POULTRY SLAUGHTERHOUSE WASTEWATER TREATMENT USING A STATIC GRANULAR BED REACTOR (SGBR) COUPLED WITH A HYBRID SIDESTREAM MEMBRANE BIOREACTOR

by

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DECLARATION

I, **Zainab Rinquest**, declare that the contents of this thesis represent my own unaided work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

19th December 2017

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ABSTRACT

An increase in the demand for poultry products coupled with the potable water shortages currently experienced in South Africa (SA), attributed to climate change among other factors, makes it crucial for SA to develop water conservation strategies to minimize potable water consumption by water-intensive industries, such as the poultry industry. The development of innovative wastewater treatment processes is therefore paramount in attempting to counteract the large quantity of wastewater generated as well as to manage the environmental health concerns arising from poultry slaughterhouse wastewater (PSW) discharge into the environment. Moreover, increasing wastewater treatment costs and the implementation of increasingly stringent government legislation to mitigate environmental pollution whilst minimizing fresh water source contamination, requires that wastewater such as PSW, be adequately treated prior to discharge.

This study, investigated the feasibility of treating PSW from a poultry slaughterhouse to: 1) a water quality standard compliant with industrial wastewater discharge standards and 2) for possible re-use purposes. The performance of a lab-scale PSW treatment system consisting of an anaerobic static granular bed reactor (SGBR) followed by single stage nitrification-denitrification (SSND) bioreactor and sidestream ultrafiltration membrane module (ufMM) post-treatment systems, were evaluated, with the objective being to: assess the treatment efficiency of the individual treatment systems namely; the SGBR, SSND bioreactor, and ufMM, under varying operational conditions, as well as to determine the performance of the overall designed PSW treatment system.

The down-flow SGBR (2 L) was used to reduce the organic matter (COD, BOD_5, and FOG) and total suspended solids (TSS) in the PSW. Anaerobic granules from a full-scale mesophilic anaerobic reactor treating brewery wastewater were used to inoculate the SGBR, and the PSW used as feed was obtained from a local poultry slaughterhouse (Western Cape, South Africa). The SGBR was operated continuously at mesophilic temperature (35-37 ˚C) without pH modification and under varying HRTs (24, 36, 48, 55, and 96 h) and OLRs (0.73 to 12.49 g COD/Lday), for a period of 138 days. The optimization of the SGBR, with regard to a suitable HRT and OLR, was determined using response surface methodology (RSM) and Design Expert® 10.0.3 statistical software. Periodic backwashing of the SGBR system was performed using stored effluent, i.e. treated PSW.

The SSND bioreactor and ufMM systems were operated at ambient temperature (23-25 ˚C). The SSND bioreactor (10 L) was used for total nitrogen (TN) removal from the SGBR effluent. Two operating strategies (down-flow mode without aeration and up-flow mode with aeration) were assessed and three types of packing material (gravel, gravel-integrated-with-

sponge, and sponge cubes) were simultaneously used as biofilm immobilization and supporting media, during the continuous operation of the SSND bioreactor. Hollow-fibre, ceramic ultrafiltration (UF) membranes (pore size of 100 nm, surface area of 0.0055 m^2) were used as a final polishing stage for the reduction of residual organic matter and TSS from the SGBR-SSND bioreactor effluent. The sidestream ufMMs were operated in dead-end filtration mode at constant feed flow rate (0.0282 L/h). Maintenance cleaning of the ufMMs were performed periodically using sodium hypochlorite (NaOCl) and citric acid solutions.

The SGBR demonstrated adequate performance and stability at HRTs ranging from 24 to 96 h and OLRs up to 12.49 g COD/Lday. It was observed that neither an increase nor decrease in the HRT or OLR had an adverse effect on the performance of the SGBR in terms of its overall removal efficiencies. The COD, TSS, BOD_5 and FOG removal efficiencies of the SGBR averaged 80%, 95%, 89% and 80%, respectively. The SSND bioreactor performed satisfactorily with regard to the TN removal in the anaerobically pre-treated effluent, without the addition of carbon sources and the use of activated sludge. The SSND bioreactor achieved optimum results when operated in up-flow mode with a continuous air supply. The TN removal achieved during this stage was 79%. Furthermore, the ufMMs were able to further reduce the residual COD and TSS by 65% and 54%, respectively.

The lab-scale PSW treatment system designed for this study was successfully employed with the resultant effluent being compliant with the CCT wastewater and industrial effluent by-law limits and the SANS 241 (2011) potable water quality limits; although, both the PO_4^{3-} and NH_4^+-N require further monitoring. Therefore, the poultry slaughterhouse from which the PSW was obtained will be able to safely discharge their wastewater; however, the treated PSW will not be suitable for re-use.

Keywords: Organic matter; Poultry slaughterhouse wastewater; Single stage nitrification-denitrification; Static granular bed reactor; Total suspended solids; Ultrafiltration membrane modules.

DEDICATION

This thesis is dedicated to Allah (swt),

And my parents,

Nabeweyah Rinquest and Ebrahim Rinquest

"And my success (in my task) can only come from Allah. In Him I trust and unto Him I look."

(Quran, 11:88)

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RESEARCH OUTPUTS

- **Published DHET accredited article(s) and conference proceeding(s):**

Basitere, M., Njoya, **Rinquest,** Z M, Sheldon, M.S., & Ntwampe, S.K.O. 2017. Performance and kinetic analysis of a static granular bed reactor treating poultry slaughterhouse wastewater*.* Frontiers International Conference on Wastewater Treatment and Modelling, 225-229. Springer, Cham (Available Online 5 May 2017; DOI: 10.1007/978-3-319-58421-8_35).

Basitere, M., **Rinquest, Z.**, Njoya, M., Sheldon, M.S. & Ntwampe, S.K.O. 2017. Treatment of poultry slaughterhouse wastewater using a static granular bed reactor (SGBR) coupled with ultrafiltration (UF) membrane system. *Water Science and Technology*, 76(1):106-114. DOI: 10.2166/wst.2017.179.

- **International conference presentations:**

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Basitere, M., **Rinquest, Z.**, Njoya, M., Sheldon, M.S. & Ntwampe, S.K.O. 2016. Treatment of poultry slaughterhouse wastewater using a static granular bed reactor (SGBR) coupled with ultrafiltration (UF) membrane. 13th IWA Specialized Conference on Small Water and Wastewater Systems (SWWS), Athens, Greece, 13 – 18 Sep 2016.

- **Article(s) and conference proceeding(s) which are under review:**

Rinquest, Z., Basitere, M., Ngongang, M.M., Njoya, M. & Ntwampe, S.K.O. 2018. Optimization of the COD removal efficiency for a static granular bed reactor treating poultry slaughterhouse wastewater. 10th Eastern Europe Young Water Professionals Conference, Zagreb, Croatia, 7 – 12 May 2018.

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LAYOUT OF THESIS

This thesis consists of the following 5 chapters:

- **Chapter 1** is an introductory chapter which provides general background information about the South African poultry industry, poultry slaughterhouse wastewater (PSW) generation, and the treatment of PSW. It includes the research problem, questions, aim and objectives, as well as the significance and delineation of this study.
- **Chapter 2** is a literature review of the anaerobic, nitrification-denitrification and membrane filtration processes, used for the treatment of wastewater. It contains a brief overview of the current water situation in South Africa, the water usage of poultry slaughterhouses, PSW characterization, and the legislation governing the discharge of PSW.
- **Chapter 3** describes the materials, equipment and methods used for the setup and operation of the anaerobic, nitrification-denitrification and membrane filtration treatment systems used in this study. It specifies the operating conditions of each treatment system as well as the sampling, analytical and statistical methods and equipment used to analyse the experimental data.
- **Chapter 4** presents the results relating to the performance of the individual treatment systems and that of the overall designed PSW treatment system; and includes a detailed discussion thereof.
- **Chapter 5** provides the overall conclusions of this study with recommendations for further research.

All references used in this study are listed in accordance with the guidelines for research theses for a CPUT masters qualification.

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LIST OF SYMBOLS

Symbol	Description	Unit
A	OLR	g COD/Lday
$A_{membrane}$	Membrane surface area	m^2
B	HRT	day
C	Weight of glass fibre filter and sample residue	mg
C_1	Initial concentration	g/L
C_2	Final concentration	g/L
C_i	Feed concentration	mg/L
C_o	Effluent concentration	mg/L
D	Weight of glass fibre filter	mg
e	Error of quadratic model	Dimensionless
E	Volume of sample filtered	ml
F	Weight of ignited glass fibre filter	mg
J	Permeate flux	L/m^2h
k	Number of factors	Dimensionless
%	Percentage	Dimensionless
Q	Volumetric flow rate	L/h
R^2	Determination coefficient	Dimensionless
RE	Removal efficiency	%
V_1	Initial volume	L
V_2	Final volume	L
V_w	Working volume	L
X_I	Factor/Independent variable 1	Dimensionless
X_j	Factor/Independent variable 2	Dimensionless
Y_i	Response variable	Dimensionless

Greek letters

β_o	Constant coefficient	Dimensionless
β_i	Linear coefficient	Dimensionless
β_{ii}	Quadratic coefficient	Dimensionless
β_{ij}	Interaction coefficient	Dimensionless

LIST OF ABBREVIATIONS

Abbreviation	Definition
AC	Anaerobic contact
AD	Anaerobic digestion
AF	Anaerobic filter
AFFR	Anaerobic fixed film reactor
AFP	Aerated facultative pond
Al_2O_3	Aluminium oxide
ANAMMOX	Anaerobic ammonium oxidation
ANOVA	Analysis of variance
AS	Activated sludge
AOB	Ammonium-oxidising bacteria
BOD_5	Biological oxygen demand
CA	Cellulose acetate
$CaCO_3$	Calcium carbonate
CCD	Central Composite Design
CCT	City of Cape Town
CH_4	Methane
CIP	Cleaning-in-place
cm	Centimetre
COD	Chemical oxygen demand
d	Day
DAF	Dissolved air flotation
DI	De-ionized
DO	Dissolved oxygen
EBPR	Enhanced biological phosphorous removal
EC	Electrical conductivity
ESS	Environmental sciences section
FOG	Fats, oils and grease
g	Grams
GAC-SBBR	Granular activated carbon sequencing batch biofilm reactor
GF/C	Glass fibre/Circles
h	Hour
HF	Hollow-fibre
HRT	Hydraulic retention time
IAASBR	Integrated anaerobic aerobic sequencing batch reactor

kg	Kilograms
kPa	Kilopascal
L	Litres
LMH	Litres per m^2 per hour
m	Metres
m^3	Metres cubed
m^2	Metres squared
MBR	Membrane bioreactor
MF	Microfiltration
mg	Milligram
mg/L	Milligram per litre
min	Minutes
ml	Millilitres
mm	Millimetres
MPa	Megapascal
N	Nitrogen
NaOCl	Sodium hypochlorite
NaOH	Sodium hydroxide
NF	Nanofiltration
NH_3	Ammonia
NH_4^+	Ammonium
NH_4^+-N	Ammonium-nitrogen
ni	Not indicated
NLR	Nitrogen loading rate
nm	Nanometre
NOB	Nitrite-oxidising bacteria
NO_3^-	Nitrate
NO_3^--N	Nitrate nitrogen
NO_2^-	Nitrite
NO_2^--N	Nitrite nitrogen
NRF	National Research Foundation
NTU	Nephelometric turbidity units
OLR	Organic loading rate
P	Phosphorous
PA	Polyamide
PE	Polyethylene
PES	Polyethersulfone
PAO	Polyphosphate accumulating organisms

PHA	Polyhydroxyalkanoanes
PhAC	Pharmaceutical active compounds
PO_4^{3-}	Phosphate
PO_4^{3-}-P	Ortho-phosphate
PP	Polypropylene
ppm	Parts per million
PPW	Poultry processing wastewater
PS	Polysulfone
PSW	Poultry slaughterhouse wastewater
PTFE	Polytretrafluoroethylene
PVC	Polyvinyl chloride
PVDF	Polyvinylidene fluoride
RBC	Rotating biological contactor
RO	Reverse osmosis
RSM	Response surface methodology
SA	South Africa
SANAS	South African Nation Accreditation System
SANS	South African National Standard
SASBR	Static anaerobic sludge bed reactor
SBR	Sequencing batch reactor
SD	Standard deviation
SGBR	Static granular bed reactor
SND	Simultaneous nitrification and denitrification
SRT	Solids retention time
SSND	Single stage nitrification-denitrification
TDS	Total dissolved solids
TKN	Total Kjeldahl nitrogen
TMP	Transmembrane pressure
TSS	Total suspended solids
TN	Total nitrogen
UAF	Up-flow anaerobic filter
UASB	Up-flow anaerobic sludge blanket
ufMM	Ultrafiltration membrane module
UF	Ultrafiltration
v/v	Volume/volume %
VFA	Volatile fatty acids
VSS	Volatile suspended solids
WWTP	Wastewater treatment plant

GLOSSARY

Term	Explanation
Aerobic	Conditions where biochemical reactions are dependent on oxygen which acts as the electron donor (Judd, 2006).
Anaerobic	Conditions where biochemical reactions occur in the absence of oxygen (Judd, 2006).
Anoxic	Conditions where biochemical reactions occur and a species other than oxygen acts as the electron donor (Judd, 2006).
Biological Oxygen Demand (BOD)	The amount of oxygen required by aerobic micro-organisms for the biodegradation of organic matter (Latif & Dickert, 2015).
Chemical Oxygen Demand (COD)	The amount of oxygen used for the oxidation of organic matter (Judd, 2011).
Fats, oils and grease (FOG)	Includes the fats, oils, waxes and other related constituents found in wastewater. Fats and oils are compounds (esters) of alcohol or glycerol with fatty acids (Srinivas, 2008).
Hydraulic Retention Time (HRT)	The duration or time period that the wastewater remains within the reactor (Judd, 2011).
Membrane Bioreactor (MBR)	A biological treatment process integrating a perm-selective membrane with a biological process (Judd, 2011).
Organic Loading Rate (OLR)	The rate at which organic materials enter the reactor (Judd, 2011).
Poultry slaughterhouse wastewater (PSW)	The wastewater generated by slaughterhouses during the slaughtering and processing of poultry products including by-products (Del Nery et al., 2007).
Static Granular Bed Reactor (SGBR)	A high-rate down-flow anaerobic digester which utilizes a bed of active anaerobic granules for the treatment of wastewater (Oh et al., 2015).

Solids Retention Time (SRT)	The duration or time period that the solids or biomass remains within the reactor (Judd, 2011).
Single stage nitrification-denitrification (SSND)	The simultaneous nitrification and denitrification (SND) process and oxidation of organic matter occurring under aerobic conditions within a single bioreactor (Seifi & Fazaelipoor, 2012)
Total suspended solids (TSS)	The portion of the total solids (TS) retained on a filter (with a specified pore size), measured after being dried at 105 °C; which can lead to sludge deposits and anaerobic conditions in the aquatic environment (Metcalf & Eddy, 2003).
Ultrafiltration (UF)	A pressure-driven process that separates on the basis of molecular diameter (5-100 nm) (Avula et al., 2009).
Ultrafiltration membrane module (ufMM)	The ultrafiltration (UF) membrane unit including the housing through which the water flows (Judd, 2011).

CHAPTER 1

INTRODUCTION

CHAPTER 1
1. INTRODUCTION

1.1 Background

The poultry industry is the largest industry within the agricultural sector in South Africa (SA) and is the largest poultry product producer in the Southern African Development Community (SADC) region. The growth in poultry product consumption in SA has steadily increased due to the products being inexpensive; and therefore, the preferred source of animal protein for majority of the population (Davids & Meyer, 2017). Apart from the significant growth in poultry production over the last 20 years, there has also been transformation in the manner in which poultry products are processed. During slaughtering and processing operations, poultry industries consume a substantial quantity of water in order to maintain the high standards of quality and hygiene required for the production of poultry products (De Nardi et al., 2011; DARD, 2009). South African poultry slaughterhouses are estimated to use an average of 15 to 20 litres of water per processed bird (Meissner et al., 2013; CSIR, 2010). This water is predominantly used in the scalding, de-feathering, bird cleansing prior and subsequent to eviscerating, and chilling operations, as well as for the disinfection of production equipment and facilities (Yornadov, 2010; Plumber, 2009; Molapo, 2009).

The large quantities of poultry slaughterhouse wastewater (PSW) generated as a by-product of the slaughtering and processing operations has proven to be a constant concern for poultry slaughterhouses. The composition of the PSW generated varies considerably among poultry slaughterhouses (Mohamed, 2014; Yordavov, 2010). However, it is generally classified as medium-strength wastewater due to the high concentrations of organic materials contained within such wastewater which contribute to the chemical oxygen demand (COD) and biological oxygen demand (BOD); suspended solids; colloidal matter such as fats, carbohydrates, and proteins; nutrients (i.e. nitrogen and phosphorous); and pathogens (Del Nery et al., 2007; Avula et al., 2009; Debik & Coskun, 2009). These contaminants result in heightened organic matter loads for wastewater treatment plants (WWTPs) and the environment if discharged untreated, which can culminate in eutrophication and de-oxygenation of fresh water bodies (Oh et al., 2015; Mohamed, 2014). Thus, the treatment of such wastewater prior to discharge is critical.

On-site wastewater treatment systems employed by poultry slaughterhouses generally consist of screening, followed by an equalisation tank and a dissolved air flotation (DAF) system (Avula et al., 2009; DARD, 2009). In addition to these wastewater treatment processes, several other processes have been reported for PSW treatment, with anaerobic biological processes being the most commonly used process due to their high overall treatment efficiency (up to 90%) and suitability for treating wastewater with a high organic load (Debik & Coskun, 2009). However, due to increasingly stringent environmental regulations with regard to high nitrogen and phosphorous levels as well as growing interest in water re-use and reclamation, membrane bioreactor (MBR) technology, which integrates biological treatment with membrane filtration, can be considered as an attractive alternative to conventional anaerobic treatments for achieving compliance with regulatory standardized limits, herein referred to as discharge standards, and to recover process water for re-use (Avula et al., 2009).

The use of static granular bed reactor (SGBR) anaerobic digester, single stage nitrification-denitrification (SSND) bioreactor and sidestream ultrafiltration membrane module (ufMM) systems has proven to be successful in the treatment of various industrial wastewaters. However, few studies have been reported on the performance and efficiency of the combined use of these processes for the treatment of PSW. The selection of treatment processes used in this study was based on the characteristics of the PSW under investigation, the economic feasibility of the available technology, and the compliance of the effluent with local and national legislation.

1.2 Research problem statement

The large quantities of PSW generated by poultry slaughterhouses contains high concentrations of organic matter i.e. chemical oxygen demand (COD) and biological oxygen demand (BOD_5); suspended and colloidal matter i.e. total suspended solids (TSS) and fats, oils and grease (FOG); as well as nutrients i.e. nitrogen ($NH4^+$-N) and phosphorous ($PO4^{3-}$-P). As a result, this medium-strength wastewater cannot be directly discharged into fresh water sources or municipal sewer systems as it does not comply with the wastewater and industrial effluent discharge standards. South African poultry slaughterhouses are therefore required to use advanced treatment methods for their wastewater, which pose severe risks to municipal sewer systems and the environment if it is not efficiently treated and appropriately discharged. If adequately treated to a suitable water quality standard, the reclaimed water can be used as process water, to mitigate the effect of current water shortages.

1.3 Research questions

- How efficient is the anaerobic SGBR coupled with SSND bioreactor and sidestream ufMM systems in treating PSW?
- What effect does varying the hydraulic retention times (HRTs) and organic loading rates (OLRs) have on the removal efficiency of the organic matter and suspended solids by the SGBR?

- Can total nitrogen (TN) removal from the anaerobically pre-treated PSW be achieved in a SSND bioreactor system?
- Is a sidestream ufMM system an efficient polishing stage for the SGBR-SSND treated PSW?
- Does the final effluent from the overall PSW treatment system comply with the wastewater and industrial effluent discharge standards including standards used to characterize potable water, and can it be re-used as process water?

1.4 Research aim and objectives

The aim of this study was to evaluate the feasibility of treating PSW to a water quality standard compliant with the wastewater and industrial effluent discharge standards and safe for re-use purposes, using an anaerobic SGBR followed by both SSND bioreactor and sidestream ufMM systems.

The specific objectives of this study were to:

- Evaluate the treatment efficiency of the anaerobic SGBR system for organic matter and suspended solids removal at varying HRTs and OLRs.
- Optimize the SGBR operating conditions and develop a model to predict the COD removal efficiency of the SGBR using response surface methodology (RSM).
- Assess the total nitrogen (TN) removal by the SSND bioreactor system.
- Evaluate the effectiveness of the ufMM system as a final polishing stage.
- Determine the performance of the overall PSW treatment system for the removal of organic matter, suspended solids and nutrients from the PSW.

1.5 Significance of the research

The lab-scale PSW treatment system used in this study was successfully employed for the treatment of medium-strength PSW under varying operating conditions. The use of the anaerobic SGBR pre-treatment coupled with the SSND bioreactor and sidestream ufMM systems for the treatment of PSW was operationally and economically advantageous since it combined the benefits of both anaerobic and aerobic processes, with the inclusion of ultrafiltration (UF) membranes as a final polishing stage. The findings obtained from this study provided information based on the combined biological and membrane processes, its performance and efficiency in removing organic matter, suspended solids, and nutrients from PSW. Furthermore, the implementation of this type of system for the treatment and recovery of PSW may benefit poultry slaughterhouses by reducing the volume of wastewater discharged and potable water consumed, minimizing discharge costs and penalties whilst improving the efficiency of their processes through energy savings.

1.6 Delineation of the research

This research study does not include the following aspects (which may form part of a subsequent research study):

- The quantitative and qualitative evaluation of biogas production, although the SGBR design used in this study had a gas collection port;
- The growth kinetics and identification of micro-organisms within each treatment system;
- The testing of various types, materials or sizes of membranes;
- Membrane fouling effects; and
- Kinetic/process modelling; scale-up and costing.

CHAPTER 2

LITERATURE REVIEW

CHAPTER 2
2. LITERATURE REVIEW

2.1. Introduction

Increasing water scarcity is a major concern worldwide, especially in developing countries such as South Africa (SA) which experienced its worst drought during 2015 and 2016 since the year 1904 (GreenCape, 2017; Jaiyeola & Bwapwa, 2016). SA is classified as a water-stressed country due to its limited fresh water resources and the Western Cape Province in particular, situated in the south-western corner of SA, is classified as a water-stressed region within SA. According to projections, SA will not be able to provide for the water needs of its people and industrial sector by the year 2030, as the demand for water will exceed its availability by 17%. The projected water demand for the Western Cape will exceed supply as soon as 2020 (GreenCape, 2017; Jaiyeola & Bwapwa, 2016; CSIR, 2010). Population growth, increased industrialisation and urbanisation, climate and rainfall fluctuations, water pollution (attributed to agriculture, industry, urban runoff and inadequate wastewater treatment), and the unsustainable use of fresh water sources, are some of the contributing factors to SA's status as a water-scarce country and its rank as the 30^{th} driest country worldwide (GreenCape, 2017; CSIR, 2010). It is therefore crucial for SA to develop water conservation strategies, especially during the drought, in order to minimize the consumption of potable water by water-intensive industries such as the poultry industry.

2.2. Water consumption of poultry slaughterhouses

The production of poultry products is a water-intensive process with the largest volume of water being used for slaughtering and processing operations. These operations generally include slaughtering and bleeding, cleaning of slaughter areas, scalding and de-feathering, evisceration, chilling, and rinsing of poultry products including carcasses and by-products in between processing stages. A considerable quantity of water is also used for utilities (i.e. steam generation, cooling water and refrigeration, and hot water), sanitizing and disinfecting of slaughterhouse facilities and equipment, and for transporting poultry products including by-products for further processing (Molapo, 2009; Plumber, 2009). The quantity of water consumed by poultry slaughterhouses vary according to the processes and equipment used, size and productivity/capacity of the slaughterhouse, and water and wastewater management practices implemented on-site (Molapo, 2009; Amorim et al., 2007). South African poultry slaughterhouses generally utilize an average of 15 to 20 L of water per processed bird, of which approximately 80–90% of this process water is discharged as poultry slaughterhouse wastewater (PSW) (DEA & DP, 2015; CSIR, 2010).

An estimated volume of 6 m^3 of water is utilized in the production of 1 kg of fresh poultry products (CSIR, 2010). To ensure that the poultry products are fit for human consumption, it is imperative that poultry slaughterhouses utilize clean, potable water that is free of suspended material and harmful substances (South Africa, 1992). The average water consumption of a typical South African poultry slaughterhouse is shown in Figure 2.1.

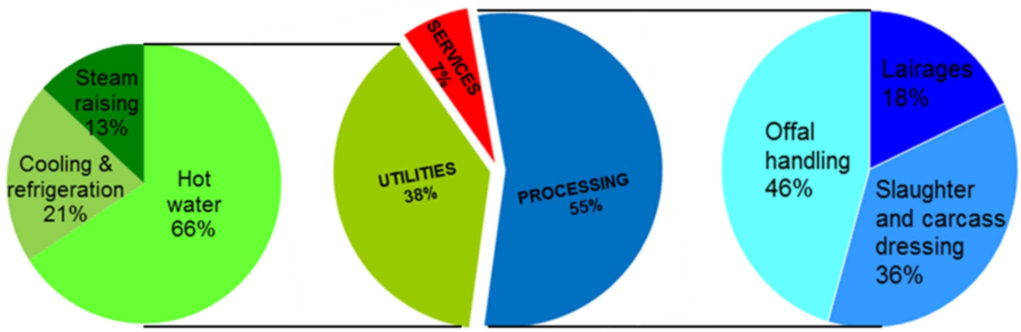

Figure 2-1: Analysis of the average water consumption of a typical South African poultry slaughterhouse (Adapted from Molapo, 2009; South Africa, 1992)

2.3. Characterization of poultry slaughterhouse wastewater (PSW)

Poultry slaughterhouses generate large quantities of highly biodegradable wastewater, as is expected of most food processing industries (Kiepper, 2003). Common PSW characteristics as reported in the literature reviewed are summarized in Table 2.1. The composition of the PSW differs significantly for each poultry slaughterhouse depending on the type, size and number of birds processed, the water consumption per processed bird, the efficiency of the blood collection process, and the type of industrial processes implemented (Debik & Coskun, 2009; Del Nery et al., 2007). PSW is classified as medium-strength wastewater since it generally contains a high concentration of contaminants in the form of soluble and particulate organic and inorganic matter. These contaminants are as a result of the accumulation of residual blood, carbohydrates, fats, faeces, feathers, and proteins (Avula et al., 2009; Debik & Coskun, 2009; Del Nery et al., 2007). Furthermore, PSW contains significant quantities of chemical constituents which originate from the cleaning and disinfecting operations, as well as pathogens (Basitere et al., 2017; Bayar, 2011; Avula, 2009). The high concentrations of organic matter in the form of biological oxygen demand (BOD_5) and chemical oxygen demand (COD); colloidal matter such as fats, oils & grease (FOG); total suspended solids (TSS); and nutrients i.e. total Kjeldahl nitrogen (TKN) and total phosphorous (TP) among others, as seen in Table 2.1, distinguish PSW from other industrial wastewaters.

Table 2-1: PSW characteristics from the literature reviewed

Treatment method	pH	COD (mg/L)	BOD_5 (mg/L)	TSS (mg/L)	FOG (mg/L)	TP (mg/L)	TKN (mg/L)	References
UF & MF membrane	-	1300-3772	200-2341	200-2446	100-970	14-50	120-250	Abboah-Afari, 2011
Hybrid-UASB, UAF	7.0-7.6	3000-4800	750-1890	300-950	800-1385	-	109-325	Rajakumar et al., 2011
UF membrane	-	3610-4180	1900-2200	2280-2446	289-389	-	-	Yordanov, 2010
SGBR & SASBR	5.6-8.1	4200-9100	-	1850-3750	-	5.8-12.1	565-785	Debik & Coskun, 2009
DAF & UASB	6.3-7.0	2060-4380	1559-2683	480-1230	131-261	-	-	De Nardi et al., 2008
DAF & UASB	6.5-7.0	2360-4690	1190-2624	-	249-702	33-128	147-233	Del Nery et al., 2007
UASB	6.1-7.1	5800-11,600	4524-8700	726-1462	147-666	7.17-12.74	-	Chavez et al., 2005
Electrocoagulation	6.73	2171	1123	-	143.1	-	-	Bayar et al., 2011
Electrocoagulation	6.7	26000-29000	10000-12000	840-1200	1500-1800	-	-	Kobya et al., 2006
DAF, UASB & Chemical-DAF	6.8-7.8	2790-5520	1558-2988	-	72-202	-	-	Del Nery et al., 2016
SGBR & UF membrane	6.31-7.26	1223-9695	734-4992	986-1450	-	-	-	Basitere et al., 2017
IAASBR	6.8	2711	930	835	281	-	-	Rajab et al, 2017
UF, RO & NF membrane	6.6	7970	-	2760	-	-	-	Coskun et al., 2015
DAF & UASB	-	3102	-	-	375	76	186	Amorim et al., 2007
EGSB, anoxic & aerobic bioreactors	6.5-8.0	2133-4137	1100-2750	315-1273	131-684	8-27	77-352	Basitere et al., 2016

2.4. Legislation governing the discharge of PSW in South Africa (SA)

Legislative guidelines are necessary for poultry slaughterhouses to manage their operations sustainably and to alleviate their environmental impact. The standards and regulations governing the discharge of PSW vary significantly worldwide (Bustillo-Lecompte & Mehrvar, 2017). In SA in particular, regulations relating to water and wastewater management practices and the discharge of wastewater and industrial effluent are governed by the National Water Act (Act 36 of 1998) and the Water Services Act (Act 108 of 1997). Poultry slaughterhouses generally discharge their wastewater to municipal sewer treatment plants subsequent to effective pre-treatment on-site. The compliance with national legislation prior to discharge may provide economic relief with regard to wastewater discharge costs, whilst ensuring the sustainable use of potable water.

Poultry slaughterhouses which have been granted permission to discharge wastewater directly into municipal sewer treatment systems are required to abide by the municipal by-laws within each municipality as prescribed by the Water Services Act of 1997; although, monitoring is limited (CSIR, 2010; Molapo, 2009). There are 278 municipal departments within SA and 24 local municipalities in the Western Cape Province (GreenCape, 2017). Poultry slaughterhouses located in the Cape Town district municipality (Western Cape, SA) must therefore comply with the City of Cape Town (CCT) Wastewater and Industrial Effluent By-law (2013). The discharge rates associated with industrial effluent are calculated in accordance with Schedule 1 of this by-law and the Tariff by-law of the CCT. The chemical oxygen demand (COD) is the main parameter used to calculate the discharge rate. In the event that the COD concentration of the industrial effluent is less than 1000 mg/L, the COD factor used to calculate the discharge rate is negligible (City of Cape Town, 2014).

In addition, municipalities enforce surcharges or poultry slaughterhouses are penalised when discharged PSW does not meet the required discharge limits with regard to water quality standards (Table 2.2) and volumes as set out in accordance with Schedule 1 of the CCT By-law (2013). In addition to developing and enforcing by-laws, local municipalities regulate potable water standards as set out by South African National Standards (SANS) (GreenCape, 2007). The maximum limits of permitted discharge into municipal sewers, including limits regulated by the SANS 241:2011 potable water quality requirements, applicable to this study, are presented in Table 2.2 (City of Cape Town, 2014; DWA, 2011).

Table 2-2: Maximum limits of permitted discharge in accordance with the CCT by-law (2013) and limits regulated by the SANS 241:2011 potable water quality requirements

		CCT Industrial Effluent By-law (2013)	**Sans 241: 2011 (Potable Water Quality)**
Parameter	**Unit**	**Maximum limits**	
General parameter limits			
Temperature	°C	40	ni
Electrical conductivity at 25 °C	mS/m	500	170
pH at 25 °C	-	12	9.7
Chemical Oxygen Demand (COD)	mg/l	5000	ni
Turbidity (operational/aesthetic)	NTU	ni	1/5
Chemical substances limits			
Total dissolved solids at 105 °C	mg/l	4000	1200
Suspended Solids	mg/l	1000	ni
Oils, greases, waxes and fat	mg/l	400	ni
Ammonium as N	mg/l	ni	1.5
Nitrates as N	mg/l	ni	11
Nitrites as N	mg/l	ni	0.9
Total phosphates as P	mg/l	25	ni

ni– not indicated

2.5. Treatment of PSW

The types of treatment processes commonly involved in PSW treatment are: physical, chemical, and biological treatment processes (Kiepper, 2003; Cheremisinoff, 2002). Each treatment type has unique advantages as well as process limitations. PSW treatment plants generally comprise a combination of these treatment processes depending on the quality and quantity of the PSW generated (Cheremisinoff, 2002). It is desired that treated wastewater is low in organic and inorganic contaminants, as well as free from biological contaminants such as pathogens and viruses; therefore, treatment processes that are reliable and effective in removing an extensive range of wastewater contaminants are required. Moreover, the costs and regulations associated with water supply and wastewater disposal need to be considered when evaluating wastewater treatment options (Bustillo-Lecompte & Mehrvar, 2017). Figure 2.2 represents some of the treatment processes used for PSW (Molapo, 2009; Mittal, 2005; Kiepper, 2003; Massé & Massé, 2000; Johns, 1995).

Physical separation technologies used in wastewater treatment represents a group of solid-liquid separation processes of which membrane filtration is the most commonly applied process for PSW treatment (Cheremisinoff, 2002). Ultrafiltration (UF), microfiltration (NF), nanofiltration (NF) and reverse osmosis (RO) processes have been employed for the treatment of PSW and the recovery of valuable by-products such as the proteins found in PSW (Abboah-Afari, 2011; Yordanov, 2010; Coskun et al., 2005).

Chemical treatment processes used in wastewater treatment involves the use of chemicals that aid in the separation, destruction or neutralization of contaminants present in wastewater such as PSW; thus, synthetic chemical usage depends on the chemical interactions with the contaminants. Chemical treatment may be applied independently or it may be coupled with physical processes, i.e. physio-chemical treatment (Cheremisinoff, 2002). The chemical supported dissolved air flotation (chemical-DAF) system, is one of the physio-chemical treatments available, which may be implemented as a pre- or post-treatment process, and is commonly used for the on-site treatment of PSW (Del Nery et al., 2016). Moreover, electrocoagulation processes have been also been used for PSW treatment (Bayar et al., 2011; Kobya et al., 2006).

For environmentally benign wastewater treatment, biological treatment processes are suitable to address problems associated with the biodegradable organic content of PSW and involves the degradation of organic matter by micro-organisms in a controlled environment (Cheremisinoff, 2002). The use of high-rate anaerobic reactors (e.g. the UAF, UASB reactor, EGSB reactor, and SGBR), and hybrid versions of these reactors have been studied extensively for PSW treatment (Rajakumar et al., 2011; Debik & Coskun, 2009; Chavez et al., 2005). Combined treatment of PSW; i.e. integrated processes, include DAF systems coupled with UASB reactors (De Nardi at al., 2008; Del Nery et al., 2007; Amorim et al., 2007), SGBR anaerobic digester pre-treatment followed by UF membrane post-treatment (Basitere et al., 2017), and the integration of both aerobic and aerobic processes in a single reactor, i.e. the integrated anaerobic aerobic sequencing batch reactor (IAASBR) (Rajab et al., 2017).

Treatment type

	Physical	Chemical/Physio-chemical	Biological
Processes	Screening, filtration, fat traps, catch basins, settling, sedimentation, dissolved air	Chemical-DAF, chemical flocculation, electrocoagulation	Activated sludge systems, anaerobic and aerobic systems
Application	Removal of suspended solids, fats, oils and grease	Removal of fats, suspended solids, nutrients	Removal of organic matter (COD and BOD), pathogens

Figure 2-2: Types of treatment processes used for PSW

2.6. Biological treatment of PSW

Biological wastewater treatment involves the conversion of biodegradable organic, inorganic and pathogenic material into stable biomass and other by-products, by means of suitable micro-organisms (Molapo, 2009; Mittal, 2005). Biological processes used for the treatment of wastewater require sufficient contact time between the wastewater and micro-organisms for effective treatment with regard to the biodegradation of organic matter. These biological processes are classified into three categories namely, aerobic, anaerobic, and anoxic processes. Aerobic and anaerobic treatment processes occur in the presence and absence of free-oxygen, respectively; whereas, anoxic processes utilise nitrate (NO_3^-) or nitrite (NO_2^-) ions for the biodegradation of organic matter (Gerardi, 2003).

Aerobic treatment systems include aerobic lagoons, activated sludge (AS) processes, aerobic membrane bioreactors (MBRs), extended aeration processes, oxidation ponds, sequencing batch reactors (SBRs), trickling filters and rotating biological contactors (RBCs) (Kiepper, 2001). These wastewater treatment systems are limited by the high volumes of sludge produced and high energy requirements needed for aeration; coupled with the high costs associated with aeration and the pre-treatment and disposal of the sludge including acquisition of land, i.e. for lagoons/ponds (Molapo, 2009; Massé & Massé, 2000; Johns, 1995).

Anaerobic systems commonly used for the treatment of highly biodegradable wastewaters such as PSW includes anaerobic lagoons, anaerobic contact (AC) processes, anaerobic MBRs, up-flow anaerobic sludge blanket (UASB) reactors, expanded granular sludge bed (EGSB) reactors, static granular bed reactors (SGBRs), and anaerobic filter (AF) processes. The biodegradation of organic matter by means of micro-organisms in these systems, results in the production of biogas as a by-product during anaerobic treatment (Debik & Coskun, 2009; Mittal, 2005). Anaerobic treatment is considered as the most feasible process for PSW to date due to its numerous advantages such as low initial and operational costs and minimal space requirements and high treatment capacity. Anaerobic treatment systems are also useful in achieving high organic removal efficiencies and a high degree of biomass stabilization coupled with low sludge production. Furthermore, anaerobic treatment systems are preferred due to its ability to treat high-strength wastewater and perform efficiently after long periods without re-inoculation and/or feeding despite their longer start-up time required as opposed to aerobic systems. Anaerobic digesters are generally utilized as a primary treatment process in wastewater treatment systems; thus, anaerobic effluents require further treatment to meet discharge standards for wastewater disposal (Rittmenn & McCarty, 2012; Metcalf & Eddy, 2003).

2.7. Anaerobic digestion (AD) process

The AD process involves the biodegradation of organic matter by means of micro-organisms in an oxygen-free environment (Mittal, 2005; Gerardi, 2003). The biogas generated as a by-product contains 50-70% methane which can be recovered as a clean source of energy (Abbasi et al., 2012; Rittmann & McCarty, 2012; Wang et al., 2010). There are four sequential stages carried-out by a diverse group of micro-organisms during the AD process, namely: 1) hydrolysis, 2) acidogenesis, 3) acetogenesis, and 4) methanogenesis (Wang et al., 2010). Considering that the four stages are co-dependent, the biodegradation rates at all the stages must be equivalent for the AD process to proceed efficiently (Gerardi, 2003). The inhibition at any stage limits the subsequent stage and ultimately leads to the reduction in methane production. The most common setbacks of AD result from the inhibition of methane-forming micro-organisms i.e. methanogens (Gerardi, 2003).

In the initial hydrolysis stage, micro-organisms convert macromolecules into simpler monomers i.e. fatty acids and glycerol, monosaccharides, amino acids, nitrogenous by-products. These monomers are then converted into organic acids during the acidogenesis stage. Subsequently, during the acetogenesis i.e. fermentation stage, the organic acids are converted to acetate and hydrogen. In the final stage, i.e. methanogenesis, methanogens utilise the acetate and hydrogen formed to produce methane (Kelleher et al., 2002). A simplified mechanism of the biodegradation of organic matter including different intermediates obtained during AD process is shown in Figure 2.3.

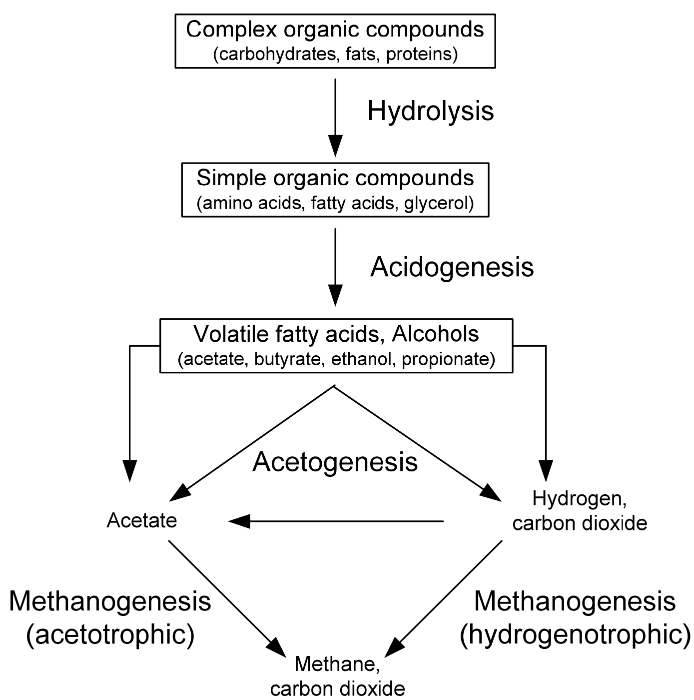

Figure 2-3: Anaerobic digestion and biogas production mechanism (Adapted from Mohamed, 2014; Gerardi, 2003)

2.7.1. Hydrolysis stage

During this initial stage, insoluble complex organic compounds such as particulate and colloidal matter consisting of carbohydrates, fats, oils and grease (FOG), and proteins are solubilised for the consumption by micro-organisms (Nayona, 2010). The hydrolysis of these large insoluble organic compounds by hydrolytic facilitated mechanisms of anaerobes, results in the formation of simpler, soluble organic matter which are further hydrolysed to easily absorbable monomers (Wang et al., 2010). These hydrolytic reactions and size reductions are facilitated using extracellular enzymes, referred to as hydrolases (Gerardi, 2003).

2.7.2. Acidogenesis stage

During the acidogenesis stage, the soluble organic compounds formed by the hydrolytic reactions are biodegraded by a diverse group of anaerobes generating a mixture of volatile fatty acids (VFAs) including acetate, alcohols such as ethanol and propionate, including new microbial biomass (Gerardi, 2003). Acetate is the most significant of the organic acids produced since it can be formed through both the acidogenesis and acetogenesis stages (Mohamed, 2014). Failure in the biodegradation of the products formed at this stage, may lead to the accumulation of acid within the system which can result in an undesirable loss of alkalinity, culminating in the decrease in pH in the bioreactor (Chen et al., 2007).

2.7.3. Acetogenesis stage

The intermediates, i.e. butyrate, ethanol, and propionate, accumulated during the acidogenesis stage undergo further transformation into acetate, carbon dioxide and hydrogen during this stage (Mohamed, 2014; Gerardi, 2003). These products can be used as a substrate by methanogens. Acetogenic or acid-forming micro-organisms are responsible for the production of acetate (Mohamed, 2014).

2.7.4. Methanogenesis stage

In this final stage, methanogens convert acetate, hydrogen gas, carbon dioxide, and other organic matter to methane (Wang et al., 2010; Gerardi, 2003). There are primarily two types of methanogens involved in this process namely, acetotrophic and hydrogenotrophic methanogens (Nayona, 2010; Gerardi, 2003). The acetotrophic methanogens produce methane from acetic acid while the hydrogenotrophic methanogens oxidize hydrogen and reduce carbon dioxide to methane. These methanogens are crucial to the AD process, since they facilitate the removal of COD in the form of methane and carbon dioxide (Mohamed, 2014; Metcalf & Eddy, 2003). This stage is considered as the rate-limiting stage because of the slow growth of methanogens in anaerobic digesters. In addition, there exists an optimal pH range, i.e. 6.5 to 8.5, in which methanogens are able to function efficiently (Chen et al., 2007). The chemical reactions describing methane production using acetotrophic and hydrogenotrophic methanogens are shown in Eq.(s) 2.1 – 2.2 (Mohamed, 2014).

Acetotrophic methanogenesis:

$$CH_3COOH \rightarrow CO_2 + CH_4 \quad (2.1)$$

Hydrogenotrophic methanogenesis:

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (2.2)$$

2.8. High-rate anaerobic reactors

High-rate anaerobic reactors are considered suitable for treating wastewaters containing high organic matter and suspended solids concentrations. These reactors operate at long hydraulic retention times (HRTs) and short solids retention times (SRTs) which facilitates the treatment of a large quantity of wastewater using highly-active biomass in the form of anaerobic sludge or granules (Lim & Fox, 2014; Chong et al., 2012). Despite the improvement of sludge stabilization in high-rate anaerobic systems, the development of a system capable of withstanding varying environmental conditions, such as the temperature fluctuations experienced in full-scale systems, has proven to be a constant challenge in addition to the frequent loss of solids from such systems (Ellis, 2008). These systems include the anaerobic filter (AF), anaerobic fixed film reactor (AFFR), anaerobic sequencing batch reactor (ASBR), up-flow anaerobic sludge blanket (UASB) reactor, expanded granular sludge bed (EGSB) reactor, static granular bed reactor (SGBR) and hybrid reactor systems, which have been extensively used for the treatment of different wastewaters (Chong et al., 2012; Park et al., 2012).

2.9. Static granular bed reactor (SGBR)

The SGBR is a relatively new high-rate anaerobic reactor developed at the Iowa State University in 2000 (Park et al., 2012). The SGBR was developed with the purpose of simplifying the design, operation, and maintenance of high-rate anaerobic reactors whilst still maintaining high-quality influent treatment and maximizing the biogas production (Ellis, 2008; Evans, 2004). It has proven to be successful in the treatment of various low- to medium-strength wastewaters, including PSW (Table 2.4). Pilot-scale SGBR systems are being used for wastewater treatment with the intention of studying the effect of reactor operating conditions such as the hydraulic retention time (HRT), solids retention time (SRT), and organic loading rate (OLR). The data collected from such pilot-scale studies are to be used for the design of full-scale SGBR systems and the commercialization of this new technology (Park et al., 2012).

The design of the SGBR is based on the UASB reactor design; however, the distinguishing feature of the SGBR is its down-flow configuration which eases the separation of the wastewater, solids, and biogas (Figure 2.4). Unlike other down-flow anaerobic reactors, such as the AF, the SGBR uses highly active anaerobic granules instead of synthetic media (Debik & Coskun, 2009; Evans, 2004). In addition, the down-flow configuration of the SGBR enables the accommodation of higher suspended solids concentrations in comparison to up-flow anaerobic reactors, such as the UASB and EGSB, which frequently experiences solids washout, i.e. the loss of granular biomass due to the high up-flow velocities used. Furthermore, granule buoyancy due to its flocculent nature does not inhibit the SGBR performance as experienced in the UASB and EGSB reactors (Ellis, 2008).

A schematic diagram of the SGBR is shown in Figure 2.4. The SGBR is relatively easy to operate with no mixing and mechanical equipment required other than a feed pump and an overflow line used to remove granules trapped in the under-drain system during backwashing (Evans, 2004). The wastewater fed into the SGBR flows downwards enabling it to filter through the dense anaerobic granular bed whilst the biogas produced rises to the headspace at the top of the reactor, from which it is removed, providing pneumatic mixing within the SGBR (Debik & Coskun, 2009). The gravel underdrain system helps to retain the granules within the reactor which ensures that there is sufficient microbial activity for the degradation of the organic matter present in the feed wastewater; thereby, facilitating the production of increased volumes of biogas which may in turn be used as a clean energy resource (Ellis, 2008).

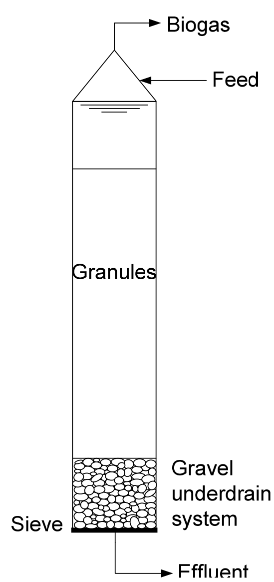

Figure 2-4: Schematic diagram of SGBR anaerobic digester

2.9.1. Advantages and disadvantages of the SGBR

There are many significant advantages and few performance limitations associated with the SGBR system which distinguishes it from other anaerobic systems, as seen in Table 2.3 (Basitere et al., 2017; Oh et al., 2015; Debik & Coskun, 2009; Ellis, 2008; Evans, 2004).

Table 2-3: Advantages and disadvantages of the SGBR

Advantages	Disadvantages
▪ Operates as a bioreactor and biofilter (high organic matter and suspended solids removal and excellent effluent quality) ▪ High retention of biomass (sufficient microbial activity and smaller reactor volume requirement) ▪ No oxygen required (reduced operational costs) ▪ No mixing, heating or circulation equipment required (significant energy savings) ▪ Counter-current flow between wastewater and biogas (easy separation) ▪ Simple reactor operation due to influent flow distribution design and down-flow configuration ▪ Maximum contact achieved between wastewater and granules with minimal granules washout	▪ Clogging and drainage problems due to occasional increases in head loss ▪ Periodic backwashing requirement to eliminate accumulated solids ▪ Ideally suited to low- to medium-strength wastewater

2.9.2. Application of the SGBR for the treatment of different wastewater types

The SGBR has been used to treat a wide variety of synthetic and actual wastewaters due to its operational simplicity, high COD removal efficiency (>90%) and excellent effluent quality (<50 mg/L TSS and BOD_5) (Ellis, 2008; Evans, 2004). According to the literature reviewed, mainly laboratory- and pilot-scale SGBRs operating at ambient temperature (i.e. approximately 25˚C), have been researched (Table 2.4). Variations of the SGBR operating conditions such as the temperature, OLR and HRT have proven to have relatively minimal effect on the stability of the SGBR and do not significantly affect the effluent quality (Ellis, 2008). Industrial wastewaters treated by the SGBR include wastewater from food processing industries such as dairy processing, pork slaughterhouses, meat slaughterhouses, and poultry slaughterhouses (Basitere et al., 2017; Oh et al., 2015; Park et al., 2012; Oh, 2012; Lim & Fox, 2011; Debik & Coskun, 2009). These wastewaters are characterised as non-toxic and highly biodegradable due to the high concentrations of organic matter and suspended solids; thus, making it ideally suited for anaerobic treatment by SGBRs (Mohamed, 2014).

Oh et al. (2015) and Park et al. (2012) evaluated the performance of a pilot-scale SGBR for the treatment of dairy processing wastewater under varying OLR, HRT, and operating temperatures. Oh et al. (2015) achieved average COD, BOD_5, and TSS removal efficiencies greater than 90% at temperatures as low as 11 °C, at OLRs up to 7.31 kg COD/m^3day with an HRT of 9 h. Similarly, Park et al. (2012a) achieved high organic removal efficiencies of 94, 97 and 89% for COD, BOD_5 and TSS, respectively, at temperatures ranging from 10 to 29 °C, HRTs from 9 to 48 h and OLRs from 0.63 to 9.72 kg COD/m^3day. It was further reported that increases in head loss and reactor clogging occurred throughout the study due to the accumulation of non-biodegradable solids in the system at HRTs of less than 18 h and OLRs of more than 3.5 kg COD/m^3day which impacted the physical operation of the SGBR. However, a backwashing system was implemented to alleviate this challenge and to maintain system stability including operability (Park et al., 2012a). Park et al. (2012b) further investigated the performance of an on-site pilot-scale SGBR for pork slaughterhouse wastewater for a range of OLRs and HRTs and achieved COD, TSS, and VSS removal efficiencies exceeding 95%.

Debik and Coskun (2009) performed a comparative study based on a SGBR using anaerobic granules and a static anaerobic sludge bed reactor (SASBR) using non-granular sludge for PSW treatment at 22 °C. The average COD removal efficiency in both the SGBR and SASBR was greater than 94%. The study concluded that: 1) neither an increase nor decrease in the HRT and OLR had an adverse effect on the efficiency of the reactors, and 2) non-granular sludge may be used in the SGBR resulting in high COD removal and methane production (Debik & Coskun, 2009). Basitere at al. (2017) evaluated PSW treatment by a SGBR on the basis of Debik and Coskun's (2009) results. The lab-scale SGBR was operated under mesophilic conditions (35-37 °C) as a pre-treatment stage. The results showed average COD, TSS and FOG removal efficiencies of 93, 95 and 90%, respectively; achieved over a period of 64 days at HRTs of 55 and 40 h, which were considered to be comparatively high to those reported in other studies.

Lim & Fox (2011) achieved an average COD removal efficiency of 87% for a SGBR operated at 24 °C for the treatment of swine wastewater. The study concluded that the COD removal efficiency was a function of the OLR, which ranged from 0.8 to 5.5 kg/m^3day. A similar trend was observed by Oh (2012) for two pilot-scale SGBRs operated under different HRTs and OLRs for the treatment of meat slaughterhouse wastewater. Average COD removal efficiencies of 94 and 95% were achieved at OLRs ranges of 1.01 to 3.56 kg COD/m^3day (HRT from 28 to 48 h) and 0.94 to 12.76 kg COD/m^3day (HRT from 20 to 96 h), respectively. According to Oh (2012), high organic removal efficiencies were maintained at the maximum OLR applied to each reactor.

Other industrial wastewaters treated using the SGBR include: pulp and paper, textile, landfill leachate, simple- (non-fat dry milk) and complex-synthetic (non-fat dry milk and sucrose), and sulphate-rich wastewaters (Turkdogan, 2013; Debik et al., 2012; Debik et al., 2005; Mach, 2004). Although the SGBR consistently demonstrated excellent performance in the treatment of these various wastewaters, further treatment of SGBR effluent is required prior to discharge into receiving water bodies or the environment (Lim & Kim, 2014). Thus, the application of tertiary treatment systems such as membrane bioreactors (MBR) or membrane filtration systems are implemented for the removal of the residual organic matter and suspended solids, nutrients (i.e. nitrogen and phosphorous), as well as pathogens (Lim & Kim, 2014). Table 2.4 summarises the operational results for the SGBR in the treatment of various types of wastewaters.

Table 2-4: Operational results of the SGBR for the treatment of various types of wastewaters

Type of Wastewater	Scale of plant	Temperature (˚C)	HRT (h)	OLR (kg COD/m^3d)	COD removal (%)	Methane yield	References
Poultry slaughterhouse	Lab	35-37	40-55	1.01-3.14	>90	-	Basitere et al., 2017
Dairy processing	Pilot	11-21	9-60	0.6-9.7	>90	0.26 (L CH_4/g $COD_{removed}$)	Oh et al., 2015
Pulp & paper	Pilot	35	4-24	1.2-3.6	67-92	-	Turkdogan et al., 2013
Dairy processing	Pilot	10-29	9-48	0.63-9.72	>90	-	Park et al., 2012
Pork slaughterhouse	Pilot	24-26	20-48	0.77-12.76	>95	-	Park et al., 2012
Textile	Lab	22	24, 48	1-1.7	70-93	0.3 (L CH_4/g $COD_{removed}$)	Debik et al., 2012
Meat slaughterhouse	Pilot	ni	28-48	1.09-2.91	93.4-94.9	-	Oh, 2012
Meat slaughterhouse	Pilot	ni	20-96	1.14-6.19	92.1-96.6	-	Oh, 2012
Swine	Lab	24	24	0.8-5.5	77.2-94.5	2.62 (m^3/day)	Lim & Fox, 2011
Poultry slaughterhouse	Lab	22	36-60	0.64-4.97	85-97.8	0.25 (L CH_4/g $COD_{removed}$)	Debik & Coskun, 2009
Landfill leachate	-	ni	-	-	90	0.017-0.051 (kg/day)	Debik et al., 2005
Municipal	Lab	25	8-48	-	74-84	-	Evans, 2004
Simple synthetic (Non-fat dry milk)	Pilot	ni	5-36	0.7-4.8	91.7-97.3	-	Mach, 2004
Simple synthetic (Non-fat dry milk)	Pilot	ni	5-36	0.7-4.0	93.9-96.6	-	Mach, 2004
Complex synthetic (Non-fat dry milk + sucrose)	Pilot	ni	18-48	2.5-5.0	93.5-95.3	-	Mach, 2004
Pork slaughterhouse	Pilot	ni	16-48	1.3-4.6	91.8-94.2	-	Mach, 2004
Pork slaughterhouse	Lab	ni	8-48	0.4-7.1	83.7-95.7	-	Mach, 2004
High sulphate waste-stream	Pilot	ni	18	4.0	97.3	-	Mach, 2004
Municipal	Pilot	23	8-48	0.08-0.8	56.5-81.6	-	Mach, 2004

2.10. Membrane bioreactor (MBR) technology for PSW treatment

An MBR system is essentially an enhanced biological activated sludge process which utilizes membranes for solid-liquid separation; thus, it provides the combined benefits of biological degradation of waste and membrane filtration (Jaiyeola & Bwapwa, 2016; Avula et al., 2009, Radjenović et al., 2008). MBR technology which was developed due to the limitations of conventional activated sludge processes has proven to be effective in the removal of organic matter, inorganic matter, pathogens and nutrients from various industrial wastewaters (Lin et al., 2012; Radjenović et al., 2008). MBRs provide several advantages over conventional activated sludge processes which include: smaller footprint (i.e. compact process), high quality effluent, reduced sludge production, process flexibility towards influent variations, effective separation of bacteria and viruses, complete biomass retention, and improved nitrification (Lin et al., 2012; Judd, 2011; Radjenović et al., 2008). The disadvantages of MBRs are mainly associated with the membrane filtration unit of the MBR. These disadvantages include: membrane fouling, high costs due to membrane cleaning, maintenance and replacement, and high energy requirement for aeration to pressurize or create a vacuum in the system for effective solid-liquid separation (Judd, 2011; Radjenović et al., 2008). However, the development of new membrane materials with anti-fouling properties and improved module design configurations make MBR technology a viable PSW treatment option (Jaiyeola & Bwapwa, 2016; Avula et al., 2009).

2.11. Biological nutrient removal using MBRs

The design of an MBR system can be based on the required nutrient removal from the wastewater (Radjenović et al., 2008). The removal of excess nutrients such as nitrogen and phosphorous is crucial for successful wastewater treatment as they have the potential to accelerate eutrophication in receiving water bodies (Phan et al., 2014; Radjenović et al., 2008). The removal of phosphorous can be achieved by either a chemical process (i.e. precipitation) or an enhanced biological phosphorous removal (EBPR) process which involves alternating between anaerobic and aerobic conditions (Phan et al., 2014; Radjenović et al., 2008). The aerobic/anoxic MBR configuration is commonly used for the biological nitrogen removal from wastewater facilitated by nitrification (oxidation of ammonium ions to nitrate with nitrite as the intermediate) and denitrification (reduction of nitrate to molecular nitrogen and other gaseous nitrogen compounds) processes. According to Phan et al. (2014), anoxic/aerobic MBRs can achieve complete nitrogen removal and greater than 90% phosphorous removal.

2.11.1. Nitrification

Nitrification is an autotrophic process which requires free molecular oxygen for aerobic treatment. During the nitrification process, ammonium (NH_4^+) and nitrite (NO_2^-) ions undergo oxidation by strict aerobic nitrifying bacteria, resulting in the formation of nitrate (NO_3^-) (Gerardi, 2003). The nitrification process occurs in two sequential steps, each step utilising a distinct type of aerobic nitrifying bacteria which require free molecular oxygen as the electron acceptor. During the first step known as nitratation, NH_4^+ is oxidised to NO_2^- by *Nitrosomas* or ammonium-oxidising bacteria (AOB) as shown by Eq. 2.3 (Gerardi, 2003).

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+ + H_2O + \text{energy} \quad (2.3)$$

The NO_2^- formed by the first step serves as an energy source for nitritation. Nitritation involves the oxidation of NO_2^- to NO_3^- by *Nitrobacter* or nitrite-oxidising bacteria (NOB), as shown in Eq. 2.4 (Gerardi, 2003).

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- + \text{energy} \quad (2.4)$$

The free molecular oxygen acts as the electron acceptor for both AOB and NOB. The degradation of proteins, amino acids, and other nitrogenous compounds results in the formation of high concentrations of inorganic nitrogen in the form of NH_4^+ (Oh, 2015). However, due to the rapid rate at which nitrifying bacteria oxidises NH_4^+ to NO_3^-, NO_3^- is present in much higher concentrations than NH_4^+ in most aerobic environments. The NO_3^- produced is considered less toxic than NH_4^+ and can undergo further reduction to molecular nitrogen (N_2) by denitrifying bacteria (Wang et al., 2010). The overall nitrification process is shown in Eq. 2.5 (Gerardi, 2003).

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O \quad (2.5)$$

Challenges associated with the full-scale application of nitrification in wastewater treatment processes include the washout of nitrifying bacteria from aerobic bioreactors due to the relatively slow rate at which the autotrophic AOB and NOB grow in comparison to heterotrophs, as well as the high sensitivity demonstrated by nitrifying bacteria in the presence of toxic materials (Wang et al., 2010).

2.11.2. Denitrification

During the denitrification process, NO_3^- is used as the electron acceptor for the biological reduction of NO_3^- to nitrogen gas (N_2) (Gerardi, 2003). This reduction of NO_3^- is carried out by denitrifying bacteria in a series of steps during which the NO_3^- is reduced through NO_2^-, nitric oxide (NO) and nitrous oxide (N_2O) to gaseous N_2. Denitrification processes generally occur under anoxic or anaerobic conditions, i.e. in the absence of free molecular oxygen (Phan et al., 2014; Kraume et al., 2005). Denitrifying bacteria can use free molecular oxygen and NO_3^- as electron acceptors. Aerobic denitrification occurs when the denitrifying bacteria utilise the free molecular oxygen when the wastewater has sufficient free molecular oxygen concentrations (Seifi & Fazaelipoor, 2012). There are also instances where the denitrifying bacteria, which are mainly facultative aerobes, are capable of converting the NO_3^- to NH_4^+ when in the presence of free molecular oxygen. This process, referred to as nitrate ammonification, only occurs when NO_3^- is available in place of free molecular oxygen (Wang et al., 2010).

2.11.3. Simultaneous nitrification and denitrification (SND)

The biological removal of nitrogen from wastewater can be achieved through the unconventional heterotrophic nitrification and aerobic denitrification processes, by means of nitrifying and denitrifying micro-organisms which exist in equilibrium (Chen at al., 2009). These micro-organisms are capable of carrying out nitrification and aerobic denitrification simultaneously during which neither nitrite (NO_2^-) nor nitrate (NO_3^-) are used as electron acceptors (Shoda et al., 2017). This process is known as simultaneous nitrification and denitrification (SND). The oxidation of organic matter and the SND process can occur under aerobic conditions within a single bioreactor; i.e. single stage nitrification-denitrification (SSND), provided that the operating conditions are suitable for the three groups of micro-organisms involved namely, aerobic heterotrophs, nitrifying, and denitrifying micro-organisms, as shown by Figure 2.5 (Seifi & Fazaelipoor, 2012).

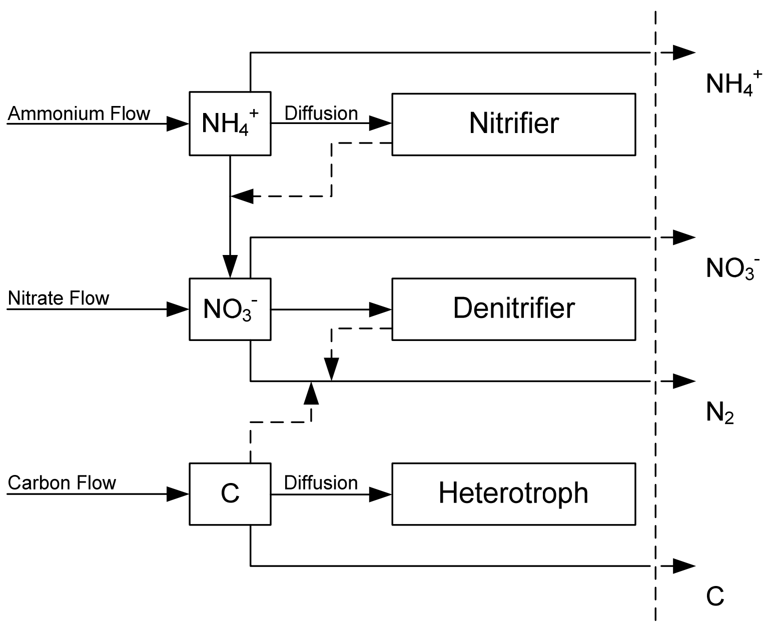

Figure 2-5: SND mechanism (Adapted from Halling-Sorensen & Nielson, 1996)

The reactions for heterotrophic nitrification (Eq. 2.6) and aerobic denitrification (Eq. 2.7) are shown below (Shoda et al., 2017).

$$NH_4^+ \rightarrow NH_2OH \rightarrow NO_2^- \rightarrow NO_3^- \qquad (2.6)$$

$NO_3^- \rightarrow NO_2^- \rightarrow N_2O \rightarrow N_2$ (2.7)

During the SND process, nitrogen removal occurs through the oxidation of NH_4^+ to the intermediates NO_2^- and NO_3^-, prior to the reduction of NO_2^- and NO_3^- to nitrogen gas (N_2). This suggests that the initial oxidation of ammonium (i.e. nitratation) is the rate-limiting step in nitrogen removal via SND (Holman & Wareham, 2005). For this reason, the implementation of an HRT which is sufficient for the development of a nitrifying population is of great importance, especially in continuous biofilm reactor systems as the growth rate of these micro-organisms is slow in comparison to other micro-organisms competing for the same substrates. The aeration rate and ratio of organic carbon to nitrogen (i.e. C/N) also play a critical role in facilitating the SND process as the different micro-organisms present within the bioreactor compete for substrates, i.e. organic carbon, ammonium, and free molecular oxygen, in order to perform their specific functions. The autotrophs and nitrifying micro-organisms compete for oxygen, whereas, the heterotrophs and denitrifying micro-organisms compete for organic carbon (Seifi & Fazaelipoor, 2012).

The combined reaction for the SND process is shown in Eq. 2.8 (Shoda et al., 2017).

$NH_4^+ \rightarrow NH_2OH \rightarrow N_2O \rightarrow N_2$ (2.8)

The SND process offers several advantages over the conventional nitrification and denitrification processes. These advantages include: minimal sludge production, reduced carbon source requirement, lower energy consumption due to the reduced aeration requirement, and smaller footprint due to the combined nitrification and denitrification processes simultaneously occurring within a single bioreactor, i.e. SSND (Seifi & Fazaelipoor, 2012; Yoo et al., 1999). The most common application of SND processes are in sequencing operation processes (i.e. SBRs). In biofilm bioreactors, the competition for substrates occurs within the biofilm. Apart from competing for free molecular oxygen, the heterotrophic aerobes and nitrifying bacteria which are prevalent in the outer layers of the biofilm, i.e. where the oxygen is concentrated; consume the organic carbon and ammonium, respectively. The denitrifying micro-organisms exist within the inner layers of the biofilm (Seifi & Fazaelipoor, 2012; Holman & Wareham, 2005; Yoo et al., 1999).

The efficiency of the SND process can be estimated using Eq. 2.9 (Fu et al., 2009).

$$SND\ (\%) = \left(1 - \frac{NO_x{}^- - N_{remained}}{NH_4{}^+ - N_{produced}}\right) \times 100 \qquad (2.9)$$

Where, $NO_x{}^- - N_{remained}$ is the sum of $NO_2{}^- - N$ and $NO_3{}^- - N$ present in the effluent subsequent to the SND process in mg/L and $NH_4{}^+ - N_{produced}$ is the difference between the feed and effluent $NH_4{}^+ - N$ concentrations in mg/L.

2.12. MBR configurations

There are two main MBR configurations: immersed and sidestream MBRs (Figure 2.6). The direction of flow and the pressure difference across the membrane, i.e. the transmembrane pressure (TMP), are the distinguishing factors of the MBR configurations (Judd, 2011). In the immersed MBR, the membrane is placed directly into the bioreactor and separation is achieved through vacuum-driven filtration (Lin et al., 2012; Radjenović et al., 2008). The immersed MBR is commonly used in wastewater treatment due to is being less energy intensive and prone to membrane fouling compared to the sidestream MBR (Judd, 2011; Radjenović et al., 2008). In the sidestream MBR, the wastewater is passed through the membrane and is then recycled back to the bioreactor. The recycling of the wastewater requires additional pumping which in turn affects the TMP and permeate flux; thus, the TMP is the driving force of sidestream MBRs (Lin et al., 2012; Radjenović et al., 2008). Sidestream MBRs offer the benefit of easier membrane cleaning, using chemical cleaning-in-place (CIP) processes; and replacement (Lin et al., 2012; Judd, 2011).

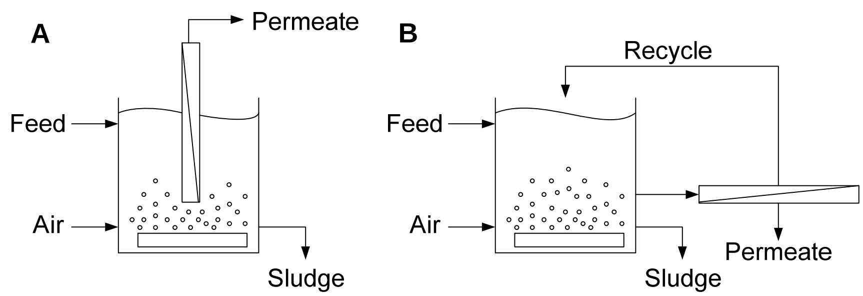

Figure 2-6: (A) Immersed and (B) sidestream MBRs (Adapted from Judd, 2011)

2.13. Membrane filtration processes

Membrane filtration refers to the pressure-driven process which utilizes semi-permeable membranes to separate particulate matter from wastewater (Judd, 2011). Microfiltration (MF) and ultrafiltration (UF) are the two most commonly applied membrane filtration processes used by MBRs for wastewater treatment (Judd, 2011; Radjenović et al., 2008). However, MF, UF, nanofiltration (NF), and reverse osmosis (RO) processes have been previously investigated for the treatment of PSW (Yordanov, 2010; Lo et al., 2005; Coskun et al., 2005). These membranes are characterized according to their separation capability or pore size i.e. 100-1000 nm and 5-100 nm for MF and UF, respectively (Avula et al., 2009; Radjenović et al., 2008). Despite the high capital costs associated with membrane filtration processes, the use of MF and UF membranes is considered to be viable treatment option due to the membrane life cycle costs being comparable to costs associated with conventional treatments (Avula et al., 2009).

The two most commonly used materials for the construction of membranes are polymeric (organic) or ceramic (inorganic) materials. These membrane materials are generally classified according to their hydrophilic and hydrophobic properties. Polymeric materials used for commercial MF and UF membrane applications include: hydrophobic polymers such as polyproylene (PP), polyethylene (PE), and polytretrafluoroethylene (PTFE); semi-hydrophobic polymers such as polyamide (PA), polysulfone (PS), polyethersulfone (PES), and polyvinylidene difluoride (PVDF); and hydrophilic polymers such as cellulose acetate (CA) which is generally not used in commercial MBRs. Ceramic materials are the most commonly used inorganic material for industrial membrane applications due to their high performance in comparison to polymeric membranes, high chemical and thermal resistivity, greater hydrophilicity, inert nature and the ease of cleaning (Judd, 2011). Although the use of ceramic membranes in MBRs is limited due to its high capital costs, it has proven to be suitable for the treatment of PSW (Basitere et al., 2017).

There are two primary flow configurations utilised in conventional pressurised membrane processes: cross-flow and dead-end flow (Figure 2.6). The passage of the feed across the membrane is the distinguishing characteristic by which these flow configurations differ. In the cross-flow configuration, the feed flows parallel to the membrane surface whilst the retentate flows continuously from the module outlet; thus, only a portion of the feed is converted to permeate. The ratio of permeate to feed flow, i.e. the recovery, may further be reduced when the permeate is utilised for backwashing, i.e. reversing the flow for cleaning. In the dead-end configuration, the feed flow is perpendicular to the membrane surface and there is no retentate stream. Filtration by means of the dead-end configuration results in higher resistance to flow which is proportional to the degree of fouling formed on the membrane surface (Judd, 2011). The cross-flow configuration is generally preferred to dead-end filtration as it causes minimal fouling on the membrane surface and therefore it does not experience significant reductions in the permeate flux; thereby, requiring periodic cleaning, which occurs considerably in the dead-end flow configuration (Zhou et al., 2015). However, the cross-flow configuration required up to 20 times the volume of feed in order to maintain a relatively high flux which ultimately increases the energy requirement for MBRs (Zhang et al., 2005).

Flux reduction is primarily experienced due to a build-up of rejected solutes on the membrane surface, resulting in blockages of the membrane pores (Zhou et al., 2015). In UF processes, the resistance of the gel-polarised layer formed and the resistance of the boundary layer are attributed to be the largest influences on flux reduction. Fouling of membranes which is prevalent in UF applications is considered undesirable as it results in reduced membrane performance and an increase in operational costs. These costs are associated with the increase in the pressure requirements, which requires more energy input;

and the increase in the frequency of membrane cleaning processes which in turn increases the consumption of the chemicals used for cleaning (Zhou et al., 2015).

2.13.1. Ultrafiltration (UF) membrane processes for PSW treatment

UF membrane processes facilitate the removal of colloidal matter, suspended solids and macromolecular matter including the separation of large solutes such as proteins on the basis of their molecular weight, physical shape and chemical structure by means of a semi-permeable membrane (Judd, 2011). These processes generally operate at an applied pressure of 1 MPa for the removal of particulate matter in the range of 5 to 100 nm. UF membranes have proven to be efficient in the removal of particulate matter, pathogens and environmentally hazardous nutrients from PSW (Yordanov, 2010; Avula et al., 2009; Lo et al., 2005); and has also demonstrated its suitability as a post-treatment system for anaerobically treated PSW (Basitere et al., 2017). Moreover, UF membranes are capable of recovering the proteins, nutrients and potential by-products from the manufacturing of consumer goods in the food, chemical, and pharmaceutical industries (Avula et al., 2009; Lo et al., 2005). However, since the treatment of PSW is significantly influenced by stringent environmental regulations and not necessarily by the economic feasibility of designed treatment processes, limited research studies have been conducted in an attempt to successfully recover valuable by-products from the PSW.

According to Lo et al. (2005), by-products containing 30 to 35% protein could be produced from PSW by integrating UF with dehydration processes. The high content of both proteins and fats present in the PSW could potentially cause the membrane to experience severe fouling which would ultimately reduce the feasibility of commissioning the process to an industrial scale (Lo et al., 2005). The effect of different organic membrane materials and pore sizes, i.e. PS (100 nm), PVDF (300 nm) and ultrafilic (50 nm), was investigated to evaluate the efficiency of UF membranes for the treatment of PSW. The PVDF membrane achieved a significant reduction in the COD concentration of the PSW with retention coefficients of 87% at 345 kPa and 89% at 552 kPa. The ultrafilic membrane (smallest pore size) and the PS membrane demonstrated the highest and lowest reduction in COD concentration with retention coefficients of 88% (345 kPa) and 91% (552 kPa), and 79% (345 kPa) and 82% (552 kPa), respectively (Abboah-Afari, 2011).

2.13.1.1. Operating parameters affecting the flux during UF

Membrane filtration processes are generally applied to PSW as a secondary and/or tertiary treatment process (Yordanov, 2010). The major operating parameter considered during UF is the permeate flux and its susceptibility to fouling and pore structure (Avula et al., 2008). During membrane filtration, the permeate flux experiences a gradual decrease and membrane fouling becomes evident when all other operating parameters such as pH, TMP, temperature, wastewater solute concentration, and feed flow rate remains constant (Yordanov, 2010; Avula et al., 2008). Since protein molecules do not have a fixed structural conformation, it is common to experience an initial decrease in permeate flux when using UF membranes for processing protein laden wastewater, such as PSW. Moreover the pH plays an influential role on the volumetric flux; a decrease in the pH of PSW results in a reduction of the colloidal properties of PSW and a subsequent increase in the volumetric flux. A further increase in the volumetric flux can be achieved by maintaining the pH and feed flow rate and increasing the TMP (Avula et al., 2009).

2.14. Operating conditions of the combined anaerobic, aerobic, and membrane filtration system

There are bioreactor conditions which must be monitored and maintained to ensure that anaerobic and aerobic bioreactors operate optimally and more importantly to avoid process inhibition and system failure. Firstly, the micro-organisms used must have sufficient time for the biodegradation of organic matter and the sorption of unreactive species, and secondly, the organic loading must be controlled in such a manner that overfeeding of the micro-organisms is prevented (Nayona, 2010). The HRT, OLR, and SRT are some of the key operating conditions used to ensure that an appropriate balance is achieved in bioreactors treating PSW (Wellinger et al., 2013). These conditions can be optimized using statistical methods, such as Response Surface Methodology (RSM). Similarly, the permeate flux is the main operating condition which is monitored in membrane filtration processes as it serves as an indication of the overall membrane performance with regard to membrane pressure, resistivity, permeability and fouling (Judd, 2011; Avula et al., 2009).

2.14.1. Hydraulic retention time (HRT)

The HRT is the theoretical time that the wastewater spends inside the anaerobic bioreactor (Nayona, 2010). The shortest HRT is dictated by the rate of the slowest growing, essential micro-organisms of the anaerobic bacterial community within the anaerobic bioreactor (Zaher et al., 2007). The HRT has a direct influence on the conversion of volatile solids or organic matter to biogas during the anaerobic digestion process; therefore, it influences the rate and extent of biogas production (Nayona, 2010).

The HRT can be estimated using Eq. 2.10 (Wellinger et al., 2013; Bwapwa 2010).

$$HRT = \frac{V_w}{Q} \tag{2.10}$$

Where, HRT is measured in h; bioreactor working volume (V_w) in L; and feed flow rate (Q) in L/h.

2.14.2. Organic loading rate (OLR)

The OLR is defined as the quantity of organic matter (i.e. volatile solids or COD) introduced into the bioreactor, which requires treatment by a defined volume of an anaerobic bioreactor for a specific retention time (Nayona, 2010). The higher the OLR, the more sensitive the system becomes to environmental conditions; hence stringent monitoring is required (Wellinger et al., 2013). To determine the effect of the OLR on the quality of the effluent produced by the bioreactor, the HRT must be maintained at a steady-state for considerable periods.

The OLR can be estimated using Eq. 2.11 (Wellinger et al., 2013; Bwapwa 2010).

$$OLR = \frac{COD_f \times Q}{V_w} \qquad (2.11)$$

Where, *OLR* is measured in g COD/Lday; feed COD (COD_f) in g/L; bioreactor working volume (V_w) in L; and feed flow rate (Q) in L/h.

If the concentration of the organic matter in the feed is kept constant, reducing the HRT will result in a higher OLR. Conversely, if the organic matter in the feed varies the value of the OLR will vary at the same HRT.

The ratio between the feed flow rate and bioreactor working volume is equivalent to the HRT; therefore, Eq. 2.11 reduces to Eq. 2.12 (Wellinger et al., 2013; Bwapwa 2010).

$$OLR = \frac{COD_f}{HRT} \qquad (2.12)$$

Where, *OLR* is measured in g COD/Lday; feed COD (COD_f) in g/L; and *HRT* in day.

2.14.3. Membrane (permeate) flux

The permeate flux refers to the quantity of permeate which passes through a unit of the actual filtering membrane surface area per unit of time (Judd, 2011). A higher permeate flux results in the formation of fouling on the membrane surface, which poses a problem as a higher operational permeate flux is desirable for process economics. It is therefore necessary to operate below the threshold flux in order to control the rate and degree of fouling (Zhou et al., 2015).

The permeate flux can be estimated using Eq. 2.13 (Wang et al., 2010; Avula et al., 2009).

$$J = \frac{Q}{A_{membrane}} \qquad (2.13)$$

Where, permeate flux (J) is measured in L/m^{2}h or indicated as LMH; permeate flow rate (Q) in L/h; and membrane surface area ($A_{membrane}$) in m^2.

CHAPTER 3

MATERIALS AND METHODS

CHAPTER 3
3. MATERIALS AND METHODS

3.1 Introduction

In this study, an anaerobic pre-treatment stage coupled with an aerobic bioreactor and sidestream membrane filtration post-treatment stage was used for the treatment of poultry slaughterhouse wastewater (PSW) (Figure 3.1). The lab-scale PSW treatment system consisted of: 1) an anaerobic static granular bed reactor (SGBR) for organic matter and suspended solids removal; 2) a single stage nitrification-denitrification (SSND) aerobic bioreactor for total nitrogen (TN) removal via simultaneous nitrification and denitrification (SND); and 3) an ultrafiltration membrane module (ufMM) system as a final treatment stage for residual organic matter and suspended solids removal.

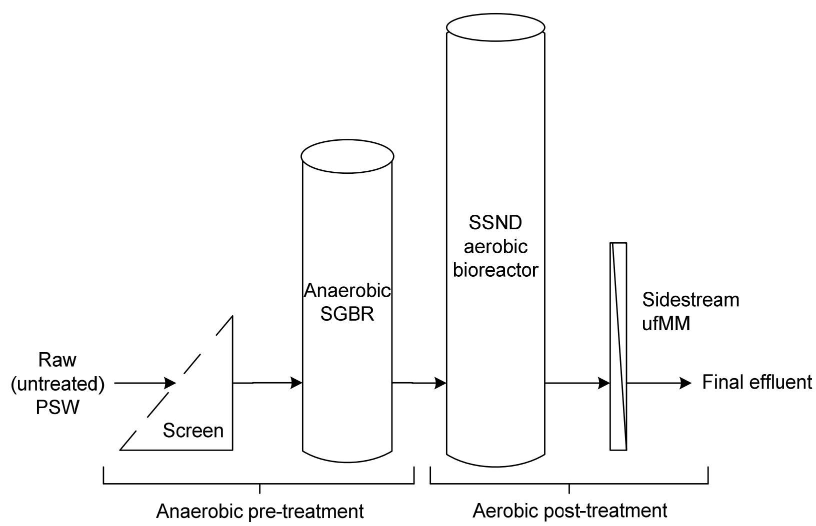

Figure 3-1: Schematic diagram of PSW treatment system

3.2 Poultry slaughterhouse wastewater (PSW)

PSW sourced from a poultry slaughterhouse located in the Western Cape Province (South Africa) was used in this study. The raw (untreated) PSW samples were collected in 25 L plastic containers from the sump of the existing wastewater treatment facility of poultry slaughterhouse, during the slaughtering and cleaning operations. The raw PSW samples were refrigerated at 4 ˚C throughout this study. The characteristics of the raw, unfiltered PSW used in this study are listed in Table 3.1. The SGBR feed was prepared by filtering the PSW through a 2 mm fine mesh screen to remove floating solids such as feathers, fats and particulate matter from the PSW.

Table 3-1: Characteristics of the raw, unfiltered PSW used in this study

Parameter	Unit	Average
Chemical oxygen demand (COD)	mg/L	5216
Biological oxygen demand (BOD_5)	mg/L	2875
pH at 25 ˚C	-	5.6
Conductivity at 25 ˚C	mS/m	159
Ammonia (as N)	mg/L	25.2
Ortho-phosphate (PO_4^{3-})	mg/L	24.3
Alkalinity (as $CaCO_3$)	mg/L	170
Fats, oils & grease (FOG)	mg/L	1110
Nitrate (NO_3^-)	mg/L	1.0
Total dissolved solids (TDS)	mg/L	1208
Total suspended solids (TSS)	mg/L	1580
Volatile suspended solids (VSS)	mg/L	1533
Volatile fatty acids (VFA)	mg/L	421

3.3 Anaerobic pre-treatment of PSW

3.3.1 Static granular bed reactor (SGBR) set-up and inoculation

The lab-scale SGBR used in this study was made from a cylindrical glass column with a height and inner diameter of 0.62 m and 0.065 m, respectively. The SGBR had a working volume of 2.0 L. A perforated feed tube was positioned at the top of the SGBR for the uniform distribution of the SGBR feed. A second perforated tube was positioned in the centre of the underdrain bed as part of a backwashing system, which also had an overflow line. Pumice (average diameter of 5-20 mm) was used as the underdrain bed to prevent the washout of the anaerobic granules. A stainless steel sieve (pore size: 2 mm) was fixed to the bottom of the SGBR to retain the pumice, which occupied a volume of 0.3 L. The SGBR system consisted of a 5 L feed storage tank, a 5 L effluent storage tank, polyvinyl chloride (PVC) and glass fittings, silicone tubing, a MINIPULS® Evolution peristaltic pump (Gilson, USA) for feeding the PSW into the SGBR and discharging the effluent from the SGBR, and a circulating water bath used to supply the water jacket (Figure 3.2). The biogas produced was collected through an off-gas exit port at the top of the SGBR into a 0.5 L Tedlar® bag.

The SGBR was inoculated with 0.4 L anaerobic granules obtained from a full-scale mesophilic up-flow anaerobic sludge blanket (UASB) reactor operated at a local brewery (Newlands, South Africa) and fed with 1.6 L of raw PSW. A dry milk solution of 0.01 L (1427±65 mg COD/L) prepared with potable water (50% (v/v)) was used for the acclimatization of the granules to the new environment. The specifications of the anaerobic granules and PSW used are presented in Table 3.2.

Table 3-2: Specifications of the PSW and anaerobic granules

Parameter	Unit	PSW	Anaerobic granules
pH	-	7.5	7.0
COD	mg/L	2800 ±36	-
TSS	mg/L	675	42867
VSS	mg/L	1625	51391
NH_4^+-N	mg/L	215	-
NO_3^--N	mg/L	1.9	-
PO_4^{3-}-P	mg/L	31	-

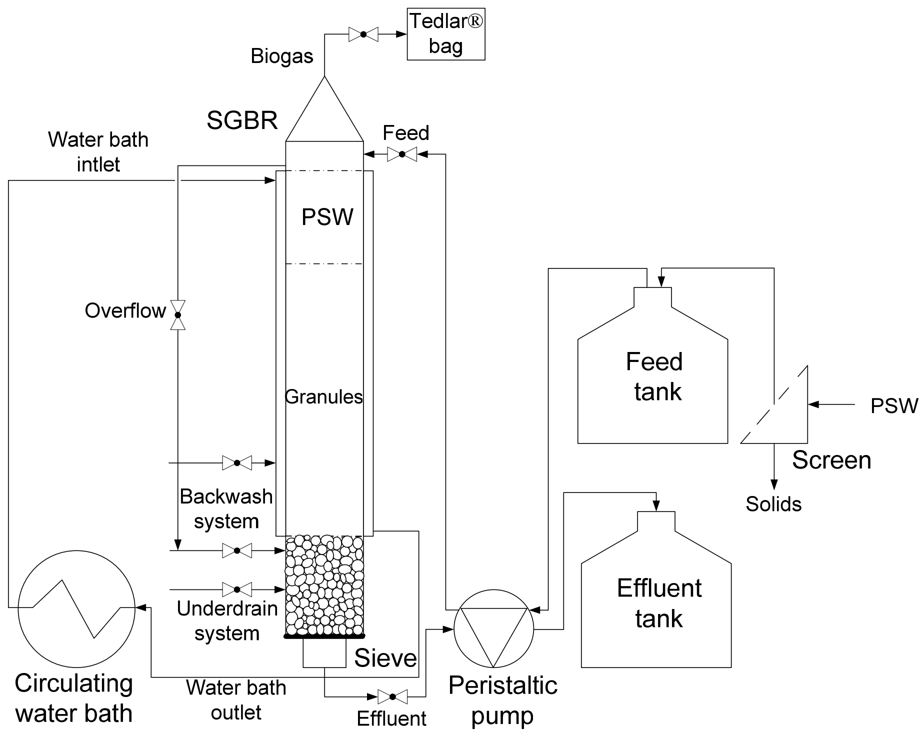

Figure 3-2: Schematic diagram of the SGBR system

3.3.2 SGBR operation and operating conditions

The SGBR was maintained at mesophilic temperature (i.e. 35-37 ˚C) throughout the 138 days of operation. The filtered PSW was diluted (50% (v/v) and subsequently 25% (v/v)) with potable water to prevent overloading of the SGBR during the start-up period. The SGBR was fed with 50% (v/v) and 25% (v/v) diluted PSW for a period of 43 and 8 days, respectively. The hydraulic retention times (HRTs) at which the SGBR operated was based on conditions reported in Debik & Coskun (2009) and Basitere et al. (2017). The SGBR operating conditions are summarised in Table 3.3. After the acclimatization period of 48 h during which the granules were allowed to acclimatize to the substrate, i.e. PSW, the HRT was set to 55 h to initiate the start-up organic loading rate (OLR) of 1.18 g COD/Lday, and the SGBR was operated in continuous mode under pseudo-steady state conditions. The HRT was decreased step-wise after the start-up period to simulate variations in the feed flow rate and to adequately assess the performance of the SGBR i.e. to a maximum average OLR of 5.74 g COD/Lday. The SGBR was backwashed on an as needed basis (weekly, biweekly or approximately 2-3 times per week). During backwashing, a portion of the stored effluent, i.e. treated PSW, was pumped back into the underdrain system and an equal quantity of supernatant was removed through the discharge port/overflow line in order to maintain a constant water level in the reactor.

Table 3-3: Summary of SGBR operating conditions

Operating time (days)	HRT (h)	Flow rate (Q) (L/h)	Average OLR (g COD/Lday)	Dilution (v/v) (%) [number of days]
28	55	0.0364	1.18	50 [28]
29	96	0.0208	0.78	50 [15] 75 [8] None [6]
27	48	0.0364	1.96	None
28	36	0.0486	4.10	None
26	24	0.0729	5.74	None

3.4 Aerobic post-treatment of SGBR (anaerobic) effluent: SSND and ufMM systems

3.4.1 Single stage nitrification-denitrification (SSND) bioreactor system set-up and operation

A lab-scale SSND aerobic bioreactor with a total volume of 19 L and a working volume of 10 L, was used in this study. The bioreactor was made from a cylindrical PVC column with a height and inner diameter of 2 m and 0.11 m, respectively. Two types of immobilized packing material in the form of gravel (average diameter of 20-30 mm) and gravel-integrated-with-sponge (average diameter of 25-35 mm) were used as immobilization and supporting media for the micro-organisms in the bioreactor, respectively. The total packed bed height was 1 m. A perforated PVC cylinder (height and inner diameter of 0.52 m and 0.09 m, respectively), holding 20 mm sponge cubes for microbial attachment, was positioned at the top of the bioreactor. Four sampling ports were installed along the length of the bioreactor at heights of 0.345 m, 0.9 m, 1.4 m and 1.855 m from the base of the bioreactor. The HRT was controlled by a MINIPULS® Evolution peristaltic pump (Gilson, USA). During the aeration stage, a Resun® AC-9906 air pump (Resun®, China) was used to supply air into the column using a diffuser (air stone) positioned at a height of 0.85 m from the top of the bioreactor.

The bioreactor was filled with 10 L of raw, unfiltered PSW (Table 3.4) and inoculated with 100 ml basal medium periodically at 24 intervals for 7 consecutive days. The composition of the basal medium is provided in Table 3.5. A quantity of 100 ml of the PSW was removed prior to adding the basal medium. Yeast extract (7.8 g/L) was supplemented in the basal medium which was filter-sterilised using a 0.22 µm filter and adjusted to a pH of 7 using sodium hydroxide (0.1 M NaOH). After the acclimation period (7 days), the SGBR (anaerobic) effluent was introduced as feed into the SSND bioreactor.

Table 3-4: Specifications of the raw, unfiltered PSW used in the SSND bioreactor

Parameter	PSW specifications
COD	5694 ±135 mg/L
NH_4^+-N	210 ±10 mg/L
NO_3^--N	3.8 ±0.35 mg/L
NO_2^--N	8.7 ±0.12 mg/L
PO_4^{3-}-P	53.7 ±0.58 mg/L

Table 3-5: Specifications of the nutrient solution

Chemical	Specifications
	Basal medium
KH_2PO_4	1.5 g/L
Na_2HPO_4	7.9 g/L
$MgSO_4.7H_2O$	0.5 g/L
Trace element	1 ml
	Trace elemental solution
EDTA	50 g/L
$ZnSO_4.7H_2O$	2.2 g/L
$CaCl_2$	5.5 g/L
$MnCl_2.4H_2O$	5.06 g/L
$FeSO_4.7H_2O$	5.0 g/L
$(NH_4)_6Mo_7O_2.4H_2O$	1.1 g/L
$CuSO_4.5H_2O$	1.57 g/L
$CoCl_2.6H_2O$	1.61 g/L

In this study, two operating strategies were assessed during the continuous operation of the SSND bioreactor in order to determine the optimal bioreactor configuration, i.e. down-flow or up-flow, and to facilitate TN removal via a simultaneous nitrification and denitrification (SND) process (Figure 3.3). During stage 1 (33 days excluding the start-up period), the bioreactor was operated in a down-flow configuration without aeration (Figure 3.3 A). During stage 2 (33 days), the configuration was changed to an up-flow mode and air was sparsely continuously supplied into the column (Figure 3.3 B). The dissolved oxygen (DO) concentration was maintained in the range of 1.8 to 3.2 mg/L in the aerobic zone. The bioreactor was operated at ambient (i.e. 23-25 ˚C) temperature throughout the 87 days of operation. The HRT was varied step-wise between 6 days and 11 days in stage 1 and stage 2, respectively. The SSND bioreactor operating conditions are summarised in Table 3.6.

Table 3-6: Summary of the SSND bioreactor operating conditions

SSND Operating stage	Operating time (days)	HRT (days)	Flow rate (Q) (L/h)	Average OLR (g COD/Lday)	Average NLR[a] (g TN/Lday)
Start-up	12	6	0.066	0.13	0.03
1	33	6	0.066	0.12	0.04
2	33	11	0.039	0.08	0.03

NLR[a]: TN loading rate

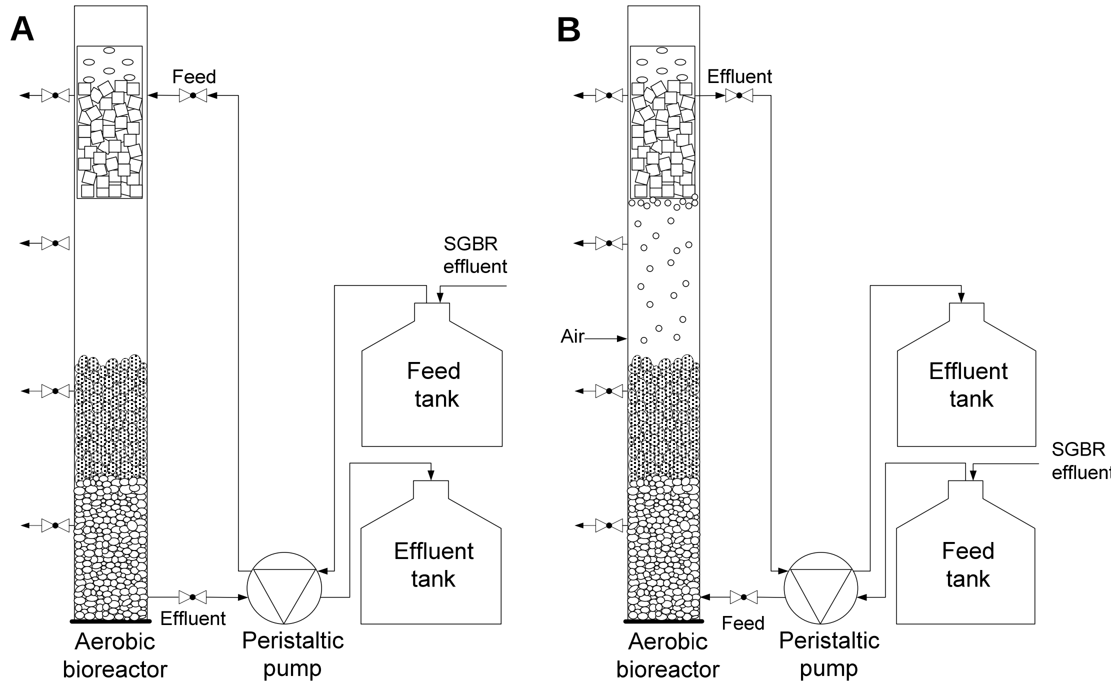

Figure 3-3: Schematic diagram of the SSND aerobic bioreactor system (A) down-flow configuration without aeration and (B) up-flow configuration with aeration

3.4.2 Ultrafiltration membrane module (ufMM) setup, operation and maintenance cleaning

This study used hollow-fibre (HF), ceramic UF membranes with a pore size of 100 nm, a length of 0.25 m and an active surface area of 0.0055 m^2. The membranes were enclosed in glass, cylindrical modules, each with a volume of 45 ml. The inner and outer diameters of the membrane were 6 mm and 8 mm, respectively. The UF membrane specifications are presented in Table 3.7.

The UF membrane module was operated under dead-end filtration mode (Figure 3.4). The feed (i.e. SSND bioreactor effluent) was pumped into the bottom of the module into the lumen side of the membrane using a MINIPULS® Evolution peristaltic pump (Gilson, USA). The permeate was collected from the shell side of the membrane module. A weekly composite sample from the SSND bioreactor effluent was fed to the membrane module at a constant feed flow rate (Table 3.8). The UF membranes were started-up on day 27 of the SSND bioreactor operation and were operated at ambient temperature (i.e. 23-25 ˚C). The operating conditions of the ufMM are summarised in Table 3.8.

Maintenance cleaning was performed on the UF membranes (on an as needed basis i.e. weekly) using sodium hypochlorite solution (400 mg/L, NaOCl) followed by citric acid solution (1 %). The effluent from the SSND bioreactor was allowed to accumulate during the cleaning of the UF membranes. The UF membrane modules were flushed with de-ionized (DI) water subsequent to the UF membranes being soaked with sodium hypochlorite solution and citric acid solution for 24 h for the removal of organic matter and 4 h for the removal of inorganic contaminants, respectively. The preparation procedures for the cleaning solutions are provided in Appendix C.

Table 3-7: UF membrane specifications

Specification	Description
Type of membrane	Hollow-fibre (HF)
Material	Alpha aluminium oxide (Al_2O_3)
Surface properties/Hydrophobicity	Neutral, hydrophilic
Surface area	0.0055 m^2
Pore size	100 nm
Membrane diameter	6 mm (inner), 8 mm (outer)

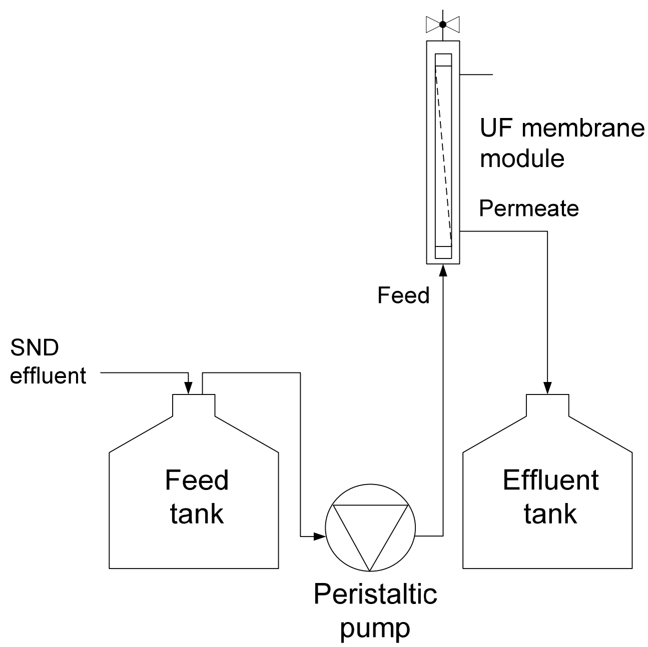

Figure 3-4: Schematic diagram of the ufMM system

Table 3-8: Summary of the ufMM operating conditions

MBR operating stage	Operating time (days)	Feed flow rate (L/h)
1	19	0.0282
2	33	0.0282

3.5 Sampling and analysis

The performance of each treatment system i.e. anaerobic SGBR, SSND aerobic bioreactor and ufMM, was monitored using the analyses of the feed and effluent samples for the parameters listed in Table 3.9, 3.10 and 3.11, respectively. All analyses for the duration of this study were completed in triplicate according to standard methods (APHA). The samples were stored at 4 ˚C prior to analysis to minimize biological activity.

3.5.1 SGBR sampling and analysis

The SGBR feed and effluent samples were collected and analysed for the following parameters: temperature; pH; conductivity; total dissolved solids (TDS); salinity; turbidity; chemical oxygen demand (COD); total suspended solids (TSS); volatile suspended solids (VSS); ammonium-nitrogen (NH_4^+-N); nitrate-nitrogen (NO_3^--N) and ortho-phosphate phosphorous (PO_4^{3-}-P). A weekly composite sample of the SGBR feed and effluent were sent to a South African National Accreditation System (SANAS) accredited lab (Scientific Services, South Africa), for alkalinity; biological oxygen demand (BOD_5); fats, oils and grease (FOG); and volatile fatty acids (VFA) analyses.

Table 3-9: Schedule of SGBR sampling and analysis

Frequency	Parameter
Daily	Temperature pH Conductivity TDS Salinity Turbidity
Thrice per week (Monday, Wednesday and Friday)	COD TSS VSS NH_4^+-N NO_3^--N
Weekly	PO_4^{3-}-P BOD_5 FOG VFA Alkalinity

3.5.2 SSND aerobic bioreactor sampling and analysis

The SSND aerobic bioreactor feed and effluent samples were collected and analysed for the following parameters: NH_4^+-N; NO_3^--N; nitrite-nitrogen (NO_2^--N); COD and PO_4^{3-}-P. The COD and PO_4^{3-}-P were measured for a weekly composite sample. The dissolved oxygen (DO) concentration, inside the bioreactor, was measured during the aeration stage.

Table 3-10: Schedule of the SSND aerobic bioreactor sampling and analysis

Frequency	Parameter
Thrice per week (Monday, Wednesday and Friday)	NH_4^+-N NO_3^--N NO_2^--N DO
Weekly	COD PO_4^{3-}-P

3.5.3 ufMM system sampling and analysis

The ufMM feed and permeate samples were collected and analysed for the following parameters: temperature; pH; conductivity; TDS; salinity; turbidity; COD; TSS; NH_4^+-N; NO_3^--N; and PO_4^{3-}-P.

Table 3-11: Schedule of the ufMM sampling and analysis

Frequency	Parameter
Thrice per week (Monday, Wednesday and Friday)	Temperature pH Conductivity TDS Salinity Turbidity COD TSS NH_4^+-N NO_3^--N PO_4^{3-}-P

3.6 Analytical methods and equipment

The temperature, pH, conductivity, TDS, and salinity were measured using a calibrated PCSTestr 35 multiparameter (Wirsam Scientific and Precision Equipment (Pty) Ltd.). The turbidity was measured using a calibrated TN-100 Turbidimeter (ISO 7027 compliant nephelometric method). The TSS and VSS were determined according to the ESS Method 340.2, using glass fibre filter paper (Whatman GF/C, 1.2 µm pore size). The COD samples were prepared using Merck COD solutions (solution A: Cat. No. 1.14538.0065 and 1.14679.049; solution B: Cat. No. 1.14539.0495 and 1.14680.1495), digested in a preheated Spectroquant TR420 Thermoreactor and measured using the Merck Spectroquant Nova60. The NH_4^+-N; NO_3^--N; and NO_2^--N were measured using Merck Spectroquant test kits (NH_4^+-N: Cat. No. 1.00683.0001; NO_3^--N: Cat. No. 1.14773.0001; and NO_2^--N: Cat. No. 1.14776.0001). The PO_4^{3-}-P was measured using the Merck Spectroquant cell test (Cat. No. 1.14729.0001). The NH_4^+-N; NO_3^--N; NO_2^--N; and PO_4^{3-}-P were all measured using the Nova60 Spectroquant. The DO was measured using an 820 portable DO meter equipped with a DO probe. Details of the above analytical methods are provided in Appendix A1 to A9.

The alkalinity; BOD_5; FOG; and VFA were analysed by a SANAS accredited lab (Scientific Services, South Africa), according to standard methods.

3.7 Statistical analysis of data

The analyses for each parameter measured were completed in triplicate. An average for each parameter was calculated from the measurements recorded. Averaged standard deviation (±SD) values were used to compare the concentrations and removal efficiencies of the different parameters. The removal efficiency (*RE*) was calculated using Eq. 3.1.

$$RE\ (\%) = \frac{(C_i - C_o)}{C_i} \times 100 \tag{3.1}$$

Where, C_i is the feed concentration in mg/L and C_o is the effluent concentration in mg/L.

3.8 Optimization using response surface methodology (RSM)

RSM was used for the optimization of the COD removal efficiency for the SGBR treating PSW, as the primary treatment bioreactor which can be treatment system limiting. A central composite design (CCD) was used to determine the optimum operating conditions i.e. hydraulic retention time (HRT) (B) and organic loading rate (OLR) (A), and the interaction between these independent variables, with the COD removal efficiency (%) being the response variable. The OLR and HRT were chosen based on their direct impact on the COD removal efficiency and the chosen ranges were based on the operating conditions used for the SGBR in this study, i.e. conditions reported in Debik & Coskun (2009) and Basitere et al. (2017). Design Expert® 10.0.3 statistical software (Stat-Ease, Inc., USA) was used for the experimental design. A two-factor (i.e. HRT and OLR), two-level (i.e. low (-1) and high (+1)) CCD was applied, with a total of 15 experimental runs generated. Table 3.12 shows the list of factors and their levels in the CCD.

Table 3-12: Factors and levels based on a two-factor, two-level CCD

		Coded Factor Levels	
Factor	**Symbol**	**Low (-1)**	**High (+1)**
OLR (g COD/Lday)	A	0.73	12.5
HRT (day)	B	1	4

Analysis of variance (ANOVA) was used to evaluate the significance of the response surface quadratic model, the individual variables and their interactions. The response surface quadratic model is described by Eq. 3.2 (Bustillo-Lecompte & Mehrvar, 2017).

$$Y_i = \beta_o + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=i,j=2}^{k-1,k} \beta_{ij} X_i X_j + e \tag{3.2}$$

Where Y_i is the response variable, X_i and X_j are the independent variables, β_o, β_i, β_{ii} and β_{ij} are the constant, linear, quadratic, and interaction coefficients, and *k* and *e* are the number of factors and the error, respectively. The determination coefficient (R^2) was calculated to evaluate the adequacy of the model.

CHAPTER 4

RESULTS AND DISCUSSION

CHAPTER 4
4. RESULTS AND DISCUSSION

4.1 Introduction

The aim of this study was to evaluate the performance of a lab-scale poultry slaughterhouse wastewater (PSW) treatment system consisting of an anaerobic static granular bed reactor (SGBR) pre-treatment followed by single stage nitrification-denitrification (SSND) aerobic bioreactor and sidestream ultrafiltration membrane module (ufMM) post-treatment systems.

4.2 Poultry slaughterhouse wastewater (PSW) characterization

The PSW used in this study which was obtained from a local poultry slaughterhouse (Western Cape, South Africa) was analysed for its characteristics; with the minimum, maximum and average values of all parameters measured for the duration of this study (i.e. in-house and external analyses) being summarised in Table 4.1. Generally, PSW with a high fats, oils and grease (FOG) content requires pre-treatment prior to undergoing biological treatment (Del Nery et al., 2007). In this study, the PSW was filtered through a mesh screen (2 mm) to remove suspended solids such as feathers, including colloidal particles, i.e. FOG, and particulate matter, prior to being fed to the SGBR. The variation in the PSW characteristics can be attributed to the wastewater samples being collected from the poultry slaughterhouse at irregular intervals. Generally, the composition of PSW generated can vary significantly according to the number of birds processed, the water consumption per bird, and the management of the water used by the poultry slaughterhouse (Debik & Coskun, 2009; Del Nery et al., 2007). The high standard deviation for the parameters further emphasized the variation in the PSW characteristics.

The presence of organic materials such as blood, carbohydrates, fats, and proteins in the PSW contributes to the high concentrations of chemical oxygen demand (COD) and biological oxygen demand (BOD_5) (Debik & Coskun, 2009). The average BOD_5/COD ratio of 0.56, of the PSW used in this study, falls within the organic matter biodegradability range of 0.4 to 0.8 reported by Oh (2015), and thus verified the biodegradability of the constituents present in the PSW. The characteristics of the wastewater used in this study are similar to the PSW characteristics reported in other literature studies (Basitere et al., 2017; Abboah-Afari, 2011; Rajakumar et al., 2011; Debik & Coskun, 2009; Chavez et al., 2005) with regard to the pH, COD, BOD_5, total suspended solids (TSS), and FOG, as seen in Table 4.1.

Table 4-1: PSW characterization

		PSW				
		This study			Literature studies	
Parameter	Unit	Minimum	Maximum	Average ±SD	Minimum	Maximum
pH	-	6.13	7.24	-	5.6	8.1
Conductivity	μS/cm	973	2405	1604 ±414	-	-
TDS	Ppm	691	1693	1138 ±294	-	-
Salinity	ppm	529	1413	916 ±179	-	-
Turbidity	NTU	237	997	719 ±201	-	-
TSS	mg/L	313	8200	1654 ±1695	200	3750
VSS	mg/L	239	8920	1906 ±1498	-	-
COD	mg/L	2517	12490	5216 ±2534	1223	11600
NH_4^+-N	mg/L	135	447	216 ±56	-	-
NO_3^--N	mg/L	0.63	22.7	3.33 ±4.45	-	-
PO_4^{3-}-P	mg/L	29	54	38 ±6	-	-
VFA	mg/L	105	898	375 ±213	-	-
Alkalinity	mg/L	322	923	499 ±158	-	-
BOD_5	mg/L	925	5000	2477 ±1347	200	8700
FOG	mg/L	156	1710	715 ±506	100	1385

4.3 Performance of the static granular bed reactor (SGBR) anaerobic digester

The SGBR was used as the primary treatment stage for organic matter and suspended solids reduction from the PSW prior to undergoing further treatment, i.e. nutrient removal and reduction of residual contaminants using the combined SSND bioreactor and sidestream ufMM systems. Since the lab-scale SGBR was operated continuously at mesophilic temperature (i.e. 35-37 ˚C) under varying operating conditions using a well-defined starting procedure, the change in operational parameters, i.e. hydraulic retention times (HRTs) and organic loading rates (OLRs), were used to demarcate the discussion into sections highlighting the SGBRs performance during the 138 days of operation.

4.3.1. Start-up of the SGBR

After the acclimation period, the HRT was set to 55 h and the SGBR was fed with 50% (v/v) PSW to initiate the start-up OLR of 1.18 g COD/Lday. Generally, a start-up OLR of about 1 g COD/Lday is recommended for SGBRs based on previous studies (Park et al., 2012; Lim, 2008). The HRT was maintained at 55 h and the SGBR was operated in continuous mode under pseudo-steady state conditions, throughout the start-up period (28 days). The SGBR feed COD averaged at 2661 mg/L after diluting the filtered PSW with potable water to maintain low OLRs and thus prevent overloading of the slow growing micro-organisms within the anaerobic granules. The washout of sloughed-off fine granules hypothesized herein as dead biomass based on its discolouration when compared to the rest of the biomass, i.e. via the effluent stream, occurred during the start-up period and resulted in a reduction in the volume of the granular bed. In order to restore the bed to its original volume, granule re-supplementation into the SGBR was implemented on day 16, allowing for a further 24 hours for acclimatization. The addition of the granules caused an increase in the pH of the system. The HRT was then increased from 55 to 96 h (average OLR of 0.78 g COD/Lday) to enable the biomass to restabilize whilst preventing further washout of granules. At the end of the start-up period, the effluent COD and TSS averaged 693 and 53 mg/L, respectively. As the performance of the SGBR stabilized with regards to the percentage COD removal, the HRT was varied step-wise to simulate variations in the influent flow rate and thus the OLR so as to adequately assess the performance of the SGBR in treating the PSW.

4.3.2. Treatment efficiency of the SGBR at varying operating conditions

The hydraulic retention time (HRT) and organic loading rate (OLR) are significant operating conditions that must be monitored and maintained to ensure the optimum performance of an anaerobic digester. The treatment efficiency of the SGBR used in this study was evaluated under varying HRTs and OLRs for 138 days of operation, as illustrated in Figure 4.1. According to the literature reviewed, SGBRs treating slaughterhouse wastewaters are capable of operating under HRTs ranging from 8 to 96 h (Oh, 2012; Mach, 2004) and OLRs as high as 12.76 g COD/Lday (Park et al., 2012). The operating conditions of the SGBR used in this study were based on conditions reported in Debik & Coskun (2009) and Basitere et al. (2017). During the start-up period, the HRT was maintained at 55 h with an average OLR of 1.18 g COD/Lday. After the start-up period, the SGBR was operated by varying the HRT to evaluate the treatment efficiency of the system up to a maximum OLR of 12.5 g COD/Lday. The OLR was gradually increased as the HRT was decreased step-wise after pseudo-steady state was achieved for each HRT. The HRTs applied to the system were 96, 48, 36 and 24 h with average OLRs of 0.78, 1.96, 4.10 and 5.74 g COD/Lday, respectively. Fluctuations in the feed COD, and thus the OLR, were due to the organic matter concentrations varying according to the operation in the poultry slaughterhouse at the time of sampling (as seen in Figure 4.1). The feed COD concentrations ranged between 2195 and 12490 mg/L and averaged at 4344±2312 mg/L, with corresponding average OLRs of 0.96, 12.5 and 2.75±2.11 g COD/Lday, respectively.

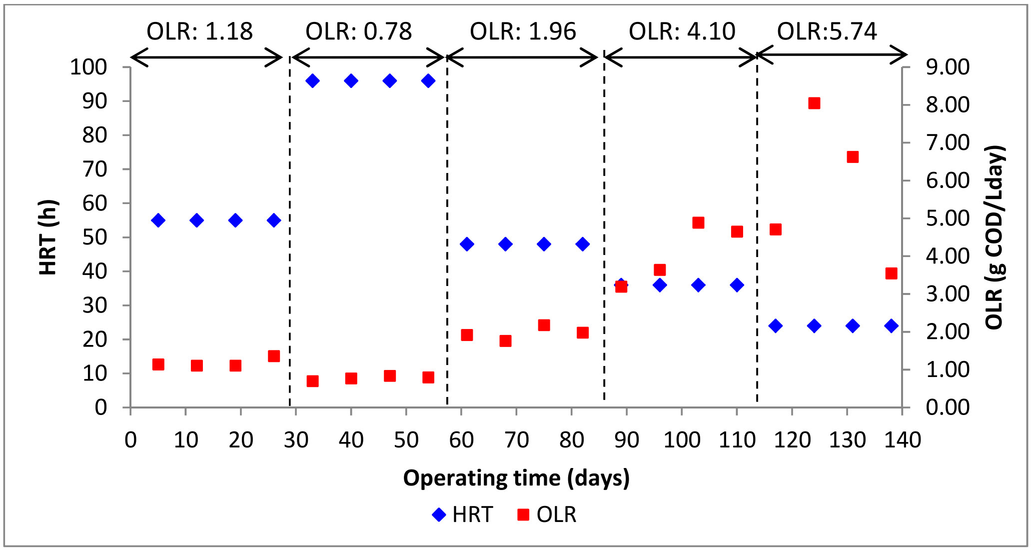

Figure 4-1: Relationship between the HRT and OLR for 138 days of operation of the SGBR

The COD, TSS, FOG, and BOD_5 removal efficiencies in relation to the varying HRTs and average OLRs are shown in Figure 4.2. The COD, TSS, and BOD_5 removal efficiencies were consistently above 70%, 80% and 90%, respectively, throughout the duration of this study including the start-up period (Figure 4.2). The FOG removal efficiencies, however, were relatively low during the start-up period; with increases remaining consistently above 80% after the start-up period. The COD, TSS, FOG and BOD removal efficiencies reached a maximum of 86%, 95%, 94%, and 99%, respectively, at an OLR of 4.10 g COD/Lday, when the HRT was reduced to 36 h. Overall, the lowest removal efficiencies were observed during the start-up period which may be due to the reduced granules activity.

Accordingly, the COD, TSS, and BOD_5 removal efficiencies increased as the OLR increased (see Figure 4.2); whereas, the FOG removal efficiencies were not a function of the OLR. A similar trend was reported by Lim & Fox (2011) in the treatment of swine wastewater using a lab-scale SGBR operated at 24 ˚C. The increase in the removal efficiencies at higher OLRs was as a result of biological degradation and the physical filtration and retention of the organic matter, suspended solids, fats and proteins including other contaminants. The combination of a 48 h HRT and an average OLR of 1.96 g COD/Lday significantly contributed to the quantity of accumulated suspended solids in the reactor. This ultimately led to clogging of the granular bed which impacted on the operability of the SGBR. However, neither an increase nor decrease in the HRT or OLR had an adverse effect on the overall biological performance of the SGBR in terms of its removal efficiencies. This indicated that the high biomass concentration in the SGBR was sufficient to maintain the required high removal efficiencies for a wide range of HRTs and OLRs. Debik & Coskun (2009) operated a SGBR at HRTs of 48 and 36 h for the treatment of PSW; and achieved COD removal efficiencies higher than 90% at OLRs ranging between 2.53 and 4.97 g COD/Lday. Similarly, Basitere et al. (2017) reported COD, TSS and FOG removal efficiencies of 93%, 95% and 90%, respectively, when operating a SGBR for PSW treatment at HRTs of 55 and 40 h with average OLRs of 1.01 and 3.14 g COD/Lday.

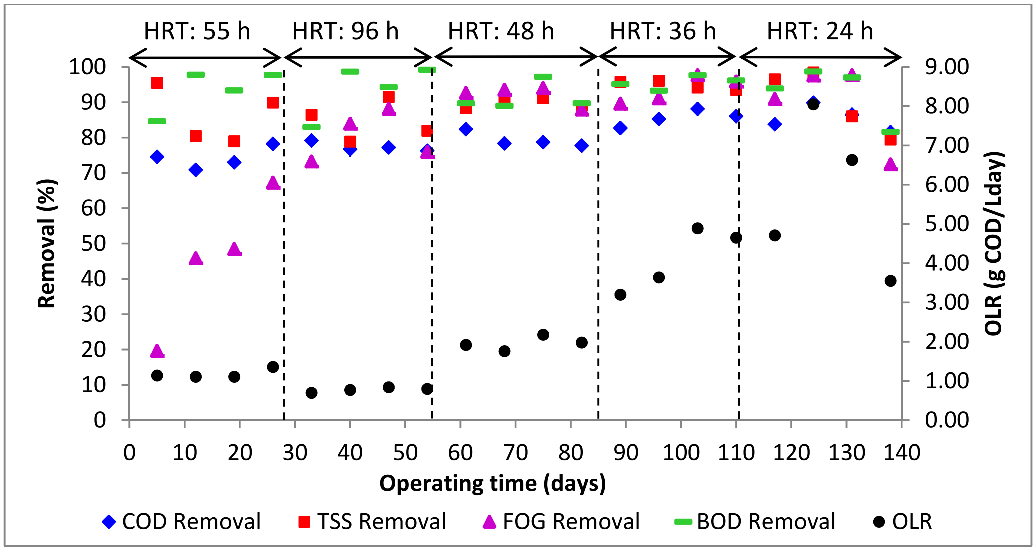

Figure 4-2: Treatment efficiency of the SGBR at varying HRTs and OLRs

4.3.3. Organic matter removal in the SGBR

The organic matter removal by the SGBR was evaluated based on the COD, BOD_5, and FOG reduction efficiency. The SGBR feed COD, effluent COD and COD removal efficiencies are shown in Figure 4.3. The COD of the feed averaged at 4311±2312 mg/L and the OLR at 2.75±2.11 g COD/Lday. Similarly, Debik & Coskun (2009) operated a lab-scale SGBR at an average OLR of 2.73 g COD/Lday, during which the COD concentrations of the PSW feed ranged between 1600 and 9100 mg/L. The COD concentrations of PSW as reported in literature ranges between 1223 and 11600 mg/L (Basitere et al., 2016; Abboah-Afari, 2011; Rajakumar et al., 2011; Chavez et al., 2005). The effluent COD was consistently low throughout this study, which was attributed to the high activity and suitability of the granules used in the SGBR. The SGBR efficiently reduced the COD to an average effluent value of 729±98 mg/L, which correlates to values of 735±288 mg/L and 746±195 mg/L, obtained for PSW treatment in UASB reactors (Del Nery et al., 2007; Del Nery et al., 2016); whereas, average effluent COD concentrations of 263 mg/L, 301 mg/L and 291 mg/L were observed for SGBRs treating different slaughterhouse wastewaters (Basitere et al., 2017; Park et al., 2012; Lim & Fox, 2011). In this study, the COD of the effluent varied between 482 and 974 mg/L and the removal efficiencies corresponded to a RE% range of between 67% and 95%.

During the 138 days of operation, the COD removal efficiencies exceeded 80%, with the intermittent performance reduction on several days, attributed to backwashing operations. Similarly, the low initial COD removal efficiencies (average 74.1±3.6%) observed during the start-up period could be as a result of reduced FOG hydrolysis and suspended solids interaction with the anaerobic granules which contributed to an increase in soluble COD non-biodegradation. Del Nery et al. (2007) reported 65% COD and 85% soluble COD removal efficiencies for the treatment of PSW in a UASB at an average OLR of 1.64 g COD/Lday. As observed in Figure 4.3, the average COD removal efficiencies of 78±4%, 79±5%, 86±5% and 85±5%, for the corresponding HRTs of 96, 48, 36 and 24 h, respectively, was attributed to the increased functionality of the biomass with increasing bioreactor operation time, thus the SGBRs ability to effectively tolerate increasing OLRs as the biomass matured. Several studies using the SGBR to treat a variety of wastewaters showed a similar trend (Debik & Coskun, 2009; Lim & Fox, 2011; Park et al., 2012; Oh et al., 2015). Despite fluctuations in the COD of the feed, an OLR of up to 12.5 g COD/Lday, and step-wise decreases in the HRT, culminated in the COD removal efficiencies remaining relatively consistent. Overall, the SGBR design used in this study demonstrated a stable performance with COD removal efficiencies greater than 95% observed towards the end of the study at the 24 h HRT. Comparatively, the SGBR is known to produce COD removal efficiencies exceeding 90% for a wide range of HRTs, i.e. 9 to 55 h and OLRs i.e. 0.63 to 9.72 g COD/Lday, under varying temperature conditions (Debik & Coskun, 2009; Lim & Fox, 2011; Park et al., 2012; Oh et al., 2015; Basitere et al., 2017), which is indicative of the robustness of the chosen primary treatment technology for PSW reclamation.

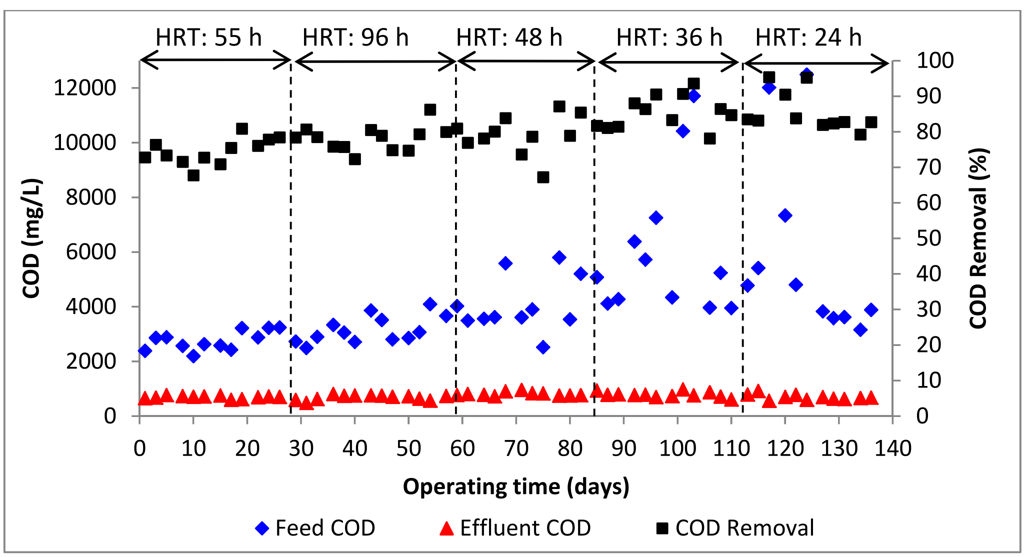

Figure 4-3: COD removal efficiency of the SGBR

The SGBR feed BOD_5, effluent BOD_5 and BOD_5 removal efficiencies are shown in Figure 4.4. The BOD_5 concentration was reduced from an average of 1859±1385 mg BOD_5/L in the PSW to 95±62 mg BOD_5/L. Park et al. (2012) treated pork slaughterhouse wastewater in a pilot-scale SGBR, at HRTs ranging from 24 to 48 h, achieving a BOD_5 concentration of 87 mg/L (maximum) in the effluent irrespective of the influent quality. The BOD_5 removal efficiencies were relatively consistent throughout this study at values exceeding 90%, indicating that majority of the biodegradable matter was removed from the PSW as seen in Figure 4.4. The maximum BOD_5 removal efficiency of 99% was observed at an HRT of 96 h and OLR of 0.3 g BOD_5/Lday. Park et al. (2012) further evaluated the performance of a pilot-scale SGBR, for the treatment of dairy wastewater at various OLRs (0.63 to 9.72 kg/m^3 day), and achieved COD and BOD_5 removal efficiencies exceeding 90%; which concurred with the observations in this study, whereby, a BOD_5 removal efficiency of 93±5% at HRTs between 24 and 96 h was achieved, which was attributed to the relative biodegradable nature of the PSW used, as indicated by a BOD_5/COD ratio of 0.56. Similarly, the performance of a UASB reactor treating PSW with a BOD_5/COD ratio of 0.75 was investigated by Chavez et al. (2005), achieving a 95% reduction in BOD_5 at an HRT of 4 h; whereas, Del Nery et al. (2016) obtained an lower BOD_5 removal efficiency of 71±6% in a similar UASB reactor treating PSW.

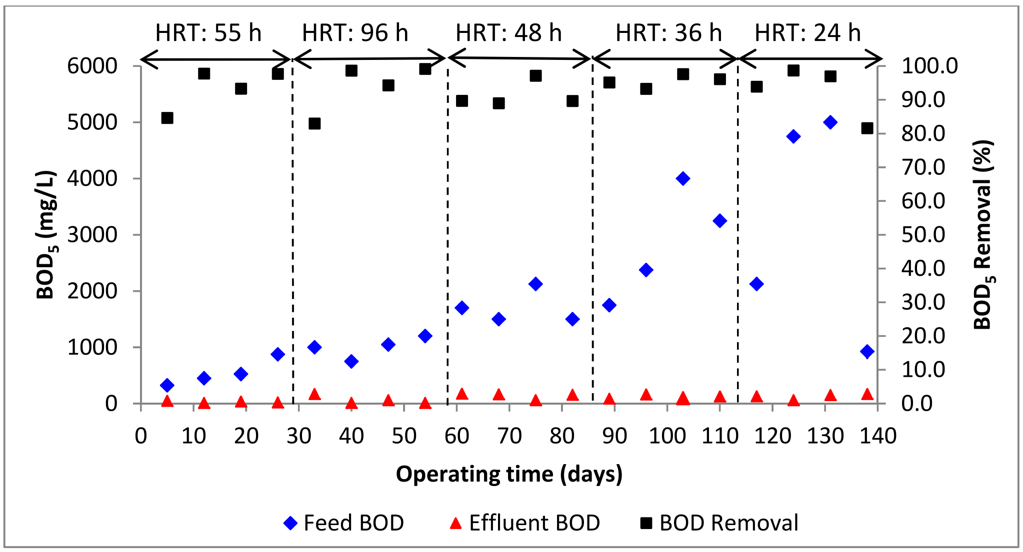

Figure 4-4: BOD_5 removal efficiency of the SGBR

Similarly, the FOG removal efficiency was expected to be high for the PSW used in this study (Figure 4.5), due to the colloidal matter contained within the PSW which facilitated its retention within the porous granular bed resulting in low effluent FOG concentrations of between 24 and 100 mg/L (average 51±22 mg/L). PSW typically has a high FOG (up to 1385 mg/L) and suspended solids content which may inhibit the biological process efficiency and bioreactor stability (Rajakumar et al., 2011). Furthermore, FOG has the potential to cause operational issues in wastewater treatment systems such as blockages and clogging of piping systems, when the environmental temperature is reduced; whilst the formation of an emulsion can further contribute to the souring of the bioreactor. For this reason, the raw (untreated) PSW used in this study was filtered through a fine mesh screen to reduce the colloidal matter, i.e. FOG, and particulate matter prior to undergoing biological treatment in the SGBR. The FOG concentration of the PSW, i.e. SGBR feed, averaged at 537±474 mg/L subsequent to the screening process. Overall, the FOG removal from the PSW was sufficient for the SGBR effluent to comply with the CCT wastewater and industrial effluent by-law limit (i.e. 400 mg/L), with minimal reactor challenges being observed.

Average FOG removals efficiencies of 83±8%, 92±3% and 94±4%, and 90±12% were observed at HRTs of 96, 48, 36 and 24 h, respectively, and at OLRs up to 12.49 g COD/Lday. Rajakumar et al. (2012) reported a reduction in the efficiency of an up-flow anaerobic filter (UAF) treating PSW due to FOG and suspended solids entrapment which caused substrate transport limitations at an OLR exceeding 10.98 kg/m^{3}day. Due to the insoluble nature of FOG and the inept biodegradable proficiency of the granular bed during the initialization of the SGBR operation, an average FOG removal efficiency of 45±20% was observed during the start-up period at an OLR of 1.14 g COD/Lday. This was most likely a period dominated by slow hydrolytic mechanisms for the digestion process. Subsequent to the start-up period, the FOG removal efficiencies increased to above 80% on average and remained consistently high for the remainder of the SGBR operation. As shown in Figure 4.5, the FOG removal was insignificantly affected by the OLR. Moreover, the down-flow mode of operation of the SGBR prevented the occurrence of sludge washout commonly experienced in up-flow anaerobic reactors treating FOG laden wastewater, such as the UASB and EGSB reactors, due to high FOG and suspended solids loading which encapsulates the biomass making it easier to float due to bed fluidization facilitated by an upward movement of the wastewater being treated (Basitere et al, 2016; Rajakumar et al., 2012).

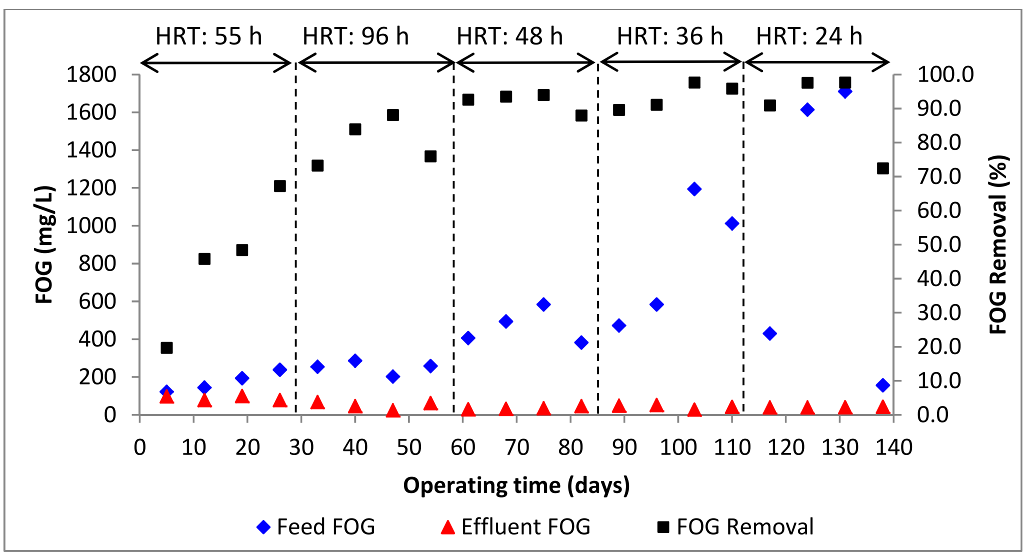

Figure 4-5: FOG removal efficiency of the SGBR

4.3.4. Suspended solids removal in the SGBR

Since the SGBR operates as both a bioreactor and biofilter, the TSS is an important parameter used to evaluate the suspended solids retention. The variation in the SGBR feed TSS, effluent TSS and TSS removal efficiencies are shown in Figure 4.6. The TSS of the feed which fluctuated throughout this study according to the variability of the PSW characteristics, averaged at 1192±1456 mg/L, which was within the range for the TSS concentration of PSW, i.e. between 200 and 3750 mg TSS/L, as reported in literature (Del Nery et al., 2007; Debik & Coskun, 2009; Rajakumar et al., 2011). The effluent TSS; however, was relatively stable in comparison to the fluctuating feed TSS, which was efficiently reduced to a minimum of 13 mg/L. Overall, the SGBR achieved an average effluent TSS concentration of 63±38 mg/L and TSS removal efficiency of 89±10%. Similar averaged effluent TSS values, i.e. 84 mg/L, were observed by Park et al. (2012) treating pork slaughterhouse wastewater in a pilot-scale SGBR operating at ambient temperature and HRTs of 20 to 48 h. Park et al. (2012) futher investigated the SGBR performance for dairy processing wastewater treatment and reduced the TSS by 89% using an HRT range of 9 to 48 h. These results verify the consistent treatability with regard to the TSS of a variety of wastewaters in SGBRs operated under different operating conditions; thus, its classification as a biofilter.

In this study, the average TSS removal efficiencies for the HRT of 96, 48, 36 and 24 h were 85±10%, 90±8%, 95±6% and 90±11%, respectively. A similar trend was observed in another study treating PSW in a mesophilic SGBR operated at HRTs of 55 and 40 h, achieving average TSS removal efficiencies of 93 and 98%, respectively (Basitere et al., 2017). Based on the high removal efficiencies obtained in this study, it can be deduced that the reduction in the TSS resulted from the biodegradation of the organic solids as well as the physical entrapment of the inorganic suspended solids in the dense granular bed, since the SGBR operated as both a bioreactor and biofilter. Rajakumar et al. (2012) investigated the performance of a hybrid UASB reactor using pleated polyvinyl chloride rings as filter media for the treatment of PSW and observed that an increase in feed TSS due to increasing organic loading, enhanced the physical retention of suspended solids due to densification of the sludge bed and its increased filtration potential.

An increase in headloss attributed to the accumulation of suspended solids was observed during this study, specifically when the SGBR was fed with undiluted PSW, which was as a result of the down-flow configuration of the SGBR and the subsequent settling of the suspended solids within the granular bed of the SGBR. This entrapment of suspended solids resulted in the need for a backwashing procedure to eliminate a portion of the accumulated solids and maintain a constant quantity of biomass within the SGBR. The backwashing procedure had insignificant and/or no adverse impact on the TSS removal efficiencies achieved in this study; which was comparable to a study by Lim & Fox (2011), in which it was concluded that the recovery of the TSS removal after backwashing was not a function of the recovery time or the OLR. Thus, backwashing should have minimal impact on the biofilter (SGBR granular bed and underdrain system) efficacy.

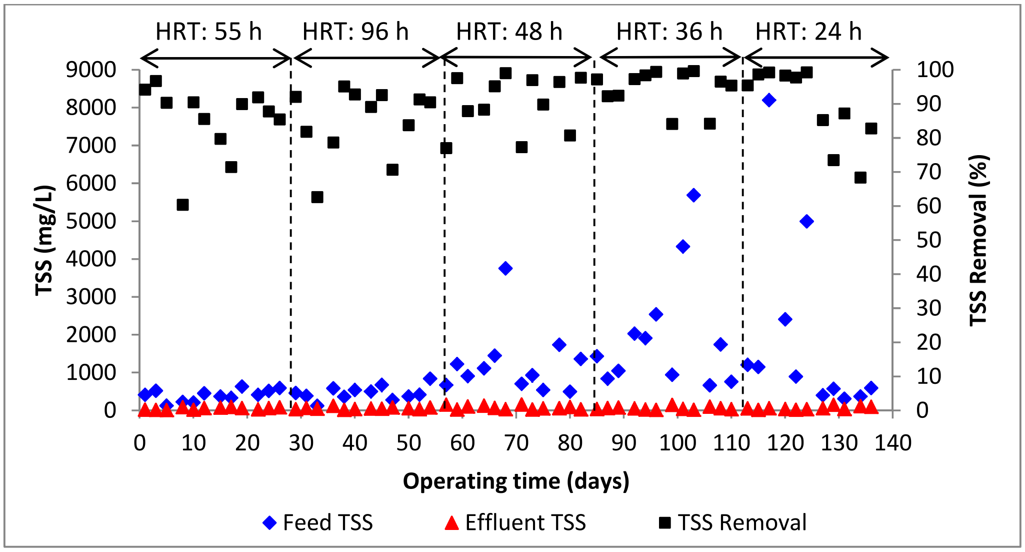

Figure 4-6: TSS removal efficiency of the SGBR

4.3.5. Stability of the SGBR: pH, alkalinity and volatile fatty acids (VFA)

The pH and VFA/alkalinity ratio are important parameters used to ensure proper conditions for anaerobic digestion and to monitor process stability in the digester. The pH, VFA and alkalinity of the SGBR feed fluctuated due to the variability of the PSW collected from the poultry slaughterhouse. The average alkalinity values varied considerably throughout the operation of the SGBR; whereas, the VFA values were stable despite the rapid increments from days 54 to 68, with stabilization being observed on day 75 (Figure 4.7). The SGBR effluent pH ranged between 6.29 and 8.59 and remained relatively consistent with values within the optimum range for acceptable activity of methane-forming micro-organisms, i.e. pH between 6.2 and 8.5 (Sakar et al., 2009). The VFA/alkalinity ratios obtained in this study ranged from 0.03 which was indicative of system stability (i.e. VFA/alkalinity ratio less than 0.4), to a maximum value of 0.99 which exceeded the limit of 0.5; thus, suggesting that corrective action was required to avoid system failure attributed to an excess of VFAs (Figure 4.8). Rajakumar et al. (2011) reported comparatively lower VFA/alkalinity ratios of between 0.13 and 0.19 for a hybrid UASB operated under mesophilic conditions for PSW treatment. VFA/alkalinity ratios below 0.3 was also obtained for a lab-scale SGBR used for treating PSW (Debik & Coskun, 2009).

During days 54 and 68, VFA/alkalinity ratios exceeding 0.5 was observed subsequent to a decrease in the HRT from 96 to 48 h and an increase in the average OLR from 0.78 to 1.96 g COD/Lday. This is expected for industrial scale systems; therefore, a suitably designed bioreactor will effectively mitigate the effects of such changes and/or variability. In this study, a maximum ratio of 0.99 was observed at an HRT of 48 h and OLR of 1.84 g COD/Lday, indicating unfavourable conditions for the methane-forming micro-organisms due to the hypothesized accumulation of VFAs. This result indicated toward the need for corrective action so as to increase the alkalinity of the feed. However, the VFA/alkalinity ratio decreased to values below the failure limit after day 68 without the addition of alkalinity, as shown in Figure 4.8 – an added design advantage of the SGBR, as self-correction of the bioreactor might be a positive design attribute whereby plant personnel are not equiped to intervene, a challenge prevalent in developing countries such as South Africa, wherein training is inadequate in the wastewater treatment sector.

On average, the alkalinity was reduced by 11±25%. This reduction of alkalinity can be attributed to the reduced organic matter concentrations (Bwapwa, 2010), as a result of the decrease in the OLR from 1.18 to 0.78 g COD/Lday. Yetilmezsoy and Sakar (2008) observed a similar average reduction in alkalinity of 16±9% in the treatment of poultry manure wastewater. Despite the decrease in alkalinity and high VFA/alkalinity ratios, the SGBR demonstrated stable performance due to the sufficient buffering capacity of the digestate. This was validated by the constant increase in the pH values from the feed to the effluent (Bwapwa, 2010).

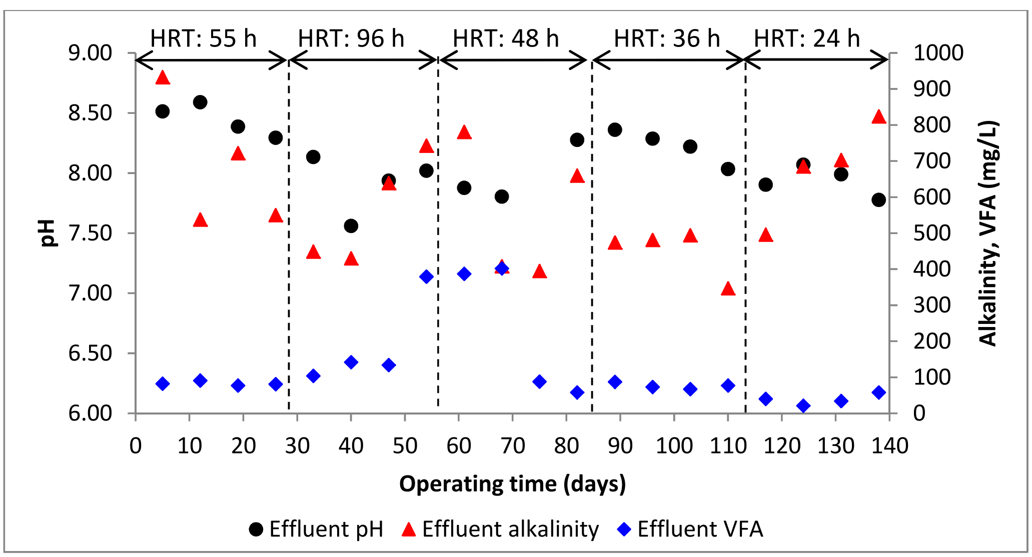

Figure 4-7: Variation in the SGBR effluent pH, alkalinity and VFA

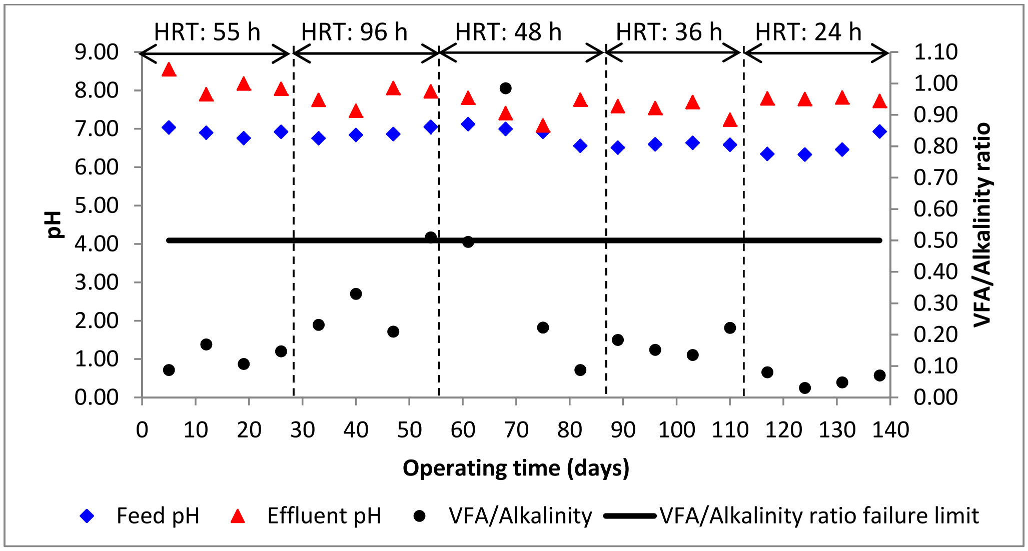

Figure 4-8: SGBR pH and VFA/Alkalinity ratio

4.3.6. Nutrient removal in the SGBR

Figure 4.9, 4.10 and 4.11 illustrates the SGBR feed and effluent concentrations for ammonium-nitrogen (NH_4^+-N), nitrate-nitrogen (NO_3^--N), and ortho-phosphate (PO_4^{3-}-P), respectively. As expected from anaerobic digesters, there was insignificant nutrient removal observed throughout the 138 days of SGBR operation. Anaerobically treated effluents generally require post-treatment in order to achieve compliance with discharge regulations pertaining to nutrient levels in the effluents intended for discharge into local fresh water bodies. Marginal variations in the NH_4^+-N of the SGBR feed and effluent were observed (Figure 4.9). The NH_4^+-N of the SGBR feed ranged between 79 and 443 mg/L with an average of 183±64 mg/L. The initial increase in the NH_4^+-N during the first 57 days of operation (HRTs of 55 and 96 h) may be attributed to the degradation of proteins and amino acids present in the PSW and the conversion of organic nitrogen into NH_4^+-N (Krakat et al., 2017; Oh, 2012). The decrease in the NH_4^+-N which occurred during the last 81 days of operation (HRTs of 36 and 24 h) may have been the result of the consumption of VFAs by the methane-forming micro-organisms. Overall, the NH_4^+-N was reduced suggesting that an ammonification process occurred, which facilitated the conversion of organic nitrogen to ammonium (Wang et al., 2010).

The SGBR feed NO_3^--N averaged at 2.33±3.76 mg/L. An increase in the SGBR effluent NO_3^--N concentration was observed from 1.07 to 24 mg/L, with an average of 12±6.98 mg/L (Figure 4.10). Overall, the NO_3^--N consistently increased by 81±24% on average, suggesting that an anaerobic ammonium oxidation (ANAMMOX) process may have occurred within the SGBR; however, analyses of the biomass within the SGBR would have to be performed in order to confirm the presence of the ANAMMOX micro-organisms. The PO_4^{3-}-P was measured from days 61 to 138, during which low reductions in PO_4^{3-}-P was observed (Figure

4.11). The average PO_4^{3-}-P removal was 19±10%. Average PO_4^{3-}-P removals of 29±9 %, 13±4% and 15±6% were achieved at HRTs of 48, 36 and 24 h, respectively. Under anaerobic conditions, polyphosphate accumulating organisms (PAOs) degrade polyphosphate and stored glycogen and synthesize polyhydroxyalkanoanes (PHAs) from VFAs. The SGBR feed PO_4^{3-}-P concentration ranged between 29 and 54 mg/L with an average of 38±6 mg/L. The SGBR effluent PO_4^{3-}-P concentration was reduced from 21 and 46 mg/L with an average of 31±6 mg/L.

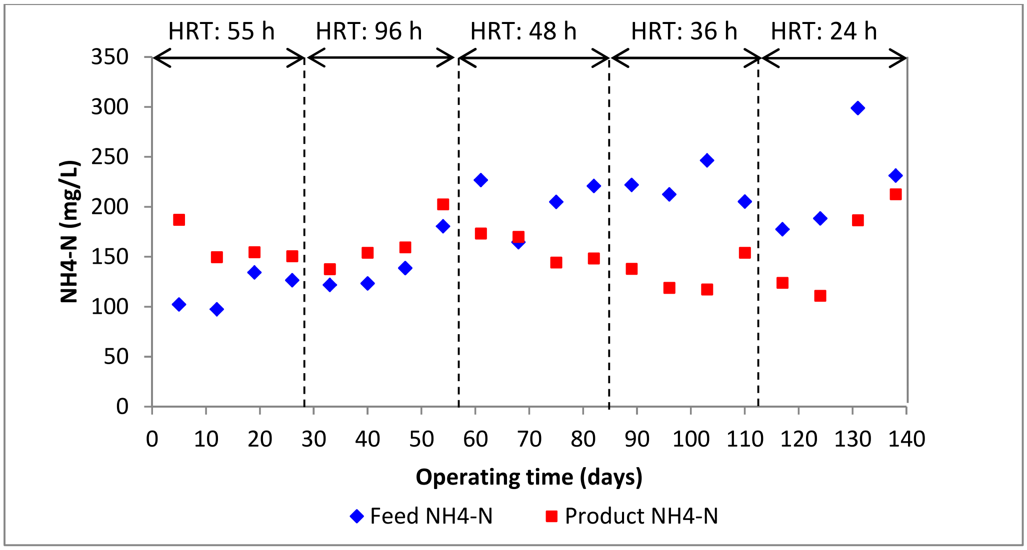

Figure 4-9: Variation in the SGBR feed and effluent $NH4^{+}$-N

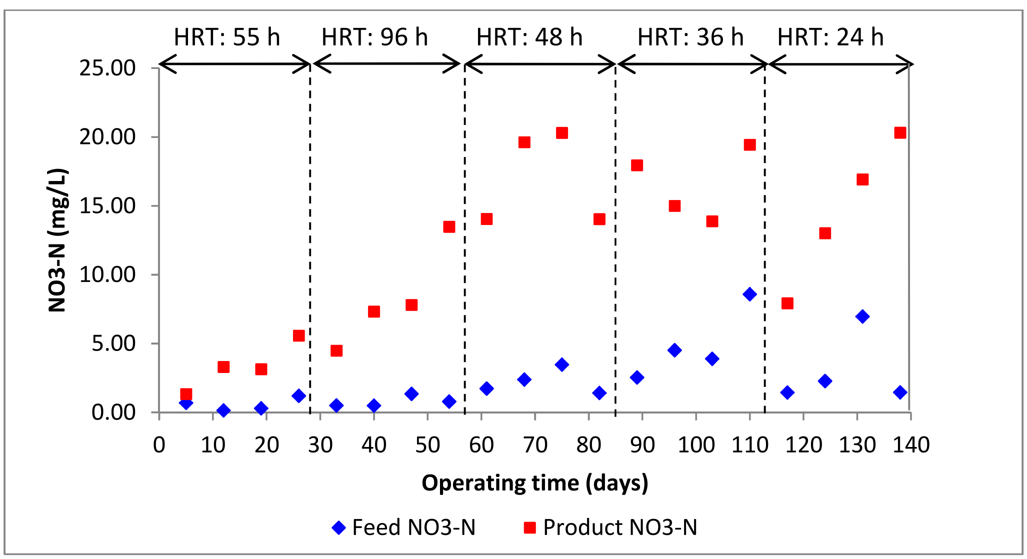

Figure 4-10: Variation in the SGBR feed and effluent NO_3^{-}-N

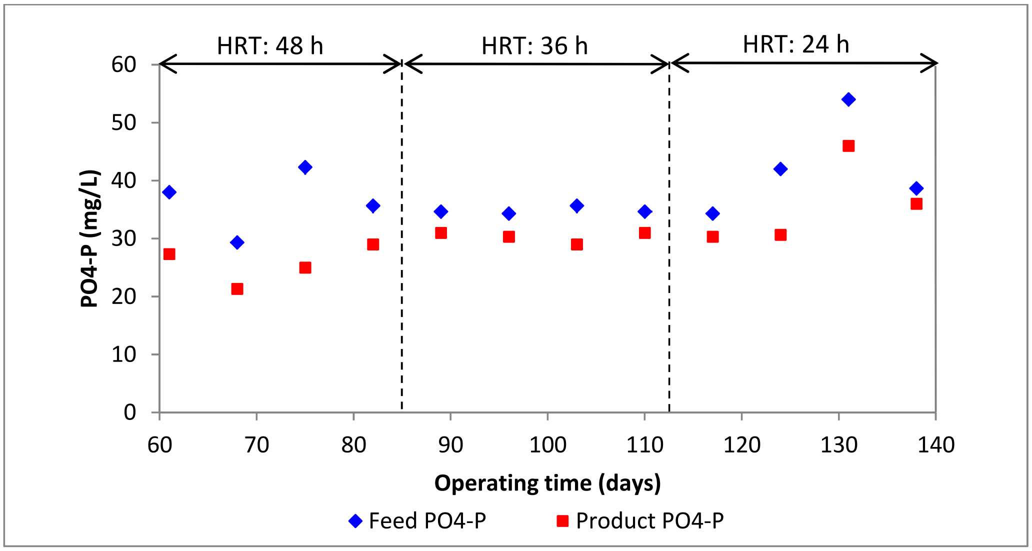

Figure 4-11: Variation in the SGBR feed and effluent $PO4^{3-}$-P

4.3.7. Overall performance of the SGBR and efficiency in relation to effluent discharge quality and regulations

Table B1 (Appendix B) summarises the feed and effluent characteristics as well as the removal efficiencies of the SGBR operated under different HRTs and OLRs for a period of 138 days. The SGBR demonstrated adequate performance and stability at HRTs ranging from 24 to 96 h and an overall OLR of 2.75 g COD/Lday. Overall, the SGBR performed efficiently with regard to the soluble and particulate organic matter removal. The COD, TSS, BOD_5 and FOG removal efficiencies in the SGBR averaged at 80%, 95%, 89% and 80%, respectively, showing variations over the operating period. Furthermore, average removal efficiencies of 97±5% and 48±29% were observed for the turbidity (Figure 4.15) and VSS (Figure 4.16). The overall conductivity (Figure 4.12), TDS (Figure 4.13) and salinity (Figure 4.14) effluent concentrations were 1608±328 mg/L, 1142±232 mg/L and 882±134 mg/L, respectively.

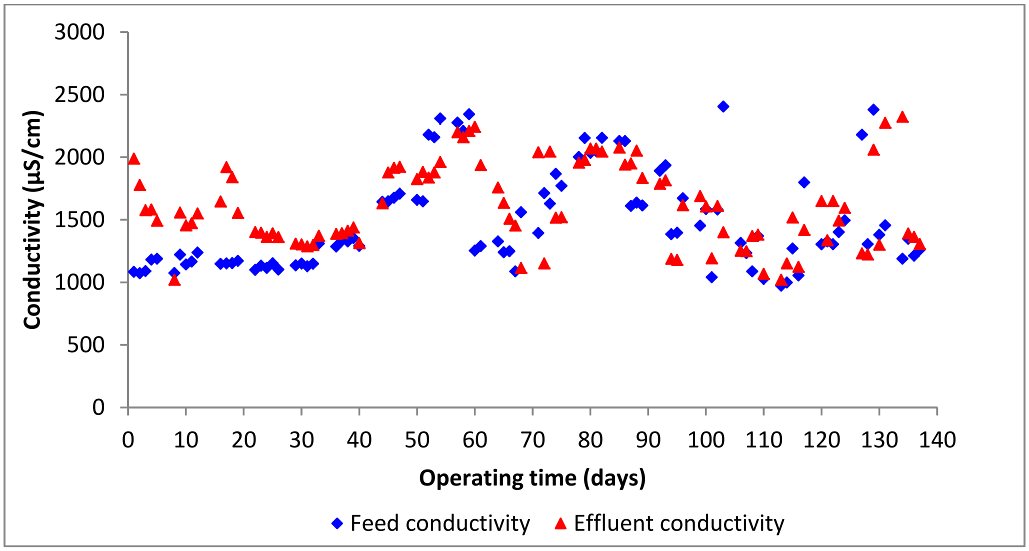

Figure 4-12: Variation in the SGBR feed and effluent conductivity

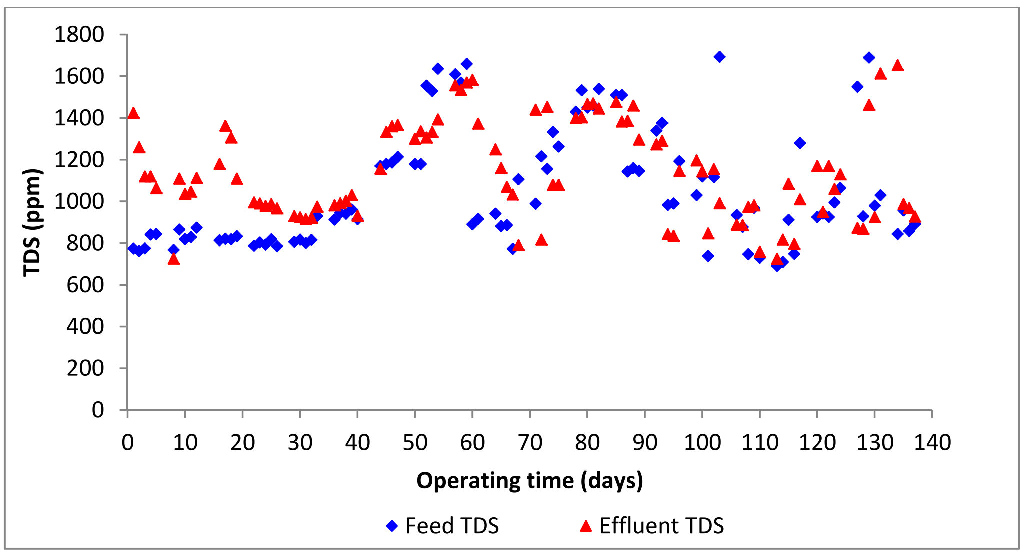

Figure 4-13: Variation in the SGBR feed and effluent TDS

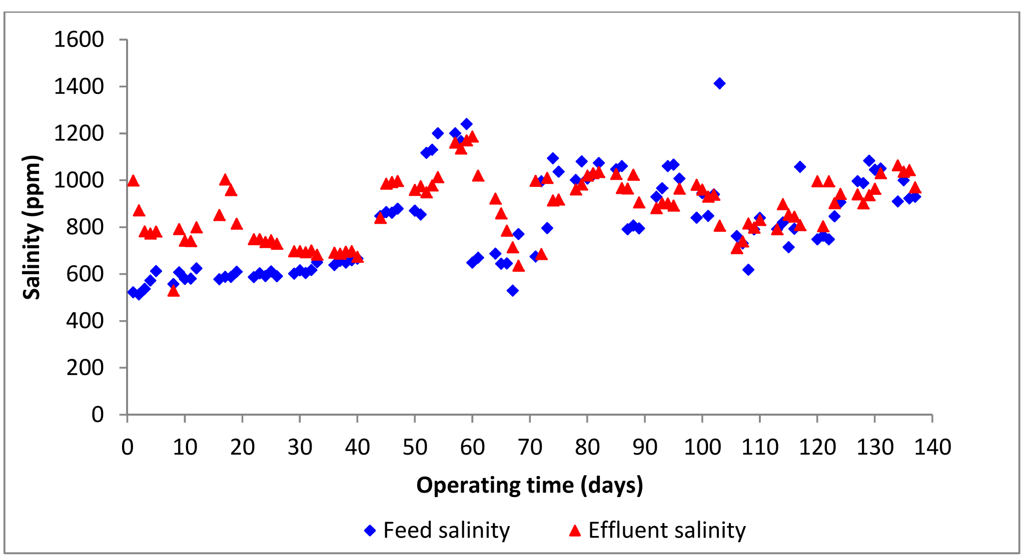

Figure 4-14: Variation in the SGBR feed and effluent salinity

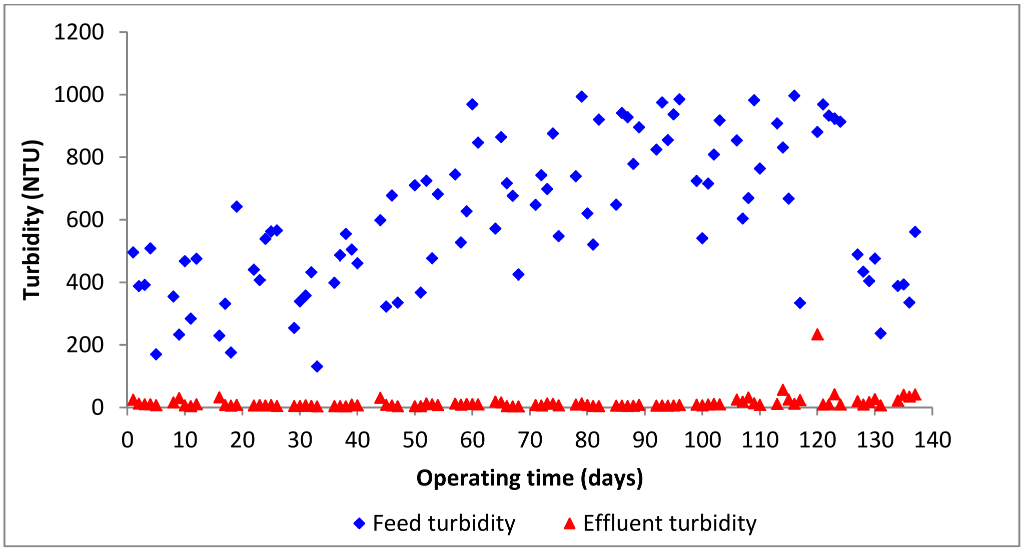

Figure 4-15: Variation in the SGBR feed and effluent turbidity

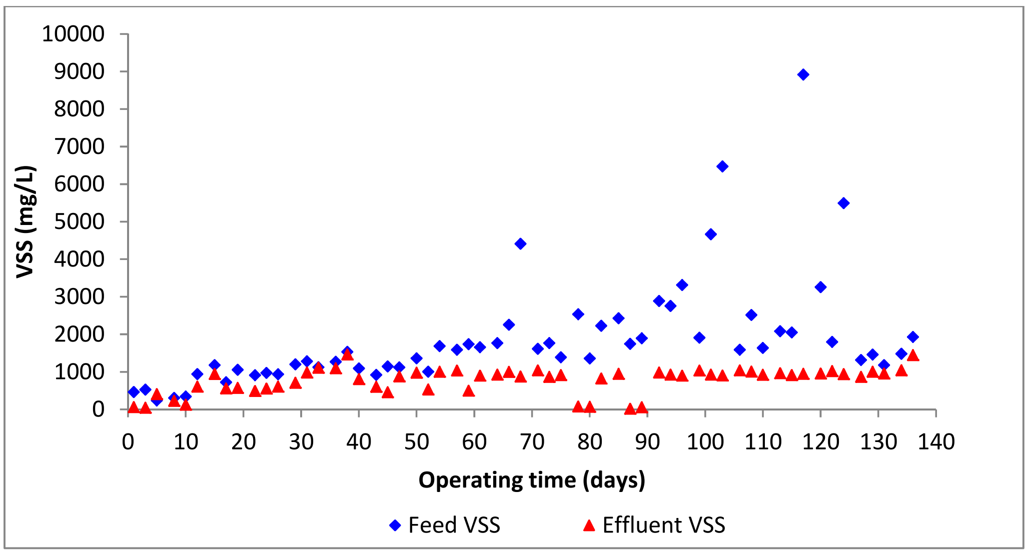

Figure 4-16: Variation in the SGBR feed and effluent VSS

In summary, the SGBR successfully reduced the COD content of PSW to a maximum value of 974 mg/L (average COD of 729±98 mg/L) which is less than the maximum limit of permitted discharge to municipal sewers as specified by the CCT, i.e. 5000 mg/L. Despite the PSW COD averaging at 4344 mg/L, further reduction of COD to a value below 1000 mg/L eliminates the penalties imposed on industries, including poultry slaughterhouses. The maximum effluent TSS and FOG concentrations of 160, and 100 mg/L were well below the acceptable limit for municipal discharge of 1000 and 400 mg/L, respectively. The maximum PO_4^{3-} concentration of 95 mg/L, however, exceeded the maximum limit for permitted discharge, i.e. 10 mg/L, thus indicating the need for further treatment of the SGBR effluent. The effluent pH ranged between 6.29 to 8.59 which was within the acceptable range for both the CCT by-law (i.e. 5.5–12) as well as the SANS 241 potable water standards (i.e. >5–9.7).

The average NH_4^+-N and NO_3^--N concentrations of 177 and 12.12 mg/L, respectively, were non-compliant with the SANS 241 potable water standards of 1.5 and 11 mg/L, respectively; whereas, the average TDS concentration of 1142 mg/L was below the limit of 1200 mg/L. However, the effluent TDS concentrations exceeded the discharge limit on several days (Figure 4.18C). Overall, further treatment of the SGBR effluent was required with regard to the PO_4^{3-}, NH_4^+-N, NO_3^--N, and TDS levels.

Table 4-2: Characteristics of the SGBR effluent

Parameter	**Unit**	**Minimum**	**Maximum**	**Average (±SD)**	**CCT By-law limit[a]**	**SANS 241: 2011[b]**
pH (25 ˚C)	-	6.29	8.59	-	5.5-12	5-9.7
Conductivity (25 ˚C)	mS/m	1021	2323	1608 ±328	500	≤170
TDS	mg/L	725	1653	1142 ±232	4000	≤1200
Salinity	mg/L	529	1187	882 ±134	-	-
Turbidity	NTU	3.57	234	14.7±24.6	-	≤1
TSS	mg/L	13	160	63 ±38	1000	-
VSS	mg/L	20	1465	744±343	-	-
COD	mg/L	482	974	729 ±98	5000	-
Ammonium (as N)	mg/L	103	283	177±36	-	<1.5
Nitrate (as N)	mg/L	1.07	24	12.12±6.98		≤11
Ortho-phosphate (PO_4^{3-})	mg/L	65	141	94 ±18	10	-
VFA	mg/L	21	402	124±118	-	-
Alkalinity (as $CaCO_3$)	mg/L	347	933	588 ±163	-	-
BOD_5	mg/L	10	175	95 ±62	-	-
FOG	mg/L	24	100	51 ±22	400	-

[a]CCT: City of Cape Town wastewater and industrial effluent by-law (Cape Town, South Africa, 2014).
[b]SANS 241:2011: South African National Standards (SANS 241) potable water standards (Department of Water Affairs, 2011).

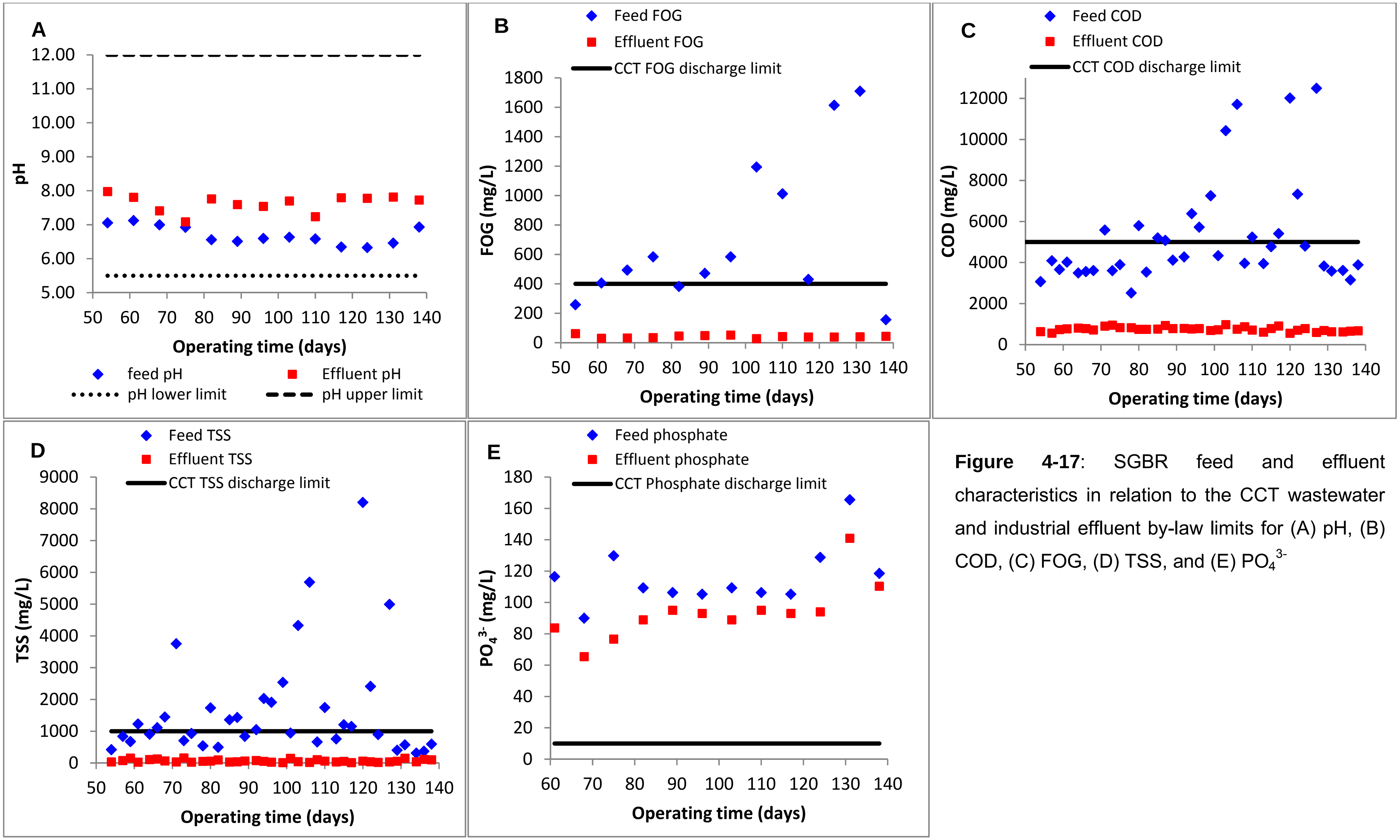

Figure 4-17: SGBR feed and effluent characteristics in relation to the CCT wastewater and industrial effluent by-law limits for (A) pH, (B) COD, (C) FOG, (D) TSS, and (E) PO_4^{3-}

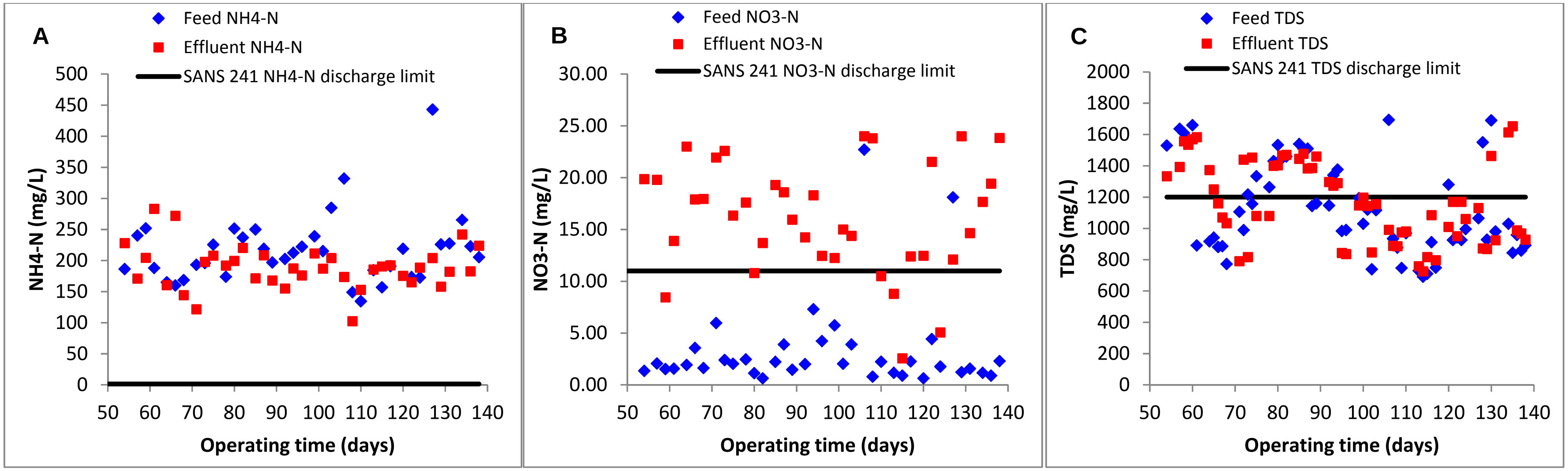

Figure 4-18: SGBR feed and effluent characteristics in relation to the SANS 241 potable water limits for (A) NH_4^+-N, (B) NO_3^--N and (C) TDS

4.3.8. Operation strategy of the SGBR: Mitigation of clogging

Due to the simplistic design and down-flow configuration of the SGBR, daily operation merely included monitoring the reactor outlet to detect clogging of the under-drain system. Increases in headloss occurred periodically due to reduced anaerobic granular bed porosity as a result of the build-up of non-biodegradable solids in the system and the compression of the granular bed. The frequency at which increases in headloss occurred increased after the SGBR was fed with undiluted PSW and the HRT was decreased, thereby increasing the OLR and resulting in the need for periodic backwashing. Backwashing was performed on a weekly basis by recirculating a portion of the stored effluent back into the underdrain system. During the backwashing procedure, an equal quantity of the supernatant containing the solids dislodged from the granular bed was removed through the discharge port in order to maintain the water level in the reactor. When increases in headloss occurred more frequently, backwashing was performed biweekly and at a later stage, several (n= 2–3) times per week. The backwashing procedure allowed for the separation of the accumulated solids from the granular bed and hence unclogging of the SGBR system. In addition to dislodging the entrapped solids, backwashing resulted in the redistribution of the granular bed thus allowing it to settle better which may have had a beneficial effect. Although the backwashing procedure hindered the physical operation of the SGBR, it had minimal adverse effects on the performance of the SGBR with regard to organic matter reduction and suspended solids retention. The SGBR was thus able to maintain its stability at high OLRs after backwashing since the effluent COD and TSS concentrations were a function of the OLR and not the recovery time.

4.4 Optimization of the chemical oxygen demand (COD) removal efficiency for the SGBR

Response surface methodology (RSM) was used for the optimization of the SGBR operating conditions through the development of a quadratic model used to predict the COD removal efficiency of the SGBR for the treatment of PSW. The hydraulic retention time (HRT) and organic loading rate (OLR) were selected as the two independent variables which were evaluated in order to determine their effect on the COD removal efficiency. SGBRs treating slaughterhouse wastewaters are capable of operating under HRTs ranging from 8 to 96 h (0.33 to 4 days) (Oh, 2012; Mach, 2004) and OLRs as high as 12.76 g COD/Lday (Park et al., 2012). The chosen ranges used were based on the operating conditions used for the SGBR in this study, i.e. conditions reported in Debik & Coskun (2009) and Basitere et al. (2017). The SGBR system used in this study was able to consistently reduce the organic content of the PSW, resulting in an overall COD RE of 80% for HRTs of between 1 to 4 days (24 h to 96 h) and an average OLR of 2.75 g COD/L.day. Table 4.3 shows the central composite design (CCD) of the independent variables used to optimize the COD removal efficiency. The predicted results from the CCD indicated that an OLR of 12.49 g COD/Lday (A) and a HRT (B) of 1 day were the optimum conditions for attaining the maximum COD removal efficiency (95.5%) for the SGBR used for the treatment of the poultry slaughterhouse wastewater (PSW). The overall results suggest that the COD removal efficiency attained by the SGBR increased as the organic strength of the PSW increased and the HRT was decreased. Conversely, Basitere et al. (2017) reported a decrease in the COD removal efficiencies of an SGBR treating PSW when the OLR increased subsequent to a decrease in the HRT.

Table 4-3: CCD of the independent variables, (A) OLR and (B) HRT

	Factors		**COD Removal efficiency (%)**	
Run	**OLR (A) (g COD/Lday)**	**HRT (B) (day)**	**Experimental**	**Predicted**
1	1.04	2.29	72.8	72.4
2	0.96	2.29	67.7	71.7
3	1.26	2.29	76.0	74.1
4	0.73	4	78.5	77.8
5	0.88	4	78.9	80.3
6	0.77	4	79.3	78.5
7	2.01	2	80.9	79.5
8	1.81	2	80.1	78.2
9	2.79	2	83.8	84.5
10	3.47	1.5	85.4	86.0
11	4.26	1.5	88.0	89.0
12	3.49	1.5	86.4	86.1
13	12.0	1	95.4	95.0
14	7.33	1	90.4	90.2
15	12.49	1	95.2	95.5

Figure 4.19 represents the relationship between the predicted and experimental results for the COD removal efficiency obtained from the CCD using RSM.

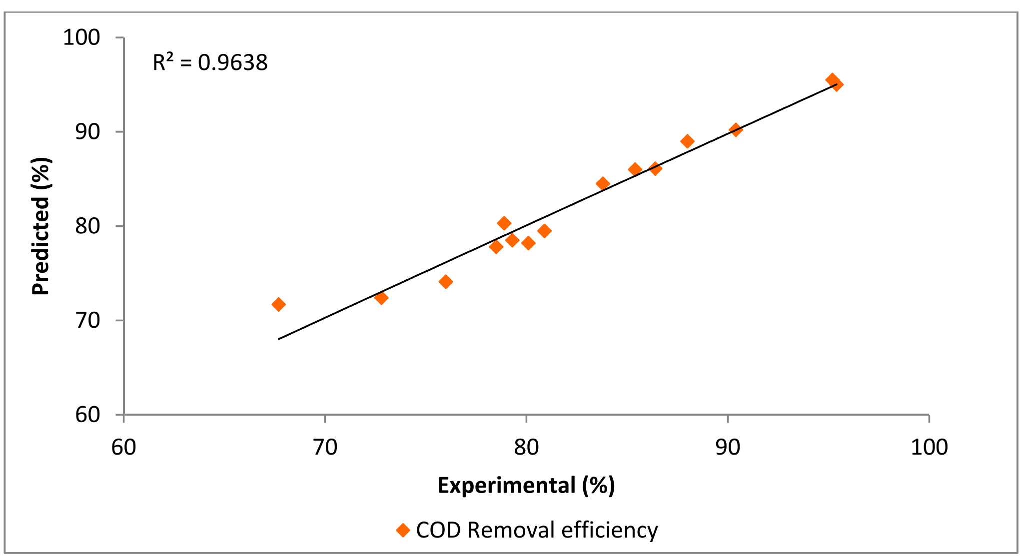

Figure 4-19: Predicted vs. experimental COD removal efficiency based on the CCD

The interaction between the operating conditions, i.e. HRT and OLR, and the COD removal efficiency was determined using polynomial regression. Table 4.4 summarises the analysis of variance (ANOVA) results for the quadratic model used to predict the chemical oxygen demand (COD) removal efficiency of the SGBR. ANOVA was used as a means to evaluate the statistical significance of the model equation, the individual variables and factor interactions (Bustillo-Lecompte & Mehrvar, 2017). Eq. 4.1 represents the resultant quadratic model which best fit the regression results.

$$COD\ Removal\ efficiency\ = 121.64 + 51.38A + 42.57B + 44.44AB - 0.84A^2 + 10.31B^2 \quad (4.1)$$

The adequacy of the proposed model was determined according to the determination coefficient (R^2), F-value and p-value. An R^2 of at least 0.80 is indicative of the good fit of a model (Dahunsi et al., 2016). The F-value is based on the comparison between the variance related with all terms and the residual variance; whereas, the p-value refers to the probability value which is related to the F-value for all terms (Dahunsi et al., 2016). The model R^2, F-value and p-value of 0.9638, 47.93 and <0.0001, respectively, obtained for this optimization study indicate that the model is suitable to predict the COD removal efficiency. The adjusted determination coefficient (R^2 Adj) and predicted determination coefficient (R^2 Pred) values obtained were 0.9437 and 0.9097, respectively. The R^2 values are similar to those reported in literature for the optimization process using RSM for wastewater treatment (Bustillo-Lecompte & Mehrvar, 2017).

The significance of the individual variables and their interactions in the model were determined by p-values less than 0.05. According to Table 4.4, the A^2 and B^2 terms in Eq. 4.1 had an insignificant effect on the COD removal efficiency. Therefore, Eq. 4.1 was reduced to Eq. 4.2, which represents the final quadratic model equation used to estimate the COD removal efficiency. Eq. 4.2 correlates the experimental results and the predicted results and is thus considered as a good fit – see Figure 4.19, with the response being graphically illustrated in Figure 4.20.

$$COD\ Removal\ efficiency\ = 121.64 + 51.38A + 42.57B + 44.44AB \quad (4.2)$$

Table 4-4: ANOVA of the quadratic model for COD removal efficiency

Source	Sum of squares	Degree of freedom	Mean square	F-value	p-value Prob>F
Model	818.36	5	163.67	47.93	<0.0001
A	75.90	1	75.90	22.22	0.0011
B	54.42	1	54.42	15.93	0.0031
AB	34.03	1	34.03	9.96	0.0116
A^2	0.058	1	0.058	0.017	0.8995
B^2	7.78	1	7.78	2.28	0.1656
Residual	30.74	9	3.42		
R^2	**0.9638**	**R^2 Adj**	**0.9437**	**R^2 Pred**	**0.9097**

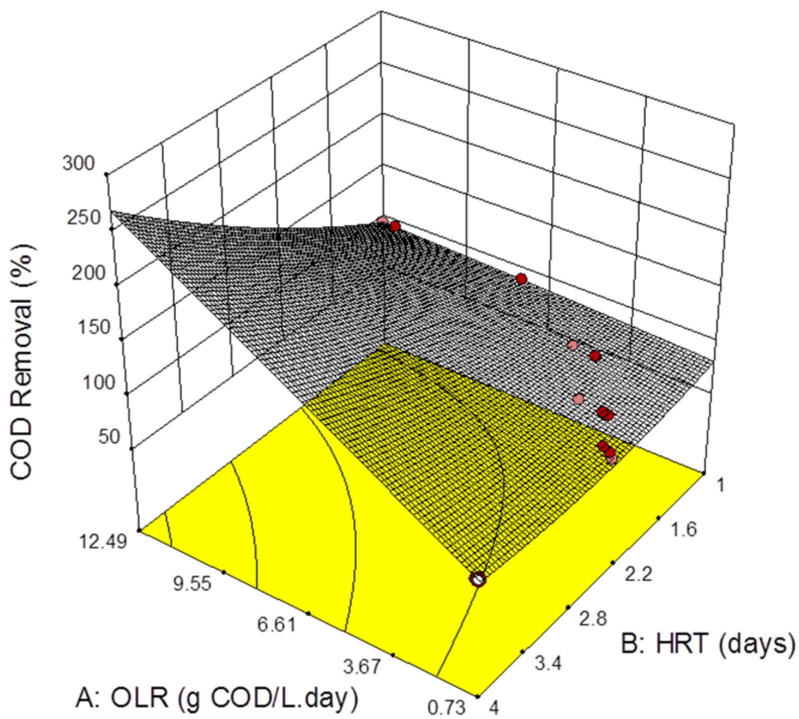

Figure 4-20: 3-D response surface for the COD removal efficiency as a function of variables A: OLR and B: HRT

Figure 4.20 illustrates the 3-D response surface plot representing the effect of the OLR and HRT on the COD removal efficiency achieved by the SGBR during the treatment of PSW. It is evident that the SGBR efficiency is affected by both the organic and hydraulic loading of the reactor. Thus, the correct balance between the two operating conditions must be maintained in order to ensure the stable and efficient operation of the SGBR as well as to prevent reactor failure. In other words, in order to allow the micro-organisms sufficient time for the biodegradation of the organic matter (i.e. COD) present in the PSW, certain conditions must not be exceeded so as to prevent overfeeding of the micro-organisms (Nayona, 2010). The optimal SGBR performance with regard to the maximum COD removal efficiency was predicted for an OLR of 12.49 g COD/Lday and a HRT of 1 day (24 h), resulting in a 95.5%

COD removal efficiency. Similarly, Muhamad et al. (2013) reported a COD removal efficiency of 9% at an HRT of 1 day (24 h) for the optimization of the COD removal of a granular activated carbon sequencing batch biofilm reactor (GAC-SBBR) treating recycled paper wastewater. Ideally, it is desired that the SGBR be operated at a maximum OLR, for the shortest HRT, in order to achieve the highest COD removal efficiency possible without adversely impacting the SGBR performance and operation for high throughput rates of PSW. However, since the SGBR is a biological system there might be other factors other than the organic and hydraulic loading, which might affect the SGBR performance such as the activity of the methanogens (Oh et al., 2015); however, these were not identified and investigated in this study.

4.5 Performance of the single stage nitrification-denitrification (SSND) bioreactor and ultrafiltration membrane module (ufMM) post-treatment systems

The SSND aerobic bioreactor and sidestream ufMM systems were operated at ambient temperature (i.e. 23-25 ˚C) for a period of 87 days, in order to reduce the nutrients and residual organic matter and suspended solids contents of the anaerobically pretreated PSW.

4.5.1. Performance of the SSND bioreactor

The SSND bioreactor was used to reduce the nutrient content of the anaerobically treated PSW, specifically the nitrogenous by-products (i.e. NH_4^+-N, NO_3^--N, and NO_2^--N) from the primary treatment stage, via the SND process. The SSND aerobic bioreactor was operated at ambient temperature (i.e. 23-25 ˚C) under two different operating strategies for a period of 87 days.

4.5.1.1 Nitrogen removal in the SSND bioreactor

After the acclimation period, the SSND bioreactor was fed with the SGBR effluent which was characterized by average ammonium-nitrogen (NH_4^+-N), nitrate-nitrogen (NO_3^--N) and nitrite-nitrogen (NO_2^--N) concentrations of 241±47 mg/L, 19±6.1 mg/L and 9.2±2.1 mg/L, respectively. It took approximately 10 days for the untreated PSW to be replaced by the SGBR effluent. Stage 1 (operating strategy 1) was characterized by the down-flow operational mode without aeration (anoxic conditions), thus relying on the air entering at the top of the column (nitrification zone) and the sponge packing media for the development of a nitrifying population. During stage 1 (day 11 – 45), the nitrification zone preceded the denitrification zone and the hydraulic retention time (HRT) was maintained at 6 days.

Figure 4.21 shows the total nitrogen (TN) removal obtained for 87 days. The effluent TN (i.e. the sum of NH_4^+-N, NO_3^--N and NO_2^--N) concentration ranged from 139 mg/L to 194 mg/L during stage 1, with an average TN removal efficiency of 33±10%. During the first week of stage 1, low NH_4^+-N removal efficiencies (<12%) were observed which may have been due to the system lacking a readily available biodegradable carbon source and the slow growth of ammonium-oxidising bacteria (AOB) (Wang et al., 2010).

The bioreactor was therefore inoculated with raw PSW on day 17 (i.e. one week after the first operating stage was initiated). The re-inoculation with raw PSW resulted in an increase in the TN removal efficiency from 20% to 37% (Figure 4.21). Consequently, the NH_4^+-N removal efficiency increased from 9% (in the first week) to 29% after the re-inoculation. However, the NH_4^+-N concentrations were remained unchanged and significantly high throughout stage 1 (Figure 4.22). The effluent NH_4^+-N concentration averaged at 168±18 mg/L with an average removal efficiency of 25±13%, indicating that nitrification was inhibited. The low NH_4^+-N oxidation may have been the due to the AOB and nitrite-oxidising bacteria (NOB) competing for dissolved oxygen (DO) (Seifi & Fazaelipoor, 2012). Whereas, the NO_3^--N and NO_2^--N removal efficiencies exceeded 90%, resulting in effluent NO_3^--N and NO_2^--N concentrations of 0.69±0.52 mg/L and 0.66±0.88 mg/L, respectively. The high NH_4^+-N and relatively low effluent NO_3^--N and NO_2^--N effluent concentrations (<1 mg/L) suggest that the initial oxidation of NH_4^+-N was the rate-limiting step in the removal of nitrogen (Holman & Wareham, 2005). The significant denitrification process, i.e. reduction of NO_3^--N and NO_2^--N to nitrogen gas, may be attributed to oxygen limitations within the biofilm thereby providing the oxygen-free conditions for heterotrophic denitrifying bacteria activity (Seifi & Fazaelipoor, 2012; Holman & Wareham, 2005; Yoo et al., 1999).

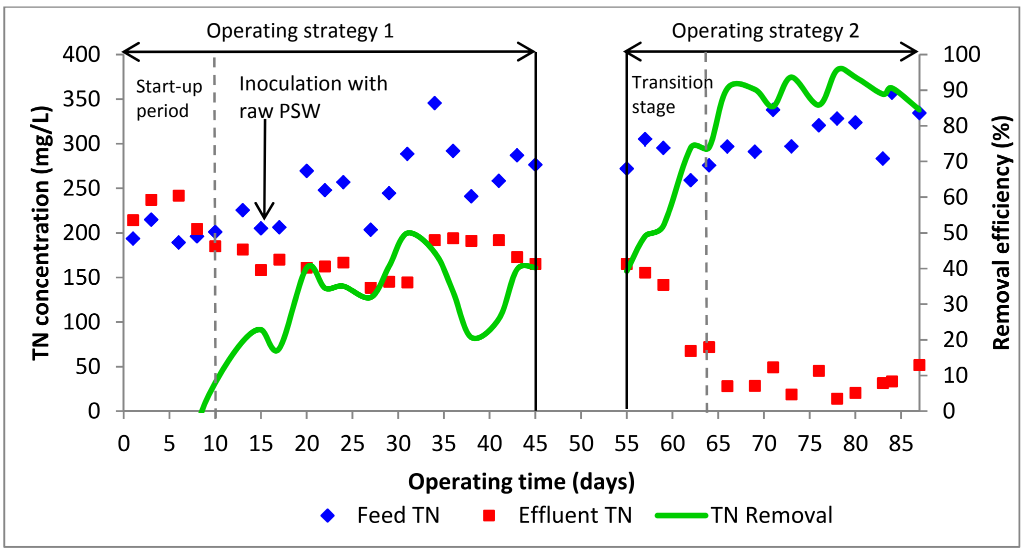

Figure 4-21: SSND bioreactor feed and effluent TN concentrations and removal efficiencies

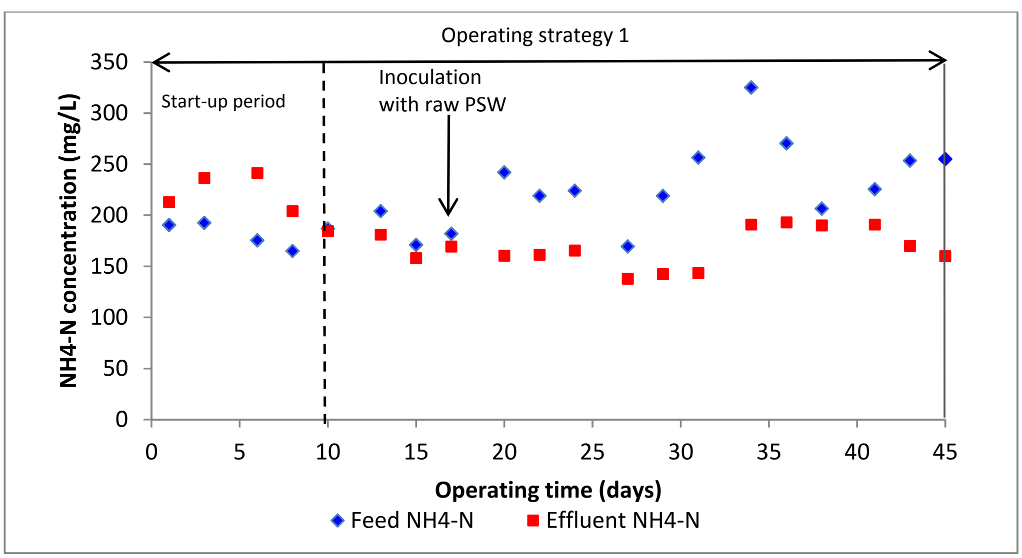

Figure 4-22: SSND bioreactor feed and effluent NH4+-N) concentrations during operating strategy 1

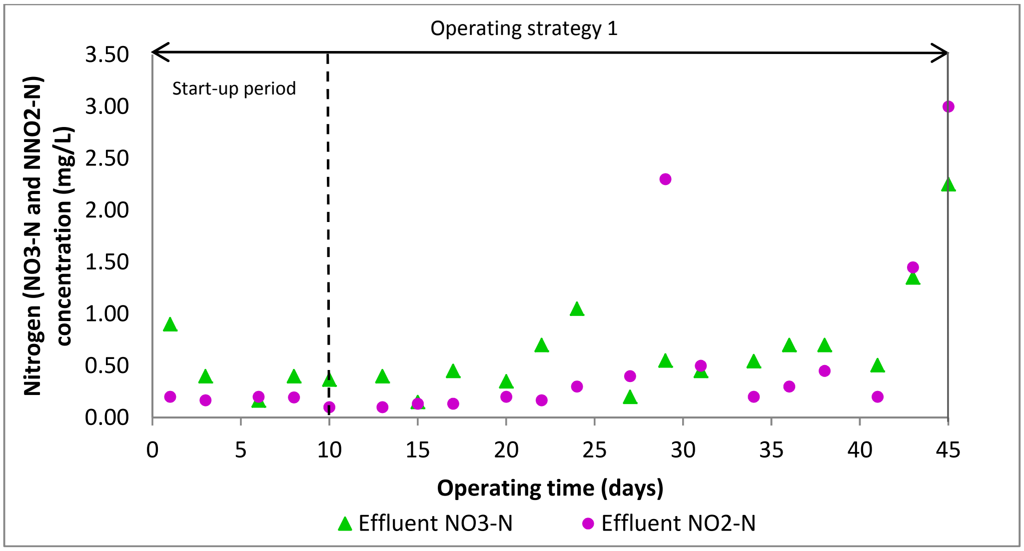

Figure 4-23: SSND bioreactor effluent NO_3^--N and NO_2^--N concentrations during operating strategy 1

Due to the high NH_4^+-N concentrations in the effluent from stage 1 (Figure 4.22) and the spike in the NO_3^--N and NO_2^--N concentrations from day 43 to 45 (Figure 4.23), an air supply was introduced into the bioreactor (on day 51) and the bioreactor was re-configured (on day 54) to operate in up-flow operational mode. During stage 2 (day 55-87), the DO concentration in the aerobic zone was maintained between 1.8 and 3.2 mg/L. As a result of the up-flow configuration, the nitrification zone followed the denitrification zone and the HRT was therefore increased to 11 days to minimize the washout of the nitrifying micro-organisms (Seifi & Fazaelipoor, 2012). via the effluent stream exiting the top of the bioreactor, and to provide sufficient time for the adjustment of the biofilm to the up-flow configuration. Complete denitrification may not have been achieved by this configuration since a portion of the aerobic bioreactor effluent was not recycled through the system.

The TN concentration ranged from 14 mg/L to 165 mg/L with an average TN removal efficiency of 79±18%, which was significantly higher than the TN removal efficiency observed in stage 1 (Figure 4.21). The effluent NH_4^+-N concentration averaged at 28±12 mg/L with a removal efficiency of 81±17%, indicating that nitrification was the dominant process occurring within the bioreactor. Furthermore, the higher NH_4^+-N removal efficiency obtained in stage 2 verified that the oxygen supply is an important factor for efficient NH_4^+-N oxidation (Seifi & Fazaelipoor, 2012). The decreasing denitrification may have been due to the shift in the micro-organisms' population distribution as a result of the bioreactor reconfiguration and the increase in the DO concentration. During the 2 weeks transition stage, i.e. the first 12 days of stage 2 (day 55 to 64 of), the average effluent NO_3^--N concentration increased from 0.69 mg/L (stage 1 average) to 13 mg/L with an average removal efficiency of 45±7%. The substantial increase in the NO_3^--N may have been due to the suppression of the ability of the biofilm to completely reduce the oxidized nitrogen. Whereas, the effluent NO_2^--N concentrations remained constant during this period. An increase in the NH_4^+-N oxidation occurred immediately after the sparse sparging (Figure 4.24). From day 66 onwards (after 11 days), average NH_4^+-N, NO_3^--N, and NO_2^--N removal efficiencies of 90±4%, 87±8% and 79±14% were achieved, respectively. The reduction in the NO_3^--N after day 66, suggested that the SSND biofilm acclimatized to the increasing DO concentrations (Figure 4.25). Furthermore, the low average NH_4^+-N, NO_3^--N, and NO_2^--N concentrations in the effluent i.e. 28±12 mg/L, 2.3±0.5 mg/L and 2.0±1.3 mg/L (Figure 2.24 and 2.25) and high TN removal efficiency (i.e. 79±18%) observed, suggest that a significant SND process occurred during this stage. However, the presence of NO_2^--N in the effluent indicated that complete denitrification was not achieved within the bioreactor; thus, the oxidation of the NH_4^+-N may have been the dominating step.

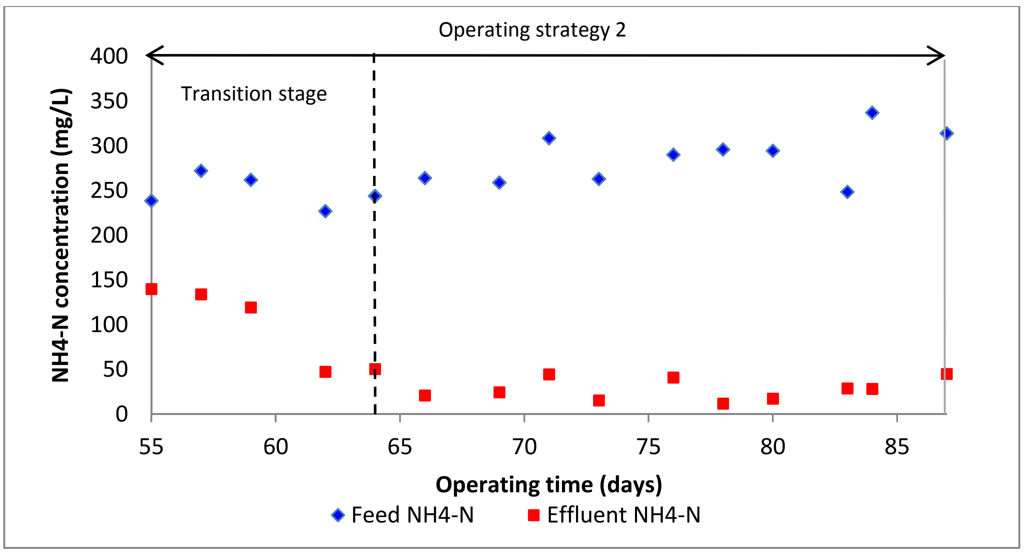

Figure 4-24: SSND bioreactor feed and effluent NH_4^+-N concentrations during operating strategy 2

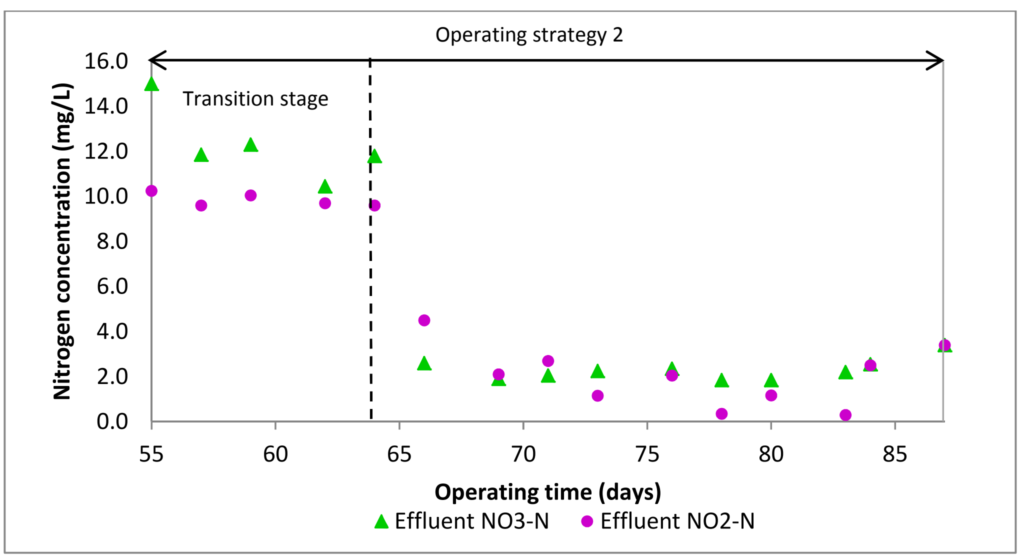

Figure 4-25: SSND bioreactor effluent NO_3^--N) and NO_2^--N concentrations during operating strategy 1

4.5.1.2 Organic matter removal in the SSND bioreactor

Since the SSND bioreactor used in this study was implemented after the SGBR primary treatment stage, the SSND bioreactor feed (i.e. SGBR effluent) contained low COD and high inorganic nitrogen (i.e. NH_4^+-N) concentrations due to the biological degradation of organic matter and proteins, respectively. In such instances, the addition of an external biodegradable organic carbon source is required for the growth of microbial communities suitable for nitrification-denitrification processes. In a study performed by Mpongwana (2016), it was proven that bacterial isolates (i.e. *Enterobacter* sp., *Yersinia* sp. and *Serratia* sp.) from PSW, obtained from the same source as the PSW used in this study, are capable of carrying out simultaneous nitrification and aerobic denitrification (SND). Therefore, the biological potential exists for SND without supplemental organic carbon sources.

During stage 1 (day 11-45) of the SSND bioreactor operation, the feed COD/TN ratio was 2.86 with a low corresponding OLR of 0.12 g COD/Lday. Under these conditions, the SSND bioreactor achieved satisfactory denitrification but not efficient organic matter removal with an average COD removal efficiency of 6%. This may have been as a result of the feed containing residual COD in the form of slowly biodegradable and/or non-biodegradable organic matter and insufficient DO within the bioreactor. The COD removal efficiency increased to 19% during stage 2 (day 66-87), which may have been due to the increase in the HRT and DO. Despite the slight increase in the COD removal, the feed COD/TN ratio of 2.71 and OLR of 0.08 g COD/Lday was still proven to be insufficient for simultaneous nitrogen and organic matter removal. This may be attributed to the growth of competitive bacteria and the absence of activated sludge (AS) which is commonly used for biofilm development in aerobic bioreactors. Furthermore, the low COD/TN ratios may have resulted in a rapid carbon deficit causing an unbalanced SND process. The influent and effluent COD concentrations of the SSND bioreactor averaged at 786 mg/L and 688 mg/L (i.e.11% overall COD removal efficiency).

4.5.1.3 Overall performance of the SSND bioreactor and efficiency in relation to the effluent discharge quality and regulations

Under oxygen limiting conditions and the down-flow configuration (stage 1), the nitrogen removal was relatively low and the initial oxidation of NH_4^+-N was the rate-limiting step in the nitrogen removal. The nitrogen removal increased significantly to satisfactory levels after the reconfiguration of the bioreactor from down-flow to up-flow operational mode and the addition of a sparging system (stage 2). The NH_4^+-N, NO_3^--N, and NO_2^--N removal efficiencies achieved were 25%, 96%, and 93% during stage 1, and 90%, 87% and 78% during stage 2, respectively. These results indicated that the air supply promoted the growth of the AOB and NOB; however, the bioreactor setup was not yet optimized with respect to the oxygen transfer. Although complete nitrogen removal was not achieved, the aim was to assess the possibility of a SND process occurring within a single stage bioreactor without the addition of external carbon sources. The nitrogen removal due to SND was 97.8% and 97.9% during stage 1 and 2, respectively. Furthermore, the bioreactor was not designed as an activated sludge process for COD and PO_4^{3-}-P removal; hence, there was no significant COD and PO_4^{3-}-P removal observed. However, there was an increase in both the COD and PO_4^{3-}-P removal from stage 1 to stage 2, as shown in Figure 4.27 and 4.28.

Table 4-5: Overall performance of the SSND aerobic bioreactor for a period of 87 days

	Operating strategy 1		**Operating strategy 2**	
	HRT (day)			
	6		**11**	
Operating period (days)	13-45		66-87	
OLR (g COD/Lday)	0.12		0.08	
NLR[a] (g TN/Lday)	0.04		0.03	
COD/TN	2.86		2.71	
	Effluent	*Removal (%)*	*Effluent*	*Removal (%)*
NH_4^+-N (mg/L)	168 ±18	25	28 ±12	90
PO_4^{3-}-P (mg/L)	38 ±5	2	20 ±5	39
COD (mg/L)	691 ±50	6	685 ±48	19
NO_3^--N (mg/L)	0.69 ±0.53	96	2.3 ±0.5	87
NO_2^--N (mg/L)	0.66 ±0.88	93	2.0 ±1.3	78

NLR[a]: TN loading rate

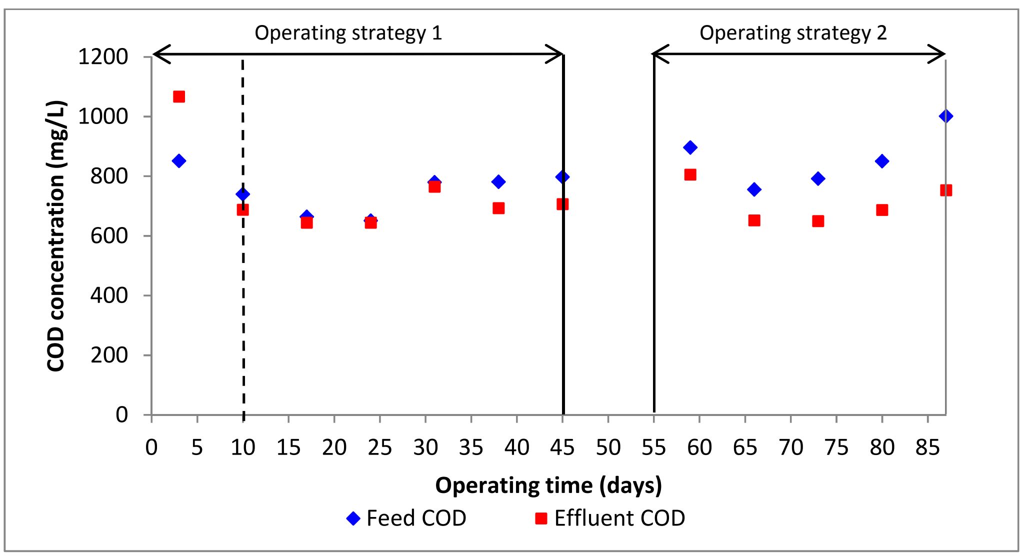

Figure 4-26: SSND bioreactor effluent COD concentrations

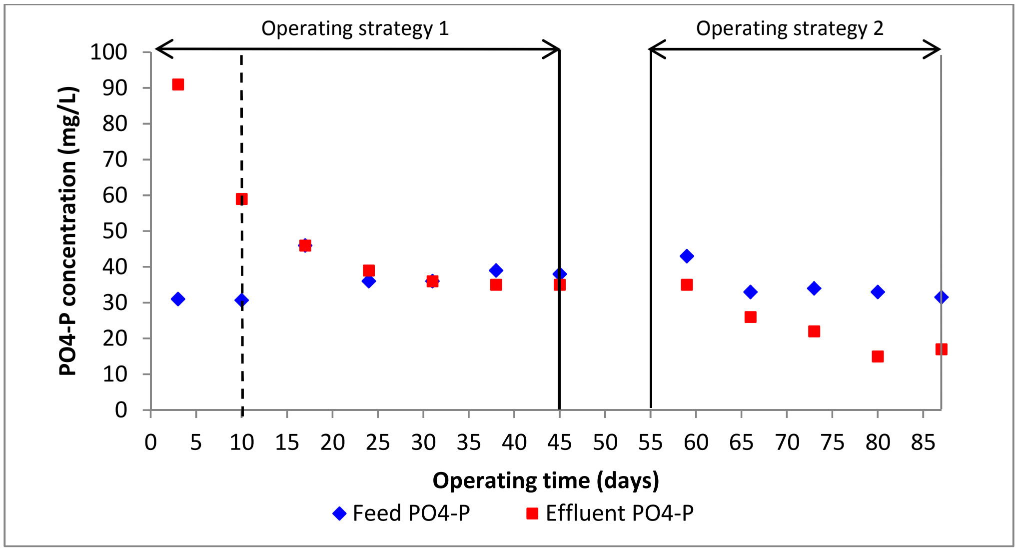

Figure 4-27: SSND aerobic bioreactor effluent PO_4^{3-}-P concentrations

Further treatment of the anaerobically treated PSW was required with regard to the nutrient content (i.e. nitrogen and phosphorous) in order to achieve compliance with environmental discharge requirements. The SSND bioreactor effluent characteristics were compared to the SANS 241 (2011) potable water specifications (Figure 4.28 and 4.29). During stage 1, the SSND bioreactor successfully reduced the NO_3^--N concentration to 0.66±0.88 mg/L which is below the limit of 11 mg/L. Whereas, the NH_4^+-N concentration of 168±18 mg/L was significantly higher than the NH_4^+-N maximum limit of permitted discharge of 1.5 mg/L. Conversely, during stage 2, the NH_4^+-N and NO_3^--N concentrations were reduced to effluent values of 28±12 mg/L and 2.3±0.5 mg/L, respectively. The NH_4^+-N concentrations obtained during both stage 1 and 2 were therefore not compliant with the SANS 241 standard of 1.5 mg/L. Furthermore, the average PO_4^{3-} concentration of 117 mg/L (stage 1) and 85 mg/L (stage 2), exceeded the maximum limit for permitted discharge i.e. 10 mg/L. Thus, further treatment of the SSND bioreactor effluent was required with regard to NH_4^+-N and PO_4^{3-}.

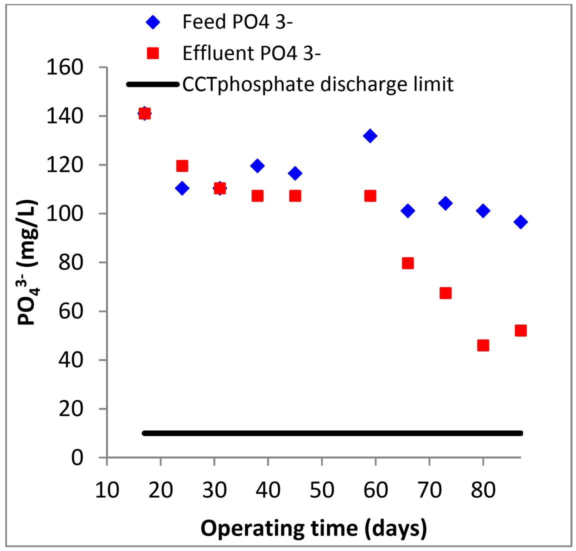

Figure 4-28: SSND bioreactor feed and effluent PO_4^{3-} characteristics in relation to the CCT wastewater and industrial effluent by-law limit

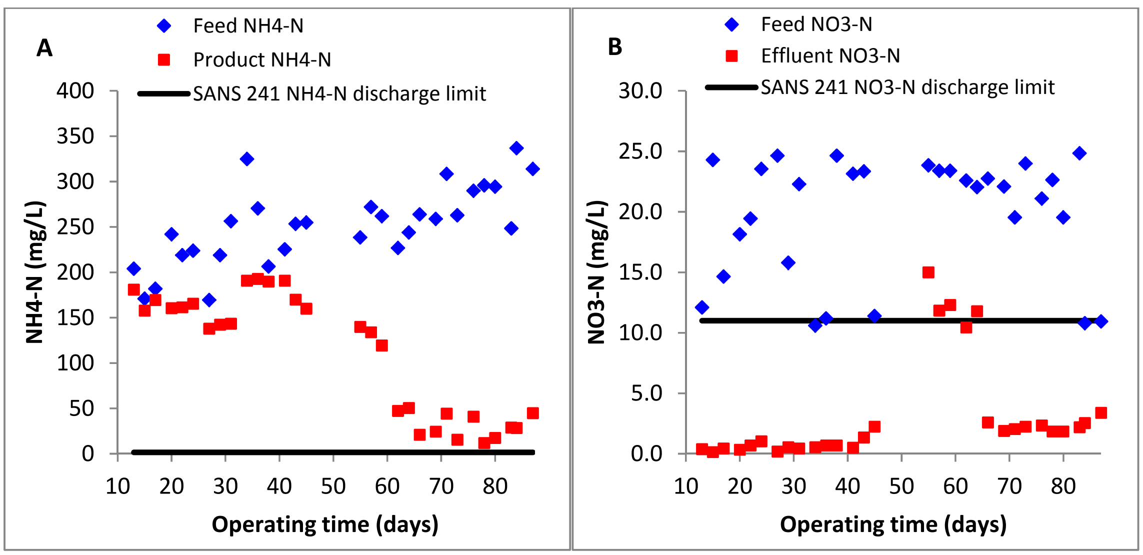

Figure 4-29: SSND bioreactor feed and effluent characteristics in relation to the SANS 241 potable water limits for (A) NH_4^+-N and (B) NO_3^--N

4.5.2. Performance of the ultrafiltration (UF) membrane module (ufMM) system

The ufMM system was operated subsequent to the SSND aerobic bioreactor, at ambient temperature (i.e. 23-25 ˚C), for a period of 87 days. The ufMM system was implemented as a final treatment stage for further reduction of the residual COD, TSS, including the turbidity and conductivity.

4.5.2.1. ufMM system treatment efficiency

The operation of the ufMM system was initiated on day 27 of the SSND aerobic bioreactor operation. A weekly composite sample of the SSND bioreactor effluent was fed to the ufMM at a constant feed flow rate of 0.0282 L/h in order to demonstrate the long-term performance of the SSND-ufMM system for an operational period of 52 days. The ufMM performance was evaluated based on the permeate quality in accordance with the COD, TSS, turbidity, and conductivity. The SSND bioreactor effluent was characterized by average COD and TSS concentrations of 714±56 mg/L and 61±24 mg/L, respectively. The average turbidity and conductivity of the SSND bioreactor effluent was 24±5 NTU and 1506±484 mS/cm, respectively. The ufMM feed (i.e. SSND bioreactor effluent) COD, ufMM permeate COD, and COD rejection efficiencies are shown in Figure 4.32. The ufMM efficiently reduced the COD to an average permeate COD concentration of 288±138 mg/L and 373±254 mg/L during stage 1 and 2 of the SSND-ufMM system, respectively, with corresponding rejection efficiencies of 59±21% and 71±17%. The overall COD rejection efficiency was observed as 65±18%. Similarly, Basitere et al. (2017) reported an average COD rejection efficiency of 64% for UF membranes with a pore size of 40 nm, used as a post-primary treatment system for PSW which was pre-treated by a SGBR anaerobic system.

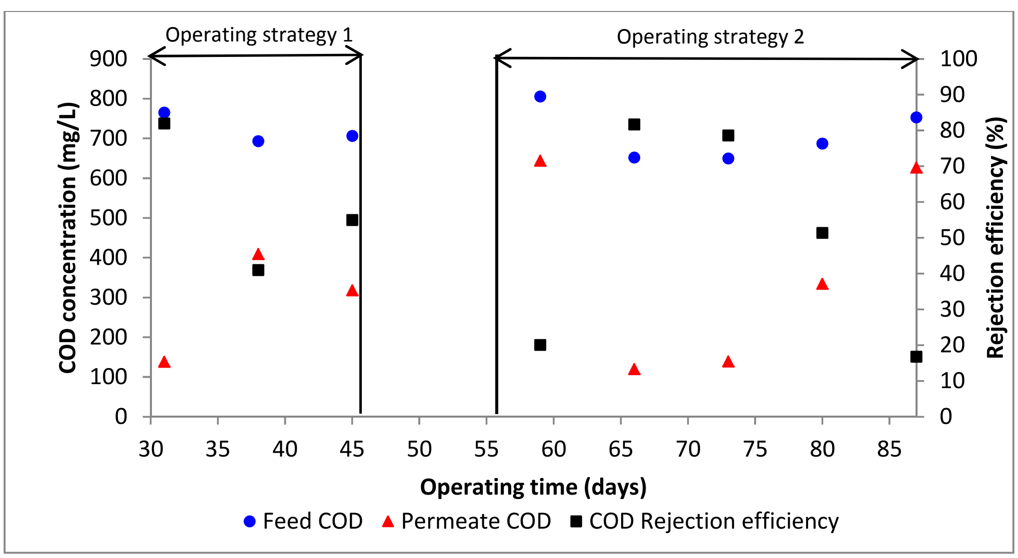

Figure 4-30: COD rejection efficiency of the ufMM system

The ufMM feed TSS, ufMM permeate TSS, and TSS rejection efficiencies are shown in Figure 4.31 to 4.32. The ufMM system efficiently retained 57±8% and 52±10% of the TSS from the SSND bioreactor effluent resulting in average permeate TSS concentrations of 30±10 mg/L and 33±8 mg/L, during stage 1 and 2 of the SSND-ufMM system operation, respectively. The marginal reduction in the TSS rejection efficiency from stage 1 to 2 may be attributed to fouling of the UF membranes since the permeate flux observed during stage 2 (4.77 L/m^{2}h) was lower than in stage 1 (5 L/m^{2}h), in spite of the constant feed flow rate. The overall TSS rejection efficiency was observed as 54±9%. The retention of suspended solids by the ufMM was mainly dependent on the pore size of the membrane with respect to the size and shape of the suspended solids. The average TSS rejection efficiencies observed in this study were relatively low in comparison to the 94% TSS rejection efficiency obtained by Yordanov (2011) for the treatment of raw PSW with a TSS concentration range of 2280 to 2446 mg/L, and the 88% TSS rejection efficiency achieved by Basitere et al. (2017) for the treatment of anaerobically pre-treated PSW characterized by a TSS concentration of 29±3 mg/L. The presence of suspended solids in the PSW increased the turbidity by obstructing the transmittance of light through the PSW. The turbidity of the SSND-ufMM system effluent was reduced by an average of 90±8%.

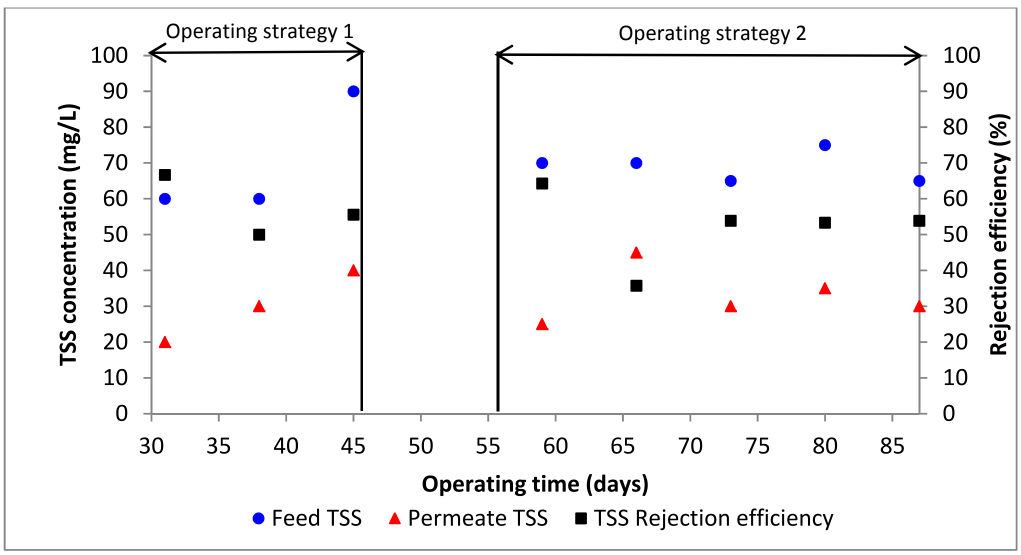

Figure 4-31: TSS rejection efficiency of the ufMM system

The conductivity is another important parameter used to evaluate the performance of membrane processes as it serves as an indicator of the soluble organic and inorganic matter or dissolved solids concentration present in the PSW (Coskun et al., 2015). The reduction in the ufMM permeate conductivity varied by 50% from stage 1 to 2, as seen in Figure 4.34. Overall, the conductivity was reduced by an average of 11±6%. This insignificant reduction in the conductivity was expected since UF membranes are used for the removal of insoluble organic matter and suspended solids.

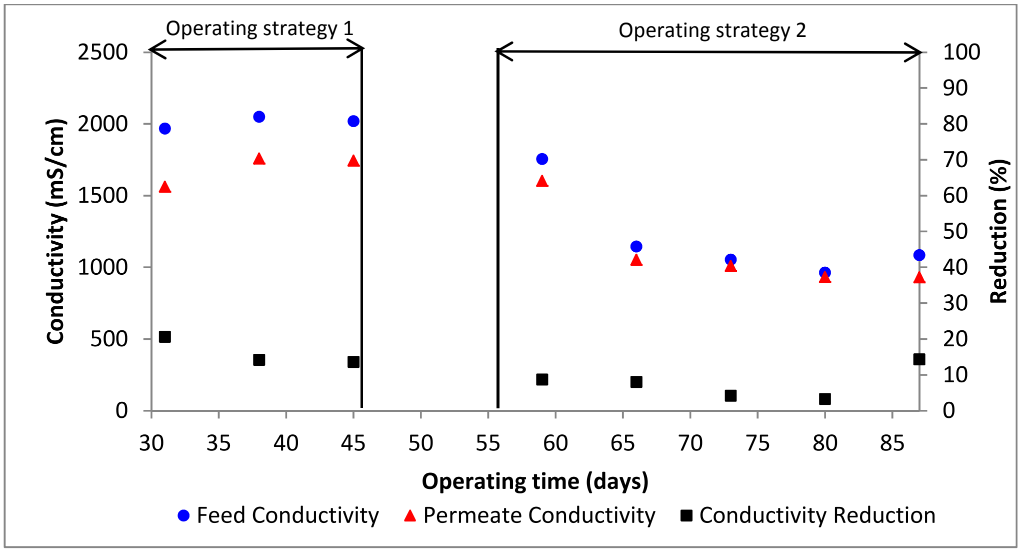

Figure 4-32: Conductivity reduction efficiency of the ufMM system

4.5.3. Overall performance of the ufMM system and efficiency in relation to effluent discharge quality and regulations

Table 4.6 summarises the overall performance of the ufMM used as a final treatment stage for the SSND aerobic bioreactor effluent over a period of 52 days. The ufMM demonstrated adequate performance at a constant feed flow rate and permeate flux of between 4.77 and 5 L/m^{2}h. Under operating stage 1 of the SSND-ufMM system operation (19 days), the ufMM achieved removal efficiencies of 59% for COD, 61% for TSS, 88% for turbidity with an average ufMM permeate TDS concentration of 1198 mg/L (see Figure 4.33 (B)). The permeate produced by stage 2 of the SSND-ufMM system operation (33 days) was characterized by an average TDS concentration of 785 mg/L and removal efficiencies of 78% for COD, 50% for TSS, and 92% for turbidity. Although the complete removal of the residual COD and TSS was not achieved, the results indicated that the overall performance of the ufMM was more efficient during stage 2, with the exception of the TSS removal efficiency which was greater for stage 1.

Table 4-6: Overall performance of the ufMM system for a period of 52 days

	Operating strategy 1		**Operating strategy 2**	
	HRT (day)			
	6		**11**	
Operating period (days)	27-45		55-87	
Feed flow rate, Q (L/h)	0.0282		0.0282	
Permeate flux (L/m^{2}h)	5		4.77	
	Effluent	*Removal (%)*	*Effluent*	*Removal (%)*
COD (mg/L)	288 ±138	59	373 ±254	71
TSS (mg/L)	37±26	61	47±18	50
Turbidity (NTU)	2.48±2.1	88	2.34±2.43	92
Conductivity (mS/cm)	1198±77		785±202	

The ufMM further reduced the COD and TSS of the SSND aerobic effluent to average concentrations of 341 and 43 mg/L, respectively, which is significantly lower than the corresponding industrial effluent discharge limits of 5000 mg/L and 1000 mg/L. The permeate had a neutral pH of between 7.15 and 7.72. The average TDS concentration of 940 mg/L was below the limit of 1200 mg/L; however, the TDS concentrations observed during the first operating strategy of the SSND-ufMM system did not meet the required discharge limit. The average PO_4^{3-} concentration of 69 mg/L exceeded the maximum limit for permitted discharge i.e. 10 mg PO_4^{3-}/L, thus indicating further treatment optimization of the ufMM is still required for the removal of PO_4^{3-}.

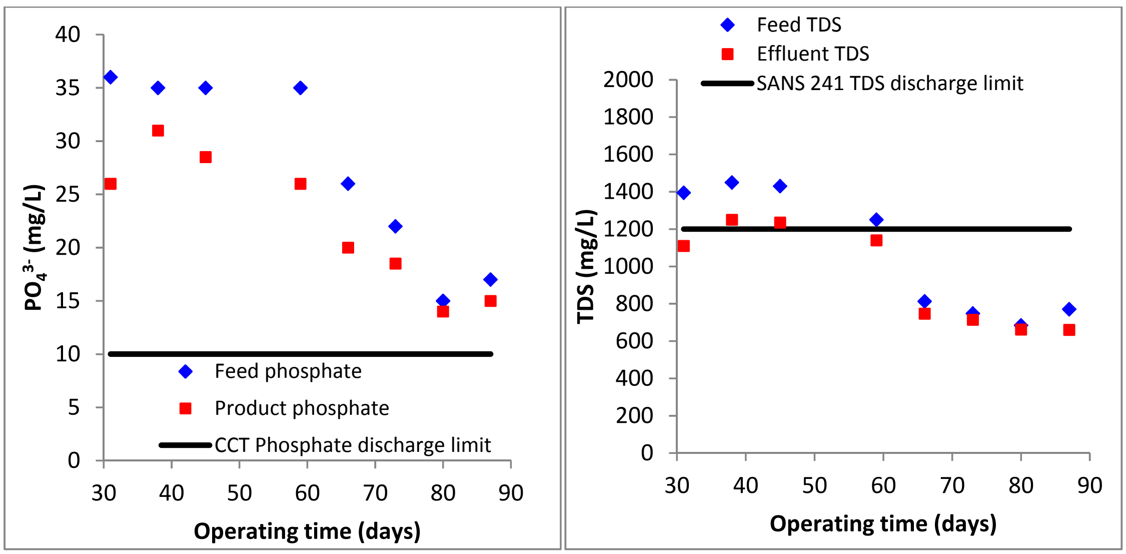

Figure 4-33: ufMM feed and effluent characteristics (A) PO_4^{3-} in relation to the CCT wastewater and industrial effluent by-law and (B) TDS in relation to the SANS 241 potable water requirements

4.6. Performance of the overall PSW system

Prior the discharging their PSW, poultry slaughterhouses are required to remove majority of the soluble, particulate, organic and inorganic matter as well as macronutrients (i.e. nitrogen and phosphorous) in order to achieve compliance with municipal and environmental discharge requirements. Physiochemical analyses of the PSW used in this study indicate that most parameters of the untreated PSW exceeded the permissible discharge limits for industrial effluent. To ensure that the contaminants present within the PSW do not adversely impact surface water by directly discharging the wastewater into the environment, there are treatment requirements prior to discharge as well as limits for the constituents that may allowably be discharged after treatment. Moreover, municipalities enforce surcharges and/or penalties when wastewater or industrial effluent does not meet the required discharge standards or volumes.

The characteristics of the overall PSW treatment system effluent were compared to the CCT wastewater and industrial effluent by-law (2013) discharge standards and the SANS 241 (2011) potable water specifications to determine its suitability for discharge into municipal sewer systems and its potential for re-use on-site. Table 4.7 represents the average performance of the combined SGBR and SSND-ufMM system compared with the local discharge and potable water standards. The performance of the overall PSW system was evaluated for a period of 87 days during which it was successfully employed for the treatment of the PSW under different operating conditions, i.e. HRT, OLR and flux, and varying operating configurations.

The SGBR and SSND-ufMM systems combined the benefits of both the biological and physical treatment processes and achieved average removal efficiencies of 91% for COD and 51% for PO_4^{3-}, whilst retaining 97% and 52% of the TSS and TDS, respectively. The resultant treated effluent was characterized by conductivity and salinity values of 1325 mS/cm and 1417 mg/L on average, respectively, and a pH of between 7.77 and 8.08. The overall results achieved in this study indicated that the PSW treatment system was effective at producing a high quality permeate with a turbidity of 2 NTU which is close to potable water standards and was compliant with industrial effluent discharge standards; however, further treatment is required in order to qualify the treated PSW safe for discharge into receiving water bodies or for re-use purposes.

Similarly, Basitere et al. (2017) treated PSW using an SGBR pre-treatment coupled with a UF membrane post-treatment and achieved overall COD and TSS removal efficiencies of 98.7% and 99.8%, respectively, with the final effluent being compliant with the local discharge standards. Del Nery et al., 2016 evaluated the performance of a full-scale PSW treatment plant consisting of both biological and physical treatment systems, i.e. a DAF system, UASB reactors, and an aerated facultative pond (AFP) followed by a chemical-DAF system. The overall PSW treatment plant achieved a COD removal efficiency of 98% and a final effluent PO_4^{3-} concentration of 1.34±0.93 mg/L, which indicated that the combination of treatment technologies used were suitable to produce a final effluent safe for discharge into water bodies.

Table 4-7: Average performance of the combined SGBR and aerobic SSND-ufMM system compared with the local industrial effluent discharge standards and potable water standards for 87 days of operation

Parameter	Unit	SGBR Feed	SGBR Effluent	% SGBR	SSND-ufMM Effluent	% SSND-ufMM	% Overall System	CCT By-law limit[a]	SANS 241: 2011[b]
pH	-	6.94	7.46	-	8.08	-	-	5.5-12	5-9.7
Conductivity	mS/cm	2761	2244	19	1325	11	52	5000	≤1700
TDS	mg/L	1956	1588	19	940	11	52	4000	≤1200
Turbidity	NTU	710	19	97	2	90	99.7	ni	≤1
TSS	mg/L	1084	64	94	32	54	97	1000	ni
COD	mg/L	4245	832	80	341	53	91	5000	ni
Ortho-phosphate (PO_4^{3-})	mg/L	45	36	19	22	18	51	10	ni

ni– not indicated

[a]CCT: City of Cape Town wastewater and industrial effluent by-law (Cape Town, South Africa, 2014).

[b]SANS 241:2011: South African National Standards (SANS 241) potable water standards (Department of Water Affairs, 2011).

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

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5. CONCLUSION AND RECOMMENDATIONS

5.1. Conclusions

The following conclusions were drawn based on the results of this study.

The SGBR was able to consistently reduce the organic matter and suspended solids content of the pre-filtered PSW throughout its 138 days of operation. The SGBR efficiently reduced the COD, BOD_5, and TSS by 80%, 95%, and 89%, respectively, for HRTs ranging from 24 to 96 h and average OLRs of between 0.78 and 5.74 g COD/Lday. Neither an increase nor decrease in the HRT or OLR had an adverse effect on the treatment efficiency of the SGBR in terms of its biological performance. Using RSM, the optimal SGBR performance with regard to the maximum COD removal efficiency was predicted for an OLR of 12.49 g COD/Lday and a HRT of 1 day (24 h), resulting in a 95.5% COD removal efficiency. Treatment with the SGBR achieved an FOG removal efficiency of 80%; hence, it was concluded that the use of a pre-treatment system, such as a dissolved air flotation (DAF) system, for FOG removal from the untreated PSW was not required when using the SGBR. However, post-treatment of the SGBR effluent was required with regard to the PO_4^{3-}, NH_4^+-N, NO_3^--N, and TDS concentrations in order to meet the CCT wastewater and industrial effluent discharge standards and the SANS 241 potable water standards.

The SSND bioreactor was able to achieve TN removal efficiencies of 33% and 79% at HRTs of 6 and 11 days, with aeration (DO concentration >2.0 mg/L, but <3.2 mg/L) and without aeration (DO concentrations <2.0 mg/L), and with down-flow and up-flow bioreactor configurations for stages 1 and 2, respectively. Furthermore, the SSND bioreactor achieved a 98% SND efficiency for both stage 1 and 2. It was concluded that the initial oxidation of NH_4^+-N was the rate-limiting step in the nitrogen removal via SND during stage 1. Whereas, the presence of NO_2^--N in the effluent obtained from stage 2 indicated to an incomplete denitrification process which requires further research. In addition, low COD and PO_4^{3-}-P removals (<20% on average) were observed since the SSND bioreactor operated without the addition of refined carbon sources and without the use of activated sludge, a strategy required to mitigate operational costs at an industrial scale.

The ufMM system operated efficiently as a sidestream and final treatment stage for the SGBR-SSND treated PSW. The UF membranes were able to further reduce the COD and TSS by an average of 65% and 54%, respectively. Whilst the COD removal efficiency was similar to that reported in the literature reviewed, the TSS removal efficiency achieved in this study was comparatively lower despite the high average turbidity reduction of 90%.

The SGBR, SSND bioreactor, and ufMM systems were successfully employed for the treatment of the PSW. The overall PSW treatment system was evaluated for a period of 52 days at HRTs of 6 and 11 days, OLRs of 0.08 to 0.12 g COD/Lday, and an average NLR of 0.04 g TN/Lday. The overall PSW treatment system demonstrated the combined benefits of utilizing both biological and physical treatment processes, with averaged removal efficiencies of 91% for COD, 51% for PO_4^{3-}-P, 97% for TSS and 52% for TDS being achieved. The final effluent (i.e. ufMM permeate) was compliant with the CCT wastewater and industrial effluent by-law limits and the SANS 241 (2011) potable water limits with regard to the physiochemical parameters, with the exclusion of the PO_4^{3-} and NH_4^+-N. The poultry slaughterhouse from which the PSW was obtained will therefore be able to safely discharge their wastewater; although, refinement of the design might still be required.

5.2. Recommendations

The following recommendations are suggested for further research:

- Further experiments should quantitatively and qualitatively evaluate the biogas produced by the SGBR treating PSW. The possibility of using this biogas as an energy source for the self-sustainability of the PSW treatment system should be investigated.
- Further study should determine a possible scale-up of the SGBR system to be adapted as a pilot-scale on-site treatment for local poultry slaughterhouses.
- Further investigation should focus on the growth kinetics and identification of the micro-organisms present in the anaerobic granules and the raw PSW.
- Further experiments should investigate the application of a down-flow SSND bioreactor configuration with intermittent aeration and the use of activated sludge.
- Further analyses should be done on the micro-organisms within the biofilms formed in the SSND bioreactor.
- A comparative study of the ufMM permeate and the potable water used by the poultry slaughterhouse from which the PSW was obtained should be performed in order to determine the potential on-site use of the ufMM permeate i.e. treated PSW.
- The ability of the UF membranes to reduce pathogens (i.e. bacteria) and nutrients should be further studied.
- An optimization study of the overall PSW treatment system including that of the individual SSND bioreactor and ufMM systems in addition to the SGBR system should be performed.
- Further research should consider the examination of emerging contaminants present in PSW, such as pharmaceutical active compounds (PhACs), cleaning agents, and other pollutants.

- Further work may consider additional post-treatment processes subsequent to the ufMM system for the removal of pathogens and PO_4^{3-}, such as UV/ozone processes or chemical/biological phosphorous removal processes, respectively.

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APPENDICES

APPENDIX A: Analytical methods

APPENDIX A1: Method to determine the pH, conductivity, TDS and salinity using the PCSTestr 35 multiparameter meter

Calibration procedure of the PCSTestr 35 multiparameter meter:

1. Switch on the PCSTestr 35 meter.
2. Place the PCSTestr 35 probe into 100 ml of distilled water for 2 minutes.
3. Take the PCSTestr 35 probe out of the distilled water and pat dry using tissue paper. Do not rub in order to avoid damaging the probe.

pH calibration:

1. Press the MODE key on the PCSTestr 35 meter until the pH screen is reached.
2. Press the CAL key. The calibration screen is opened on the display and the bottom row flashes 4.01, 7.00 and 10.00.
3. Place the PCSTestr 35 probe in the pH 4 buffer solution and wait until the top reading on the screen stabilizes.
4. Press the MODE/ENT key. The pH 4 calibration is completed.
5. Rinse the probe with distilled water and pat dry.
6. The bottom row on the display will flash 7.00 and 10.00 to prompt for the pH 7 and 10 calibration.
7. Repeat steps 3, 4 and 5 for pH 7 and pH 10 buffer solutions.
8. Once the calibration is completed, press the CAL key to exit the pH calibration mode.

Conductivity calibration:

1. Press the MODE key on the PCSTestr 35 meter until the conductivity screen is reached.
2. Press the CAL key. The calibration screen is opened on the display and the bottom row flashes 1,413 μS/cm.
3. Place the PCSTestr 35 probe in the 1,413 μS/cm conductivity buffer solution and wait until the top reading on the screen stabilizes.
4. Press the MODE/ENT key. The 1,413 μS/cm conductivity calibration is completed.
5. Rinse the probe with distilled water and pat dry.

TDS calibration:

1. Press the MODE key on the PCSTestr 35 meter until the TDS screen is reached.
2. Place the PCSTestr 35 probe in the 300 ppm TDS buffer solution and press the CAL key.

3. Press the HOLD key to increase the value in the top digital display screen and the CAL key to decrease the value in the top digital display screen until the value is set to the known concentration of the buffer i.e. 300 ppm.
4. Once the desired value is reached, press the CAL key to confirm the calibration and exit the TDS calibration screen.
5. Rinse the probe with distilled water and pat dry.

pH, conductivity, TDS and salinity measuring procedure:

1. Place approximately 50 ml to 100 ml of the sample into a 250 ml beaker.
2. Press the ON key to switch on the PCSTestr 35 meter.
3. Press the MODE key until the desired parameter for measuring is reached (i.e. pH, conductivity, TDS or salinity)
4. Submerge the front 3 cm of the PCSTestr 35 probe in the sample and keep it there until the reading the stabilizes.
5. Record the measurement displayed on the screen.
6. Rinse the probe with distilled water and pat dry after each sample.

APPENDIX A2: Method to determine turbidity

Calibration procedure of the TN-100 turbidimeter:

1. Place the TN-100 turbidimeter on a flat level surface.
2. Insert the CAL 1 (800 NTU) calibration standard into the sampling well, aligning the mark on vial with the mark on the meter.
3. Press the vial down until it snaps into the meter.
4. Cover the vial with the light shield cap.
5. Press the ON/OFF key to switch on the meter. The meter will go into the measurement mode after the start up sequence.
6. Press the CAL key to switch to calibration mode. The meter will prompt for the CAL 1 standard to be inserted.
7. Press the READ/ENTER key. The annunciator will blink for 12 seconds and then prompt for the CAL 2 (200 NTU) calibration standard to be inserted.
8. Repeat steps 2, 3, 4 7 and 8 for CAL 2, CAL 3 (100 NTU) and CAL 4 (0.02 NTU) calibration standards.
9. After CAL 4 (0.02 NTU) calibration standard is calibrated, the display will show STbY. The meter is now ready for measurement.

Turbidity measuring procedure:

1. Obtain a clean dry sample vial. Handle the sample vial by the lid.

2. Rinse the sample vial with approximately 10 ml of the sample, capping the vial with a black screw cap and inverting gently several times. Discard the used sample and repeat the rinsing procedure twice.
3. Fill the sample vial with approximately 10 ml i.e. up to the mark indicated on the sample vial. Cap the vial with a black screw cap.
4. Place the sample vial inside the sample well of the meter aligning the mark of the vial with the mark of the meter.
5. Press the vial down until it snaps into the meter.
6. Cover the vial with the light shield cap.
7. Turn the meter on by pushing the ON/OFF key. Following the power up sequence, the meter will go into measurement mode and the display will blink "Rd" approximately 10 times.
8. Record the measurement displayed on the screen.

Reference
ISO 7027 compliant nephelometric method

APPENDIX A2: Method to determine total suspended solids (TSS)

ESS Method 340.2 for TSS determination:

1. Preparation of the glass fibre filter paper:

- Insert the glass fibre filter paper into a Büchner funnel attached to a collection flask. While vacuum is applied, wash the filter with three successive 20 ml volumes of Milli-Q water. Remove all traces of water by continuing to apply vacuum after the Milli-Q water has passed through.
- Remove the Büchner funnel from the collection flask and place the filter in an aluminium dish and ignite in a muffle furnace at 550°C ± 50°C for 30 minutes. Rewash the filter with an additional three successive 20 ml volumes of Milli-Q water, and dry in an oven at 103 - 105°C for one hour. When needed, remove the aluminium dish from the oven, desiccate, and weigh the glass fibre filter.

2. Select a sample volume, a maximum of 200 ml that will yield no more than 200 mg of TSS.
3. Place the filter in the Büchner funnel attached to a collection flask and apply vacuum. Wet the filter with a small volume of Milli-Q water to seal the filter against the Büchner funnel.
4. Shake the sample vigorously and quantitatively transfer the sample to the glass fibre filter using a large orifice, volumetric pipette. Remove all traces of water by continuing to apply vacuum after the sample has passed through.

5. Rinse the pipette and Büchner funnel onto the glass fibre filter with a small volume of Milli-Q water. Remove all traces of water by continuing to apply vacuum after the Milli-Q water has passed through the filter.
6. Carefully remove the glass fibre filter from the Büchner funnel. Dry the filter at 103 – 105 °C for at least one hour. Cool the filter in a desiccator and weigh.
7. The TSS is calculated using Eq. A1:

$$TSS\ (mg/L) = \frac{(C-D) \times 1000}{E} \tag{A1}$$

Where, C is the weight of the glass fibre filter and sample residue in mg; D is the weight of the glass fibre filter in mg; and E is the volume of sample filtered in ml.

ESS Method 340.2 for VSS determination:

1. After weighing the glass fibre filter and calculating the TSS, place the glass fibre filter in a ceramic crucible and ignite in a muffle furnace at 550°C ± 50°C for 30 minutes.
2. Cool the filter in a desiccator and weigh.
3. The VSS is calculated using Eq. A2:

$$VSS\ (mg/L) = \frac{(C-F) \times 1000}{E} \tag{A2}$$

Where, C is the weight of the glass fibre filter and sample residue in mg; F is the weight of the ignited glass fibre filter in mg; and E is the volume of sample filtered in ml.

APPENDIX A4: Method to determine chemical oxygen demand (COD)

1. Switch on the Spectroquant thermoreactor TR420 and select the pre-set setting of 148 ˚C for 2 hours. It will take approximately 10 minutes for the thermoreactor TR420 to heat up to 148 ˚C.
2. For the 500 to 10 000 mg/L measuring range (using Merck COD Solution A, Cat. No. 1.14679.0495 and Merck COD Solution B, Cat. No. 14680.0495):

- Pipette 2.2 ml of COD solution A into an empty test tube.
- Pipette 1.8 ml of COD solution B into the test tube.
- Pipette 1 ml of the sample into the test tube.
- Close the test tube with the screw cap and mix using a vortex mixer.
- Heat the test tubes in the Spectroquant thermoreactor TR420 at 148 ˚C for 2 hours.
- Remove the test tubes after 2 hours and place in a test tube rack to cool down for 10 minutes.
- Mix the test tube contents using a vortex mixer.
- Allow the test tubes to cool down to room temperature for 30 minutes.

- Place the test tubes in the Nova 60 Spectroquant and enter the code **024 (**500 to 10 000 mg/L measuring range).
- Record the measurement displayed on the screen.

3. For the 100 to 1500 mg/L measuring range (using Merck COD Solution A, Cat. No. 1.14538.0065 and Merck COD Solution B, Cat. No. 1.14539.0495):

- Follow the same procedure as for COD solutions A and B for the 500 to 10000 mg/L measuring range with the exception of:
- Pipette 0.30 ml of COD solution A into the empty test tube.
- Pipette 2.85 ml of COD solution B into the test tube.
- Pipette 3 ml of sample into the test tube.
- Place the test tubes in the Nova 60 Spectroquant and enter the code **023 (**100 to 1500 mg/L measuring range).

APPENDIX A5: Method to determine ammonium (NH_4^+-N)

Measuring range: 2.0 – 75 mg/L NH_4^+-N (2.6 – 96.6 mg/L NH_4^+)

1. Ensure that the pH of the sample is in the specified range: 4 – 13.
2. Pipette 5.0 ml of NH_4-1 reagent into an empty test tube.
3. Add 0.20 ml of the sample into the test tube.
4. Add 1 level blue micro-spoon of the NH_4-2 reagent into the test tube.
5. Close the test tube with the screw cap and shake vigorously to dissolve the NH_4-2 powder.
6. Leave the test tube to stand in the test tube rack for 15 minutes.
7. Transfer the test tube contents into a 10 mm curvette.
8. Insert the NH_4 Autoselector (measuring range: 2.0 – 75 mg/L NH_4^+-N) into the Nova 60 Spectroquant.
9. Place the curvette into the 10 mm compartment of the Nova 60 Spectroquant and record the measurement displayed on the screen.

Reference

Merck Spectroquant ammonium test kit (Cat No. 1.00683.0001)

APPENDIX A6: Method to determine nitrate (NO_3^--N)

Measuring range: 0.5 – 20.0 mg/L NO_3^--N (2.2 – 88.5 mg/L NO_3^-)

1. Place 1 level blue micro-spoon of the NO_3-1 reagent into a dry, empty test tube.
2. Pipette 5.0 ml of the NO_3-2 reagent into the test tube.
3. Close the test tube with the screw cap and shake vigorously for 1 minute to dissolve the NO_3-1 powder completely.

4. Add 1.5 ml of the sample very slowly; close the test tube with the screw cap, and mix.
5. Leave the test tube to stand in the test tube rack for 10 minutes.
6. Transfer the test tube contents into a 10 mm curvette.
7. Insert the NO_3-1 Autoselector (measuring range: 0.5 – 20.0 mg/L NO_3^--N) into the Nova 60 Spectroquant.
8. Place the curvette into the 10 mm compartment of the Nova 60 Spectroquant and record the measurement displayed on the screen.

Reference

Merck Spectroquant nitrate test kit (Cat No. 1.14773.0001)

APPENDIX A7: Method to determine nitrite (NO_2^--N)

Measuring range: 0.02 – 1.00 mg/L NO_2^--N (0.01 – 3.28 mg/L NO_2^-)

1. Ensure that the pH of the sample is in the specified range: 2 – 10.
2. Pipette 5.0 ml of the sample into an empty test tube.
3. Add 1 level micro-spoon of NO_2-1 reagent into the test tube.
4. Close the test tube with the screw cap and shake vigorously to dissolve the NO_2-1 powder completely.
5. Leave the test tube to stand in the test tube rack for 10 minutes.
6. Transfer the test tube contents into a 10 mm curvette.
7. Insert the NO_2-1 Autoselector (measuring range: 0.02 – 1.00 mg/L NO_2^--N) into the Nova 60 Spectroquant.
8. Place the curvette into the 10 mm compartment of the Nova 60 Spectroquant and record the measurement displayed on the screen.

Reference

Merck Spectroquant nitrite test kit (Cat No. 1.14776.0001)

APPENDIX A8: Method to determine ortho-phosphate (PO_4^{3-}-P)

Measuring range: 0.05 – 5.00 mg/L PO_4^{3-}-P (0.2 – 15.3 mg/L PO_4^{3-})

1. Ensure that the pH of the sample is in the specified range: 0 – 10.
2. Pipette 1 ml of the sample into the reaction test tube; close the test tube with the screw cap and mix.
3. Add 5 drops of the P-2K reagent into the test tube; close the test tube with the screw cap and mix.
4. Add 1 dose of P-3K reagent into the test tube.
5. Close the test tube with the screw cap and shake vigorously to dissolve the P-3K powder completely.

6. Leave the test tube to stand in the test tube rack for 5 minutes.
7. Place the test tube into the test tube compartment of the Nova 60 Spectroquant aligning the mark on the test tube with the mark on the Nova 60 Spectroquant and record the measurement displayed on the screen.

Reference

Merck Spectroquant ortho-phosphate cell test kit (Cat No. 1.14543.0001)

APPENDIX A9: Method to determine dissolved oxygen (DO)

Calibration (in % saturation mode) procedure of the DO meter:

1. Wait 10 minutes for the probe to polarize, press the MODE key to select the % saturation mode.
2. Press the SETUP key. The meter enters the setup menu.
3. Press the ENTER key to confirm. The meter enters the calibration mode and the display shows "100.0%/CAL1"
4. Hold the DO probe in the air and wait for the measured value to stabilize.
5. Press the ENTER key to confirm calibration. The meter returns to the measurement mode and the calibration is completed.

DO measuring procedure:

1. Rinse the probe with distilled water and pat dry. Set the atmospheric pressure and salinity coefficient factor before use.
2. Dip the probe into the sample (ensure that the probe is immersed in enough solution) and wait for the reading to stabilize.
3. Record the measurement displayed on the screen.
4. Rinse the probe with distilled water and pat dry after each sample.

APPENDIX B:

APPENDIX B1: SGBR summary for 138 days of operation

Table B-1: Summary of the SGBR feed and effluent characteristics for 138 days of operation

	HRT (h)											
	55		**96**		**48**		**36**		**24**		**Overall study**	
Operating period (days)	1-28		29-57		58-84		85-122		113-138		138	
OLR (g COD/Lday)	1.18		0.78		1.96		4.1		5.74		2.75	
	Feed	*Effluent*	*Feed*	*Effluent*	*Feed*	*Effluent*	*Feed*	*Effluent*	*Feed*	*Effluent*	*Feed*	*Effluent*
Parameter												
pH	6.90	8.13	6.89	7.8	6.89	7.51	6.57	7.51	6.56	7.64	6.76	7.71
Conductivity (mg/L)	1144	1555	1510	1581	1708	1820	1632	1512	1384	1475	1480	1608
TDS (mg/L)	814	1107	1073	1123	1214	1291	1156	1145	983	1048	1051	1142
Salinity (mg/L)	581	801	778	817	890	953	924	907	897	927	818	882
Turbidity (NTU)	394	12.17	469	7.57	703	9.60	825	10.27	642	33.16	610	14.7
COD (mg/L)	2713	693	3126	678	3935	802	6143	795	5738	685	4344	729
BOD_5 (mg/L)	544	29	1000	63	1706	164	2844	116	3200	153	1859	105
TSS (mg/L)	385	51	473	60	1231	85	2045	58	1822	61	1192	63
VSS (mg/L)	697	419	1206	865	2008	747	2868	799	2718	999	1906	774
FOG (mg/L)	175	89	250	50	467	36	816	43	998	40	537	51
Alkalinity (mg/L)	387	686	563	565	574	561	-	449	-	588	499	588
VFA (mg/L)	161	83	510	190	464	234	-	76	-	38	375	124
NH_4^+-N (mg/L)	116	162	149	160	201	200	222	175	224	191	183	177
NO_3^--N (mg/L)	0.57	3.52	0.88	9.16	2.26	16.75	4.88	16.57	3.04	14.54	2.33	12.12
PO_4^{3-}-P (mg/L)	-	-	-	-	36	26	35	30	42	36	38	31
Removal (%)												
COD	74.1		78		78.6		85.5		85.4		80.4	
BOD_5	93.4		93.8		94		95.5		97.4		94.8	
TSS	85.3		85.1		89.7		94.9		90.1		89	
FOG	45.3		80.3		92		93.6		89.7		80.2	

APPENDIX C: Membrane cleaning solutions preparation procedures

APPENDIX C1: Citric acid (1%) preparation procedure

Steps for preparing 1 L of 1% citric acid:

1. 1% citric acid = 1 g/100 ml = 10 g/L
2. Therefore, 10 g of citric acid must be added to 1 L of water to obtain a 1% citric acid solution.

APPENDIX C2: Sodium hypochlorite (NaOCl) preparation procedure

Steps for preparing 1 L of 400 mg/L NaOCl from 12.5% NaOCl

1. 12.5% NaOCl = 12.5 g/100 ml = 125 g/L ($\mathbf{C_1}$)
2. 400 mg/L NaOCl = 0.4 g/L ($\mathbf{C_2}$)
3. Using the equation, $\mathbf{C_1V_1 = C_2V_2}$
   Where, C_1 = 125 g/L, C_2 = 0.4 g/L, V_2 = 1 L and V_1 = ?
4. $V_1 = C_2V_2/ C_1$ = (0.4 x 1)/125 = 0.0032 L (3.2 ml)
5. Therefore, 3.2 ml of 12.5% NaOCl must be added to 1 L of water to obtain a 400 mg/L NaOCl solution.