Cape Peninsula
University of Technology

# Treatment of Poultry Slaughterhouse Wastewater Using An Expanded Granular Sludge Bed Anaerobic Digester Coupled with Anoxic/Aerobic Hybrid Side Stream Ultrafiltration Membrane Bioreactor

**by**

**Yasheemah Williams**

**Thesis submitted in fulfilment of the requirements for the degree**

**Master of Engineering: Chemical Engineering**

**in the Faculty of Engineering**

**at the Cape Peninsula University of Technology**

**Supervisor: Prof. Seteno Karabo Obed Ntwampe**

**Co-supervisor: Dr. Moses Basitere**

**Cape Town**

**December 2017**

## DECLARATION

I, **Yasheemah Williams**, declare that the contents of this dissertation/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.

**31st December 2017**

**Signed** **Date**

## ABSTRACT

For more than a decade, poultry product consumption increased in developed and developing countries, with more than 470 new slaughterhouses being constructed in South Africa (SA). Customer demand for poultry products resulted in a rapidly growing poultry industry, with consequential increases in the quantity of organic solid and liquid waste being produced from the poultry slaughterhouses. Annually, the productivity and profitability within the livestock production sector has increased, an evaluation based on the number of slaughtered and sold animals. Potable water is required for these animals, resulting in the generation of high strength wastewaters. Instantaneous disposal of such wastewaters into the environment is concerning as it results in odour and the spreading of diseases in local rivers and freshwater sources. The generated poultry slaughterhouse wastewater (PSW) contains a high quantity of biodegradable organic, suspended and colloidal matter in the form of proteins, fats, oil and grease (FOG), protein from meat, blood, skin, and feathers, resulting in high Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD), which can contribute to environmental deterioration if not treated adequately before discharge.

On average, PSW contains a high concentration of BOD, COD, nitrogen, pathogenic and non-pathogenic viruses, bacteria and parasites, including their eggs. These characteristics make PSW highly polluted with a large quantity of bird carcass debris including FOG. Due to the high concentration of organic matter and suspended solids in the wastewater, it is necessary to pre-treat the PSW prior to sequential anaerobic treatment. Most of the contaminants present in the PSW can be reduced by means of numerous treatment steps, i.e. physical, chemical and biological treatment.

For this study, biological treatment methods, physical separation methods, and a membrane bioreactor system, were used to treat PSW. The biological treatment methods used were an anaerobic digester (AD) followed by a single stage nitrification/denitrification reactor and then a third stage in which an ultrafiltration (UF) and Microfiltration (MF) membrane bioreactor (MBR) was used. The AD used was an Expanded Granular sludge Bed Reactor (EGSB) as anaerobic digestion is one of the most effective biological wastewater treatment methods used, as it reduces the organic matter to even produce biogas as a renewable energy source. The basis of anaerobic treatment method relies on suitable bacteria cultivated in the absence of dissolved oxygen, facilitating decomposition of organic matter into a renewable source such as biogas. Similarly, biological nitrification/denitrification processes for the removal of total nitrogen (TN) in wastewater has become one of the most commonly used processes within the wastewater treatment sector. Nitrification and denitrification processes can be performed by some microorganisms within the wastewater in Wastewater Treatment Plants (WWTPs)

The PSW used was collected at different times from a local poultry slaughterhouse in the Western Cape (South Africa) and stored in a refrigerator at 4°C until it was fed to the first stage of the treatment which was the EGSB. Before being fed to the EGSB, the PSW was filtered with a sieve to remove feathers and agglomerated FOG to avoid clogging of the tubing. The EGSB was inoculated with 0.747 L anaerobic granular sludge, had a working volume of 2.7 L, an inner diameter of 0.065 m and a height of 0.872 m respectively. Ceramic marbles with an average diameter of 0.0157m were placed at the bottom of the bioreactor as packing for the underdrain and to maintain the granular sludge within the heated section of the bioreactor. The EGSB was fed with three types of PSW: 50% (v/v), 70% (v/v), which was diluted with distilled water. Thereafter once the system stabilised the reactor was fed with undiluted PSW (100%). Each dilution was operated at different Hydraulic Retention Times (HRTs) and Organic Loading Rates (OLRs), with average HRTs used being 62.5, 57.5 and 49.65 h. Furthermore, the average OLRs were 1, 2 and 3 g tCOD/L.day respectively. The performance of the EGSB was determined using tCOD, Total Suspended Solids (TSS) and FOG, with overall averaged removal rates for these constituents being 69%, 98% and 92% respectively. The highest tCOD removal of 93 % (optimal efficiency) was obtained at an average HRT of 57.5 h with a corresponding average OLR of 2 g tCOD/L.day.

The product of the EGSB was then fed to the single stage nitrification/denitrification (SSND) bioreactor with a diameter and height of 0.11m and 2m as well as working volume of 12.7 L. Gravel stone covered with sponge was placed at a height of 0.945 m from the bottom of the column as a bacterial support matrix. At the top of the column, a cylinder of diameter 0.09 m was submerged at 0.543 m into the column with holes punched through it and was filled with sponge blocks as packing for biomass retainment including growth. The column was operated using two types of configurations whereby the EGSB effluent was fed downwards (down-flow) for 48 days and then upwards (up-flow) for 31 days, with sparging introduced in the column for nitrification and aerobic denitrification in the up-flow configuration. The SSND was used for the removal of Total Nitrogen (TN) only and was operated at three HRTs of 11.54, 7.72 days (down-flow) and 13.74 (up-flow) days. The results obtained for the down-flow system indicated an average, $NH_4^+$- N and TN removal efficiency of, 21% and 7% at an HRT of 11.54 days; with a slightly improved performance of 21% and 15% removal at HRT of 7.72 days. The maximum and average $NH_4^+$- N removal efficiency was 87% and 58%, whereas the TN removal efficiencies were 85% and 57% respectively were achieved during the up-flow configuration. The Dissolved Oxygen (DO) concentration across the column was maintained between 0.40 – 4.36 mg/L with an average DO of 1.38 mg/L, conditions which can be considered anoxic.

The third stage of the PSW treatment process consisted of MBR, with Aluminium oxide ceramic ultrafiltration and microfiltration membranes with pore size of 100 nm (UF) and 1.9

µm (MF) being evaluated. The membranes had an inner diameter of 0.0068 m, an outer diameter of 0.012 m and a length of 0.25 m. The MBR system was fed at 0.6 mL/min (MF) and 0.65 mL/min (UF) using composite samples from the EGSB and SSND product. Permeate from each MBR using different membranes was analysed and the performance was determined using TSS and Total Chemical Oxygen Demand (tCOD) removal efficiency. The average TSS and tCOD removal for the MF system was 42% and 60% respectively, while that of an UF system was 47% and 62% respectively when the EGSB permeate was used. For the SSND permeate, the average TSS removal efficiencies were 46% for the MF pore size membrane and 57% for the UF. However, the average tCOD removal was 17% for the MF and 19% for the UF membranes. When the inlet flow rate for the MBR was increased (1.22 mL/min), the EGSB-UF system TSS removal was reduced to 42%, with that observed for the tCOD being 56%, whereas the SSND-UF system removal efficiency was 10% TSS removal and 27% tCOD removal respectively. The overall system efficiency (EGSB-UFMBR and EGSB-SSND-UFMBR) for tCOD and TSS removal efficiency was 92% and 99% respectively.

**Keywords:** Chemical oxygen demand; Expanded Granular Sludge Bed reactor; Ultrafiltration; Poultry slaughterhouse wastewater; Single stage nitrification and denitrification.

## ACKNOWLEDGEMENTS

**I wish to thank:**

- God/Allah the almighty for the health, strength and knowledge throughout my journey, without him nothing would have been possible.
- My mother Ms Farieda Ely for all the patience, support and encouragement, as well as believing in me. Without your guidance and encouragement, I would have never been where I am today. You have supported me financially as well as emotionally. Thank you for all your prayers and tolerating my moods and emotions when I was under pressure. You have never given up on me no matter what and always pushed me to do better than I was doing. There is no better support and love than a mother's love. I love and appreciate you more each day of my life.
- My supervisor Prof. S.K.O. Ntwampe for the support, guidance and advice through this journey, for listening to my issues and understanding my personal problems. You believed in me, pushed me to never give up on myself and always guiding me into the right direction.
- My co-supervisor Dr. M. Basitere for technical advice as well as support, guidance and motivation. You have been an excellent mentor and supervisor throughout my academic life and always believed in me to do great things.
- My partner Gakeem Williams for all the patience and support through my journey and acknowledging my strengths, even in tough times. For sticking with me despite everything.
- My team-members, Zainab Rinquest, Mahomet Njoya and Ephraim Kaskote for all the assistance and support.
- The *BioERG* team for their assistance, support and knowledge passed on to me.
- My friends and family who continuously supported me through my stressful times.
- The technical staff of Cape Peninsula University of Technology mainly Mrs Hannelene Small and Mr Alwyn Bester.
- The financial assistance of the National Research Foundation (NRF) towards this research is acknowledged.

## DEDICATION

To my beautiful, intelligent daughter

**Farah Williams**

## TABLE OF CONTENTS

## LIST OF FIGURES

## LIST OF TABLES

## GLOSSARY

**Activated sludge** - The biomass produced in wastewater by the growth of organisms in the presence of organic matter.

**Aerobic** - Conditions where oxygen acts as electron donor for biochemical reactions.

**Anaerobic** - Conditions where biochemical process occurs in complete absence of oxygen.

**Anoxic** - Conditions where oxyanion instead of oxygen acts as an electron donor for biochemical reactions.

**Biochemical oxygen demand (BOD)** - The amount of oxygen required or consumed for the decomposition of microbial reactions within wastewater.

**Chemical oxygen demands (COD)** - The amount of oxygen required to chemically oxidise substances in the wastewater.

**Conductivity** - The measure of the ability of a solution to conduct electricity.

**Expanded granular bed reactor (EGSB)** - a reactor that is a variant of the UASB reactor which uses up-flow velocity through a sludge bed.

**Hydraulic retention time (HRT)** - a measure of the average length of time that a soluble compound remains in a bioreactor.

**Membrane** - an interphase separating two phases and selectively controlling the transport of the material between these two phases.

**Membrane Bioreactor (MBR)** - Combination of a membrane process with a bioreactor to separate particles and/or chemical compounds.

**Organic loading rate (OLR)** - The rate of organic compounds being fed to a reactor.

**Total suspended solids (TSS)** - The total number of particles that are in suspension in water/wastewater.

**Total dissolved solids (TDS)** - The combined content of all inorganic and organic substances contained in a liquid which are present in a molecular, ionized or micro-granular suspended form.

**Turbidity** - An expression of the optical property of a liquid medium and its ability to transmit light. This is a measure of relative sample clarity, and not colour.

## ABBREVIATIONS

| Abbreviation | Description |
|---|---|
| **AOB** | Ammonium oxidising bacteria |
| **AFBR** | Anaerobic fluidised bed reactor |
| **BOD** | Biological oxygen demand |
| **CCT** | City of Cape Town |
| **COD** | Total chemical oxygen demand |
| **CPUT** | Cape Peninsula University of Technology |
| **CSTR** | Continuous stirred tank reactor |
| **DAF** | Dissolved air flotation |
| **DWA** | Department of Water Affairs |
| **EGSB** | Expanded Granular Sludge Bed reactor |
| **FA** | Free ammonia |
| **FH** | Free hydroxylamine |
| **FOG** | Fats, oil and grease |
| **HRT** | Hydraulic retention time |
| **MBR** | Membrane bioreactor |
| **MF** | Microfiltration |
| **NF** | Nano filtration |
| **NOB** | Nitrogen oxidising bacteria |
| **NWA** | National Water Act |
| **OLR** | Organic loading rate |
| **P** | Phosphorous |
| **pH** | Potential of hydrogen |
| **PSW** | Poultry slaughterhouse wastewater |
| **RSM** | Response surface methodology |

| | |
|---|---|
| **RTI** | Research, Technology and Innovation |
| **RO** | Reverse osmosis |
| **SA** | South Africa |
| **SAB** | South African Breweries |
| **SANS** | South African National Standards |
| **sCOD** | Soluble chemical oxygen demand |
| **SGBR** | Static Granular Sludge Bed Reactor |
| **SHBR** | Sequence hybrid biological reactor |
| **SND** | Simultaneous nitrification and denitrification |
| **Sp** | Species |
| **SRT** | Solids retention time |
| **SS** | Suspended solids |
| **SSND** | Single stage nitrification/denitrification |
| **tCOD** | Total chemical oxygen demand |
| **TDS** | Total dissolved solids |
| **TKN** | Total nitrogen |
| **TP** | Total phosphorous |
| **TSS** | Total suspended solids |
| **TS** | Total Solids |
| **TVS** | Total volatile solids |
| **UASB** | Up-Flow Anaerobic Sludge Bed |
| **UF** | Ultrafiltration |
| **US** | United States |
| **UF** | Ultrafiltration |
| **VFA** | Volatile fatty acids |
| **VSS** | Volatile suspended solids |
| **WDCS** | Water discharge system |

| | |
|---|---|
| **WWTP** | Wastewater treatment plant |

## LIST OF SYMBOLS

| Symbol | Description | Units |
|---|---|---|
| $°C$ | Degrees Celsius | |
| $V$ | Volume | $m^3$ |
| $Q$ | Flow rate | $m^3$/day |

| Chemical Formulae | Compound name |
|---|---|
| $C_6H_{12}O_6$ | Glucose |
| $CH_4$ | Methane |
| $CO_2$ | Carbon dioxide |
| $H_2$ | Hydrogen |
| $H_2O$ | Water |
| $H_2S$ | Hydrogen sulphide |
| $NH_3$ | Ammonia |
| $NH_3$-N | Ammonia nitrogen |
| $NH_4^+$ | Ammonium |
| $NH_4$-N | Ammonium nitrogen |
| $NO_2^-$ | Nitrite |
| $NO_2$-N | Nitrite nitrogen |
| $NO_3^-$ | Nitrate |
| $NO_3$-N | Nitrate nitrogen |
| $PO_4^{3-}$ | Phosphate |

# CHAPTER 1

# INTRODUCTION

# CHAPTER ONE

# 1. INTRODUCTION

## 1.1. Background

One of the most current and common global challenges is the limited access to clean, safe and potable water, as well as sanitation. One of the reasons for this is that most water is contaminated and has the potential to cause illness (Shannon et al., 2008).

Contaminant levels in fresh water sources are increasing in both developing and industrialised nations. These contaminants have a potential to affect human health and aquatic life (Shannon et al., 2008). According to Shannon et al. (2008), more than 80% of potable water is consumed through agriculture, livestock and energy production for human use. Therefore, with the global population increase affecting water consumption, there is a need to use fresh water sources effectively or develop wastewater treatment strategies suited for all industries. This lays a heavy burden on science, technology and engineering to meet the challenges related to water treatment to secure future water supply (Rao et al., 2014).

A common challenge facing the poultry processing industry worldwide is the consumption of a large quantity of potable water for the process of cleaning, scaling, and defeathering of slaughtered birds, as well as for the packaging of poultry products (Del Nery et al., 2008; Avula et al., 2009). This consumption of large quantities of potable water then produces large volumes of wastewater, which contain high concentrations of organic matter, nitrogen and phosphorus (Del Nery et al., 2008; Avula et al., 2009). This wastewater generated is at least significantly more contaminated than domestic sewage wastewater (Keller et al., 2013). An estimated 2% to 5% of carcass proteins are also lost in to the wastewater (Lo et al., 2005), resulting in wastewater that contains 35% protein. This in turn results in higher chemical and biological oxygen demand than domestic wastewater (Avula et al., 2009; Lo et al., 2004).

Despite the fact that the poultry industry is a large consumer of water and thus generates large quantities of high strength wastewater, minimal attention has been given to the management of the wastewater with minimal practices being in place (De Nardi et al., 2011), especially in South Africa (SA). Instantaneous disposal of such wastewaters into the environment is raising a concern as it results in odor and the spreading of diseases, culminating in the pollution of local rivers, and other fresh water sources (Nasir et al., 2012). By effectively treating this wastewater, it can be reused for moving heavy solids in eviscerating troughs, scalding tanks, feather flow-away, feather picking facilities, and for washing packer aprons (Avula et al., 2009). Therefore it is important to treat this wastewater for reuse to reduce the unsustainable usage of potable water.

The local poultry industry in SA faces pressure with regard to developing advanced treatment processes for the wastewater produced, with strict national legislation, municipal by-laws, and continuing drought providing the primary motives to ensure implementation of sustainable wastewater treatment technologies to reduce potable water consumption. This then challenges the industry to come up with new solutions and to contain the burden on limited water resources (Basitere et al., 2016).

Previous studies on the treatment of poultry slaughterhouse wastewater (PSW) conducted using an EGSB coupled with an anoxic/aerobic tanks, which resulted in 65% tCOD removal (Basitere et al., 2016). Other treatment systems involving the EGSB coupled with a membrane bioreactor achieved 95% tCOD removal by treating soft drink manufacturing wastewater (Sheldon and Erdogan, 2016). This resulted in a research study undertaken for this study, which focused on the treatment of PSW using the EGSB reactor coupled with a single stage nitrification/denitrification (SSND) and membrane system – an overall system variation for the EGSB studied by both Basitere et al. (2016) and Sheldon and Erdogan (2016) whereby the SSND stage focused on total nitrogen (TN) reduction.

Furthermore, Nunez and Martinez (1999) reported achieving 67% tCOD removal using an EGSB to treat slaughterhouse wastewater. The performance of an anaerobic bioreactor coupled with a MBR was conducted by Chu et al. (2005) using an EGSB and a hollow fibre membrane to treat domestic wastewater, achieving a tCOD removal of 90% at an HRT of 3.5 h and a temperature of 25 °C.

Designing and optimising biological processes has been addressed by many different approaches to achieve optimum conditions, of which RSM is one. Engineers uses RSM for control, prediction and to solve problems within process industries by determining and observing factors that influence processes and responses such as the pH, temperature, COD, HRT etc. (Ngongang, 2016), which were determined to be crucial in this study. Zinatizadeh et al. (2011) used RSM for the design of experiments for the treatment of diary wastewater using a sequence batch reactor (SBR), with Sathian et al. (2014) using a SBR for the treatment of textile dye wastewater for a study in which RSM was used to optimise parameters such as the air flow rate, SRT and the cycle period.

In SA, the treatment of PSW has not been applied frequently and consistently by slaughterhouses. This study therefore was conducted to assess the efficiency and performance of an EGBB coupled with a SSND system as well as an MBR for implementation by the slaughterhouses. As well as investigating if RSM software can be used to predict the performance of the EGSB by developing a model Seeing that the poultry industry is expanding in SA, it is important that a treatment process which does not need

highly skilled personnel to operate is developed as the wastewater is discharged into local rivers.

### 1.2. Problem Statement

SA is a water scares country and the poultry slaughterhouse industry is one of the largest producers of PSW due to cleaning, bird slaughtering and packaging of their products. This PSW generated contains a high concentrations of organic matter, suspended solids, nitrogen and phosphorous. Due to the prevalence of these contaminants, the wastewater does not meet the South African industrial discharge standard. This means that it has the potential to contaminate other receiving water sources if discharged untreated. As such, a suitable wastewater treatment facility needs to be designed and evaluated for use, even in developing countries such as SA.

### 1.3. Hypothesis

An EGSB followed by a SSND coupled with a MBR can effectively treat PSW to meet South African and local municipal discharge standards.

### 1.4. Research Questions

- How efficient and effective is the anaerobic digester (EGSB) in treating PSW?
- How effective and efficient is the SSND with regards to nitrification and denitrification?
- How efficient is the UF in comparison to a MF membrane system in reducing particulate matter in the form of suspended solids and tCOD in the EGSB/SSND treated PSW?
- Does the quality of treated wastewater leaving the EGSB and the MBR system meet the prescribed discharge standard?
- Is there any significant quantity of biogas being produced from the EGSB reactor?
- How efficient and effective is the overall performance of the EGSB-SSND-MBR system designed for PSW treatment?
- For process control purposes, can a suitable model be developed to predict the EGSB performance in terms of tCOD reduction during PSW treatment?
- Can RSM be used to predict the model for the EGSB performance?

### 1.5. Research aims and Objectives

The aim of the study was to evaluate the possibility of reducing the consumption of fresh water in poultry slaughterhouse facilities by treating PSW for reuse using a lab-scale EGSB coupled with a single stage nitrification/denitrification bioreactor and a UF and MF membrane bioreactor. This study was divided into phases: Phase 1: Evaluate performance and operational stability of the EGSB treatment system in removing suspended solids, FOG, BOD and COD in the PSW. Phase 2: Design a single stage nitrification/denitrification system and evaluate its efficiency, effectiveness, and performance. Phase 3: Evaluate the efficiency, effectiveness and performance of the UF and MF membrane in reducing the high level of soluble and particulate matter in treated PSW as well as the overall system efficiency, subsequent to Phase 4: Model development for tCOD reduction prediction for the EGSB. The phases and their individual objectives are detailed below:

**Phase 1: Objective 1:** Evaluate performance and operational stability of the EGSB treatment system in removing suspended solids, FOG, BOD and COD.

**Objective 2** Evaluate performance and operational stability of the EGSB treatment system at three different organic loading rate (OLR) and hydraulic loading rate (HRT).

**Objective 3:** Determine if biogas is produced during the anaerobic digestion stage.

**Phase 2: Objective 1:** To design a lab-scale single stage nitrification/denitrification bioreactor and assess its efficiency in removing total nitrogen and COD in the PSW.

**Phase 3: Objective 1**: Evaluate the performance of the UF membrane in reducing the high level of soluble and particulate matter in the poultry slaughterhouse wastewater, TSS, FOG, Cond, TDS, pH and COD.

**Objective 2**: Evaluate the overall performance of the treatment system in removing suspended solids, FOG, BOD and COD.

**Phase 4: Objective 1:** To develop a model to predict EGSB performance in terms of tCOD reduction using RSM.

### 1.6. Significance of the study

Due to increasingly stringent national legislation, including penalties levied for non-compliance, the implementation of the water discharge levy system (WDCS), potable water supply security, as well as ongoing water scarcity in South Africa, poultry processing industries are required to develop advanced treatment technologies of their wastewater. They are thus required to provide solutions to water resource limitations and to take steps toward achieving the sustainability of a cleaner environment and the preservation of fresh water sources. The treatment of PSW will benefit the poultry industry in South Africa by: 1) reducing their potable water demand by reusing treated PSW; 2) reducing the volume of wastewater discharged into local receiving bodies; and 3) meeting industrial wastewater discharge standards. This research project is aligned to the Cape Peninsula University of Technology (CPUT) Research, Technology and Innovation (RTI) strategy for which a 10 year blueprint was developed to strengthen research and innovation across the institution for the benefit of the local community and industry. It aims at developing capacity to mitigate effects of climate change and environmental deterioration, thus to promote ecological sustainability, as well as the development of process systems for the bio-economy and the development of the biotechnology industry. It is also aligned with the Sustainable Development Goals of the 2030 agenda for Sustainable Development adopted by world leaders in 2015; with regard to clean water and sanitation, which focusses on the assurance and contribution to the availability and sustainable management of water and wastewater globally which is to be achieved by 2030.

### 1.7. Delineation of the study

This research project will not focus on the following:

- The use of different membranes, such as Nanofiltration (NF), and reverse osmosis (RO),
- Scale-up studies and costing, which can be undertaken in subsequent studies, and
- The use of pre-treatment systems such chemical supported or/and conventional Dissolved Air Flotation systems, which can add to the operational cost of the proposed system.
- The wastewater treatment using CCD will not focus on ammonia, colour or biogas production but only on COD.

# CHAPTER 2
# LITERATURE REVIEW

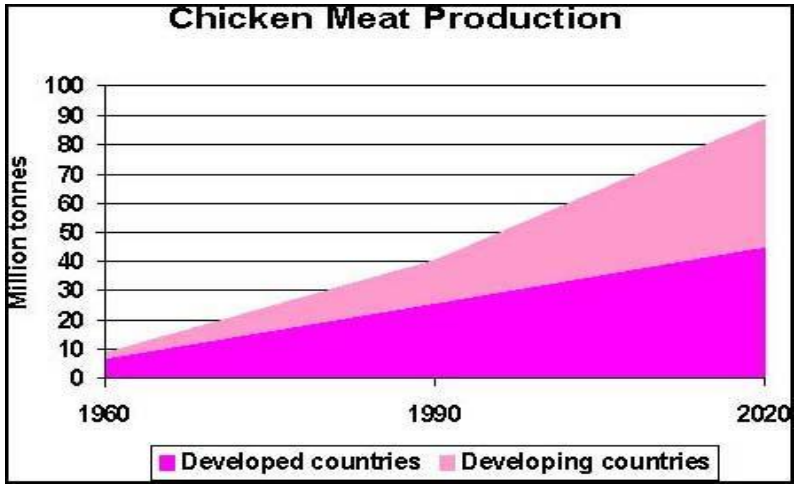
# CHAPTER TWO

# 2. LITERATURE REVIEW

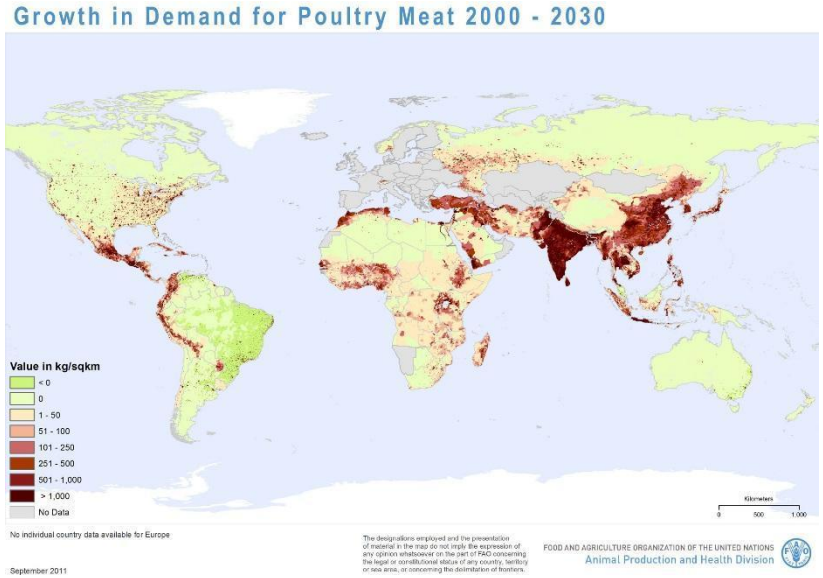
## 2.1. Poultry Product Processing: Demand and Potable Water Usage

For more than a decade, poultry product consumption has been increasing internationally, both in developed and developing countries such as South Africa (Department of Agriculture and Rural Development, 2009). This resulted in a rapidly growing poultry industry, with consequential increases in the quantity of organic solid by-products or wastes including wastewater being generated, in particular, from local poultry slaughterhouses (Salmien et al., 2001; Del Nery et al., 2007). The estimated production is 270 tonnes annually and in the year 2005, poultry product production increased up to 9.4 million tonnes per annum (Del Nery et al, 2007). In 2015, according South African Poultry Association (2015), the poultry industry remained one of the largest single contributors to the agricultural sector in South Africa, contributing 20.6% of the total agricultural gross value (South African Poultry Association, 2015). Furthermore, in the same year, the production of chicken meat was 1.5 million tonnes compared to 900 000 tonnes in 2013 (South African Poultry Association, 2015). This resulted in a 66.7% increase over a period under evaluation. Within the industry, 76% of the birds are used for meat production while the remainder is used for egg production (South African Poultry Association, 2015).

Due to population growth and the rising demand in poultry products, slaughterhouses and poultry processing plants worldwide increased (Cammarota and Friere, 2006). However, in South Africa there are challenges within the industry such as poultry product imports from the United States of America resulting from trade agreements, the implementation of regulations to meat imports by the European Union countries, limited export opportunities, and changes to the legislation on brining levels within chicken products (South African Poultry Association, 2015). Figure 2.1 describes the population demand and production for poultry products globally.




(a)




(b)

**Figure 2-1**: (a) Poultry production globally; (b) Demand for poultry products (Speedy, 2004; Robinson and Pozzi, 2011)

In 2015, the South African government and the United States (US) had agreed on 65 000 tonne of American frozen leg quarters imports per annum in to South Africa; the US government demanded to export 50% more tonnes to SA. This resulted in 97.5 000 tonnes per annum of US bone-in portions being imported (South African Poultry Association, 2015). This challenge was further increased by the unbanning of imports from the Netherlands, Germany, Hungary and the UK which further increased imports of frozen poultry products into South Africa. This resulted in the poultry industry in South Africa reducing production of chicken, which resulted in a loss of jobs in the local poultry industry. Most personnel were retained only to perform key functions, which did not include the proper implementation of systems to operate and maintain the functionality of wastewater plants, culminating in their neglect.

Size variations in poultry processing plants, is influenced by the quantity of birds to be processed. Larger processing plants can easily process birds at a rate of 100000 or more birds per hour. This approximates to one million birds per week, with smaller plants only processing a few batches of birds per week (Burton et al., 2010). In SA during 2015, 1.0045 million broiler chickens were produced for slaughtering, which resulted in a prediction of 19.97 million production of broiler chickens per week (South African Poultry Association, 2015). Numerous studies state that poultry processing plants can consume an average of 26.5 L/bird of potable water during the primary and secondary processing of live birds to meat. This quantity ranges from 18.9 to 37.8 L/bird based on the processing plant capacity. For turkey processing, the volume of water used is considerably higher and is largely based on average live weight of slaughtered turkeys which exceed 12 Kg. In some cases, such as the processing of large tom turkeys, bird weight can reach up to 18 Kg, with water consumption for processing such birds being in the range of 130 to 150 L/bird (Avula et al., 2008). Figure 2.2 illustrates water usage at each bird processing stage (+) while highlighting the cumulative (overall) used.

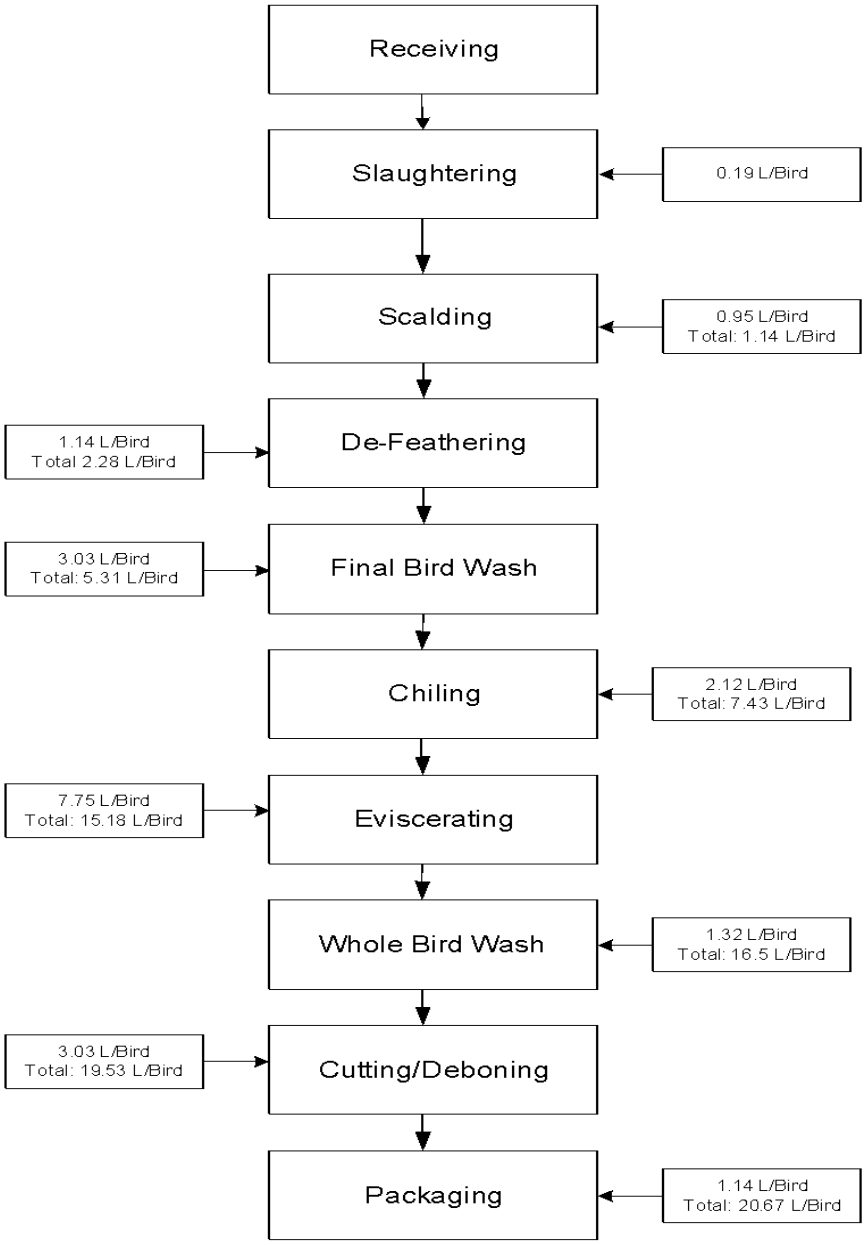



**Figure 2-2:** Average Water usage in a typical poultry processing plant (L/b: litres per bird) (adopted from Avula et al., 2009).

Overall, the poultry product processing industry requires a large quantity of potable water, which consequently leads to the generation of high strength wastewater in large volumes from bird processing (De Nardi et al., 2011); as well as wastewater from cleaning and sanitation processes (Keller et al*.,* 2013).

### 2.2. Wastewater Treatment: Inadequacies, contamination and regulatory constraints

Generally, the treatment of wastewater may comprise several stages, namely: pre-treatment for removal of suspended solids, primary treatment for the reduction of organic matter to reduce tCOD and BOD; secondary treatment such as nitrification and denitrification for nitrogenous compound removal; and tertiary treatment for disinfection including reduction of non-biodegradable matter including residual chemical contaminants (Binne et al., 2002). However, not all wastewater requires treatment using all these treatment stages. In any given case, the treatment required has to be decided based on the strength of the wastewater prior to plant design considerations. This has a direct impact on reducing environmental degradation and pollution, specifically surface water sources, and including the contamination of the ecosystem (Sarkar et al., 2006). Furthermore, unsuitable process design can affect natural water resources, as most treated wastewater is released to such receiving bodies with a potential to cause strain on further industrial growth due to contamination, culminating in the deterioration of the living standards for humans (Sarkar et al., 2006). Furthermore, as humans depend on the ecosystem, its further degradation will impact directly on human life by reducing the reliance and dependence of humans on the ecosystem and water sources (Ng and Jern, 2006).

The global population is increasing, resulting in a high increase in water consumption and water shortages exacerbated by drought and fresh water contamination challenges (Rao et al., 2014). Currently, disease outbreaks can occur due to untreated water or contaminated fresh water sources, and because of the daily challenges related to clean water supply. It is therefore important to treat the available wastewater adequately for reuse. The reuse of treated wastewater is a critical factor when considering the development of sustainable water use strategies, and to abide to regulatory disposable guidelines (Driessen and Vereijken, 2003). Wastewater produced by a community is called sewage and can be classified into three different categories namely: (1) domestic wastewater generated from domestic use, (2) industrial wastewater from industrial operations and (3) rain water (Seghezzo et al., 1998). Economic activity associated with industrial production, climate change conditions, and social behaviour related to water usage all contribute to wastewater generation, and affect the quantity of the wastewater produced as well as its composition, i.e. quality characteristics (Seghezzo et al., 1998). However, a large contribution towards such wastewater is from industrial water use and wastewater generation. This type of wastewater contains pathogens,

heavy metals and organic materials which are also harmful, and thus cannot be discharged directly into the environment. Therefore, it is necessary for the wastewater to have a primary treatment stage and secondary treatment stage prior to discharging it to either the municipal sewers and/or fresh water bodies (Pontes and Pinto, 2006). For some systems, a tertiary treatment stage is not necessary for the removal of residual nitrogen and phosphorus, which are primarily removed by an anaerobic digester used as a primary treatment stage (Pontes and Pinto, 2006).

A contributory factor to the poor quality of fresh water sources is the discharge of improperly treated industrial and municipal (domestic) wastewater into fresh water sources. These wastewaters contain a high quantity of inorganic pollutants, total suspended solids (TSS), including Chemical Oxygen Demand (COD) and/or Biological Oxygen Demand (BOD) which then in turn pollute freshwater sources as well as the ecosystems (Ng and Jern, 2006). Furthermore, untreated wastewater endangers aquatic life and affects the environment if not treated before disposal. Various government bodies are implementing strict rules and regulations to prohibit such illegal discharges, with regulatory monitoring and enforcement taking place to reduce the burden on the aquatic environment and to reduce wastewater pollution to our ecosystem (Chan et al., 2009).

Due to operational costs associated with wastewater treatment, wastewater treatment plant systems should be simplified and be efficient in removing pollutants. Suitable, adequate and best designs should include low energy consumption rates and a low plant footprint, reduction of the use of expensive and sophisticated equipment (Seghezzo et al., 1998).

There are advantages associated with regulations as non-abidance results in increases in capital expenditure and running costs including negative economic returns when contaminated fresh/raw water has to be treated. One advantage of such compliance is that water usage costs may be reduced, which in turn will benefit the user as most water is directly sold to consumers. Inadequate monitoring is disadvantageous, as the resulting penalties have a direct economic impact on both private and public entities and individuals. The principle that “the polluter pays” forces industries to comply with regulatory wastewater discharge standards (Sarkar et al., 2006). Therefore, the quality of wastewater being discharged is solely the responsibility of the source industry. This then requires the implementation of effective wastewater treatment and water use and reuse strategies.

### 2.3. Conventional wastewater treatment

Conventional wastewater treatment operations consists of a combination of physical, chemical, and biological processes to degrade organic matter and remove solids, whilst

reducing nutrients from the wastewater (Pescod, 1992). Different degrees of wastewater treatment are classified as preliminary, primary, secondary, and tertiary or advanced treatment stages.

### 2.3.1. Pre-treatment Stage

The primary objective of the preliminary treatment stage is the removal of coarse solids and other large particle matter often found in raw wastewater that could possibly block or interfere with the other process stages/units (equipment) such as pumps etc., further downstream.. Removal of these solids is necessary to enhance the operation and maintenance of subsequent treatment units using systems such as screening and grit removal (Templeton and Butler 2011).Some of these methods are described and listed below:

- **Screening**

The screening process is usually the first step in wastewater treatment processes as it removes large and heavy particles such as rags, paper and plastic including solidified FOG and carcass debris. Such material is usually removed by screens such as bar screens, drum screens and band screens are used (Templeton and Butler 2011).

- **Grit removal**

Grit removal is usually the second step in a wastewater treatment process. Grit involves insoluble particles such as sand, gravel. Grit removal is also important to prevent clogging of pipes and to protect mechanical equipment as well due accumulation within a treatment plant. Common grit channels are velocity grit channels, aerated grit channels, and gravity separators (Templeton and Butler 2011).

### 2.3.2. Primary treatment stage

The primary treatment stage involves some form of a separation of undissolved solids, either by mechanical screening or sedimentation (Burton et al., 2010). The primary objectives of this stage are to remove settleable organic and inorganic solids by sedimentation, and floccuable suspended solids (SS) by skimming. Approximately 25 to 50% of the incoming BOD, 50 to 70% of the SS, and 65% of FOG are removed during this stage (Pescod, 1992).

Some examples of primary treatment processes are:

- **Sedimentation/Clarification**

Sedimentation is also known as settling or clarification and is used to separate solids from the wastewater. There are four types of settling namely discrete settling, flocculent settling, hindered settling and compression settling It works by concentrating suspended solids in a

single unit with the resultant wastewater being clarified, thus achieving reduced turbidity (Templeton and Butler 2011).

- **Dissolved air flotation (DAF) and flocculation**

DAF uses air bubbles to remove solids from wastewater. The sparged air which forms bubbles is fed from the bottom of the DAF tank so that it can rise and attach solid particles to it as it floats to the surface. The diameter of the bubbles is 10 – 100 µm. A skimmer is then attached to the DAF at the top of the tank, skimming flocculated matter from the wastewater surface. The pre-treated wastewater is then removed for collection through tubes to a primary treatment stage, i.e. an anaerobic digester. A DAF system removes particles that are difficult to settle. The particles that are removed are of a density lower than or similar to that of the wastewater (Templeton and Butler 2011).

- **Lamella plate settlers**

Lamella plate settlers, also known as high rate settlers or parallel plate settlers, remove suspended solids. The plates are inclined an angle of 40° - 60°. The spaces between the plates are between 50 to 200mm. The particles (settled solids) will shear off when the plates are turned upwards as water enters horizontally (Templeton and Butler 2011).

### 2.3.3. Secondary treatment stage

During the secondary treatment stage, dissolved organic matter is removed by a biological process which is either aerobic or anaerobic. This process follows the pre-treatment/primary treatment stages such as sedimentation in which activated sludge or other consortia are used for the biodegradation of organic matter, and inorganic constituents in the wastewater (Burton *et al.*, 2004). Such biological treatment processes are important due to their capacity to treat wastewater with differentiated quality characteristics from a variety of sources (Chan et al., 2009). Wastewater that contains biodegradable constituent have a BOD/COD ratio > 0.5, which is an indication that the wastewater can be treated easily by biological means if appropriate environmental conditions are implemented (Chan et al., 2009). This will therefore effectively reduce treatment and operational costs and minimise secondary pollution of the environment (Chan et al., 2009), while minimising charges and abiding with government regulations. Examples of secondary processes are (Templeton and Butler, 2011):

- Mesophilic and thermophilic aerobic treatments,
- Mesophilic and thermophilic anaerobic digestion and energy recovery,
- Composting, and
- Activated sludge treatment.

### 2.3.4. Tertiary treatment stage

If after the secondary treatment stage minute concentration or residual organic matter is present, then a tertiary process is implemented, also known as the polishing stage (Burton et al., 2010). Examples of tertiary processes include:

- Membrane filtration systems, and
- Thermal and/ or disinfection processes.

## 2.4. Quality characteristics of poultry slaughterhouse wastewater

Poultry slaughterhouse wastewater (PSW) can contribute to numerous environmental challenges if not appropriately treated (Atuanya and Aigbiror, 2002). On average, it contains high concentrations of BOD (151 000 – 200 000 mg/L), COD (385 000 mg/L), nitrogen, pathogenic and non-pathogenic viruses, bacteria and parasites including their eggs. These characteristics, together with a large amount of bird carcass debris and FOG, makes slaughterhouse wastewater highly polluted, (Cao and Mahrvar, 2011). According to Chen (1992), poultry by-products and waste may contain over 100 different species of micro-organisms including pathogens such as *Salmonella* sp., *Staphylococcus* sp. and *Clostridium* sp., often found in contaminated feathers, feet, intestinal contents, and processing equipment. As such, the tCOD of PSW is four times higher than that of domestic sewage wastewater. However, the quality characteristics of such wastewater may differ between varieties of processing plants, as this is influenced by the production processes used (Del Nery et al., 2006).

The generated PSW contains biodegradable organic matter, and suspended and colloidal matter in the form of proteins, fats, and carbohydrates from meat, blood, skin, and feathers, resulting in high BOD and tCOD. This challenge is exacerbated when rinse-off water is used to move heavy solids in eviscerating troughs and scalding tanks, and when the water is used as a feather flow-away agent, or is used for washing of picker aprons in cutting and picking rooms (Avula et al., 2009).

Due to possible final product contamination by pathogenic organisms, facilities that process poultry products must continuously utilize large volumes of clean drinking water to rinse the products as they are cut and packaged. Residual blood, animal fat from skin, oils that are desorbed during scalding for feather removal, and processed edibles from the bird and faeces, contribute to organic matter in the PSW, whilst residual blood, urine, cleaning and sanitising agent contributing to the phosphorous, nitrogen concentrations (Del Nery et al., 2007). A fair quantity of grit containing other inorganic matter is also released (Avula et al, 2009). Due to the high concentration of organic matter and suspended solids in the

wastewater, it is necessary to pre-treat the PSW prior to sequentially primary treatment (Keller et al., 2013). Most of the contaminants present in the PSW can be reduced by means of processing steps highlighted in section 2.3, i.e. physical, biological and chemical treatment stages (Del Nery et al., 2007). Table 2.1 illustrates typical effluent characteristics of PSW with Table 2.2 highlighting legislated South African industrial discharge and drinking water standards for various districts and municipalities (Department of Water Affairs, 2012; South African National Standards 241-2, 2015).

The primary reason as to why the PSW must be treated prior to its disposal into the environment is to minimise or eliminate any adverse effects on the receiving environment and for the protection of human health (Cao et al., 2011; Cuetos et al., 2010). Additionally, factors such as wastewater disposal restrictions, treatment costs, and an environmentally aware populace, which prefers products from facilities that support environmental sustainability, make slaughterhouses accountable for environmentally benign operations. For these reasons, PSW treatment is not only a major concern for poultry slaughterhouses and processors but also for service providers in the supply chain of the poultry products industry (Kobya et al., 2006). In the case of non-compliance, associated human and environmental health problems include odour generation and infestation by rodents, insects and other pests, proliferation of pathogens, and groundwater contamination (Yetilmezsoy and Sakar, 2008b). Therefore, it is both a legal and moral requirement to treat PSW for environmental sustainability. Figure 2.3 illustrates some FOG and feathers within the PSW.

**Figure 2-3**: FOG and feathers within the PSW

**Table 2-1**: Characteristics of the raw wastewater from industrial poultry slaughtering process and from cleaning and sanitation of equipment and facilities (adopted from Zhang et al., 1997; Del Nery et al., 2007; Yordanov 2010; Yetilmezsoy et al., 2011)

| | **Poultry slaughtering processes** | | **Cleaning and sanitation processes** | |
|---|---|---|---|---|
| **Parameter (mg/L)** | **Min.** | **Max.** | **Min.** | **Max.** |
| $COD_T$ | 2360 | 4690 | 1004 | 1745 |
| $BOD_T$ | 1190 | 2624 | 436 | 1350 |
| O&G | 249 | 702 | 76 | 166 |
| pH | 6.5n | 7.0 | 6.5 | 6.9 |
| TKN | 147 | 233 | 93 | 141 |
| $NH_3$-N | 20 | 68 | 21 | 71 |
| TP | 33 | 128 | 22 | 102 |
| TS | 2032 | 3139 | 1207 | 2004 |
| TVS | 1397 | 2379 | 756 | 1084 |
| SS | 640 | 1213 | 180 | 473 |
| VFS | 617 | 1548 | 498 | 689 |

**Table 2-2:** South African industrial discharge (Department of Water Affairs (DWA) 2010), and South African National Standards (SANS) 241-2 (2015) drinking and municipal discharge standards (Western Cape and Mangaung)

| Parameters | Units | DWA 2010 | City of Cape Town discharge by laws | National Water Act 2013 | SANS 241-2:2015 |
|---|---|---|---|---|---|
| | | General Limit | | | |
| pH | | 5.5-9.5 | 5.5≤12 | 6≤ 9 | 5≤9.7 |
| Temperature | °C | | 0≤40 | | |
| Conductivity | µs/cm | 70-150 | ≤500 | ≤200 | ≤170 |
| TDS | ppm | | 4000 | | |
| Salinity | ppm | | | | |
| Turbidity | NTU | | | | ≤5 |
| tCOD | mg/L | 75 | ≤5000 | ≤5000 | 1000-2400 |
| sCOD | mg/L | | | | |
| TSS | mg/L | 25 | 1000 | | |
| VSS | mg/L | | | | |
| FOG | mg/L | 2.5 | 400 | | |
| BOD | mg/L | | | | |
| VFA | mg/L | | | | |
| Alkalinity | mg/L | | | | |
| $NH_4$ | mg/L | | <25 | ≤3 | ≤ 1.5 |
| Nitrate | mg/L | | | ≤15 | ≤11 |
| TP | mg/L | 10,0 | <25 | ≤10 | |

### 2.5. Effective treatment of poultry slaughterhouse wastewater

Few wastewater treatment plants (WWTP) can treat PSW completely in a single stage. Often, when there are challenging wastewater treatment (WWT) targets, two or more sequential treatment stages are required as discussed in 2.3. PSW has been treated using physical, chemical and biological processes prior to discharge into receiving surface water sources. This is due to its relatively biodegradable characteristics (BOD/COD > 0.5). It has been suggested that an anaerobic biological treatment process is one of the most suitable and effective treatment processes available (Cao and Mehrav, 2011). However, prior to using this type of biological treatment process, an efficient pre-treatment system such as a suspended solids separator is needed, as the wastewater contains a high concentration of TSS and FOG which can lead to the failure or instability of the biological treatment process (Manjunath et al., 2000).

Other pre-treatment systems are used to remove TSS and FOG from the influent prior to the downstream treatment of the PSW, such as grease-traps, tilted plate separators, or a DAF system supported by chemical and/or biological agents. However, this increases operational costs due to the reagents and personnel needed to operate numerous process units (Del Nery, 2007). It can be advantageous to use pre-treatment methods to minimise sludge flotation and the clogging of PSW treatment units which is caused by the FOG, feathers and blood (Del Nery, 2007). Prior to PSW discharge from the WWTP, poultry product processors are required to remove a majority of the soluble and particulate organic matter present in the wastewater in order to achieve compliance with environmental discharge regulations. The majority of poultry processors use some form of screening application to reduce suspended particulates, including internally and externally fed rotary screens, shakers and bar type screens (Avula et al., 2009).

Most PSW treatment systems utilize activated sludge in anaerobic reactors as their primary biological treatment stage. The high energy demand requirements for aeration of aerobic reactors, considering the large quantity of sludge generated, increases overall disposal costs of the excess sludge for WWTPs. This therefore limits the potential of aerobic technology as the primary biological treatment stage of high strength industrial wastewater such as PSW. The energy savings and mitigation of unnecessary activities associated with anaerobic digestion processes can culminate in minute excess sludge production, thus reducing disposal costs. Del Nery et al., (2007) showed that utilizing a combination of reactors, i.e. an up-flow anaerobic sludge blanket reactor (UASB) and a stirred tank reactor coupled with a membrane filtration unit, achieved >90% organic matter removal. This treatment strategy can be adopted with minimal modification to treat PSW collected for this study. Possibly to a similar degree of effectiveness. According to Avula et al. (2009), some physical methods

have also been reported in the reviewed literature as effective, with 3 major categories being identified, namely: (a) the destruction of pollutants by an electrical change or UV radiation, (b) the combination of biochemical and chemical destruction using oxidants such as ozone, chemical separation or biochemical degradation systems, and (c) physical separation processes using technology such as DAF and membrane filtration systems. However, all these processes produce recalcitrant by-products which can further contaminate available natural water sources. According to Avula et al. (2009), the advantage of existing physical PSW treatment processes are: (1) only minimal efforts can be made to reclaim nutrients, and (2) other valuable constituents in PSW can be degraded during the biological treatment process to produce biogas. Previous studies of PSW treatment included the utilisation of a DAF and a UASB. According to Del Nery et al*.* (2008), treatment comprising a DAF system and two UASB reactors in series can achieve complete organic matter degradation rates.

A full-scale DAF system was determined to accomplish unsatisfactory removal efficiencies of 15% for SS and only 8% for FOG, suggesting operational inadequacies (Del Nery et al., 2008). However, a lab scale DAF system showed that flocculation agent addition and the implementation of air pressurization, including a 40% recycled effluent can increase TSS and FOG removal by up to 74% and 99% respectively (Del Nery et al*.,* 2008). Furthermore, a study by Del Nery et al*.* (2007) in which long-term operation performance was monitored over 4 years using rotary and static screens, an equalization tank, a DAF system and two UASB reactors showed an average of 51% FOG removal and 37% TSS removal. The operational parameters of this system included an OLR, applied to the UASB that ranged between 0.9 to 2.7 kg tCOD/$m^3$.day with up-flow velocities varying from 0.2 to 0.5 m/h. The system showed the satisfactory performance of UASB reactors, with a tCOD removal efficiency of 85% (Del Nery et al., 2007). For the recovery of essential compounds, recovery processes can be implemented; for example, Lo et al*.* (2005) recovered protein from PSW using membrane ultrafiltration after the primary treatment stage, which included two DAF systems in parallel for the removal of 90% of FOG in the PSW. This resulted in retainment of the crude proteins, which subsequently reduced the tCOD to less than 200 mg/L. However, the membranes were fouled severely, resulting in the implementation of cleaning-in-place processes to restore performance (Lo et al., 2005).

For this current study, a lab-scale expanded granular sludge bed reactor (EGSB) was used as the primary anaerobic digester, with filtration implemented as a pre-treatment stage, followed by a post EGSB treatment using single-stage nitrification and aerobic denitrification process attached to a hybrid side-stream UF membrane bioreactor (MBR).

## 2.6. Efficiency of anaerobic digestion

Anaerobic digestion is one of the most effective biological wastewater treatment technologies available as it not only reduces the organic waste volume in the water being treated, but also produces biogas as a renewable energy source (Niclas et al., 2017) Moreover, anaerobic digestion requires low energy input due to its classification as a low performance process. The basis of anaerobic treatment method relies on suitable bacteria cultivated in the absence of dissolved oxygen, facilitating the decomposition of organic matter into by-products such as biogas (Majd et al., 2017). Anaerobic digestion is based on the use of manure and/or sludge as the primary driver in the treatment process. It is seen to be beneficial and advantageous when compared to other treatment process (Yetilmezsoy and Sakar 2008a). It has been recognised as sufficiently suitable for PSW treatment as its functionality is independent of dissolved oxygen to degrade organic matter, although it is both temperature and oxygen sensitive. This then makes anaerobic treatment criteria and design different in different regions (Yetilmezsoy and Sakar, 2008a). Anaerobic digesters involve the initial stabilization and degradation of organic material under anaerobic conditions (Burton et al., 2004). This results in the formation of biogas, a mixture of $CO_2$ and $CH_4$, and the generation of minute quantities of biomass, which makes it suitable for pollutant reduction from a variety of industrial operations.

Generally, anaerobic digestion enables low volumes of sludge production culminating in reduced spent sludge disposal cost and the recovery of biogas for energy production, making it one of the most suitable wastewater treatment systems available for developing countries (Girault et al., 2012). When compared to mesophilic digestion, thermophilic anaerobic digestion has additional benefits, which includes a high degree of organic matter degradation. This can be achieved if the bioreactors used are stabilised, which culminates in improved post-treatment sludge dewatering (Chen et al., 2008). Acid and methane forming microorganisms differ widely in terms of physiology, nutritional needs, growth kinetics and sensitivity to environmental conditions. Failure to maintain a balance between these two groups of microorganisms is the primary cause of anaerobic digestion reactor instability, attributed to digester souring (Chen et al., 2008). Anaerobic digestion consists of several treatment stages, namely hydrolysis, acidogenesis, acetogenesis and biogas (methane) formation (Karakat et al, 2017). Furthermore, some of the pathogens that are likely to be present in wastewater from poultry slaughterhouses (Sarkar et al., 2006; Demirer and Chen, 2005) can be destroyed in the digester environment.

The evolution of new high-rate anaerobic digesters is due to understanding of the functions and improved designs of anaerobic processes (Ghangrekar et al., 2003). There have also been reports of anaerobic treatment digestion for poultry waste, according to Sakar et al. (2006), which is suitable also for effluents from different kinds of processing plants, i.e. meat processing plants, culminating in the high removal efficiency of the organic load with low operational costs (Seghezzo et al., 1998).

Another advantage of this type of treatment system is that it is technologically and economically feasible for the treatment of liquefied influent using recently developed systems such as the UASB, EGSB and AFBR (Saravanan and Sreekrishnan, 2006). It also yields high methane gases and has the potential for net energy production.

### 2.6.1. Advantages of anaerobic digestion

Anaerobic digestion has been recognised as suitable for PSW treatment due to its efficiency in treating high strength wastewater. One advantage of using anaerobic digesters over sewage sludge systems is that they enable reduction in biomass generation (Girault et al., 2012), enhancing substrate (organic matter) biomass interactions and thus decomposition; and generation of biogas which increases pneumatic mixing within the treatment system, thus rapid stabilisation. The list below indicates other advantages associated with anaerobic digestion (Borja et al., 1988; Seghezzo et al., 1998; Zakkour et al., 2001; Gerardi; 2003):

- Material recovery and energy production from high methane yields,
- Resource recovery and utilization while achieving the objective of pollutant degradation,
- Can be used for both domestic and industrial wastewater,
- Although temperature can negatively influence anaerobic bacteria, such organisms can adapt to low temperatures,
- Can tolerate a wide variety of toxicants, and
- Is suitable for high strength wastewater with biodegradable COD of >4000 mg/L, such as that of PSW.

### 2.6.2. Disadvantages of anaerobic digestion

Similarly, there are numerous disadvantages associated with anaerobic digestion (Borja et al., 1998; Seghezzo et al., 1998; Zakkour et al., 2001; Masse and Masse 2000, Gerardi; 2003), which are:

- Slow or impaired system efficiency due to accumulation of TSS and FOG which can lead to the reduction in the methanogenic activity, thus biogas production reduction and biomass washout,

- Sensitivity to high OLR ,
- Requires long HRT to minimise sludge wash-out,
- Low growth rate of microorganisms, and the
- Need for post-treatment systems to reduce ammonium and hydrogen sulphide and other residual contaminants.

For this study, the anaerobic stage consisted of an EGSB reactor, which can reduce the influence of organic loading, prior to an anoxic and aerobic hybrid post treatment system (Lerner et al., 2007). This mitigates some of the listed disadvantages while trying to retain some of the advantages. Table 2.3 lists some of the features associated with anaerobic reactors and the treatment outcomes and/or requirements when using an anaerobic digester.

**Table 2-3**: Features associated with anaerobic systems and the treatment outcomes and/or requirements (Chan et al., 2009)

| Feature | Treatment |
|---|---|
| Organic Removal Efficiency | High |
| Effluent Quality | Moderate to poor |
| OLR | High |
| Sludge Production | Low |
| Nutrient Requirement | Low |
| Alkalinity Requirement | High for certain industrial use |
| Energy Requirement | Low to moderate |
| Temperature sensitivity | High |
| Start-up time | 2-4 months |
| Odour | Potential odour problems |
| Bioenergy and nutrient recovery | Yes |
| Mode of treatment | Pre-treatment required |

### 2.7. Anaerobic digester: Types and functionality

Anaerobic digesters can be categorized according to two main criteria: 1) whether the biomass is fixed to a surface, i.e. attached or mobilised to support growth or can mix freely with the reactor liquid (wastewater), i.e. suspended growth; and 2) by the organic loading rate, i.e. the influent mass rate of chemical oxygen demand required per unit volume. Some examples of anaerobic digesters are (Shannon et al, 2002):

- Batch system anaerobic digester,
- Continuous stirred-tank reactor (CSTR),
- Expanded granular sludge bed digestion (EGSB), and
- Up-flow anaerobic sludge blanket digestion (UASB).

#### 2.7.1. Upward-flow anaerobic sludge blanket reactor (UASB)

Anaerobic granular sludge bed technology refers to a reactor concept in which a high rate of anaerobic treatment can be achieved. The concept was initiated with an upward flow anaerobic sludge blanket (UASB) reactor (Chan, 2009). The UASB operates in three distinct phases: liquid, solid and gas phases. The liquid phase is whereby the wastewater is being treated, while the solid phase is the sludge or biomass present in the reactor. The gas phase consists of the biogas formed during the anaerobic digestion process (Caixeta et al., 2002). The wastewater flow is directed upward through an anaerobic sludge bed whereby the sludge comes into contact with the organic matter in the wastewater. The sludge bed is composed of microorganisms that naturally form granulates of 0.5 to 2 mm (Chan, 2009), and that have a high sedimentation velocity and thus resist upward movement by pneumatic forces, which prevents a biomass wash-out from the system even at high hydraulic loading rates, and therefore results in low HRT. The resulting organic matter anaerobic degradation process is responsible for the production of biogas containing $CH_4$ and $CO_2$. The upward motion of the generated biogas causes hydraulic turbulence that provides pneumatic mixing. At the top of the reactor, the sludge and gas are separated in a three-way phase separator, i.e. a biogas-liquid-solid separator. The three-phase separator consists of a biogas cap with a settler unit being situated above the biogas collection port. Below, the opening of the biogas cap, baffles are used to divert the biogas to the gas-port opening (Chan, 2009). Figure 2.4 illustrates the set-up of the UASB.

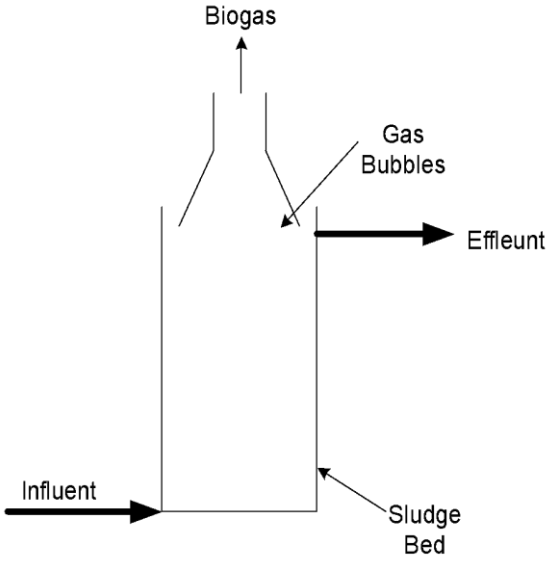



**Figure 2-4**: Schematic diagram of a UASB (Adopted from Chan, 2009)

The success of a UASB depends on the pre-treatment of the wastewater prior to anaerobic treatment to reduce fats and suspended solids. It is important to apply suitable influent up-flow velocity, i.e. surface speed, in UASB to minimise sludge washout. A suitable average up-flow velocity is between 0.5 – 0.7 m/h which is also dependant on reactor volume and configuration. Overall, UASB reactors have been demonstrated to be robust (Seghezzo et al., 1998), with experiments showing COD removal rates >60% for most wastewater from different industries (von Sperling et al., 2001). However, like all other bioreactors, the UASB has some disadvantages.

#### 2.7.1.1. Disadvantages of UASB

The successful operation of a UASB is largely dependent on overcoming the following disadvantages (Chan et al., 2009):

- Accumulation of suspended solids and FOG,
- Reduction in methanogenic activity biomass washout, and
- Periodic re-inoculation.

To reduce the impact of these advantages, the UASB was modified into an EGSB.

### 2.7.2. Expanded granular sludge bed reactor (EGSB)

An EGSB is a variant/modified design of the UASB concept, which includes a recirculation stream for the reactor (Seghezzo et al., 1998) in order to improve dissolved organic matter biomass contact (von Sperling and de Lemos Chernicharo, 2017). The achievement of biomass contact of an expanded granular sludge bed with a high up-flow velocity, i.e. >4 m/h, which was determined to improve the reactor performance and hydraulic mixing, when compared to the UASB (von Sperling and de Lemos Chernicharo, 2005).

The distinguishing feature of the EGSB is that a higher rate of flow velocity can be implemented (Beddow, 2010), particularly for systems with a height/diameter ratio of >20 (von Sperling and de Lemos Chernicharo, 2005). The increased flux permits partial anaerobic bed expansion, i.e. fluidization of the granular sludge bed, resulting in improved wastewater-sludge contact, while enhancing the segregation of inactive suspended particles from the sludge bed into a wash-out port. The EGSB design is appropriate for low strength wastewater having 1 - 2 g soluble COD/L that contains inert or poor/partially biodegradable SS which should not be allowed to accumulate in the sludge bed (Chan, 2009). Figure 2.5 illustrates the EGSB described.

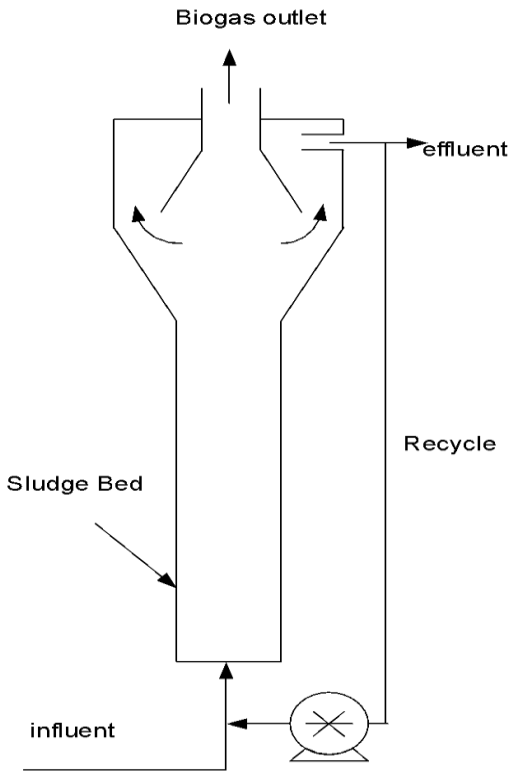



**Figure 2-5**: Schematic diagram of an EGSB (Chan, 2009)

Operationally, the EGSB has demonstrated better performance than the UASB; however, a UASB can handle high strength wastewater when compared to the EGSB, which can only handle low soluble COD containing wastewater (Chan 2009). According to Zhang et al. (2008), the EGSB can achieve a reported 91% COD removal for an HRT of 48h with the feed content containing 80 g soluble COD/L, which is larger than the maximum cited in Chan (2009). To demonstrate versatility, the EGSB design concept has been applied in many other treatment plants, i.e. breweries, starch processors, molasses producers, as well as in domestic and municipal WWTP (Seghezzo et al., 1998; Zhang et al., 2008). Research reports on EGSB are rare, but based on reports of the UASB, the EGSB can be assessed for its suitability to treat PSW, a focus of this study. Furthermore, EGSB has been proven to be suitable for the treatment of low strength wastewater, i.e. dilute PSW at ambient temperatures (Chu et al., 2003). Like the UASB, an EGSB has some disadvantages.

#### 2.7.2.1. Advantages of an EGSB

The advantages below listed are some of the operational attributes for the EGSB (Seghezzo et at., 1998; Chu et al., 2005; Chan et al., 2009; Saravanan and Sreekrishnan 2006; van Haandel and van der Lubbe, 2007):

- Effective removal of soluble pollutants,
- The design can be optimised to treat high strength organic wastewater up to an OLR of 30 kg COD/$m^3$d,
- Minimal accumulation of flocculating/excess sludge,
- The recycle can be used to alter the concentration of the influent supplied to the unit,
- Higher biogas production, pneumatic mixing, up-flow velocities (thus treatment capacity), and a small plant footprint,
- Expanded sludge bed, resulting in improved organic matter-biomass contact and
- Active sludge remains granular, with excellent settleability characteristic, which effectively provides operational longevity.

However, with such high up-flow velocities some disadvantages associated with such an operational strategy are not abnormal.

#### 2.7.2.2. Disadvantages of an EGSB

Due to the high up-flow velocities, there are numerous disadvantages associated with the EGSB (van Haandel and van der Lubbe, 2007), which include:

- Reduced ability to remove particulate organic matter due to high up-flow velocities,
- The Suspended Solids are not retained by the granular bed thus exit with the effluent to downstream units, and
- High sludge washout when granule activity is reduced.

### 2.8. Anaerobic digester: metabolic process and mechanisms

The anaerobic digestion process begins with bacterial hydrolysis of organic matter. Insoluble organic polymers, such as carbohydrates, are broken down into soluble derivatives that become available for other bacteria. Acidogenic bacteria then convert the sugars and amino acids into carbon dioxide, hydrogen, ammonia, and organic acids. These bacteria convert these resulting organic acids further into acetic acid, along with additional ammonia, hydrogen, and carbon dioxide. Finally, methanogens convert these by-products to methane and carbon dioxide. Generally, methanogens population play an indispensable role in anaerobic digestion in WWTP (Tabatabaei et al., 2010). However, many other microorganisms do affect the performance of anaerobic digestion. These are include acetic acid-forming bacteria (acetogens) which can influence and/or hamper biogas production rates. The anaerobic digestion process produces biogas consisting of methane, carbon dioxide and traces of other 'contaminant' gases. This biogas can be used directly as a combustible fuel, in combined heat and power gas engines or upgraded to natural gas-quality biomethane. The nutrient-rich digestate also produced can be used as fertilizer for agricultural soil bio-augmentation (Tabatabaei et al., 2010).The anaerobic digestion process represents an integrated system which has suitable physiological traits including microbial energy metabolism, as illustrated in Figure 2.6 (Mao et al., 2015).

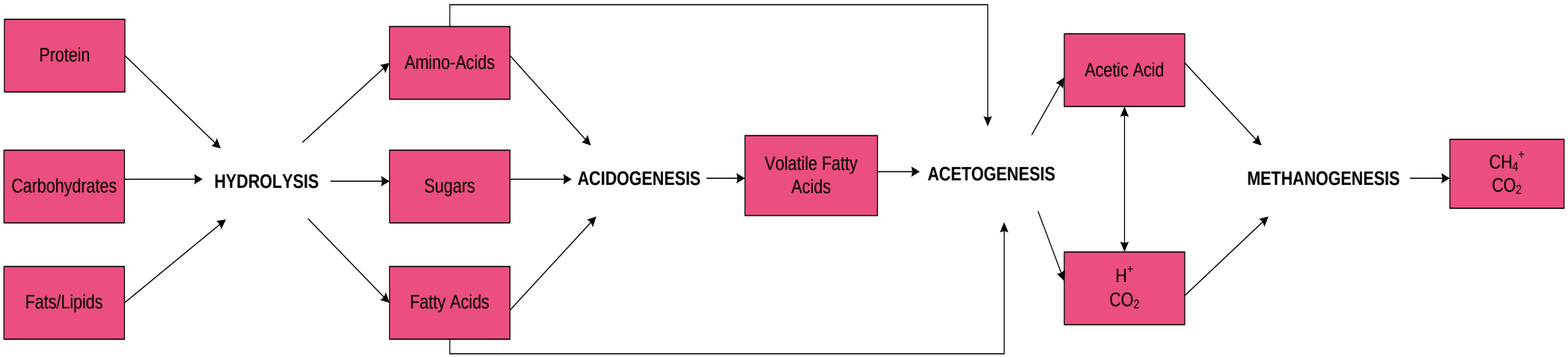



**Figure 2-6**: Anaerobic digester process (Mao et al., 2015)

There are four key biological and chemical stages of anaerobic digestion (Mao et al., 2015):

- Hydrolysis,
- Acidogenesis,
- Acetogenesis, and
- Methanogenesis

### 2.8.1. Hydrolysis of Polymeric Organic Matter

In most cases, the wastewater introduced into the system is made up of organic polymers. For the bacteria in an anaerobic digester to access the energy potential of these polymers, their chains must first be broken down into their smaller constituent parts. These constituent parts, or monomers, such as sugars, can then be readily available to other bacteria. The process of breaking these chains and dissolving the monomers into solution is called hydrolysis. Therefore, hydrolysis of these high-molecular-weight polymeric components is an essential and necessary preliminary step in anaerobic digestion (Sleat and Mah, 1987). Through hydrolysis, the complex organic molecules in the wastewater are broken down into monomers such as amino acids and fatty acids. Thereafter, acidogenesis ensues.

### 2.8.2. Acidogenesis of monomeric constituents

Monomeric constituents produced in the first stage of hydrolysis can be used directly in the acidogenesis stage. Other molecules, such as volatile fatty acids (VFAs) with a chain length greater than that of acetate must first be catabolized into compounds that can be directly used by methanogens. During this stage, VFAs are created, along with ammonia, carbon dioxide, and hydrogen sulphide, as well as other by-products (Gerardi, 2003). The by-product from the acidogenesis can further be broken down, in a process classified as acetogenesis.

### 2.8.3. Acetogenesis of by-products of acidogenesis

The third stage of anaerobic digestion is acetogenesis. The process involves the digestion of simple and low molecular weight molecules created through the acidogenesis phase of anaerobic digestion, which are further digested by acetogens to produce acetic acid, as well as carbon dioxide and hydrogen (Gerardi, 2003). Acetate acid is used as a substrate for methanogenesis. The $CO_2$ and $H_2$ produced from the acetogenesis stage can be converted directly to acetate or $CH_4$. Acetate being one of the most important compounds within the anaerobic stages, it is most commonly used during the methanogenesis stage. However, acetate can be degraded when there are sulphate-degrading organisms present. Further, if

$H_2$ produced in the acetogenesis stage accumulates within the reactor bed, the pressure resulting from such an accumulation can hamper acetate formation. However, methane-forming bacteria uses $H_2$ to produce $CH_4$ (Gerardi, 2003). Following the acetate formation (acetogenesis), the methanogenesis stage occurs which is described in the subsequent section.

### 2.8.4. Methanogenesis: Biogas formation

For this process, methanogens use the intermediate products of the preceding stages and convert them into methane, carbon dioxide, and water. These end-products make up the majority of the biogas produced from anaerobic digestion. Methanogenesis is sensitive to both high and low pHs and occurs between pH 6.5 and pH 8 (Gerardi, 2003). The remaining indigestible material that the sludge consortium cannot use, including any dead bacterial biomass, remains the constituent of the digestate. A simplified generic stoichiometric equation (Eq. 2.1) for the overall processes is as follows:

$$C_6H_{12}O_6 + \text{Bacteria} \rightarrow 3CO_2 + 3CH_4 \qquad (2.1)$$

## 2.9. Biogas generation from anaerobic digestion

The organic matter in anaerobic digestion is easily biodegradable and is therefore easily converted to methane and carbon dioxide gases (Ruiz et al., 2009). The biogas from anaerobic digesters is useful when addressing global energy needs and it can also help with the mitigation of environmental pollution and global warming (Mao et al., 2015). Table 2.4 below illustrates some beneficial environmental outcomes of biogas harvesting from anaerobic digestion.

**Table 2-4**: Biogas environmental benefits (Mao et al., 2015)

| Biogas | Corresponding benefits | References |
|---|---|---|
| Green energy production | Electricity<br>Heat<br>Fuel | Rehl and Muller (2011) |
| Organic waste disposal | Biodegradation of Industrial waste<br>Household waste<br>Municipal solid waste<br>Liquefied Organic waste | Cuéllar and Webber (2008) |
| Environmental protection | Pathogen reduction through sanitation<br>Less nuisance from insects<br>Forest vegetation conservation<br>Replacing inorganic fertilisers | Cuéllar and Webber (2008)<br><br><br><br>Tambone et al. (2010) |
| Biogas linked to agro systems | Livestock-biogas- fruit system<br>Biogas-livestock and poultry farms system | Qi et al.(2005)<br><br>Jiang et al. (2011) |
| GHG emission reduction | Substituting conventional energy sources | Cuéllar and Webber (2008) |

Another benefit of biogas in terms of socio-economic benefits is that it reduces input costs of plants when it is used as an energy source (Holm-Nielsen et al., 2009), due to its renewable energy source classification when compared to other sources of energy (Mao et al., 2015). Biogas production is seen as an adaptable way of providing continuous power generation (Mao et al., 2015), even in impoverished communities. According to an EU policy, as reported in Holm-Nielsen et al. (2009), it is estimated that 25% of bio-energy can come from biogas. Currently, bio-energy is ranked as the fourth largest energy resource globally due to its abundance (Chen and Lee, 2014). However, for maximum biogas generation from anaerobic digestion, environmental effects associated with reactor performance, i.e. pH, temperature, OLR, and HRT must be minimised.

### 2.9.1. Factors affecting biogas production from anaerobic digesters

There are various factors that affect the anaerobic digestion process including biogas production, namely temperature, pH, OLR, HRT, and the type of reactor used.

### 2.9.1.1. Temperature effects on biogas production

Thermophilic and mesophilic temperatures are used in anaerobic digestion. Thermophilic temperature range is from 55 - 70 °C, whereas mesophilic temperature is at 37° C. Comparing the two temperatures, thermophilic facilitates faster reaction rates including higher-load bearing capacities of the digester being used, culminating in higher productivity, compared to mesophilic temperatures (Mao et al., 2015). However, there are advantages and disadvantages associated with thermophilic temperature conditions (see Table 2.5).

**Table 2-5**: Advantages and disadvantages of temperature regimes for biogas production and anaerobic digestion (Mao et al., 2015)

| **Advantage of thermophilic temperature** | **Advantage of mesophilic temperature** |
|---|---|
| • Acidification may occur reducing biogas production | • Better process stability<br>• Higher richness in bacteria |
| **Disadvantages of thermophilic temperature** | **Disadvantages of mesophilic temperature** |
| • Decreased stability thus low quality effluent<br>• Increased toxicity<br>• Susceptibility sensitivity to sudden changes environmental conditions<br>• Larger operational costs due to higher energy input requirement.<br>• Poor methanogenic activity | • Low biogas yield<br>• Poor biodegradability rates<br>• Nutrient imbalance |

Therefore, it is clear that for methanogenesis, mesophilic conditions are suitable for biogas production, due to the sensitivity of microorganisms to sudden temperature changes.

### 2.8.1.2. pH effects on anaerobic digestion

Anaerobic bacteria, especially methanogens, are sensitive to the acidic conditions within the digester as their proliferation can be inhibited by acid accumulation, herein referred to as souring of the anaerobic digester (Verma, 2002). It has been observed that the proliferation of microbial species increases at a pH 4 – 7 (Mao et al., 2015), with the optimum pH value for anaerobic digestion being between 5.5 and 8.5 (Verma, 2002). As such, microbial consortium catalysing methanogenesis require a pH range between 6.5 and 8.2 whereas the acid-producing bacteria that facilitate acidogenesis have an optimum pH between 5 and 6 for sustainability and metabolic stability. The optimal pH for proliferation and maintenance of bacterial growth under anaerobic conditions should therefore be in range of 6.5 to 7.5, a range suitable for sludge granulation. Low pH levels below 6.6 result in the deactivation and metabolic redundancy of methanogens (Mao et al., 2015).

#### 2.9.1.3. OLR effects on anaerobic digestion

The quantity of volatile solids fed into a digestive reactor using a continuous influent supply strategy, i.e. OLR, can result in high biogas yield; although, the operational equilibrium of the process can easily become unsteady. Furthermore, high OLRs facilitate bacterial inhibition due to dominance of hydrolysis/acidogenesis rather than methanogenesis bacterial activity (Mao et al, 2015).

#### 2.9.1.4. HRT/SRT effects on anaerobic digestion

Hydraulic retention time is the time required for the complete biodegradation of a known concentration of organic matter. For such a biodegradation, temperature effects are influential, and must be considered when the HRT is considered, as temperature has a direct effect on microbial growth rate. Retention time, namely HRT and Solid Retention Time (SRT) (Mao et al., 2015), can also be influential, as a high SRT can culminate in some solids being degraded while biomass is attached to the solids. SRT is defined as the average time the solids remain in the reactor and HRT is defined as the time the liquid spends in the reactor (see Eq. 2.2).

$$\mathrm{HRT} = \frac{\mathrm{V}}{\mathrm{Q}} \qquad (2.2)$$

Where *V* is the volume of the reactor and *Q* is the influent flow rate.

An HRT between 15 – 30 days was reported to be the average retention time required to treat wastewater under mesophilic conditions. As HRT is highly dependent on the OLR to obtain and sustain the proliferation of biomass within the reactor, a balance must be achieved, taking into consideration both the HRT and OLR. Generally, in order to achieve maximum biogas yields, a low OLR and a long HRT would be advantageous. Moreover, increasing the SRT can culminate in destabilisation which will thus reduce biodegradation rates, in particular when a large proportion of the solids are inert. This would affect reactor performance (Nges and Liu, 2010), as observed from previous studies whereby an increase in SRT from 10 – 20 days resulted in a 25% decrease in biogas production (Mao et al., 2015).

### 2.10. Efficiency of aerobic treatment processes: a focus on nitrification

In aerobic treatment systems, adequate aeration involves dissolving sufficient oxygen in the wastewater in order to adequately substitute the anaerobic system with an aerobic

environment in order to facilitate the microbial activity of aerobes oxidising organic matter to relatively harmless products such as carbon dioxide ($CO_2$) and water ($H_2O$). Removal of this organic matter also reduces odours associated with WWTP and many of the anaerobic pathogens are destroyed (Burton et al., 2004). Aerobic treatment is nutrient-limited; therefore it is dependent on both temperature and aeration levels, i.e. the temperature should be kept within the range of 15 – 40 °C while the dissolved oxygen consideration should be between 2 – 2.5 mg/L, conditions which are classified as anoxic (Yoo et al., 1999). At higher temperatures, thermophilic activity prevails which could lead to reduced system performance (Burton et al., 2004). Generally, aerobic treatment is suitable for low strength wastewaters (COD < 1000 mg/L). A higher removal of biodegradable organic matter at a lower COD concentration, and thus a lower OLR, can be achieved using aerobic processes culminating in reduced flocculated biomass, which results in lower suspended solids in the effluent post treatment (Chan et al., 2009). In aerobic systems, nitrification also prevails, whereby ammonia is oxidised to nitrates and subsequently nitrates are oxidized to nitrites (Templeton et al., 2011; Tchobanoglous et al., 2004). This process involves two distinct autotrophic bacteria, i.e. nitrosomonas and nitrobacter sp., which oxidise ammonia to nitrites and then to nitrates respectively (Metcalf and Eddy et al., 1972). The two-step nitrification process can be represented by the following biostoichiometric reactions; Eq. 2.3 to 2.5 (von Sperling, 2007).

*Nitrosomonas* sp. driven conversion of ammonium:

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O \quad (2.3)$$

Nitrobacter sp. driven bioreaction:

$$2NO_2{}^- + O_2 \rightarrow 2NO_3{}^- \quad (2.4)$$

The overall bio oxidation processes is as follows- Eq. 2.5.

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

Aerobic processes were determined to be reliable as a secondary treatment subsequent to anaerobic digestion for the treatment of PSW; however, the efficiency of the process can be reduced at a high concentration of COD, $BOD_5$, TN and pathogens from anaerobic digestion.

Other associated problems include high energy input for aeration, excessive biomass production and stripping of pseudo-halogenic gasses (Cao and Mehrvar, 2011; Kobya et al., 2006). Table 2.6 illustrates advantages and disadvantages of an aerobic treatment process.

**Table 2-6**: Advantages and disadvantages of aerobic treatment (Chan et al., 2009)

| Feature | Treatment |
|---|---|
| Organic matter removal | High |
| Effluent quality | Excellent |
| OLR | Moderate |
| Biomass production | High |
| Nutrient requirements | High |
| Alkalinity requirement | Low |
| Energy requirement | High |
| Temperature sensitivity | Low |
| Start-up time | 2-4 weeks |
| Odour generation | Minimised opportunity for odour |
| Bioenergy and nutrient recovery | Minimal |

### 2.10.1. Factors affecting nitrification

There are many factors that affect nitrification/denitrification, namely pH, free ammonia (FA) concentration, free hydroxylamine (FH) concentration, pH, temperature and DO. The bacteria nitrosomonas and nitrobacter can be affected and be inhibited by non-ionised forms of ammonia ($NH_4$) and nitrite as nitrous acid ($HNO_2$). It was reported that nitrobacter reacts sensitively to low concentrated $NH_4$ ions which causes inhibition. Also concentrations as high as 35 mg/L $NH_4$-N cause inhibition. The inhibition then leads to an accumulation of nitrite (Yoo et al., 1999). This then indicates that the FA must be kept low (1.0 mg/L) to prevent inhibition of nitrosomonas. The FA must also be kept low to prevent the inhibition of nitrification.

Similarly, temperatures between 10 – 20°C proved that there was low nitrite accumulation, whereas at temperatures between 20°C and 30°C the nitrating activity was observed to be slow. However, the optimal temperature for which nitrification does occur is between 20 - 30°C. Furthermore, low DO concentrations were determined to be effective for nitrification as previously reported, with a DO lower than 5.0 mg/L facilitating nitrite accumulation whereas a DO of higher than 5.0 mg/L culminated in minimal residual nitrite concentrations (Yoo et al., 1999). Therefore, for effective nitritation, the optimal DO should be less than 5.0 mg/L. The nitrification process is normally followed by an anoxic process, i.e. denitrification.

### 2.11. Anoxic treatment process (denitrification)

The anoxic treatment process (absence of oxygen but in the presence of nitrates), also known as the denitrification process, involves biological reduction of nitrates to nitrogen gas (Barana et al., 2013) in the presence of heterotrophic bacteria. This process involves biological oxidation of the wastewater using nitrates as electron donors instead of DO, and reduces nitrates to nitrogen gas. The denitrification process can be described as follows, Eq. 2.6 (Metcalf and Eddy et al., 1972).

$$C_{10}H_{19}O_3N + 10NO_3^- \rightarrow 5N_2(gas) + 10CO_2 + 3H_2O + NH_3 + 10OH^- \quad (2.6)$$

Or

$$2NO_3{}^- + 2H^+ \rightarrow N_2 + 2.5\ O_2 + H_2O \quad (2.7)$$

This process is independent of DO concentration in the wastewater, although, oxygen atoms are present within the nitrate molecule. In order to reduce energy requirements, operational costs and space, i.e. footprint, single stage sequential nitrification and aerobic denitrification systems are preferable (Tchobanoglous et al., 2004). In this process, it is difficult to remove DO completely, due to the heterotrophic denitrifying bacteria which need minute quantities of DO. Furthermore, high DO will suppress the activity of most of the anaerobic denitrifying bacteria as well (Mpongwana, 2016). These denitrifying micro-organisms require reduced organic compounds for energy, which can be obtained from a primary anaerobic treatment stage, making an anoxic treatment process suitable as the secondary treatment process.

### 2.12. Single stage/simultaneous nitrification and denitrification

The biological nitrification/denitrification process for the removal of total nitrogen in wastewater has become one of the most commonly used processes within the wastewater treatment sector (Wang et al., 2009). Single stage nitrification and denitrification was described by other authors before the millennium for different processes/systems such as anaerobic-aerobic sequencing batch reactors (Münch et al., 1996) and an intermittently aerated and decanted single-reactor process (Yoo et al., 1999).

Nitrification and denitrification processes are performed by microorganisms and are significantly influential in removing total nitrogen (TN) in wastewater treatment in WWTPs (Mpongwana et al., 2016). The rate limiting step of TN removal is the nitrification step (Suwa et al., 1992). Microorganisms involved in this process have the ability to oxidise ammonia ($NH_4$-N) and nitrite ($NO_2$-N) and are known as nitrogen and ammonium oxidising bacteria (NOB and AOB) (Mpongwana et al., 2016). However, there has been an evaluation of TN

removal in various biological treatment systems (Bishop et al., 1976), with their primary disadvantage being that they have high energy demand due to sparging (Taskan, 2016).

According to Bishop et al. (1976), both nitrification and denitrification can occur within a single stage process, but only if the nitrifying organisms are developed by using a sufficient HRT. Additionally, Rittmann and Langeland (1985) also discovered that both nitrification and denitrification can occur simultaneously in a single stage process. This is one of the advantages of simultaneous nitrification and aerobic denitrification Such a process is associated with the reduction of plant size, thus its footprint, for TN removal (Suwa et al., 1992), by AOB and NOB respectively, with aerobic denitrification being facilitated in the presence of organic matter as an electron donor (Mpongwana et al., 2016; Suwa et al., 1992). In conventional aerated biological systems, a two-stage process for nitrification and efficient oxidation of the carbonaceous and nitrogenous compounds is normally used (Suwa et al, 1992). Under aerobic conditions, the nitrate produced, as well as organic matter, can act as electron donors for denitrification (Suwa et al., 1992); although this is dependent on the wastewater being treated, as nitrification and aerobic denitrification under saline and cyanogenic conditions is difficult (Mpongwana et al., 2016). Overall, salinity can reduce the effectiveness of the single stage nitrification and aerobic denitrification process (Campos et al., 2002).

According to Yoo et al. (1999), SND can be achieved by controlling the DO concentration. Yan et al. (2007) also achieved SND via a nitrite decomposition mechanism with a maximum SND efficiency of 54.6 % by controlling the DO concentration. This then proves that SND is achievable if certain parameters are controlled.

Similarly, the effect of OLR on denitrification was reported by Suwa et al. (1992), with the concentration of the biomass increasing with BOD loading increases culminating in outcomes associated with increase in denitrification rates. In this study, i.e., whereby effluent treatment of PSW is required, the application of a single-stage nitrification and aerobic denitrification is proposed. Mpongwana et al. (2016) successfully isolated and screened bacteria from PSW to assess their ability to sequentially nitrify and denitrify under high salinity and cyanogenic conditions. The isolates achieved 75% $NH_4$-N removal rates within 72 h in the presence of cyanide (as a toxicant) and sodium chloride of 4.5% (w/v), which facilitated sufficient denitrification rates. There have been other studies in which algae was used for nitrification (Taskan, 2016). This method has the advantage of low input costs. However, in some instances, 57% of TN and 52% of TP were removed using algae (Maroneze et al., 2014), with recent studies such as Taskan (2016) reporting TN removal of 70.2% in an algal photo-bioreactor, rates which are desirable. Another study done by Hernández et al. (2016) also succeeded in treating wastewater containing TN using algae.

Wang et al. (2009) investigated the change of DO on a Sequence hybrid biological reactor (SBR), achieving 85% nitrogen removal and 92% COD removal. They investigated the DO concentrations and discovered that a DO <1.0 mg/L favours ammonium oxidisers; partial nitrification was also achieved at a DO of <2.0 mg/L and the optimum DO was at 0.50 mg/L with a maximum removal at 0.30 mg/L.

Other researchers such as Chung et al. (2006) and Mosquera et al. (2005) in the last few years have found and implemented the following rapid method for nitrogen removal: the process of nitrification and denitrification can be achieved using an anaerobic ammonium oxidation process called anammox. This process saves energy by minimal use of DO during the nitrification and for denitrification, reducing the need of organic matter as an energy source. This then saves and reduces the costs of nitrogen removal (Wand et al., 2009).

### 2.13. Membrane reactor usage in tertiary treatment systems

Membrane processes are capable of removing suspended solids as well as microorganisms, making these processes ideal for disinfection and water polishing, such that the treated water has potable water quality characteristics. Since 1971, membrane processes have been used widely in the dairy, food, fruit, vegetable, fat, oil and grease, meat and sugar processing industries (Avula et al., 2009). Membrane processes have exceptional efficiency in the removal of small particles which also includes the removal of pathogens and dissolved hazardous compounds in wastewater. Membrane processes can be used in the place of chemical oxidation processes such as chlorination and assist in the removal of residual nitrogen and phosphorous. As noted above, membrane filtration technology can produce water with potable water quality for household use, and industrial application (Avula et al., 2009), even if the source waster is from WWTPs. However, membrane life cycle costs are much higher, especially for microfiltration (MF) and ultrafiltration (UF) membranes, although, the carbon footprint of these membrane processes is less than conventional filters that use chemicals. Furthermore, the input costs associated with chemical usage in conventional system are higher than the costs of membrane processes (Avula et al., 2009). Another advantage of membrane filtration is that it does not require temperature monitoring, although residual FOG can cause membrane clogging (Avula et al., 2009).

Pressure-driven membranes are classified into four categories. These pressure-driven membranes are usually used for dissolved contaminants by means of solid-liquid separations. These four categories are distinguished by the mean pore size of the membranes used, which are (Avula et al., 2009):

- Reverse osmosis (RO) for removal of species (<1nm) for the removal of monovalent salts,
- Nanofiltration (NF) species (1-5 nm) for the removal of sugars,
- Ultrafiltration (UF) species (5 – 100 nm) for the removal of proteins and pathogens, and
- Microfiltration (MF) species (100 – 10 000 nm) for the removal of microbial cells.

There are different types of configurations for membrane processes which include configurations such as the plate and frame, spiral wound, tubular, and hollow fibre (Backhurst and Harker, 2002). The cheapest and simplest configuration is the plate and frame configuration. However, the spiral wound processes configuration provides higher packing density, although, it results in increased opportunity for clogging (Mannapperuma, 1997). Some membrane processes are usually operated in semi-batch cycles whereby the wastewater is added at the same rate as the permeate is withdrawn. This operational strategy ensures that there is a constant level of wastewater in the holding tank at all times to prevent membrane drying. Some advantages of using membrane filtration type process are discussed in the ensuing subsections.

### 2.13.1. Advantages of membrane treatment processes

As a key compound for tertiary treatment systems, membrane bioreactors has several key advantages, which include (Judd, 2010):

- The technology can be widely used with different wastewater,
- The membrane can be charged to reject similar charged components in the wastewater,
- Permeate quality characteristics is uniform,
- Minimal additional chemicals are needed for the process, and
- It is highly automatable, and the system doesn't require highly qualified personnel.

Although this advantages provide adequate support for membrane processes/units use in WWTPs, there are some disadvantages associated with their use.

### 2.13.2. Disadvantages of membrane treatment processes

For the treatment of highly contaminated wastewater, tertiary treatment systems such as membrane processes have the following disadvantages (Cheryan and Rajagopalan, 1998):

- Capital costs which are high with scale-up costs being linear,
- High energy input to sustain pressure in the systems, and
- The need to replace membranes frequently due to membrane fouling and degradation.

Overall, membrane processes can be added to other primary/secondary treatment processes despite these disadvantages, as they are easier to operate. Shih and Kozink (1980), conducted studies for the treatment of PSW using UF membranes, achieving 85% removal of TS and 95% for COD. Additionally, the total nitrogen (TN) removal and reduction was lower than that of protein, with a removal efficiency of 86% and 94% of TN and protein respectively being reported. However, microorganisms present in the feed stream of the UF membrane were found to form a layer on the membranes used which affected the membranes porosity due to fouling and culminating in the reduction of flux (Avula et al., 2009).

## 2.14. Response Surface Methodology (RSM) for system outcomes prediction and optimisation

Response Surface Methodology (RSM) is software developed for the purpose of optimising processes and the generation of efficient designs using mathematical modelling and statistical techniques. RSM is used mainly for the purpose of optimisation of process conditions as well as maximising the production of essential products in a process. The software has been used productively and widely in different fields, namely food technology, environmental biotechnology, and enzyme production (Sathian et al., 2014). It was also used and adapted in the chemical industry since its development in the 1950s (Ngongang, 2016).

RSM uses Central Composite Design (CCD) which designs a set of experiments to generate efficient and optimum process conditions for bioprocesses.

The software uses experimental data and is depended on the input data provided to make the process efficient and optimal. The nature of the experiment is also dependent on what type of response required. Whether it is first or second order, etc., a surface response model is then used to describe the results and optimisation can ensue thereafter. RSM also depends on the approximation of the response developed from the data (Osman et al., nd).

RSM is successful because it adapts a mathematical model for the process optimisation which is generated by a few practical steps. These steps are listed below (Ngongang, 2016):

- Identification of variables (dependant),
- Design of statistically oriented experiments,
- Estimation of coefficients of the statistics of the designed experiment,
- Prediction of the response,
- Checking the adequacy of the designed model,
- Regression analysis,
- Interpretation of mathematical model resulting from the results, and
- Plotting the response on 3D surface plots.

# CHAPTER 3

# MATERIALS AND METHODS

# CHAPTER 3

# 3. MATERIALS AND METHODS

## 3.1. Poultry Slaughterhouse Wastewater (PSW) characteristics

The PSW was collected from a slaughterhouse located in the Western Cape Province, South Africa and stored at 4°C before use. The characteristics of the wastewater are summarised in Table 3.4, which lists averaged values parameters quantified over a 3 week sampling period. All measurements were performed according to Standard Methods.

**Table 3-1**: Typical poultry slaughterhouse wastewater characteristics tabulated from previous studies in the Western Cape South Africa, Adopted from Basitere et al., 2016 ; Basitere et al., 2017).

| | | Poultry Slaughterhouse wastewater | |
|---|---|---|---|
| **Parameter** | **Unit** | **Range** | **Average** |
| pH | °C | 6.5-8 | 6.88 |
| Alkalinity | mg/L | 0-489 | 489 |
| $_{t}$COD | mg/L | 2133-9695 | 2903 |
| $_{s}$COD | mg/L | 595-1526 | 972 |
| $BOD_5$ | mg/L | 1100-2750 | 1667 |
| TKN | mg/L | 77-352 | 211 |
| $NH_4$-N | mg/L | 29-51 | 40 |
| $PO_4$-P | mg/L | 8-27 | 17 |
| FOG | mg/L | 131-684 | 406 |
| TDS | mg/L | 372-936 | 654 |
| TSS | mg/L | 315-4992 | 794 |
| VSS | mg/L | 275-1200 | 738 |
| Soluble proteins | mg/L | 0-368 | 72 |
| VFA | mg/L | 96-235 | 235 |
| $NO_3$-N | mg/L | 0-2.903 | 2.903 |

## 3.2. Analyses used for the Poultry Slaughterhouse Wastewater (PSW) for each phase

The following parameters were analysed in triplicate: pH, temperature, conductivity, total dissolved solids (TDS), salinity and turbidity, with total chemical oxygen demand (tCOD), total suspended solids (TSS), volatile suspended solids (VSS), ammonium-nitrogen, ortho-phosphates, soluble chemical oxygen demand (sCOD), nitrate-nitrogen and nitrite-nitrogen being analysed at 48h intervals. These parameters were quantified for each of the bioreactor streams, i.e. the effluent and influent, to ascertain the PSW quality characteristics. A weekly

composite sample was analysed at a South African National Accreditation System (SANAS) accredited laboratory (Scientific Services, City of Cape Town, South Africa) for confirmatory analyses, focussing on tCOD, $BOD_5$, FOG, Volatile Fatty Acids (VFA) and alkalinity. All analyses and sample testing were measured according to standard methods (APHA, 2012).

Furthermore, pH, temperature, conductivity, TDS and salinity were measured using a PSCTestr 35 multi parameter (Wirsam Scientific, Malaysia), while the turbidity of the samples was quantified using a Turbidmeter TN-100 (Wirsam Scientific, Indonesia). The tCOD was quantified using Merck solutions A (1.14679.0495) and B (1.14680.0495). A Merck spectroquant NOVA 60 was used to measure the tCOD concentrations (see Appendix 1 and 2). The TSS was measured using the ESS method 340.2 (see Appendix A3) while the VSS was measured using a furnace at 550°C.

### 3.2.1. Single stage nitrification/denitrification (SSND)

For the SSND process feed and product, samples were collected every consecutive day and analysed for TN (total Nitrogen), to monitor the changes within the column with respect to ammonium-nitrogen, nitrite-nitrogen and nitrite-nitrogen. TN measurements included $NH_4$-N, $NO_2$-N and $NO_3$-N analyses using a NOVA 60 spectroquant for test analyses facilitated by the use of Merck test kits. The DO was measured as well every day to monitor the DO across the column at each sampling point with a DO meter.

### 3.2.2. Membrane systems analyses

For the membrane systems, the following parameters were measured: pH, conductivity, TDS, TSS, turbidity and tCOD. Each of these parameters was measured for the feed and the product to determine the efficiency of the membrane systems. For comparative analysis, two types of membranes, namely a UF and MF membrane, were used.

## 3.3. Phase 1 experiments

**Phase 1: Objective 1:** Evaluate performance and operational stability of the EGSB treatment system in removing suspended solids, FOG, BOD and COD.

**Objective 2** Evaluate performance and operational stability of the EGSB treatment system at three different organic loading rate (OLR) and hydraulic loading rate (HRT).

**Objective 3:** Determine if biogas is produced during the anaerobic digestion stage.

### 3.3.1. EGSB experimental setup and equipment

The purpose of the EGSB anaerobic digester was to effectively reduce the organic load of the influent, i.e. PSW, subsequent to the treated effluent produced being disposed into the environment. The EGSB consisted of a cylindrical shape glass column with a total working volume of 2.7 L, an inner diameter of 0.065 m and a height of 0.872 m. Ceramic marbles with an average diameter of 0.0157m were placed at the bottom of the bioreactor as packing for the underdrain and to maintain the granular sludge within the heated section of the bioreactor. PVC containers (5 L, n = 2) were used for feed and product storage for the EGSB. The EGSB was fed with influent at the bottom of the bioreactor with a Gilson (Germany) multi-head peristaltic pump with the effluent produced being drawn at the same rate. Silicon tubing with an inner diameter of 0.8 cm was used to connect the bioreactor streams. A recycle stream was connected to the feed/influent stream for sludge suspension and for hydraulic mixing in the bioreactor. The bioreactor was operated at mesophilic temperature (35 to 37 °C), conditions maintained using a water jacket in which warm water was supplied from a thermostatic water bath. Figure 3.1 below describes the EGSB set-up.

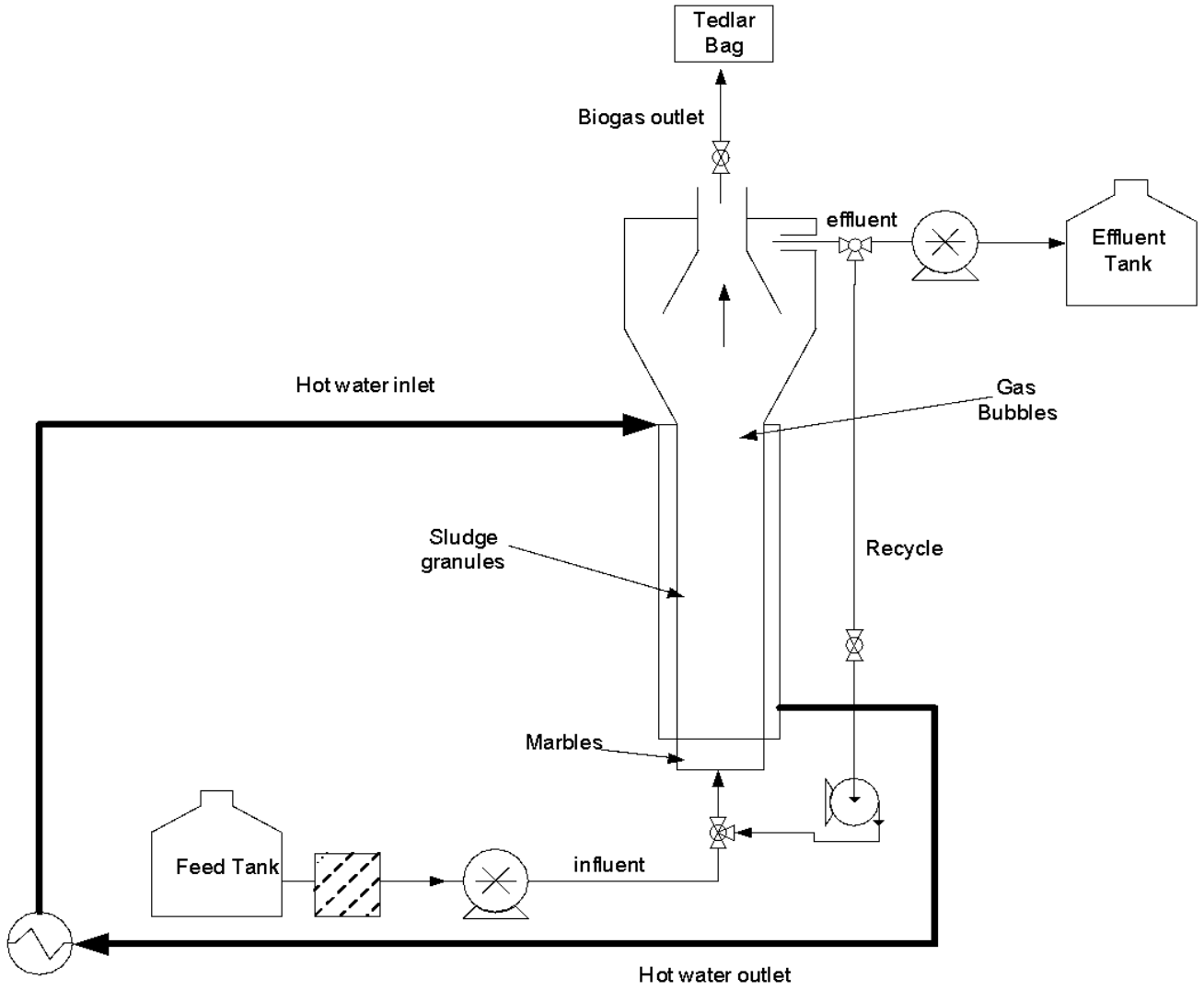



**Figure 3-1**: Schematic diagram of the EGSB set-up

### 3.3.2. EGSB Inoculation

The EGSB was inoculated with 0.747 L of anaerobic granular sludge collected from a full-scale up-flow anaerobic sludge bed (UASB) reactor operated at SABMiller PLC (Newlands Brewery, South Africa). PSW was collected from a poultry slaughterhouse located in the Western Cape, South Africa. Furthermore, a dry milk solution (10 mL, 50% v/w) was prepared, and used as a feed during the acclimation period (48 h).

### 3.3.3. EGSB operating conditions

The influent was filtered (2 mm mesh sieve size) to remove feathers and suspended solids, which might clog the tubes. The PSW was initially diluted to minimise shock loading, using dilution ratios of 50% and 30% (v/v) with undiluted PSW being used thereafter. The bioreactor was operated at average HRTs of 57.5 h, 49.8 h and 62.5 h respectively. During the start-up phase, a 50% dilution feed was supplied to the EGSB operated at an averaged HRT of 62.5 h for 43 days, which corresponded to an averaged OLR of 1.0 g tCOD/L day. Subsequently, the bioreactor was fed with 70% PSW at an average HRT of 57.5 h for 49 days which corresponded to OLRs of 2 g tCOD/L day. To ascertain the treatment systems performance, an undiluted PSW was fed to the reactor for a period of 81 days at an HRT of 49.8 h and an OLR of 3. g COD/L day. The HRT was calculated on the selected OLRs. Table 3.1 lists the operating conditions of the EGSB over a period of 173 days.

**Table 3-1**: Operating conditions (HRT and OLR) for the EGSB system

| Dilution (%) | Days | HRT(h) | OLR (g COD/L day) |
|---|---|---|---|
| 50% | 43 days | 62.5 | 1. |
| 30% | 49 days | 57.5 | 2 |
| Undiluted | 81 days | 49.8 | 3 |

## 3.4. Phase 2 experiments

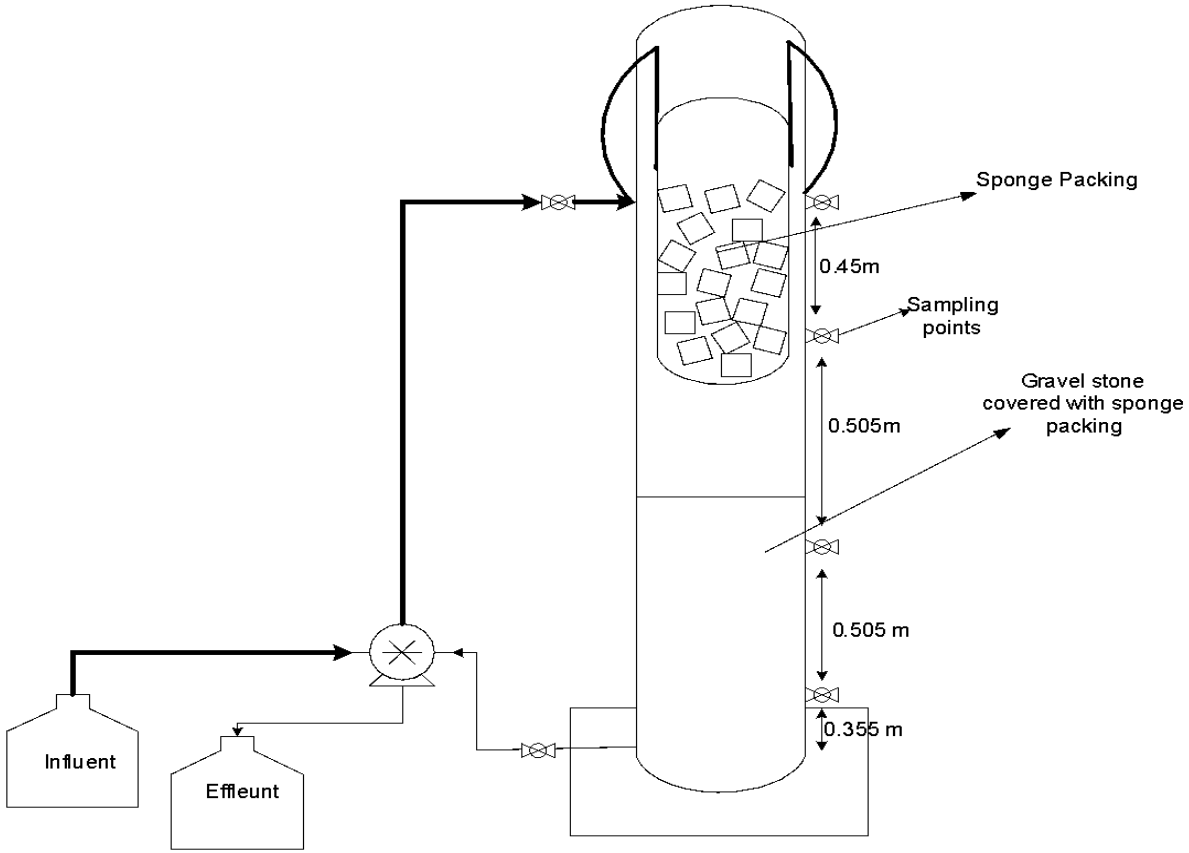
**Phase 2: Objective 1:** To design a lab-scale single stage nitrification/denitrification bioreactor and assess its efficiency in removing total nitrogen and COD in the PSW.

### 3.4.1. Single stage nitrification and denitrification experimental setup and methods

The purpose of the Single stage nitrification and denitrification (SSND) was to effectively reduce the nitrogenous compounds ($NH_4$-N, $NO_3$-N, and $NO_2$-N) of the influent, i.e. EGSB effluent, subsequent to the effluent produced being treated in a side stream MBR. The SSND

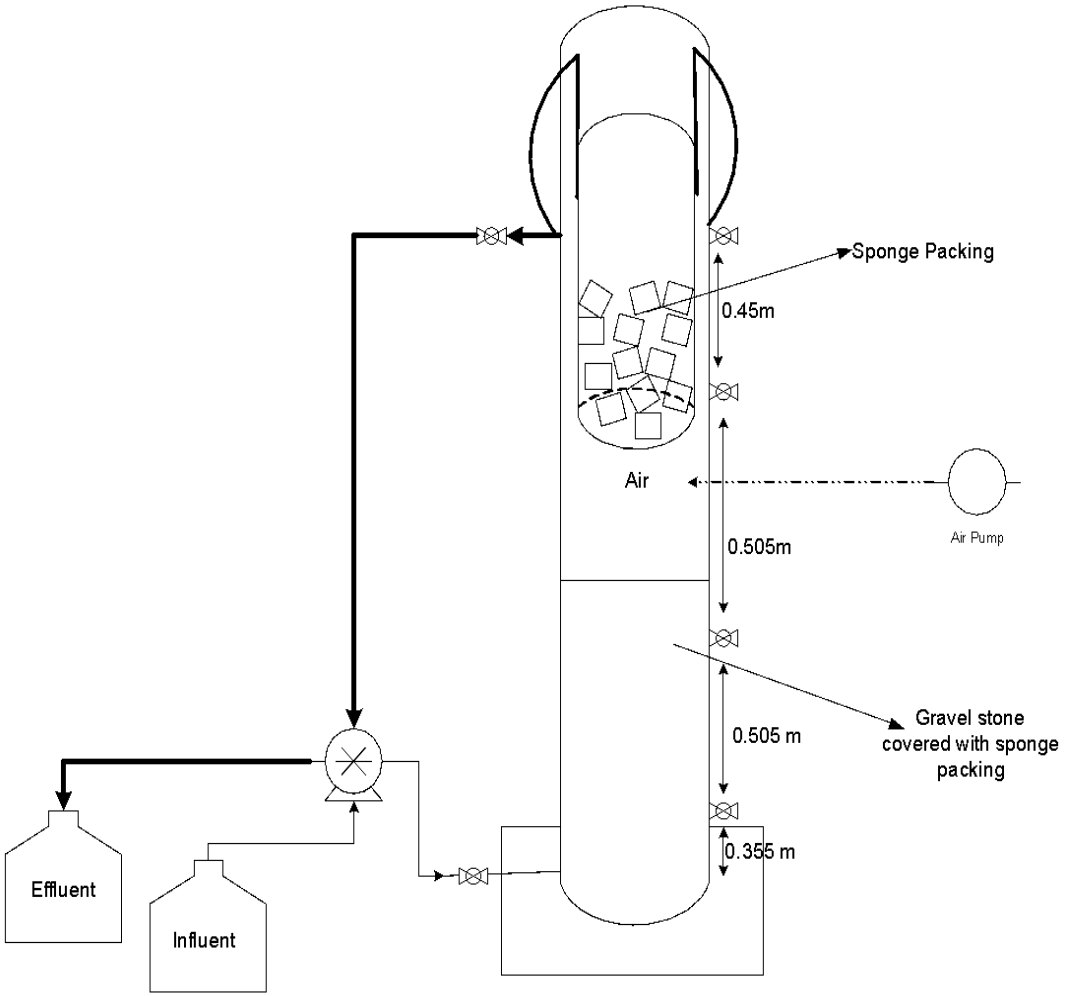
consisted of a cylindrical reactor, consisting of a PVC column, with a total working volume of 12.7 L, an inner diameter of 0.11 m and a height of 2 m (see Figure 3.2). The column had 4 sampling points on the side of the reactor to monitor the change in nitrogenous compounds within the reactor with the first three sampling points from the bottom being 0.51 m apart whereas the top/forth sampling point was 0.45 m from the third sampling point. PVC containers (5 L, n = 2) were used for feed and product storage for the SSND. The SSND was initially fed with influent (EGSB effluent) at the top of the bioreactor with a Gilson (Germany) multi-head peristaltic pump with the effluent produced being drawn at the same rate on top for a period of 45 days.

After a period of 48 days, the feed configuration was changed, with the feed now located at the bottom and the effluent being drawn from the top. Sparging was introduced into the column using an air stone inserted into the column at a height of 0.1 m from the bottom while a bacterial immobilisation surface made of sponge was used in the cylinder at an insertion height of 0.45m from the top of the reactor. Silicon tubing with an inner diameter of 0.8 cm was used to connect the bioreactor streams. The bioreactor column was operated at ambient temperature (24°C). Figure 3.2 below illustrates the SSND set-up.




(a)

**Figure 3-2**: (a) Single Stage nitrification/denitrification setup (down flow);




(b)

**Figure 3-3 cont:** (b) Single Stage nitrification/denitrification (up-flow)

### 3.4.2. Single stage nitrification and denitrification (SSND) Inoculation

Gravel stones with an average length ranging between 0.01- 0.1 m, covered with sponge, (Figure 3.3) were placed at the bottom of the bioreactor as packing and to maintain the biomass within the bioreactor. The packing covered 0.95 m of the column from the bottom. At the top of the column, a perforated cylinder of diameter 0.09 m and 0.43 m long was submerged 0.543 m into the column, suspended from the top of the column. This cylinder was filled with square sponge blocks to retain the biomass within the bioreactor. The column was inoculated with raw unfiltered PSW for 48 hr at 24 hr intervals, and 100 mL of basal media was added to the top of the column. After 15 days of operations the column was topped up with raw 100 mL filtered PSW.

**Figure 3-4:** Sponge used for packing in column

### 3.4.3. SSND Operating Conditions

The influent was the product of the EGSB. The column was operated at average HRTs of 11.5 days, 7.7 days and 13.7 days respectively. These HRTs were dependents on the EGSBs HRT as it was a continuous flow system. During the start-up phase, the reactor was topped up with 100 ml of basal media for 48 hr at 24 hr intervals. The column was operated at ambient temperature. Air was introduced after 48 days of operations with a Dissolved Oxygen (DO) ranging 0.40 mg/L – 4.36 mg/L with an average DO of 1.38 mg/L.

**Figure 3-5**: Single stage nitrification and denitrification Set-up

Table 3.2 illustrates the feed used for the SSND which is the EGSB product.

**Table 3-2**: Feed parameters of the SSND

| Parameters | Units | EGSB Product | | |
|---|---|---|---|---|
| | | **Max.** | **Min.** | **Ave.** |
| $NO_2$-N | mg/L | 0,75 | 0,04 | 0,26 |
| $NO_3$-N | mg/L | 1,55 | 0,05 | 0,67 |
| $NH_4$-N | mg/L | 378,50 | 165,00 | 295,10 |
| TN | mg/L | 380,40 | 165,35 | 296,03 |
| tCOD | mg/L | 2202,50 | 1315,00 | 1657,01 |

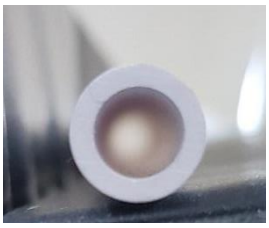
### 3.5 Phase 3 experiments

**Phase 3: Objective 1**: Evaluate the performance of the UF membrane in reducing the high level of soluble and particulate matter in the poultry slaughterhouse wastewater, TSS, FOG, Cond, TDS, pH and COD.

**Objective 2**: Evaluate the overall performance of the treatment system in removing suspended solids, FOG, BOD and COD.

#### 3.5.1. Ultrafiltration membranes setup and methods

Aluminium oxide ceramic membranes in membrane bioreactors (n = 2) with pore size of 1.9 µm (MF) and 100 nm (UF) were used. The membranes had an inner diameter of 0.0068 m, an outer diameter of 0.012 m and a length of 0.25m as shown in Figure 3.5. Glass SCHOTT bottles (1 L) were used for the feed and permeate storage for the UF membranes systems. A dead-end filtration configuration was used for the membrane systems. The membranes were fed with influent of the SSND product and that of the EGSB, for comparative analysis, using a Gilson (Germany) multi-head peristaltic pump with permeate produced being drawn at the same rate. Silicon tubing with an inner diameter of 0.4 cm was used to connect the membrane bioreactor streams. The bioreactor column was operated at ambient temperature (24°C). Figure 3.6 and 3.7 illustrate the UF membrane system set-ups.



(a)

(b)

**Figure 3-6**: (a) Diameter of UF membrane; (b) UF membrane used

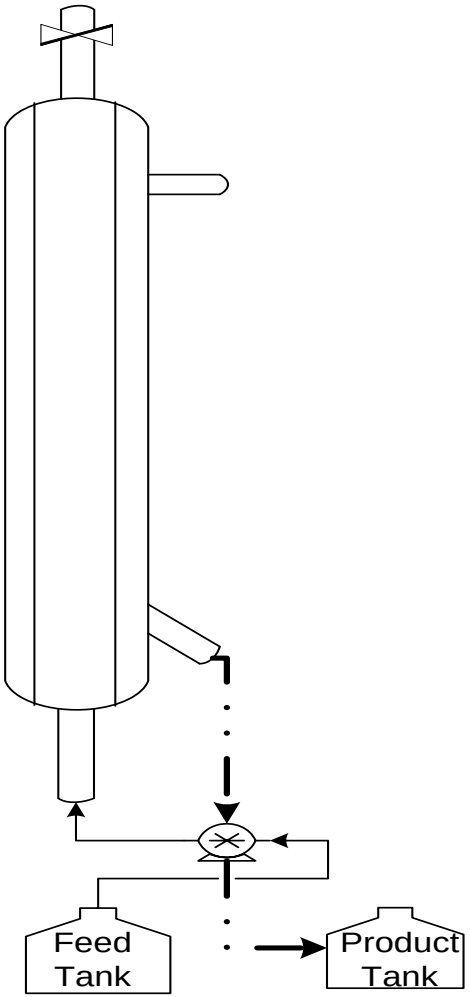



**Figure 3-7**: Schematic diagram of the UF membrane system

**Figure 3-8**: UF membrane system set-up

### 3.5.2. Tertiary treatment of the SSND and EGSB using the UF membrane systems

All of the feed samples used for the membrane systems were products of the previous treatment stages, namely the EGSB and the SSND. A weekly composite sample was collected of each treatment phase and analysed prior to feeding to the membrane systems. Table 3.3 highlights the feed quality characteristics of the PSW treated using the EGSB and SSND prior to tertiary treatment using the UF membrane systems. The product of the EGSB and SSND was fed through the 0.00738 $m^2$ through the lumen of the ceramic aluminium oxide membranes while permeate was retrieved from the shell side of the membrane modules.

**Table 3-3**: Quality feed characteristics used for the membrane systems

| | | EGSB Product | | | SSND Product | | |
|---|---|---|---|---|---|---|---|
| **Parameters** | **Units** | **Max.** | **Min.** | **Ave.** | **Max.** | **Min.** | **Ave.** |
| $NO_2$-N | mg/L | 0.75 | 0.04 | 0.26 | 10.55 | 0.04 | 3.16 |
| $NO_3$-N | mg/L | 1.55 | 0.05 | 0.67 | 30 | 0.10 | 8.41 |
| $NH_4$-N | mg/L | 378.50 | 165 | 295,10 | 258 | 32 | 173.19 |
| TN | mg/L | 380.40 | 165.35 | 296.03 | 260.87 | 52.60 | 184.76 |
| tCOD | mg/L | 2202.50 | 1315 | 1657.01 | 2315 | 1232.50 | 1532.61 |
| TSS | mg/L/l | 320 | 260 | 279.8 | 375 | 190 | 284 |

### 3.5.3. Membrane operating conditions

Weekly composite samples (n = 2, 500 mL) of the EGSB and SSND product were collected and stored in a fridge at 4°C. The samples were then fed through the membrane systems at 0.6 mL/min and 0.65 mL/min. Furthermore, the UF membrane system with a pore size of 100 nm was compared to the MF membranes with a pore size of 1.9 µm, aluminium oxide ceramic membranes using a similar operational and assessment strategy. Thereafter, the flow rate was doubled to determine the difference in efficiency when the influent into the membrane systems was increased. The following parameters were analysed to characterise the efficiency of the membrane systems; TSS, TDS, Turbidity, tCOD, conductivity, pH, and temperature.

## 3.6. Phase 4 experiments

**Phase 4: Objective 1:** To develop a model to predict EGSB performance in terms of tCOD reduction using RSM.

### 3.6.1 Modelling and optimisation of the EGSB as a primary PSW treatment process

Response Surface Methodology (RSM) was used to determine the best model that would fit the experiment data as well as to find the best optimum conditions for COD removal using the EGSB. In this study, HRT and OLR were the parameters/variables used for optimisation using central composite design (CCD) in RSM. Design-Expert® software version 10.0.0 (Stat-Ease Inc., Minneapolis, USA) was used to generate 15 experimental runs. Each factor had a high (+1), and low (-1) level. Table 3.5 illustrates describes the values and variables used. An OLR range of 1, 2, and 3 g COD/L day was selected respectively and the HRT corresponds with the selected OLR as it is dependent on the OLR.

CCD was used because it the most common response surface design tool (Sufiate et al.,2018). It also demands a smaller number of experiments as well as at the same time it provides comparable results. With it being the most commonly used design tool it is also the most popular of all second-order designs and consists of three portions which includes a 2K factorial design whose factors are coded as -1 and 1. (Amrou et al., 2018). CCD is used for two factorial/ fractional designs instead of three factorial/fractional designs such as Face central composite design (FCCD), Full Factorial designs (FDD) and Box- Behnken Designs (BBD) as our experiment has two factors namely HRT and OLR (Stamenković et al., 2018)

**Table 3-4**: Factors used in the experimental design

| Factors | Units | Code | Low (-1) | High (+1) |
|---|---|---|---|---|
| **OLR** | gCOD/L.day | A | 1.01 | 4.82 |
| **HRT** | Day | B | 1.50 | 2.71 |

A model which was determined to be a suitable representation of the EGSB system's COD reduction (removal) is in the form highlighted in Eq. 3.1.

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j + \varepsilon \quad (3.1)$$

# CHAPTER 4

# RESULTS AND DISCUSSION

# CHAPTER 4

# 4. RESULTS AND DISCUSSION

This chapter was divided into 4 phases.

- **Phase 1:** To evaluate performance and operational stability of the EGSB treatment system in removing suspended solids, FOG, BOD and COD and biogas production.
- **Phase 2:** To design a lab-scale single stage nitrification/denitrification bioreactor and assess its efficiency in removing total nitrogen and COD in the PSW.
- **Phase 3:** To evaluate the efficiency, effectiveness and performance of the UF membrane in reducing the high level of soluble and particulate matter in the poultry slaughterhouse wastewater, TSS, FOG, Cond, TDS, pH and COD.
- **Phase 4**: To develop a model to predict EGSB performance in terms of tCOD reduction using RSM.

## 4.1. Phase 1: Expanded Granular Sludge Bed (EGSB) reactor performance

### 4.1.1. Poultry slaughterhouse wastewater characteristics

Table 4.1 represents the results for the feed and product of the EGSB compared to the City of Cape Town (CCT) by-laws, National Water Act (NWA) 2013 and South African National Standards (SANS) 241. The feed tCOD with a maximum concentration of 11068 mg/L exceeded the limit of <5000 mg/L; however, the average feed tCOD concentration was 4981 mg/L which was barely within the by-laws of the CCT discharge standards. Furthermore, the product had an average tCOD concentration of 1359 mg/L, which was also within the discharge standards. It was observed that the $NH_4^+$-N, turbidity, conductivity, TSS and FOG of the feed exceeded the by-laws of the City of Cape Town. It was hypothesised that the fact that the wastewater contained FOG, organic matter, and nutrients contributed to the limits being exceeded. The C:N:P ratio of the PSW was determined to be 167:6:1.

**Table 4-1**: Poultry Slaughterhouse wastewater characteristics and results gathered from this study compared to standards

| | | EGSB Feed | | | EGSB Product | | | City of Cape Town discharge by laws | NWA 2013 | SANS 241:2015 |
|---|---|---|---|---|---|---|---|---|---|---|
| **Parameters** | **Units** | **Min.** | **Max.** | **Ave.** | **Min.** | **Max.** | **Ave.** | | | |
| pH | | 6 | 8 | 7 | 6 | 8,6 | 7,6 | 5.5≤12 | 6≤ 9 | 5≤9.7 |
| Temperature | °C | 16 | 25 | 20 | 16 | 26 | 20 | 0≤40 | | |
| Conductivity | µs/cm | 798 | 2360 | 1479 | 524 | 3495 | 1515 | ≤500 | ≤200 | ≤170 |
| TDS | ppm | 567 | 2145 | 1059 | 372 | 2470 | 1073 | 4000 | | |
| Salinity | ppm | 390 | 926 | 772 | 238 | 1790 | 718 | | | |
| Turbidity | NTU | 99 | 1847 | 749 | 4 | 487 | 48 | | | ≤5 |
| tCOD | mg/L | 1423 | 11068 | 4981 | 550 | 2798 | 1359 | ≤5000 | ≤5000 | 1000-2400 |
| sCOD | mg/L | 129 | 1389 | 637 | 90 | 564 | 225 | | | |
| TSS | mg/L | 60 | 5165 | 1399 | 10 | 520 | 173 | 1000 | | |
| VSS | mg/L | 0 | 5655 | 1991 | 65 | 3000 | 927 | | | |
| FOG | mg/L | 312 | 1542 | 795 | 30 | 189 | 60 | 400 | | |
| BOD | mg/L | 850 | 6125 | 3090 | 10 | 275 | 112 | | | |
| VFA | mg/L | | | | 27 | 837 | 243 | | | |
| Alkalinity | mg/L | | | | 243 | 891 | 499 | | | |
| $NH_4$ | mg/L | 67 | 294 | 180 | 32 | 533 | 168 | <25 | ≤3 | ≤ 1.5 |
| Nitrate | mg/L | 0,1 | 11,4 | 2,0 | 0,5 | 18,7 | 6,3 | | ≤15 | ≤11 |
| Ortho-phosphate | mg/L | 13,4 | 46,2 | 29,1 | 11,9 | 33,5 | 24,1 | <25 | ≤10 | |

**Ave: All average values are reported from a set of triplicate measurements**

### 4.1.2. Bioreactor tolerance test: Variation of OLR and HRT

During the EGSB commissioning, the PSW was diluted with distilled water to prevent shock loading, a period classified as an acclimatization phase (48 h). Thereafter, an average HRT of 62.5 h was used for an additional 43 days at an average OLR of 1.0 g tCOD/L.day, with further operational changes being an average HRT of 57.5 h for an additional 49 days, with an average OLR of 2 g tCOD/L.day. The 50% diluted PSW was used as influent which was altered to 30% diluted PSW, and eventually to undiluted (100%) PSW feed. At undiluted PSW, HRTs of 60, 55, 48 and 36 h with corresponding OLRs of 2.12, 1.93, 4.07 and 5.32 g tCOD/L.day were evaluated respectively. The influence of the variation of the up-flow velocity on the undiluted (100%) PSW was not investigated as the up-flow velocity was constant at 0.1 m/h. As illustrated in Figure 4.1, the HRT varied between 36 h and 65 h for the 172 days of bioreactor operation with an overall average HRT of 54 h. The OLR ranged from 0.9–7.4 g tCOD/L.day with an average OLR of 2.4 g tCOD/L.day. For the undiluted PSW, the average HRT and OLR was 49.4 h and 3.4 g tCOD/L.day. The high HRT during the period of acclimatization was to prevent sludge washout. It was also observed that at 50% dilution the EGSB operated proficiently at two HRTs of 65 h and 60 h with corresponding average OLRs of 0.93 and 1.17 g tCOD/L.day respectively. Thereafter, at 70% PSW, two HRTs of 60h and 55 h with corresponding OLRs of 1.84 and 2.01 g tCOD/L.day respectively were evaluated.

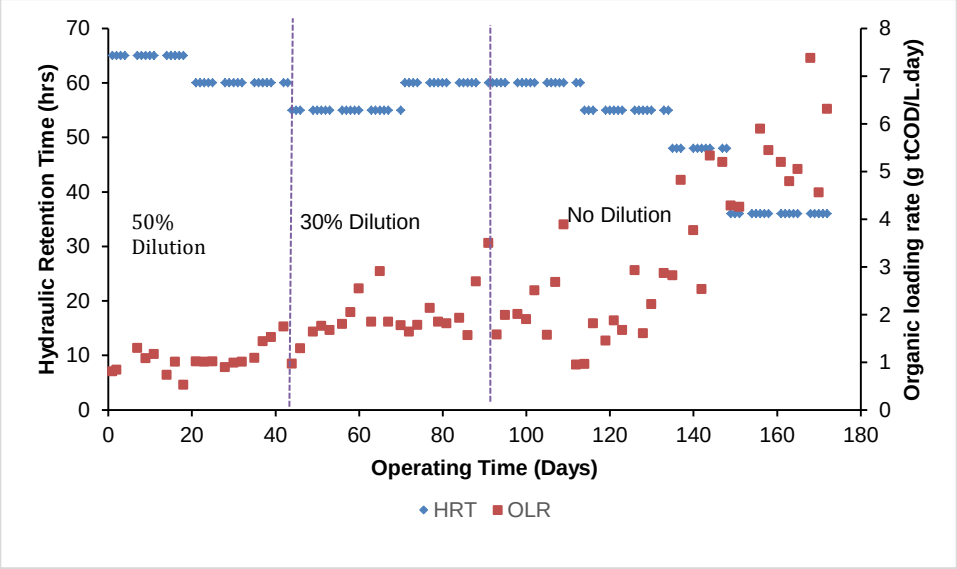



**Figure 4-1**: Variation of HRTs and OLRs during the 172 days

The tCOD concentration in the EGSB feed ranged from 1422 mg/L (min) to 11068 mg/L (max) with an average tCOD in-flow concentration of 4981 mg/L. Initially, the tCOD was used as a comparative parameter to quantify system performance and to monitor the effect of the

OLR for the anaerobic digester designed. For the EGSB, the product tCOD range was between 550 to 2798 mg/L, averaging a tCOD concentration of 1359 mg/L, which was lower than the required discharge standards prescribed (South African National Standards 2015).

### 4.1.3. EGSB performance based on COD removal

Figure 4.2 illustrates the overall tCOD removal using the EGSB. At 50% PSW dilution, it was observed that the tCOD removal fluctuated between 10% and 60% due to system and granular sludge stabilisation at an average HRT and OLR of 62.5 h and 1 g tCOD/L.day respectively. Thereafter, the tCOD removal at 70% (30% diluted) PSW fluctuated between 50% and 70%, which was influenced by the averaged HRT/OLR used i.e. of 57.5 h and 2 g tCOD/L.day. When the PSW was used without dilution, the tCOD removal increased from 60 % to 93% at an average HRT and OLR of 49.8 h and 3 g tCOD/L.day. The system when fed with undiluted PSW was determined to be effective due to the system stabilising with regard to tCOD removal, which remained constant.

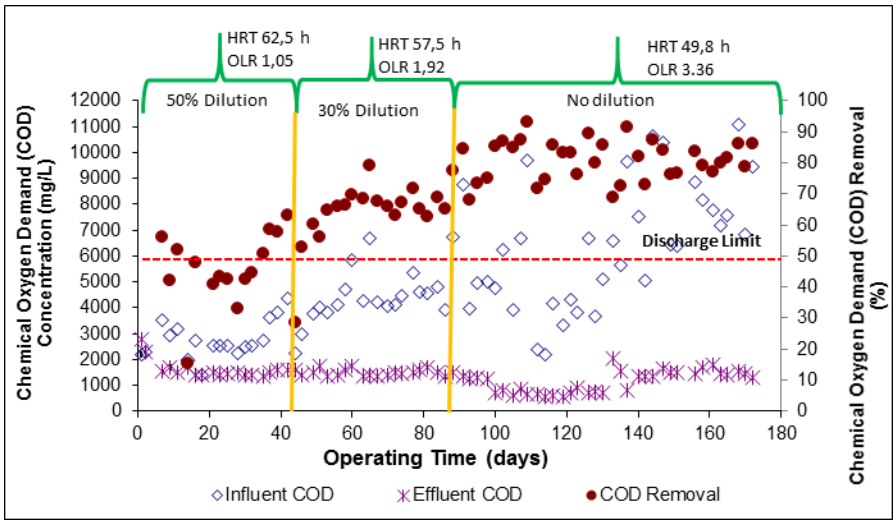
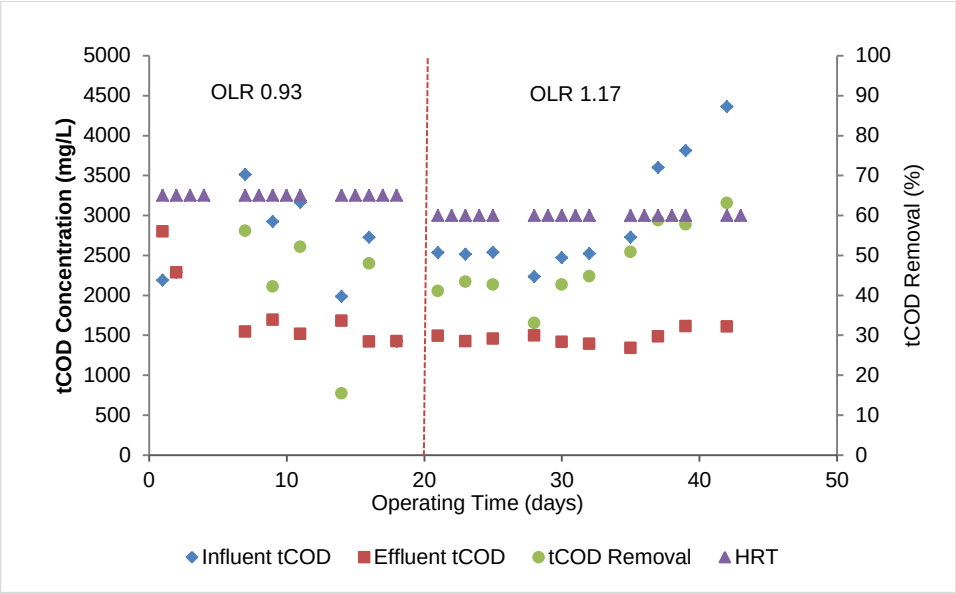



**Figure 4-2**: tCOD removal efficiency of the EGSB at different averaged HRTs, OLRs and dilutions

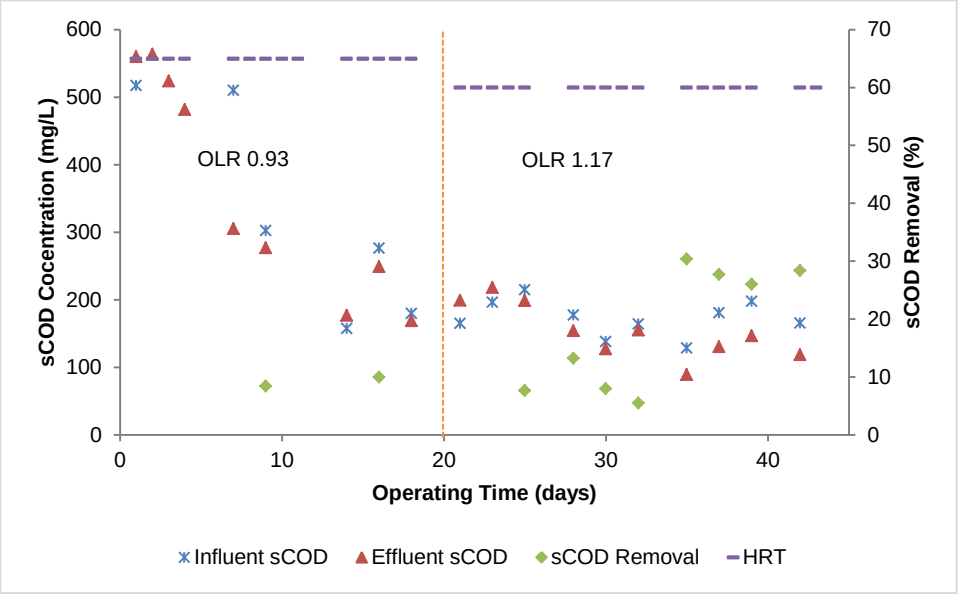
Overall, the initial influent to the EGSB was above the discharge standard limit of the City of Cape Town, (City of Cape Town Wastewater and Industrial Effluent By-Law, 2013) which prescribes that tCOD concentration should not exceed 5000 mg/L, while the effluent tCOD concentration post-treatment from the EGSB was below this discharge limit.

#### 4.1.3.1. Diluted PSW as feed

Figure 4.3 indicates the different tCOD concentrations at numerous experimental HRTs and average OLRs when using a 50% diluted PSW. The following HRTs and averaged OLRs were assessed: HRTs of 65 h for 19 days and 60 h for 23 days; and average OLRs of 0.95 and 1.17g tCOD/L.day respectively. Figure 4.3 (a) indicates the tCOD concentration and removal efficiency and Figure 4.3 (b) illustrates the sCOD removal efficiency and concentrations. The sCOD removal was lower than the tCOD removal, which is an anomaly, as it was expected that sCOD would be easier to biodegrade. Such a phenomenon can only ensue as a result of the system's instability. It was observed that at 50% diluted PSW, the highest tCOD removal was 60% at an HRT of 60 h and an average OLR of 1.17 g tCOD/L.day and the average tCOD removal was 46%; whereas the maximum sCOD removal was 30% with an average sCOD removal of 16.5%. For the 50% diluted PSW feed, the highest tCOD concentration quantified was 3500 mg/L which was still, below the discharge standard but only due to the feed being diluted with distilled water. Furthermore, FOG removal was 83% when a 50% diluted PSW was used.




(a)

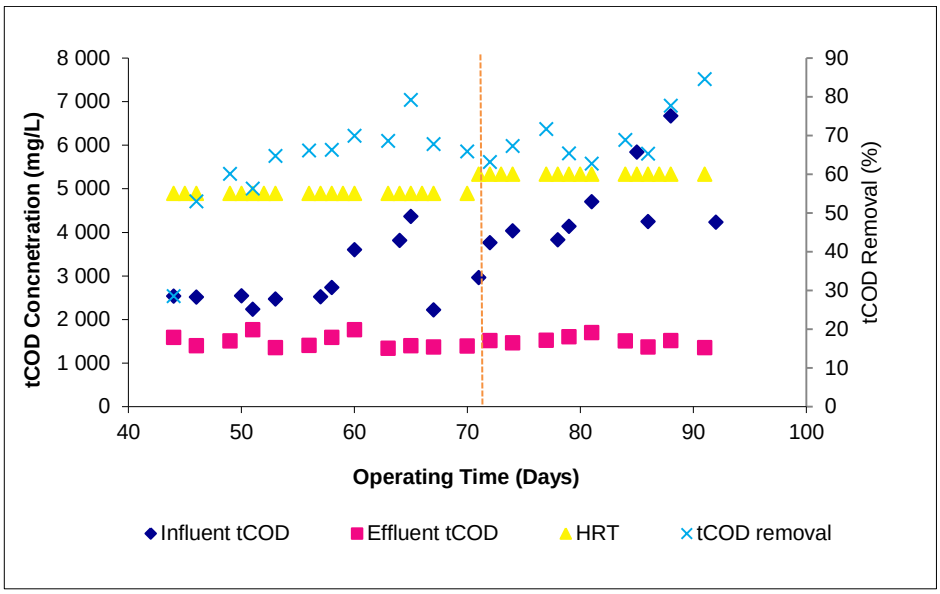



(b)

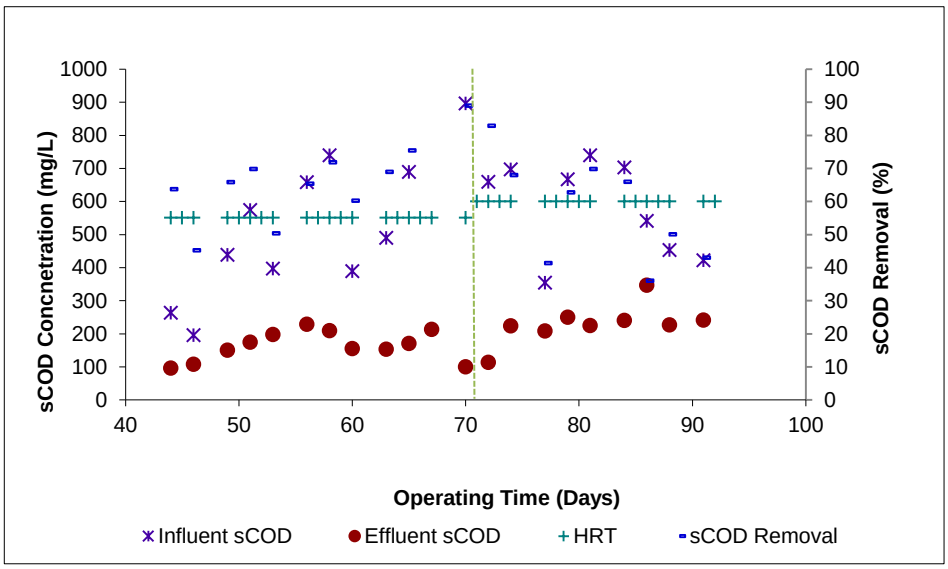
**Figure 4-3**: (a) EGSB tCOD concentrations and removal at the two HRTs and average OLRs using 50% PSW; (b) EGSB sCOD concentrations and removal at the HRTs and average OLRs using 50% PSW.

After the 50% diluted PSW was fed to the system, the feed concentration for the system was changed, for which a 30% dilution of PSW at two HRTs of 55 h and 60 h was used (see Figure 4.4). The corresponding average OLRs were 1.84 and 2.01 g tCOD/L.day respectively. The maximum and average sCOD removal (Figure 4.2 (b)) increased to 89% and 62.2% respectively. The average tCOD feed concentration was 4663 mg/L during this

stage, averaging at 1492 mg/L. During this stage, the overall tCOD removal was 65.3% at an average HRT of 57.5 h and an average OLR of 2 g tCOD/L.day. Furthermore, the FOG removal efficiency was still high at 87%.




(a)



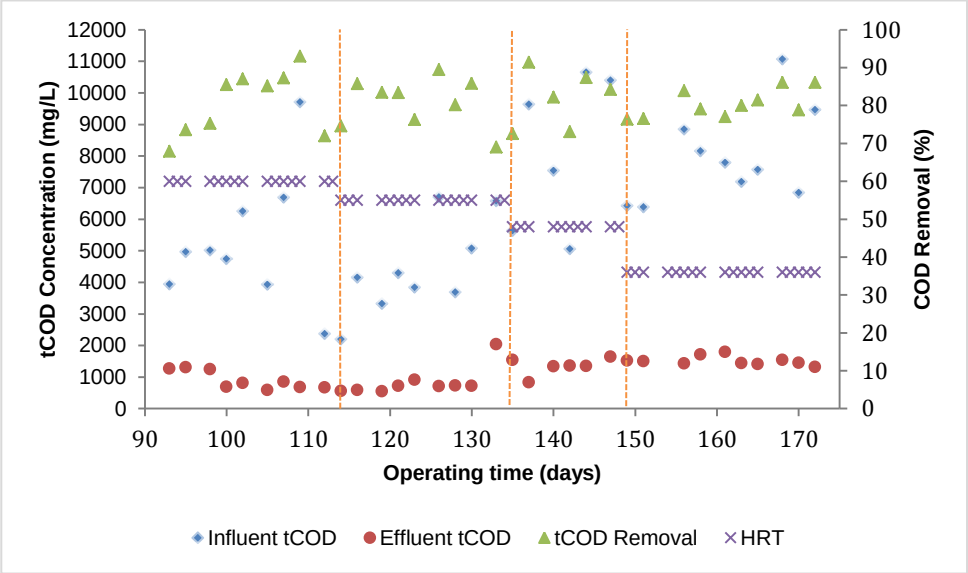

(b)

**Figure 4-4**: (a) EGSB tCOD concentrations and removal at the two HRTs and average OLRs at 30% diluted PSW; (b) EGSB sCOD concentrations and removal at the HRTs and average OLRs at 30% diluted PSW

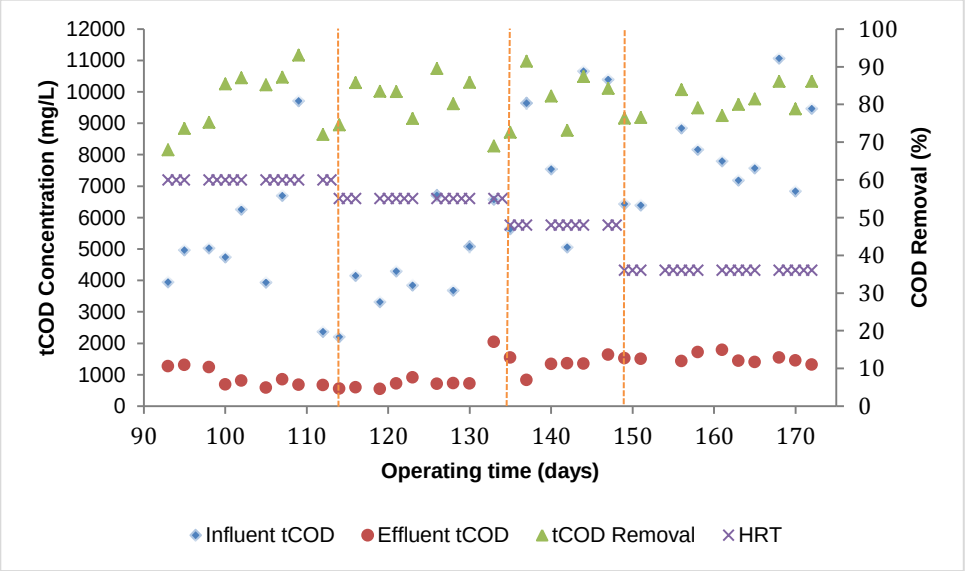
#### 4.1.3.2. Undiluted PSW as feed

Figure 4.5 (a) illustrates the performance of the EGSB when undiluted (100%) PSW was used, a phase in which four different HRTs and averaged OLRs were assessed. The four HRTs were 60, 55, 48 and 36 h respectively; this corresponded to average OLRs of 2.11,

1.93, 4.08 and 5.03 g tCOD/L.day. The highest and average tCOD influent concentration of the EGSB was 11068 mg/L and 6359 mg/L during this phase. Furthermore, it was observed from these values that the undiluted PSW was above the City of Cape Town discharge standard of <5000 mg/L. However, the highest and average post-treatment EGSB effluent tCOD concentrations were 2040 mg/L and 1149 mg/L respectively. The overall tCOD removal using the undiluted PSW was 81% at an average HRT and average OLR of 49.8 h and 3 g tCOD/L.day. Furthermore, increases in FOG removal were observed to be above 93%, although sCOD with undiluted PSW only averaged 72% (Figure 4.5 b).




(a)



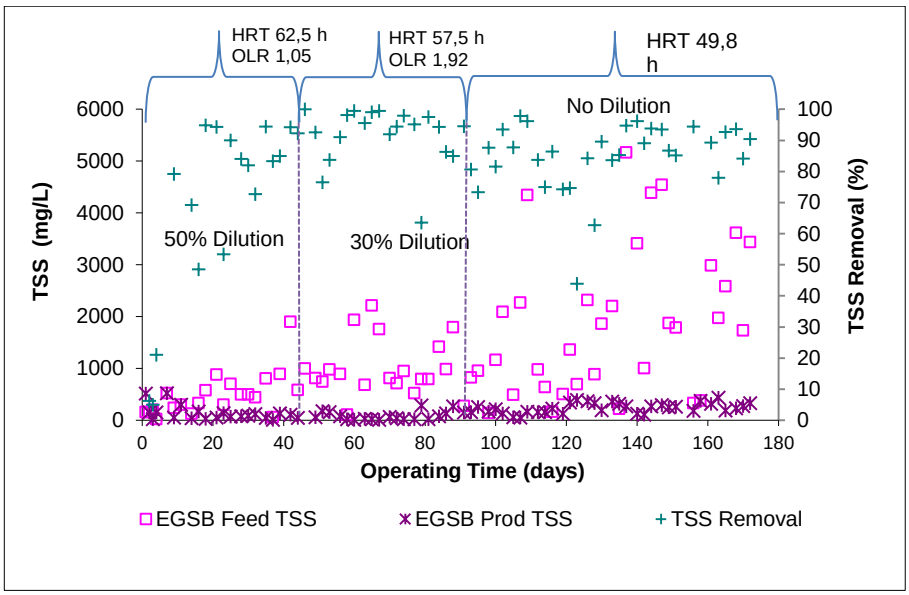

(b)

**Figure 4-5**: (a) EGSB tCOD concentrations and removal at the two HRTs and average OLRs at 100% PSW; (b) EGSB sCOD concentrations and removal at the HRTs and average OLRs at 100% PSW.

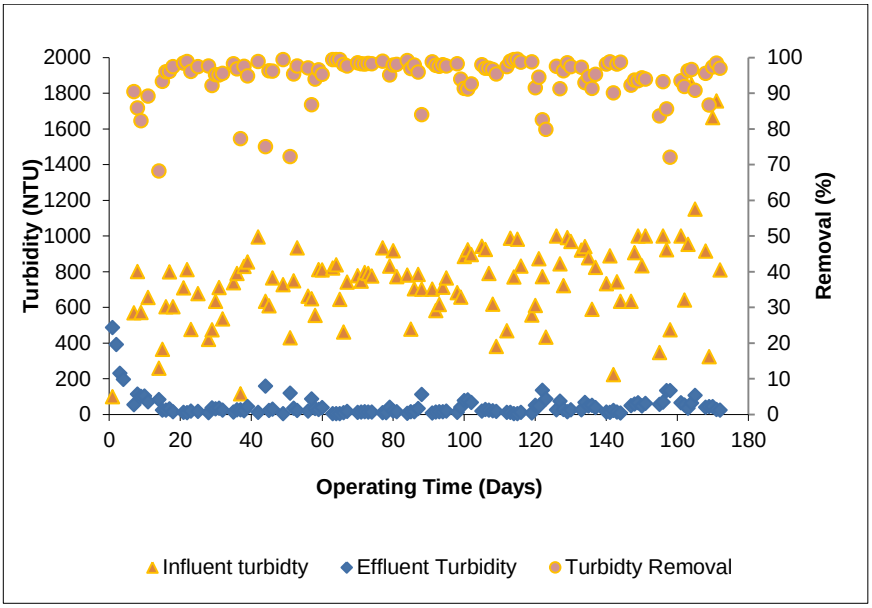
### 4.1.4. Total Suspended Solids (TSS) removal and turbidity reduction

The TSS was quantified to determine the concentration of organic and inorganic matter and suspended solids within the PSW (EGSB feed), and to evaluate TSS removal efficiency by the EGSB, in which the anaerobic bed acted as a biofilter under various HRTs, OLRs and up-flow velocities (see Figure 4.6 (a), which indicates variations for TSS in the influent and effluent, highlighting the overall TSS removal efficiency by the EGSB). Pre-treatment using filtration was conducted prior to the PSW being fed to the EGSB. It was observed that reduction of the TSS took 10 min to 520 mg/L (max), with subsequent TSS removal in the EGSB being 50 to 99%, averaging 83% during the system's operation. Minimum TSS removal was observed during the start-up period. For undiluted PSW, TSS removal stabilised at 70% removal, which is an indication of system efficiency when an appropriate start-up procedure is followed. At 50% dilution, an HRT and OLR of 62.5 h and 1 g tCOD/L.day was observed. The average removal was only 68%, followed by an average removal of 92% at an HRT of 57.5 h and OLR of 2 g tCOD/L.day. Furthermore, for undiluted PSW, the removal was observed to be 88% at 60 h HRT and 1.95 g tCOD/L.day averaged OLR.

The turbidity was measured, as the clarity of the wastewater influent and effluent is an important quantifiable parameter if the treated PSW is to be discharged into the environment (see Figure 4.6 (b), which illustrates the turbidity reduction of the EGSB effluent during the period of 172 days). As highlighted in Table 4.1 and SANS 241-2:2015, the optimal turbidity for drinking water should be <5 NTU; however, the effluent turbidity exceeded this limit. This is an indication that perhaps tertiary treatment systems are required for further treatment of the wastewater. The maximum turbidity reduction observed was 99%, averaging 94%. The turbidity reduction ranged between 68 and 99%, which indicated good reduction by the EGSB. The difference in turbidity reduction was attributed to the feed quality characteristics being varied throughout the study. The influent turbidity was in a range of 99 – 1847 NTU, whereas the effluent ranged from 4.2 – 487 NTU with an average turbidity of 47.6 NTU for effluent, and 749 NTU for the influent.




(a)




(b)

**Figure 4-6**: (a) Total Suspended Solids (TSS) results of the EGSB; (b) Turbidity results of the EGSB

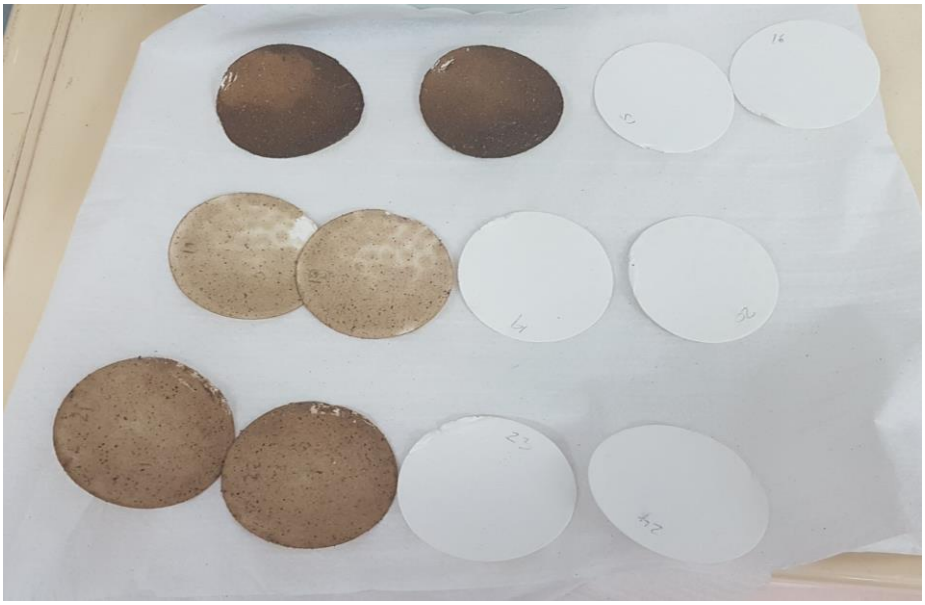


**Figure 4-7**: TSS results of the feed and product of the EGSB

It was observed that turbidity is directly influenced by TSS (see Figures 4.6 and 4.7), turbid PSW having a high concentration of TSS; although, the presence of blood in the PSW could have contributed significantly to the high NTU observed.

### 4.1.5. VFA/Alkalinity Ratio

The VFA/Alkalinity ratio is a parameter used to evaluate the stability of an anaerobic process (Cuetos et al., 2010). It is a measurement/parameter used to foresee if an anaerobic system failure is pending or not, before a change in pH occurs (Cuetos et al., 2010). A ratio that is less than 0.3 - 0.4 best describes the stability of the system (Liu et al., 2012), whereas anything greater suggests instability in the anaerobic bed, which could culminate in the system's failure. Figure 4.8 indicates an average VFA/Alkalinity ratio range between 0.09 (low) to 3.4 (max), which indicated that the system was initially unstable and gradually became stable as the ratio slowly reduced to <0.3 after 60 days of operation. The tCOD removal within the 60 days fluctuated between a lowly 28% and 60%, yielding an average tCOD removal of 50% which was indicative of the anaerobic bed's reduced functionality. There was also minimal biogas production within the first 60 days which was indicative of souring in the system. During the instability phase, it was observed that the turbidity of the EGSB effluent fluctuated and there was sludge washout, i.e. sloughing, resulting in dead biomass in the effluent. Additionally, the tCOD, TSS and FOG removal efficiencies were low at 50%, 75% and 86% respectively, when compared to when the EGSB was operating at steady state. All these observations can be attributed to 1) molecular oxygen exposure

during inoculation and start-up period, and 2) the slow growth rate of microbial biomass constituting the anaerobic bed.

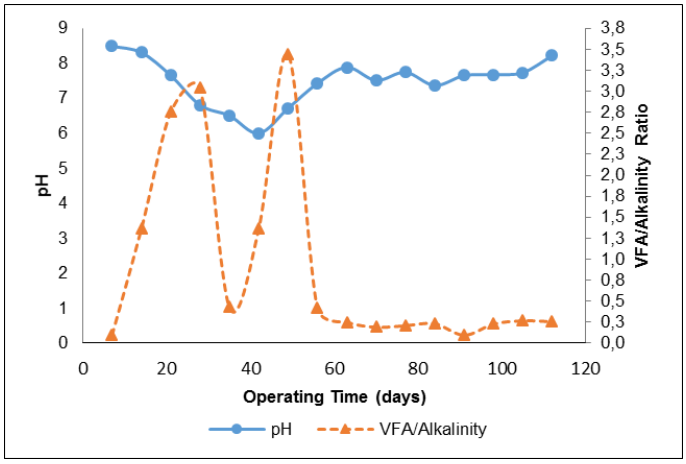



**Figure 4-8**: Average weekly pH and VFA/Alkalinity ratio of the EGSB

### 4.1.6. Temperature and pH

The influent pH varied from 6 – 7.5 with an average pH of 6.7, while the effluent pH varied from 6 – 8.6 and an average 7.6 respectively, as shown in Figure 4.9. Furthermore, the temperature varied, fluctuating between 16 and 25 °C with an average temperature of 20 °C measured within the bioreactor, which was indicative of the unsuitability of the water bath used to supply heated water to the heating jacket, even though the system was jacketed to minimise heat loss. It is important that the temperature of a system such as an EGSB is kept constant as temperature affects bacteria production and organic matter digestion. Generally, when the temperature decreases or increases, it affects bacterial activity, whereas with high temperatures some bacteria can be deactivated, which will affect the production of biogas (Samani Majd et al., 2017), further affecting the microbial diversity of the digester. Furthermore, as pH plays an important role with regard to methane (biogas) generation within an anaerobic digester, very low and/or high pH can be detrimental to the digester. For good methane generation, a pH of 6.5 – 7.8 is recommended, and for acid-forming bacteria, a pH of 5 – 6 is required (Samani Majd et al., 2017). Zhai et al. (2015), observed that at pH 7.5, the highest quantity of methane can be produced, and also at a maximum pH of 8, with a pH below 7 resulting in decreased methane.

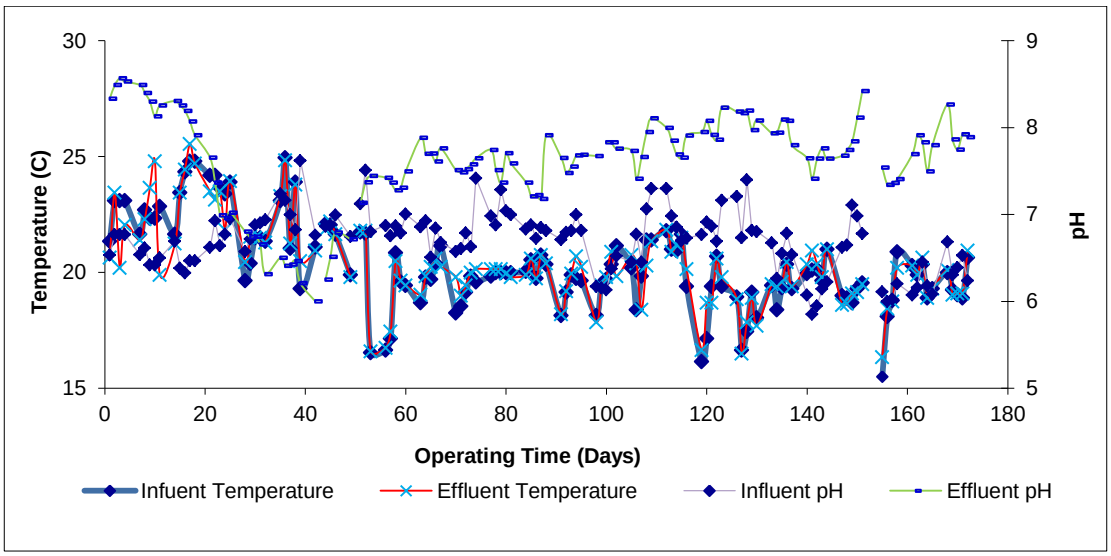



**Figure 4-9**: Temperature and pH of effluent and feed

### 4.1.7. Nutrient removal: ammonium-nitrogen and ortho-phosphate

Table 4.2 lists ammonium-nitrogen and ortho-phosphate removal within the EGSB. The overall nutrient removals were 0.1%, 24.2% and 0.2% for ammonium nitrogen, nitrate-nitrogen and ortho-phosphate respectively. However, from the values reported herein, it was observed that the nitrate-nitrogen had a higher removal than the ammonium nitrogen and ortho-phosphate. The nitrate-nitrogen removal seemed to be directly proportional to the HRT. The higher the HRT, the higher the nitrate-nitrogen removal, i.e. at 36h it was 82% and at 48h it was 95%. The removal of this nitrogen constituent is normally achieved by the retention of organic N by biomass for growth purposes. As highlighted in Table 4.2 (a) it was observed that there was a higher ammonium-nitrogen removal only at 30% PSW with removal efficiency of 44%. However, when undiluted PSW was fed to the EGSB, it was observed that at a higher HRT (60) there was a higher ammonium-nitrogen removal of only 26% compared to the other HRTs. With regard to the low removal rates for the system, oxidation of $NH_4^+$ via the nitrification mechanism seemed to be low, due to minimal presence of dissolved oxygen, which led to the low removal efficiency observed (Ge et al., 2013). Furthermore, the accumulation of $NH_3$-N within the system as observed in the effluent, was attributed to the conversion of organic N into $NH_4$-N ions and nitrogen gas. The low treatment efficiencies are expected for nitrogenous bacteria, particularly ammonia and phosphates, since anaerobic reactors are known to reduce a negligible quantity of these nutrients (Yetilmezsoy and Sakar, 2008b). This necessitates the commissioning of a secondary treatment process, i.e. Single stage nitrification and denitrification. However, for ortho-phosphate removal, the efficiencies were much higher at 30% diluted PSW with an average removal of 28%, with the highest average removal of 69.6% at HRT of 55 h. The slightly

better performance regarding phosphorus removal indicated that phosphorus removal was indeed occurring.

**Table 4-2**: (a) $NH_4$-N removal (b) Ortho-phosphate removal

(a)

**NH4-N Removal**

| Dilution | Removal (%) | HRT(h) | Average removal per dilution |
|---|---|---|---|
| 50% | 30 | 60 | 33% |
| 30% | 55 | 55 | 44% |
| | 31 | 60 | |
| Undiluted | 26 | 60 | 13% |
| | 15 | 55 | |
| | 13 | 48 | |
| | -1 | 36 | |

**Ave: All average values are reported from a set of triplicate measurements**

(b)

**Ortho-phosphate removal**

| Dilution | Removal (%) | HRT (h) | Average removal per dilution |
|---|---|---|---|
| 50% | 0.01 | 55 | 0.01 % |
| 30% | 69.6 | 55 | 28% |
| | 22 | 60 | |
| Undiluted | 27 | 60 | 26% |
| | 19 | 55 | |
| | 30 | 48 | |
| | 29 | 36 | |

**Ave: All average values are reported from a set of triplicate measurements**

### 4.1.8. Biogas production

During the 172 days of operation, only a 0.5 L Tedlar bag containing biogas was produced at an HRT of 55 h and an average OLR of 1.84 g tCOD/L.day when 30% diluted PSW was fed into the bioreactor, which is indicative of leakages within the system designed and the low temperature measured in the EGSB. The composition and concentrations of $CH_4$, $CO_2$, $O_2$, $H_2$, $H_2S$ and $N_2$ in the biogas were 40.4 %, 3.4%, 12.9%, 0.005%, 0.0092% and 41.21% respectively. Parameters affecting biogas production include, amongst others temperature, pH, OLR, and HRT, with thermophilic temperatures being suitable for biogas production whereas mesophilic temperatures were indicated to produce low methane yields (Mao et al., 2015). From the raw data, the system was operated at mesophilic temperature as seen in Figure 4.9. Similarly, pH also affects biogas production whereby pH below 6.3 decreases methane production rates (Sheldon and Erdogan, 2016). According to Mao et al. (2015), the

optimum pH for acidogenesis was between 5.5 and 6.5, and between pH 6.5 – 8.2 for methanogenesis, with the growth rate of methanogens being at a pH below 6.6. Figure 4.9 shows that the system was at optimal pH levels with the lowest influent pH of 5.9, and the lowest effluent pH of 6 which is in the range for acidogenesis and methanogenesis to occur. The highest influent pH of 7.6 and 8.6 for the effluent was observed. The average pH for influent and effluent was 6.7 and 7.6, which was within a range suitable for biogas production. With regard to the HRT and OLR, it was stated that a low OLR and a high/low HRT can culminate in the production of high methane yields (Mao et al., 2015). Ammonium-nitrogen ($NH_4$-N) adversely affects methane production as well, as it is one of the inhibitors involved in reduced methane production (Samani Majd et al., 2017). Ammonia-nitrogen is a nutrient for bacterial growth; however, it can also prevent and reduce growth if its concentration is high, with concentration exceeding 150 mg/L being reported to have an inhibitory effect on anaerobic digestion. This is because methane-generating and hydrogen consuming bacteria are sensitive to ammonium ions (Samani Majd et al., 2017; Cao et al., 2011). Therefore, a high removal of ammonium-nitrogen can significantly increase the rate of methane production. For this study, the ammonium-nitrogen concentration was estimated to be an average of 168 mg/L, as observed in the effluent stream of the EGSB, exceeding the reported 150 mg/L maximum concentration required for biogas production.

Similarly, the C/N ratio for the EGSB was between 10 and 61 with an average C/N ratio of 28. According to Mao et al. (2015), the optimal C/N ratio for anaerobic digesters should be between 20 and 35, with 25 being the most suitable ratio. Values out of the range could results in ammonia-nitrogen inhibition. The EGSB had an average C/N ratio of 28.1 with a maximum of 68.1 and minimum of 10.7. These values are all out of the required range. The fluctuation between the ratios is one of the reasons for ammonia-nitrogen inhibition.

### 4.1.9. Volatile Suspended Solids (VSS)

Figure 4.10 illustrates the efficiency of the VSS within the EGSB over a period of 172 days. The highest VSS removal efficiency was found to be 88%, which occurred at 36 h HRT when undiluted feed was used. Furthermore, the overall VSS removal efficiency was 55%. The maximum VSS concentration was 5655 mg/L (feed), which was reduced to a minimum of 65 mg/L (product). With a 50% dilution feed at an averaged HRT of 60 h and averaged OLR of 1.17 g tCOD/L day, the maximum VSS removal efficiency was 88%, averaging a VSS removal efficiency of 48%. The average removal efficiency increased to 65% when a 70% PSW was used, at an averaged HRT and OLR of 57.5 h and 2 g tCOD/L.day, with the highest removal efficiency being 87% at 55 h HRT. Furthermore, at a 55h HRT, the average removal efficiency was only 70%. For undiluted PSW, the average removal efficiency was

found to be 51% at 49.8 h HRT and a higher OLR of 3.65 g tCOD/L.day. Overall, the highest removal efficiency was 82% during this period, i.e. at a 48 h HRT.

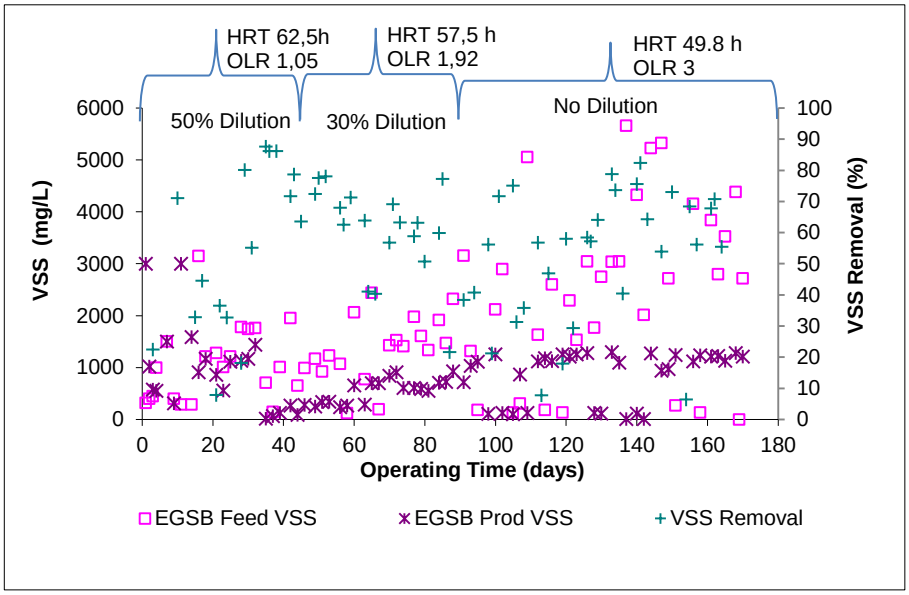



**Figure 4-10**: Volatile Suspended Solids (VSS) of EGSB feed and product as well as the removal

## 4.2. Phase 2: Single stage nitrification/denitrification efficiency and effectiveness

As numerous parameters can describe the performance of the Single Stage simultaneous nitrification and denitrification (SSND) system, several parameters were quantified, i.e. tCOD, ammonium-nitrogen, nitrate-nitrogen, nitrite-nitrogen, with and without sparging, under two operational configurations, i.e. a down-flow and an up-flow configuration.

### 4.2.1. Total nitrogen removal

#### 4.2.1.1. Down-flow configuration: Effect of no sparging

During the SSND commissioning, unfiltered and undiluted PSW (v=0.018m$^3$), including 100 mL Basal media, were decanted into the column bioreactor during the acclimatization period (48 h) at 24 hr intervals, without being continuously fed with the PSW. Thereafter, the PSW was fed into the column in a down-flow configuration with no sparging, with only the feed being exposed to the atmosphere. The system operated for 26 days at an HRT of 11.54 days and thereafter, an HRT of 7.72 days was used for 22 days. After 16 days of operation, the system was supplemented with 100mL of raw unfiltered PSW to rejuvenate the biomass cultures within the SSND column, as the feed and product total nitrogen seemed to have minimal variations, i.e. reached equilibrium, with minimal tCOD concentration and ammonium-nitrogen removal rates.

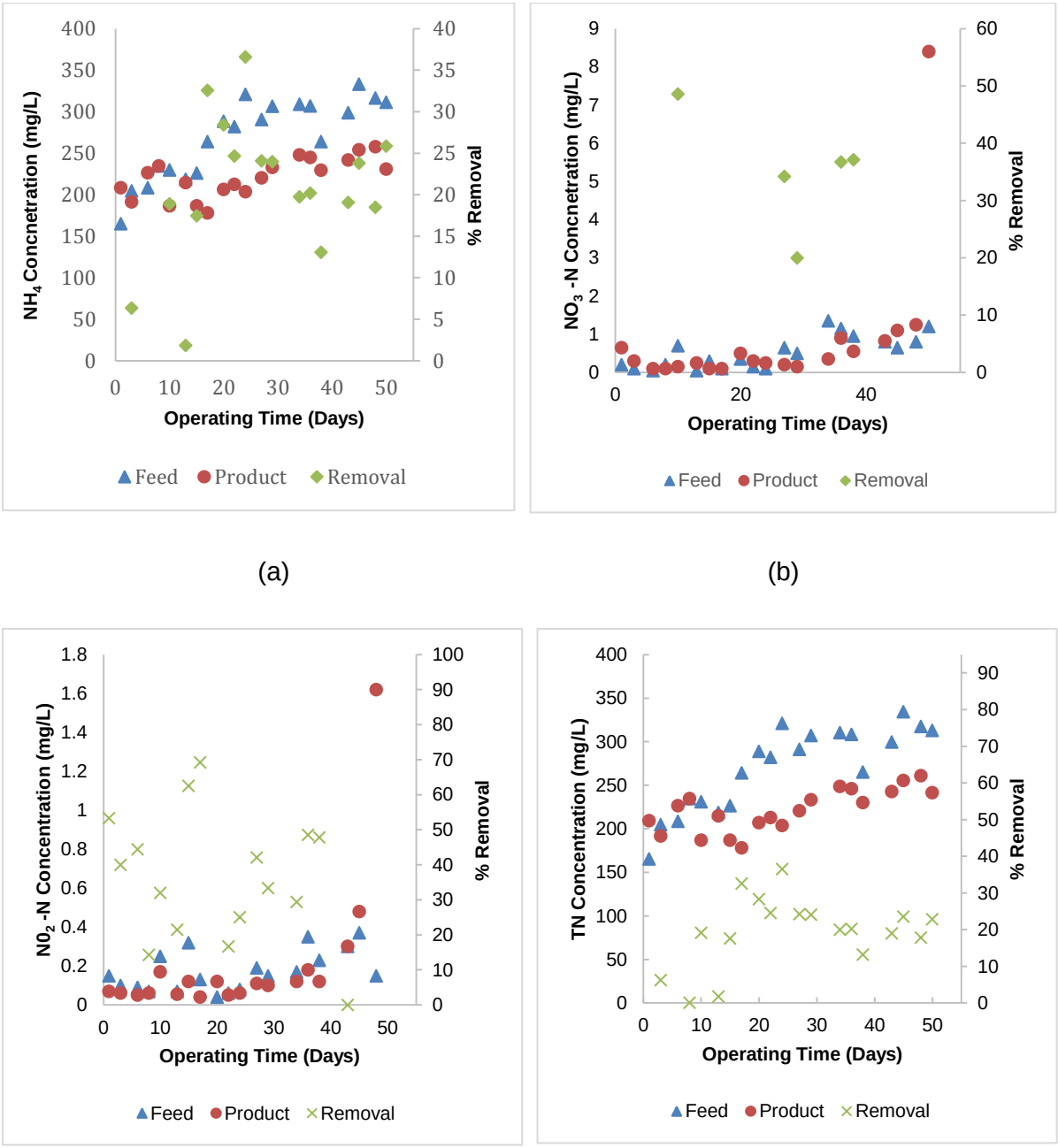



**Figure 4-11**: (a) Ammonium-nitrogen removal efficiency of the SSND; (b) Nitrate-nitrogen removal efficiency of the SSND; (c) Nitrite-nitrogen removal efficiency of the SSND, (d) Total nitrogen removal of the SSND

Figure 4.11, illustrates the differentiation in total nitrogen removal efficiencies using the SSND. It was observed that the ammonium-nitrogen removal (see Figure 4.11(a)) efficiency was low with a maximum removal of 37% and an average removal of 21% at an HRT of 11.54 days being observed. The maximum removal efficiency occurred at 72 h, subsequent to the supplementation of the cultures with raw unfiltered PSW. Thereafter, at an HRT of 7.72

days, the average and maximum efficiency was 21% and 25% respectively, indicating a reduction in the ammonium-nitrogen reduction.

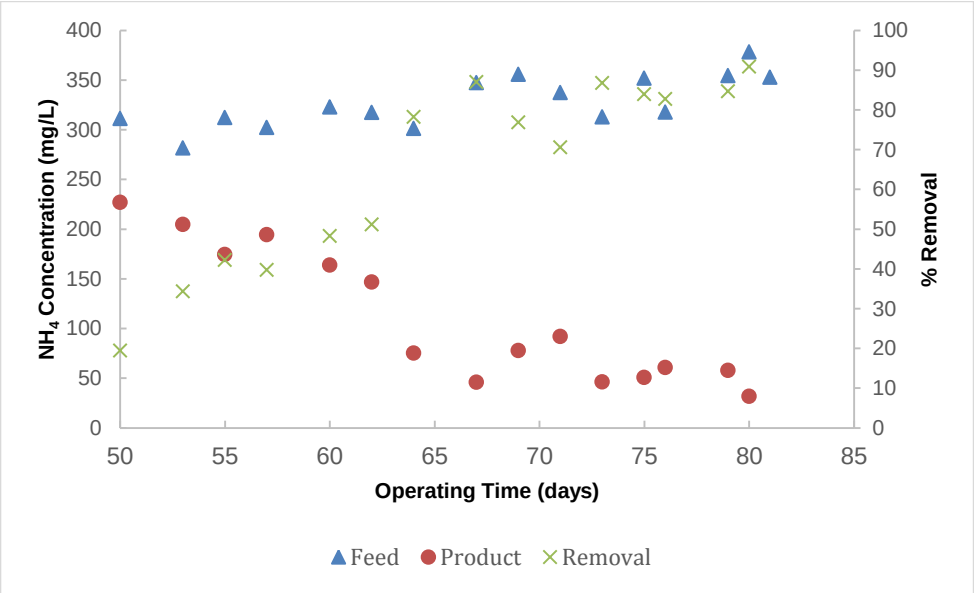
Similarly, Figure 4.11(b) illustrates the nitrate-nitrogen removal efficiency within the SSND. The highest removal efficiency was 34%, at an HRT of 11.54 days. This lowly removal efficiency indicated that there was minimal conversation of nitrate-nitrogen, i.e. denitrification, which in turn resulted in the accumulation of the contaminant within the system. As explained by von Sperling and Ponds (2007), denitrification occurs when nitrates are reduced to nitrogen gas, and without such a reduction, toxicity, and thus culture inhibition, can ensue. By supplementing the cultures of the SSND with the unfiltered PSW, the highest removal efficiency occurred within 96 h at a 7.72 days HRT, with the maximum removal efficiency of 37%.

Furthermore, as indicated in Figure 4.11(c), nitrite-nitrogen removal efficiency was at a maximum of 69%, averaging 41% respectively, at an HRT of 11.54 days. However, after the supplementation of the cultures with the raw unfiltered PSW (100 mL), minimal nitrate-nitrogen removal/reduction was observed. However, after 49 h of system stabilisation, further reduction of nitrite-nitrogen ensued, albeit minute rates, with further increases at an HRT of 7.72 days, achieving maximum and average removal of 49% and 32% respectively.

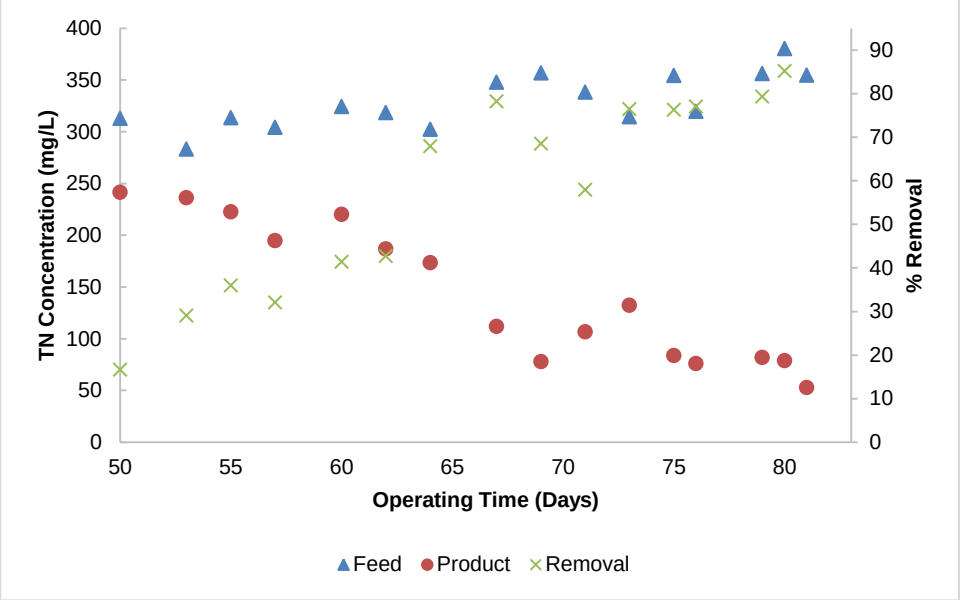
For total nitrogen removal (Figure 4.11(d)) assessed for 48 days, the maximum total nitrogen removal was a lowly 29% with the SSND system only starting to achieve higher total nitrogen removal rates only after 14 days of operation. This was attributed to the systems' instability at the initiation of the SSND operation. After such an evaluation, a reactor re-configuration strategy was developed, in which an up-flow feeding strategy and sparging were introduced. The disadvantage of sparging is associated with ammonium-nitrogen stripping; therefore, low sparging rates were deemed sufficient to maintain DO culture requirements within the SSND.

#### 4.2.1.2. An Up-flow configuration effect on total nitrogen removal: influence of sparging

After 48 days of operation the SSND systems' configuration was changed to an up-flow configuration and sparging was introduced into the column. The SSND was operated at an HRT of 13.74 days for a further 27 days. Figure 4.12 illustrates the ammonium-nitrogen and total nitrogen removal efficiency over the 27 days used for the up-flow configured system.




(a)




(b)

**Figure 4-12**: (a) Ammonium-nitrogen removal of SSND; (b) Total nitrogen removal of SSND

Figure 4.12 (a); illustrates the ammonium-nitrogen removal of the SSND at a HRT of 13.74 days over a period of 27 days. For this strategy, the maximum removal efficiency was 91% with an average of 65%. The lowest ammonium-nitrogen in the product was 31 mg/L. From the removal efficiency, it was observed that nitrification had occurred as the ammonium-nitrogen was transformed to nitrates. According to von Sperling and Ponds (2007), nitrification occurs when ammonia is transferred to nitrites and these nitrites to nitrates. However, there was no nitrates-nitrogen or nitrite-nitrogen removal within the SSND after air

was introduced into the reactor to contribute to ammonia transformation, as this requires anoxic conditions. From Figure 4.12 (a), it can be observed that by introducing sparging, the transformation of the ammonium nitrogen to other nitrogenous by-products did occur. As nitrate-nitrogen and nitrite-nitrogen had accumulated in the SSND reactor, it was obvious that the oxidised forms ammonium nitrogen, i.e. nitrate-nitrogen and nitrite-nitrogen, were not removed but merely transformed as converted by-products from ammonium nitrogen.

As observed in Figure 4.12 (b), the removal efficiency increased by 27.4% when compared to the initial configuration whereby a down-flow feeding scheme was used. For this configuration, the maximum TN removal efficiency was 85% with an average removal efficiency of 57% for the SSND system. It was observed from the removal efficiency that the total nitrogen was reduced which concludes that a simultaneous nitrification and aerobic denitrification took place in the SSND system. The dissolved oxygen measured across the column was at a maximum of 4.36mg/L, with a minimum of 0.40 mg/L being observed. The average DO across the SSND column was 1.38 mg/L.

Comparing the two configurations with regard to nitrification and aerobic denitrification, it was observed that sparging induced nitrification, albeit at a slow pace, with aerobic denitrification ensuing at a similar rate. Nevertheless, with the introduction of sparging, nitrification and aerobic denitrification did indeed occur in the SSND via the nitrite decomposition mechanism; however, nitrate oxidation was suppressed. When the DO concentrations were low, the $NH_4$ - N effluent concentrations from the SSND were high, while the opposite was observed at high DO concentration. Therefore, a recycle should have been implemented to reduce the nitrates to nitrogen gas for sustainable aerobic denitrification to occur.

A justifiable juxtaposition for the high nitrate concentrations was probably due to the inhibition of *Nitrobacter* sp. and the high DO concentration levels.

### 4.2.2. Single stage nitrification/denitrification based on tCOD removal

Table 4.3 lists the analysed findings on tCOD removal efficiency of the SSND. For the down-flow configuration, the average tCOD removal was a lowly 7% (ave.) with a maximum of 12% and a minimum of 3% at an HRT of 11.54 days for the 27 days used for the reactor operation. Thereafter, at an HRT of 7.72 days (24 days), a slightly higher averaged tCOD removal rate of 15.7% was observed, computed by using the observed maximum removal efficiency of 29% and a minimum of 7.72%. For the up-flow configuration, the average and maximum tCOD removal was 17.3% and 28% respectively. It was observed that the SSND column was not efficient in removing tCOD because the maximum tCOD for the SSND system was low at 30% removal. However, the product tCOD concentration of the column was still below the discharge standard of <5000 mg/L. The tCOD concentration of the

product from the SSND ranged between 2177 mg/L (max) and 1232 mg/L (min) with an average concentration of 1477 mg/L.

**Table 4-3:** tCOD removal efficiency of the SSND

| Configuration | HRT (days) | Highest (%) | Lowest (%) | Average (%) |
|---|---|---|---|---|
| Down-flow | 11.54 | 12 | 3 | 7 |
| | 7.72 | 29 | 5.8 | 15.7 |
| Up-flow | 13.74 | 28 | 6.5 | 17.3 |

**Ave: All average values are reported from a set of triplicate measurements**

### 4.3. Phase 3: Membrane effectiveness and efficiency

#### 4.3.1. Comparison between UF and MF membrane bioreactor performance

Table 4.4 and 4.5 shows the averaged results obtained from the membrane systems in which both MF and UF membrane systems were used. Table 4.4 shows the averaged results obtained from the EGSB product that was fed through the membrane systems at two flow rates of 0.6 and 0.65 mL/min, whereas, Table 4.5 indicates the comparison of both UF and MF membrane systems for the SSND product at a similar flow rate to that used for the EGSB product, i.e. 0.6 and 0.65 mL/min. The tCOD, conductivity, TSS, TDS and turbidity were used to determine the performance and effectiveness of the membrane systems. For the EGSB product, it was seen that the turbidity decreased from 12.53 NTU to 1.63 NTU when the MF membrane systems were used and from 18 NTU to 0.51 NTU when UF membrane systems were implemented, which translated into turbidity reduction of 86% for MF systems and 97% reduction for UF modules. Significant changes with regard to conductivity did not occur for both the UF and MF membrane systems under evaluation. The tCOD decreased from 200 mg/L to 79 mg/L for the MF membrane systems and from 168 mg/L to 62 mg/L for the UF membrane systems. However, the removal efficiency variation between the differently sized membranes was only 2%, with the UF membrane having the higher average removal efficiency of 62%. The suspended solids had a low average of <50% removal but the concentration of the permeate TSS was less than the discharge standards.

Table 4.4 (a) and (b) indicates the comparison of the two membrane systems evaluated with regard to filtering the SSND product and the EGSB product. The values tabulated are averaged values. From the results obtained, it was observed that the turbidity from the UF permeate was less than that of the MF systems. The conductivity and TDS removal were determined to be insignificant for either membrane systems. Tentatively, the turbidity for the UF membrane systems indicated a 95% decrease in NTU compared to the MF membrane

systems. The TSS removal efficiency for the UF membrane systems was only 57% whereas for the MF systems, a lowly 46% removal efficiency was achieved. For both the UF and MF membrane systems, the tCOD removal was low.

Table 4.4 lists analyses results of both the EGSB and SSND product filtered through the UF membrane systems at a higher flow rate, i.e. 1.1 mL/min. From the results obtained regarding the tCOD and TSS removal efficiency, the EGSB permeate results seemed more promising than the SSND permeate. However, comparative analyses indicated that the tCOD removal efficiency decreased by 4% while the TSS removal efficiency remained unchanged. Furthermore, the SSND tCOD removal efficiency increased to 27%.

Overall, all the pollutants indices, except for conductivity, were below the discharge standards of CCT as well as the SANS 241-2:2015.

**Table 4-4**:(a) Comparison of the UF and MF systems using the EGSB product; (b) Comparison of the UF and MF systems using the SSND product

(a)

| | | MF systems | | | UF systems | | |
|---|---|---|---|---|---|---|---|
| | | Product | Permeate | Removal | Product | Permeate | Removal |
| Parameter | Units | Ave. | Ave. | (%) | Ave. | Ave. | (%) |
| Temp | °C | 25.6 | 25.3 | n/a | 23 | 24 | n/a |
| pH | | 8 | 8.2 | n/a | 8.27 | 8.54 | n/a |
| Conductivity | µs | 2300 | 2110 | 8.2 | 2330 | 2079 | 10 |
| TDS | ppm | 1595 | 1381 | 13.4 | 1655 | 1478 | 10 |
| Turbidity | NTU | 12.53 | 1.63 | 86 | 18 | 0.51 | 97 |
| TSS | mg/L | 280 | 163 | 42 | 290 | 153 | 47 |
| tCOD | mg/L | 200 | 79 | 60 | 168 | 62 | 62 |

**Ave: All average values are reported from a set of triplicate measurements**

(b)

| | | MF systems | | | UF systems | | |
|---|---|---|---|---|---|---|---|
| | | Product | Permeate | Removal | Product | Permeate | Removal |
| Parameter | Units | Ave. | Ave. | (%) | Ave. | Ave. | (%) |
| Temp | °C | 25.9 | 25.1 | n/a | 23 | 24 | n/a |
| pH | - | 8 | 8.1 | n/a | 7.5 | 7.8 | n/a |
| Conductivity | µs | 1989 | 1884. | 5.2 | 1565 | 1474 | 6.2 |
| TDS | ppm | 1400 | 1287.5 | 7.9 | 1113 | 1047 | 6.3 |
| Turbidity | NTU | 10 | 6.5 | 56 | 6.9 | 0.37 | 95 |
| TSS | mg/L | 330 | 177.5 | 46 | 285 | 123 | 57 |
| tCOD | mg/L | 165 | 138 | 16.6 | 151 | 121 | 19.3 |

**Ave: All average values are reported from a set of triplicate measurements**

**Table 4-5**: Results of both EGSB and SSND at increased flow rate

| | | EGSB | | | SSND | | |
|---|---|---|---|---|---|---|---|
| | | Product | Permeate | Removal | Product | Permeate | Removal |
| Parameter | Units | Ave. | Ave. | (%) | Ave. | Ave. | (%) |
| Temp | °C | 27 | 27.5 | - | 27.3 | 27.5 | - |
| pH | | 8.1 | 8.6 | - | 7.0 | 7.4 | - |
| Conductivity | µs | 2325 | 1550 | 6 | 1094 | 1042 | 4.8 |
| TDS | ppm | 1650 | 1550 | 6 | 777 | 140 | 4.8 |
| Turbidity | NTU | 31 | 0.82 | 97 | 6.38 | 0.15 | 98 |
| TSS | mg/L | 260 | 150 | 42 | 190 | 170 | 10 |
| tCOD | mg/L | 190 | 84 | 56 | 128 | 94 | 27 |

**Ave: All average values are reported from a set of triplicate measurements**

### 4.3.2. Overall TSS, tCOD removal of the EGSB and SSND coupled with the membrane bio-reactor systems

Table 4.6 illustrates the overall turbidity, TSS and tCOD removal of the EGSB-MBR system as well as the EGSB-SSND-MBR system. Both systems had similar removal efficiencies which were 99%, 92% and 99% for the turbidity, TSS and tCOD respectively. For the overall efficiency results of the AD and MBR (EGSB-MBR) system, the results of EGSB feed and permeate of the UF and MF membrane bio-reactor was used. Whereas with regard to the overall system of the EGSB followed by the SSND and side stream MBR (EGSB-SSND-MBR), each product of the individual treatment stages i.e. EGSB, SSND and MBR (UF and MF) as well as the feed of the EGSB was used to determine the removal efficiencies of the overall continuous system.

**Table 4-6**: Overall Turbidity, TSS and tCOD removal of the overall system

| | | EGSB-MBR | | | EGSB-SSND-MBR | | |
|---|---|---|---|---|---|---|---|
| | | EGSB | MBR | | SSND | MBR | |
| | | feed | permeate | Removal | feed | permeate | Removal |
| Parameter | Units | Ave. | Ave. | (%) | Ave. | Ave. | (%) |
| Turbidity | NTU | 642 | 1.02 | 99 | 18.5 | 2.7 | 99 |
| TSS | mg/L | 2491 | 156 | 92 | 280 | 154 | 92 |
| tCOD | mg/L | 9008 | 73 | 99 | 185 | 122 | 99 |

**Ave: All average values are reported from a set of triplicate measurements**

(a) (b)

**Figure 4-13**: (a) Colour change of EGSB feed (left) and SSND-MBR product (Right); (b) Colour change of the overall system, EGSB feed (Left), EGSB-MBR product (Middle), SSND-MBR product (Right).

## 4.4. Phase 4: Response Surface Methodology (RSM) experiments

### 4.4.1. EGSB performance predicted using RSM and CCD

The effect of HRT and OLR on the EGSB using tCOD removal was investigated. A Central Composite Design (CCD) was used to determine and evaluate the effect of HRT and OLR on tCOD removal in the EGSB reactor. Furthermore, the optimum operating conditions with regard to HRT (B) and OLR (A) was determined. The experimental results for the tCOD removal using RSM and CCD are listed in Table 4.7. A total of 15 runs were investigated for the experiment. The response variable was the % tCOD removal.

The interaction between HRT, OLR and tCOD removal was analysed and the fitness of the model reduced to a two factorial (2FI) regression was determined such that tCOD removal efficiency could be modelled. The model was based on the sum of squares and proved to be statistically significant. The 2FI model was built to fit the results and the final equation for tCOD removal from the model was found to be (Eq. 4.1):

$$\text{tCOD removal (\%)} = 79.21 + 15.20*A + 0.72*B + 17.48*AB \quad (4.1)$$

Where *A* is OLR (g tCOD/L.day) and *B* is HRT (days).

**Table 4-7**: CCD results for COD removal

| Run | Factors | | COD Removal (%) | |
|---|---|---|---|---|
| | A (g tCOD/L.day) | B (day) | Actual | Predicted |
| **1** | 2.71 | 1.17 | 52,0949 | 51.8239 |
| **2** | 2.71 | 1.01 | 47,9377 | 49.2411 |
| **3** | 2.5 | 1.44 | 58,7500 | 60.0823 |
| **4** | 2.5 | 1.53 | 57,7049 | 61.2113 |
| **5** | 2.29 | 1.85 | 68,5142 | 68.2625 |
| **6** | 2.5 | 1.64 | 63,1258 | 62.7122 |
| **7** | 2.5 | 1.78 | 67,2462 | 64.6382 |
| **8** | 2.5 | 2.14 | 71,6090 | 69.3534 |
| **9** | 2.5 | 1.93 | 68,7792 | 66.5243 |
| **10** | 2.5 | 2.70 | 77,6335 | 76.765 |
| **11** | 2.5 | 3.50 | 84,5384 | 87.4308 |
| **12** | 2.5 | 3.89 | 93,0245 | 92.5578 |
| **13** | 2 | 4.82 | 91,4160 | 91.5918 |
| **14** | 1.5 | 4.29 | 76,3516 | 78.2855 |
| **15** | 1.5 | 4.79 | 79,9652 | 78.2109 |

Table 4.8 tabulates and illustrates the Analysis of Variance (ANOVA) of the empirical model for tCOD removal (%). The $R^2$, F and P values were analysed and determined from the experimental results given. A mean square regression and mean square residual ratio was used to calculate the F-value of the model, whereas, the significance of the coefficients was described by the P-value. Knowing the interrelatedness of the test variables was necessary to calculate the P-value. It was observed that the Prob> F value for the model was < 0.0001 which was very low, which indicated that the model was significant. According to Sathian et al. (2015), the lower the P-value (<0.05), the more significant the model is. The $R^2$, $R^2$ adjusted and the $R^2$ predicted were 0.98, 0.98 and 0.96 respectively. These values are observed to be numerically similar. This indicates that a good correlation obtained between the experimental values and the values predicted. These values were similar to that of Osman et al. (n.d.) that optimised COD removal from a paper mill using RSM. RSM using CCD was employed to optimize HRT and organic loading rate (OLR) in modelling the COD concentration. Sathian et al. (2014) also optimised parameters such as air flow rate and SRT in modelling COD removal in the treatment of textile dye wastewater. Both Osman et al. (n.d.) and Sathian et al. (2014) obtained $R^2$ values between 0.996 – 0.994 and 0.87 – 0.94 respectively. For statistical reasons, a co-relation coefficient ($R^2$) value of >0.90 can be assumed to suitably describe experimental results when compared with modelled values. Overall, an $R^2$ value closer to unity is preferred. The model produced had $R^2$ values >0.95

which proved that the model developed described the experimental results well, within significant statistical parameters.

**Table 4-8**: Analysis of variance (ANOVA) of the quadratic model for tCOD removal

| Source | Sum of Squares | Degree of freedom | Mean Square | F Value | p-value Prob > F | |
|---|---|---|---|---|---|---|
| **Model** | 2489.98 | 3 | 829.99 | 185.43 | < 0.0001 | significant |
| ***A*** | 294.06 | 1 | 294.06 | 65.70 | < 0.0001 | |
| ***B*** | 0.53 | 1 | 0.53 | 0.12 | 0.7372 | |
| ***AB*** | 234.87 | 1 | 234.87 | 52.47 | < 0.0001 | |
| ***Residual*** | 49.24 | 11 | 4.48 | | | |
| $R^2$ | 0.98 | **$R^2$Adj** | 0.98 | **$R^2$ Pred** | 0.96 | |

The relationship between the HRT and OLR for optimised tCOD removal was plotted on a 3D graph (see Figure 4.14). The 3D model provides the best representation of the influences of HRT and OLR on tCOD removal. This 3D plot is very useful in determining the behaviour of the system within the known environmental parameter variations representation (Sathian et al., 2015). From the 3D plot, it was seen that the optimum conditions for OLR and HRT for a maximum tCOD removal was 2 g tCOD/L.day OLR and 4.82 days HRT for which a maximum tCOD removal of 93% was obtained. The OLR was influenced and not HRT therefore B was not significant.

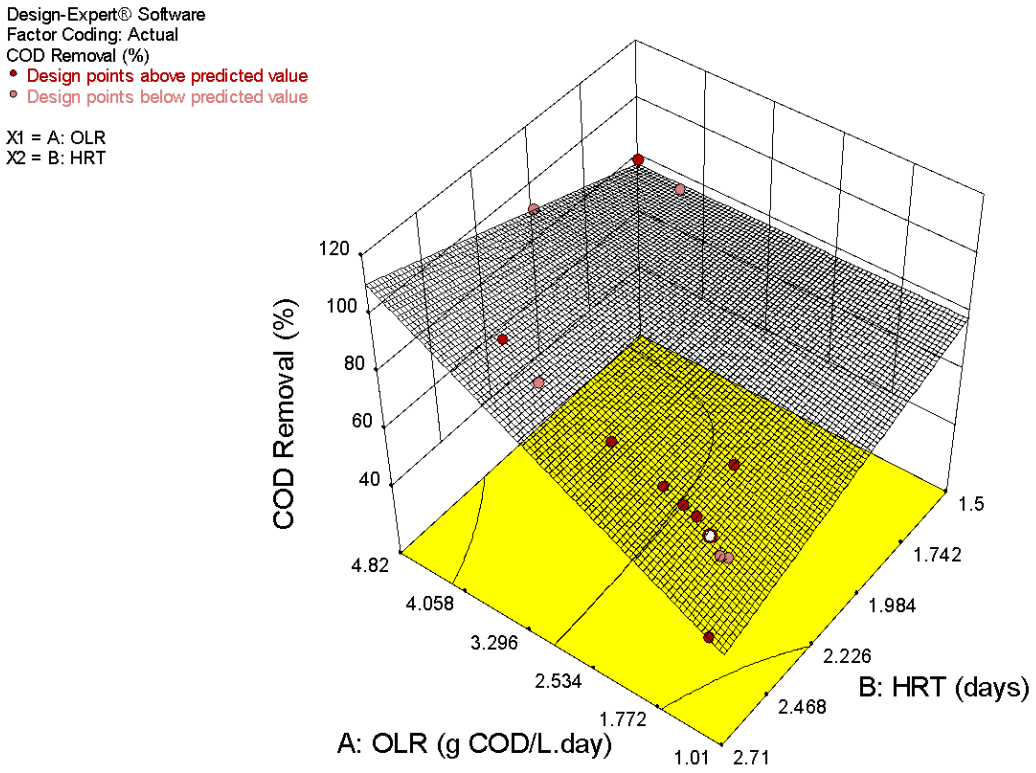



**Figure 4-14**: 3D plot of the response between OLR (A) and HRT (B) and their effect on tCOD removal for EGSB

# CHAPTER 5

# CONCLUSION

# AND

# RECOMMENDATIONS

# CHAPTER 5

# 5. CONCLUSION AND RECOMMENDATIONS

## 5.1. Expanded Granular Sludge Bed (EGSB) reactor: Efficiency and Operatability

The laboratory-scale EGSB anaerobic digester was successfully operated over a period of 172 days for the treatment of PSW, using different HRTS and OLRs. The average tCOD, TSS and FOG removal efficiencies over 172 days were 69%, 98% and 92% respectively. An average tCOD, sCOD and TSS, removal of 46%, 16.5% and 68% was observed at an average OLR of 1 g COD/L.day and at HRT of 62.5 h. Furthermore an average FOD and BOD removal was observed to be 83% and 96% at the same HRT and OLR. While an average tCOD, sCOD TSS removal of 65%, 62.3%, 92%, 87% was observed at OLR 2 g tCOD/L.day and HRT 57.5 h. As well as a FOG and BOD removal of 95.5% was observed at the above mentioned HRT and OLR. A further increase in tCOD average removal of 81%, sCOD average removal of 72%, TSS removal of 88% as well as an average FOG and BOD removal was found to be 93% and 95% for an OLR of 3 g tCOD/L.day and HRT of 49.8 h. The highest tCOD removal of 93 % was obtained at an HRT of 60 h and OLR of 1.95 g tCOD/L. day, which was considered to be low with the highest sCOD removal being 92.3% at HRT and OLR of 36h and 5.32 g tCOD/L.day. The maximum feed tCOD was 11068 mg/L which was reduced to a minimum effluent tCOD of 550 mg/L. The highest FOG and BOD removal were 97% and 99% at an HRT of 36h and OLR of 5.32 g tCOD/L.day.

The biogas constituents were, i.e. in terms of concentration and/or compositions: 40.4% ($CH_4$), 3.4% ($CO_2$), 12.9% ($O_2$), 0.005% ($H_2$), 0.0092% ($H_2S$) and 41.21% ($N_2$) respectively. These results were obtained at low OLR, with ammonium nitrogen being determined to inhibit biogas production at high concentration.

## 5.2. Single stage nitrification/denitrification (SSND)

Two flow configurations were considered for the SSND set-up, namely an up-flow and down-flow configuration. The down-flow configuration had no sparging while during the up-flow, sparging was used. For the down-flow configuration with an average of 9.63 days HRT, the system had an average TN removal of 15.8%; whereas, for the up-flow configuration with sparging, the system was operated at HRT of 13.74 days, achieving an average TN removal efficiency of 58%. There was minimal removal of $NO_2^-$-N, $NO_3$–N, whereas, the maximum TN removal efficiency was 78% which was 20% higher in the up-flow configuration. This therefore indicated that the up-flow configuration with sparging worked better than the down-

flow configuration initially used. An average 1.38 mg/L DO concentration was observed across the SSND column when sparging was introduced into the system.

### 5.3. Optimisation of the EGSB operation using RSM

The optimisation of the EGSB was conducted using RSM. From the ANOVA results, it was observed that the P-value was <0.001 for the model developed, achieving a $R^2$ value of 0.98, when experimental and modelled results were compared. This indicated that the model (2FI) was significant in predicting tCOD removal. The optimum conditions were found to be 2 g tCOD/L/day OLR and 4.82 days HRT, to obtain a maximum tCOD removal of 93%.

### 5.4. Utilisation of membrane systems

Two membrane types with different pore sizes were used to filter product streams from the EGSB product and SSND product. The parameters measured were averaged pH, conductivity, TDS, TSS and tCOD. The removal efficiencies of the MFMBR for the EGSB were 86%, <50% and 60% for turbidity, TSS and tCOD respectively. A better performance was observed for the UFMBR with the EGSB product being process to achieve removal efficiencies of 97%, <50%, and 62% for turbidity, TSS and tCOD. Similarly, the SSND product processed through the MF membrane systems achieved a removal efficiency of 46%, 95% and <20% for the turbidity, TSS and tCOD respectively. Furthermore, the TSS removal efficiency was observed to increase by 57% when UF systems were used in comparison to mF systems. At a higher flow rate, the tCOD for the EGSB product was reduced by 56% for the UF systems in comparison to that of MF systems, with an increase for the SSND-UF systems being only 27%.

### 5.5. Combined system performance: EGSB-MBR and EGSB-SSND-MBR systems

For the overall system (EGSB-MBR and EGSB-SSND-MBR) the removal efficiencies were found to be 99%, 92% and 99% for turbidity, TSS and tCOD respectively. However, the removal efficiency for conductivity was minimal when using the membrane systems; albeit, the effluent quality characteristics were within the discharge standards of the CCT and SANS241.

### 5.6. Recommendations for future research studies

- Pre-treatment stage should be implemented such as a DAF before entering the EGSB for removing all the FOG and other organic material

- Focusing on the effects of different higher up-flow velocities and its affects should be implemented as a research study on the EGSB in treating PSW.
- Consistent and proper biogas equipment needs to be implemented instead of the Tedlar bag method, such as the displacement of gas method for future projects as the method used was very unreliable and inconsistent.
- The recovery of nutrients such as phosphorous, sulphates and proteins can be focused or instigated in future PSW projects.
- A stripper could be installed on the SSND design which focusses on the recovery of the $NH_4$.
- Improvement on the design of the SSND with regard to nitrogen removal as the nitrogen within the product of the column was high and above discharge standards.
- A smaller pore size MBR should be implemented to use in removing smaller organics and particles which would increase the removal percentages of the TDS, conductivity, TSS and tCOD.
- A project should be implemented to focus on the biogas production and how it could be used as a source of energy when being produced from the AD.
- All three stages should be operated continuously and simultaneously and monitored to check the performance and efficiency of the system.
- Improvement on the EGSB design should be implemented with regard to the water jacket covering the whole EGSB and not excluding the bottom, as this kills the microorganisms within the reactor.
- For the SSND a recycle should have been implemented to reduce the nitrates to nitrogen gas for sustainable aerobic denitrification to occur.
- Wastewater treatment using CCD should focus on ammonia, colour, biogas production as well.

# REFERENCES

## REFERENCES


Amrou, M.H.E., Hamouda, K. and Keddam, M., 2018. Modelling and optimisation of treatment parameters using response surface methodology: application to superficial hardening of 2017A alloy using the rods-balls device. *International Journal of Materials Engineering Innovation*, *9*(1), pp.46-63.

Atuanya, E. I. and Aigbirior, M. 2002. Mesophilic biomethanation and treatment of poultry waste-water using pilot scale UASB reactor. *Environmental monitoring and assessment* 77(2): 139-147.

Avula, R. Y., Nelson, H. M. and Singh, R. K. 2009. Recycling of poultry process wastewater by ultrafiltration. *Innovative Food Science & Emerging Technologies* 10(1): 1-8.

Backhurst, J. and Harker, J. 2002. *Coulson & Richarson's Chemical Engineering*. Oxford: Butterworth Heinneman.

Barana, A.C., Lopes, D.D., Martins, T.H., Pozzi, E., Damianovic, M.H.R.Z., Del Nery, V. and Foresti, E. 2013. Nitrogen and organic matter removal in an intermittently aerated fixed-bed reactor for post-treatment of anaerobic effluent from a slaughterhouse wastewater treatment plant. *Journal of Environmental Chemical Engineering*, *1*(3): 453-459.

Basitere, M., Rinquest, Z., Njoya, M., Sheldon, M.S. and 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.

Basitere, M., Williams, Y. Sheldon, S. Ntwampe, S., De Jager, D. and Dlangamandla, C. 2016. Performance of an expanded granular sludge bed (EGSB) reactor coupled with anoxic and aerobic bioreactors for treating poultry slaughterhouse wastewater. *Water Practice and Technology*,11(1): 86-92.

Bishop, D.F., Heidman, J.A. and Stamberg, J.B. 1976. Single-stage nitrification-denitrification. *Journal (Water Pollution Control Federation)*: 520-532.

Burton, C.H., Cumby, T.R. and Tinker, D.B. 2004. Treatment and disposal of poultry processing waste. In Mead, G.C. (ed). P*oultry Meat Processing and Quality.*. Cambridge. Woodhead Publishing Limited.

Borja, R., Banks, C.J., Wang, Z. and Mancha, A. 1998. Anaerobic digestion of slaughterhouse wastewater using a combination sludge blanket and filter arrangement in a single reactor. *Bioresource technology*, *65*(1-2):125-133.

Caixeta, C.E., Cammarota, M.C. and Xavier, A.M. 2002. Slaughterhouse wastewater treatment: evaluation of a new three-phase separation system in a UASB reactor. *Bioresource Technology*, 81(1): 61-69.

Cammarota, M.C. and Freire, D.M.G. 2006. A review on hydrolytic enzymes in the treatment of wastewater with high oil and grease content. *Bioresource technology*, 97(17): 2195-2210.

Campos, J.L., Mosquera-Corral, A., Sanchez, M., Méndez, R. and Lema, J.M. 2002. Nitrification in saline wastewater with high ammonia concentration in an activated sludge unit. *Water Research*, *36*(10): 2555-2560.

Cao, W., Mehrvar, M. 2011. Slaughterhouse wastewater treatment by combined anaerobic baffled reactor and UV/H 2 O 2 processes. *Chemical engineering research and design*, 89(7): 1136-1143.

Chan, Y.J., Chong, M.F., Law, C.L. and Hassell, D.G. 2009. A review on anaerobic-aerobic treatment of industrial and municipal wastewater. *Chemical Engineering Journal,* 155(1): 1-18.

Chen, H.H. And Lee, H.H. 2014. Comprehensive overview of renewable energy development in Taiwan. *Renewable and Sustainable Energy Reviews,* 37: 215-228.

Chen, T.C. 1992. Poultry meat microbiology. *Encyclopedia Food Science Technology*, *4*: 2140-2145.

Chen, Y., Cheng, J.J. and Creamer, K.S. 2008. Inhibition of anaerobic digestion process: a review. *Bioresource technology,* 99(10): 4044-4064.

Cheryan, M. and Rajagopalan, N. 1998. Membrane processing of oily streams. Wastewater treatment and waste reduction. *Journal of membrane science*, *151*(1): 13-28.

Chu, C.P., Lee, D.J., Chang, B.V., You, C.H., Liao, C.S. and Tay, J.H. 2003. Anaerobic digestion of polyelectrolyte flocculated waste activated sludge. *Chemosphere*, *53*(7): 757-764.

Chu, L.B., Yang, F.L. and Zhang, X.W. 2005. Anaerobic treatment of domestic wastewater in a membrane-coupled expended granular sludge bed (EGSB) reactor under moderate to low temperature. *Process Biochemistry*, *40*(3-4):1063-1070.

Chung, J., Shim, H., Park, S.J., Kim, S.J., Ba, W. 2006 Optimization of free ammonia concentration for nitrite accumulation in shortcut biological nitrogen removal process", *Bioprocessing. Biosystems. Engineering.,* 28 (4), 275-282

City of Cape Town. 2013. Wastewater and Industrial Effluent By-Law, Province of the Western Cape: Provincial Gazette

Cuéllar, A. D. And M. E. Webber. 2008. Cow power: the energy and emissions benefits of converting manure to biogas. *Environmental Research Letters,* 3(3): 1-8.

Cuetos, M., Gómez, X. Otero, M. and Morán, A. 2010. "Anaerobic digestion and co-digestion

of slaughterhouse waste (SHW): influence of heat and pressure pre-treatment in biogas yield." *Waste management,* 30(10): 1780-1789.

De Nardi, I.R., Del Nery, V. Amorim, A.K.B. Dos Santos, N.G. and Chimenes F. 2011. Performances of SBR, chemical–DAF and UV disinfection for poultry slaughterhouse wastewater reclamation. *Desalination,* 269(1): 184-189.

De Nardi, I.R., Fuzi, T.P. and Del Nery, V. 2008. Performance evaluation and operating strategies of dissolved-air flotation system treating poultry slaughterhouse wastewater. *Resources, Conservation and Recycling*, 52(3): 533-544.

Del Nery, V., De Nardi, I.R. Damianovic, M.H.R.Z. , Pozzi, E. Amorim, A.K.B. and Zaiat, M. 2007. Long-term operating performance of a poultry slaughterhouse wastewater treatment plant. *Resources, conservation and recycling,* 50(1): 102-114.

Del Nery, V., Pozzi, E. Damianovic, M.H. Domingues, M.R. and Zaiat, M. 2008. Granules characteristics in the vertical profile of a full-scale upflow anaerobic sludge blanket reactor treating poultry slaughterhouse wastewater. *Bioresource technology* 99(6): 2018-2024.

Department of Agriculture and Rural Development. 2009. Guideline manual for the management of abattoirs and other waste animal origin. Gauteng.

Department of Water Affairs (DWA). 2010 Integrated Water and Waste Management Plan for the preparation of the Water Quality Management Technical Document to support the Application for Licences for Mining and Industries in Terms of the Requirements of the National Water Act, 1998 (Act 36 of 1998). Pretoria

Department of Water Affairs and Forestry (DWAF). 2013. Revised National Water Act (Act 36 of 1998). Pretoria

Demirer, G.N. and Chen, S. 2005. Two-phase anaerobic digestion of unscreened dairy manure. *Process biochemistry*, *40*(11): 3542-3549.

Driessen, W. and Vereijken, T. 2003. March. Recent developments in biological treatment of brewery effluent. *The Institute and Guild of Brewing Convention, Livingstone, Zambia, March*: 2-7

Ge, H., Batstone, D.J. and Keller, J. 2013. Operating aerobic wastewater treatment at very short sludge ages enables treatment and energy recovery through anaerobic sludge digestion. *Water research*, 47(17): 6546-6557.

Gerardi, M.H. 2003.*The microbiology of anaerobic digesters*. John Wiley & Sons.

Ghangrekar, M.M., Kahalekar, U.J. and Takalkar, S.V. 2003. Design of Upflow Anaerobic Sludge Blanket reactor for treatment of organic wastewaters. *Indian journal of environmental*

*health,* 45(2): 121-132.

Girault, R., Bridoux, G., Nauleau, F., Poullain, C., Buffet, J., Peu, P., Sadowski, A.G. and Béline, F. 2012. Anaerobic co-digestion of waste activated sludge and greasy sludge from flotation process: batch versus CSTR experiments to investigate optimal design. *Bioresource technology*, *5*: 1-8.

Hernández, D., Riaño, B., Coca, M., Solana, M., Bertucco, A. and Garcia-Gonzalez, M.C. 2016. Microalgae cultivation in high rate algal ponds using slaughterhouse wastewater for biofuel applications. *Chemical Engineering Journal*, 285: 449-458.

Holm-Nielsen, J.B., Al Seadi, T. and Oleskowicz-Popiel, P. 2009. The future of anaerobic digestion and biogas utilization. *Bioresource technology* 100(22): 5478-5484.

Judd, S. 2010. *The MBR book: principles and applications of membrane bioreactors for water and wastewater treatment*, Elsevier.

Jiang, H.L., Akita, T., Ishida, T., Haruta, M. and Xu, Q. 2011. Synergistic catalysis of Au@ Ag core− shell nanoparticles stabilized on metal−organic framework. *Journal of the American Chemical Society*, *133*(5):.1304-1306.

Kobya, M., Senturk, E. and Bayramoglu, M. 2006.. Treatment of poultry slaughterhouse wastewaters by electrocoagulation. *Journal of hazardous materials* 133(1): 172-176.

Keller, J., Batstone, D.J., Ge, H. 2013. Operating aerobic wastewater treatment at very short sludge ages enables treatment and energy recovery through anaerobic sludge digestion. *Water Research*, 47: 6546-6557

Krakat, N., Demirel, B., Anjum, R. and Dietz, D., 2017. Methods of ammonia removal in anaerobic digestion: a review. *Water Science and Technology*, *76*(8): 1925-1938.

Lerner, M., Stahl, N. and Galil, N. 2007. Aerobic vs. anaerobic-aerobic biotreatment: paper mill wastewater. *Environmental Engineering Science*, *24*(3): 277-285.

Lo, Y. M., Cao, D. Argin-Soysal, S. ,Wang J. and Hahm, T.S. 2005. Recovery of protein from poultry processing wastewater using membrane ultrafiltration. *Bioresource Technology,* 96(6): 687-698.

Liu, X., Gao, X., Wang, W., Zheng, L., Zhou, Y. and Sun, Y. 2012. Pilot-scale anaerobic co-digestion of municipal biomass waste: focusing on biogas production and GHG reduction. *Renewable Energy*, *44*: 463-468.

Mao, C., Feng, Y. Wang X. and Ren, G. 2015. Review on research achievements of biogas from anaerobic digestion. *Renewable and Sustainable Energy Reviews,* 45: 540-555.

Mannapperuma, J.D. 1997. Design and performance evaluation of membrane systems.

*Handbook of food engineering practice*, 167-210.

Mosquera, C.A., González, F., Campos, J.L., Méndez, R. 2005. Partial nitrification in a SHARON reactor in the presence of salts and organic carbon compounds. *Process Biochemistry*., 40 (9), 3109-3118.

Manjunath, N.T., Mehrotra, I. and Mathur, R.P. 2000. Treatment of wastewater from slaughterhouse by DAF-UASB system. *Water Research*,*34* (6): 1930-1936.

Maroneze, M.M., Barin, J.S., Menezes, C.R.D., Queiroz, M.I. Zepka, L.Q. and Jacob-Lopes, E. 2014. Treatment of cattle-slaughterhouse wastewater and the reuse of sludge for biodiesel production by microalgal heterotrophic bioreactors. *Scientia Agricola* 71(6): 521-524.

Masse, D. and Masse, L. 2000. Bioprocessing Engineering-characterization of wastewater from hog slaughterhouses in eastern canada and evaluation of their in-plant wastewater treatment systems. *Canadian Agricultural Engineering,* 42(3): 139-146.

Metcalf, L. and Eddy, H.P. 1972. *Wastewater engineering collection treatment disposal*, McGraw-Hill Interamericana.

Mpongwana, N., Ntwampe, S.K.O.,.Mekuto, L., Akinpelu, E.A., Dyantyi S, and Mpentshu, Y. 2016. Isolation of high-salinity-tolerant bacterial strains, Enterobacter sp., Serratia sp., Yersinia sp., for nitrification and aerobic denitrification under cyanogenic conditions. *Water Science and Technology,* 73(9): 2168-2175.

Münch, E.V., Lant, P. and Keller, J. 1996. Simultaneous nitrification and denitrification in bench-scale sequencing batch reactors. *Water Research*, *30*(2): 277-284.

Nasir, I. M., Mohd Ghazi, T.I. and Omar, R. 2012. Anaerobic digestion technology in livestock manure treatment for biogas production: a review. *Engineering Life Science*, 12: 258-269.

Ng, W.J. 2006. *Industrial wastewater treatment.* No. 628.30950 N5.

Nges, I.A. and Liu, J. 2010. Effects of solid retention time on anaerobic digestion of dewatered-sewage sludge in mesophilic and thermophilic conditions. *Renewable Energy,* 35(10): 2200-2206.

Ngongang, M. 2016. Production of biopreservation compounds from non-Saccharomyces yeast using a single-stage bioreactor. Doctoral dissertaion, Cape Peninsula University of Technology.

Nunez, L.A., Fuente, E., Martinez, B. and Garcia, P.A. 1999. Slaughterhouse wastewater treatment using ferric and aluminium salts and organic polyelectrolites. *Journal of Environmental Science & Health Part A*, 34(3): 721-736.

Nunez, L. A. and Martinez, B. 1999. "Anaerobic treatment of slaughterhouse wastewater in an expanded granular sludge bed (EGSB) reactor." *Water Science and Technology*, 40(8): 99-106.

Osman, W.H.W., Sanusi, S.N.A., Mohd, N., Yassim, M.M. and Abdullah, S.R.S. n.d. Application of response surface (RSM) for optimization of cod removal from paper mill wastewater using granula methodology r activated carbon sequencing batch biofilm reactor. Available: https://www.researchgate.net/profile/Norzila_Mohd/publication/283042780_APPLICATION_OF_RESPONSE_SURFACE_METHODOLOGY_RSM_FOR_OPTIMIZATION_OF_COD_REMOVAL_FROM_PAPER_MILL_WASTEWATER_USING_GRANULAR_ACTIVATED_CARBON_SEQUENCING_BATCH_BIOFILM_REACTOR/links/5627058d08aed3d3f138a84e/APPLICATION-OF-RESPONSE-SURFACE-METHODOLOGY-RSM-FOR-OPTIMIZATION-OF-COD-REMOVAL-FROM-PAPER-MILL-WASTEWATER-USING-GRANULAR-ACTIVATED-CARBON-SEQUENCING-BATCH-BIOFILM-REACTOR.pdf

Pontes, R.F. and Pinto, J.M. 2006. Analysis of integrated kinetic and flow models for anaerobic digesters. *Chemical Engineering Journal*, *122*(1-2): 65-80.

Pescod, M.B. 1992. Wastewater treatment and use in agriculture: irrigation with wastewater. In *FAO. Irrigation and Drainage*. Available: http://eprints.icrisat.ac.in/8638/1/RP_07946_wastewater_treatment.....pdf.

Qi, X., Zhang, S., Wang Y. and Wang R. 2005. Advantages of the integrated pig-biogas-vegetable greenhouse system in North China. *Ecological Engineering,* 24(3): 175-183.

Rao, A.N.S. and Venkatarangaiah, V.T. 2014. Metal oxide-coated anodes in wastewater treatment. *Environmental Science and Pollution Research*, *21*(5): 3197-3217.

Rehl, T. and Muller, J. 2011. Life cycle assessment of biogas digestate processing technologies. *Resources, Conservation and Recycling,* 56(1): 92-104.

Rittmann, B.E. and Langeland, W.E., 1985. Simultaneous denitrification with nitrification in single-channel oxidation ditches. *Journal (Water Pollution Control Federation)*: 300-308.

Ruiz, I., Blã¡zquez, R. and Soto, M 2009. Methanogenic toxicity in anaerobic digesters treating municipal wastewater. *Bioresource technology,* 100(1): 97-103.

Robinson, T.P. and Pozzi, F. 2011. Mapping supply and demand for animal-source foods to 2030. *Animal Production and Health Working Paper*, *2*: 1-154.

South African National Standards 241-2. 2015. South African National Standard Drinking water - Part 2: application of SANS 241-1:2011. ISBN 978-0-626-29841-8

Majd, S.S., Abdoli, M.A., Karbassi, A., Pourzamani, H.R. and Rezaee, M. 2017. Effect of

physical and chemical operating parameters on anaerobic digestion of manure and biogas production: a review. *Journal of Environmental Health and Sustainable Development,* 2(1): 235-247.

Saravanan, V. and T. R. Sreekrishnan. 2006. Modelling anaerobic biofilm reactors: a review. *Journal of Environmental Management*, 81(1): 1-18.

Sarkar, B., Chakrabarti, P.P., Vijaykumar, A. and Kale, V. 2006. Wastewater treatment in dairy industries—possibility of reuse. *Desalination*, *195*(1-3): 141-152.

Sathian, S., Rajasimman, M., Radha, G., Shanmugapriya, V. and Karthikeyan, C. 2014. Performance of SBR for the treatment of textile dye wastewater: Optimization and kinetic studies. *Alexandria Engineering Journal*, *53*(2): 417-426.

Seghezzo, L., Zeeman, G., Van Lier, J.B., Hamelers, H.V.M. and Lettinga, G. 1998. A review: the anaerobic treatment of sewage in UASB and EGSB reactors. *Bioresource technology,* 65(3): 175-190.

Shannon, M. A., Bohn, P.W., Elimelech, M., Georgiadis, J.G., Mariñas, B.J. and Mayes, A.M.. 2008. Science and technology for water purification in the coming decades. *Nature,* 452(7185): 301-310.

Sheldon, M.S. and Erdogan, I.G., 2016. Multi-stage EGSB/MBR treatment of soft drink industry wastewater. *Chemical Engineering Journal*, *285*: 368-377.

Shih, J.C. and Kozink, M.B., 1980. Ultrafiltration treatment of poultry processing wastewater and recovery of a nutritional by-product. *Poultry Science*, *59*(2): 247-252.

Sleat, R. & Mah, R. 1987. Hydrolytic Bacteria in Anaerobic digestion of biomass. New York: Elserivier Science Publishing Company 15-33

South African Poultry Association (SAPA). 2015. Industry Profile. Available: https://www.sapoultry.co.za/pdf-docs/sapa-industry-profile.pdf

Speedy, A.W. 2002. Overview of world feed protein needs and supply. In *FAO expert consultation and workshop on protein sources for the animal feed industry, April 29-May 3, 2002, Bangkok, Thailand*.

Stamenković, O.S., Kostić, M.D., Radosavljević, D.B. and Veljković, V.B., 2018. Comparison of Box-Behnken, Face Central Composite and Full Factorial Designs in Optimization of Hempseed Oil Extraction by n-Hexane: a Case Study. *Periodica Polytechnica Chemical Engineering*, *62*(3), pp.359-367.

Sufiate, B.L., Soares, F.E., Gouveia, A.S., Moreira, S.S., Cardoso, E.F., Tavares, G.P., Braga, F.R., ARAÚJO, J.V. and QUEIROZ, J.H., 2018. Statistical tools application on

dextranase production from Pochonia chlamydosporia (VC4) and its application on dextran removal from sugarcane juice. *Anais da Academia Brasileira de Ciências*, *90*(1), pp.461-470.

Suwa, Y., T. Suzuki, H. Toyohara, T. Yamagishi and Y. Urushigawa.1992. Single-stage, single-sludge nitrogen removal by an activated sludge process with cross-flow filtration. *Water Research,* 26(9): 1149-1157.

Tabatabaei, M., Rahim, R.A., Abdullah, N., Wright, A.D.G., Shirai, Y., Sakai, K., Sulaiman, A. and Hassan, M.A. 2010. Importance of the methanogenic archaea populations in anaerobic wastewater treatments. *Process Biochemistry*, *45*(8): 1214-1225.

Taşkan, E. 2016. Performance of mixed algae for treatment of slaughterhouse wastewater and microbial community analysis. *Environmental Science and Pollution Research* 23(20): 20474-20482.

Tchobanoglous, G., Burton, F.L., H. and Stensel D. 2004. *Wastewater Engineering: Treatment and Reus*e. London: McGraw Hill.

Templeton, M.R. and Butler, D., 2011. *Introduction to wastewater treatment*. London: Bookboon.

Von Sperling, M., Freire, V.H. and de Lemos Chernicharo, C.A. 2001. Performance evaluation of a UASB-activated sludge system treating municipal wastewater. *Water Science and Technology*, *43*(11): 323-328.

Von Sperling, M. and de Lemos Chernicharo, C.A. 2005. Biological Wastewater Treatment in Warm Climate Regions Vol. 1. London: IWA publishing.

Von Sperling, M. And W. S. Ponds. 2007. Biological Wastewater Treatment Series. Volume five: Activated sludge and aerobic biofilm reactors. London: IWA Publishing.

van Haandel, A. and van der Lubbe, J. 2007. *Handbook biological waste water treatment-design and optimisation of activated sludge systems*. Webshop Wastewater Handbook.

Verma, S. 2002. Anaerobic digestion of biodegradable organics in municipal solid wastes. M.Sc. thesis, Columbia University.

Wang, J., Lu, H., Chen, G.H., Lau, G.N., Tsang, W.L. and van Loosdrecht, M.C. 2009. A novel sulfate reduction, autotrophic denitrification, nitrification integrated (SANI) process for saline wastewater treatment. *Water research*, *43*(9): 2363-2372.

Yan, J., Wang, S.Y., Gao, S.Y. and Cheng, Y.R. 2007. Simultaneous nitrification and denitrification of wastewater with low C/N ratio under low DO. *China Water & Wastewater*, *23*(3): 44-48.

Yetilmezsoy, K., Ilhan, F., Sapci-Zengin, Z., Sakar, S. and Gonullu, M.T. 2009.

Decolorization and COD reduction of UASB pretreated poultry manure wastewater by electrocoagulation process: A post-treatment study. *Journal of hazardous materials,* 162(1): 120-132.

Yetilmezsoy, K. and Sakar, S. 2008a. Development of empirical models for performance evaluation of UASB reactors treating poultry manure wastewater under different operational conditions. *Journal of Hazardous materials,* 153(1): 532-543.

Yetilmezsoy, K. and Sakar, S. 2008b. Improvement of COD and color removal from UASB treated poultry manure wastewater using Fenton's oxidation. *Journal of Hazardous materials,* 151(2): 547-558.

Yetilmezsoy, K., Turkdogan-Aydinol, I., Gunay, A. and Ozis, I. 2011. Post treatment of poultry slaughterhouse wastewater and appraisal of the economic outcome. *Environmental Engineering & Management Journal (EEMJ)*, *10*(11): 1635-1645.

Yoo, H., Ahn, K.H., Lee, H.K. Kwak, Y.J. and Song, K.G. 1999. Nitrogen removal from synthetic wastewater by simultaneous nitrification and denitrification (SND) via nitrite in an intermittently-aerated reactor. *Water research,* 33(1): 145-154.

Yordanov, D. 2010. Preliminary study of the efficiency of ultrafiltration treatment of poultry slaughterhouse wastewater. *Bulgarian Journal of Agricultural Science,* 16(6): 700-704.

Zhai, N., Zhang, T.. Yin, D., Yang, G., Wang, X., Ren, G. and Feng, Y. 2015. Effect of initial ph on anaerobic co-digestion of kitchen waste and cow manure. *Waste Management,* 38: 126-131.

Zhang, S.Q., Kutowy, O. Kumar, A. and Malcolm, I.1997. A laboratory study of poultry abattoir wastewater treatment by membrane technology. *Canadian Agricultural Engineering,* 39(2): 99-106.

Zhang, Y., Li, Y.A.N., Lina, C.H.I., Xiuhua, L.O.N.G., Zhijian, M.E.I. and Zhang, Z., 2008. Startup and operation of anaerobic EGSB reactor treating palm oil mill effluent. *Journal of Environmental Sciences*, *20*(6): 658-663.

Zinatizadeh, A., Mansouri, Y., Akhbari, A. and Pashaei, S, 2011. Biological treatment of a synthetic dairy wastewater in a sequencing batch biofilm reactor: statistical modeling using optimization using response surface methodology. *Chemical Industry and Chemical Engineering Quarterly/CICEQ*, 17(4): 485-495.

Zakkour, P.D., Gaterell, M.R., Griffin, P., Gochin, R.J. and Lester, J.N. 2001. Anaerobic treatment of domestic wastewater in temperate climates: treatment plant modelling with economic considerations. *Water research*, *35*(17): 4137-4149.

# APPENDICES

# APPENDICES

## APPENDIX A: Analytical Methods

### A1: pH, Conductivity, Total Dissolved Solids (TDS) and salinity testing procedure and determination using PCSTestr 35

**Calibration of the PCSTestr35**

- The PCSTestr35 must be calibrated before every use
- Switch on the PCSTestr 35
- Rinse the PCSTestr35 with distilled water and pat dry with paper

**pH Calibration**

1. Press the mode button and wait until the pH screen is reached
2. Press CAL button on the PCSTest 35 and the digital display at the bottom the row will flash 4.01, 7, and 10
3. Place the PCSTest 35 in the pH 4 buffer solution and wait until the reading stabilises
4. Press MODE/ENT button and the pH 4 buffer calibration is complete
5. Rinse the PCSTest 35 with distilled water and pat dry
6. Redo steps 1 – 5 with pH 7 and pH 10 buffer solution
7. When complete the pH calibraton is complete. Rinse the PCSTest 35 with distilled water and pat dry.

**Conductivity calibration**

1. Pres MODE button until conductivity screen appears
2. Pres CAL button on PCSTest 35 and insert the PCSTest 35 into the conductivity buffer solution of 1413 µs/cm
3. Wait until the reading stabilises and the press MODE/ENT and the conductivity calibration is complete
4. Rinse PCSTest 35 with distilled water and pat dry

**TDS calibration**

1. Press MODE button until TDS screen appear
2. Insert the PCSTest 35 in the TDS 300 ppm buffer solution
3. Press CAL button and wait until the reading stabilises on 300ppm, if not press the HOLD button to increase the value in the top digital display and the CAL button to decrease the value in the top digital display until the value is set to 300 ppm. Then press CAL.
4. Once stable press MODE/ENT, calibration complete
5. Rinse PCSTest 35 and pat dry

**Determination of pH, TDS, Conductivity and Salinity**

1. When taking readings approximately 50 ml of the required sample is placed in a beaker.
2. Switch on the PCSTest 35 and press the MODE button until the desired variable/paraPCSTest 35 for measuring is reached (i.e. pH, TDS, Conductivity or salinity)
3. Keep the PCSTest 35 submerged in the sample until the required reading has stabilised.
4. Take the measurements of each desirable paraPCSTest 35 and repeat in triplicates

**A2: Turbidity Determination**

**Calibration Procedure**

- Place the TN100 turbidity meter on a flat level surface
- Press CAL button and insert the desired calibration standards ie.e CAL 1 (800 NTU), CAL 2 (200 NTU), CAL 3 (100 NTU) and CAL 4 (0.02 NTU), aligning the mark on the vial to the mark on the meter.
- Cover the vial with the light shield cap and press READ/ENTER after each CAL standard is prompted for and inserted (ie. CAL 1, CAL 2, CAL 3, CAL 4)
- 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

2. Rinse the vial with distilled water and fill the vial with approximately 10 ml (i.e. up to the mark indicated on the sample vial) of desired sample
3. Wipe the sample vial with the soft, lint-free cloth to ensure the vial is dry, clean and free from smudges
4. Place the vial inside the well of the turbidity meter and align the index mark on vial with the meter's index mark, push the sample vial down until its fully snapped in
5. Cover the vial with the light shield cap and press READ/ENTER
6. The measured reading will appear on the display
7. Repeat if necessary

**A3: Total Suspended Solids (TSS) determination**

1. Prepare a glass fibre filter disk by weighing it before placing it into a B□chner funnel attached to a collection flask. While vacuum is applied, rinse the disk with Distilled water to attach the disk to the base
2. Remove rinsed water from funnel
3. Select a sample volume of no more than 200 ml and shake vigorously before transferring in onto the filter disk in the funnel
4. Transfer the sample onto the filter paper in the funnel and allow vacuum to remove all traces of water from the sample
5. Carefully remove the glass fibre filter disk from the funnel and dry the disk at 103 – 105 °C for 1 hour
6. Cool the filter paper in a desiccator and weigh

**TSS Calculation**

$$TSS\ (mg/L)\frac{(A-B)x\ 1000}{C}$$

Where A is the weight of the filter disk before filtered (mg)

B is the weight of the filter disk with sample residue (mg)

C is the volume of sample filtered (ml)

**A4: Ammonium ($NH_4^+$) determination**

**Method for determining ammonium (2 – 75 mg/L range)**

Using a Merck Spectroquant $NH_4^+$ test kit, Cat No. 1.00683.0001

1. Pipette 5 ml of the $NH_4$-1 solution into a test tube

2. Add 0.2 ml of the sample to the test tube
3. Add 1 level blue microspoon of the $NH_4$-2 powder to the test tube. The microspoon is located in the cap of the $NH_4$-2 bottle
4. Place a cap on the test tube and mix vigorously until the $NH_4$-2 powder is completely dissolved
5. Leave to react for 15 min
6. Add mixture to a 10mm cuvette
7. Place the Autoselector tube for the 2- 75 mg/L range into the Nova 60 Spectroquant
8. Place the 10mm cuvette sample in the slot of the Nova 60 Spectroquant and record the measurement displayed

**Notes**

- Make sure test cells are dry and clean
- All samples must be tested in triplicate
- Turbid samples need to be filtered first
- Do not allow samples to stand for longer than 15 minutes after all reagents have been mixed
- The measurements obtained from the Nova 60 Spectroquant are $NH_4$-N and therefore these needs to be converted to $NH_4^+$ using the following equation

$$NH_4^+ \left(\frac{mg}{L}\right) = 1.2887\ (NH_4^+ - N\frac{mg}{L}) + \ 0.0247$$

**A5: Nitrate ($NO_3^-$) Determination**

**Method for determination (0-0.5 mg/L $NO_3$-N)**

Using a Merck Spectroquant Nitrate cell test, Cat No. 1.14773.001

1. Place 1 level blue microspoon $NO_3$-1 powder into a test tube. The microspoon is located in the cap of the bottle
2. Add 5.0 ml of the $NO_3$-2 solution to the test tube
3. Place the cap on the test tube and mix vigorously until the reagent has completely dissolved
4. Slowly add 1.5 ml of the sample to the test tube
5. Place cap back on and mix vigorously. CAUTION: Test cell will become hot!
6. Leave to react for 10 minutes
7. Add to a 10mm cuvette

8. Place Auto selector tube for 0.0 – 5.0 mg/L nitrate range in Nova 60 Spectroquant
9. Place 10 mm cuvette into slot of the Nova 60 Spectroquant and take measurement

**A6: Phosphate ($PO_4$) determination**

**Method for orthophosphate determination**

Using Spectroquant Phosphate cell test for orhtophosphates and total phosphorous, Cat No. 1.14543.0001

1. Add 1.0 ml of the sample to a barcoded test cell
2. Place a cap on and mix vigorously
3. Add 5 drops of P-2K to the cell
4. Add 1 dose of P-3K to the cell using the blue dose-metering cap
5. Place the cap on the test cell and mix vigorously until all reagents are completely mixed
6. Wait 5 minutes for reaction to occur
7. Place the test call into the Nova 60 Spectroquant to measure for orthophosphate

**A7: Chemical Oxygen Demand (COD) Determination**

**A7.1 Method for determining total COD (tCOD)**

- Switch on the Spectroquant thermoreactor TR 420 to the preset setting of 148°C for 2 hours and allow it to heat up to the desired temperature
- When Using COD solutions A and B for 500 – 1000 mg /L range:
    - Pipette 2.2 ml of COD solution A into the test cell
    - Pipette 1.8 ml of COD solution B into the same test cell
    - Pipette 1 ml of the sample into the same test cell
    - Tightly attach the cap and mix vigorously
    - Place the test cell into the thermoreactor at 148°C for 2 hours
    - Carefully remove the test cell after 2 hrs and place in a test rack to cool. Do not cool with water
    - After 10 minutes mix the contents of the call again
    - Allow test cell to cool for another 30 minutes
    - Place the test cell in the Nova 60 Spectroquant
    - Enter the code 024 for the COD readings in the 500 – 1000 mg/L range and the concentration of the sample will be displayed on the screen
- When using COD solutions A and B for the 100 – 1500 mg/L range:

- This procedure is exactly the same as for COD solution A and B for 500-10 000mg/L with exception of:
- Pipette 0.30mL of COD solution A into the cell using
- Pipette 2.30mL COD solution B into the cell using
- Pipette 3mL of the sample into the cell

**A7.2 Method for determining soluble COD (sCOD)**

- The B chner funnel is placed into 500ml suction flask.
- The suction flask is either connected to a water or vacuum pump.
- The glass microfiber filters discs, 5.5 cm, without organic binder, Whatman type GF/F (0.7 µm) is placed inside the Buchner funnel.
- Raw sample is filtered using the vacuum pump.
- The filtered sample is then used to run a COD test.
- The procedure for the COD test is the same as for the total COD test, the difference is that only filtered samples are used.

**APPENDIX B: COMPOSITION OF BASAL MEDIUM USED FOR SND**

**B1 :BasalB1: Basal medium**

| | |
|---|---|
| KH2PO4 | 1.5 g |
| Na2HPO4 | 7.9 g |
| MgSO4.7H2O | 0.5 g |

1 mL traces elemental per litre

**B2: Trace elemental solution**

| | |
|---|---|
| EDTA | 50 g |
| ZnSO4.7H2O | 2.2 g |
| CaCl2 | 5.5 g |
| MnCl2.4H2O | 5.06 g |
| FeSO4.7H2O | 5.0 g |
| (NH4)6Mo7O2.4H2O | 1.1 g |
| CuSO4.5H2O | 1.57 g |

| | |
|---|---|
| CoCl2.6H2O | 1.61 g |

## APPENDIX C: FORMULAS USED FOR CALCULATING OPERATION PARAMETERS

### C1: HRT CALCULATION

$$HRT\ (h) = \frac{working\ volume\ of\ reactor\ (m^3)}{flowrate\ of\ influent\ (\frac{m^3}{hr})}$$

### C2: OLR CALCULATION

$$OLR\ (mg\frac{COD}{L}h) = \frac{influent\ COD(\frac{mg}{L})}{HRT(h)}$$

### C3: UPFLOW VELOCITY CALCULATION

$$V_{up}(\frac{m}{h}) = \frac{Q\ (\frac{m^3}{h})}{A(m^2)}$$