K while operating at lower ISR (=2.5). Moderate to strong negative linear correlation existed between SO_4^{2-} with μ_m (r = −0.74), K (r = −0.61) and A (r = −0.60). In reactors operating at near optimal ISR (=3–4), increases in SO_4^{2-} from 665 to 710, 710 to 1335 and

1335 to 1960 mg/L led to an increase in A and K with a decrease in μ_m and λ; decrease in A, K and μ_m with an increase in λ; and an increase in K with a decrease in A, μ_m, and λ respectively (Figure 3-5). The results demonstrated a non-monotonic relationship between process parameters and kinetics. Therefore, the optimal SO_4^{2-} that promoted a higher A, K, and μ_m and lower λ was determined to be 922 mg/L at ISR = 3.7. This was applied in the setup of anaerobic sequential batch reactors (AnSBRs).

Generally, AD process instability is caused by a metabolic imbalance between acidogenesis and methanogenesis and/or sulfidogenesis. In order to understand the hydrolytic-methanogenic balance, it was important to study the relationship between K and μ_m by converting the units of μ_m to d^{-1}. The K/μ_m ratio was >1.0 in all reactors, showing that the rate of hydrolysis rate was faster than methanogenesis rate, and the imbalance increased (r = 0.65, (F test, $p < 0.05$)) with SO_4^{2-}. This consolidates the hypothesis that hydrolysis and acidogenesis proceeded faster than methanogenesis. Thus, methanogenesis was the rate-limiting step in this study.
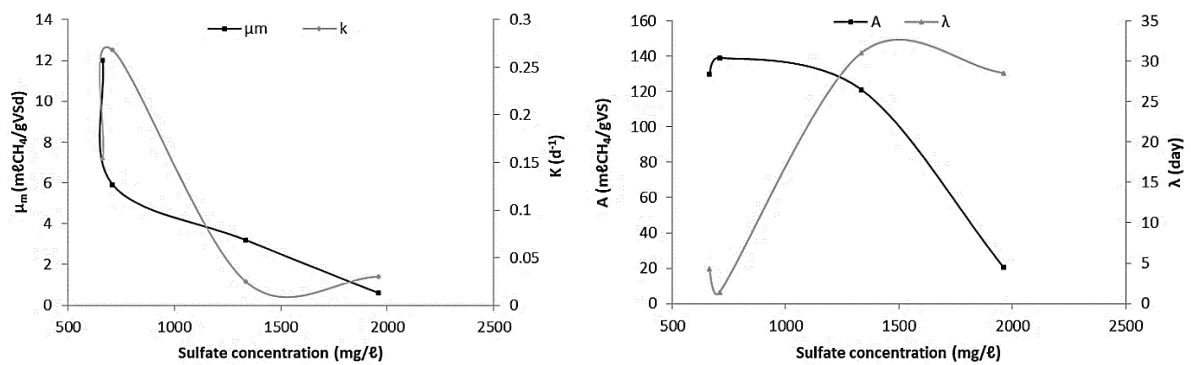
Figure 3-5: Correlation between kinetic parameters with influent sulfate concentrations for reactors operating at near optimum inoculum to substrate ratio (3.0–4.0).

3.7 Resource recovery from anaerobic sequencing batch bioreactor experiments

3.7.1 Cumulative methane generation and anaerobic biodegradability

The results from the BMP experiments were used to inform the ISR and $[SO_4^{2-}]$ applied in a scaled-up (20 L) anaerobic sequential batch bioreactor (AnSBR) experiment at different mixing conditions [(50, 100, 200 and 300 revolutions per minute (rpm)] using a pitched four blade marine impeller. In this case, ISR = 4 and $[SO_4^{2-}] \approx 680$ mg/L were used (i.e. no additional SO_4^{2-} was added to the SOTE). The bioreactor desirably operated under anaerobic conditions with an ORP that ranged between -445 and -543 mV, which was ideal for best methanogenic activity (Amani et al., 2010; Zupančič et al., 2012).

The cumulative CH_4 yields from the four runs were 51.3, 209, 58 and 31 mL/gVS at 300, 200, 100 and 50 rpm, respectively (Figure 3-6). The average CH_4 qualities were 31.2, 43.9, 30.9 and 30.9 %CH_4, respectively. The AnSBRs suffered inhibition and experienced about 14 days' lag phase. This was likely NH_3, HS^- and VOA inhibition. The AnSBRs achieved maximum CH_4 production after 15 days from the end of the lag phase. The yields from this study were comparable to those reported in previous studies (Table 2-2) and from the BMP experiments. These cumulative CH_4 yields clearly demonstrated the positive effect of continuous mixing on the process efficiency.
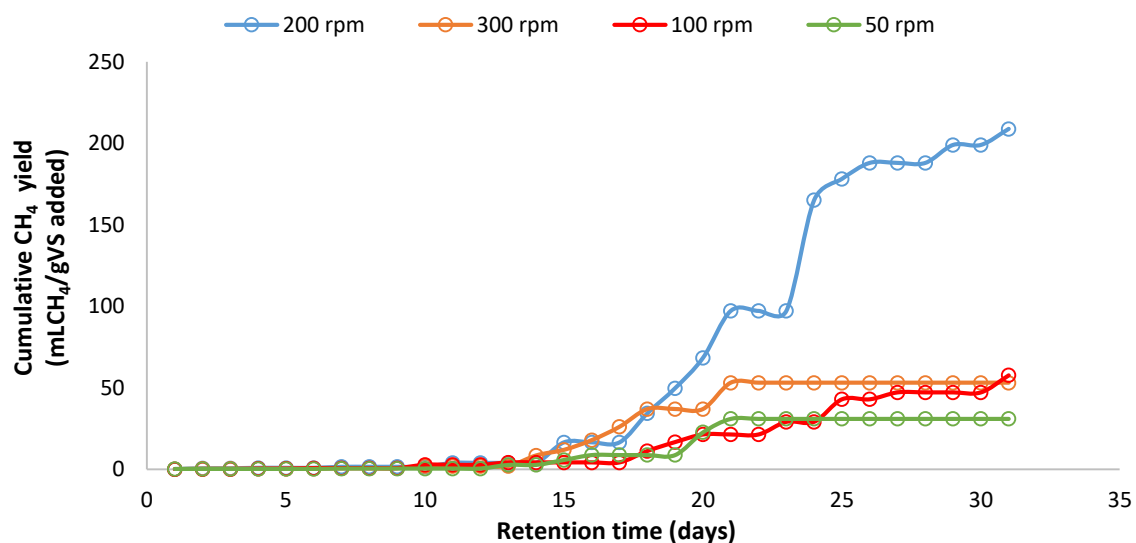
Figure 3-6: Cumulative methane yields from the pilot scale anaerobic sequencing batch bioreactor during the treatment of ostrich tannery effluent

The positive effect of mixing on CH_4 yield was observed when mixing was increased from 50 rpm to 200 rpm. The CH_4 yields from AnSBRs at 200 rpm were higher than the optimal from the BMP experiments while an increase to 300 rpm led to lower CH_4 yields. Continual mixing at 300 rpm may have (i) played a role in promoting the transfer of toxicants (HS^-, NH_3 and VOAs), and/or (ii) prevented ppt. of metals leading to their increased (toxic) bioavailability in the bioreactor, and/or (iii) that there was washout of functional biomass due to inadequate settling. The biomass in the BMP bioreactors settled well, in contrast to the biomass in the AnSBR. During the settling phase, a significant volume of biogas was generated in the AnSBR (7–24% of the totals). This suggested that sedimentation may have also provided closer microbial consortia proximity (juxtapositioning) as in the (unmixed) BMPs, which is essential for syntrophic microorganisms such as syntrophic sulfur reducing bacteria (SRB) and hydrogenotrophic methanogens (HMs) (Kim et al., 2002). It was also postulated that the presence of micro-niches at the bottom of the bioreactors may have protected microbes against high levels of inhibitory substances in the bulk liquid of the bioreactors. Additionally, higher mixing rates may have ruptured bacterial cells particularly non-coccoid shaped methanogens.

Hydrolysis seemingly limited the settling capacity of the SS as settling times of up to 5 days were inadequate. Nonetheless, mesophilic bioreactors have highly diverse and complex methanogenic communities, which enhance their adaptability, inhibition resistance, and resilience, leading to their operation at inhibited steady state conditions (Deng et al., 2014; Nozhevnikova et al., 2007).

3.7.2 Inhibition of methane production in anaerobic sequential batch reactors

3.7.2.1 *Ammonia and sulfate/sulfide inhibition*

The measured weekly $[NH_3]$ were above the minimum inhibitory concentration (53 mg/L) reported in literature for AD in all the mixing conditions and mostly climaxed (79 – 233 mg/L) on week 3 (day 21) (Figure A-1, appendice). These $[NH_3]$ may have led to the ultimate termination of methanogenesis together with HS^-inhibition. As discussed for BMPs, the high $[SO_4^{2-}]$ promoted the activity of SRB that became involved in the syntrophic degradation of organic substances (acidogenesis) and in biological sulfate (BSR) to HS^-. The weekly HS^-concentrations also fell within the inhibitory range (IC_{50} =14 – 125 mg/L at pH 7–8) found to inhibit methanogenesis during AD of suspended sludge (Koster et al., 1986; McCartney & Oleszkiewicz, 1991; O'Flaherty et al., 1998). An increase in $[SO_4^{2-}]$ was also observed between week 3 and 4, and this coincided with an increase in alkalinity, and NO_2^- while there was a significant decrease in NO_3^- . It was assumed that autotrophic denitrification had taken place (Equation 3-1 – Equation 3-4). However, the pathways involved in autotrophic denitrification integrated with HS^- oxidation are still obscure.

3.7.2.2 *Volatile organic acid inhibition*

The [VOA] increased markedly drastically over the first week of stable digestion (VOA:ALK>0.4) and this coincided with a slight decrease in [TOC] and [COD$_s$]. This was likely due to the effect of mixing that enhanced hydrolysis and acidogenesis by SRB in bioreactors that were assumed to already be inhibited by high $[NH_3/NH_4^+]$ and $[HS^-]$. The presence of a lag phase in CH_4 generation (Figure 3-6) indicated that the methanogens were inhibited. It was plausible that AnSBRs operated under an inhibited steady state condition [(VOA:ALK>0.4) as evidenced by the high $[NH_3]$ and $[HS^-]$, but stabilised after the second week with significant improvement in B$_o$. This agrees with the regeneration time of 5–16 days for methanogens (Deublein & Steinhauser, 2008; Zupančič et al., 2012).

3.7.3 Kinetic study of cumulative methane production in anaerobic sequential batch reactors

The kinetic models best fitted the data in the order Logistic>Cone>modified Gompertz>first order (Figure 3-7). Statistical parameters showed a similar trend to those obtained from the kinetic data for the BMP experiments. The Cone (Adj R^2 = 0.977–0.988) and Logistic (Adj R^2 = 0.976–0.989), and modified Gompertz (Adj R^2 = 0.664–0.997) models gave better estimations of the kinetic constants.

The kinetic constants obtained in this study were A (53.2–210 mLCH$_4$/gVS), μ_m (4.37 − 22.4 mLCH$_4$ /gVSd^{-1}), K (0.02–0.06 day^{-1}) and λ (13.6–17.2 days) (Table 3-5). The A and μ_m obtained in this study were higher than those achieved in the BMP experiments. The reaction rate constants (K) were lower than those achieved in the BMPs due to inhibition exacerbated by mixing. Nonetheless, higher maximum specific growth rates were achieved in the AnSBRs, which translated into an inhibited steady-state during AD. The A and K increased with mixing speed to a maximum at 200 rpm before decreasing when mixing speed was increased to 300 rpm. This demonstrated a parabolic relationship in the investigated mixing speed range (0–300 rpm) while the relationship between μ_m and mixing speed was positively linear.
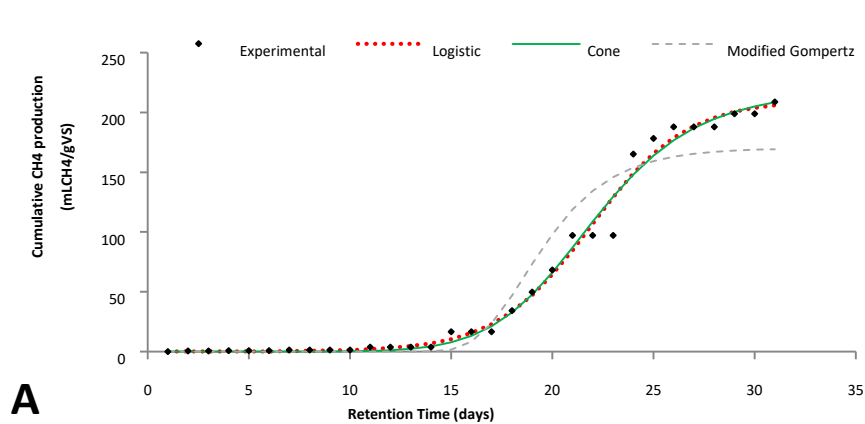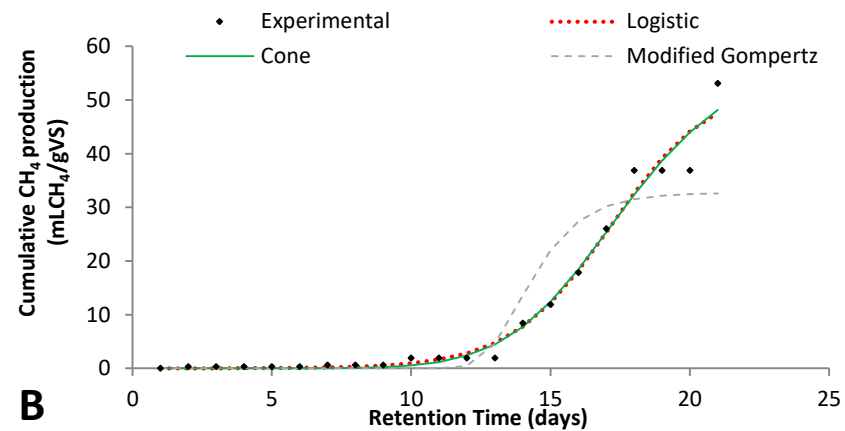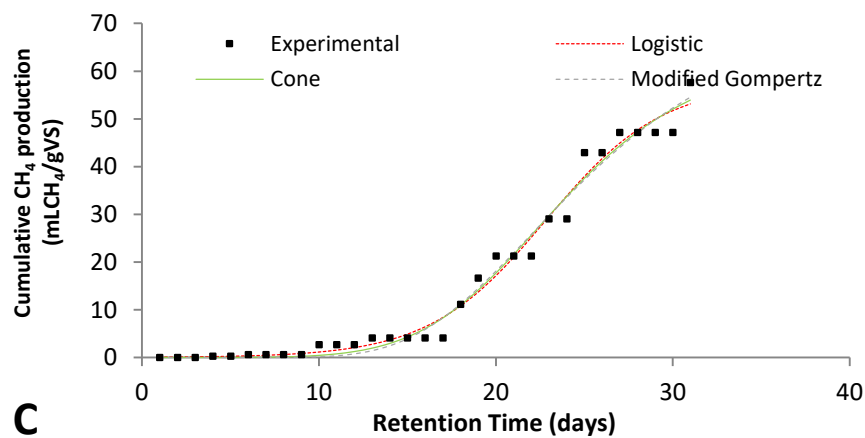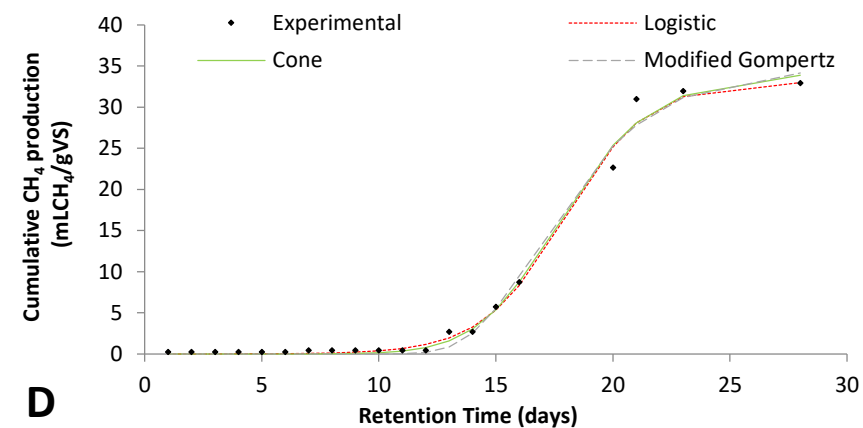
Figure 3-7: Graphs depicting the experimental and kinetic model curves for cumulative methane yields during anaerobic digestion of ostrich tannery effluent in an anaerobic sequencing batch bioreactor (A) 200 rpm, (B) 300 rpm, (C) 100, and (D) 50 rpm

Table 3-5: Kinetic parameters and goodness of fit of models fitted on to methane yields of anaerobic sequencing batch

Mixing speed	Model	A (mLCH$_4$/gVS)	μ$_m$ (mLCH$_4$/gVSd^{-1})	ʎ (d)	K	n	Adj R^2	p value Prob>F	AIC	RMSE
200 rpm	Logistic	210	22.4	17.2	ND	ND	0.989	0.4961	225	7.98
	Cone	220	ND	ND	0.045	8.6	0.988	0.4936	227	8.30
	Gompertz	63	9.80	18.6	ND	ND	0.789	0.3271	316	34.9
	C&H	4735411	1.66	ND	2E+06	ND	0.674	0.0023	331	44.2
	First order	48875	ND	ND	1E-04	ND	0.659	0.0014	332	45.2
300 rpm	Cone	59	ND	ND	0.057	8.3	0.977	0.4939	103	2.30
	Logistic	53	7.42	13.6	ND	ND	0.976	0.4848	104	2.36
	Gompertz	12	3.54	13.8	ND	ND	0.664	0.2610	159	8.83
	C&H	3746074	0.22	ND	4E+05	ND	0.679	0.0830	160	8.86
	First order	10914	ND	ND	1E-04	ND	0.531	0.0014	167	10.7
100 rpm	Gompertz	26	1.44	22.1	ND	ND	0.997	0.4841	75.1	1.20
	Logistic	58	4.37	16.3	ND	ND	0.982	0.4890	112	2.88
	Cone	66	ND	ND	0.042	5.8	0.981	0.4910	113	2.95
	C&H	2179699	0.13	ND	1E+05	ND	0.893	0.3315	150	7.19
	First order	15779	ND	ND	8E-05	ND	0.852	0.0027	158	8.57
50 rpm	Logistic	33	4.64	14.4	ND	ND	0.998	0.4830	16.8	0.28
	Cone	34	ND	ND	0.056	9.5	0.999	0.4904	19.6	0.30
	Gompertz	13	1.65	16.7	ND	ND	0.998	0.4845	41.3	0.44
	C&H	1975621	0.16	ND	2E+05	ND	0.822	0.2782	165	4.02
	First order	9898	ND	ND	9E-05	ND	0.755	0.0038	175	4.76

A = ultimate CH$_4$ yield μ$_m$ = maximum CH$_4$ production rate (specific microbial growth rate) ʎ = lag phase
K = specific rate constant n = shape factor constant ND = no data AIC = Akaike's information criterion
RMSE = root mean square error R = reactor R^2 = coefficient of determination RT = retention time

3.8 Microbial analysis

Microbial analysis was performed on the BMP bioreactors and AnSBRs by the authors and reported on a separate study (Kibangou et al., 2021). This study demonstrated that the initial [SO$_4^{2-}$] and ISR did not have significant (p>0.05) effects on the methanogenic and sulfidogenic community structure in the BMP tests. However, *Desulfofustis glycolicus,* known to reduce SO$_4^{2-}$ to H$_2$S was found at higher relative abundance (RA) in the bioreactor operating at SO$_4^{2-}$ ≥ 1960 mg/L (RA=15.91% v/s <0.003% in other bioreactors). It was postulated that H$_2$S may have inhibited some methanogens in the former than their competition for substrate with SRB. The selection of the sulfidogenic and methanogenic community structures in the BMPs were mainly driven by combinations of [NH$_3$], [VOA], [TOC], and [alkalinity], as well as VOA:alkalinity and *dsrB* copy numbers. In the ASBRs, continuous mixing promoted better survival and higher RA abundance of *Methanosarcina mazei* (15–32%) (Figure 3-8).

It was postulated that this species may be a hyper-producer of CH_4, as the specific CH_4 generation was significantly higher (0.0–208 mLCH_4/gVS) than BMPs (0.0–146 mLCH_4/gVS).

Quantitative PCR results showed that in the BMP tests, the abundance of the *mcrA* gene ranged from 3.63×10^5 to 6.46×10^6 copy numbers/ng DNA and were 1 to 2 orders of magnitude higher than the *dsrB* gene copy numbers (5.13×10^4 to 8.44×10^5/ng DNA) indicating the dominance of the former. Similarly, in ASBRs, the copy numbers of *mcrA* ranged from 9.32×10^6 to 1.32×10^7/ng DNA while *dsrB* gene copy numbers varied between 2.27×10^5 to 6.72×10^5/ng DNA, indicating the dominance of methanogenesis.
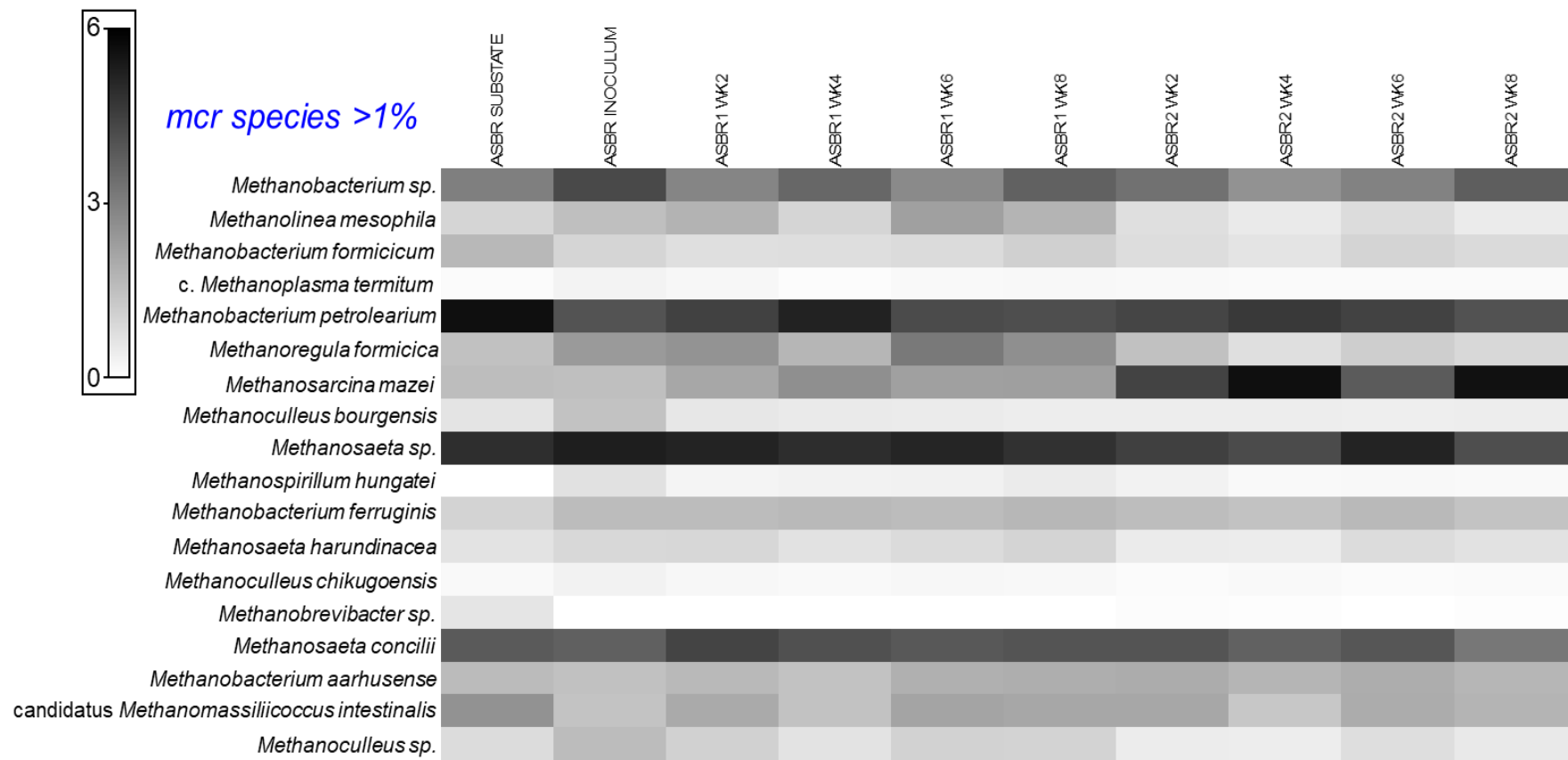
Figure 3-8: Shade plot of square root transformed data from mcrA amplicon sequencing of samples from anaerobic sequencing batch bioreactors. Inclusive of all species >1% of population in any one sample (18 of 42 species)

3.9 Summary

Efficient AD of SOTE is capable of reducing solids by up to 49% (dry mass) while recovering irrigation water, elemental sulfur, and up to 146 mLCH$_4$/gVS. It was also apparent that at least a fraction of the HS$^-$ formed from sulfidogenesis was oxidised into S^0 and a white yellowish layer formed at the interface of the bulk liquid and head space. It was postulated that chemolithotrophic SOB that reduce oxidised N compounds (NO$_2^-$ and NO$_3^-$) and oxidise HS$^-$ and other reduced S compounds such as thiosulfate (S$_2$O$_4^{2-}$) to form S^0. Methanogenic activity was highest (K = 13.4–17.5 d^{-1} and μ$_m$ = 0.15 − 0.27 mLCH$_4$ /gVSd^{-1}) when reactors were operated at ISR ≥ 3 and/or lower [SO$_4^{2-}$] ≤ 710 mg/L while high [SO$_4^{2-}$] ≥ 1960 mg/L and ISR < 3.0 caused almost complete inhibition regardless of corresponding ISR and SO$_4^{2-}$. The initial [SO$_4^{2-}$] and ISR did not have significant (p>0.05) effects on the methanogenic and sulfidogenic community structure in the BMP tests.

The BMP tests demonstrated that based on the SOTE [SO$_4^{2-}$] ≈646±417 mg/L at the study site, there was no need for pre-treatment to remove sulfur species and recover them as S^0. Therefore, the anaerobic sequential batch reactor (AnSBR) operated at ISR = 4 and 50–300 rpm were used to treat SOTE with [SO$_4^{2-}$] ≈680 mg/L. The setup achieved 31–208 mLCH$_4$/gVS depending on the mixing regime. The optimal conditions at 200 rpm achieved 208 mLCH$_4$/gVS and 49% TS (dry solids mass) reduction while recovering irrigation water in 21 days. It is acknowledged that while [SO$_4^{2-}$] will vary by tannery, and on a temporal basis at each tannery, concentrations measured in this study were ideal for AD and resource recovery. This presents integrated tanneries and slaughterhouses as potential biorefineries that can promote the sustainable economic development of developing countries.

CHAPTER 4

ANAEROBIC CO-DIGESTION OF BOVINE-OVINE TANNERY EFFLUENTS: PROCESS OPTIMISATION FOR RESOURCE RECOVERY, RECYCLING AND REUSE IN A CIRCULAR BIOECONOMY

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[This chapter investigated the AcoD of bovine-ovine beamhouse and tanyard effluents at different compositions (%v/v) in order to alleviate inhibition, supplement deficient nutrients and promote resource recovery (methane, irrigation water, biofertiliser and elemental sulfur)]

4.1 Introduction

The leather industry is a strategically important sector for the socioeconomic development of third world countries that dominate the industry. Leather products are amongst the most commonly traded commodities globally (Buljan & Král, 2015). The industry prevents the disposal of skins/hides from the meat and dairy industry by recycling them into leather. However, the industry is generally regarded as a heavy polluter due to its linear economy that dictates the disposal of about 20–40 m³ of complex effluents, 450–730 kg of solid waste, and 500 kg of wet wastewater sludge laden with residual processing chemicals per tonne of tanned raw skins/hides (Buljan & Král, 2015; Buljan & Král, 2019). The process wastes about 380 kg (84%) of the feed chemicals per tonne of processed skins/hides (Buljan & Král, 2019). The final quantitative and qualitative characteristics of tannery effluents depend on the tannery and wastewater treatment operations (Table A-2, appendix A1).

Physicochemical treatments, coupled with the conventional activated sludge process are mostly used to remediate tannery effluents (TE). Tanneries generally separate effluent streams into soaking, beamhouse (BHE), tanyard (TYE) and general effluent in order to maintain simplicity in the tannery wastewater treatment plant (WWTP). The beamhous operations contribute more than 80% of the organic pollution load and about 40-70% of ammonia nitrogen (NH_3N) in TE while tanyard processes contribute more than 90% chrome (Cr) and 60% sulfate (SO_4^{2-}) (Buljan & Král, 2019). The chromium contained in TY effluents (TYE) is mainly Cr (III) and/or Cr (VI) which is more toxic, carcinogenic, and mutagenic even in trace quantities. High sulfide levels contained in BHE may be released as a gas if mixed with acidic effluents such as TYE. Other microbial toxicants such as ammonia (NH_3), toxic metals, sulfate (SO_4^{2-}), sulfide (H_2S) and volatile organic acids (VOA) may be diluted and/or exacerbated by mixing streams. Nonetheless, tanneries still struggle to effectively treat separated streams to comply with the stipulated discharge standards, and to deal with enormous excess sludge. There are a number of studies that have reported on environmental pollution by tanneries in the developing world, where the quest for economic development seems to have outweighed the need for environmental protection to varying extents.

In line with the current circular economy principles and sustainable development, tanneries are capable of preventing waste generation and environmental pollution by promoting resource conservation, recovery, recycling and reuse. However, the adoption of cleaner production techniques in developing countries is traditionally slow as tanners are reluctant to adapt their tanning processes due to their perception that these may jeopardise the quality of the leather produced (Buljan & Král, 2019). Additionally, sophisticated equipment and specialty chemicals are generally expensive and require higher capital investments. A review by Mpofu et al. (2021a) reported on the potential of

converting tannery WWTPs into biorefineries through implementation of AD for the recovery of reusable, and recyclable value-added products such as bioenergy (methane, hydrogen or biomass), organic acids, sulfur, metals, construction aggregate, biofertiliser/compost, and/or 'fit for purpose' water reuse. However, the application of AD is affected by nutrient imbalance, deficiency and/or inhibition and this can be potentially abated through co-digestion (AcoD) of segregated TEs. This eliminates additional costs for the transportation of co-substrates from other industries.

Previous studies by Agustini et al. (2019); Berhe and Leta, (2018); Mekonnen et al. (2016) and Vazifehkhoran et al. (2018) successfully codigested general (combined) TEs with tannery solid wastes (leather shavings, sludge and/or fleshings), cow dung, and fresh wheat straw (*Triticum aestivum*), respectively, for biogas (methane) recovery. Achouri et al. (2017) investigated the AcoD of BHE and a combined TYE and dyehouse effluent at 50% volumetric composition after their pre-treatment with ferric chloride (coagulation). Majority of the previous studies including mono digestion did not explicitly describe the type or source of the TE used, and only provided a limited number of effluent physicochemical parameters (Mpofu et al., 2021a). To the authors' knowledge there are no studies investigating the AcoD of bovine/ovine BHE and TYE at varying volumetric (%v/v) and inoculum to substrate (ISR) ratios for resource recovery other than biogas (methane) and/or the determination of process kinetics. Therefore, the purpose of this chapter was to optimise the AcoD of BHE and TYE, respectively for the recovery of recyclable/reusable resources at varying volumetric substrate to substrate ratios [SSR (v/v)], and inoculum to substrate ratios (ISR). The study also sought to bridge the research gap by (i) comprehensively characterising the BHE and TYE from a bovine/ovine tannery (ii) modelling and determining the kinetics of the AcoD process and (iii) demonstrating the feasibility of a circular bioeconomy that promotes net positive tannery operations with potential cost savings through resource recovery.

4.2 Materials and methods
4.2.1 Sampling

Tannery effluent samples were collected from a local tannery in South Africa that processes about 3500 – 4000 bovine hides and 5000 – 8000 ovine skins per day via wet-blue tanning. Grab samples of BHE and pre-treated TYE streams were taken to form six monthly composites of about 50 L over the course of 6 months to allow for fluctuations in effluent quality.

4.2.2 Analytical methods

A Merck Spectroquant Pharo® Spectrophotometer (Darmstadt, Germany) together with Merck cell tests/kits, ion-chromatography, inductively coupled plasma (ICP) atomic emission spectroscopy (AES) and loss of mass on ignition standard methods were used to determine the physicochemical characteristics of TEs as described in section 3.2.2. The quantification of CH_4, CO_2, O_2 and H_2S contained in biogas was described in section 3.2.5.

4.2.3 Biomethane Potential experiments

The biomethane potential protocol (BMP) described by Holliger et al. (2016) was followed in this study. Thirteen bioreactors for AcoD experiments were set up following a full factorial central composite design (CCD) for ISR (1–4) and SSR (0–100%BHE) as variables. Three duplicate sets of four bioreactors were set up for (i) duplicate axial points for AcoD experiments, (ii) mono anaerobic digestion experiments and (iii) negative control experiments. This experimental design was created using Design-Expert® Software Version 10 (Stat-Ease, Inc., Minneapolis, USA). Validation experiments were set up to validate the determined theoretical optimum conditions. A viable acclimated inoculum was prepared by feeding a 50/50 (v/v) of BHE and TYE to digestate obtained from a mesophilic batch reactor treating ostrich TE. A constant volume of inoculum was added in all reactors, while the volume of blended BHE and TYE varied depending on the desired SSR and ISR. Detailed information on the experimental set up and operation is described elsewhere by Mpofu et al. (2021b).

4.2.4 Kinetic study

The kinetics of the BMP reactors were evaluated by fitting the modified Gompertz, first-order, Logistic, Cone, and Chen and Hashimoto (C&H) models onto the cumulative CH_4 yield data using non-linear regression method. These kinetic models were selected as they display a sigmoidal shape which adequately describes the lag, exponential and stationary phases expected during the AD of TE. Statistical measures that were used to test for models' goodness of fit were adjusted coefficient of determination (Adj R^2), F test, Akaike's Information Criterion (AIC), and the root mean square error (RMSE).

4.3 Results and discussion

4.3.1 Characterisation of tannery effluents

It is known that there is considerable intra and inter-site variation in TE characteristics due to differences in tannery operations and in wastewater treatment processes. The characteristics of BHE and TYE were within the concentration ranges reported in literature (Mpofu et al., 2021a) and their variation over time are discussed below. This demonstrates the need to mention the type or source of the characterised TE as the concentrations vary.

4.3.1.1 *Chemical and biological oxygen demand and solids concentrations in the bovine/ovine tannery effluents*

The characteristics of the bovine/ovine (BOTE) exhibited a batch-to-batch variation (Table 4-1a and Table 4-2b). Variations in TS, TVS, BOD and COD of both BHE and TYE were insignificant (ANOVA, p>0.05). The TS, TVS, BOD and COD concentrations of BHE were notably higher than those in the TYE, but the BOD:COD ratios of the TYE (0.36–0.61) were higher than those of the BHE (0.18– 0.32). The BOD:COD ratios of batch 4 and 5 of the TYE (0.58 and 0.61, respectively), suggested that these batches contained a higher fraction of biodegradable organics (Table 4-1a) compared to BHE with BOD:COD (≤0.32) (Table 4-2b) which suggested that BHE had a higher fraction of inorganics and/or recalcitrant organics. Generally, BHE processes are the main source of suspended (79%) and dissolved solids (61%), BOD (75%), and COD (75%) in TEs (Buljan & Král, 2019). In contrast to the BOD:COD ratio, the BHE exhibited a higher TVS:TS (0.18–0.41) compared to TYE (0.09–0.14). The TS, BOD and COD concentration ranges of all the batches were comparable to the ranges 10.1–31.5, 0.1– 4.33, and 0.51 – 55 g/L reported in similar studies, respectively (Mpofu et al., 2021b). There is however limited literature on TOC, TVS, TS concentrations and TVS:TS ratios of TEs.

4.3.1.2 *Concentration of nitrogen, carbon and volatile organic acids in the bovine/ovine tannery effluents*

Expectedly, the total nitrogen (TN) concentrations of BHE and TYE batches were >1000 mg/L, and >500 mg/L, respectively. Unhairing contributes to the high nitrogen (N) and HS^- content in BHE (Table 4-2b). These concentrations were notably higher than those reported in literature (112 – 915 mg/L) (Mpofu et al., 2021a). In contrast to TN in TE, which has been widely reported, only one literature report was found where TOC was included, and a few where C:N ratios were included as measured parameters (Mpofu et al., 2021a). The TOC concentrations of BHE (3540-9250 mg/L, Table 4-2a) and TYE (594–1330 mg/L, Table 4-2b) were higher than the 514 mg/L, reported in literature. The AD of TEs with low C:N ratios is susceptible to instability due to the potential accumulation of NH_3 and VOAs. Berhe and Leta, (2018) reported C:N ratios between 6–9 as optimal for the AD of TE. The C:N ratios (2.49–8.94) of some BHE batches were optimal, while those of TYE (1.02–1.46) were below optimal, and therefore potentially susceptible to NH_3 inhibition during AD.

The total VOA (VOA$_t$) concentrations of BHE (2.01–4.17 g/L) and TYE (0.53–1.46 g/L) were mostly below the inhibitory thresholds reported in literature (VOA$_t$=5.80–6.90 g/L) (Mpofu et al., 2021a). The VOA:ALK ratios of BHE (0.64–1.38) and TYE (0.11–0.73) were >0.4, except for batch 2, 5 and 6 of TYE. This indicated the likelihood of bioreactor instability and failure during AD (Gao et al., 2015). However, the speciation of $NH_3-NH_4^+$ and $CO_2-HCO_3^--CO_3^{2-}$ during AD serves as a buffer for maintaining pH within the optimal range.

4.3.1.3 *Inorganic characteristics of bovine/ovine tannery effluents*

The BHE concentrations of nitrite (NO_2^-), nitrate (NO_3^-) and sulfide (S^{2-}) were higher than those of the TY, while their NH_3 and Cl^- concentrations were comparable (ANOVA, p>0.05). The NH_3 concentrations in all the batches were within the inhibiting range of 53–1450 mg/L reported in previous studies (Mpofu et al., 2021a). Beamhouse processes are the main source of inorganic substances such as nitrogen compounds (85%), and sulfide (80%) in BOTE (Buljan & Král, 2019).

There were significant batch-to-batch SO_4^{2-} differences (ANOVA, p<0.05) which were assumed to emanate mainly from the tanning operations. As expected, TYE batches exhibited higher SO_4^{2-} concentrations (2090–4200 mg/L) than BHE ($1120 - 2400$ mg/L) as chrome sulfate $[Cr_2(SO_4)_3]$ is used as a tanning agent. These were comparable to the 450–3900 mg/L concentration range from previous studies (Mpofu et al., 2021a). According to Buljan & Král, (2019), TYE and BHE are expected to contribute 16% and 52% SO_4^{2-} to final TE emissions. The COD: SO_4^{2-} ratios (5.3–22) of BHE batches were likely to favour methanogenesis, while those of TYE (0.75–1.96) were likely to support sulfidogenesis (Chou et al., 2008). Nonetheless, the HS^- levels in BHE (276–476 mg/L) were inhibitory to methanogens and sulfidogenesis was likely to be favoured. The IC_{50} ranges for H_2S determined by O'Flaherty et al. (1998) for methanogenic archaea were 43–125 mg/L at pH 7–8, $57 - 184$ and $14 - 60$ mg/L at pH 7.2–7.4 and 8.0–8.5 for aceticlastic methanogens (AMs), respectively.

4.3.1.3.1 *Metal profiles*

The metal concentrations of BHE and TYE batches displayed similar trends (r = 0.73–0.99, F test, p<0.05) except for Ni, Pb and Cr. The total Cr concentration range was 39–283 µg/L and was on the lower end of the 0.26–7000 mg/L concentration range from literature (Mpofu et al., 2021a). As was expected, concentrations of K (45.6–129 and 85.7–130 mg/L), Na (5.55–7.18 and 5.93–6.66 g/L), Ca (0.2 –1.42 and 0.18–0.33 g/L) and Mg (3.7–375 and 186–263 mg/L) in BHE and TYE, respectively, were equally high as they are added in different forms to preserve skin/hides and/or throughout the tanning process. These concentrations ranges were comparable to the 0.14–17.4 g/L Na, $0.06 - 2.98$ g/L Ca, and $3.19 - 48$ mg/L Mg reported in previous studies (Mpofu et al., 2021a). There is a lack of studies reporting on the metal concentrations in TEs. Metabolic co-factors Ni, Zn, Co, Cu and Ca were either within or below the optimal literature range for AD (Mpofu et al., 2021a).

Nonetheless, a wide range of inhibitory concentrations of metals have been reported in literature due to variability in bacterial acclimation, substance synergism, and antagonism on different reactor operating conditions (Mpofu et al., 2021b), and the fact that microbial communities may adapt and prevent metal toxicity or deficiency (Thanh et al., 2016). The full-scale application of this study is expected to succeed provided the versatile microorganisms and suitable operational parameters are

maintained. Work by Kibangou et al. (2021) on the AcoD of TE and slaughterhouse wastewater demonstrated the preferential selection of metabolically versatile methanogenic genus *Methanosarcina spp*, *Methanobacterium*, and *Methanosaeta* in both unmixed and continuously mixed reactor regimes. They further reported *Desulfovibrio*, *Desulfomicrobium*, *Desulfobacterium* and a member of the order *Clostridiales* as the most abundant SRB genera in both reactor regimes. Similar to this work, their work demonstrated the syntrophic coexistence of SRB and methanogens. Based on the inhibitory concentrations reported in literature, Na in BHE and TYE (Table 4-2a and Table 4-2b) was within the inhibitory range whereas other metals were below the inhibitory range reported by previous studies (Mpofu et al., 2021a).

Table 4-1a: Characteristics of beamhouse bovine/ovine tannery effluents

Parameter	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Mean	SD±
TOC (mg/L)	9250	7240	5160	4710	4220	3540	5687	2150
COD (mg/L)	24650	20350	27725	21675	23180	26200	23963	2776
BOD (mg/L)	7500	6500	5000	6000	6000	7000	6333	876
VOA$_t$ (mg/L AAE)	2637	3109	4173	2674	2007	2691	2882	724
TN (mg/L)	1035	1320	1020	1315	1600	1430	1287	226
TAN (mg/L NH_3-N)	865	96	136	230	176	313	303	286
NO_3^- (mg/L)	5.90	4.7	3.15	5.85	5.15	4.1	4.81	1.06
NO_2^- (mg/L)	0.30	12.2	10.6	1.10	1.30	1.60	4.50	5.40
TP (mg/L $PO_4^{2-}-P$)	63.2	4.95	5.9	7.5	10.6	40.9	22.2	24.3
SO_4^{2-} (mg/L)	1120	2400	2200	1130	1450	1850	1692	545
HS$^-$ (mg/L)	0.40	476	276	456	1.88	0.60	202	231
Cl (mg/L)	2500	3175	9025	6825	8125	8840	6415	2885
TS (g/L)	31.4	30.5	38.0	46.5	34.0	32.7	35.5	6.01
TVS (g/L)	11.6	9.83	14.4	18.9	10.9	11.8	12.9	3.30
Proteins (mg/L)	1562	2610	2090	3931	4184	2871	2875	1024
K (mg/L)	45.6	78.5	99.6	129	126	ND	95.7	34.8
Na (mg/L)	5552	6446	6058	6820	7184	ND	6412	638
Fe (mg/L)	0.05	0.03	0.08	0.26	0.13	ND	0.111	0.09
Ca (mg/L)	497	254	199	1091	1419	ND	692	539
Mg (mg/L)	375	60.3	151	3.7	11.8	ND	120	154
Zn (µg/L)	169	432	158	739	996	ND	499	366
Cu (µg/L)	21.8	13.8	10.9	26.7	168	ND	48.2	67.2
Co (µg/L)	1.79	2.57	2.84	4.36	2.74	ND	2.86	0.93
Cd (µg/L)	0.00	0.00	0.00	0.09	0.32	ND	0.083	0.14
Ni (µg/L)	26.48	30.9	28.9	21.9	25.9	ND	26.8	3.40
Cr (µg/L)	136	62.6	155	66.0	38.8	ND	91.8	50.8
Pb (µg/L)	2.90	2.31	2.15	4.57	8.25	ND	4.04	2.55
Al (µg/L)	42.7	8.51	8.61	52.0	55.5	ND	33.5	23.2
Alk (mg/L $CaCO_3$)	2425	4425	3030	4200	2200	2770	3175	929
EC (mS/cm)	29.8	29.2	33.2	33.1	34.8	ND	32.0	2.16
pH	7.28	10.9	9.56	14.2	14.3	11.2	11.2	2.71
TVS:TS	0.371	0.323	0.38	0.41	0.32	0.36	0.36	0.037
BOD:COD	0.30	0.32	0.18	0.28	0.26	0.27	0.27	0.054
C:N	8.94	5.48	5.06	3.58	2.64	2.48	4.70	2.41
VFA:Alk	1.09	0.70	1.38	0.64	0.91	0.97	0.95	0.30
COD: SO_4^{2-}	22.0	8.5	12.6	19.2	16.0	14.2	15.7	5.30
COD:TVS	2.12	2.07	1.92	1.15	2.13	2.23	1.94	0.40

ND=not determined   SD=standard deviation

Table 4-2b Characteristics of tanyard bovine/ovine tannery effluent.

Parameter	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Mean	SD±
TOC (mg/L)	930	594	1330	875	700	1208	940	285
COD (mg/L)	3880	4625	6570	1725	1955	4795	3925	1842
BOD (mg/L)	1500	1650	2443	900	1200	1800	1575	517
VOA_t (mg/L AAE)	1458	531	1985	1062	167	1102	1051	647
TN (mg/L)	765	580	910	545	595	980	729.2	185
TAN ($mg/LNH_3 - N$)	760	102	153	315	422	730	414	281
NO_3^- (mg/L)	0.70	0.61	0.80	<0.5	1.1	0.75	0.79	0.19
NO_2^- (mg/L)	0.01	5.50	5.65	0.30	0.32	0.34	2.01	2.76
TP ($mg/L\ PO_4^{2-} - P$)	3.2	5.05	8.05	10.4	7.90	8.60	7.20	2.61
SO_4^{2-} (mg/L)	2740	4200	3400	2310	2090	2450	2865	796
HS⁻ (mg/L)	0.1	5.70	4.80	0.65	0.68	0.48	2.07	2.57
Cl (mg/L)	7725	7500	9400	2870	4620	7460	6595	2387
TS (g/L)	20.1	16.9	17.5	22.5	12.9	20.1	18.3	3.34
TVS (g/L)	2.34	1.50	2.51	2.02	1.29	1.79	1.91	0.47
Proteins (mg/L)	256	282	204	377	435	560	352	132
K (mg/L)	85.7	ND	80.3	105	130	ND	100	22.5
Na (mg/L)	6235	ND	6072	6664	5928	ND	6225	319
Fe (mg/L)	0.27	ND	0.04	0.34	0.11	ND	0.19	0.14
Ca (mg/L)	198	ND	212.0	331	183	ND	230	67.6
Mg (mg/L)	218	ND	215.2	263	186	ND	221	31.9
Zn (µg/L)	110	ND	78.6	189	383	ND	190	137
Cu (µg/L)	20	ND	25.1	22.6	236	ND	75.8	107
Co (µg/L)	0.00	ND	1.36	1.99	4.65	ND	2.00	1.95
Cd (µg/L)	0.00	ND	0.00	0.11	0.06	ND	0.04	0.05
Ni (µg/L)	20	ND	14.6	15.1	15.5	ND	16.3	2.5
Cr (µg/L)	130	ND	119	283	269	ND	200	87.7
Pb (µg/L)	0.00	ND	3.30	3.49	5.64	ND	3.11	2.33
Al (µg/L)	30	ND	15.0	25.3	42.0	ND	28.1	11.2
Alk (mg/L $CaCO_3$)	2010	1730	2705	1960	1590	5420	2569	1449
EC (mS/cm)	31.4	ND	31.1	33.9	29.4	ND	31.5	1.59
pH	8.38	8.80	8.52	8.04	8.48	7.82	8.34	0.35
TVS:TS	0.12	0.09	0.14	0.09	0.10	0.09	0.11	0.02
BOD:COD	0.39	0.36	0.37	0.52	0.61	0.38	0.44	0.11
C:N	1.22	1.02	1.46	1.61	1.18	1.23	1.29	0.21
VOA:Alk	0.73	0.31	0.73	0.54	0.11	0.20	0.44	0.27
$COD:SO_4^{2-}$	4.17	7.79	4.94	1.97	2.79	3.97	4.27	2.02
COD:TVS	1.66	3.09	2.62	0.85	1.51	2.67	2.07	0.86

Alk = alkalinity   BOD/COD = biological/chemical oxygen demand EC = electrical conductivity   ND = not determined

SD = standard deviation   TAN = total ammonia nitrogen   TOC = total organic carbon   TP = total phosphate

TN = total nitrogen TVS = total volatile solids   VOA = volatile organic acids

4.4 Optimisation of anaerobic biodegradability and resource recovery

The experimental CH_4 (0 to 357 mL/gVS) and biogas (0 to 692 mL/gVS) yields and B_o [% reduction TOC (12.2–58.8%), SO_4^{2-} (33.5–84.6%), COD (12.3–84.4%), VS (18.9–52.5%), and TS (16.9–37.6%) (Table 4-3) were modelled using a general quadratic polynomial with up to second degree interaction terms, and linear equations (Table 4-4 and Figure 4-1).

Table 4-3: Experimental design matrix showing the gas yields and biodegradability results

Reactor	A:Composition BHE	B:ISR	Biogas Yield	Methane Yield	Average Methane	Biodegradability indicators (% Reduction)				
						TOC	Sulfate	TS	VS	COD
	(%)		(mL/gVS)	(mL/gVS)	%	(%)	(%)	(%)	(%)	(%)
R1	50	2.5	473	196	41	51.8	37.1	32.2	46.0	67.4
R2	25	2.0	139	25	18	56.7	68.0	16.9	18.9	49.0
R3	75	3.0	600	218	36	37.7	38.8	26.0	34.3	65.9
R4	50	2.5	415	161	39	42.0	63.3	32.5	49.3	64.7
R5	0	2.5	0	0	ND	29.3	40.5	19.6	34.8	55.3
R6	50	1.0	57	7.6	13	13.1	44.5	17.9	29.7	12.3
R7	75	2.0	0	0	ND	44.2	58.9	29.9	42.8	70.2
R8	100	2.0	692	357	52	39.3	53.9	37.6	52.5	68.4
R9	50	2.5	460	162	39	56.0	53.7	30.3	44.7	64.6
R10	50	4.0	296	79	27	29.8	45.2	30.6	42.2	50.2
R11	25	3.0	153	39	25	37.9	84.6	25.4	36.7	84.4
R12	50	2.5	440	173	39	25.5	65.9	28.0	46.1	68.8
R13	50	2.5	329	120	36	58.8	69.5	27.3	45.7	64.3
R14	100	3.0	393	150	38	12.2	75.0	13.4	45.6	62.5
R15	100	4.0	257	89.7	35	17.0	37.1	24.7	54.5	57.0
R16	0	2.0	0	0	ND	45.3	48.9	17.1	24.3	65.5
R17	0	4.0	0	0	ND	21.9	33.5	33.9	49.5	47.8

COD=chemical oxygen demand   Conc=concentration   ISR=inoculum to substrate ratio   R=reactor
TOC=total organic carbon   TS=total solids   VS=volatile solids

The fractional design space (FDS = 0.99) and the signal:noise ratios were sufficiently greater than the recommended 0.8, while the adequate precision for all the empirical models were desirably >4 (Stat- Ease, Inc., Minneapolis, USA). The F test showed that %BHE composition was a significant ($p<0.05$) model term for gas yield (biogas and CH_4) and B_o (TS and TOC), whereas ISR and its interactions [$(ISR)^2$ and (%comp)(ISR)] were the only significant terms (F test, $p<0.05$) on %TS and %COD reduction, respectively. However, both terms were not significant on B_o (SO_4^{2-}, VS and TOC reduction) as all the reactors were somewhat active.

All the models were significant (F test, $p<0.05$), and there was only 0.01–4.0% chance that this may have occurred due to noise (Table 4-4). The correlation coefficients (R^2) of the models (Table 4-4) indicated that 53; 49; 35 and 5% of the variability in biogas yield, CH_4 yield, %TS and %COD reduction was not explained by the models, respectively. The models' adj. R^2 values which corrected the R^2 values with respect to the sample size and number of terms in the models were 0.58 and 0.93 for %TS and %COD reduction, suggesting the models' moderate and good predictability, respectively. The high

variability in biogas and CH_4 yields (adj. R^2 = 0.37 and 0.41) was an indication of the complexity of reactor contents, consortium and the biochemical reactions that were taking place leading to gas production.

The general quadratic polynomial and linear equations (Equation 4-1–Equation 4-7) generated by Design-Expert® Software Version 10 (Stat-Ease, Inc., Minneapolis, USA) were statistically tested for significance and their accuracy in fitting the experimental data of gas yields and B_0 (Table 4-4). The CH_4 yield, %COD and %VS reduction models (Equation 4-2, Equation 4-4 and Equation 4-6) did not fit the data very well (Lack of fit: F test, p≤0.05) (Table 4-4). Therefore, excluding these three, other models (Equation 4-1 –Equation 4-3, and Equation 4-5 –Equation 4-7) were used to navigate the design space and to optimise the cumulative biogas yields, TOC, SO_4^{2-}, and %TS reduction as plotted in Figure 4-1. Based on the interest to maximise gas yields and B_o, the theoretical optimum operating conditions were found to be at %BHE = 100% and ISR=2.5 with a desirability of 0.73. These optimum conditions were expected to generate 562 mL biogas/gVS, 262 mLCH_4/gVS, with reduction efficiencies of 40.8%, 55.7%, 67.6%, 49.4%, and 35.6%, for TOC, SO_4^{2-}, COD, VS, and TS, respectively.

Table 4-4: Summary of the statistical results of the fitted models

Models	Std dev	p-value	F test (LOF) p value	R^2	Adj R^2	Adeq Prec	AIC
Biogas Linear	189	0.04	0.55	0.47	0.37	6.21	178
CH_4 Linear	2.99	0.03	0.01	0.51	0.41	6.91	70.6
TOC Quadratic	0.03	0.01	0.61	0.63	0.55	8.73	-52.5
Sulfate Mean	ND	ND	0.42	ND	ND	ND	107
TS Linear	3.97	0.01	0.10	0.65	0.58	8.67	78.0
VS 2FI	0.17	0.05	0.001	0.56	0.42	6.08	-0.32
COD Quadratic	4.66	<0.0001	0.03	0.95	0.93	22.4	89.2

Adeq Prec=adequate precision   Adj=adjusted   AIC=Akaike's Information Criterion   LOF=lack of fit
COD=chemical oxygen demand   ND=not determined   Pred=predicted   Std   dev=standard   deviation
TS=total solids   TOC=total organic carbon   VS=volatile solids

$$\text{Biogas yield} = 121\text{ISR} + 5.64\%\text{Comp} - 308 \qquad \text{Equation 4-1}$$

$$\text{CH}_4 \text{ yield} = 3.13\text{ISR}^2 + 0.01(\%\text{Comp})^2 0.05\text{ISR} - 55.4 \qquad \text{Equation 4-2}$$

$$\% \text{TOC}_\text{reduction} = \frac{1}{0.176 - 0.04\text{ISR}^2 + 0.001\text{ISR}^4} \qquad \text{Equation 4-3}$$

$$\% \text{VS}_\text{reduction} = 1.04\%\text{Comp} + 2.33\text{ISR} - 1.01\text{Comp(ISR)} - 1.61 \qquad \text{Equation 4-4}$$

$$\% \text{TS}_\text{reduction} = 3.88\text{ISR} + 0.16\%\text{Comp} + 9.27 \qquad \text{Equation 4-5}$$

$$\% \text{COD}_\text{reduction} = -15.4\text{ISR}^2 - 0.79\%\text{Comp(ISR)} + 130\text{ISR} + 2.08\%\text{Comp} - 167 \qquad \text{Equation 4-6}$$

$$\% \text{SO}_{4\text{reduction}} = 55.7 \qquad \text{Equation 4-7}$$
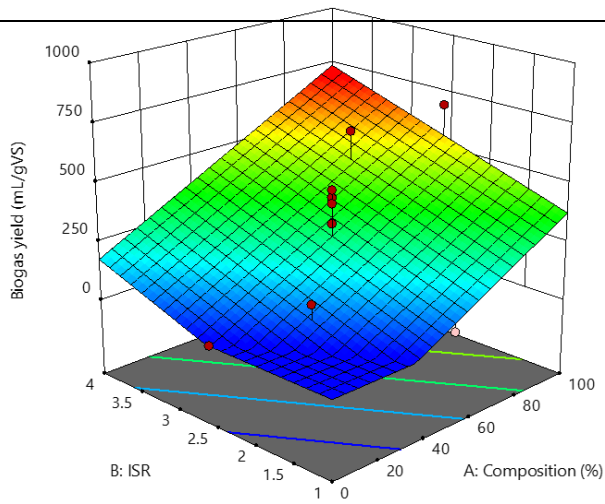
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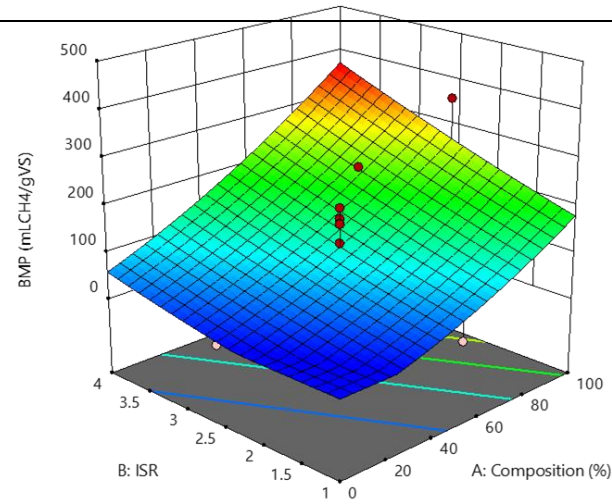
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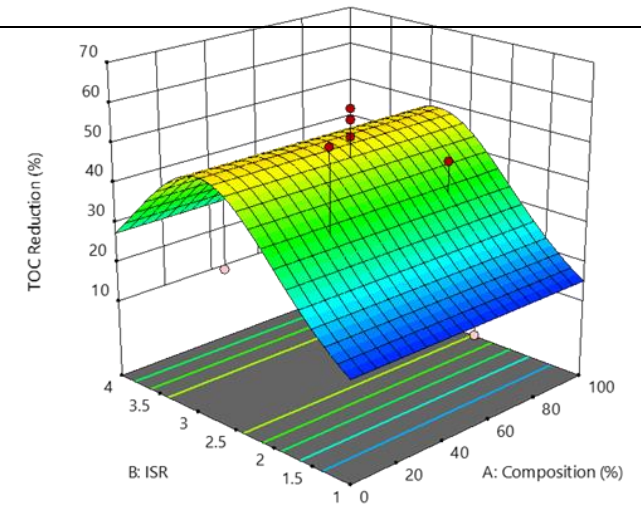
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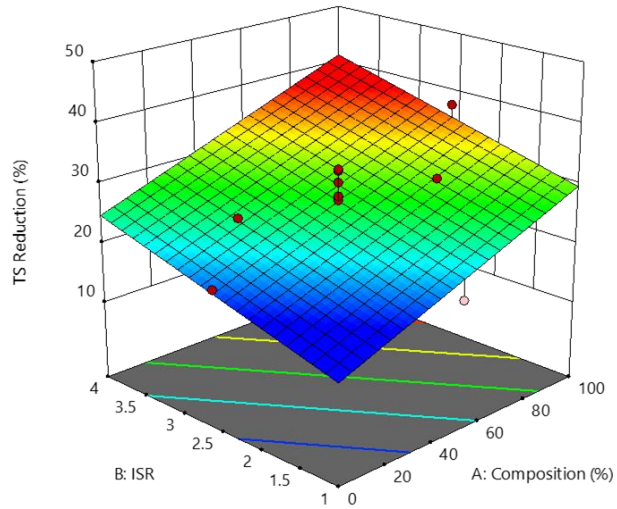
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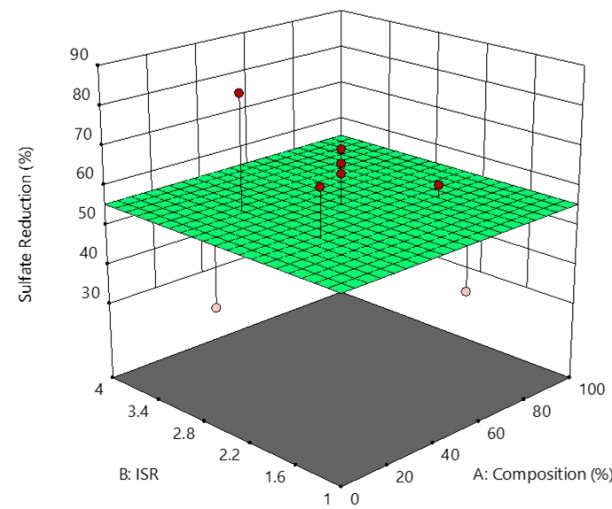
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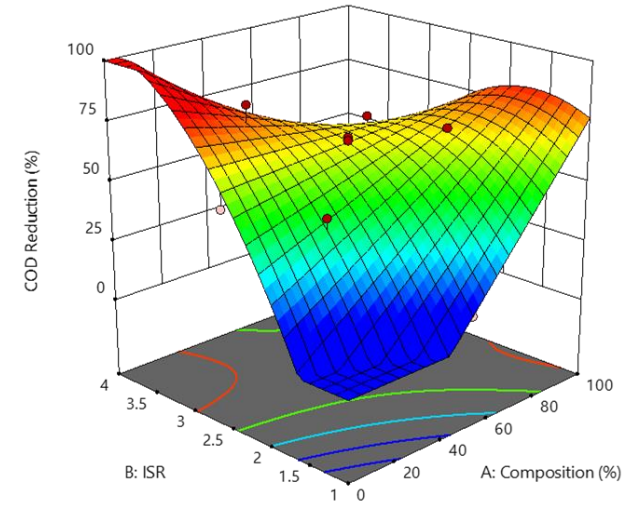
F

Figure 4-1: Effect of BHE concentration and inoculum to substrate ratio on: A - biogas yield; B - cumulative methane yield; C -total organic carbon reduction; D - total solids reduction; E- sulfate reduction; and F-COD reduction during anaerobic co-digestion of segregated b/ovine tannery effluents

4.4.1 Biomethane recovery

The repeat experiments to confirm the theoretical optimum conditions yielded 639 mL biogas/gVS, 377 mL CH_4/gVS (59% average CH_4), with reduction efficiencies of 42.2%, 57.8%, 62.6%, 64.5%, and 48.3%, for TOC, SO_4^{2-}, COD, VS, and TS, respectively. The highest COD removal (62.6%) was comparable to the $43\% - 95\%$ removal efficiencies reported by previous studies (Mpofu et al., 2021a). The cumulative CH_4 yields were higher than optimum yields obtained by Saxena et al. (2019), Berhe and Leta, (2018) and Mpofu et al. (2021b) for AD of general TEs with and/or without pre-treatment (7.6–27 mL/gVS, 56–61 mL/gCOD, and 0–146 mL/gVS after 35, 20, and 52 days, respectively. These results were also lower than the 406–753 mLCH_4/gVS and comparable to the 653–737 mL biogas/gVS determined by Achouri et al. (2017) after 37 days. Achouri et al. (2017) reported higher CH_4 quality (v/v) of 53–84% presumably due to the higher dilutions (34–53%) using tap water, addition of optimal micronutrients, and use of a blend 50:50 (v/v) of BHE and a combination of TYE and dye house effluents at ISR=1.5. The 13–59% average CH_4 quality measured in this study was due to parallel reactions that produced other gases such as CO_2, CO, H_2S, and other gases that could not be quantified using the biogas 5000 analyser. It was theorised that the main gas was most likely N_2 (Equation 4-8–Equation 4-15), and the rest of the gases were NH_3, H_2 and/or VOAs produced by AD processes. Nonetheless, the CH_4 yields from this study were comparable to $261 - 437$ and 314 mL/gVS reported by Vazifehkhoran et al. (2018) while co-digesting general TWW with cleaning (drainage) effluent and wheat straw (1:9 w/w) at ISR=3, respectively.

The performance of the reactors improved with increasing %BHE and up to ISR=3 (Figure 4-1A–D). At ISR>3 reactor performance decreased significantly at all %BHE. This strongly suggested that the characteristics of BHE were more suitable for AD than TYE, notably due to lower concentration of toxicants, and higher concentrations of essential macro- and micro-nutrients (Table 4-1a and Table 4-2b). The inhibition of reactors that led to the long lag phases and lack of CH_4 production is discussed in Section 3.3. In practical terms, these results suggest that bioreactors must be operated at 100% BHE and ISR (2≤ISR≤3) by optimising the solids retention time (SRT) and/or desludging rate.
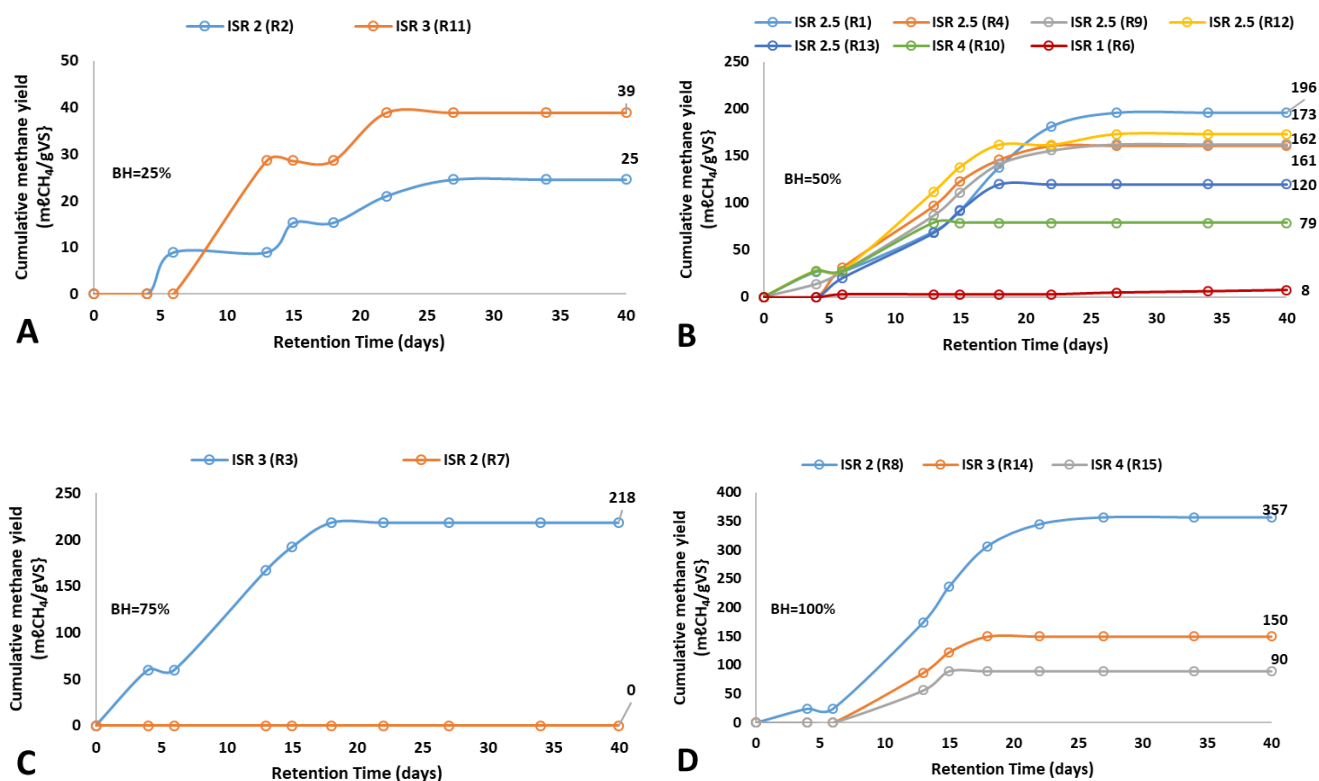
Figure 4-2: Cumulative methane yields of reactors operating at different inoculum to substrate ratios (A-D) and beamhouse effluent composition: 25% (A), 50% (B), 75% (C), and 100% (D)

4.4.1.1 *Kinetic study of cumulative methane production*

The fitted models displayed perfect fit to the experimental data with high precision and suitability [F test, $p < 0.05$ and $0.823 \leq$ Adj $R^2 \leq 0.999$). The models fitted the cumulative CH_4 production data in the order: Logistic>modified Gompertz>Cone>Chen and Hashimoto>First-order (Table 4-4; Figure 4-3). According to the statistical parameters (Table 4-4), the first-order ($0.823 \leq$ Adj $R^2 \leq 0.950$) and the Chen and Hashimoto (C&H) models ($0.825 \leq$ Adj $R^2 \leq 0.970$) were the worst predictors of cumulative CH_4 yields of most reactors. This was unexpected as all the reactors experienced none to shorter lag phases (0–6 days) and the exponential rise to a maximum curve portrayed by the first-order model should have accurately fitted the data. However, the inhibition of microbial activity affected the proportionality between CH_4 production and substrate depletion. Mpofu et al., (2021b) also reported the first-order model as the overall worst performer, particularly for all the reactors that experienced a lag phase and the best fit for reactors that did not experience any lag phase.

The Logistic, Cone and modified Gompertz model were the best performers ($0.827 \leq$ Adj $R^2 \leq 0.999$) for most reactors as they assume proportionality between CH_4 production and microbial activity or growth rate which is proportional to inhibition. Additionally, the models resemble a sigmoidal shape (Figure 4-3) which is adequate for predicting the lag phase, and exponential rise to a maximum. This was the case in this study as (i) the proportion of BHE detected the initial amount of biodegradable

substances added into the reactor; (ii) the amount of stimulating or inhibiting substances, and (iii) the initial reactor ISR determined the starting microbial population.

The highest μ_m were found in reactors R8, R14 and R15 operating at 100% BHE and ISR = 2, 3 and 4 respectively, while the highest K values were found in R3 and R10 operating at 50% and 75% BHE and ISR = 3 and 4, respectively (Table 4-5). These results confirm that from the range of parameters tested, higher % BHE and ISR≥3 provided the most ideal environment for the proliferation of methanogens. The μ_m = 0.04–74.2 mLCH$_4$/gVSd^{-1} and K = 0.06–0.16 day^{-1} results in this study were somewhat comparable to μ_m = 0.171–17.5 mLCH$_4$/gVSd^{-1} and K = 0.025–0.268 day^{-1} reported by Mpofu et al., (2021b), while digesting slaughterhouse-ostrich TE. The authors reported that the kinetics from their study were higher than those reported while co-digesting tannery sludge with other organic solid wastes. Nonetheless, there is a lack of studies investigating reaction kinetics during AcoD of TWW.
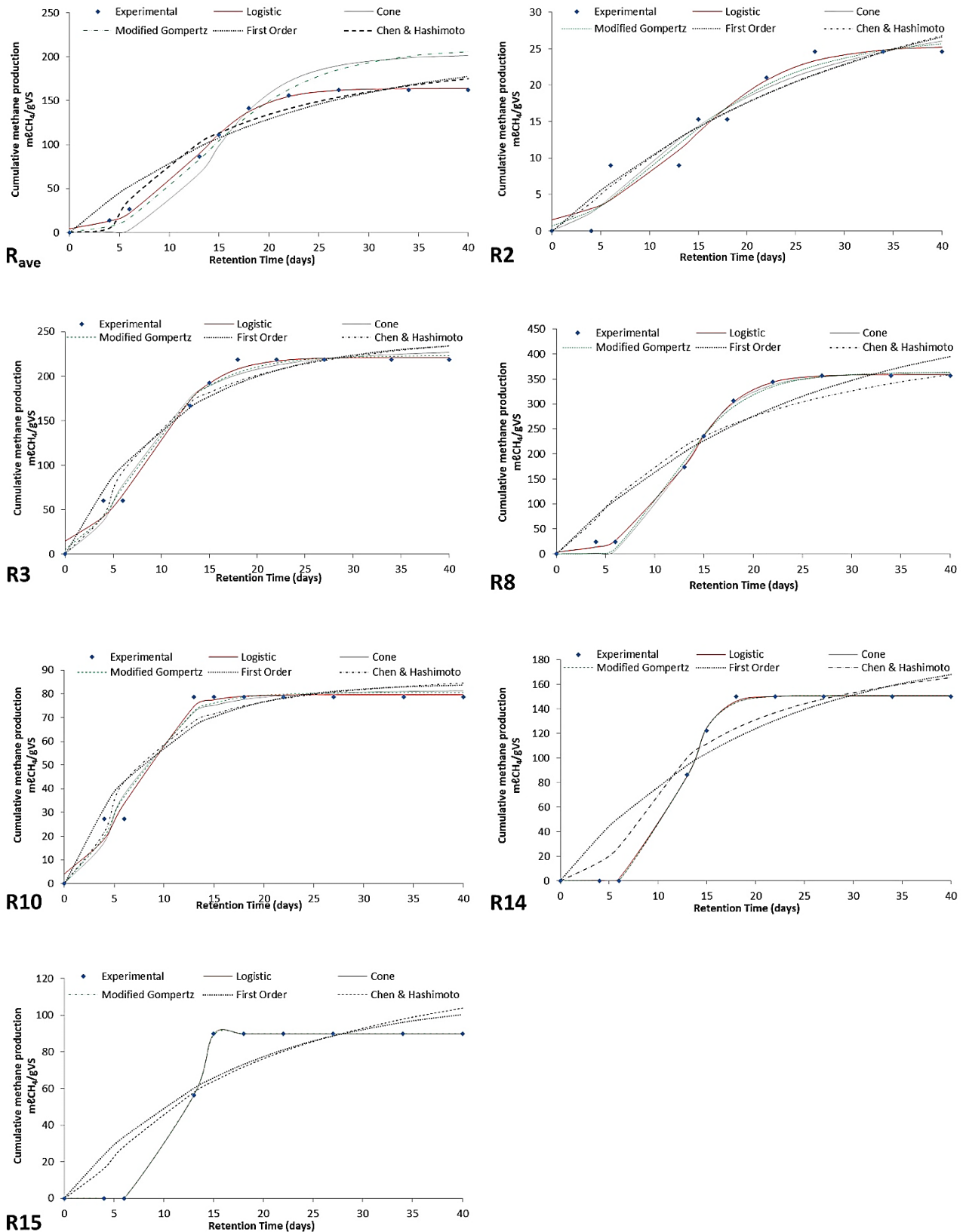
Figure 4-3: Graphs depicting the experimental and model curves for cumulative methane yields during the anaerobic co-digestion of beamhouse and tanyard b/ovine tannery effluents at different operating conditions: Rave – 50%BHE, ISR = 2.5; R2 – 25%BHE, ISR = 2; R3 – 75%BHE, ISR = 3; R8 – 100%BHE, ISR = 2; R10 – 50%BHE, ISR = 4; R14 – 100%BHE, ISR = 3; and R15 – 100%BHE, ISR = 4.

Table 4-5: Kinetic parameters and goodness of fit of models fitted onto cumulative methane yields of biomethane potential bioreactors

(%BHE /ISR)		A (mLCH$_4$/gVS)	μ_m (mLCH$_4$/gVSd)	Λ (d)	K (d^{-1})	n				
R1	Logistic	201	12.7	7.17	ND	ND	0.989	0.478	228	3.77
(50/1.0)	Gompertz	77.2	4.09	12.5	ND	ND	0.973	0.471	265	5.99
	Cone	204	ND	ND	0.07	4.56	0.972	0.413	266	6.07
	C & H	394	49.0	ND	9.98*	ND	0.937	0.464	298	9.10
	First-order	330	0.027	ND	ND	ND	0.923	0.388	298	10.1
R2	Logistic	25.4	1.23	4.00	ND	ND	0.941	0.450	128	1.08
(25/2.0)	Gompertz	9.77	0.42	11.1	ND	ND	0.939	0.456	129	1.09
	Cone	31.9	ND	ND	0.06	1.73	0.935	0.466	131	1.13
	C & H	52.8	18.1	ND	35.4*	ND	0.925	0.456	138	1.23
	First-order	36.2	0.033	ND	ND	ND	0.924	0.398	139	1.24
R3	Logistic	221	16.7	2.16	ND	ND	0.988	0.480	238	4.27
(75/3.0)	Gompertz	82.2	6.34	6.43	ND	ND	0.986	0.492	244	4.63
	Cone	232	ND	ND	0.13	2.37	0.978	0.490	261	5.74
	C & H	273	38.9	ND	2.15*	ND	0.959	0.476	288	7.97
	First-order	241	0.088	ND	ND	ND	0.950	0.407	296	8.83
R8	Logistic	359	31.6	7.43	ND	ND	0.999	0.496	173	1.91
(100/2.0)	Cone	364	ND	5.03	0.08	5.03	0.995	0.457	250	4.99
	Gompertz	134	11.5	11.3	ND	ND	0.994	0.465	255	5.30
	First-order	486	0.042	ND	ND	ND	0.910	0.334	366	21.2
	C & H	500	105	ND	9.67*	ND	0.888	0.355	375	23.7
R1; R4; R9; R12	Logistic	164	12.0	5.50	ND	ND	0.998	0.496	143	1.30
& R13 (50/2.5)	C & H	232	38.4	ND	3.18*	ND	0.970	0.483	255	5.28
	First-order	207	0.049	ND	ND	ND	0.933	0.364	279	7.92
	Gompertz	77.2	4.09	12.5	ND	ND	0.888	0.285	308	10.2
	Cone	204	ND	ND	0.07	4.56	0.863	0.237	316	11.3
R10	Logistic	79.7	8.63	2.09	ND	ND	0.981	0.481	175	1.95
(50/4.0)	Gompertz	29.6	3.16	5.18	ND	ND	0.974	0.498	186	2.24
	Cone	81.8	ND	ND	0.16	2.843	0.965	0.491	199	2.63
	First-order	84.3	0.119	ND	ND	ND	0.928	0.398	227	3.74
	C & H	92.7	43.5	ND	1.23*	ND	0.935	0.463	223	3.56
R14 (100/3.0)	Logistic	150	24.6	9.52	ND	ND	0.999	0.493	104	0.81
	Gompertz	55.4	11.2	12.0	ND	ND	0.999	0.500	110	0.86
	Cone	151	ND	ND	0.08	9.11	0.999	0.500	114	0.91
	C & H	207	23.1	ND	1.93*	ND	0.941	0.466	286	7.85
	First-order	192	0.052	ND	ND	ND	0.851	0.264	324	12.5
R15 (100/4.0)	Cone	89.7	ND	ND	0.08	49.1	0.999	0.497	-270	0.008
	Logistic	89.7	74.2	12.2	ND	ND	0.999	0.499	-244	0.01
	Gompertz	33.1	24.0	12.6	ND	3.44	0.999	0.499	-48	0.12
	C & H	155	53.9	ND	10.8*	ND	0.825	0.395	290	8.18
	First-order	110	0.06	ND	ND	ND	0.823	0.243	291	8.33

A = ultimate CH$_4$ yield BHE = beam house effluent C&H = Chen and Hashimoto K = specific rate constant

Λ = lag phase n = shape factor ND = not determined P = cumulative CH$_4$ yield R = bioreactor

μ_m= maximum CH$_4$ production rate (specific microbial growth rate) ISR = inoculum to substrate ratio

d = day

4.4.2 Sulfur recovery

It was apparent that at least a fraction of the HS^- formed from sulfidogenesis was oxidised into elemental sulfur (S^0). A white-yellowish layer was formed at the interface of the bulk liquid and head-space in the reactors. White-yellowish flakes were also observed settling to the bottom of the reactors and were most apparent in R5, R7, R8, R11, and R16 that operated at higher/lower BHE composition (0, 75, 100, 25, and 0%) and ISR≤3 (2.5, 2.0, 2.0, 3.0, and 2.0), respectively. These reactors also suffered methanogenesis inhibition as they had high initial SO_4^{2-} concentrations that supported sulfidogenesis. Chemolithotrophic sulfur oxidising bacteria (SOB) can facilitate the formation of S_0 from the oxidation of HS^- and H_2S by nitrate (NO_3^-) and nitrite (NO_2^-) during AD following the reactions in Equation 4-8– Equation 4-11 (Moraes et al., 2012). The consumption of H^+ by SOB could explain the anomalous increase in alkalinity in some reactors (section 3.4.4). The biological formation of S^0 presents an ideal opportunity for S^0 and nitrogen gas (N_2) recovery from TE.

$$5HS^- + NO_3^- + 3H^+ \rightarrow 5SO_4^{2-} + 4N_2 + 4H_2O \qquad \text{Equation 4-8}$$

$$3HS^- + 8NO_2^- + 5H^+ \rightarrow 3SO_4^{2-} + 4N_2 + 4H_2O \qquad \text{Equation 4-9}$$

$$5HS^- + 2NO_3^- + 7H^+ \rightarrow 5S_0 + N_2 + 6H_2O \qquad \text{Equation 4-10}$$

$$3HS^- + 2NO_2^- + 5H^+ \rightarrow 3S_0 + N_2 + 4H_2O \qquad \text{Equation 4-11}$$

A notable NO_3^- decrease (7–81%) in all reactors performing mono AD of TYE or BHE and in the severely or permanently inhibited reactors supported the hypothesis that it was readily utilised as an electron donor by SOB. This confirmed the conspicuous accumulation of S^0, NO_2^- (10–133%) and utilisation of HS^- particularly in reactors that operated at higher/lower BHE composition (0, 25, 75 and 100% BHE) and ISR≤3. The accumulation of NO_2^- may have partly inhibited methanogenesis (Moraes et al., 2012). It is also possible that the observed NH_4^+ accumulation was partly due to dissimilatory process of NO_3^- reduction to NH_4^+ on top of protein hydrolysis. However, NO_3^- accumulation in the rest of the reactors (12–255% increase) suggested occurrence of nitrification by microorganisms responsible for anaerobic ammonium oxidation (ANAMMOX). Whereas, in R2 (25% BHE and ISR = 2) and R9 (50% BHE and ISR = 2.5) it coincided with 13 and 46% NO_2^- reduction respectively, as it may have been used as the preferred electron donor over NO_3^- for HS^- oxidation.

ANAMMOX reactions (Equation 4-12–Equation 4-15) may have been a contributing pathway for S_0 and N_2 formation. These reactions were also postulated by Sabumon, (2008a, 2008b) who injected air at the bottom of the reactor and observed the formation of S_0 from the oxidation of HS^- and H_2S in the anoxic region of an up-flow hybrid anoxic reactor treating primary treated combined TE. The author also reported that 20–23% of the inlet sulfur escaped in treated TE while 63–66% was recovered as S_0. Mpofu et al. (2021b) observed the possibility of producing S_0 during AD of ostrich TE

combined with slaughterhouse effluent. The authors also observed re-formation of SO_4^{2-}, ostensibly from the anaerobic oxidation of S_0 by NO_3^- and NO_2^-. However, the dominant pathway for S^0 was not postulated. A sulfur mass balance on reactors operating at optimum conditions indicated that about 13% of influent sulfur is recoverable as S^0, 12% in the sludge and 22% in biogas, while 53% escapes in the effluent. Sabumon, (2008a, 2008b) achieved higher SO_4^{2-} removal (64.2–83.1%) and S^0 recovery (63–66%) as a sulfidogenic culture was used and oxygen (air) was added while limited NO_3^- and NO_2^- acted as oxidising agents in this study. The recovered S^0 can be harvested by skimming or retrofitting the batch reactor with a fine mesh filter.

$$3SO_4^{2-} + 4NH_4^+ \rightarrow 3S^{2-} + 4NO_2^- + 4H_2O + 8H^+ \qquad \text{Equation 4-12}$$

$$3S^{2-} + 2NO_2^- + 8H^+ \rightarrow 3S_0 + N_2 + 4H_2O \qquad \text{Equation 4-13}$$

$$2NO_2^- + 2NH_4^+ \rightarrow 2N_2 + 4H_2O \qquad \text{Equation 4-14}$$

$$SO_4^{2-} + 2NH_4^+ \rightarrow S_0 + N_2 + 4H_2O \qquad \text{Equation 4-15}$$

Nonetheless, the autotrophic denitrification and anaerobic ammonia nitrification integrated with sulfide oxidation occur via complex biochemical reactions that depend on various factors and the dominant pathway/s (species) were inconclusive in this study. Microbial analysis must be conducted in future studies to ascertain the dominant species and biochemical pathways. It was therefore hypothesised that (i) sulfidogenesis, methanogenesis, anaerobic ammonia oxidation and mixotrophic denitrification occurred concurrently, and (ii) sulfidogenesis initially dominated, followed by methanogenesis (particularly hydrogenotrophic) in the active reactors. This was supported by qualitative analysis of the biogas which showed very low %CO_2 compared to %CH_4 (CH_4: CO_2>1) and high H_2S >1610–9999 ppm during the first 2–6 days HRT, which gradually increased over time. Further research is needed to maximise sulfur production as a pre-treatment step to remove sulfide using a modified bioreactor such as the hybrid linear flow channel reactor to make BOTE more amenable for AD (Marais et al., 2020).

4.4.3   Nitrogen recovery

The final NH_3 concentrations in the soluble fraction (filtrate) in all the reactors ranged from 264 - 596 mg/L. The TE can be recycled and further processed downstream to recover nitrogen as a fertiliser. This could be accomplished by adding Mg and P to precipitate struvite ($MgNH_4PO_4. 6H_2O$), or through NH_3 stripping. Additional downstream ANNAMOX can be employed to further remediate the BHE and convert the NH_3 to N_2. However, sustainable and economically feasible options must be explored. A mass balance on nitrogen suggested that the optimal reactor conditions recovered about 18% of the influent nitrogen as N_2 in biogas. The CO_2 and N_2 in the biogas most likely resulted from the autotrophic denitrification and/or ANNAMOX integrated with sulfide

oxidation (Equation 4-8–Equation 4-15) can be recovered through absorption and stripping. Pilot studies will focus on using gas chromatography to ascertain biogas constituents.

4.4.4 Water reuse

Apart from recycling the TE for N_2 or NH_3 recovery, it can be used for irrigation purposes. The BOTE characteristics met the stipulated irrigation standards of various developing countries that lead in bovine leather production (Table 4-6). The treated BOTE did not meet the discharge standards for Na, Mg, B, Cr, Cl, nitrogen, and NH_3/NH_4 in many of the countries (Table 4-6). In the South African context, the TE characteristics met the discharge standards for irrigating up to 500 m^3/day (Department of Water Affairs, 2013). This is suitable for a medium-sized tannery processing about 100 tonnes/day of raw bovine hides (Swartz et al., 2017). Nonetheless, the TE can be mixed with other water sources to meet the 2000 m^3/day irrigation standards for COD (75 mg/L), Cl (0.25 mg/L), phosphorous (10 mg/L), nitrogen (15 mg/L) and NH_3 (3.0 mg/L). Maqbool et al. (2018) reported 50:50 (v/v) of TE and tap water as optimal for irrigating vegetables. Additionally, it can be further treated and/or diluted for reuse within the tanning process e.g. in soaking, de/liming and tanning (Buljan & Král, 2019). Alternatively, the treated effluent can be used by other neighbouring industries within the eco-industrial park for non-potable purposes such as construction, cooling and smelting. The use of TE for irrigation must be carefully considered, and the soil and plants being irrigated must be selected based on the less risk for contamination.

4.4.5 Biofertiliser and compost

The digestate contained the contained essential macronutrients: nitrogen, phosphorus and potassium (N:P:K=2:1:1 for optimal conditions), calcium, sulfur, magnesium and micronutrients such as boron, manganese, iron, and molybdenum for use as a biofertiliser (Table 4-6). The recovery of P, Mg and N in the digestate is assumed to have been aided by the precipitation of struvite while other metals most likely precipitated as sulfide and/or hydroxide complexes. These were observed as white flakes that settled to the bottom of the reactors, particularly under optimal conditions. The optimal conditions produced a digestate with 22.9% carbon, 2.94% hydrogen, 2.09% nitrogen and 1.84% sulfur. The metal concentrations of the digestate were within the recommended limits for class A1a sludge suitable for agricultural use in South Africa and other developing countries leading in bovine leather production, except for Cr (Table 4-6). Chrome is insoluble in water and it generally attaches to the suspended solids and hence its high concentration in the sludge. Nonetheless, the digestate can be further composted with stabilised organic waste that has minimal Cr concentrations. The type of organic substrate/s and mixing ratios can be optimised depending on the type of soil and the cultivated plant/s. Application of such compost will be beneficial for sandy soils that mainly lack the essential elements for plant growth.

Table 4-6: Allowable limits for wastewater and sludge for agriculture application in leading bovine leather producing countries including South Africa

Country	Brazil	China	Egypt	Ethiopia	India	Mexico	South Africa	South Korea	Turkey	Limit Range	This study (range)
pH	6-9|ND	5.5–8.5| ND	ND|ND	ND|ND	5.5–9.0|ND	5-10|ND	5.5–9.5|ND	5.5–8.5|ND	6–9|ND	5.5–9.5|ND	7.76–8.52|ND
(TSS)/TDS	450–2000|ND	(60-100)/2000|ND	20–250|1000	ND|ND	(200)/2100|ND	20–30|ND	25|ND	ND|ND	(20–60)|ND	(20–100)/20–2100|ND	ND|5.9–12.9
COD	ND|ND	40–200|ND	ND|ND	500|ND	ND|ND	ND|ND	75–400|ND	ND|ND	ND|ND	40–500|ND	270–3310|ND
BOD$_5$	60–120|ND	40–100|ND	20–400|ND	200|ND	100|ND	20–30|ND	ND|ND	8.0|ND	0–200|ND	8–400|ND	ND|ND
(NO$_3$)/TN	/20–70|ND	ND|ND	ND|ND	(50)/60|ND	ND|ND	ND|ND	15|ND	ND|ND	(0–50)|ND	(0–50)/15–70|ND	(0.3–7.1)/321–551|ND
Coliform	ND|ND	2000–40000|ND	1000–5000|ND	ND|ND	ND|ND	1000|1000	100–100000|ND	200|ND	0–1000|ND	100–100000|ND	ND|ND
NH$_3$/NH$_4$	ND|ND	ND|ND	ND|ND	30|ND	ND|ND	ND|ND	3.0|ND	ND|ND	0–50|ND	0–50|ND	256–596|ND
TP	4–30|ND	ND|ND	30|ND	ND|ND	ND|ND	ND|ND	10|ND	0.2–2.0|ND	ND|ND	0.2–30|ND	6.5–20.8|4569–7290
EC	0.5–2.7|ND	ND|ND	ND|ND	ND|ND	ND|ND	ND|ND	70–200|ND	0.7–2.0|ND	0–3|ND	0.5–200|ND	ND|ND
Se	0.02–0.05|100	0.02|ND	0.02|36	ND|ND	ND|ND	ND|ND	ND|ND	0.02|ND	0.02|ND	0.02–0.05|36-100	ND|0.89–2.15
Mo	ND|40	0.05|ND	0.01|18	ND|ND	ND|ND	ND|ND	ND|ND	ND|ND	0.01|ND	0.01–0.05|18–40	ND|6.88–12.4
As	0.05–1.0|51	0.05|75	0.1|41	ND|ND	0.2|10	0.4|41–75	ND|ND	0.05|50	0.1|ND	0.05–0.4|10–75	ND|1.64–6.63
Cd	0.01|39	0.01|5–20	0.01|39	ND|ND	ND|5	0.1|39–85	ND|40–85	0.01|30	0.01|3	0.01–0.1|3–85	ND|0.27–0.71
Cu	0.2–1.0|1500	1.0|800–1500	0.2|1500	ND|ND	ND|300	6.0|1500–4300	ND|1500–4300	0.2|500	0.2|450	0.2–6.0|300–4300	0–0.34|167-333
Hg	0.002|17	0.001|5–15	0.002|17	ND|ND	ND|0.15	0.01|17–57	ND|15–55	0.001|40	ND|5	0.001–0.01|0.15–57	ND|1.86–50.0
Ni	0.2–0.5|40	0.1|100–200	0.2|420	ND|ND	ND|50	4.0|420	ND|420	0.2|ND	ND|120	0.1–4.0|40–420	0.09–0.59|18.5–32.9
Pb	0.1–5.0|300	0.2|300–1000	5.0|300	ND|ND	ND|100	10|300–840	ND|300–840	0.1|1000	5.0|150	0.1–10|150–1000	0.0|18.8-35.6
Zn	2–5|2800	2.0|2000–3000	5.0|2800	ND|ND	ND|1000	20|2800–7500	ND|2800–7500	2.0|ND	2.0|1100	2–20|1000–7500	0.08–0.75|1620–2870
Ca	80–400|ND	ND|ND	230|ND	ND|ND	ND|ND	ND|ND	ND|ND	ND|ND	ND|ND	80–400|ND	93–341|31746–76873
Mg	50–120|ND	ND|ND	100|ND	ND|ND	ND|ND	ND|ND	ND|ND	ND|ND	ND|ND	50–120|ND	71–583|2668–5413
Cr	0.05–0.1|1300	0.1|ND	0.1|1200	2|ND	ND|50	1.0|1200–3000	ND|1200–3000	0.05|30	0.1|350	0.05–2|50–3000	0.42–1.86|2803–6950
Fe	0.2–5|ND	1.5|ND	5.0|ND	ND|ND	ND|ND	ND|ND	ND|ND	ND|ND	5.0|ND	0.2–5.0|ND	0.67–2.89|1243–2707
B	0.5–3.0|ND	1.0|ND	1.0|ND	ND|ND	2.0|ND	ND|ND	ND|ND	0.75|ND	0–2.0|ND	0.5–3.0|ND	1.58–7.03|47.3–83
Cl	70–530|ND	350|ND	400|ND	1000|ND	600|ND	ND|ND	0.25|ND	ND|ND	0–710|ND	0–1000|ND	2050–6900|ND
SO$_4$/S	100–1000|ND	ND|ND	500|ND	/1|ND	1000|ND	ND|ND	ND|ND	ND|ND	0–960|ND	0–1000|ND	75–785|ND
Na	50–70|ND	ND|ND	230|ND	ND|ND	#60|ND	ND|ND	ND|ND	ND|ND	#20–80|ND	50–230|ND	*2.73–18.9|6.58–41.8
Reference	1; 2	1; 2; 3	2;3;4	2; 10	5	1; 2; 7	2; 8; 9	9;10	2; 11		

water (mg/L)\|sludge (mg/kg) * water (g/L)\| sludge (g/kg) #% pH (dimensionless) EC=electrical conductivity (mSm^{-1}) Coliform (cfu/100mL) ND=not determined TDS=total dissolved solids

T/SS=total suspended solids (De Morais Ferreira et al., 2019)[1] (Leblanc et al., 2008)[2] (Shoushtarian & Negahban-Azar, 2020)[3] (Elbana et al., 2019)[4] (IL&FS Ecosmart Limited, 2009)[5]

(India Central Pollution Control Board. Ministry of environment and forest, 2006)[6] (Gutiérrez, 2008)[7] (Snyman & Herselman, 2006)[8] (Department of Water Affairs, 2013)[9] (Alemu et al., 2016)[10]

(Jeong et al., 2016)[11]

4.4.6 Concrete and ceramic aggregate

The high concentration of carbon (229–252 g/kg), Si (5.61–2.75 g/kg) and metals (Table 4-6) indicated that the digestate may be suitable as a concrete and/or ceramic aggregate. Abreu and Toffoli (2009) successfully incorporated tannery sludge in the manufacture of ceramic tiles and reported a gain in aesthetics (brightness, color, and texture).. Furthermore, this has the benefit of reducing the production costs as less cement and energy will be used. Juel et al. (2017) achieved 15–47% energy savings after adding 10–40% tannery sludge in clay bricks while Kavouras et al. (2015) reported the incineration (500°C) of tannery sludge to recover Cr (III) and conditioned ash suitable for vitrification. However, co/combustion of digestate offsets volatile toxic heavy metals such as Cr (VI) (Abreu & Toffoli, 2009). The total metal emission limits applicable to digestate combustion/co-combustion in South Africa are 0.05 mg/m^3 (Cd and Cr), 0.5 mg/m^3 (Sb, As, Pb, Cr, Co, Cu, Mn, Ni, V, Be, Ba, Ag, Sn), 1 mg/m^3 (HF), 10 mg/m^3 (HCl and TOC), 50 mg/m^3 (SO$_2$), and $200-800$ mg/m^3 (NO$_x$) (Herselman et al., 2009).

4.5 Process efficiency and inhibition

A review of previous studies on the AD of TE by Mpofu et al. (2021a) reported that NH_3 resulting from protein hydrolysis, metals, VOAs, SO_4^{2-} and/or H_2S from sulfidogenesis are the main inhibitors of methanogenesis. Methanogens dictate the minimum S/HRT as they are the slowest-growing and the most sensitive functional microbes in the consortium.

The control reactors (R18, R19, R20 and R21) and reactors (R5, R16 and R17) treating 100% TYE at different ISR generated negligible biogas that was less than the control, regardless of the operating ISR. All the permanently inhibited reactors, including the acetate control reactor (R19) were seemingly active proving that it was only methanogenesis that was inhibited. The reversible inhibited reactors (R2, R11, R14 and R15) operating at 25 or 100 %BHE suffered short lag phases of 4–6 days (Figure 4-2A–D) and were in agreement with the typical regeneration time (5–16 days) for methanogens (Zupančič et al., 2012). All the active reactors produced >85% of their ultimate CH_4 yield within 20 days HRT except for R6 that only produced 40% and required 34-days to achieve this (Figure 4-2).

4.5.1 Ammonia, volatile organic acids inhibition and pH changes

The lack of CH_4 generation in some of the reactors reflected poor or absent methanogenic activity, but not necessarily a lack of other metabolic processes. The primary mechanism for NH_3 release and accumulation (2.3–40.4% after 40 days), was through protein hydrolysis as evidenced by the decrease in protein concentration (1.4–46.2% after 40 days, data not shown). The increase of hydrolysis metabolites concentrations, namely NH_3 and VOAs, in some reactors was assumed to be due to an imbalance of AD processes. Achouri et al. (2017) attributed the observed inhibition between

day 4 and 12 to the fast formation of VOAs, particularly considering the low operating ISR (=1.5). There was an overall decrease 11–97% in VOAs initial concentrations (149–1283 mg CH_3COOH/L) except for R6 (50%BHE, ISR = 1) that suffered an 85% increase to 2382 mg CH_3COOH/L (Figure A-1, appendix A2). This may have been the cause of permanent inhibition of methanogenesis and dominance of sulfidogenesis in R6. In general, SRB are known to be more resilient to inhibition and they have a higher affinity for hydrogen (H₂) and CH_3COOH compared to methanogens. However, Berhe & Leta, (2018) reported steady-state operations at VOAs concentrations as high as 8300 mg CH_3COOH/L.

The NH_3 concentration at day 0 (228–484 mg/L) and day 40 (264–620) in all reactors fell within the inhibitory concentration range of 53–1450 mg/L reported by previous studies (Mpofu et al., 2021a). The accumulation of NH_3/NH_4^+ led to pH increases above the optimal range of 6.5–7.8 for methanogens in some reactors. The NH_3/NH_4^+ buffering capacity most likely played a huge role in maintaining reactor pH within the optimal range. Apart from R6, the VOA:ALK ratios of all the reactors were within optimal range (<0.3-0.4) for stable reactors (Gao et al., 2015). The alkalinity ($CaCO_3$) in all the reactors increased and suggested microbial utilisation of produced H^+ by oxidising homoacetogens, chemolithotrophic sulfur oxidising bacteria (SOB) and/or hydrogenotrophic methanogens (HMs). The alkalinity concentrations (1550–4030 mg$CaCO_3$/L) were within the optimal range (1000–5000 mg$CaCO_3$/L) for steady system (Berhe & Leta, 2018).

4.5.2 Sulfate and sulfide inhibition

Sulfidogenesis occurred in all the reactors and led to reductions in SO_4^{2-}, with concomitant increases in HS^- concentrations over the 40 days S/HRT (Table 4-3). The majority of the initial reactor COD: SO_4^{2-}(1.1–6.3) suggested the likelihood of competition between sulfidogenesis and methanogenesis. The theoretical optimum COD: SO_4^{2-} ratio for significant COD removal by SRB was reported as <0.67. However, pro-methanogenic COD: SO_4^{2-} ratios as low as 0.5 have been reported under different operating conditions (Omil et al., 1996). The overall decrease in VOAs (11–97%) and SO_4^{2-} (34–85%) from initial concentrations of 300–3800 to 75–2180 mg/L in all reactors (Figure A-1, appendix A1) proved that both acidogenesis and acetogenesis occurred during the lag phase, and in permanently inhibited reactors. This proved that SRB did not only dominate H_2S generation (SO_4^{2-} reduction), but also utilised hydrolysis metabolites and VOAs in reactors operating at high SO_4^{2-} concentrations (820 mg/L≤SO_4^{2-}≤1390 mg/L), particularly R5, R6, R16 and R17 (Figure A-1, appendix A1). The syntrophic degradation of VOAs by SRB played a major role in improving B₀ even without CH_4 generation.

The H_2S was mostly solubilised to HS^- at pH >7 and some of it theoretically precipitated with metals. Precipitation of HS^- with metals can reduce direct HS^- toxicity on functional microbial species, but it can also reduce the bioavailability of essential methanogenic micronutrients. Achouri et al. (2017) and Berhe and Leta, (2018) experienced steady-state conditions using substrates with high initial sulfide concentrations of 320 and 132 mg/L at initial pH 7 and pH 6.2 (approximately 180 and 109 mg H_2S /L), respectively. The H_2S concentrations for R1, R2, R4 and R6 (operating at 25–50% BHE and ISR=1–2.5) at day 40 fell close/within the methanogenesis inhibitory range (IC$_{50}$ =43–125 mg/L at pH 7–8) for suspended sludge (O'Flaherty et al., 1998).

4.5.3 Metal inhibition and stimulation

The concentrations of metals were measured in the soluble (filtered) fraction of the reactor contents, which was assumed to represent the bioavailable fraction. The bioavailability of essential metals (Cu, Ni and Zn) collectively reduced in R1, R2 (except Ni), R3, R4, R6 and R8 (except Cu), R14, R15, and R17 (except Ni) while they increased in R5 and R10. Similarly, Ca, Mn, Cr (except R5), Fe (except R5, R11 and R16), K and Na (except R1, R4, R7, R10, R13) and Mg (except R1, R3, R4, R5, R7, R10 and R13) (Figure A-2, appendix A2) reduced in all the reactors and may have prevented HS^- and metal inhibition. However, the decrease in the bioavailability of essential metals through precipitation and increase of other metals (bracketed) may have contributed to inhibition. It is conceivable that precipitates may have reversibly inhibited functional microorganisms by blocking their access to substrates (Utgikar et al., 2002). Notably, most metals increased in the permanently inhibited reactors (R5, R16 and 17) that mono digested TYE.

Additional experiments conducted by the authors at pilot scale with mechanical mixing over a period of 6 months have demonstrated the selection of specific methanogenic archaea *(Methanobacterium, Methanosaeta and Methanosarcina)* and specific genera of sulfur reducing bacteria *(Desulfovibrio, Desulfomicrobium and Desulfobacterium)* (Kibangou et al., 2021).

4.6 Circular bioeconomy in tanneries

The application of optimum conditions at full-scale would produce about 20.8 ML (mega litres) of biogas with 12.32 ML recoverable as CH_4, when treating 2300 m³ of BHE. Alternatively, this can be used to produce approximately 9.89 MWh of electricity that can be used to process 2300–5205 hides, while reducing electricity demand by 22.5% (Table 4-7). This is based on the assumption that biogas has an average calorific value of 6 kWh/m³ and its conversion efficiency to electricity is 35% (Agustini et al., 2019), while processing a bovine hide requires about 1.9–4.4 kWh (Swartz et al., 2017). The disposal of wastewater in the municipal sewer and landfill disposal of solid waste will be reduced by 22% and 62% while potentially saving US$ 711 and US$ 665, respectively (Table 4-7). There is a

potential revenue of US\$ 3816 from the sale of recycled sulfur and biofertiliser/compost for agricultural applications. Additionally, replacing the activated sludge process with AD would reduce carbon equivalent emissions by 38% (19 tCO_2eq) based on the assumption that BOTE treatment using the activated sludge process produces 3.15 tCO_2eq/t$COD_{removed}$ and that producing a MWh of electricity releases 0.90 tCO_2eq (Climate Transparency, 2021; Giaccherini et al., 2017). Additional tonnes of CO_2eq emissions from trucking sludge to disposal sites will be avoided.

This study demonstrated that the full-scale application of AD in tannery WWTPs of developing countries presents an opportunity for transitioning to a circular bioeconomy through the establishment of onsite biorefineries for the recovery of resources (biofuels, bioenergy, biofertiliser, sulfur and irrigation water), reduction of carbon emissions, and prevention of waste disposal. This will aid in the sustainable socio-economic development of developing countries whose economies are mainly dependent on fossil based energy and raw materials. However, each tannery must conduct a detailed technoeconomic assessment and market research in order to determine the market potential of the most economically viable recoverable resources and viability of implementing AD (Mpofu et al., 2021a). There is also a need for the upskilling of wastewater treatment practitioners as AD is a complex process that is prone to inhibition particular when treating TEs. Governments of developing countries must assist tanneries with capital funding and introduce progressive policies, regulations, incentives and subsidies to support the adoption of AD. Furthermore, governments and the private sector can sign long term off take agreements with tanneries for the reuse and recycling of products, particularly sulfur and/or biofertiliser.

Table 4-7: Evaluation of costs at full-scale application for a medium-sized tannery

Solid waste and wastewater produced	
B/ovine skins/hides processed (35% hides and 65% skins)	10000
Weight of skin/hides (tonne)	115
Weight of shavings produced (tonne)	11
Weight of chrome splits, trimmings and dust produced (tonne)	24
Weight of sludge produced (tonne)	58
Volume of water used (m³)	2471
Volume of wastewater discharged (m³)	2300
Carbon emissions (tonnes of CO_2 equivalent)	49.7
Weight of total solid waste produced (tonne)	93
Average electricity consumption (MWh)	44
Operating costs	
Cost of municipal water (US$)	4948
Cost of wastewater disposal (US$)	3230
Cost of electricity (US$)	5348
Cost of landfill disposal of solid waste produced (excluding transport) (US$)	1080
Potential savings	
Biogas generated (m³/tVS)	639
Electric energy production potential (MWh)	9.89
Reduction in wastewater disposal	22%
Reduction in landfill solid waste disposal	62%
Reduction in electric consumption (%)	22.5%
Reduction in carbon emissions (%)	38%
Potential electricity cost savings (US$)	1177
Wastewater discharge potential savings (US$)	711
Reduction in waste disposal costs (US$)	665
Potential revenue	
Potential impure sulfur sales (US$)	1064
Potential biofertiliser/compost sales (US$)	2752

4.7   Summary

Beamhouse effluent exhibited the most favourable characteristics for anaerobic digestion, and process efficiency and kinetics improved with beamhouse composition and inoculum to substrate ratio up to 100% and 3, respectively. All methanogenic inhibited reactors were active due to the activity of sulfur reducing bacteria. The logistic, modified Gompertz and cone model showed a better fit to the experimental cumulative CH_4 data (0.827≤Adj R^2≤0.999), respectively. The optimum operating conditions at ISR=2.5 and 20 days retention time were ideal for recovering 377 mL CH_4/gVS, 13% and 18% of the inlet sulfur and nitrogen, respectively. Additionally, the process recovered reusable water for irrigation and/or for recycling into the tanning process, and digestate as a biofertiliser and/or ceramic aggregate with energy recovery. The study demonstrated the feasibility of a circular bioeconomy and net positive tannery operations with potential cost savings in electricity demand (22.5%), wastewater disposal (22%) and landfilling of solid waste (62%).

CHAPTER 5

RESOURCE RECOVERY FROM TANNERY WASTEWATER USING AN INTEGRATED BIOLOGICAL SYSTEM: TOWARDS A CIRCULAR BIOECONOMY AND NET POSITIVE TANNERY OPERATIONS

[This chapter investigated the optimisation of a novel hybrid linear flow channel reactor for the pre-treatment of bovine-ovine beamhouse effluent to recover elemental sulfur while promoting the recovery of methane in a mechanically mixed secondary anaerobic sequential batch reactor. The mixing regime (continuous/intermittent and speed) in anaerobic sequential batch reactor was optimised. The final effluent and digestate were evaluated for their suitability to be reused for irrigation and as a biofertiliser, respectively]

5.1 Introduction

Tanneries are an economically important agri-processing subsector in many developing countries. Tanneries alleviate the potential environmental burden caused by the generation of skins/hides from the slaughterhouse industry and create another by generating large volumes of highly toxic effluents, particularly from beamhouse processes. Generally, beamhouse processes produce 20–25 m^3 of effluent per tonne of skins/hides processed. The beamhouse effluent (BHE) contributes about 79% suspended solids, 75% of chemical and biological oxygen demand (BOD and COD), 74% chlorides (Cl), 100% sulfide (HS^-/S^{2-}), and 85% total nitrogen (TN) to the total pollution load (Buljan & Král, 2019). The widely used activated sludge process coupled with physicochemical treatments is not effective in treating BHE to stipulated discharge standards, particularly sulfate (SO_4), sodium (Na), Chloride (Cl) and organics (typically measured as chemical oxygen demand (COD) (Mpofu et al., 2021a; Swartz et al., 2017).

The application of anaerobic digestion (AD) in the treatment of BHE is capable of recovering methane (CH_4), elemental sulfur (S^0), biofertiliser and process/irrigation water (Mpofu et al., 2021b). Mpofu et al. (2022) reported on the concurrent occurrence of methanogenesis and sulfidogenesis at COD: SO_4^{2-} = 1.1–6.3 and consequently increased sulfide concentrations that fell within the inhibitory range (IC_{50} = 43–125 mg/L at pH 7–8) for functional methanogenic archaea (Kibangou et al., 2021; O'Flaherty et al., 1998). It was postulated that a fraction of the sulfides were partially oxidised by nitrite (NO_2^-) and nitrate (NO_3^-) into S^0 by chemolithotrophic sulfur oxidising bacteria (SOB) and anaerobic ammonium oxidation (ANAMMOX) (Mpofu et al., 2022). However, maximum CH_4 and S_0 recovery could not be achieved due to inhibition by SO_4^{2-}/H_2S, ammonia (NH_3), volatile organic acids (VOAs) and oxidising agents(Kibangou et al., 2021b; Mpofu et al., 2022).

Sulfides ($S^{2-}/HS^-/H_2S$) are commonly removed through precipitation and/or oxidation, by adding coagulants, oxidising catalysts and/or extended aeration (Swartz et al., 2017). A number of studies have successfully applied coagulation ($FeCl_3$, $Ca(OH)_2$, and $Al_2(SO_4)_3$, stripping and hydrolysis as pre-treatment steps for the removal/recovery of sulfides and Cr prior to AD of TWW (Aboulhassan et al., 2008; Song & Williams, 2004; Song et al., 2001). However, pre-treatment sometimes leads to decreased biogas/CH_4 yields and/or anaerobic biodegradability (B_0) due to increased concentrations of inhibitory cations (Fe, Ca and Al) and/or anions (SO_4^{2-}, Cl^- and OH^-), and precipitation of organic matter, and nutrients (Genschow et al., 1997; Schenk et al., 1999; Wiemann et al., 1998). Additionally, these approaches are expensive due to associated high chemical and energy costs and disposal costs due to excess hazardous sludge produced (Buljan & Král, 2019; Mpofu, 2018). Efforts by tanners to eliminate sulfur-based feed chemicals such as chrome sulfate ($Cr_3(SO_4)_3$) and sodium sulfide (Na_2S)

have not been successful due to related capital costs and perception of jeopardising the quality of the leather (Mpofu et al., 2021a). Biological SO_4^{2-} reduction (BSR) is a cost-effective alternative for removing sulfur species from TWW that can be used as a pre-treatment step to enhance AD.

The majority of biological processes for $SO_4^{2-}/S^{2-}/HS^-/H_2S$ removal employ two-stage treatment for BSR and $S^{2-}/HS^-/H_2S$ oxidation (SO). However, Sabumon, (2008b) demonstrated the feasibility of recovering S^0 from pretreated TWW by integrating BSR and SO in an up-flow hybrid anoxic bioreactor while injecting air from the bottom. Additionally, Marais et al. (2020) employed a novel hybrid linear flow channel reactor (HLFCR) that integrated BSR and SO for the recovery of S^0 from synthetic wastewater (1 g/L SO_4) emulating acid mine drainage. The HLFCR is a novel semi-passive reactor design that spatially separates anaerobic and aerobic zones while maintaining a very close interaction between the microbial species. The HLFCR creates a vertical stratification which is conducive for the formation of a harvestable floating sulfur biofilm (FSB) and the settling of suspended solids. The FSB is crucial for the partial oxidation of sulfides into S^0. Complete sulfide and S^0 oxidation due to excess O_2 is undesirable as it produces SO_4^{2-}. Studies by Mpofu et al. (2022), and Mpofu et al. (2021b) suggested a two-stage treatment using a specialised bioreactor similar to the HLFCR as a pre- treatment step to maximise the recovery of sulfur species as S^0 to make BHE more amenable for CH_4 recovery.

Therefore, this study sought to build on the work by Horn et al. (2022), Mpofu et al. (2022), Mpofu et al., (2021a) and Mpofu et al. (2021b) of promoting a circular bioeconomy and net positive tanneries in developing countries. The overall aim of the study was to develop an integrated biological treatment process for the recovery of reusable/recyclable methane, sulfur, biofertiliser, and process/irrigation water from bovine/ovine beamhouse effluent using an integrated HLFCR and anaerobic sequential batch reactor (AnSBR) treatment system. To the authors' knowledge, this is the first time that an HLFCR coupled with an AnSBR has been optimised for the recovery of resources besides biogas from bovine/ovine beamhouse effluent.

5.2 Materials and methods
5.2.1 Inoculum and substrate

The BHE used in this study was collected from a South African tannery that processes bovine hides and ovine skins via wet-blue tanning. A robust, acclimated microbial seed consortium was obtained from biomethane potential (BMP) experiments used to treat BHE and TYE as described by Mpofu et al. (2022). A saline-adapted BSR culture acclimated to BHE supplemented with lactate was used as an inoculum during start-up of a HLCFR.

5.3 Set-up and operation of Hybrid Linear Flow Channel Reactors

The pre-treatment experiments were performed in 8.1 L (450 mm (l) x 200 mm (w) x 150 mm (h)) HLFCRs (Figure 5-1A). The bioreactors were fitted with airtight lids and carbon microfibers were suspended on aluminium plates in the bulk liquid. The microfibers promoted microbial biofilm attachment and prevented biomass washout (Figure A-3, appendix A1). A plastic mesh screen positioned at the bulk liquid and gas interface was used to harvest the FSB. The HLFCRs were equipped with three sampling ports at the front, at approximately half the height of the bulk liquid. Additionally, air vents on each side of the bioreactor allowed airflow in the gas interface.

The HLFCRs were initially inoculated with BSR culture blended with BHE (10% v/v) and maintained in batch mode for seven days to allow for the development of a FSB on the mesh screen. The FSB was partially harvested on day 7 and 14. The HLFCRs were thereafter operated in at 4-days HRT for 32 days as previously described by Horn et al. (2022). The FSB was partially harvested every fourth day and the bulk liquid was sampled for analysis. The HLFCRs were thereafter connected in series and maintained at 4-days HRT for an additional 24-days to ascertain the impact of two-stage treatment on process efficiency. One HLFCR was later operated at 8-days HRT for 16 days to ascertain the impact of increased HRT. The treated effluents from single and two-stage HLFCR where fed into the AnSBRs.

5.4 Set-up and operation of anaerobic sequential batch bioreactors

Two 20 L gas-tight polyethylene AnSBRs were set up (Figure 5-1B) and operated at an inoculum to substrate ratio (ISR) of 2.5 (VS/VS), 37±2°C, and pH 7±0.5 (Mpofu et al., 2022). However, an ISR of 4 was used during the AD of settled solids from the HLFCRs (Mpofu et al., 2020b). The AnSBR contents were mixed using a Heidolph Instruments (Schwabach, Germany) Hei-torque 100 programmable overhead stirrer connected to a shaft and pitched four blade marine impeller (Figure 5-1B). The effect of mixing on the treatment of pre-treated BHE using HLFCR was investigated by mixing the reactor contents at different mixing conditions: continuous/intermittently; mixing time (0–24 hrs per day) and speed (0–100 rpm). The overhead stirrer was programmed to mix intermittently or continuously at a certain speed by defining the mixing intervals corresponding to the required mixing time i.e. mixing for 6 or 12 minutes every 12 minutes which translates to mixing time of 12 and 24 hrs (continuous) per day, respectively. The operation of AnSBRs is described else by Kibangou et al. (2021).
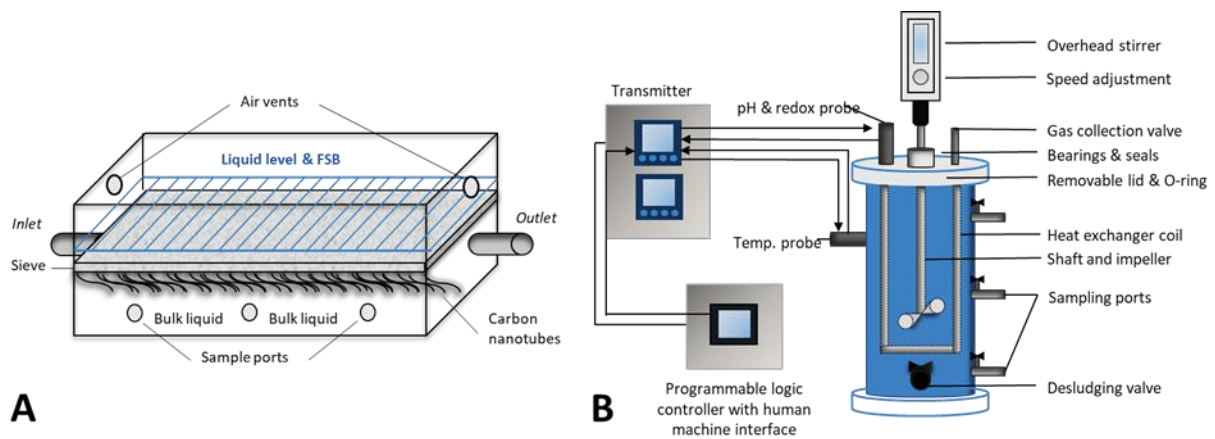
Figure 5-1: Photograph of (A) hybrid linear flow channel reactor and (B) anaerobic sequential batch reactors, and related control systems

5.4.1   Biogas sampling and analysis

The gas ports from the AnSBRs (Figure 5-1B) were connected to individual Tedler bags. The collected biogas was analysed quantitatively and qualitatively (CH_4, CO_2, O_2 and H_2S) using a graduated gas tight luer lock syringe (Lasec, South Africa) and Geotech biogas 5000 analyser (Warwickshire, England), respectively.

5.4.2   Determination of physicochemical parameters

Samples for analysis were obtained at the beginning (feed) and at the end from each reactor to determine process effiiency. A Merck (Darmstadt, Germany) Spectroquant Prove® spectrophotometer together with Merck cell tests or kits were used to determine the concentrations of different parameters shown in Table 4-1. Solids concentrations were determined using standard gravimetric methods at 105°C and 550°C for total solids and total volatile solids, respectively. Major and minor elements were quantified using a Thermo ICap 6200 ICP-AES instrument for trace analyses, and an Agilent (Santa Clara, USA) 7900 ICP-MS instrument for ultra-trace analyses. Quantification of CHNS (%wt) was done using an Elemental Vario EL cube Elemental analyser (Hamburg, Germany). Detailed information on qualitative analysis of BHE is described elsewhere Mpofu et al. (2022), and Mpofu et al. (2021b).

5.4.3   Economic analysis

An economic evaluation of the integrated treatment system was conducted based on the assumption that processing a bovine hide requires about 1.9–4.4 kWh (Swartz et al., 2017), and producing a MWh of this electricity from coal releases 0.90 tCO_2eq (Climate Transparency, 2021) while BHE treatment using ASP produces 3.15 $tCO_2eq/tCOD_{removed}$ (Giaccherini et al., 2017). Electricity generation from biogas was calculated assuming an average calorific value of 6 kWh/m^3 and a conversion efficiency of 35% (Agustini et al., 2019).

5.5 Results and discussion

5.5.1 Performance of hybrid linear flow channel reactors in recovering sulfur

5.5.1.1 *Single-stage treatment*

The formation of the FSB at the air-bulk liquid interface was observed after 24 hours. This coincided with the colonisation of the carbon microfibres in the bulk liquid. After 7 days of operation, the mesh screen was fully covered by a thin white-cream layer of FSB (Figure A-3, appendix A2). This colour resembled the physical characteristic of the expected S^0 which was partially harvested on day 7. The SO_4^{2-} concentration decreased by 78% (420 mg/L) after the first 7 days and by 70% (575 mg/L) on day 14. A similar positive trend was observed with residual ionised sulfide (S^{2-}) concentration which increased from 456 mg/L on day 7 to 594 mg/L on day 14 (Figure 5-2). After day 14 (4-day HRT), the residual SO_4^{2-} and S^{2-} concentrations stabilised at 853–1200 mg/L (745–1093 mgSO_4^{2-}/L; 38–56% reduction) and 358–471 mg/L (484–613 mgS^{2-}/L; 50–63% reduction) respectively, for the rest of the study (Figure 5-2). This translated to average residual concentrations of 907 mgSO_4^{2-}/L (45% reduction) and 461 mgS^{2-}/L (28% reduction). Horn et al. (2022a) operating an HLFCR at 4-day HRT made similar observations with residual SO_4^{2-} and S^{2-} concentrations that stabilised at around 1000 and 520 mg/L in the bulk liquid, respectively. The authors reported an overall BSR of 1230 mgSO_4^{2-}/L and SO rate of < 150 mgS^{2-}/L.day using single-stage treatment HLFCR at 4-days HRT. These results were comparable to those achieved by previous studies (Table 5-1).

Notably, SO_4^{2-} and S^{2-} removal was minimal at 4-days HRT and higher at 7 days HRT (Figure 5-2). The observed trend may have been as a result of impeded oxygen (O_2) mass transfer across the liquid surface after the formation of FSB over 7 days. Sulfide oxidation (SO) has been reported as the rate-limiting step during S^0 recovery (Xu et al., 2012). Denitrification occurred in the HLFCRs as an average reduction of 24% and 71% in nitrate (NO_3^-), 73% and 89% in nitrite (NO_2^-) was observed in single (4- days HRT) and double stage operations, respectively. This suggested the possibility of their use as electron donors by chemolithotrophic (heterotrophic) SOB (Moraes et al., 2012). However, the majority of studies using other biological reactor configurations such as expanded granular sludge bed (EGSB), upflow anaerobic sludge blanket (UASB) and upflow anaerobic filter (UAF) achieved near-complete SO, particularly those that dosed the reactors with O_2 (Table 5-1).

Elemental analysis of the harvested yellow-cream FSB for carbon (C), nitrogen (N), hydrogen (H) and sulfur (S) content showed that the dried FSB from single-stage treatment at 4-days HRT contained 18.7–28.2% C (21.8% average), 0.59–1.47% N (1.0% average), 1.65–3.79% H (2.8% average), and 0.44-2.00% S (1.2% average). The harvested FSB had a yellow-cream colour characteristic of S^0. The results were comparable to those reported by Horn et al. (2022a) who recovered 8-11% S and 38-58%

C from one HLFCR, and 62–77% S and 6.1–6.2% C from a second HLFCR, both operated at 4-days HRT. The low S^0 content may have been due to the harvesting of the formed organic C matrix while only partially formed, and/or the low relative abundance (RA) of SOB, and/or the (re)reduction of the S^0 from the biofilm. It has been shown that the metabolically versatile *Petrimonas* and *Dethiosulfovibrio* can dominate in HLFCRs treating BHE (Horn et al., 2022b). These genera are capable of reducing elemental S^0 and thiosulfate ($S_2O_3^{2-}$) (Qian et al., 2021). *Petrimonas* has also been found co-dominating synergistically with *Desulfovibrio* in a SO_4^{2-} and aromatic hydrocarbon enriched environment. Horn et al. (2022b) suggested that increased O_2 levels at the bulk liquid-FSB may discourage the growth of strictly anaerobic *Dethiosulfovibrio* and *Petrimonas* to increase S^0 recovery from the FSB.

Additionally, the subsequent high sulfide concentrations in the bulk liquid may have inhibited SRB as they were within the inhibitory range (64–448 mgS^{2-}/L) reported in literature at different pH levels (Okabe et al., 1995). However, no BSR inhibition was observed at total sulfide concentrations up to 280 mg/L at pH 7±0.2 while treating synthetic wastewater with high sulfate concentration (Huang & Khanal, 2004; Khanal et al., 2003). The increased O_2 mass transfer into the bulk liquid after the disruption of FSB oxidised sulfide into S^0 and possibly $S_2O_3^{2-}$ and polysulfide (S_n^{2-}). This made it possible to maintain anoxic conditions (−366 <ORP<−322 mV) in the bulk volume of the HLFCRs. This was slightly higher than −410 to −350 mV optimal range for SO reported by Marais et al. (2020) and lower than −275 to −265 mV reported by Huang and Khanal (2004) and Khanal et al. (2003) treating synthetic wastewater. A study by Mooruth (2013) treating acid mine water concluded that colloidal sulfur >64 mg/L at pH 8–9.5 would promote polysulfide formation and subsequently promote the dissolution of S^0. However, excess O_2 due to frequent FSB disruption (every 4-days) after day 14 may have negatively affected the activity of SRB and may have promoted the complete oxidation of formed sulfur compounds back into SO_4^{2-} (Marais et al., 2020).

The bulk liquid pH levels in this study increased from near neutral levels at the beginning to highly alkaline levels (12.6–13.3) in the effluent. In contrast, Horn et al. (2022a) and Marais et al. (2020) observed pH increases of 0.5 –1 unit to about pH 7.5 – 8.0. This can be attributed to BSR and SO that produced alkalinity (bicarbonate) and hydroxyl ions, respectively. Alkalinity increased by 3.0–40% and was generally maintained at a range of 3150–5290 $CaCO_3$ mg/L for the duration of the study. Increases in pH may have led to decreased growth rates of SRB at pH >8 as shown by Vincent O'Flaherty et al. (1998) treating synthetic substrate. The maximum net specific growth rate of pure SRB cultures (*Desulfonema magnum, Desulfobacter postgatei, Desulfotomaculum acetoxidans, Desulfovibrio vulgaris, Desulfobulbus propionicus, Desulfococcus multivorans and Desulfovibrio. sapovorans*) were

reported as 0.35–4.22 days at optimum pH 7.5–8.0 (O'Flaherty et al., 1998). Horn et al. (2022b) reported the strong selection of *Desulfobacterium autotrophicum, Desulfobacter halotolerans, Desulfomicrobium macestii* and *Desulfomicrobium orale* in the HLFCRs treating BHE. The latter remained resilient and the four species reached maximum RA of 57%, 27%, 3% and 65%, respectively (Horn et al., 2022b).

5.5.2   Increased hydraulic retention time

Single-stage treatment at 8-days HRT in this study achieved the removal of 1042–1191 mgSO_4^{2-} / L (54-61% removal) and 910–913 mgS^{2-}/L (90–94% removal). In comparison to operating at 4-days HRT, these results demonstrated an insignificant (p>0.05, F test) decrease of 5–16% in BSR, and a significant (p<0.05, F test) increase of 33–47% in SO, 18–36% in NO_3^- and 7–15% in NO_2^- reduction. It is also plausible that NH_4^+ accumulation may have been partly due to dissimilatory reduction of NO_3^-/NO_2^- from protein hydrolysis to NH_4^+ by chemoorganotrophic bacteria (Mpofu et al., 2022). The results were in close agreement with the 183 and 184 mgS^{2-}/L.day removal reported by Mooruth (2013) and Xu et al. (2012) using a linear flow channel reactor (LFCR) and an expanded granular sludge bed (EGSB) reactor for the treatment of synthetic wastewater and acid mine water, respectively. However, these studies used lactate as a source of readily available carbon unlike this study that only used BHE. In terms of BSR, previous studies achieved higher BSR of 980–5975 mgSO_4^{2-}/L (64–100% removal) due to a longer HRT and higher SO_4^{2-} feed rates (Table 5-1).

The composition of the harvested yellow-cream FSB was 10.8–14% C (12.4% average), 1.5–1.9% N (1.7% average), 1.9–4.4% H (3.1% average), and 26–39.6% S (32.8% average). A mass balance on S at optimum operating conditions (8-days HRT, pH=7.0–7.8, ambient temperature and –366<ORP<–322) using the sulfur content in the harvested FSB, influent and effluent SO_4^{2-} and S^{2-} concentration indicated that 16–25% of influent sulfur was recoverable as S_0 and 36–56% was unaccounted for. Horn et al. (2022a) reported an S recovery of 6.9–10.2% and 39–42% losses. On the other hand, Mooruth (2013), Marais et al. (2020) and Xu et al. (2012) recovered 92, 30% and 72% respectively, treating low-salinity synthetic wastewater. The plausible losses were attributed to the trapped S species in the SRB biofilms in the microfibers and the presence of colloidal S in the bulk volume as evidenced by the milky appearance. Loss due to the emission of H_2S was assumed to be negligible and this was in agreement with the observations made by Mooruth (2013). Sulfur oxidation was predominantly responsible for sulfide removal as the final bulk liquid pH was alkaline (pH=7.3–7.5) and a lack of mixing favoured ionised sulfides over the evolution of H_2S. This was advantageous as H_2S is foul-smelling and hazardous.

5.5.3 Two-stage treatment

Shifting to two-stage pre-treatment using two HLFCRs in series both operating at 4-days HRT achieved effluent concentrations of 902–1001 $mgSO_4^{2-}$/L (944–1043 $mgSO_4^{2-}$/L; 49–54% SO_4^{2-} removal) and 43-111 mgS^{2-}/L (861–929 mgS^{2-}/L; 89–96% S^{2-} removal). In comparison to single-stage treatment at 4-days HRT, this translated to an insignificant ($p > 0.05$, F test) and significant ($p < 0.05$, F test) additional removal of 54 $mgSO_4^{2-}$/L (5.3% removal) and 364 mgS^{2-}/L (82% removal) on average, respectively. However, compared to single-stage treatment at 8-days HRT, there were insignificant differences ($p > 0.05$, F test) of 2–6% in the S^{2-} removal and 0–21% in BSR. Therefore, second stage treatment mainly promoted additional SO and minimal BSR while operating at 4-days HRT. This was in agreement with observations made by Horn et al. (2022a) who observed improved SO (84% removal) in a second HLFCR in series and a lack of BSR after 56 days of operation. Nonetheless, the authors later observed an improved BSR and achieved overall average BSR of 65–70% and almost complete S^{2-} removal. The results were comparable to those achieved in this study and in a study by Genschow et al. (1996) who reported 600–680 $mgSO_4^{2-}$/L BSR (55–58% removal) using two-stage anaerobic treatment of TWW.

Similar to single-stage treatment at 4-days HRT, the composition of FSB had low S content of 1.0-1.4 % (1.2% average), 1.3–1.8% N (1.5% average) and 1.3–1.8% H (1.5% average) and a higher C content of 14.4–15.7% (15% average).
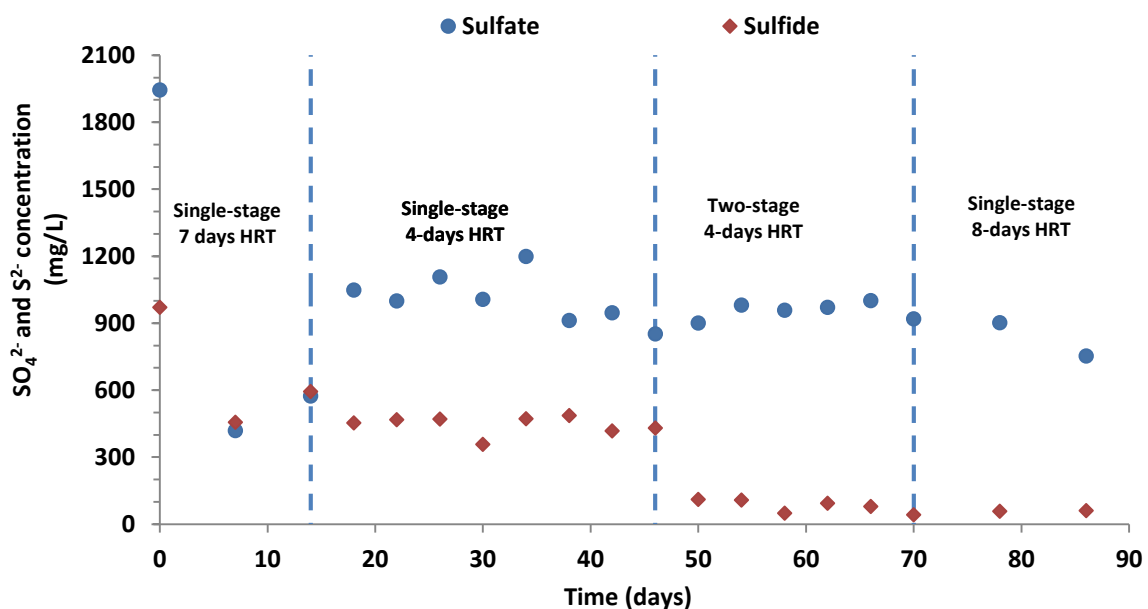

Figure 5-2: Changes in the final S^{2-} and SO_4^{2-} concentrations in hybrid linear flow channel reactors operating at different hydraulic retention times and topology

5.5.4 Performance of hybrid linear flow channel reactors in the degradation of b/ovine tannery effluent

The desirable outcome from the application of HLFCR was mainly the removal of sulfur species (H_2S and SO_4^{2-}) and metals that are toxic to AD while maintaining sufficient residual nutrients. The HLFCRs achieved reduction efficiencies of 47–71% total COD (COD_t), 34–56% total VOA (acetic acid equivalent), 10–40% total organic carbon (TOC), and 7.0–61% NH_4 (Table 5-3) after single-stage pre- treatment. This meant 29–53% COD_t and 60–90% TOC were still available for AD. As expected, higher reduction efficiencies of 62–81% COD_t, and 19–55% TOC were obtained after two-stage pre- treatment as it allowed for an increase in HRT. These were higher than the 15–44% reduction in soluble COD (COD_s) reported by Horn et al. (2022a) after two-stage treatment and comparable to the 59–69% COD reduction achieved by Genschow et al. (1996) using a BSR system during treatment of TWW. However, an increase of 40–234% in total volatile organic acids (VOA_t) (1905–4549 mg/L) and 75–231% in NH_4 (156–294 mg/L), which was theoretically attributed to increased hydrolytic activity (Table 5-3). This was undesirable as high NH_3 (53–1450 mgNH$_3$/L) and VOA_t (4000–6900 mg/L) concentrations inhibit AD (Mpofu et al., 2021a). Nonetheless, the differences in COD and TOC reduction efficiencies between single-stage and two-stage pre-treatment were insignificant (F test, p>0.05).

Coagulation pre-treatment studies by Aboulhassan et al. (2008), Song and Williams, (2004) and Song et al. (2001) reported equivalent reduction efficiencies in the range of 30–71% COD, 38–69 % suspended solids (SS), 74–99% Cr, 79–100% total sulfide and/or 85–86% colour during coagulation studies. However, coagulation is more expensive due to associated high chemical and disposal costs of excess hazardous sludge (Buljan & Král, 2019; Mpofu, 2018). This study also demonstrated the feasibility of reducing the concentration of K, Na, Ca, Mg, Mn, Pb, Fe, and Cr through precipitation as metal sulfides and/or attached to suspended solids (SS) that settled to the bottom of the reactor (Table 5-3). However, during single-stage (8-days HRT) and two-stage treatment, metals were solubilised, as evidenced by an increase in the concentrations of all the measured metals in the bulk liquid, except for Ca. This coincided with a 53–81% decrease in alkalinity ($CaCO_3$) and suggested the utilisation of produced H^+ by oxidising chemolithotrophic SOB (Mpofu et al., 2022). The respective alkalinity range of 3150–5190 and 1440–1765 mgCaCO$_3$/L for single and two-stage configurations, respectively, were within the optimal range (1000–5000 mgCaCO$_3$/L) for steady AD (Berhe & Leta, 2018). Due to the complexity of biochemical reactions taking place, it was not possible to derive a definitive relationship between SO_4^{2-} reduction and COD, VOA_t, TOC, sulfide and/or metals reduction.

The concentration of typical toxicants: VOA, metals (K, Na, Fe, Ca, Mg, Cu, and Cr) and the COD: SO_4^{2-} ratio (>5) in the HLFCR effluent (Table 5-3) were mostly theoretically below the inhibitory range for AD (Mpofu et al., 2021a). However, the respective VOA:Alk ratios of HLFCR effluents were theoretically above optimum (>0.3–0.4) while the C:N ratios were below optimal (6–9) (Berhe & Leta, 2018). The maintenance of pH at 7±0.5 in the AnSBR was likely to abate inhibition by promoting the presence of NH_4^+ and a fair distribution of H_2S and HS^-. Nonetheless, single-stage (8-days HRT) and two-stage HLFCR effluent characteristics were likely to lead to higher CH_4 yields during AD in comparison to AD of the raw BHE. The use of HLFCR as a pre-treatment step was effective in removing S^{2-} and SO_4^{2-} as S^0 and other potential toxicants rendering the effluent amenable for AD.

Table 5-1: Studies on the biological sulfate removal from tannery wastewater and synthetic wastewater

Reactors	1 stage UASB/STR/TR	1 stage LFCR	2-stage EGSB + aeration tank	1 stage HLFCR	3-stage 2xUFHR+	1-stage STR	1-stage UAF	HLFCR
Scale	Pilot	Lab-scale	Lab-scale	Lab-scale	Lab-scale	Lab-scale	Lab-scale	Pilot-scale
Reactor volume (L)	2000/2000/1500	2.13	4&5	2.0	1.8/1.2 & 0.8	1	4.5	8.1
Pre-treatment	Biological	Biological	Biological	Biological	Biological	Biological	Biological	Biological
Substrate	TWW	Synthetic	Synthetic	BHE	pTWW	Synthetic	Synthetic	BHE
Operation mode	Continuous	Continuous	Continuous	Continuous	Continuous	Batch	Continuous	Continuous
HRT (days)	4	4	0.75	4	0.44	0.5–5	3/7	4 & 8
Temperature (°C)	Ambient	28	30	Ambient	30	35	35	Ambient
pH	7.4–8.2	ND	ND	7.0– 7.7	6.0–9.0	8.0	ND	7.0–7.8
ORP (mV)	ND	−350 to−410	ND	−475 to −380	−320 to −220	ND	−300 to −250	−366 to −322
Influent COD_t (g/L)	5.32	0.70	3.00	17–28	2.5–3.9	ND	10–18	22.8±3.7
COD_t removal (%)	75–95	ND	10–88	ND	91–92	ND	ND	47–81
Influent COD_s (g/L)	ND	ND	ND	7–10	ND	ND	ND	–
COD_s removal (%)	ND	ND	ND	15–44	ND	ND	ND	–
Influent SO_4^{2-} (g/L)	1.80	1.00	1.00	1.95	2.85	1.0–10	1/3/6	1.95±0.31
BSR range (mg/L)	250–600	669–820	170–373	513–1049	ND	ND	975–5975	745–1093
BSR (%)	60–80	64–97	49–99	60–80	64–100	22–87	>98	38–78
Sulfide removal (%)	ND	95–100	82–99	>97		ND	ND	50–94
S^0 recovery (%)	ND	30	15–72	6.9-10	63–66	ND	ND	0.44–40
References	(Boshoff et al., 2004)	(Marais et al., 2020)	(Xu et al., 2012)	(Horn et al., 2022a)	(Sabumon, 2008b)	(Oyekola et al., 2010)	(Huang & Khanal, 2004)	This study

BSR = biological sulfate removal COD$_t$ = total chemical oxygen demand Cod$_s$ = soluble COD EGSB = expanded granular sludge bed reactor

Lab = laboratory LFRC=linear flow channel reactor ORP = oxidation reduction potential STR =stirred tank reactor TWW = tannery wastewater

pTWW = primary treated TWWTR = trench reactor UAF = upflow anaerobic filter UFHR = up flow hybrid reactors ND = no data

5.6 Performance of the anaerobic sequential reactor

5.6.1 Biomethane recovery and anaerobic biodegradability of b/ovine tannery effluent

The cumulative biogas (75–450 mL/COD$_{added}$) and CH_4 yields (41–243 mL/COD$_{added}$, and 64-461 mL/COD$_{removed}$) (Table 5-2) obtained in this study were comparable to 75–420 mL biogas/gCOD$_{added}$, 0.21–270 mLCH$_4$/gCOD$_{added}$ and 300–360 mLCH$_4$/COD$_{removed}$ to those reported in previous studies treating pre-treated TWW (Mpofu et al., 2021a). Operating at 50 rpm under continuous mixing achieved superior results for both single and two-stage pre-treated BHE compared to intermittent mixing and continuous mixing at 100 rpm (Table 5-2). The pre-treated BHE from single-stage (8-days HRT) and two-stage configurations achieved higher CH_4 yields of 238 and 243 mLCH$_4$/gCOD$_{added}$, respectively. Horn et al. (2022a) achieved 38.4 mLCH$_4$/gCOD$_{removed}$ from pre- treated BHE (5300 mg COD$_s$ /L) using two-stage HLFCR configuration. In contrast to this study, the authors did not use an inoculum acclimated to pre-treated BHE. The AD of raw BHE with a higher influent COD and TOC (Table 5-3) yielded 1.6 mLCH$_4$/gCOD$_{added}$ in unmixed reactors while operating at 100 and 200 rpm under continuous mixing yielded 21.3 and 4.7 mLCH$_4$/gCOD$_{added}$, respectively.

In terms of B$_0$, a further 31–63% COD$_t$, 21–60% TOC, 74–81% SO_4^{2-}, and 39–82% S^{2-} reduction was achieved. However, there was a 75–304% increase in S^{2-} concentrations while operating at 50 rpm and 12 hrs mixing time. These conditions also led to a 49–56% decrease in VOA concentration while other operating conditions led to 30–310% increase in VOA. Mpofu et al. (2022) reported the syntrophic degradation of VOAs by SRB and the inhibition of methanogens that led to the accumulation VOAs. The reduction in SO_4^{2-} in all reactors demonstrated the coexistence of SRB and methanogens, while the simultaneous NO_2^-, NO_3^- and S^{2-} decrease supported the hypothesis that they were readily utilised as electron donors by SOB. This was also evidenced by the high H_2S content (>10000 ppm) in the biogas. Mpofu et al. (2022) also made a similar observation, together with the formation of a conspicuous white-yellowish layer of S^0 at the interface of the head-space and bulk liquid in BMP experiments. Therefore, mixing conditions and BHE pre-treatment had an impact on CH_4 yields and B$_0$. Schenk et al. (1999) and Wiemann et al. (1998) treating BHE also demonstrated the feasibility of removing 70–86% H_2S using stripping and achieved 9–15% improvement in B$_0$ and/or 40–90% increase in biogas production. Similarly, other pre-treatment studies on TWW using coagulation by Achouri et al. (2017), Song et al. (2003) and Vijayaraghavan and Murthy, (1997) observed improved B$_o$, increased biogas production, reduction in HRT, and/or increased bioreactor capacity.

The majority of the metals in the AnSBR effluent were also below the inhibitory concentration ranges reported in literature except for Na (1184–7696 mg/L) (Mpofu et al., 2021a). The AnSBRs may have

experienced a deficiency of critical metabolic enzyme co-factors Ni, Mg, Zn, Co, Cu and Fe (Table 5-3). The AnSBRs may have operated under inhibited steady-state conditions, particularly due to inhibitory S^{2-} (68–308 mg/L) and NH_3 (176–461 mg/L) levels in the AnSBR effluent. Generally, inhibitory concentrations vary due to the different operating conditions, the complexity of biochemical reactions, microbial community composition, and degree of microbial acclimation. Mesophilic bioreactors have highly diverse and complex methanogenic communities, making the systems more adaptable, robust, and resilient than psychrophilic and thermophilic AD, This allows mesophilic reactor to function at inhibited steady-state conditions (Deng et al., 2014).

5.6.1.1 *Impact of mechanical mixing*

Mixing continuously (24hrs/day) at 50 rpm using the pitched four-blade marine impeller significantly improved (F test, p>0.05) the cumulative CH_4 yield by 33–65% and 63–71% compared to unmixed and intermittently mixed AnSBRs, respectively. This demonstrated the positive impact of continuous mechanical mixing conditions on the activity of microorganisms, rheology and the different biochemical reactions taking place in the AnSBRs. Continuous mixing may have prevented the precipitation of deficient metals leading to their increased bioavailability in the AnSBRs. Similarly, a study by Kibangou et al. (2021) treating ostrich TWW also reported higher CH_4 yields during continuous mixing over intermittent mixing. The authors observed the preferential selection of *Methanosarcina mazei* which made up 3.6–11% and *Methanosarcina soligelidi which made up* 10.9-21 % of the methanogen population. *Methanosarcina* archaea are widely reported as being metabolically versatile, highly resistant to NH_3 and VOAs inhibition and can facilitate hydrogenotrophic, acetoclastic, and methylotrophic methanogenesis (Amin et al., 2021). The small coccoid morphology of methanogens such as *Methanosarcina* renders them less susceptible to impeller shear forces than bacilli and larger cocci.

However, CH_4 yields from unmixed AnSBRs were higher than those achieved by intermittent mixing at 50 rpm (12 hrs) and 100 rpm (24 hrs), suggesting that mixing may have (i) played a role in promoting the transfer of toxicants (S^{2-}, NH_3 and VOAs), and/or (ii) that there was disruption of synergies and destruction of functional biomass due to excessive mixing. It was postulated that the presence of micro-niches in unmixed AnSBRs may have protected microbes against high levels of inhibitory substances in the bulk liquid. This suggested that sedimentation in the unmixed AnSBR may have also provided closer microbial consortia proximity (juxtapositioning), essential in syntrophic microorganisms such as syntrophic SRB and hydrogenotrophic methanogens (Kim et al., 2002). Long- term sedimentation in unmixed reactors led to higher B_0, 85 and 76% COD reduction for raw and pre-treated effluent (Table 5-2). However, the disruption of these microniches and incomplete

mixing of AnSBRs due to intermittent mixing may have led to lower B_0 and CH_4 yields. The AD of pre- treated BHE from the two-stage configuration achieved insignificantly higher (2%) CH_4 yields compared to effluent from single-stage 8-days HRT. In contrast, two-stage and single-stage (8-days HRT) configurations achieved significantly higher CH_4 yields compared to single-stage HLFCRs operating at 4-days HRT. As expected, all pre-treated BHE had ideal characteristics for AD and achieved higher CH_4 yields. This proved the positive impact of pre-treatment.

5.6.1.2 *Biomethane recovery from hybrid linear flow channel reactor settled solids*

As expected, settled solids (SS) from the HLFCRs were unsuitable for AD due to high concentrations of precipitated K, Na, Ca, Mg, and Fe (Table 5-3) that were theoretically inhibitory (Mpofu et al., 2021a). Nonetheless, their AD in unmixed AnSBRs suffered a lag phase of about 10 days (Figure 5-3C) and may have operated under steady-state conditions thereafter leading to longer retention time (RT). The 88 $mLCH_4/gVS$ (38.4% average CH_4) yield achieved in this study after 110 days was comparable to the 89 $mLCH_4/gVS$ (40–57% CH_4) and 84 $mLCH_4/gVS$ (56% average CH_4) reported by Akyol et al. (2014) and Mpofu et al. (2020a) after 40 and 108-days while digesting tannery wastewater sludge (TWS) at ISR = 0.1 and 4, respectively. However, Agustini et al. (2018a) and Agustini et al., (2018b) codigesting TWS with leather shavings reported lower CH_4 yields of 15 (57% average CH_4), and 8.2 $mLCH_4/gVS$ after 200 and 170 days RT, respectively. Another study by Mpofu et al. (2020b) investigating the effect of initial pH (6–9) and operating temperature (23–40°C) during the AD of TWS reported CH_4 yields in the range 12.5–176 $mLCH_4/gVS$ (40–58% average CH_4), 25.4–78.7% VS, 3.6–66.4 %TS and 22.7-51.7 % COD reduction after 108-days
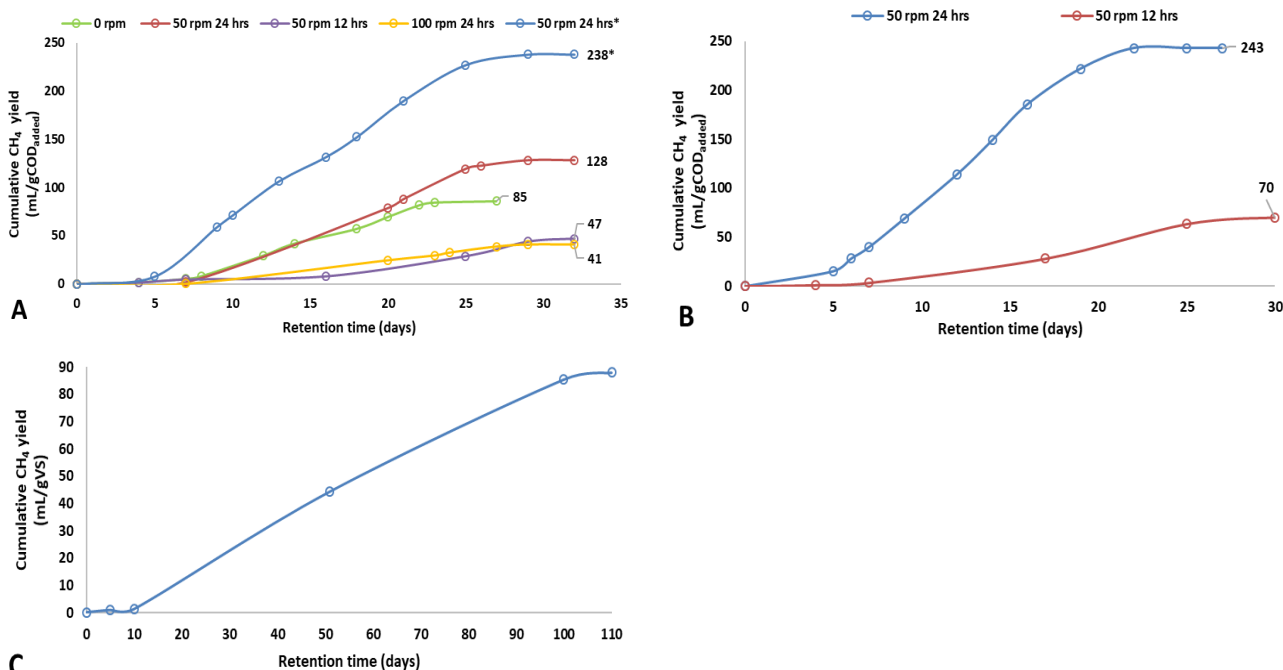
Figure 5-3: Cumulative methane yields from (A): single-stage – 4-days and *8-days HRT, (B): two-stage pre-treated bovine-ovine tannery effluent and (C): settled solids from hybrid linear flow channel reactors at different mixing conditions

Table 5-2: Process efficiency of anaerobic sequential batch reactors treating raw/pre-treated effluent and settled solids

Substrate type and mixing conditions (rpm and hrs)	Methane yields				Biogas quality	B_o
	(mL/gCOD$_{add}$)	(NmL/gCOD$_{add}$)	(mL/gCOD$_{rem}$)	(NmL/gCOD$_{rev}$)	(ave %CH$_4$)	(%COD$_{red}$)
RW–BHE 0 rpm	2.16	1.90	1.55	1.36	14.9	(85)
RW–BHE 100 rpm, 24 hrs	25.2	22.3	21.3	18.9	46.2	(72)
RW–BHE 200 rpm, 24 hrs	7.18	6.32	4.65	4.09	26.8	(65)
PT–BHE 0 rpm	111	96.6	85	74.0	29.4	76
PT–BHE 50 rpm 24 hrs	225	199	128	113	62.0	57
PT–BHE 100 rpm 24 hrs	64	55.8	41	35.7	55.9	61
PT–BHE 50 rpm 12 hrs	104	92.0	47	41.6	34.3	45
**PT–BHE 50 rpm 24 hrs	391	276	238	209	52.3	54
2 PT–BHE 50 rpm 24 hrs	403	285	243	216	58.9	53
2 PT–BHE 50 rpm 12 hrs	159	141	70	62.1	38.5	44
Settled solids 0 rpm	ND	1.90	*88	77.0	38.4	ND

*mL/gVS$_{added}$ ave = average   COD$_{ad}$ = COD added   ( ) = soluble COD   hrs = hours   CH$_4$ =methane

COD$_{rem}$ = COD removed through biological and physical means in AnSBR   rpm = revolutions per minute

RW–BHE = raw beamhouse effluent   PT–BHE = single-stage pre-treated beamhouse effluent (4-days HRT)

**PT–BHE= single-stage pre-treated beamhouse effluent (8-days HRT) B_0 = anaerobic biodegradabilty

2 PT–BHE = two-stage pre-treated beamhouse effluent   ND = not determined

NmL/gCOD = normalised CH$_4$ yield (i.e at standard temperature pressure)

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5.7 Performance of the integrated biological system

The integrated system's best operating configuration was single-stage (8-days HRT) HLFCR and AnSBR (50 rpm continuous mixing, 37±2°C and pH 7.0±0.5) or two-stage HLFCR (4-days HRT in each reactor) and AnSBR (50 rpm continuous mixing, 37±2°C and pH 7.0±0.5). These operating conditions achieved the removal of 80–91% total COD, 78–98% TOC, 89–91% SO_4^{2-}, 92–93% S^{2-}, 50–73 PO_4^{2-}, 48-60 % total nitrogen, 60–72% NO_3^- and 84–86% NO_2^- (Table 5-3). This was comparable to the 40–99% SO_4^{2-}, 82–99% S^{2-} reduction and 43–95% (68–88% soluble COD) reported in previous studies (Mpofu et al., 2021a). However, the hydrolysis process particularly in the second HLFCR (two-stage configuration) and/or in AnSBRs led to a 68–81% and 162–400% increase in VOAs and ammonia, respectively. A number of metals seemingly increased in concentration partly due to their presence in the inoculum used and the different physical, chemical and/or biochemical reactions taking place. Nonetheless, an overall decrease of 79–81% in Ca, 56–62% Sr, and 57–72% Ba was recorded.

The system achieved the production of 241±4 $mLCH_4/gCOD_{added}$ from BHE and 88±2 $mLCH_4/gVS_{added}$ from treating SS from HLFCR. The final treated effluent met many of the irrigation standards for bovine leather-producing countries (Brazil, China, Egypt, Ethiopia, India, Mexico, South Africa and South Korea Turkey) except for Na, Cl, NH_3/NH_4, total nitrogen and COD (Table 4-6). Similarly, the digestate met the stipulated standards for its application on land in most countries except for Cr. Chrome (III) is insoluble in water and hence it attaches and settles with solids. In the South African context, the treated BHE met the standards for irrigating up to 50 m³/day and the A1b sludge guidelines (Department of Water Affairs, 2013; Snyman & Herselman, 2006). Mpofu et al. (2022) recommended further treatment and/or dilution of TE for reuse e.g. in soaking, de/liming and/or tanning. Further treatment processes would promote the recovery of VOA (2284–2465 mg/L) and NH_4 (232–444 mg/L) that were still present in high concentrations (Table 5-3).

Table 5-3: Concentration of bovine/ovine tannery effluent and sludge at different stages of the integrated treatment system and South African discharge standards

	Beamhouse Effluent (mg/L)	HLFCR (mg/L) Single stage	HLFCR (mg/L) Two stage	HLFCR SS (mg/kg)	Digested SS (mg/kg)	AnSBR (mg/L) *50 rpm 24 hrs	AnSBR (mg/L) 50 rpm 24hrs	Irrigation Standard (mg/L) South Africa	Irrigation Standard (mg/L) Other countries	Land application (mg/kg) South Africa	Land application (mg/kg) Other countries
TS	32500±1650	ND	ND	123000±424	ND	ND	ND	ND	ND	ND	ND
COD	22780±3737	8723±1480	6373±1681	ND	ND	3790±154	2505±276	75–5000	40–5000	ND	ND
TOC	4603±10.6	3748±707	2925±811	ND	ND	2520±14.1	1510±78	ND	ND	ND	ND
Alk	3780±424	3892±583	1405±302	ND	ND	2003±539	2075±332	ND	ND	ND	ND
VOA	1362±31.4	721±104	2971±1171	ND	ND	2465±21	2284±98	ND	ND	ND	ND
Sulfate	1945±313	907±240	956±38	ND	ND	179±34	205±49	ND	0–1000	ND	ND
Sulfide	1119±17.7	461±59.6	81±29	ND	ND	77±10.4	68±3.5	ND		ND	ND
NH$_4$	43.6±12.4	51.9±16.5	232±53.8	ND	ND	444±55.6	232±19	0–3	0–50	ND	ND
pH	16.2	13.1	7.44	ND	ND	7±0.5	7±0.5	5.5–9.5	5–10	ND	ND
PO$_4^{2-}$	16.2±1.0	7.90±2.38	5.25±4.88	ND	ND	4.35±0.07	8.15±5.7	10	0.2–30	ND	ND
NO$_3^-$	11.5±0.9	10.5±3.14	4.04±1.57	ND	ND	3.9±0.14	5.7±0.1	15	0–50	ND	ND
NO$_3^-$	4.5±1.2	1.26±0.23	0.5±0.29	ND	ND	0.70±0.01	0.65±0.01			ND	ND
TN	1163±212	811±138	680165	ND	ND	ND	610±9.2	ND	15–70	ND	ND
Cl	9723±48	8268±2907	8950±69	ND	ND	5854±47	7850±495	0.25 as Cl$_2$	0–1000	ND	ND
Na	2141	2014±830	4827±1231	21.3±11	40.2±5.76	2769	2378	ND	50–230	ND	ND
As	ND	<0.01	<0.01	1.06±0.01	1.05±0.32	<0.01	<0.01	ND	0.05–0.4	ND	10–75
Se	ND	<0.01	0.02±0.01	0.26±0.07	0.39±0.06	0.05	<0.01	ND	0.02–0.05	ND	36–100
Mo	ND	<0.01	<0.01	0.28±0.01	1.48±0.33	<0.01	<0.01	ND	0.01–0.05	ND	18–40
Si	0.20	0.14±0.02	1.7±0.88	1.3±0.14	1.12±0.07	5.67	1.98	ND	ND	ND	ND
K	31	19.8±6.93	201±69	0.4±0.17	1.69±0.1	56.4	34.3	ND	ND	ND	ND
Ca	660	485±206	425±229	250±18	61±14.5	141	125	ND	ND	ND	80–400
Mg	1.34	0.06±0.01	6.23±2.14	6.3±0.75	2.91±0.59	13.4	6.31	ND	50–120	ND	ND
Ni	ND	0.022±0.005	0.057±0.05	3.38±0.39	5.87±2.34	0.14	0.095	ND	0.1–4.0	420	40–420
Al	0.06	0.13±0.07	0.63±0.27	8245±2347	3451±739	0.05	0.13	ND	ND	ND	ND
Cu	ND	<0.01	0.15±0.09	14.6±0.91	28.4±3.5	0.17	0.06	ND	0.2–6.0	1500–4300	300–4300
Fe	0.03	<0.01	0.08±0.03	740±2.66	2113±343	0.04	0.07	ND	0.2–5.0	0.5–3.0	ND
Zn	0.13	0.11±0.07	0.11±0.03	29.5±2.58	122±31	0.18	0.25	ND	2.0–20	2800–7500	1000–7500
Sr	1.57	1.1±0.54	2.3±0.95	669±34.7	196±40.5	0.59	0.70	ND	ND	ND	ND
Ba	0.22	0.16±0.08	0.19±0.08	78.8±2.29	41±9.35	0.06	0.09	ND	ND	ND	ND
Cd	<0.01	<0.01	<0.01	0.02±0.005	0.09±0.02	<0.01	<0.01	ND	0.01–0.1	40–85	3–85
Pb	0.018	0.11±0.03	0.11±0.02	1.78±0.09	5.29±1.62	<0.01	<0.01	ND	ND	300–840	150–1000
B	0.54±0.07	0.58±0.07	0.54±0.04	24.3±3.46	16±3.0	0.56	0.57	ND	ND	ND	ND
Cr	<0.01	<0.01	<0.01	9.1±0.76	2030±446	0.03	<0.01	ND	0.05–2.0	1200–3000	50–3000
Hg	<0.01	<0.01	<0.01	0.03±0.001	0.32±0.17	<0.01	<0.01	0.001–0.01	ND	15–55	0.15–57

AnSBR = anaerobic sequential batch reactor   HLFCR = hybrid linear flow channel reactor   *8days HRT   SS = settled sludge   rpm = revolutions per minute

5.8 Kinetic study of anaerobic sequencing batch reactor performance

The Cone (Adj R^2>0.988), Logistic (Adj R^2>0.986) and first-order (Adj R^2>0.902) kinetic models displayed the best fit of the experimental cumulative CH_4 yields. However, the first-order, Chen & Hashimoto (0.825<Adj R^2<0.984) and modified Gompertz (0.343<Adj R^2<0.993) models yielded impractical values of ultimate cumulative CH_4 yields (A) and/or maximum CH_4 production rate (microbial specific growth rates) (μ_m) (Table 5-4). The first-order and C&H models displayed similar straight line trends (Figure 5-4) leading to high A and very low μ_m. The modified Gompertz model significantly underestimated A due to its exponential rise to a maximum regression. The Cone and Logistic model were good at regressing sigmoidal cumulative CH_4 yields that included the lag, exponential rise and plateau phases generally displayed by inhibited steady-state bioreactors treating tannery effluents or sludge (Mpofu et al., 2022, 2021b, 2020a, 2020b).

The kinetic constants obtained in this study were μ_m = 2.88–19.5 mLCH$_4$/gCODd^{-1}, reaction rate (K) = 0.04–0.08 day^{-1}, and lag phase (λ) = 8.1–14.9 days depending on the pre-treatment configuration and mixing conditions. The highest μ_m (12.7–19.5 mLCH$_4$/gCODd^{-1}) and K (0.06–0.08) were obtained in continuously mixed AnSBRs operating at 50 rpm (Table 5-4). The two-stage pre-treated BHE achieved higher values probably due to relatively lower levels of inhibitors and higher levels VOAs that were below inhibitory range. However, continuously mixing at 100 rpm led to the lowest reaction kinetics (A, μ_m and K) due to the negative impact of excessive mixing (section 3.7.1.1). The kinetic constants obtained in the unmixed AnSBR were relatively similar (F test, p>0.05) to those obtained in intermittently mixed reactors (Table 5-4). Similarly, kinetics obtained in mixed AnSBRs were comparable to μ_m = 0.17–17.5 mLCH$_4$/gVSd, K = 0.025–0.27 day^1 and λ = 3–27 days reported by Mpofu et al. (2021b) while digesting ostrich tannery effluent. Additionally, BMP tests on raw BHE by Mpofu et al. (2022) reported higher μ_m (= 24.6–74.2 mLCH$_4$/gVSd^{-1}) but equivalent K (= 0.08 day^{-1}) and λ (= 5–12 days). This demonstrated the positive effect of micro-niches resulting from sedimentation in unmixed bioreactors (section 3.2.1.1). The K:μ_m ratios (>1) in this study demonstrated that methanogenesis was the rate-limiting step due to its inhibition.

The kinetics of the AD of settled solids were μ_m = 3.76 mLCH$_4$/gCODd^{-1}, K = 0.02 and λ = 39 days. This was similar to μ_m = 2.04–5.48 mLCH$_4$/gVSd^{-1} obtained by Sri Bala Kameswari et al. (2014) and K=0.0185–0.0239 d^{-1} reported by Thangamani et al. (2010, 2009) during the co-digestion of tannery sludge with tannery solid wastes. In contrast, Mpofu et al. (2020a, 2020b) reported lower K = 0.008– 0.012 day^{-1} and μ_m = 1.32–2.84 mLCH$_4$/gVSd^{-1} during the AD of waste-activated tannery sludge that was recalcitrant nature. The authors further reported improved K = 0.011–0.0291 d^{-1} and μ_m = 4.37– 18.1 mLCH$_4$/gVSd^{-1} after codigestion with slaughterhouse sludge.

Table 5-4: Kinetic parameters and goodness of fit of models fitted onto cumulative methane yields of anaerobic sequential batch bioreactors

Runs	Model	Kinetic parameters					AdjR^2	p-value Prob>F	AIC	RMSE
		A	μ_m	Λ	K	n				
		(mLCH$_4$/gCOD)	(mLCH$_4$/gCODd^{-1})	(d)	(d^{-1})					
0 rpm	Cone	104	ND	ND	0.06	3.45	0.994	0.497	112	1.65
(1 stage)	Logistic	90.2	6.42	8.1	ND	ND	0.991	0.473	120	1.94
	First-order	95	ND	ND	0.05	ND	0.974	0.488	208	9.79
	C & H	9.5E5	0.31	ND	74674	ND	0.953	0.455	167	17.0
	Gompertz	85	0.93	32.1	ND	ND	0.924	0.022	241	17.9
50 rpm	Logistic	53.8	3.02	14.9	ND	ND	0.992	0.482	93.2	0.85
12 hrs	Cone	60.1	ND	ND	0.04	4.63	0.988	0.467	108	1.13
(1 stage)	C & H	1.2E6	0.56	ND	4.7E5	ND	0.921	0.386	170	2.64
	First-order	7.9E5	ND	ND	1.7E-6	ND	0.902	0.321	178	2.94
	Gompertz	12.6	1.23	8.14	ND	ND	0.462	0.216	236	6.90
50 rpm	Logistic	130	12.7	13.9	ND	ND	0.999	0.498	41.6	0.41
24 hrs	Cone	131	ND	ND	0.05	8.51	0.999	0.492	66.6	0.61
(1 stage)	C & H	9.8E4	4.0E5	ND	9.2E9	ND	0.939	0.370	215	6.14
	First-order	1.8E5	ND	ND	2.4E-5	ND	0.937	0.370	215	6.13
	Gompertz	35.9	5.57	3.35	ND	ND	0.343	0.117	290	19.8
50 rpm	Cone	302	ND	ND	0.06	2.37	0.994	0.491	185	3.82
24 hrs	Gompertz	97.6	4.63	12.6	ND	ND	0.993	0.486	185	3.86
(*1 stage)	Logistic	247	13.2	5.67	ND	ND	0.989	0.465	201	4.93
	C & H	1.6E4	105	ND	2.0E5	ND	0.952	0.405	242	9.37
	First-order	4381	ND	ND	0.002	ND	0.962	0.401	366	9.39
100 rpm	Cone	46.5	ND	ND	0.05	4.59	0.996	0.237	49.8	0.47
24 hrs	Logistic	42.8	2.88	12.0	ND	ND	0.996	0.482	55.1	0.51
(1 stage)	C & H	4.9E5	0.35	ND	1.2E5	ND	0.961	0.481	125	1.51
	First-order	4.3E3	ND	ND	3.1E-4	ND	0.945	0.362	136	1.79
	Gompertz	11.2	1.83	3.52	ND	ND	0.348	0.12	215	6.16
50 rpm	Logistic	73.3	5.23	11.6	ND	ND	0.999	0.499	-5.3	0.19
12 hrs	Cone	75.9	ND	5.18	0.05	5.47	0.998	0.483	62.5	0.60
(2 stage)	C & H	2.0E5	0.32	ND	1.7E6	ND	0.954	0.481	153	2.71
	First-order	7.9E5	ND	ND	2.8E-6	ND	0.921	0.357	169	3.55
	Gompertz	32.0	3.60	15.0	ND	ND	0.780	0.285	263	16.9
50 rpm	Logistic	249	19.5	6.15	ND	ND	0.998	0.493	136	0.49
24 hrs	Cone	278	ND	ND	0.08	3.11	0.995	0.492	160	4.03
(2 stage)	C & H	531	0.21	ND	4.77	ND	0.984	0.490	195	7.73
	First-order	1.2E4	ND	ND	8.5E-4	ND	0.929	0.335	234	15.8
	Gompertz	69.5	10.5	7.05	ND	ND	0.507	0.203	286	41.6
0 rpm	Cone	101	ND	ND	0.02	2.85	0.999	0.496	-192	0.097
(Settled	Logistic	86.6	3.76	39.3	ND	ND	0.999	0.494	-19	0.21
Solids)	First-order	120	ND	ND	0.01	ND	0.971	0.403	412	1.52
	C & H	0.12	5.6E-5	ND	0.99	ND	0.825	0.312	610	3.74
	Gompertz	45.0	9.69	20.0	ND	ND	0.846	0.167	890	13.3

μ_m = maximum CH$_4$ production rate constant (maximum specific growth rate of microorganisms) $\qquad$ Λ=lag phase

A = ultimate CH$_4$ yield $\qquad$ K = specific rate constant $\qquad$ n = shape factor constant $\qquad$ P = cumulative CH$_4$ yield

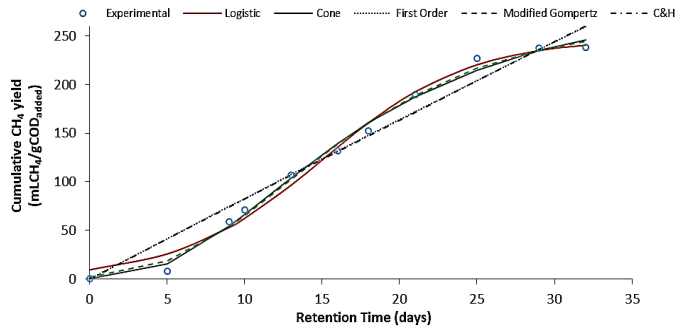C&H= Chen and Hashimoto model $\qquad$ * = single-stage 8-days HRT
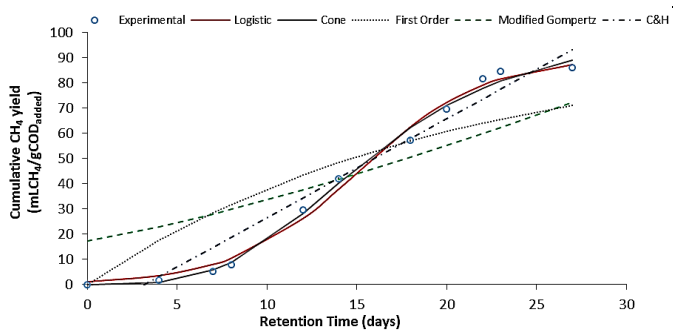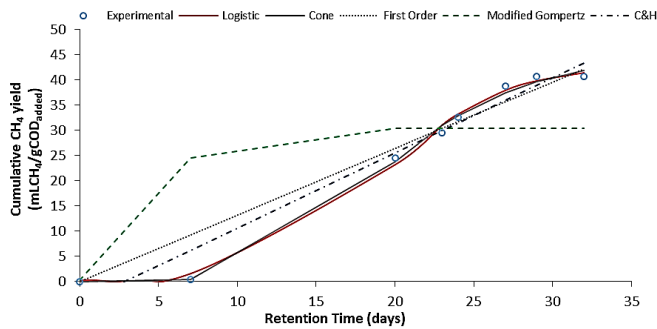
A: 0 rpm (1 stage)
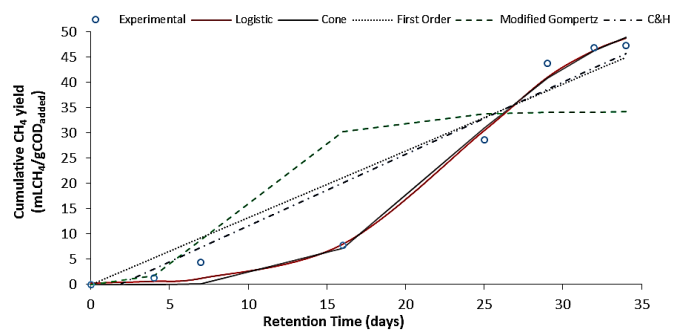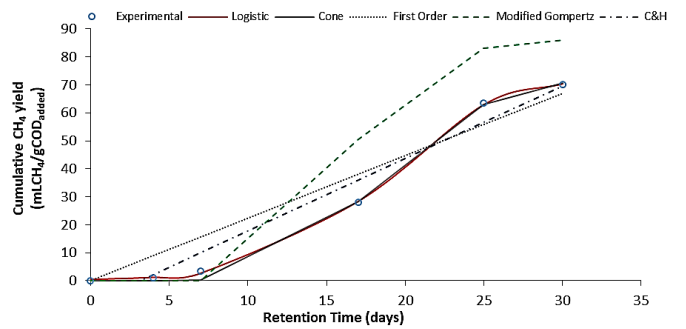
B: 50 rpm, 12 hrs (1 stage)
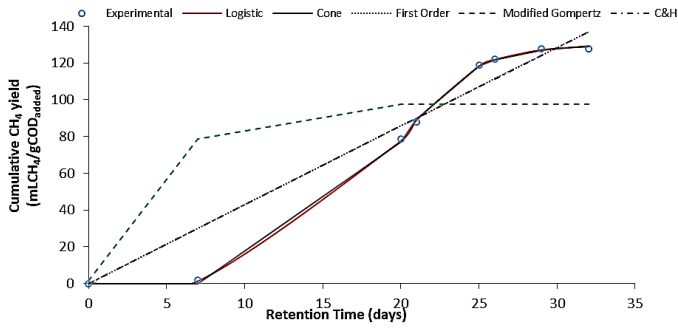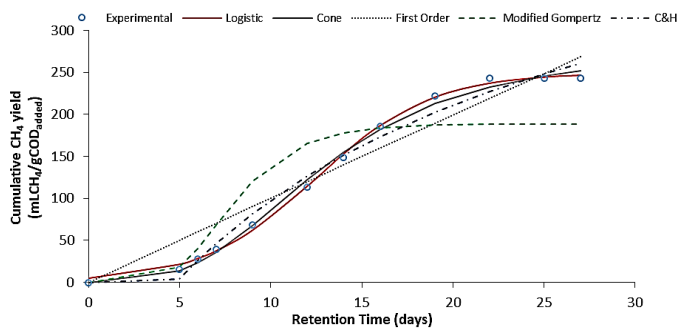
C: 50 rpm, 24 hrs (1 stage)
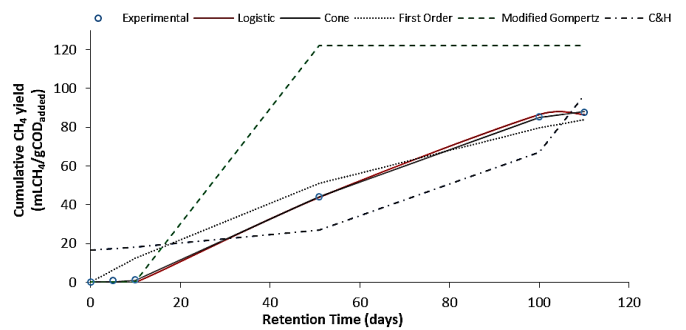
D: 50 rpm, 24 hrs (*1 stage)

E: 100 rpm, 24 hrs (1 stage)

F: 50 rpm, 12 hrs (2 stage)

G: 50 rpm, 24 hrs (2 stage)

H: 0 rpm (settled solids)

Figure 5-4: Graphs depicting the experimental and kinetic model curves for cumulative methane yields during anaerobic digestion of single and two-stage pre-treated b/ovine tannery effluent and settled solids from the hybrid linear flow channel reactor at different mixing speed and time

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5.9 Full-scale application of the integrated biological treatment system

The results from the integrated system were extended to a full-scale application at a medium-sized tannery. It was estimated that the integrated system can potentially generate US$5559 through the recovery of an impure sulfur compound with 33% S^0 (307 kg), about 5.1 mega litres (ML) of biogas (67% average CH_4) and 31 tonnes of biofertiliser/composting material when treating 2.3 ML/day of BHE (Table 5-5). Alternatively, the biogas generated can be used to produce approximately 32.4 MWh of electricity that can be used to process 7359–17042 hides, while reducing electricity demand by 72% (Table 5-5). There is a potential revenue of US$25.97 and US$1062 from the sale of the recovered FSB and biofertiliser/compost for agricultural applications. Additionally, reducing the demand for coal-generated electricity and replacing the widely used conventional activated sludge process (ASP) would reduce carbon equivalent emissions by 94% (169 tCO_2eq).

Table 5-5: Economic analysis at full-scale daily application for a medium-sized tannery

Cost analysis of waste management	
Solid waste and wastewater generated	
B/ovine skins/hides processed (35% hides and 65% skins)	10250
Weight of skin/hides (tonne)	113
Weight of sludge produced (tonne)	56.5
Volume of water used (m³)	2426
Volume of wastewater discharged (m³)	2258
Average electricity consumption (MWh)	45.1
Carbon emissions (tonnes of CO_2 equivalent)	396
Operating costs	
Cost of municipal water (US$)	4662
Cost of wastewater disposal (US$)	3043
Cost of electricity (US$)	5261
Cost of landfill disposal of solid waste produced (excluding transport) (US$)	1047
Potential savings	
Biogas (CH_4) generated (m³/m³ BHE $_{added}$)	5.1 (3.42)
Electric energy production potential (MWh)	32.4
Reduction in electricity consumption (%)	72%
Reduction in wastewater disposal (%)	*88%
Potential electricity cost savings (US$)	3777
Wastewater discharge potential savings (US$)	67.39
Reduction in landfill disposal of solid waste (%)	62%
Reduction in waste disposal costs (US$)	626
Reduction in carbon emissions (%)	**94%
Potential revenue and investment summary	
***Potential impure sulfur sales (US$)	25.97
Potential biofertiliser/compost sales (US$)	1062
Payback period (years)	5.3
Net present value (US$)	3 623 482
Internal rate of return (%)	18%

*Assuming treated effluent is reused for irrigation and other non-potable purposes

** Without taking into account CO_2 released during the generation of electricity from biogas

***Assuming that the process recovers 0.14 kgS^0/m³ of treated effluent

5.10 Summary of results

Pre-treated beamhouse effluents produced by single-stage (8-days HRT) and two-stage (4-days HRT each) HLFCR configurations exhibited the most ideal characteristics for the anaerobic digestion (AD). Their treatment in continuously mixed AnSBR at 50 rpm achieved the highest yields (238 and 243 mLCH$_4$/gCOD$_{added}$,) and kinetics (μ_m = 12.7–19.5 mLCH$_4$/gCODd^{-1} and K = 0.06 – 0.08 d^{-1}) that were determined using the Cone (Adj R^2>0.988) and Logistic (Adj R^2>0.986) models. However, the single-stage HLFCR configuration (8-days HRT) recovered the most S^0 compared to single (4-days HRT) and two-stage configurations. The CH$_4$ yields and reaction kinetics obtained in the unmixed AnSBR were relatively similar to those obtained in intermittently mixed reactors while continuously mixed at 100 rpm led to the lowest yields and kinetics due to the negative impact of excessive mixing. The novel integrated biological system with the HLFCR working at 8-days HRT and AnSBR mixed at 50 rpm continuously, demonstrated the feasibility of a circular bioeconomy and net positive tannery operations.

The integrated system operating at optimum conditions can potentially generate US$5559 through the recovery of FSB containing 33% S^0 (307 kg), about 5.1 mega litres (ML) of biogas (67% average CH$_4$) and 31 tonnes of biofertiliser/composting material when treating 2.3 ML/day of BHE. The recovered CH$_4$ can be used to generate approximately 32.4 MWh of electricity that can be used onsite to process 7359–17042 bovine hides in a medium sized tannery. In the South African context, the treated BHE met the standards for irrigating up to 50 m^3/day and the A1b sludge guidelines for agricultural application. Further treatment of BHE to promote the recovery of VOA and NH$_4$ that were still present in high concentrations would be vital in achieving an effluent that can be used within the tannery operations and/or irrigation up to 2000 m^3. Tanneries, must also promote the processing of green hides and/or separation of the soaking stream as it contributes high Na and Cl to the BHE.

CHAPTER 6

CONCLUSIONS AND

RECOMMENDATIONS

6.1 Summary of results

6.1.1 Effluent characteristics

The slaughterhouse–ostrich tanneryeffluent (SOTE), beamhouse (BHE) and tanyard effluent (TYE) batches exhibited significant inter- and intra-site variability. The variabilities could translate into unstable bioreactor performance during AD that may be overcome to some extent by using acclimated inoculum, bioaugmentation, use of a balancing tank and/or pulse feeding.

The pH, COD: SO_4^{2-}ratio, and alkalinity of all batches of SOTE were favourable for AD. The nutrient balances in the SOTE were non-ideal due to the low P, Cu, Zn, Co, Cd and Ni concentrations and the high TN concentrations. As a substrate for AD, the SOTE would theoretically require a well-acclimated inoculum, and nutrient addition to counter the deficient and/or inhibitory concentrations of metals, SO_4^{2-}, TN, and/or PO_4. In the case of BHE and TYE, nutrient balances were also non-ideal due to the low PO_4, Cu, Zn, Co, Cd and Ni concentrations and the high TN concentrations. The NO_2^- and NO_3^- were also very low and were likely to limit HS^-oxidation without the addition of oxygen. Generally, TYE had higher concentrations of essential metabolic co-factors Ca, Mg, Cu and Cr and lower concentrations of Zn, Ni, Co, and Cd than the BHE.

The SO_4^{2-} concentrations of TYE were notably higher than BH effluent. The COD:SO_4^{2-} were in the ranges (values) that support concurrent sulfidogenesis and methanogenesis for TYE and methanogenesis over sulfidogenesis for BH effluent. The HS^- concentrations in the TYE were deemed to be non-inhibitory, while those in the BH effluent fell within the inhibitory range. Based on the characteristics of TYE and BHE, their AD was predicted to suffer SO_4^{2-} and/or H_2S inhibition. Theoretically, BHE and TYE effluents could be successfully co-digested in order to balance the deficient nutrients and/or dilute inhibitors, particularly SO_4^{2-}, H_2S, and NH_3.

6.1.2 Anaerobic digestion of tannery effluents

6.1.2.1 *Ostrich slaughterhouse–tannery effluent*

The ideal operating conditions determined using the response surface methodology (RSM) for maximal CH_4 yield and anaerobic biodegradability (B_o), were [SO_4^{2-}] = 922 mg/L and ISR = 3.7. Within the range of parameters tested, methanogenesis was strongly inhibited at higher [SO_4^{2-}] ≥ 1960 mg/L and/or lower ISR < 3.0. It was deduced that at an average [SO_4^{2-}] = 646±417mg/L expected in SOTE, and high ISR ≥ 3, SOTE is an excellent candidate for AD 'as is'. There is no need for pre-treatment to remove sulfur species ($SO_4^{2-}$and $H_2S/HS^-/S^{2-}$). Methanogenic activities were determined by fitting the cone (Adj R^2 ≥ 0.977) and logistic (Adj R^2 ≥ 0.976), and modified Gompertz (Adj R^2 ≥ 0.664) models.

The reaction kinetics (K) = 13.4–17.5 d^{-1} and maximum CH_4 production rate (μ_m) = $0.15 - 0.27$ mLCH_4/gVSd^{-1}) were highest at ISR ≥ 3 and/or lower [SO_4^{2-}] ≤ 710 mg/L.

The anaerobic sequential batch reactor (AnSBR) were operated at ISR = 4 based on the optimum conditions determined during BMP tests and continuously mixed at 50–300 rpm achieved $31 - 208$ mLCH_4/gVS while treating SOTE with [SO_4^{2-}] ≈ 680 mg/L. The optimal conditions at 200 rpm, ISR = 4 and 21 days H/SRT achieved 208 mLCH_4/gVS while recovering irrigation water and a biofertiliser. The specific CH_4 yields were significantly higher than BMPs (0.0–146 mLCH_4/gVS). The treated SOTE only met the stipulated discharge standards applicable for irrigation with up to 500 m^3/day in South Africa.

The microbial analysis demonstrated that the selection of sulfidogenic and methanogenic community structures in the BMPs was mainly driven by combinations of [NH_3], [VOA], [TOC], and [alkalinity], and *dsrB* copy numbers. There was an abundance (1 to 2 times higher) of methanogens (3.63×10^5 to 6.46×10^6 copy numbers/ng DNA) than sulfate reducing bacteria (3.63×10^5 to 6.46×10^6 copy numbers/ng DNA) in the BMP bioreactors. The *Desulfofustis glycolicus,* known for SO_4^{2-} reduction was found in high RA (16%) in bioreactors operating at SO_4^{2-} ≥ 1960 mg/L compared to RA <0.003% in other bioreactors. Similarly, in ASBRs, the copy numbers of *mcrA* ranged from 9.32×10^6 to 1.32×10^7/ng DNA while *dsrB* gene copy numbers varied between 2.27×10^5 to 6.72×10^5/ng DNA, indicating the dominance of methanogenesis, particularly *Methanosarcina mazei* with higher RA of 15–32%. A fraction of the HS^- formed from sulfidogenesis was oxidised into S^0 and a white–yellowish layer was formed at the interface of the bulk liquid and head space of BMP bioreactors.

6.1.2.2 *Beamhouse and tanyard effluent*

Bioreactors with higher TYE compositions and operating at very high/low inoculum to substrate ratios (3<ISR≤2) suffered severe methanogenesis inhibition but revealed that sulfidogenesis took place. It was conclusively established that BHE had the most favourable characteristics for AD. The optimal operating conditions (ISR = 2.5, 100% BHE and 20 days H/SRT) recovered 639 mL biogas/gVS (59% CH_4, 11% CO_2 and >10000 ppm), and 13% of the inlet sulfur as S^0. The logistic, modified Gompertz and cone model showed a better fit to the experimental cumulative CH_4 yields (0.827 ≤ Adj R^2≤0.999), respectively. Process efficiency and kinetics (μ_m and K) improved with increasing BH composition and/or ISR. The highest μ_m (=24.6 and 74.2 mLCH_4/gVSd^{-1}) were found in bioreactors operating at 100% BH and ISR = 3 and 4 respectively, while highest K (0.16 and 0.13 d^{-1}) were found in bioreactors operating at 50% and 75% BHE and ISR = 4 and 3, respectively. These

conditions provided the most ideal environment for proliferation of methanogens. The kinetics were comparable to those achieved during the AD of SOTE.

The process also produced reusable process/irrigation water, recyclable digestate as a biofertiliser and/or ceramic aggregate with energy recovery. The treated BHE met the s irrigation standards of many leading bovine leather producing countries except for Na, Mg, B, Cr, Cl, nitrogen, and NH_3/NH_4 concentrations. In the South African context, it met the standards for irrigating up to 500 m^3/day while the digestate's metal concentrations were within the recommended limits for class A1a sludge suitable for agricultural application, except Cr.

6.1.3   Integrated biological system

The hybrid linear flow channel reactors (HLFCRs) were able to effectively reduce the concentration of sulfur species in the BHE, with the successful formation of FSB. This novel finding demonstrated that HLFCRs could be used for S^0 recovery from BHE and promote AD in AnSBRs. The application of single-stage and two-stage HLFCR topology operating at 8 and 4 days' HRT respectively, produced an effluent that exhibited the most ideal characteristics for AD. However, single-stage (4-days HRT) and two-stage topology recovered a FSB with low S content of 0.44–1.4% (1.2% average), 0.59–1.8% N (1.3% average), 1.3–3.79% H (2.15% average) and a higher C content of 14.4–28.2% (15% average). The low S^0 content was postulated to have been due to the low relative abundance (RA) of SOB, and/or early harvesting of the formed organic C matrix, and/or the (re) reduction of the formed S^0. Increased HRT (8-days) in single-stage HLFCRs recovered about 16–25% of the inlet sulfur as S^0 in the FSB.

The optimum configurations and operating conditions for the integrated system were single-stage HLFCR (8-days HRT, pH=7.0–7.8, ambient temperature and –366<ORP<–322) and AnSBR (50 rpm continuous mixing, pH=7.0±0.5, and 37±2°C). These conditions achieved the removal of 80–91% COD_t, 78–98% TOC, 89–91% SO_4^{2-}, 92–93% S^{2-}, 50–73 PO_4^{2-}, 48–60% total nitrogen, 60–72% NO_3^- and 84-86% NO_2^-. In terms of CH_4 yield, the system achieved the production of 241±4 $mLCH_4/gCOD_{added}$ from pre-treated BHE and 88±2 $mLCH_4/gVS_{added}$ from HLFCR settled solids. The cumulative CH_4 yields were perfectly simulated and kinetics determined using the cone (Adj R^2>0.988) and logistic (Adj R^2>0.986) model. The highest reaction kinetics (μ_m = 19.5 and 12.7 $mLCH_4/gCODd^{-1}$ and K = 0.08 and 0.06) were under optimum conditions processing pre-treated BHE in two-stage and single-stage (8 days HRT) HLFCRs, respectively. However, continuously mixing at 100 rpm led to the lowest reaction kinetics due to the negative impact of excessive mixing. The K:μ_m ratios (>1) in this study demonstrated that methanogenesis was the rate-limiting step due to its inhibition.

Similar to anaerobically treated SOTE and BHE, the treated effluent met many of the irrigation standards for most bovine leather-producing countries except for Na, Cl, NH_3/NH_4, and/or total nitrogen. The digestate also met the stipulated standards for application on land in most countries except for Cr. Chrome is water insoluble and it generally attaches to solids.

6.1.3.1 *Economic analysis*

The economic analysis of full-scale application of the integrated biological system at a local medium sized tannery in South Africa producing 2.3 ML/day of BHE demonstrated the feasibility to recovering a FSB with 33% S^0 (307kg), 3.42 mega litres of CH_4, 31 tonnes of biofertiliser and 50 m³ of irrigation water. The system has a potential revenue of US$5559 from the sale of recovered materials and potential savings from 72% reduction in electricity demand and 62% in landfill disposal of sludge. Additionally, the system will alleviate carbon equivalent emissions by 169 tonnes CO_2eq (94%).

Table 6-1: Summary of the thesis findings

	Chapter 3		Chapter 4	Chapter 5	
Reactor volume (L)	2.5	20	2.5	8	20
Reactor type	Batch	AnSBR	Batch	HLFCR	AnSBR
HLFCR pre-treatment	No	No	No	No	Yes
% substrate	100% SOTE	100% SOTE	100% BHE	100% BHE	100% BHE
ISR (TVS/TVS)	3.72	4.0	2.5	ND	2.5
H/SRT (days)	15	21	20	8 (single stage)	21
Temperature (°C)	37±2	37±2	37±2	Ambient	37±2
pH	7±0.5	7±0.5	7±0.5	7.4±4	7±0.5
ORP (mV)	ND	−543 to−445	ND	−366 to −322	ND
Continuous mixing (rpm)	ND	200	ND	ND	50
COD_t removal (%)	41.1	ND	62.6	47–71	80–91
SO_4^{2-} red (%)	75.6	ND	57.8	54–61	89–91
S^0 recovery (%)	ND	ND	13	16–25	ND
TS red (%)	27.3	49	48.3	ND	ND
TVS red (%)	27.5	ND	64.5	ND	ND
TOC red (%)	75.1	ND	42.2	10–40	78–98
Biogas (mL/gVS)	361	ND	639	ND	ND
Biogas (mL/gCOD$_{add}$)	ND	ND	ND	ND	ND
CH_4 yield (NmL/gVS$_{add}$)	235	208	377	ND	ND
CH_4 yield (NmL/gCOD$_{add}$)	ND	ND	ND	ND	276
CH_4 yield (NmL/gCOD$_{rem}$)	ND	ND	ND	ND	238
Average CH$_4$ (%)	65	44	59	ND	67
A (mLCH$_4$/gVS)	130	210	359	ND	**302
K (d^{-1})	0.15	0.05	0.08	ND	0.06
μ$_m$ (mLCH$_4$/gVSd^{-1})	17.5	22.4	31.6	ND	*12.7
Λ (days)	3.12	17.2	7.43	ND	5.67
Reuse – irrigation (m³/day)	500	500	500	ND	50
Sludge standard South Africa	A1a	A1a	A1a	ND	A1b

A = ultimate methane yield   * μ$_m$ (mLCH$_4$/gCODd^{-1})   **A (mLCH$_4$/gCOD)   K = specific reaction rate constant

ISR = inoculum to substrate ratio   L = litres   μ$_m$ = maximum CH$_4$ production rate constant   Λ = lag phase

red = reduction   ORP = oxidation reduction potential   TOC = total organic carbon   TS= total solids

TVS = total volatile solids   NmL = normalised volumes (at STP)   ND = no data

The high sulfate content in BHE requires the use of the HLFCR to recover sulfate as S^0. The pre-treated becomes more amenable for CH_4 recovery. The use of the integrated biological treatment system has proven to be technical and economically feasible with an expected payback period of about 5 year and IRR of about 18%. However, the pilot set up should be experimented over a longer term to ascertain its robustness, product yields, quality and suitability for reuse and/or recycling. The dominant microbial communities should also be identified.

6.2 Overall conclusion and recommendations

The anaerobic codigestion of beamhouse effluent and tanyard effluent at different volumetric compositions did not abate inhibition. The BMP experiments demonstrated that the mono AD of BHE was most favourable for CH_4 recovery. This partly disapproved the hypotheses of this study. Additionally, the hypothesised need for a passive pre-treatment process for the removal of pollutants (SO_4^{2-}, HS^-/H_2S, and metals) from SOTE was disapproved. The SOTE had relatively low sulfate and sulphide concentrations that could be tolerated by the methanogenic archaea. Nonetheless, the integrated system proved the hypothesised synergistic effect of upstream removal of SO_4^{2-}, HS^-/H_2S, and metals on CH_4 recovery. Furthermore, the removed sulfur species could be recovered as S^0, treated final effluent reusable for irrigation and as biofertiliser.

All the research questions were answered by the outcomes of this study except for the identification of the most dominant microbial species involved in recovering the targeted resources. This study is still ongoing and will be reported else. Nonetheless, this was partly determined in this study for the AnSBR treating SOTE and HLFCR treating BHE by (Horn et al., 2022b)

6.2.1 Recommendations for further research

Long term investigations to determine the operational parameters needed to promote the proliferation of optimal functional microbial consortia for bioaugmentation in an integrated treatment system are required. This should also coincide with investigating the consistency of recovering good quality products that can be recycled and/or reused as the robustness of the process is essential. Furthermore, the kinetics and modelling of HLFCRs should be continued to better understand the pre-treatment process. There is also a need to modify the HLFCRs to improve the harvesting of formed FSB and/or their automation to optimise the addition of air via controlling the ORP. Additional investigations should be performed to determine if CH_4 production and/or energy consumption can be improved by employing different reactor and/or mixing configurations. The recovered products should be analysed to ensure consistent quality and comparison with other products on the market.

6.2.2 Recommendations for implementation

This study demonstrated that the full-scale application of AD in tannery WWTPs of developing countries presents an opportunity for transitioning to a circular bioeconomy and net positive tannery operations. However, wastewater treatment practitioners should be upskilled as the integrated system is a complex bioprocess that is prone to inhibition. Governments may assist tanneries by providing capital funding and introducing progressive policies, regulations, incentives and subsidies to support the adoption of bio-refineries. Furthermore, governments and the private sector can assist with the sustainability of bio-refineries by signing long-term agreements with tanneries for purchasing recoverables, particularly sulfur, and/or biofertiliser for agricultural application. The integration of tanneries with cattle/ostrich farms and/or slaughterhouses would be vital in promoting a circular bioeconomy as both industries are interdependent. Integration will promote the use of green skins/hides and eliminate NaCl in SOTE and BHE. Alternatively, tanneries should separate their soaking effluent as it is the main source of over 90% of Cl in TWW. The effluents can be further treated for the recovery of VOA and NH_4 that are still present in high concentrations (2284–2465 AAE mg/L and 232-444 NH_4 mg/L). This will also aid in producing better effluent quality that can be reused within the tannery and/or in other industries within an eco-industrial park and/or special economic zone.

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APPENDICES

A1: TYPICAL POLLUTION LOAD FROM DIFFERENT TANNERY EFFLUENTS

Table A-1: Typical discharge volumes and pollutant loads of effluent from different tannery processes (adpted from Bosnic et al., 2003; Buljan & Král, 2019, 2015)

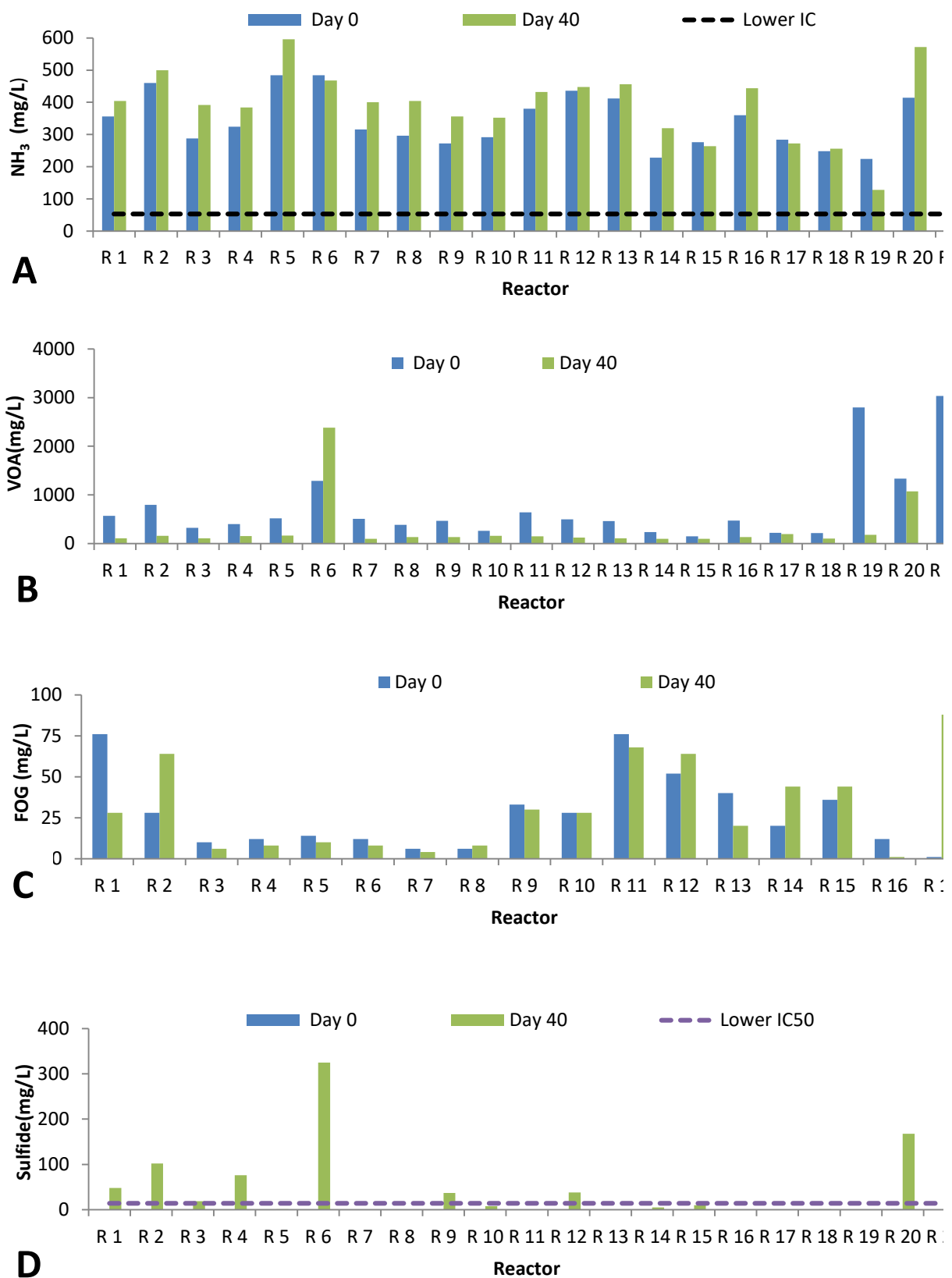| Process	Volume ($m^3/t_{raw\ salted\ hide}$)	Pollutant load ($kg/t_{raw\ salted\ hide}$)							
		SS	COD	BOD_5	S^{2-}	SO_4^{2--}	NH_3-N	TKN	Cl^-
Soaking	6-9	11-17	22-33	7-11		1-2	0.1-0.2	1-2	85-113
Liming	4-15	53-97	79-122	28-67	4-9	1-2	0.4-0.5	6-8	5-15
Deliming & Bating	7-11	8-12	13-20	5-9	<0.3	10-26	2.6-3.9	3-5	2-4
Tanning	1.5-5	5-10	7-11	2-4		30-55	0.6-0.9	0.6-0.9	40-60
Post tanning	7-13	6-11	24-40	8-15		10-25	0.3-0.5	1.2	5-10

Figure A-1: The biochemical methane potential bioreactor concentrations of (A)ammonia, (B) volatile organic acids, (C) oil and grease, (D) sulfide during anaerobic digestion of bovine/ovine tannery effluent
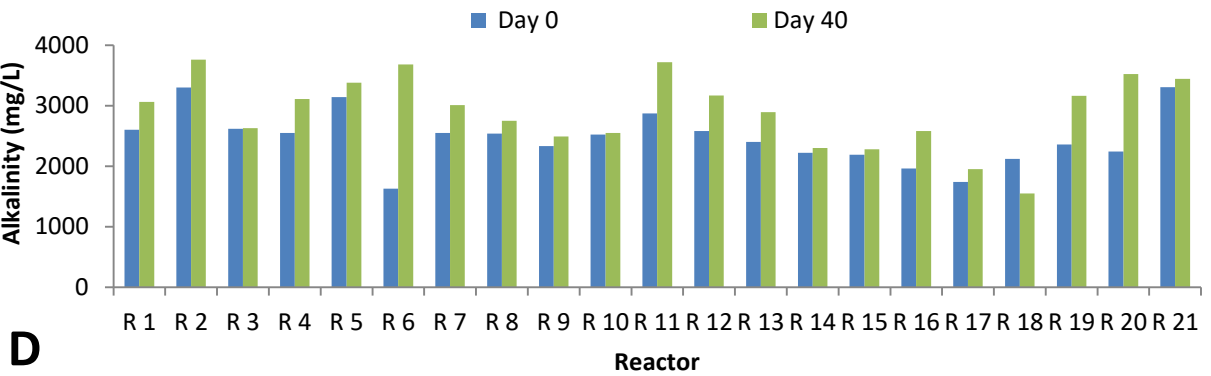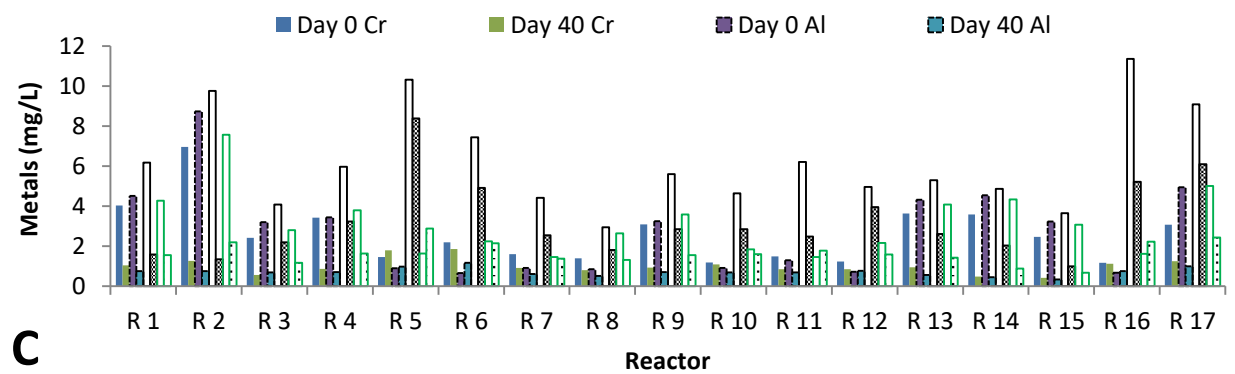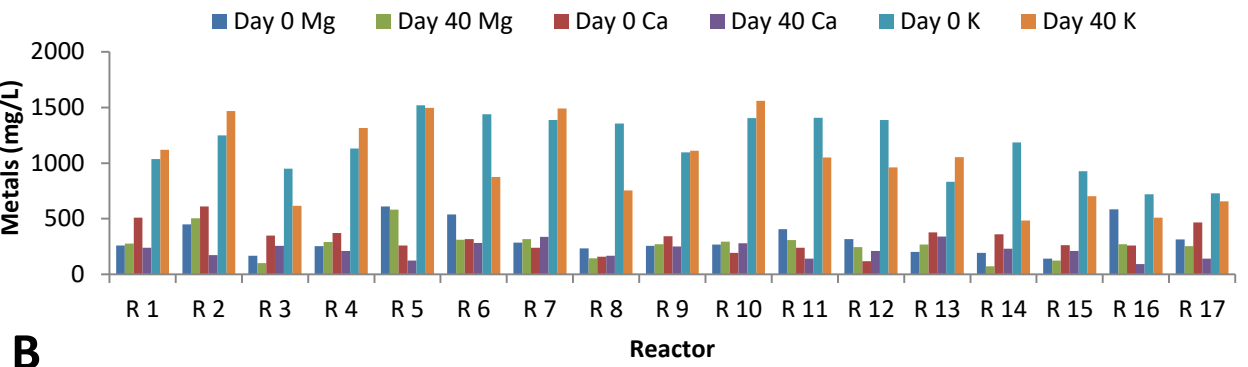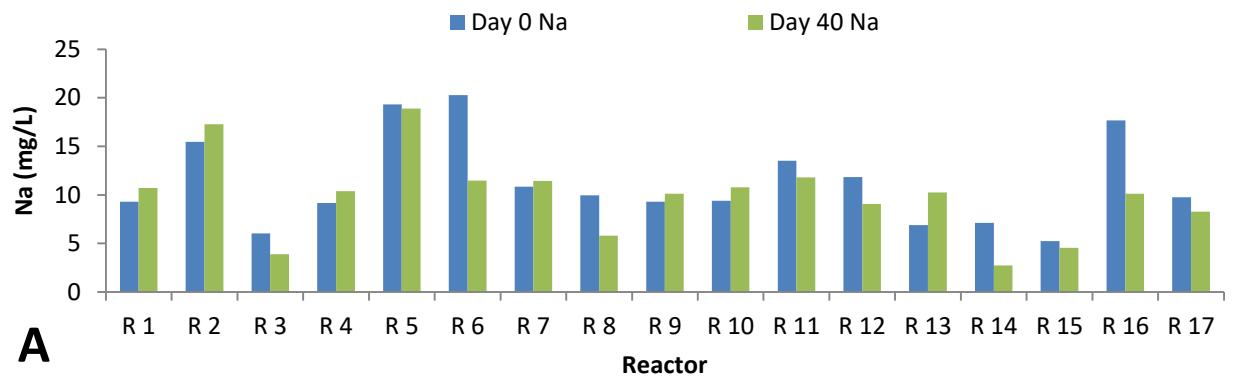
Figure A-2: The biochemical methane potential bioreactor concentrations of (A) Sodium ; (B) Mg, Ca and K ; Cr and (C) Al ; (D) Alkalinity during anaerobic digestion of bovine/ovine tannery effluent

Figure A-3: Photographs of (A) Hybrid linear floor channel reactor, (B) Floating sulfur biofilm accumulated on the mesh screen and (C) colonisation of carbon microfibers in the bulk liquid