Cape Peninsula
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Performance of a biological pre-treatment system coupled with down-flow expanded granular bed reactor (DEGBR) for poultry slaughterhouse wastewater treatment

By

Derrick Njabuliso Dlamini

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Supervisor: Dr. Moses Basitere

Co-supervisor: Prof. Seteno Karabo Ntwampe

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DECLARATION

I, **Derrick Njabuliso Dlamini**, declare that the contents of this dissertation/thesis represent my own unaided work, and that the dissertation/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.

30th June 2021

Signed **Date**

ABSTRACT

In South Africa, the poultry industry has been identified among the highest producers of wastewater. Generally, Africa and South Africa in particular, has recently faced challenges associated with the availability and access to clean, potable water, with available water sources being contaminated by anthropogenic activities which also has a negative impact on the environment. Therefore, there is a need to invent and design suitable wastewater treatment plants which can be used in the treatment of contaminants found in wastewater. Since poultry slaughterhouse wastewater (PSW) is a one of the major contributors of wastewater generated in South Africa, it is possible that the wastewater produced may pose a threat to aquatic life and it may also lead to water borne diseases if discharged untreated to minimum required standards. Therefore, this creates a need to design efficient and effective PSW treatment processes since the PSW contains a high concentration of chemical oxygen demand (COD), total suspended solids (TSSs), fats, oil and grease (FOG), proteins and carbohydrates. It is important that the wastewater is treated to acceptable environmental discharge standards. This study, evaluated the performance of a biological pre-treatment system coupled with an anaerobic down-flow granular bed reactor (DEGBR) maintained at 37˚C for PSW treatment. The biological pre-treatment system utilizes Eco-flush^{TM} as a FOG hydrolysing agent.

The results showed that the biological pre-treatment was observed to be highly effective for removal of FOG, COD and TSS with a removal efficiency of 80±6.3%, 38±8.4% and 56±7.2%, respectively. Similarly, the DEGBR showed a stable performance in terms FOG, COD and TSS removal, with average removal efficiencies of 89±2.8%, 87±9.5%, and 94±3.7%, respectively. The overall removal performance of the integrated system in terms of FOG, COD and TSS, was 97±0.8%, 92±6.3% and 97±1.2%. Furthermore, the average volatile fatty acid/alkalinity (VFA/Alkalinity) ratio of 0.2 was observed, which indicated that the DEGBR was stable throughout the operation. Overall, the biological pre-treatment system coupled with the DEGBR performed satisfactorily with regard to the removal efficiencies of FOG, COD and TSS as compared to other similar systems, i.e. UASB and EGSB.

Keywords: Chemical oxygen demand, Fats, oil and grease, Total suspended solids, Poultry slaughterhouse wastewater, Down-flow expanded granular bed reactor.

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DEDICATION

I dedicate this thesis to my lovely wife, Ntswaki Dlamini & to all of my children.

Joshua 24:15
"As for me and my house, we will serve the Lord."

RESEARCH OUTPUTS

This thesis has been published in part in the Department of Higher Education and Training (DHET) accredited article(s) and conference proceeding(s), cited below:

Published Article 1 in conference proceedings:

Derrick N. Dlamini, Moses Basitere and Seteno Karabo O. Ntwampe. 2019. Current and Functional Reactor Designs in Poultry Slaughterhouse Wastewater Treatment. 16th SOUTH AFRICA Int'l Conference on Agricultural, Chemical, Biological & Environmental Sciences (ACBES-19) Nov. 18-19, 2019 Johannesburg (S.A.). pp 295-300, https://doi.org/10.17758/EARES8.EAP1119146.

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Published Abstract and conference presentation:

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LAYOUT OF THESIS

This thesis consists of the following chapters:

Chapter 1: Is a chapter that dealt with the background, the problem statement, hypothesis, aims and objectives, and significance and delineation of this study.

Chapter 2: Focuses on the literature review of poultry demand and water usage in general. Furthermore, it focusses on the characteristics of poultry slaughterhouse wastewater (PSW). An overview of the types of biological enzymes, the types of wastewater pre-treatment processes, different types of biological pre-treatment processes and types of anaerobic bioreactors used in the treatment of poultry slaughterhouse wastewater is also presented.

Chapter 3: Outlines research methodology, experimental set-up, design and process operating conditions, PSW collection, pre-treatment, DEGBR inoculation and process sampling and analysis. Moreover, this chapter discusses results relating to the performance of the pre-treatment-DEGBR systems and that of the overall designed PSW lab scale plant.

Chapter 4: Lastly**,** this chapter concludes the study and gives some recommendations for future studies.

References: Is a list of references used for the research according to the Harvard style of referencing as per Cape Peninsula University of Technology guidelines.

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LIST OF SYMBOLS

Symbol	Description	Units
^0C	Temperature	Degrees Celsius
η	Removal efficiency	%
k	Biomethane production rate	mL/d
Q	Flow rate	m^3/day
V	Working reactor volume	m^3
T	Hydraulic retention time (HRT)	day
O	Organic loading rate	day

Chemical formula	Description
CH_4	Methane

ABBREVIATIONS

Abbreviation		**Description**
AF	-	Anaerobic filter
AL	-	Anaerobic lagoons
AS	-	Activated sludge
BOD_5	-	Biochemical oxygen demand
COD	-	Chemical oxygen demand
DAF	-	Dissolved air flotation
DAFF	-	Department of Agriculture, Forestry & Fisheries
DEGBR	-	Down-flow expanded granular bed reactor
DWS	-	Department of Water and Sanitation
EGSB	-	Expanded granular sludge-bed
HRT	-	Hydraulic retention time
MF	-	Microfiltration
OLR	-	Organic loading rate
FOG	-	Fats, oil and grease
PSW	-	Poultry slaughterhouse wastewater
RO	-	Reverse osmosis
SAPA	-	South African poultry association
SGBR	-	Static granular bed reactor
SS	-	Suspended solids
TDS	-	Total dissolved solids
TKN		Total Kjheldahl nitrogen
TN	-	Total nitrogen
TSS	-	Total suspended solids
UASB	-	Up-flow anaerobic sludge-bed
UF	-	Ultra-filtration
VSS	-	Volatile suspended solids
VFA	-	Volatile fatty acids

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 the complete absence of oxygen.

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

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

Expanded granular bed reactor (EGSB) - A reactor that is a variant of the UASB reactor that uses an up-flow feed through a sludge bed.

FOG - Fats, oil and grease substances present in effluent.

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

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

Poultry slaughterhouse wastewater (PSW) - The wastewater generated by slaughterhouses during the slaughtering and processing of poultry products including by-products.

Static Granular Bed Reactor (SGBR) - A high-rate down-flow anaerobic digester that utilizes a bed of active anaerobic granules for the treatment of wastewater.

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 that are present in a molecular, ionized or micro-granular suspended form.

Up-flow anaerobic sludge blanket (UASB) - Is a suspended-growth reactor that maintains very high concentration of microbial biomass by promoting granulation, and the wastewater enters the reactor from the bottom, and flows upward.

Volatile fatty acids (VFA) – C2 to C6 fatty acids produced during anaerobic digestion.

CHAPTER ONE

INTRODUCTION

CHAPTER ONE

1. INTRODUCTION

1.1 Background

Africa is mainly faced with challenges associated with access to clean and potable water, induced by massive contamination of surface water and climate change. Land and air pollution are among the environmental impacts which contributes to surface water contamination (Shannon *et al*., 2008). The concern of water shortage further extends to South Africa, where this concern has been addressed by the Department of Water and Sanitation (DWS). The Western Cape province is currently faced with water shortages as reported by the DWS (Mariño, 2017).

Globally, the poultry industry has been identified among the highest generators of wastewater (De Nardi, 2011)**.** Poultry slaughterhouse wastewater (PSW) was identified as being one major contributor to surface water sources contamination (DAFF, 2014). The wastewater generated during poultry processing contains solid soluble matter due to the presence of fats, oil and grease (FOG) (Klaucans, 2018). The presence of FOG in PSW has proven to be a problem due to its complex nature (Bustillo-lecompte & Merhrvar, 2014). Therefore, it was established in another study that an effective treatment process was required for PSW treatment. (Rangel *et al*., 2007).

Ordinarily, PSW is firstly pre-retreated using the conventional methods before it gets charged into a bioreactor for further treatment. Industrial pre-treatment coupled with an aerobic biological process has been found to be beneficial (Rusten *et al*., 1998). Anaerobic digestion of PSW often face a problem of suspended solids and FOG accumulation (Kobya *et al.*, 2006). The latter problem emanates from solids which bypass the pre-treatment stage. Therefore, the pre-treatment of PSW effluent helps in the alleviation of solid matter before the wastewater gets to be treated in the next treatment phase (Damasceno *et al*., 2018). In South Africa the pre-treatment of PSW has not been explored in depth to a greater extent. This study assessed the effectiveness and efficiency of a biological pre-treatment system coupled with a down-flow expanded granular bed reactor (DEGBR) for PSW treatment in the Western Cape, SA.

1.2 Problem statement

PSW mainly contains FOG, organic matter and toxic pollutants which are additional factors of concern at the pre-treatment and anaerobic bioreactor stages. The problem is that the organic matter, toxic pollutants and large particles ofin raw PSW, are not only toxic to the environment but are also a danger to humans. The production of poultry meat has been one of the leading consumable items among meat production in South Africa, which significantly contributes to nearly 20 % of the production industry within the past decade (DAFF, 2014). The nature of the activities in poultry slaughterhouses is directly associated connected to the use of high volumes of clean water during washing and bird processing. Such a vast volume of wastewater needs to be adequately treated to mitigate ecosystem degradation and limit it’s potential to harm human health.

1.3 Hypothesis

It is hypothesized that the pretreatment process utilizing Eco-flush^{TM} would contribute to the high efficiency of the DEGBR as an anaerobic system and contribute hugely to the overall performance of the lab scale system designed.

1.4 Research questions

- Can the FOG hydrolysing agent called Eco-flush^{TM}, be effective and efficient in the removal of FOG, COD and TSS in the pre-treatment of PSW?
- Can high COD, FOG and TSS removal efficiencies and a suitable VFA/Alkalinity ratio in the DEGBR be maintained?
- Is the proposed process of the biological pre-treatment coupled with DEGBR efficient enough for the treatment PSW?
- Is there any quantifiable amount of biogas generated in the DEGBR?

1.5 Aim and objectives

1.5.1 Aim

This study sought to evaluate the performance of a biological pre-treatment system coupled to a DEGBR for the removal of organic matter and toxic pollutants from PSW. The study was accomplished by addressing the following objectives stated below.

1.5.2 Objectives

Objective 1: To determine the performance of the pre-treatment stage after addition of the Eco-flush^{TM} (hydrolysing agent) in terms FOG, COD and TSS removal efficiency.

Objective 2: To determine the overall performance of the DEGBR in terms of FOG, COD and TSS removal at set operational HRT and OLR.

Objective 3: To determine the overall performance of the biological pre-treatment system coupled with the DEGBR in terms of FOG, COD and TSS removal. Moreover, evaluate the Alkalinity and volatile fatty acids (VFA) and the stability of the anaerobic DEGBR at the set operational conditions in terms of pH and temperature.

Objective 4: To determine the amount of biogas produced by the DEGBR.

1.6 Significance of the research and expected outcomes

This research can assist municipalities and the poultry slaughterhouse industries in the world by developing a new prototype design, which can enhance the treatment of PSW in a cheap, faster and effective manner. Furthermore, the research can assist researchers to further develop ideas of how efficient and effective is the employ of a biological (hydrolysis) pre-treatment system when coupled to a DEGBR for the treatment of raw PSW.

1.7 Delineation of the research

This study was conducted in the Western Cape Province, SA and the samples of PSW were collected from only one of the major poultry processing plants in the region. This study did not focus on solid, sewer waste and as well as ammonium gas generation. Moreover, the study did not focus on the cost evaluation, and energy usage which could be an important aspect for future studies.

CHAPTER TWO

LITRATURE REVIEW

Partially published as conference proceeding in 2019:

Derrick N. Dlamini, Moses Basitere and Seteno Karabo O. Ntwampe. 2019. Current and Functional Reactor Designs in Poultry Slaughterhouse Wastewater Treatment. 16th SOUTH AFRICA Int'l Conference on Agricultural, Chemical, Biological & Environmental Sciences (ACBES-19) Nov. 18-19, 2019 Johannesburg (S.A.). pp 295-300, https://doi.org/10.17758/EARES8.EAP1119146.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Poultry product demand, water usage and PSW generation

Water is an important aspect to human existence, and as well as to all other living things on earth. The South African poultry industry slaughters several million chickens a week for consumption purposes (Whitfield, 2018). Moreover, the meat processing industry is one of the largest consumers of freshwater in the world (Bustillo-Lecompte & Mehrvar, 2017) and South Africa. Poultry slaughterhouses use high volumes of water during bird processing and thus yielding high volumes of PSW (Yaakob *et al*., 2018). The rising demand of poultry produce requires an increase in poultry processing facilities which would results in further high volumes of PSW to be treated (Valta *et al.*, 2015). The poultry slaughtering process normally uses large amounts of water in all of the processing stages which include slaughtering, de-feathering, chilling, and by-product processing to mention but a few. Annually, the South African poultry industry is reported to have consumed 6 000 000 m^3 on average and about 90% of the water used during bird processing which gets to be discharged as wastewater to the environment (Kloppers *et al*., 2015). Furthermore, it was also reported that on average, a bird consumes about 17 to 20 litres of water during processing.

In 2016, South Africa slaughtered more than 935 million broilers and has also increased the production of white meat from 1 537 519 tonnes (2016) to 1 615 509 tonnes in 2018 (SAPA, 2018). The South African poultry industry dominates the animal meat production sector by providing up 65.3% of locally produced animal protein consumed in the country (DAFF, 2018). South Africa consumed 2.300 million tonnes of poultry during the period of 2018 (SAPA, 2018). Moreover, still in 2018, South Africa imported 539 297 tonnes of chicken and it produced 1 615 509 tonnes of chickens locally. This depicts that the country is under enormous pressure of fulfilling consumer demand for poultry meat. Figure 2-1 and Figure 2-2 below depict per capita consumption of protein, and the total annual boiler imports against South African production. With high poultry production values, one may draw an inference that the water usage is proportional with a high generation of PSW. This gives rise to the need of designing efficient wastewater processing plants to be used in treatment the PSW.

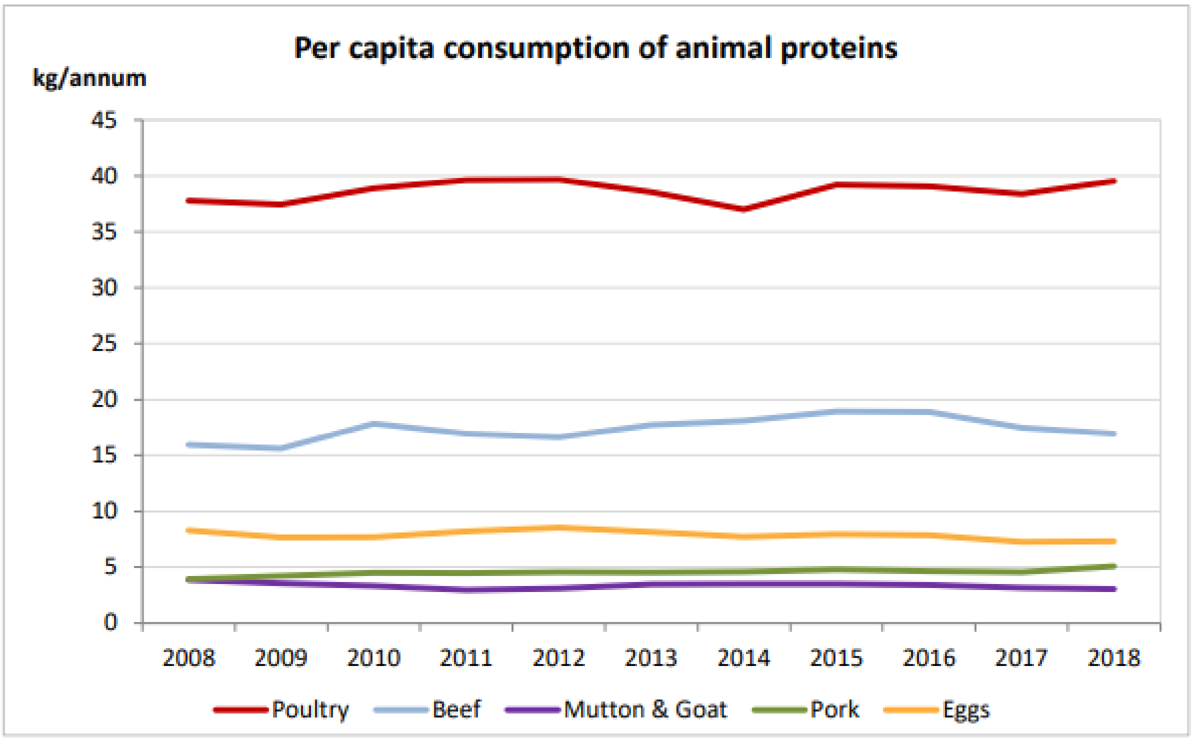


Figure 2-1: Per capita consumption of protein sources from 2008 to 2018 (SAPA, 2018)

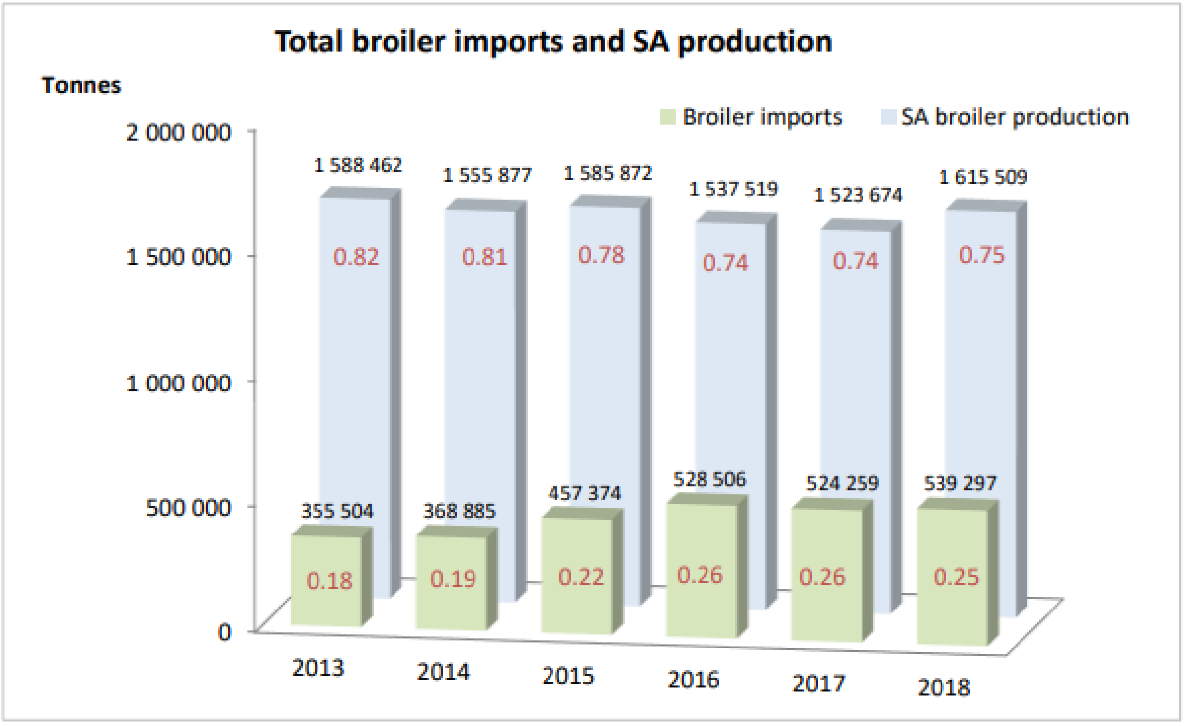


Figure 2-2: Total annual chicken imports since 2013 against local production (SAPA, 2018)

PSW is difficult to treat in nature, and it needs significant treatment before it is safely discharged to the environment, due to the high amounts of organics and nutrients contained therein (Barrera *et al*., 2012). The poultry slaughterhouse process involves a series of processing steps, from processing a live bird to converting it to edible meat fit for human consumption (see Figure 2-3). The latter processing steps require large volumes of freshwater during processing. In the past decade there has been a development for tracking water-use efficiency and monitoring within slaughterhouses (Kiepper, 2017). The calculation is based on bulk quantities of materials handled at each processing step at a poultryslaughter plant. This would translate to the measurement of gallons of water used per 1,000 (“Ib”) pounds of material handled. Commercial poultry processing plants may vary widely in the amount of portable water they use per bird and a lot of this water is used during scalding, chilling, bird washing, and plant sanitation (Kiepper *et.al.*, 2008). The high volume of water usage emanates from the fact that these steps require numerous sanitization (Meneses *et al.*, 2017). In order to avoid cross contamination, water is used at nearly all points of the poultry process plant (Park *et al*., 2015). The operations carried out at the various processing stages would involve, reception, stunning, bleeding, scalding, plucking, head pulling, hock cutting, evisceration and spray washing.

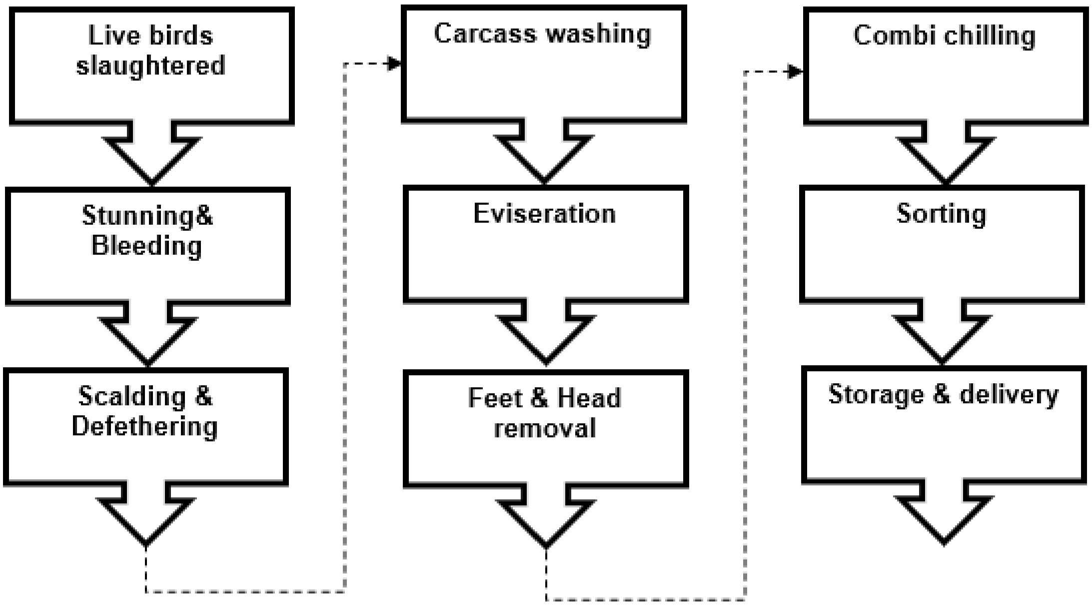


Figure 2-3: Flowchart of processing steps used in poultry plants (Park et al., 2015)

2.2 The characteristics of poultry slaughterhouse wastewater

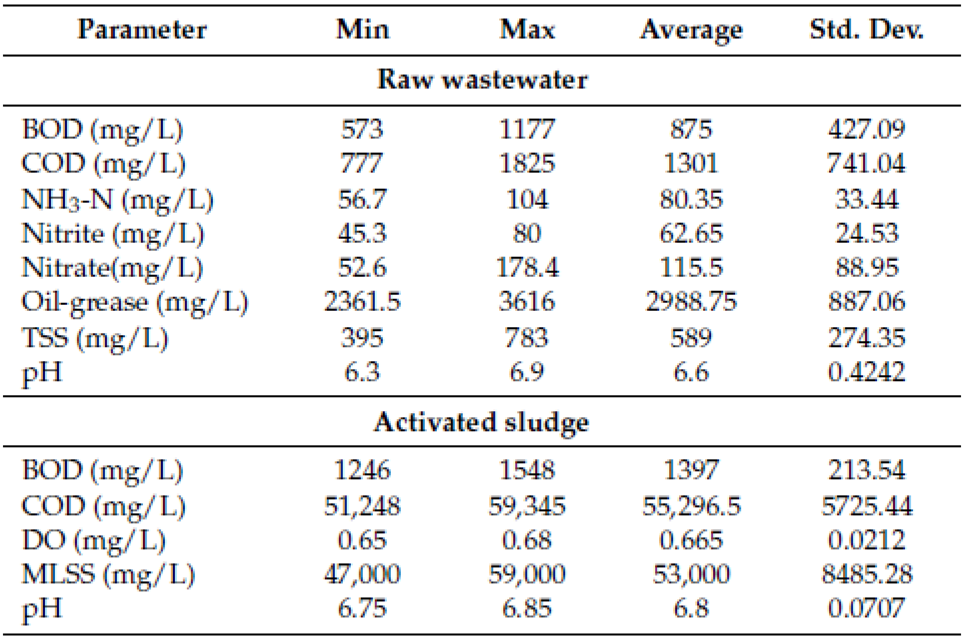
PSW gets generated from bird slaughtering, de-feathering, bird washing, deboning and bird trimming activities (Bustillo-Lecompte *et al*., 2017). Generally, PSW contains organic matter either in solid form or dissolved form (Del Nery *et al*., 2007). Wastewater generated from poultry slaughterhouse also contains high levels of COD, BOD, phosphorus, and nitrogen including suspended/dissolved solids (Sirianuntapiboon & Manoonpong, 2001). This is caused mainly by the presence of blood, FOG, meat debris, and feathers. It therefore vital to treat the wastewater generated from poultry slaughterhouse to acceptable environmental standards before being discarded to the environment. The profiling of PSW effluent was reported by researchers dating back to about five decades ago. A study on the effects of chicken blood and its components on wastewater characteristics was pursued (Garcia *et al.*, 2016). According to Garcia et al., (2016), it was discovered that blood was the most potent contributor to pollutant loads in chicken processing plant wastewater. Although, there were problems faced with the quantification of the impact of components found in the chicken blood.

In another different study, it was discovered that the direct disposal of untreated slaughterhouse wastewater into the environment led to the promotion of the growth of macroalgae (Yaakob *et al*., 2018). A research was conducted in order to compare the proximate composition of particulate matter recovered from PSW generated (Kiepper *et al*., 2008). In this study, it was discovered that FOG made up more than half of dry weight matter recovered. Moreover, there was some huge significant differences in percentage protein and ash among poultry slaughterhouse plants. It has been proven that there is no consistency of parameters of the PSW among different broiler slaughter plants within the same or different regions. These fluctuations in PSW parameters emanates from a wide range of operational factors experienced in the plants. In some broiler plants, the blood gets to be overly diluted by massive usage of freshwater used during cleaning of the carcass.

Some decades ago in one notable previous study of PSW, it was reported that there were considerable fluctuations within and among plants for each parameters measured (Singh *et al.*, 1973). In that year, Singh *et al*. (1973), reported a composition of combined poultry plant wastewater with a BOD concentration of 1475 mg/L. In the following year, PSW characteristics with a BOD concentration of 390 mg/L was reported (Carawan *et al.*, 1974). A variance between the PSW characteristic results was reported, with some BOD concentration of 1085 mg/L, which is a clear indication of fluctuations in similar effluent.

In the 1980's, COD concentration of PSW was reported to be 1968+-111 mg/L (Shih & Kozink, 1980). In the next coming years, a COD concentration range of 3610-4180 mg/L of PSW was reported (Yordanov, 2010). Therefore, there is no official defined average for raw PSW parameters. Other parameters in addition to the ones listed above would include pH, FOG (mg/L), TSS (mg/L), TSD (mg/L), VSS (mg/L), Total Kjheldahl Nitrogen, Alkalinity (mg/L), Ammonia (mg/L), Total phosphorus (mg/L), Soluble proteins (mg/L), and volatile fatty acids(mg/L). Typical results of recently published PSW parameters are as follows (see Table 1).

Table 1: Characteristics of raw wastewater and activated sludge (Aziz *et al*., 2018)



Parameter	Min	Max	Average	Std. Dev.
Raw wastewater				
BOD (mg/L)	573	1177	875	427.09
COD (mg/L)	777	1825	1301	741.04
NH_3-N (mg/L)	56.7	104	80.35	33.44
Nitrite (mg/L)	45.3	80	62.65	24.53
Nitrate(mg/L)	52.6	178.4	115.5	88.95
Oil-grease (mg/L)	2361.5	3616	2988.75	887.06
TSS (mg/L)	395	783	589	274.35
pH	6.3	6.9	6.6	0.4242
Activated sludge				
BOD (mg/L)	1246	1548	1397	213.54
COD (mg/L)	51,248	59,345	55,296.5	5725.44
DO (mg/L)	0.65	0.68	0.665	0.0212
MLSS (mg/L)	47,000	59,000	53,000	8485.28
pH	6.75	6.85	6.8	0.0707

Some of these parameters can be explained as follows:

BOD (biological oxygen demand) is a measure of biochemically oxidizable material present in wastewater of which is expressed by the amount of (O_2) oxygen is required to consume it (Metcalf & Eddy, 2003). The measurement of the contamination is denoted by the following units, mgO_2/L. BOD_5 is standard measurement method which is performed at a temperature of 20˚C for a period of 5 days.

COD (chemical oxygen demand) is a measurement of (O_2) oxygen required to breakdown organic substances and further convert them to (CO_2) carbon dioxide and (H_2O) water (Stone, 2013). It is also denoted by mgO$_2$/L, and it is worth noting that it shares similar units as the BOD but the main difference is that COD measures all organic material contained in the wastewater, while BOD only measures biologically degradable organic material. In principle, the higher the COD value the more contaminated/polluted the wastewater is.

pH is the measurement of acidity or alkalinity of a solution or effluent on a logarithmic scale. pH 7 is the neutral point while anything below 7 is termed to be acidic and anything above 7 is alkaline. PSW effluent normally contains a pH between 6-9 depending on a number of factors.

FOG has proved to be cumbersome and a growing environmental and operational concern because it leads to blockage of pipes and restrict wastewater flow (Husain *et al.*, 2014). Accordingly, FOG is composed of fatty acids, triacylglycerols and hydrocarbons. Moreover, (Yordanov, 2010) reported a range of 289 - 389 mg/L concentration of fats in raw PSW effluent.

TSS (total suspended solids) is the dry weight of suspended particles that have not dissolved in wastewater which is expressed in mg/L and it is of interest in terms of water quality control (Verma *et al.*, 2013).

Nutrients are mainly characterised by the total kjheldahl nitrogen, ammonia (mg/L), total phosphorus (mg/L) present in wastewater. Nutrients contained in PSW have a tendency of causing eutrophication which could lead to an imbalanced ecosystem (Cai *et al.*, 2013).

2.3 Biological agents used in the treatment of wastewater

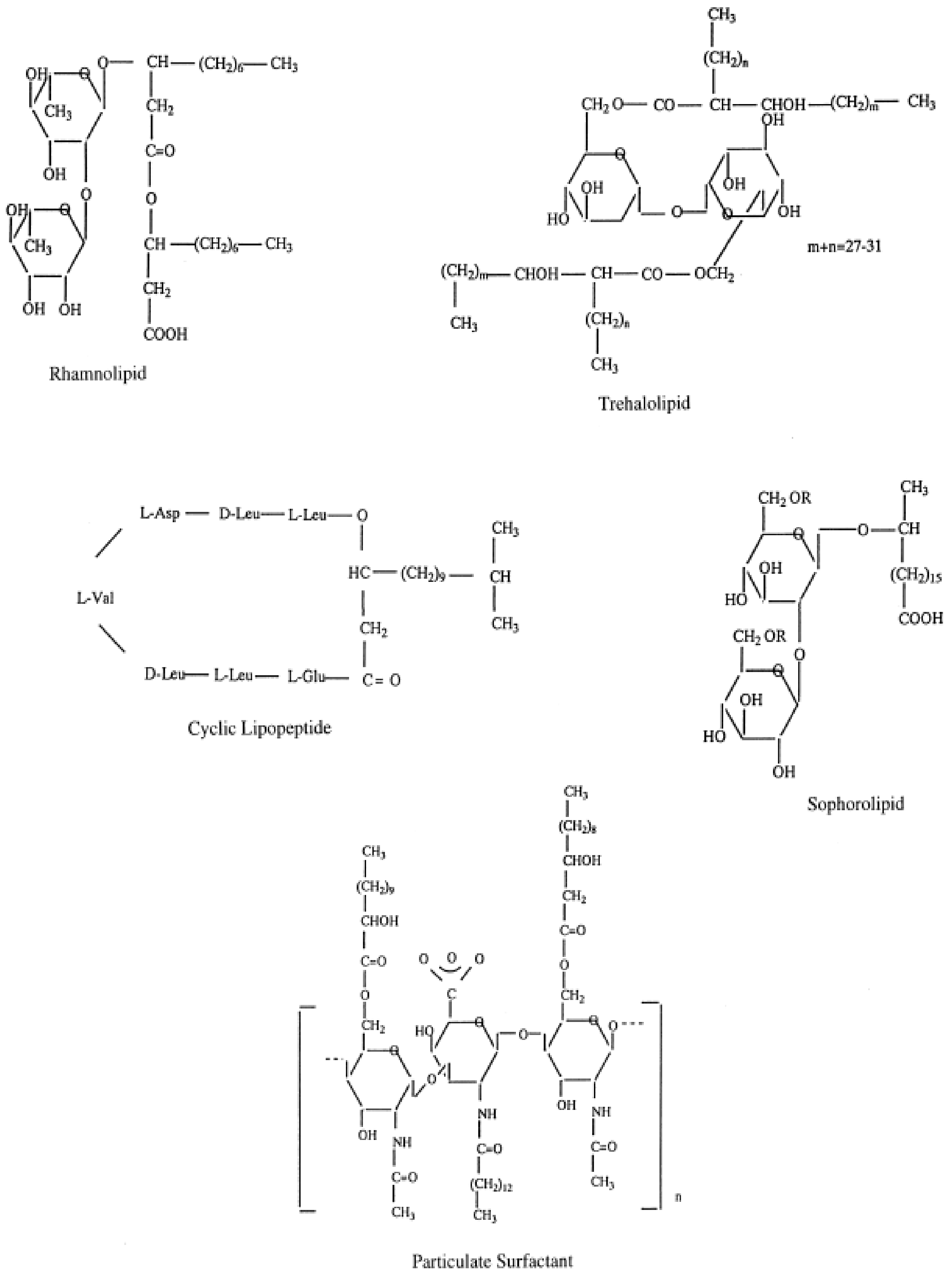
Biological enzymes are protein catalysts that speed up a reaction without being used up. Biological additives employed in wastewater treatment have been found to have significant beneficial impacts and do not harm onsite systems (Tang & Tong, 2011). According to Tang & Tong (2011), ‘Garbage enzyme’ was experimented with in the treatment of domestic wastewater and it was found to have raised the wastewater BOD in proportion to its dilution. Generally, enzymes used in wastewater treatment belong to the category of biological additives. Moreover, an enzyme called ‘pancreatic lipase’ was used for hydrolysis and to reduce FOG in slaughterhouse wastewater (Masse *et al*., 2001), and it reduced a huge amount of pork fat in the pre-treatment step. The latter, ‘pancreatic enzyme’, was isolated from a pig pancreas which contains lipase, some small amounts of amylase and protease which are primarily used in the hydrolysis of carbohydrates and protein.

Fat accumulation has established itself as being a big problem in wastewater treatment processes, thus henceforth biological pre-treatment is employed in order to reduce the amount of fat concentration (Del Nery et al., 2007). There are a few studies that have been pursued when it comes to biological pre-treatment of wastewater so far. When enzymes are employed in the pre-treatment of PSW effluent, the triglycerides are hydrolysed to fatty acids and glycerol, and this leads to faster and efficient biodegradation of fat by microorganisms (Damasceno *et al*., 2018). The efficiency of enzymic pre-treatment was reported to have shown a positive effect of COD removal when a simultaneous enzymatic hydrolysis and biodegradation of lipids in PSW effluent was used (Dors *et al*., 2013). Biosurfactants have been previously been studied in the treatment of wastewater as well. Biosurfactants are biomolecules produced by microorganisms and can also be used in the treatment of PSW effluent. An evaluation of a biosurfactant, obtained from cassava wastewater, used in the treatment of PSW effluent yielded an oil and grease removal of above 70% (Natassia *et al*., 2017).

Biosurfactants are categorized according to their microbial origin and structure. They are also divided into different classes such as phospholipids, lipoproteins, glycolipids and polymeric biosurfactant (Md, 2012). Moreover, they can move at interfaces between fluids with different polarities because they are hydrophobic and hydrophilic in nature (Karanth *et al*., 1999). Biosurfactants have good properties like biodegradability, digestibility and biocompatibility which make them appropriate for treatment of industrial wastewater (Vijayakuma & Saravanan, 2015).

Advantages of biosurfactants are enormous as compared to synthetic surfactants and they are generally considered to be less or non-toxic. A study of a biosurfactant called ‘LGP’ was conducted in comparison with a chemical surfactant called ‘Finasol’, the biosurfactant proved to be 3 times lower in terms of toxicity levels (Poremba *et al*., 1991). Furthermore, most biosurfactants are not affected by adverse environmental factors such as pH and temperature. Bio-emulsifiers isolated at pH range of 5-10 and temperature of 80 ˚C were found to be effective (De Trebbau Acevedo & Mclnerney, 1996). In terms of availability, biosurfactants have been found to be easily obtainable in large amounts and are very cheap. A pilot plant and large scale of bioremediations of soil contaminated with hydrocarbons and oil was used a source of biosurfactants (Kosaric, 2001).

In terms of surface and interfacial activity, biosurfactants become efficient and effective from a range of 1 to 2000 mg/L, while the interfacial activity and surface tension operates at a range of 1 to 30 mN/m (Santos *et al.*, 2016). Lastly, biosurfactants can either be emulsifiers or de-emulsifiers. An emulsion is a fine dispersion of droplets of one liquid immersed another in which it is not soluble or miscible. Emulsions are normally in two forms, one of water in oil and another of oil in water. Below is a list of the types of chemical structures of biosurfactants produced by microorganisms (see Figure 2-4).



Rhamnolipid

Trehalolipid

Cyclic Lipopeptide

Sophorolipid

Particulate Surfactant

Figure 2-4: Types of biosurfactants produced by microorganisms (Cameotra & Makkar, 1998)

Bacteria is divided into three types of groups in accordance to their response toward free molecular (O_2) oxygen and enzymatic ability to degrade substrates (Gerardi, 2003). The three groups are; facultative anaerobes, strict aerobes and anaerobes. According to Gerardi, (2003), strict aerobes or aerobic bacteria is used in the presence of oxygen or an aerated environment. The bacteria used in strict aerobe processes uses the oxygen found in water to degrade pollutants and further reproduce itself. The oxygen is usually supplied mechanically by aerators.

Facultative anaerobes normally operate in the presence of (O_2) oxygen but are also capable of functioning in the absence of oxygen (Stieglmeier *et al*., 2009). Anaerobic bacteria functions actively in the absence of oxygen. The anaerobic bacteria get nutritional sources from the food or nutrients instead of oxygen, and in most cases the process is advantageous in that it produces a reduced volume of sludge and methane gas is also generated from it as well. The methane gas produced from the process, if collected, can be used as a source of energy which can make it economically viable.

In the biological treatment of FOG the bacteria degrade it through enzymes. There are two types of enzymes used in the degradation of FOG, namely exoenzymes and endoenzymes (Burgess & Pletschke, 2008). An exoenzyme is an enzyme that is secreted by the cell and it operates outside of it, whereas an endoenzyme operates within the cell. In another study, an elimination of oil from enzyme pretreated wastewater was performed using activated sludge, with the process yielding a biodegradation efficiency of 90% (Cisterna, 2017).

According to Gerardi, (2003), there are three main types of bacteria groups used in the degradation of substrates in an anaerobic digester. These are acetate forming, sulfate reducing and methane forming bacteria. Acetate forming bacteria activity secrets acetate and hydrogen during degradation, sulfate bacteria degrades organic matter present in the effluent while reducing the sulfate to hydrogen sulfide (H_2S). In the process of methane forming bacteria, the microbes' activity results in the formation of methane gas as a by-product.

The role of microorganisms in biological wastewater treatment processes involves the removal of dissolved and particulate carbonaceous BOD (Metcalf & Eddy, 2003). Biological processes employed in the treatment of effluent are divided into two classes, namely; suspended growth process and attached growth process. According to Metcalf & Eddy (2003), in suspended growth processes, the micro-organisms used in the treatment are maintained in liquid suspension by appropriate mixing methods. Activated sludge processes are a typical example of suspended growth processes. The latter process utilizes aerobic microorganisms or activated sludge (AS) that can digest organic matter present in wastewater (Shchegolkova *et al*., 2016). The microorganisms grow as they tend to clamp on each other together, developing into larger particles called flocs. The flocs are then allowed to settle at the bottom of the tank and thereby creating a clear liquid which is free of organic matter. In attached growth processes, the bacteria or microorganisms treating the wastewater are attached to the media material placed in the reactor (Gavrilescu & Macoveanu, 2000). Organic matter is removed from the wastewater as the effluent passes through a biofilm. The attachment media used in this type of processes could be rocks, gravel, sand and plastics to mention but a few.

Diagrams of suspended growth process and attached growth process are shown below (see Figure 2-5):

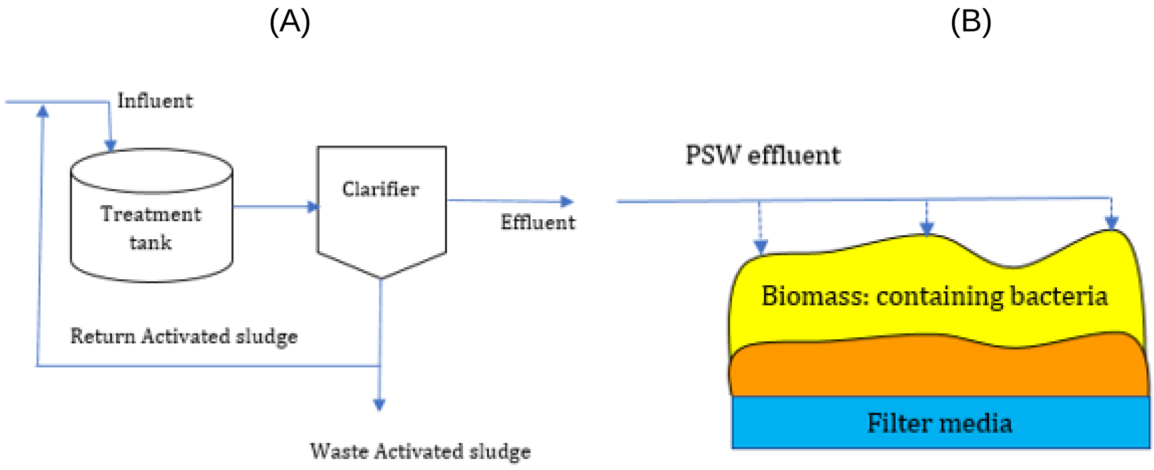


Figure 2-5: Biological treatment: Suspended growth (A) & Attached growth processes (B)

2.4 Types of wastewater pre-treatment processes

Pre-treatment is the preliminary removal of material such as FOG, and other various solid matter contained in the PSW effluent. The pre-treatment process stage of PSW effluent is highly recommended preceding the secondary treatment by using a bioreactor (De Nardi *et al*., 2011). Pre-treatment processes can either be physical, chemical, and biological, which are mainly used to remove contaminants contained in PSW. Biological processes are processes where living microbial organisms or the extracellular products are used (Wang *et al*., 2014), while chemical processes involve the addition of chemicals like coagulants and flocculants (El-gohary *et al*., 2010). Physical processes would refer to conventional process that mainly utilize screens of different aperture sizes, fat traps and grit chambers for removal of larger particles in a conventional method (Voutchkov *et al*., 2014).

2.4.1 Conventional pre-treatment methods

The pre-treatment of wastewater is done with the sole purpose of removing large organic matter that may cause clogging, block pipes and damage pumps in the subsequent stage of processing (Metcalf & Eddy, 2003). In conventional pre-treatment processes, the influent passes through a screen in order to remove large objects (EPA, 1998). Typically, screen aperture sizes for coarse particle removal is +6mm and for fine particle removal is 1,5 to 6mm (U.S. EPA, 2000). After the influent has been screened it then passes onto a grit chamber where the sand and small stones settle at the bottom and are then removed. The removal of grit normally prevents wear and tear of the equipment. It then becomes vital that grit chambers work efficiently during peak times whereby the volumetric flow rate is also high. At this point, it must be borne in mind that the influent from the grit chamber still contains a substantial amount of organic matter and suspended solids. These biosolids are then removed in a sedimentation tank where they are first allowed to settle at the bottom when the flow rate of water is reduced. A study of inorganic suspended solids removal from wastewater by using a grit chamber yielded a COD removal efficiency of 5.2% and a TSS of 12.6% (He *et al.*, 2019).

The sedimentation of biosolids is classified as the primary treatment because it utilizes gravity sedimentation as the suspended solids settle at the bottom (see Figure 2-6). At this stage of the process, BOD is greatly reduced. The solids settleable at the bottom of the sedimentation tank are removed as sludge and the resultant product is effluent. The effluent discharged is passed on to the secondary treatment step which could be in the form of a biological treatment process.

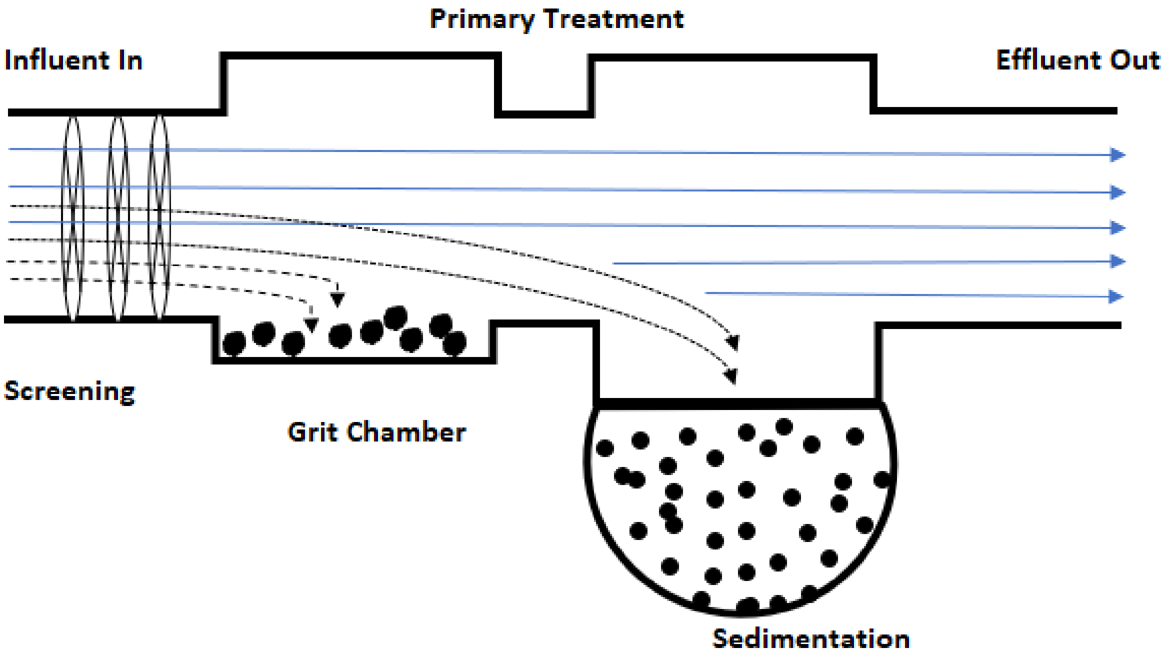


Figure 2-6: Schematic for a conventional pre-treatment process (EPA, 1998)

2.4.2 Physico-chemical pre-treatment methods

It is recommended that the effluent from the preliminary treatment is passed on to a subsequent primary and secondary treatment for further processing depending on the strength of the PSW. Physicochemical processes are predominantly used as a primary treatment facility, and it has also been undisputed for its predominance in a wide spectrum of wastewater treatment plants (Liu, 2014). A physico-chemical process employed in wastewater treatment is done so with the intention of removing suspended solids and FOG prior to the secondary treatment process. Below is a list and discussion of commonly used physicochemical methods.

2.4.2.1 Dissolved air floatation (DAF)

The dissolved air flotation (DAF) process is a method whereby liquid-solid separation typically happens by dissolving air in wastewater under pressure (Bustillo-Lecompte & Mehrvar, 2015). As the air is blown at the bottom, suspended particles like FOG are transported to the top of the flotation tank. At the top, the solids then form a sludge layer where they are constantly removed by a skimming device. The effectiveness and efficiency of the DAF process is usually supplemented by the addition of chemicals such as polymers and flocculants. The latter is performed in order to adjust pH and facilitate flocculation of the suspended particles. A DAF system was reported in a study of the treatment of PSW with a surface loading rate of 1.6±0.4 m^3/m^2, yielded a removal efficiency of oil and grease of 51±16% and suspended solids of 37±16% (Del Nery *et al*., 2007). In another study of a chemical-DAF system by using ferric chloride and cationic polymer in the treatment of PSW, nutrients removal was found to be ≥99% and TSS of 65±25% (De Nardi *et al*., 2011). The DAF process has been reported to be more appropriate for treatment of high volumes of wastewater (Schalkwyk *et al.*, 2016). Moreover, the DAF, also has additional advantages relating to its stable operation which is associated with low cost of running a wastewater treatment plant (Tian *et al*., 2018).

The theory for removal of particles in the DAF process is based on four main principles, namely; 1) gas-water equilibrium, 2) bubble formation, 3) gas precipitation, and 4) flotation of bubble particle agglomerates (Srinivasan & Viraraghavan, 2009). Firstly, the air is transferred across the air-water interface whereby particles having specific gravity almost equal to the specific gravity of water are floated. The air bubbles then float the particles to the top of the tank. Under bubble formation, the nucleation process occurs as soon as the pressurized water is realised through the nozzles. The nucleation process is followed by the enlargement of the bubbles caused by excess air build-up in the water. As the volume of the air bubbles increases in size, they also decrease in hydrostatic pressure as well. Thirdly, there are three mechanisms that occur in the bubble particle attachment phase, namely, precipitation first, bubbles get to be trapped in a floc structure as they rise through the water and the bubbles are then absorbed and formed into a floc (Palaniandy *et al*., 2017). Finally, the flocs float due to the fact that the bubbles reduce the density of the bubble-particle agglomerate. The theory behind the floatation is based on the size of the particles, if the particles are large in size then they would require more bubbles whereas if they are small in size then they would require less bubbles. The agglomerates will then form at the top surface of the wastewater where they will then be removed by a skimmer. An illustration of the DAF process is presented in Figure 2-7.

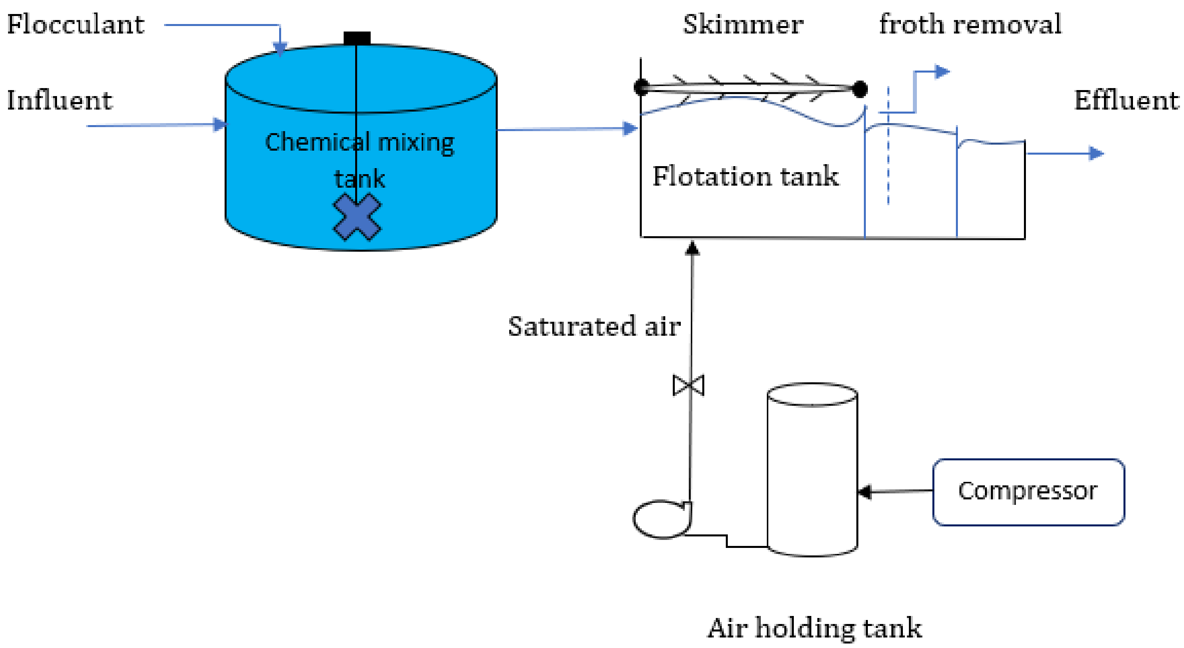


Figure 2-7: Schematic of a chemical-DAF system for PSW treatment

2.4.2.2 Coagulation and flocculation

The coagulation and flocculation pre-treatment of PSW effluent makes use of chemical reactants such as flocculants and coagulants to separate suspended and dissolved solids from water (see Figure 2-8). Coagulants destabilize particles because they have opposite charges to those of suspended solids, and flocculants prompt the destabilized particles to conglomerate into larger particles called flocs (Coca *et al*., 2011). Aluminium sulfate $Al(SO_4)_3$, ferric sulfate $Fe_2(SO_4)_3$, and ferric chloride $FeCl_3$ have commonly been used as coagulants in the treatment of PSW effluent. Results of the latter experiments showed BOD, COD, and oil and grease removal efficiency of up to 78.8%, 79.5%, and 85% respectively (de Sena *et al*., 2008).

In another study of physicochemical treatment of PSW effluent using lime, alum, ferrous sulfate and anionic polyelectrolyte as coagulants was performed. The results yielded a removal efficiency of BOD, COD and SS up to 38.9%, 36.1% and 41.9% (Satyanarayan *et al*., 2005). Even though it was found to be costly, a good removal efficiency was found from the use of lime and anionic polyelectrolyte, with removals of SS, BOD and COD reported to be 54.2%, 49.6% and 43.8%. (Dassey & Theegala, 2012). A combination of ferric chloride and floccin yielded a COD, VSS, TSS and FOG removal of up to 91%, 97%, 98% and 100%.

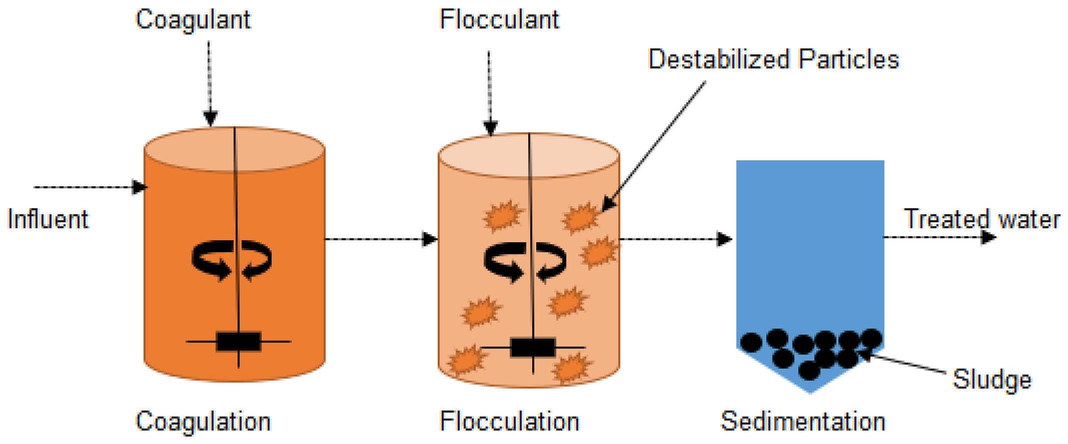


Figure 2-8: Schematic diagram for coagulation-flocculation process (Teh et al., 2016)

2.4.2.3 Electrocoagulation

Electrocoagulation, used as a pre-treatment method, removes suspended solids and other contaminants through the inducing of an electric current together with the addition of chemicals (Bayar *et al.*, 2014). The induced electric current changes the suspended solids surface charge thereby allowing the particles to form an agglomeration. (Kobya *et al.*, 2006) examined an electrocoagulation treatment of PSW by investigating the effects of electrode material, pH, and current density on the COD and FOG removal. The highest COD removal was reached with aluminium (Al) electrodes at 93% and the highest FOG removal was reached with iron (Fe) electrodes at 98%.

There are a lot of metals used as electrodes in the treatment of PSW effluent, among others, are aluminium, iron, tin dioxide, and titanium dioxide. In another study, the effect of pH was evaluated in conjunction with aluminium plate electrodes for the treatment of PSW effluent. It was established that the pH had a considerable effect on the treatment of PSW effluent and also optimum pH values were found to be within the range of 3 to 4 with a COD removal efficiency above 85% (Bayar *et al.*, 2014).

(Ahmadian *et al.*, 2012) performed a study of the treatment PSW effluent by using a batch system using iron (Fe) electrodes. In that study it was discovered that an increase in the number of electrodes or current was proportional to an increase in pollutants removal. The results of the latter study showed that at a current of 10 A/m^2, the removal efficiencies of 66% for BOD$_5$, 62% for COD, 60% for TSS and 50% for

TN was achieved as compared to the current 25 A/m^2 where the removal efficiencies of BOD_5 of 97%, COD of 93%, TSS of 81% and TN of 84% was observed respectively.

Lastly, in another study of pre-treatment of PSW effluent by using the electrocoagulation process with aluminium electrodes at a steady voltage of 9 V, and a 5min retention time, yielded removal efficiencies of 87% for BOD, 59% for COD, 84% for TSS and 94% for FOG (Sardari *et al*., 2018). However, it must noted that the metal plate electrodes and the use of electricity comes at a hefty price if the process is running for longer periods.

When Al (aluminium) and Fe (Iron) electrodes are used in the treatment of wastewater, the metal cations dissolve at the anode according to the following equations:

$$\mathrm{Fe}_{(s)} \longrightarrow \mathrm{Fe}^{n+}_{(aq)} + \mathrm{ne}^{-} \tag{2.1}$$

$$\mathrm{Al}_{(s)} \longrightarrow \mathrm{Al}^{3+}_{(aq)} + 3\mathrm{e}^{-} \tag{2.2}$$

At the cathode, the following reaction occurs:

$$2\mathrm{H}_2\mathrm{O}_{(l)} + 2\mathrm{e}^{-} \longrightarrow \mathrm{H}_2 + 2\mathrm{OH}^{-} \tag{1.3}$$

$$3\mathrm{H}_2\mathrm{O}_{(l)} + 3\mathrm{e} \longrightarrow \frac{3}{2}\mathrm{H}_2 + 3\mathrm{OH}^{-} \tag{2.4}$$

In solution, the following reaction occurs:

$$\mathrm{Fe}_{(s)} + 2\mathrm{H}_2\mathrm{O}_{(l)} \longrightarrow \mathrm{Fe(OH)}_{2(s)} + \mathrm{H}_{2(g)} \tag{2.5}$$

$$\mathrm{Al}^{3+}_{(s)} + 3\mathrm{H}_2\mathrm{O}_{(l)} \longrightarrow \mathrm{Al(OH)}_{3(s)} + 3\mathrm{H}^{+}_{(g)} \tag{2.6}$$

2.4.2.3 Membrane technology

The membrane separation technology utilizes a special porous film which is subjected to high pressure of wastewater thereby trapping particles (Zargar, *et al*. 2015). Ultrafiltration (UF), microfiltration (MF), reverse osmosis (RO) are the commonly used membrane separation technologies for the treatment of PSW. Ultrafiltration can separate compounds between the range of 0,005–10 µm, microfiltration can separate compounds between the range of 0,1-0,2 µm, and reverse osmosis can separate particles smaller than 0,1 nm in diameter. (Malmali *et al*., 2018) evaluated the performance of ultrafiltration membrane used in the treatment of PSW. The results showed significant removals of BOD, TSS, COD, and FOG of up to 93%, 100%, 94% and 100%, respectively. In another study, the effectiveness of ceramic microfiltration membranes sintered at a temperature of 1200˚C yielded a total insoluble residue rejections of up to 100% and a bacterial removal of 93% (Almandoz *et al*., 2015). Lastly, a study on the performance of reverse osmosis was conducted in the treatment of PSW whereby a COD removal of up to 90% was achieved (Coskun *et al*., 2016). It also worth noting that the membrane technology bears some numerous disadvantages which range from, high energy consumption.

2.5 Biological pre-treatment methods

Biological pre-treatment is ordinarily used as a secondary treatment step, but it may also be used as a primary pre-treatment step. Preliminary steps of PSW treatment do not completely treat wastewater to a degree of satisfaction as set out by environmental standards. The crux of this study is based on the biological pre-treatment of PSW which utilizes microorganisms to remove organics and pathogens while hydrolysing constituents in FOG. Moreover, the microorganisms used in such process would be fungi, bacteria or algae. There are different types of biological treatment processes, which include but are not limited to, anaerobic, aerobic, activated sludge and combined processes.

2.5.1 Anaerobic treatment of poultry slaughterhouse wastewater

The anaerobic treatment is a technique which is widely used in the treatment of high strength PSW (Pozo *et al*., 2003). There are advantages that come with the use of anaerobic treatment processes which range from low electricity costs, high throughput of biogas, less sludge generation, and high organic matter removal (Aziz *et al*., 2019). Usually, effluent discharged from an anaerobic treatment step needs additional treatment in order to further remove contaminants such as total kjheldahl nitrogen (TN), total phosphorous (TP) and as well as other pathogens (Gomec, 2010). Typical anaerobic processes used in the treatment of PSW effluent comprise of anaerobic lagoons (AL), anaerobic filter (AF), up-flow anaerobic sludge blanket (UASB) and anaerobic baffled reactor (Bustillo-Lecompte & Mehrvar, 2015).

(León-Becerril *et al*., 2016) evaluated the performance of an up-flow anaerobic filter (AF) used in the treatment of wastewater. The study reported a COD and BOD removal efficiency of 81% and 87% with a 15 days' period. In another study of the treatment of PSW by using an anaerobic UASB with a hydraulic retention time (HRT) of 1 day, removal efficiencies of up to COD of 70%, BOD of 73%, and FOG of 35% were obtained respectively (Del Nery *et al*., 2016). (Yousefi *et al*., 2018) conducted a study of treatment of PSW effluent by using a combined anaerobic system of 3 pilot-scale anaerobic baffled reactors (ABR) in the first stage, followed by 3 anaerobic filters (AF). An evaluation of the ABR reactor used in the latter study showed a COD removal efficiency of 83% after an HRT of 18 hours, and the AF reactor showed a removal efficiency of 63%, respectively.

2.5.2 Activated sludge processes

Activated sludge (AS) process methods utilize a combination of aeration and a biological floc composed of bacteria and protozoa. In one previous study which focused on the isolation and quantification of bacteria present in the sludge obtained from treated PSW, it was reported that by using different N-free culture media technique, it resulted in the isolation of 16 diazotrophic strains from the sludge of a PSW treatment system (Lozada *et al*., 2018). Primarily, the AS process is designed for the removal of soluble and insoluble organic pollutants present in the wastewater. The AS process uses aerobic micro-organisms that can degrade pollutants and agglomerate them by flocculation (Bustillo-Lecompte & Mehrvar, 2015). After aeration, the agglomerated particles will settle and then get separated from the clear water. The AS process has been widely and effectively used in the treatment of PSW. In another study of an evaluation of the activated process (AS) used in the treatment of PSW by kinetic model simulation (Hsiao *et al*., 2012), the AS reactor at 26˚C yielded a COD removal efficiency between 93.5% to 97.2% respectively. (Carvalho *et al*., 2013) evaluated the role of the AS system in the removal of pollutants present in the PSW. The results indicated that sorption to sludge and wastewater organic matter was responsible for the removal of drug pollutants. The study of AS reactor with 100 µg/L initial drug pollutants present in the PSW yielded removal rates of up to 68% enrofloxacin (ENR) and 77% tetracycline (TET).

2.6 Aerobic and anaerobic digesters' mechanisms

Aerobic digesters and anaerobic digesters are generally used to degrade organic matter found in wastewater. Aerobic digesters normally use a wide range of bacteria which degrade organic matter under oxygen conditions, whereas the anaerobic digesters also use a wide range of bacterium to degrade organic matter but under oxygen-free conditions (Gerardi, 2003). Anaerobic digester reactors are normally sealed and oxygen is prevented from entering the digester, whereas aerobic digesters degrade organic matter by the use of microorganisms which survive through oxygen from the atmosphere. In aerobic digesters, if the PSW influent being processed contains nitrogen, phosphorus, the end products are nitrates and phosphates (Vogts, 2007). Furthermore, in anaerobic systems the end products comprise of carbon dioxide (CO_2) and methane (CH_4). The anaerobic digesters operate in four stages when biodegrading FOG and other contaminants contained in the PSW, namely hydrolysis, acidogenesis, acetogenesis and methanogenesis (see Figure 2-9) (Manyi-Loh *et al*., 2013).

Hydrolysis involves the degrading of a large organic compound like fats into small constituents. The rate at which large organic matter like fats is broken down depends on the pH, particle size, temperature, and biomass activity (Elefsiniotis *et a*l., 2002). In the second stage, acidogenesis, the bacteria convert the small molecules typically fatty acids into carbon dioxide, ammonia, alcohols, hydrogen, and organic acids. Thirdly, acetogenesis occurs when the bacteria convert fatty acids and alcohols into acetic acid, hydrogen, and carbon dioxide. Lastly, methanogenesis is the where stage where the latter products are degraded to produce methane and carbon dioxide (Zehnder *et al*., 1983).

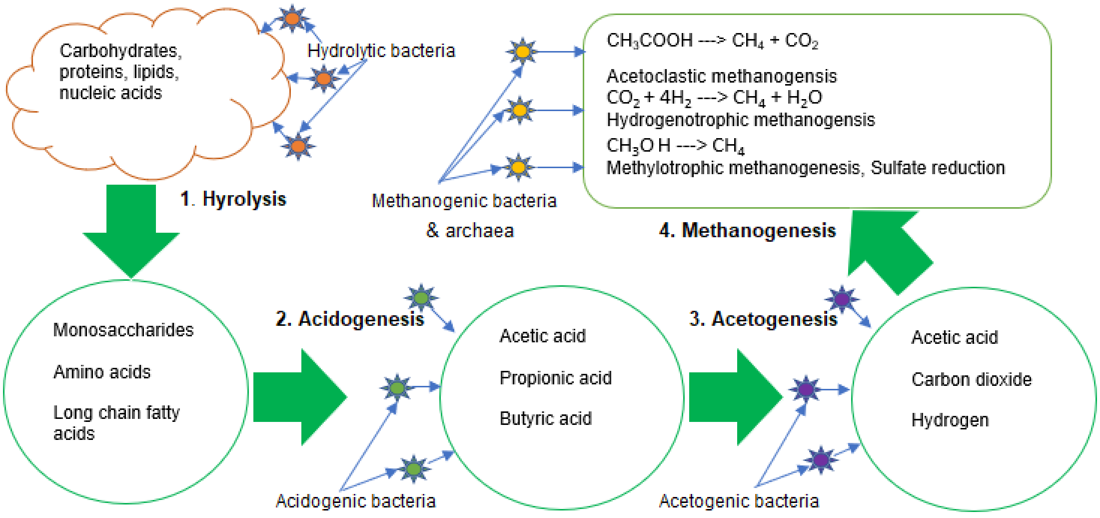


Figure 2-9: Schematic representation of anaerobic digestion steps (Sarker *et al.*, 2019)

2.7 Types of anaerobic bio-reactors

There are a wide variety of anaerobic digesters which operate in a number of different ways depending on the design specifications and material construction of each digester. Anaerobic digesters are divided into three basic categories, namely; passive systems, low rate systems and high rate system (Hamilton, 2014). According to Hamilton (2014), for passive systems, the biogas recovery is added to an existing treatment component, while for low rate systems, manure flowing through the digester is the prime source of methane forming microorganisms and lastly, in high rate systems, the methane forming microorganism are trapped in the digester in order to increase efficiency.

In addition to the three categories listed, there is also an up-flow anaerobic sludge blanket (UASB), static granular bed reactor (SGBR), and expanded granular sludge bed reactors (EGSB). In the UASB the influent enters the digester from the bottom toward the top in an upward flow direction passing a sludge granule blanket which filters and treats the wastewater as it flows through it (Lettinga *et al.*, 1980). The SGBR is a new reactor which has no mixing, but rather utilizes an anaerobic biofilter coupled with granules (Debik & Coskun, 2009) Lastly, the EGSB is similar to the UASB reactor with a high recycle ratio of the effluent stream to the influent feed stream (Zoutberg & Been, 1997) This study primarily focused on the modified DEGBR, as well as the pre-treatment processes used in the treatment of PSW.

2.7.1 Up-flow anaerobic sludge blanket (UASB)

In the up-flow anaerobic sludge blanket (UASB) process, the wastewater enters at the bottom of the reactor and flows upward (see Figure 2-10). There are micro-organisms in the sludge layer that degrade organic matter present in the PSW. Methane (CH_4) and carbon dioxide (CO_2) are normally released as products of the UASB (Del Nery *et al*., 2008). The UASB reactor has been widely used in the treatment of PSW in the secondary step. (Del Nery *et al*., 2008) evaluated the performance and stability of a PSW treatment plant which utilized the UASB reactor with organic loading rates of 1.6±0.4 kg COD/m^3 day and velocities of 0.3±0.1 m/h. The process achieved a total chemical oxygen demand (tCOD) and soluble chemical oxygen demand (sCOD) removal efficiency of up to 67% and 85%.

Also, an UASB reactor was used in the treatment of PSW effluent by adding three different types of inoculum combined with yeast extract and cow manure, where it depicted a 95% removal efficiency of BOD_5 at organic loading rates of up to 31 kg BOD_5 m^{-3} d^{-1} (Chávez *et al*., 2005). Lastly, in the treatment of PSW effluent under low up-flow velocity of about 1.38 m/day, it took about 147 days to complete a reactor start-up which yielded a tCOD and sCOD removal efficiency of up to 70% and 79%, respectively (Rajakumar *et al*., 2011).

There are advantages of UASB including, but are not limited to, low sludge production, low energy consumption, low running costs, and the reactor can handle fluctuating pH (Chen *et al*., 2011; Chong *et al*., 2012; Rajakumar *et al*., 2011).

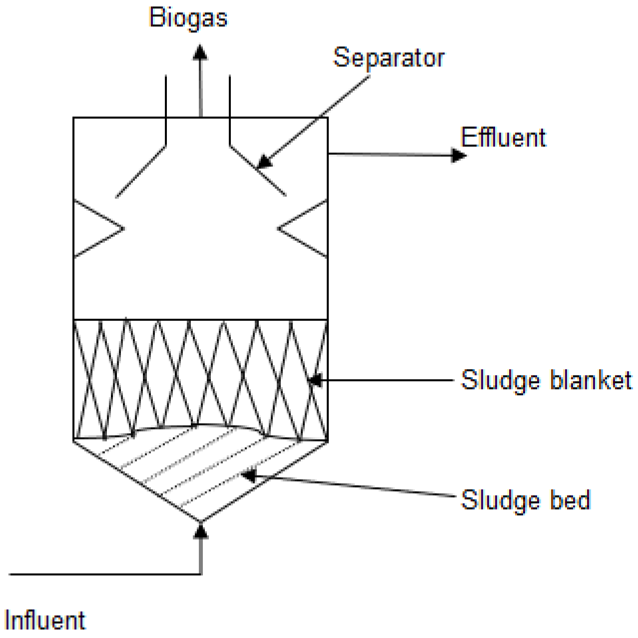


Figure 2-10: Schematic diagram of an UASB reactor (Dutta et al., 2018)

2.7.2 Expanded granular sludge bed (EGSB)

The expanded granular sludge bed (EGSB) reactor is an adaptation of the UASB reactor with a distinguishing recirculation stream of the outlet effluent to the feed influent (Kato *et al*., 1994). EGSB reactors utilize a fully or partially expanded bed granules of sludge where the wastewater gets to be treated as it passes through the bed (see Figure 2-11). The recirculation stream promotes bed fluidization of the granular sludge and like-wise there are micro-organisms present in the sludge which degenerate organic matter. (Dong *et al*., 2013) evaluated a rapid start-up of the EGSB reactor by using brewery acivated sludge in the treatment of wastewater. The study depicted that the sludge could develop faster within a period of 10 days in the EGSB reactor with little damage of granules. Removal efficiencies of up to 72.9% of COD with a hydraulic retention time (HRT) of 12.1 hrs were achieved. Yet in another study of the feasibility of ESGB reactor used in the treatment of low soluble strength wastewater using ethanol as a substrate, reported a COD removal efficiency of up to 80% at organic loading rates up to 12 g COD/L (Kato et al., 1994). It is worth noting that the performance of a biological treatment of wastewater in the ESGB by recycling half of the sludge resulted in COD removal efficiencies of up to 95% (Wang *et al*., 2015).

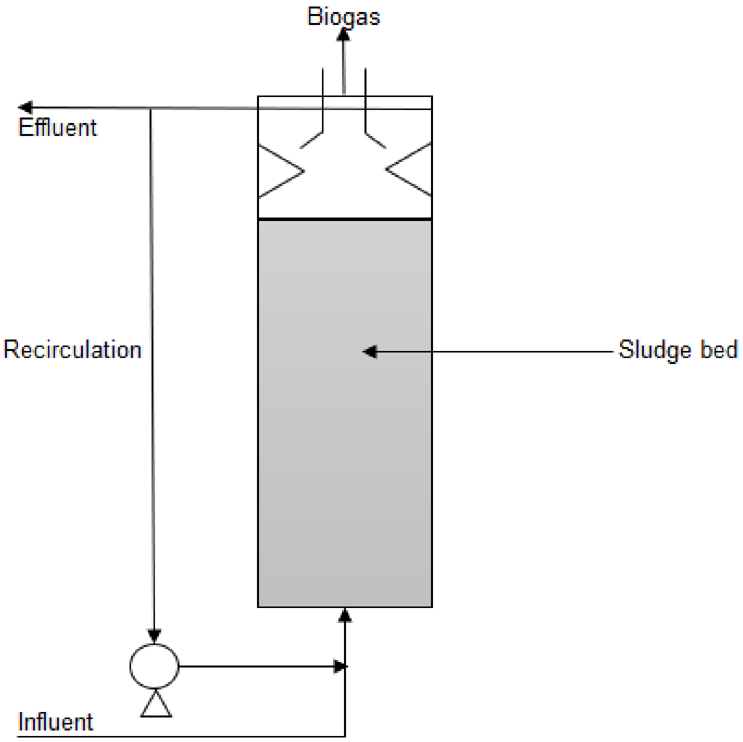


Figure 2-11: Schematic diagram of an EGSB reactor (Lim, 2011)

2.7.3 Static granular bed reactor (SGBR)

The static granular bed reactor (SGBR) is a reactor which has no mixing mechanism but rather it has a down-flow system fitted with static granules and sludge which serve as a biodegrading medium (see Figure 2-12). The SGBR reactor has a wide range of advantages which include the simplicity of operation and the production of high quality effluent (Oh *et al*., 2015). A study of the SGBR was employed in the treatment of PSW effluent with an intention of evaluating two processes using anaerobic non-granular and granular biomass (Debik & Coskun, 2009). The study showed that both processes were highly efficient in the removal of COD with values above 95%.

(Park *et al*., 2012) performed a research of the treatment of wastewater by using a pilot SGBR reactor with organic loading rates between 0.63 to 9.72 kg/m^3/d and a HRT of 9 to 48 hrs. In the latter study removal efficiencies of COD, BOD$_5$ above 90% and a TSS above 80% was achieved respectively. Moreover, in another study of a SGBR fitted with pea gravel coupled with activated sludge from brewery proved a COD removal efficiency above 90% with a HRT range of 5 to 36 hrs (Ellis & Evans, 2008). Lastly, (Oh *et al*., 2015) evaluated the performance of an SGBR with an HRT of 9 hrs under high organic loading rates of 7.3 kg/m^3/d the removal efficiencies of COD and TSS were 94% and 89% respectively.

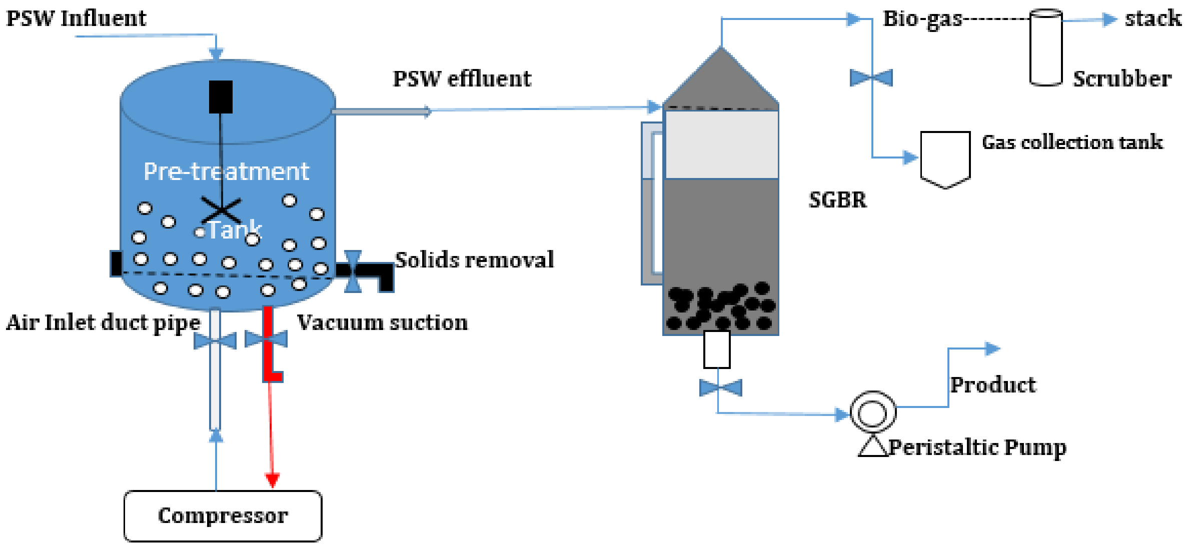


Figure 2-12: Process schematic diagram of Biological pre-treatment coupled with the Static granular bed reactor (SGBR)

CHAPTER THREE

METHODOLODY & RESULTS

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CHAPTER THREE

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Authored by:

Derrick Njabuliso Dlamini; Moses Basitere; Mahomet Njoya; Seteno karabo obed Ntwampe; Ephraim Kaskote

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3. METHODOLOGY AND RESULTS

Performance evaluation of a biological pre-treatment coupled with the Down-flow Expanded Granular Bed Reactor (DEGBR) for treatment of poultry slaughterhouse wastewater

3.1 Introduction

The meat processing industry is one of the largest consumers of freshwater in the agricultural sector (Bustillo-Lecompte & Mehrvar, 2017). Poultry slaughterhouse industries use a considerable amount of portable water during processing of live birds to consumable meat, which results in the production of high volumes of poultry slaughterhouse wastewater (PSW) (Yaakob *et al*., 2018). The rising demand of poultry produce requires an increase in poultry slaughterhouses' ability to treat high volumes of PSW (Valta *et al*., 2015). Ordinarily, poultry slaughterhouse effluent is difficult to treat, and it also requires multiple treatment steps before it can safely be discharged to the environment, due to the high amount of organic matter, FOG and nutrients contained therein (Barrera *et al*., 2012). The challenge is that the organic matter, toxic pollutants and large particles in raw PSW are not only toxic to the environment, but also cause clogging of bioreactors (Mannina, 2017). As per the South African department of Agriculture, Forestry and Fisheries (2014) report, the production of poultry meat has been one of the leading purchased items among South Africans, which significantly contributes to nearly 20% of the overall meat production in South Africa (SA) in the past decade. The nature of the activities associated with clean water usage during washing and poultry product processing culminates in the release of a tremendous volume of wastewater (De Nardi *et al*., 2011). The vast generation of PSW also presents itself as a potential threat to the ecosystem, as well as human health. Therefore, the implication of untreated organic substances, such as blood and feathers, would result in the eutrophication of receiving surface water, thereby causing environmental pollution and a health hazard on people exposed to it (Debik & Coskun, 2009). Ordinarily, poultry slaughterhouse processes involve a series of processing steps ranging from the slaughtering of live birds to the conversion of the live birds to edible meat fit for human consumption (Bustillo-Lecompte & Mehrvar, 2017). The latter processing steps require large volumes of freshwater, and this contributes to the pollution of the freshwater sources if the PSW is released untreated. The high volume of water utilized in such facilities stems from various processing steps requiring potable water because of the need to provide safe products (Meneses *et a*l., 2017). To treat the PSW, suitable bioreactors are needed. Most reactors have operational challenges which range from clogging, longer processing time and lower efficiencies (Dlamini *et al*., 2019). However, one of the most successful and effective process equipment used in the treatment of wastewater is the static granular bed reactor (SGBR) (Park *et al*., 2012). In the SGBR, PSW is fed at the top of the reactor where it flows downward via a bed of anaerobic granules. However, some problems may be encountered during the operation of the SGBR, including clogging and sludge wash-out, which are caused by high and excessive concentrations of biomass growth (Debik & Coskun, 2009). The excessive concentration of biomass growth generally leads to lower COD removal efficiencies. An important aspect that can assist in the treatment of PSW is the use of a novel bioremediation agent called Eco-flush^{TM} in the pre-treatment step, which is followed by the down-flow expanded granular bed reactor (DEGBR). The DEGBR was designed to mitigate the challenges experienced by the SGBR (Njoya *et al*., 2019). The DEGBR is a modified adaptation of the SGBR that is fitted with a recycle stream on the side of the reactor, which is used for recycling effluent from the bottom of the reactor to the top (Njoya *et al*., 2019). This is meant to prevent issues such as flow channelling, to improve the distribution of the feed to the biomass, and to implement intermittent fluidization whenever it is required using a top-down recycling strategy.

This study seeks to evaluate the performance of an integrated system containing a biological pre-treatment coupled with a DEGBR for the treatment of PSW in order to achieve high removal efficiencies of COD, TSS and FOG. Furthermore, the performance of the system was then compared to other biological systems used in previous studies for the treatment of PSW. To achieve this aim, the following objectives were developed:

Objective 1: To determine the performance of the pre-treatment stage after addition of the Eco-flush^{TM} (hydrolysing agent) in terms FOG, COD and TSS removal efficiency.

Objective 2: To determine the overall performance of the DEGBR in terms of FOG, COD and TSS removal at set operational HRT and OLR.

Objective 3: To determine the overall performance of the biological pre-treatment system coupled with the DEGBR in terms of FOG, COD and TSS removal. Moreover, evaluate the Alkalinity and volatile fatty acids (VFA) and the stability of the anaerobic DEGBR at the set operational conditions in terms of pH and temperature.

Objective 4: To determine the amount of biogas produced by the DEGBR.

3.2 Materials and Methods

3.2.1 Experimental setup

The equipment and design layout of the lab-scale plant entailed a pre-treatment process which consisted of a 20 L mixing tank and 20 L holding tank which had a magnetic stirrer. Ordinarily, the stirrer operated at a maximum of 100 revolutions per minute (RPM). The purpose of the stirrer was to create a homogenous PSW to ensure consistency in the process. The effluent produced was then screened and pumped by a peristaltic pump to the DEGBR anaerobic digester. The DEGBR was made of polyvinyl chloride (PVC) with a volume of 2 L capacity, 0.62 m height and 0.065 m in diameter as illustrated in Figure 3-13. At the bottom of the DEGBR, pumice stones were fitted as an underdrain, which rested on a 2 mm stainless sieve mash. The pumice stones aided as a sludge retaining medium and also allowed the attachments of bacteria to grow as shown in Figure 3-14. In addition, a recycle stream was fitted on the side of the reactor with the aim of preventing clogging of the bioreactor. Biogas was collected at the top of the DEGBR into 500 mL plastic storage bag.

Figure 3-13: Photograph of the modified down-flow expanded granular bed reactor (DEGBR) (source: D.N. Dlamini)

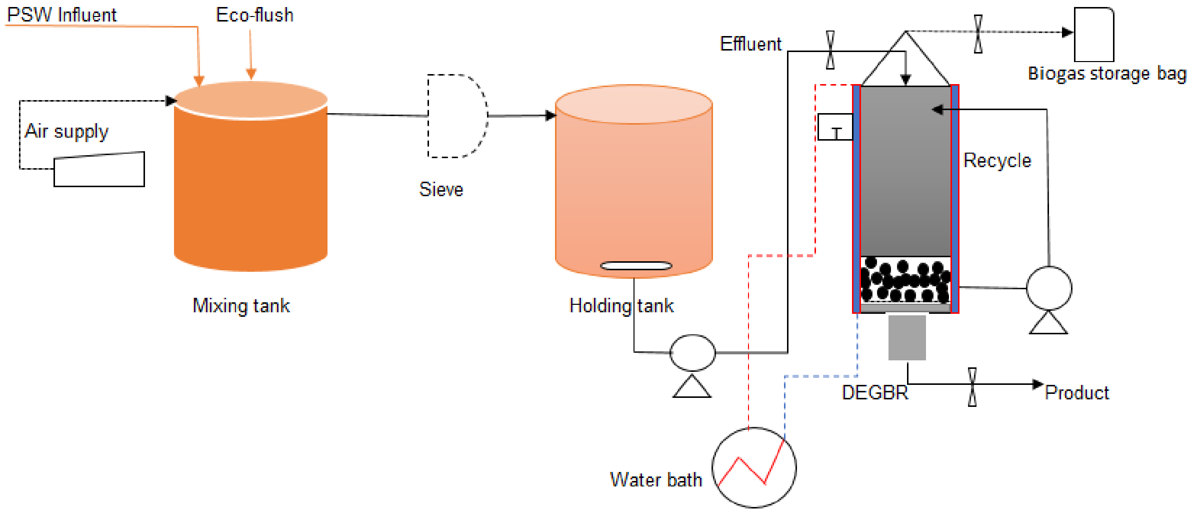


Figure 3-14: Schematic illustration of the modified DEGBR

3.2.2 Operating conditions

3.2.2.1 Pre-treatment and sample preparation

The pre-treatment was operated at ambient temperature and a mixture of a bioremediation agent known as Eco-flush^{TM} (20 mL) and 20 L of raw PSW was aerated using air stone spargers over a period of 24 h. The Eco-flush^{TM} is a commercial product used for the coagulation of FOG (Mao *et al*., 2015), which was donated for research purposes by Mavu biotechnologies (Pty) Ltd. Eco-flush^{TM} is a hydrolysis bioremediation agent, containing microorganism isolated from the soil, grown and stored in a physiological dormant state. The microorganisms are resuscitated to produce enzymes which in turn delipidate the PSW. Eco-flush^{TM} constituents are: enzymes, microbial consortia, organic nitrogen, potassium, organic carbon, water and organic matter. After aeration, the PSW was allowed to settle under anaerobic conditions for an extra 24 h. Overall, the Eco-flush^{TM} has an ability to oxidize NH_3 into NO_3^- and NO_2^- and also eliminates H_2S, including odor-producing organisms. Subsequently, after settlement, the PSW was then filtered through a 53 µm sieve screen. The reason for screening was to remove larger particulate matter including feathers.

3.2.2.2 DEGBR set-up, operation and inoculation

The DEGBR, with a working volume of 2 L, was constructed with a clear polyvinyl chloride (PVC) column with internal dimensions of 0.62 m height and 0.065 m in diameter. Pumice stones were placed at the bottom of the reactor to aid as a biomass retaining aid. Pumice stones were chosen due their inertness and thus support the accumulation of beneficial bacteria, which also help cleanse the pollutants from the PSW. A mass (0.5 kg) of pumice stones was immersed in the wastewater and had an average porosity of 0.66 (Njoya *et al*., 2019). The DEGBR was operated at a mesophilic temperature of +/−37 °C sustained using a water bath. After pre-treatment, the screened product was pumped to the DEGBR for further processing using the Antech aspendose A 5.1 L/0.5 B peristaltic pump purchased from Enelsa in Turkey. Prior to this, the inoculation of the DEGBR was done using an activated sludge obtained from a SAB brewery which is located in the Western Cape, Newlands, SA. The collected activated sludge sample was collected in the form of a mixed liquor directly from a USAB (Li & Ganczarczyk, 1991). Accordingly, the DEGBR was inoculated according to the following ratio: 1.6 L of raw PSW effluent in combination with 0.4 L of brewery activated sludge supplemented with a 50 mL solution of dry milk. The milk was added as an organic source to sustain the micro-organisms and help them to adapt to the new environment. Thereafter DEGBR was operated for 126 days (18 weeks), whereby the first 30 days were used to acclimatize the microbes, and the balance of the operational days was used to operate the DEGBR at a constant hydraulic retention time (HRT) of 106 h (Del Nery *et al*., 2008). The reactor plant operated at an influent flowrate of 0.45 L/hr.

3.2.2.3 Sampling and Analytical methods

3.2.2.3.1 Sample and analytical methods

Sampling for the process was done at the feed and exit points of every stage in the process. The samples were collected using sample bottles and stored in the cold room set at −5 °C before analyses. Thereafter, the collected samples of the wastewater were analyzed for Alkalinity, TSS, volatile fatty acids (VFA), COD, FOG, and pH. All the samples collected were analyzed using standard methods (Baird & Bridgewater, 2017). A Geotech Biogas 5000 portable gas analyser manufactured by Keison Products in the United Kingdom (UK) was used to determine the gas composition emitted that was collected in the biogas storage bag.

3.2.2.3.2 Statistical analysis

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

$$\eta = \frac{\text{Influent (mg/L)} - \text{Effluent (mg/L)}}{\text{Influent (mg/L)}} \times 100\% \qquad (3.1)$$

3.2.2.3 Procedure followed for study

Figure 3-15 shows a graphical schematic summary of the study. Initially, the study underwent a brain storming session whereby a research project design of the process was developed. A proposal of the study was subsequently done and submitted to the higher degrees committee of the Cape Peninsula University of Technology (CPUT). Once the proposal was approved and ethics clearance obtained thereof, the plant was then assembled by Malutsa (Pty) Ltd., Western Cape, Wellington Industrial Park, South Africa.

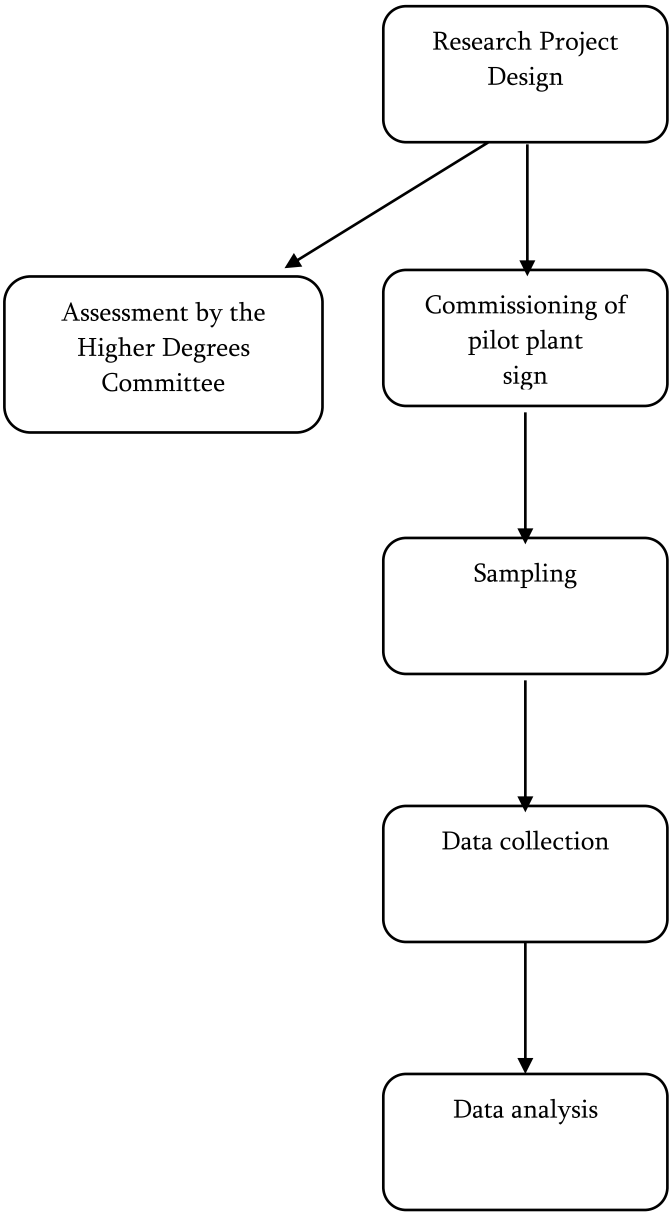


Figure 3-15: Graphical representation of the study

3.3 Results

3.3.1 Biological pre-treatment performance

3.3.1.1 Pre-treatment performance

Figure 3-16 provides an insight into the performance of the pre-treatment system with respect to COD, TSS, and FOG removal. The graphs in the first row illustrate the variation of the concentration of each water quality parameter at the inlet of the system, while the graphs in the second row provide the wastewater quality parameters at the outlet of the treatment unit, and finally the graphs in the last row provide the variation of the removal percentages for each indicated parameter. It was noticed from the COD and FOG concentrations at the inlet of the pre-treatment system that some values were outliers. To evaluate this, the inlet and outlet values of each parameter was box plotted with the aim of identifying potential outliers in each distribution.

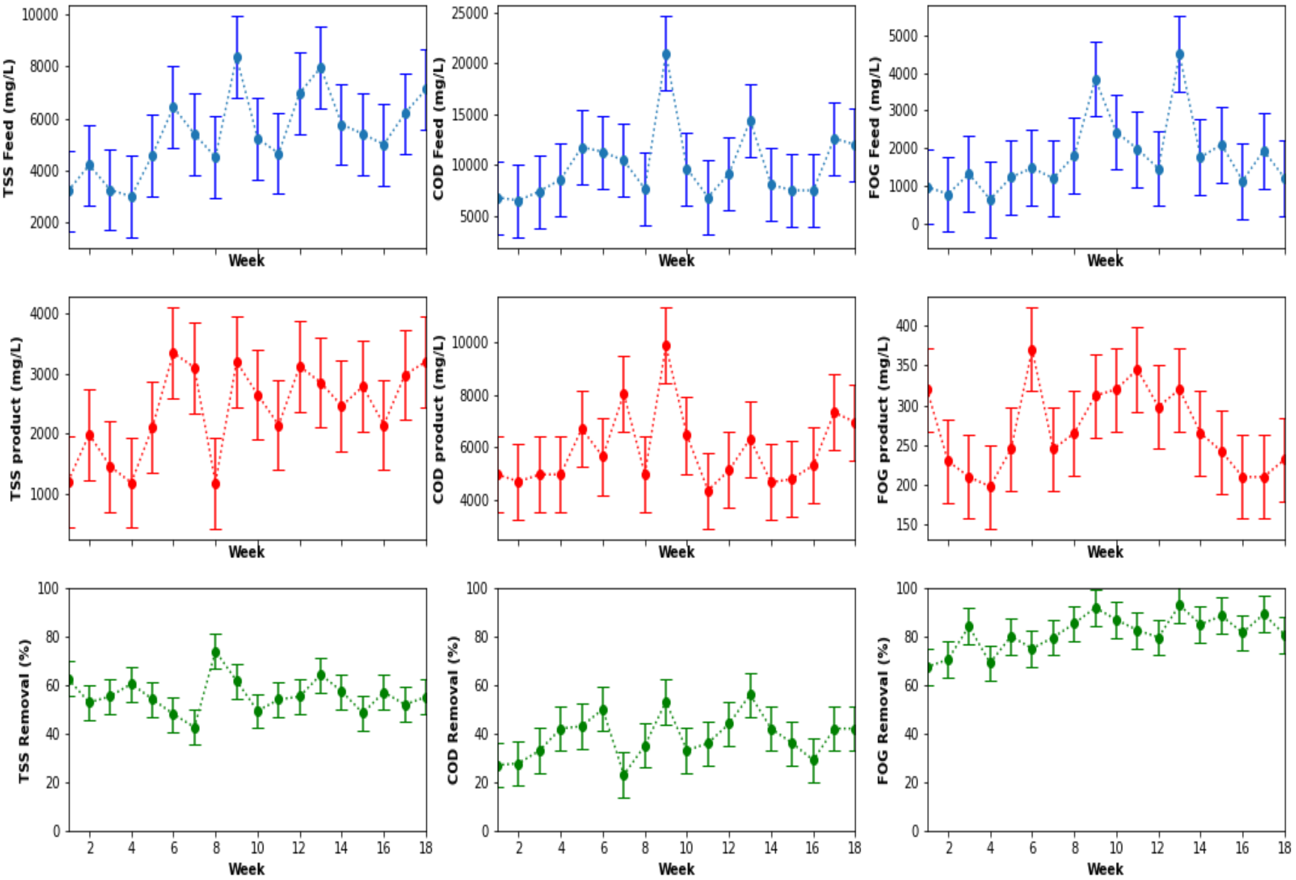
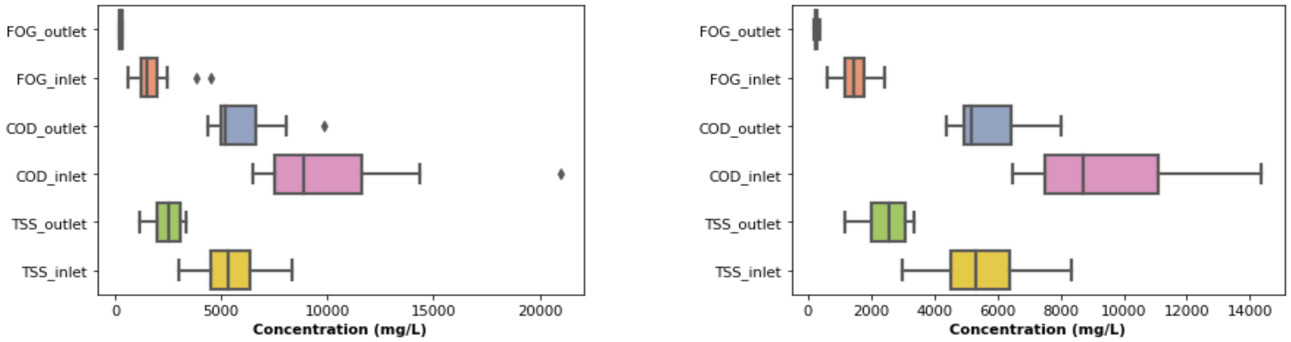


Figure 3-16: Performance of the pre-treatment unit before outlier replacement

As shown in Figure 3-17(a), the boxplot of the distribution of each parameter, assessed both at the inlet and the outlet of the pre-treatment system, indicated the presence of outliers for FOG and COD, as well as the outlet COD concentrations. For the boxplots, an interquartile rule to identify the outliers was used, with the outliers being replaced by median values which are dependent on the size of the dataset. This culminated in the boxplot in Figure 3-17(b) from which the absence of outliers can be noticed. Outliers in this experiment could originate from variations in the activities of the poultry slaughterhouse at the moment of the collection of the sample, or erroneous concentration determination of a given parameter during the experiment. These outliers were corrected for ease of data interpretation and the outcome of the experiment at each PSW treatment stage.



(a) Boxplot before outliers replacement

(b) Boxplot after outliers replacement

Figure 3-17: Boxplots of the investigated parameters during the pre-treatment stage

The performance of the pretreatment system is shown in Figure 3-18. The fluctuations in the quality of the feed and product of the system and the corresponding removal efficiencies after the data processing indicated FOG removal efficiency oscillating around 80%. Furthermore, Figure 3-18 also shows removal efficiencies of the TSS and the COD ranging between 45% to 80% for the TSS, and a variation between 25% to about 60% for the COD. These removal efficiencies fluctuated with the quality of the feed to the system. Overall, the pre-treatment system culminated in an average removal efficiency of 80±6.3%, 38±8.4% and 56±7.2% for the FOG, COD and TSS, respectively.

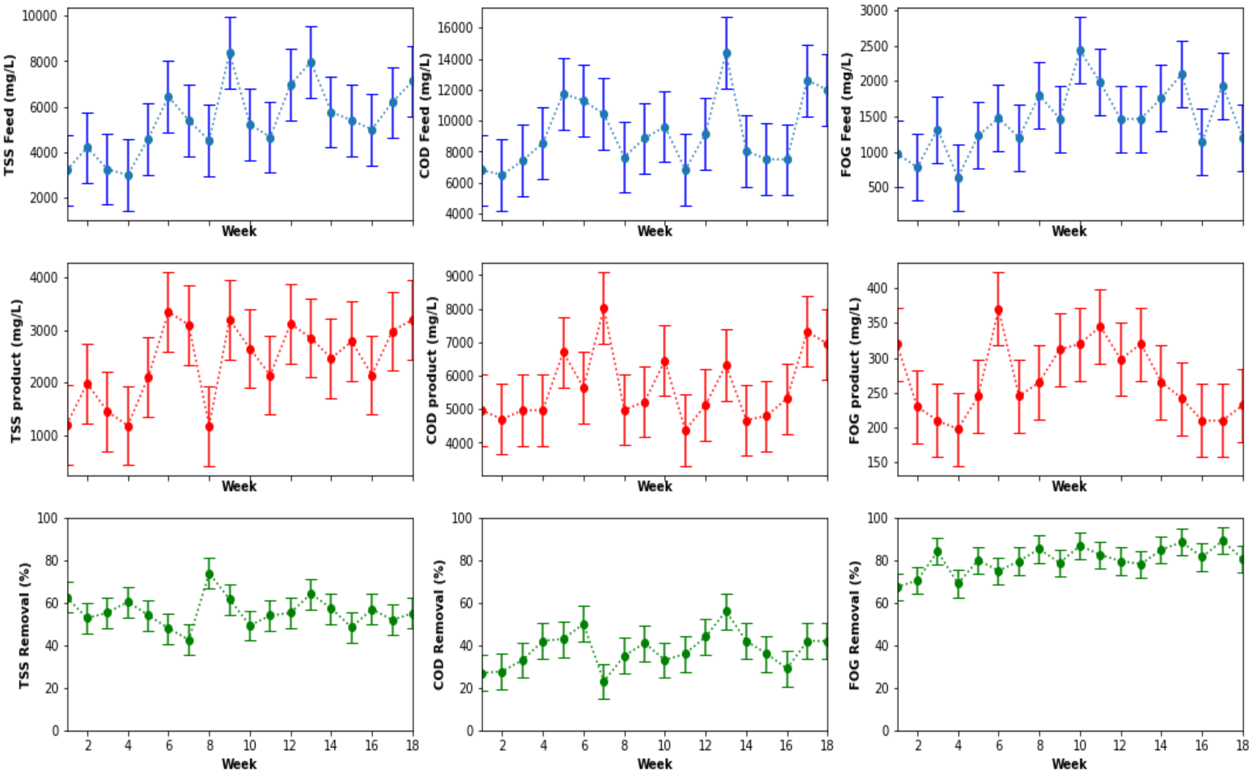


Figure 3-18: Performance of the pre-treatment system after outliers replacement

3.3.2 FOG Removal

The results of this study illustrated that the supplementation of 20 mL of the Eco-flush^{TM} for every 20 L of PSW highly influenced FOG removal. Using Eco-flush^{TM} as a FOG reduction agent was indicative of the bacterial consortia present in it, which aided in the biological degradation of the pollutants presents in the effluent. Since the raw PSW was first aerated through sparging, which supplies dissolved oxygen to the wastewater and the microorganisms, this resulted in the proliferation of the bacterial community that subsequently degraded the organic material and pollutants in the wastewater. Basically, the microbes decoupled bonds in the organic contaminants and further coagulated less soluble matter found in the raw PSW. At an average FOG removal efficiency of 80±6.3% (Table 2), the results depicted a pre-treatment unit that performed exceptionally well under the above set design and operational conditions. This study proved to perform well as compared to another study whereby an evaluation of a biosurfactant, obtained from cultures grown on cassava wastewater were used in the pre-treatment of PSW, yielding an oil and grease removal efficiency of 70% (Natassia *et al*., 2017).

Table 2: Raw and pre-treated PSW characteristics average range

Parameter	Inlet Feed	Product	Efficiency (η)
COD	9946 mg/L	5896 mg/L	39 %
TSS	5399 mg/L	2392 mg/L	56 %
FOG	1765 mg/L	269 mg/L	80 %

3.3.3 COD Removal

the effect of biological pre-treatment using Eco-flush™ indicated that the process also effectively reduced the organic matter load present in the raw PSW; albeit, with a lowly COD removal efficiency of 38±8.4%. However, this was done under ambient temperature conditions in which the biological pre-treatment can be proven to be a challenge because of the high concentration of organic matter present in the PSW. However, with an increased organic loading rate, the total removal of COD contributing constituents in the PSW can increase proportionally. As shown in Table 2, a COD product discharge of 5896 mg/L and COD removal efficiency of 38±8.4% showed that there was a need of a subsequent treatment stage that would further reduce the COD to prescribed discharge standard of wastewater (5000 mg/L) as per the City of Cape Town by-laws, 2013. Overall, this study proved to be more efficient as compared to a similar study whereby the biological pre-treatment of poultry product processing wastewater removed only 24% tCOD in an aerated equalization tank (Rusten *et al*., 1998).

3.3.4 TSS Removal

In reference to Table 2, the high values obtained at the inlet feed were attributed to solids' presence and entrapment in the animal fat which is normally observed during pick processing times (Del Nery *et al*., 2007). Furthermore, it was established that the presence of high carcass debris, might have been a contributing factor to the organic matter found in the raw PSW culminating in the high concentrations of TSS due to high FOG (Rinquest *et al*., 2019).]. However, when Eco-flush™ was added, some FOG hydrolysis ensued, which consequently resulted in the break-down of hydrocarbons, some of which were hypothesized to be converted into useful nutrients in the form of soluble fatty acids (Ergofito, 2019) for the bacterial population, releasing some solids which can be removed through filtration or a screening device. For this study a 53 µm screen was used, thus a significant portion of large particles of less soluble matter were filtered out as this will assist in the reduction of particles that could clog the DEGBR in the subsequent stage (Rustern *et al*., 1998) (Mannina, 2017).

Overall, the biological pre-treatment unit reduced COD from 9946 to 5896 mg/L, TSS from 5399 to 2392 mg/L and FOG from 1765 to 269 mg/L, respectively. A clear indication of the aesthetics of the raw PSW in comparison to the pre-treated PSW samples is shown in Figure 3-19, suggesting an initial satisfactory performance of the biological pre-treatment stage.

Raw PSW PSW after pretreatment

Figure 3-19: Photographs of the raw PSW in comparison to pre-treated PSW.

3.4 The performance of the DEBGR

3.4.1 DEGBR Performance

The product of the pre-treatment system was continuously fed to the DEGBR with the intention of further reducing the concentration of the contaminants present in the PSW. The fluctuation of the concentration of the COD, TSS and FOG during the experiments for both the inlet and the outlet of the DEGBR, including their corresponding removal efficiencies, are provided in Figure 3-20. Following the same methodology as the data processed for the pre-treatment system, the evaluation of the presence of outliers was undertaken.

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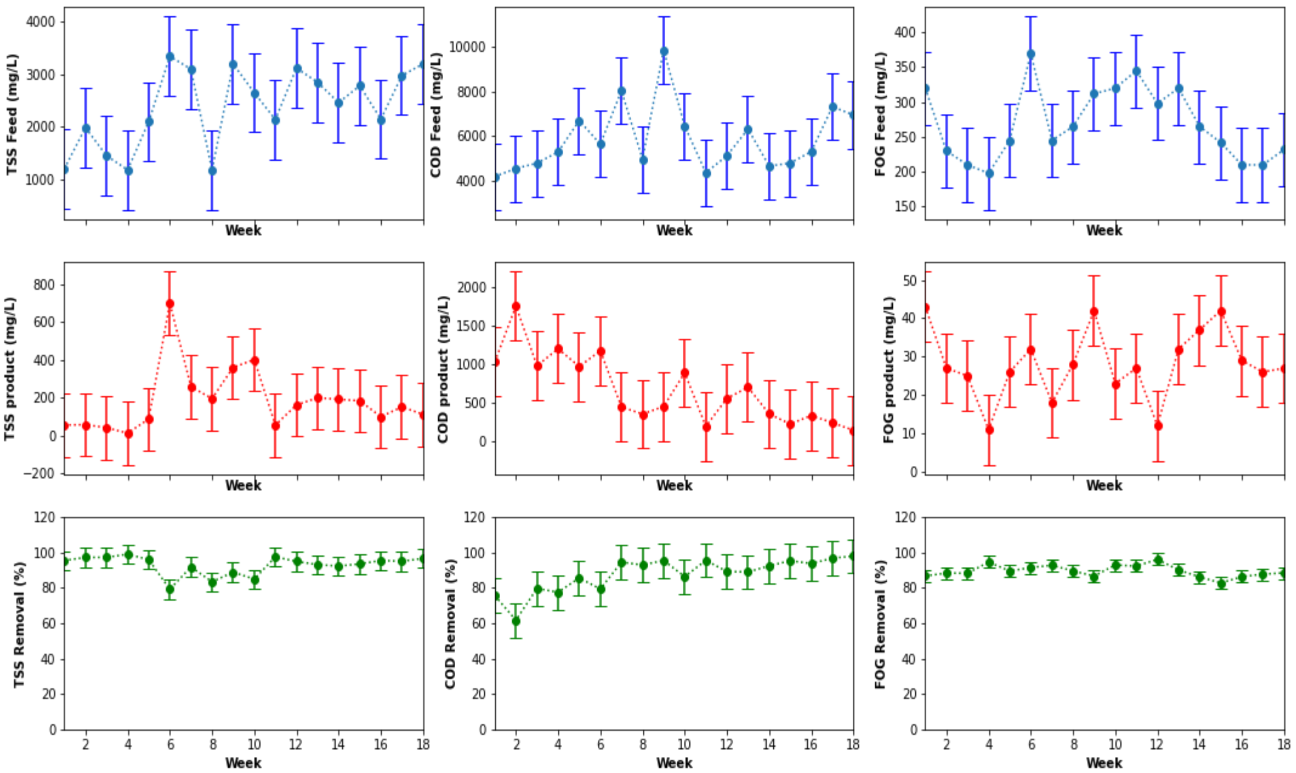
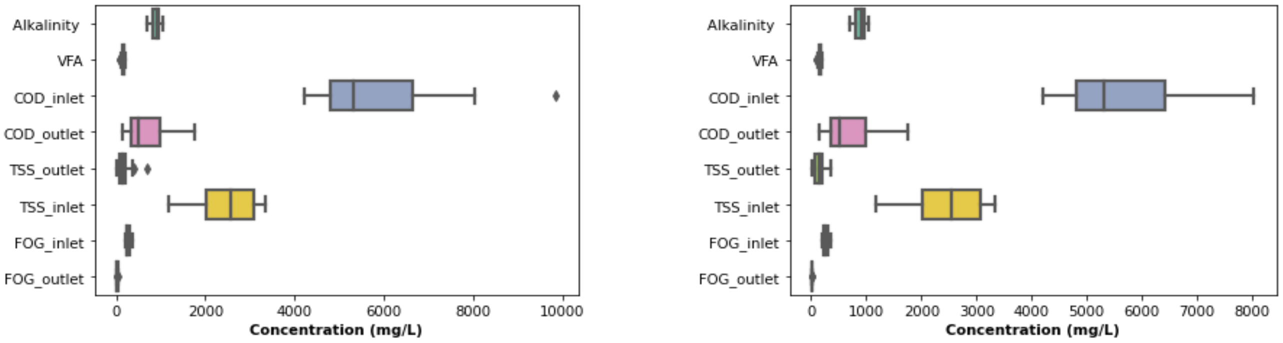


Figure 3-20: Performance of DEGBR before outliers replacement

From Figure 3-21(a), it can be observed that the distribution of the inlet COD and TSS had an outlier each. These outliers were replaced with the median value of each affected distribution, as depicted in Figure 3-21(b). From the latter, it can be observed that the data processing step addressed the challenges associated with the presence of outliers in the listed distribution and led to a further analysis of the performance of the DEGBR, as illustrated in Figure 3-22 indicating how the fluctuations varied for the concentration of TSS, FOG and COD at the inlet and outlet of the DEGBR, including their corresponding removal efficiencies.



(a) Boxplot before outliers replacement

(b) Boxplot after outliers replacement

Figure 3-21: Boxplots of the investigated parameters associated with DEGBR performance

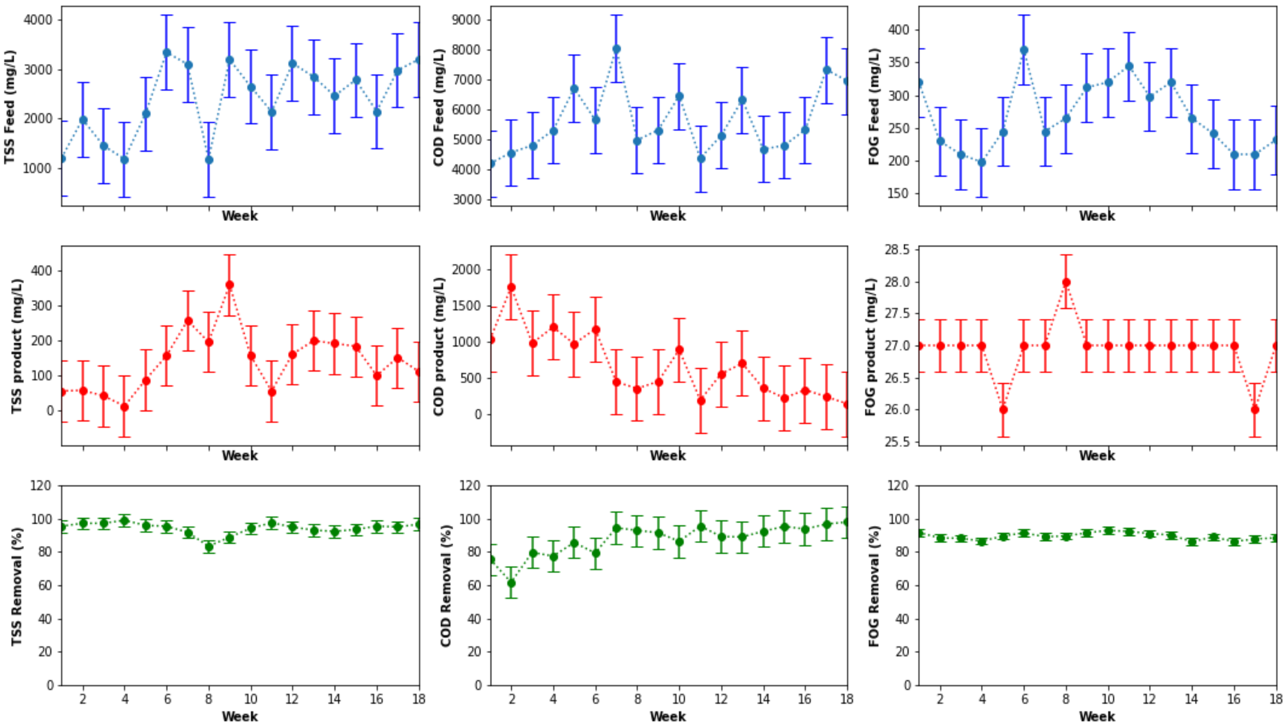


Figure 3-22: Performance of DEGBR after outliers replacement

Overall, the average removal efficiencies were 89±2.8% for the FOG, 87±9.5% for the COD and 94±3.7% for the TSS. The concentration of these contaminants was below the required discharge standards prescribed by City of Cape Town by-laws (2013) and supported the recourse undertaken suggested an efficient process system for the treatment of PSW or a wastewater having similar characteristics. The DEGBR designed for this study performed well in comparison to the EGSB used in another study, whereby it was indicated that the performance was attributed to a rapid start-up of the EGSB, in which brewery wastewater sludge was used in the treatment of slaughterhouse wastewater. It was determined that the sludge could develop faster within a period of 10 days in the EGSB reactor, with few detached sludge granules, achieving removal efficiencies of up to 72.9% of COD at an operational hydraulic retention time (HRT) of 12.1 h (Dong *et al*., 2013).

It was also observed that the performance of the DEGBR was high even at the beginning of the process, whereby the sludge micro-organisms were still acclimatizing to the new type of wastewater they were processing (i.e., PSW). This was due to the low organic loading rate used for the system throughout the experiment, which was sustained by the pre-treatment step used prior to the supply of the wastewater to the DEGBR system that significantly reduced the organic load. The management of this organic load to the DEGBR with respect to the FOG, COD, and TSS is illustrated in Figure 3-23, Figure 3-24 and Figure 3-25, respectively.

From these figures, it can be observed that the removal efficiencies of the DEGBR remained high throughout the experiment despite various fluctuations of the organic loading rate (OLR). This demonstrates the suitability of the DEGBR for the treatment of such wastewater and highlights the importance of a good pre-treatment step prior to feeding the wastewater to the anaerobic reactor. Moreover, the highest average COD removal efficiency of 92.8% was achieved in the fifteenth to eighteenth weeks, while operating at a HRT of 106 h. This could have been attributed to by the fact that the DEGBR was operating at its optimum because of an increased number of active microbes in the stabilized process. It can be said that the DEGBR performed better in terms of COD removal (Figure 3-24) as compared to a study of the treatment of PSW in an up-flow anaerobic bioreactor under a low up-flow velocity, which yielded a COD removal of only 70% (Rajakumar *et al*., 2011). Similarly, the DEGBR study proved to perform better than EGSB, which yielded a low COD removal efficiency of only 60% (Meyo *et al*., 2021).

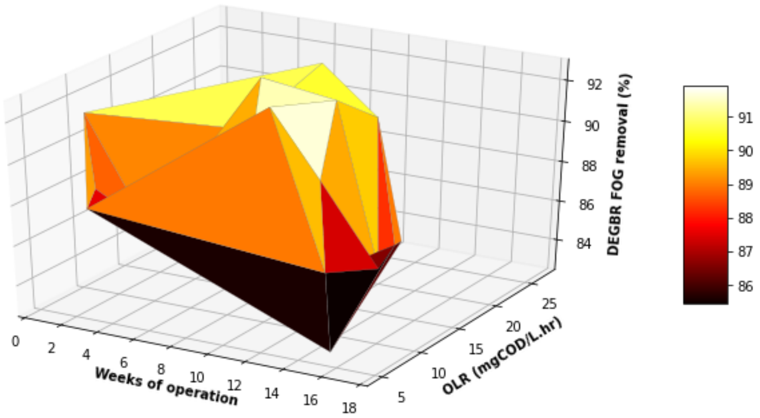


Figure 3-23: Performance of the DEGBR with respect to FOG removal under varying OLRs

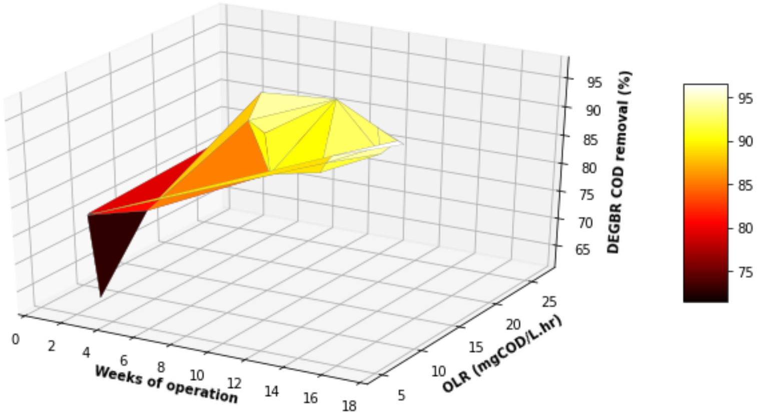


Figure 3-24: Performance of the DEGBR with respect to the COD removal under varying OLRs

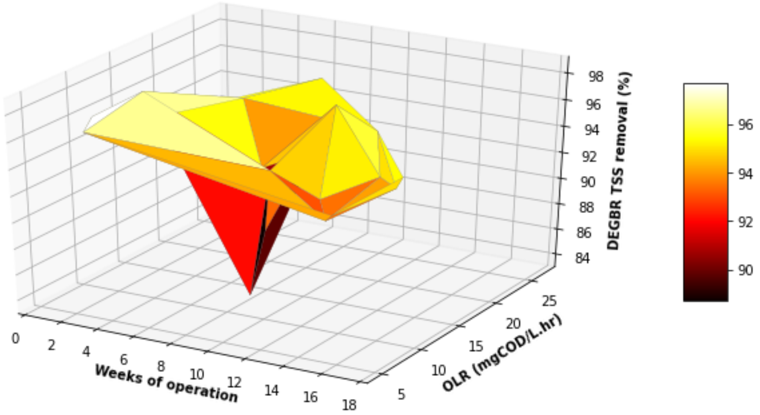


Figure 3-25: Performance of the DEGBR with respect to the TSS removal under varying OLRs

3.4.2 The effect of pH, temperature and VFA/Alkalinity on the DEGBR performance

Organic matter degradation happens in the DEGBR and it involves the decomposition of a large organic compounds into a simpler constituent for assimilation. The rate at which complex organic matter is degraded depends on the pH, temperature of the bioreactor, and molecular size of the pollutants including biomass activity (Elefsiniotis *et al*., 2002). However, there is no overall ideal operational pH, because all processes use consortia of micro-organisms, whose growth rate is differentiated at different pH values (Burgess & Pletschke, 2008). However, obviously, extreme pH can kill micro-organisms, hence there is a need to monitor and control it. This means that the operational pH is a median value which can be tolerated by most enzymes and micro-organisms. Additionally, Figure 3-26 depicts a variation of the pH and the temperature during the experiment reported herein, and these two parameters were also maintained within a mesophilic range (37 to 38 °C) for the temperature and a suitable pH range of 6.5 to 8.

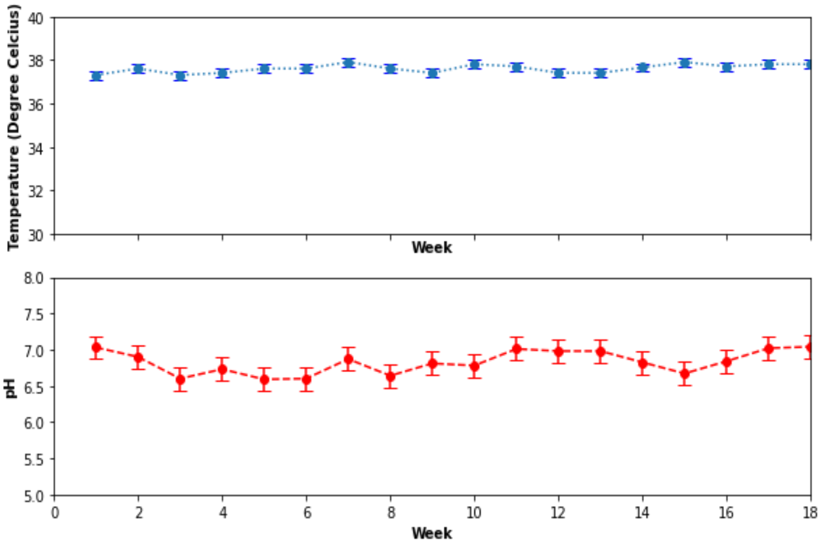


Figure 3-26: Variation of the pH and the temperature in the DEGBR during the processing of PSW

However, despite the highly appraised pre-treatment step, other parameters are essential to a conducive anaerobic digestion in the DEGBR. These included the VFA/Alkalinity ratio that reflected a good acidic and alkaline balance within the bioreactor. Ideally, this ratio should be kept under 0.3 for a stable anaerobic operation (Debik & Coskun, 2009). A VFA/Alkalinity ratio test was performed in order to establish the progression in the digestion of the PSW and the stability of the reactor.

Figure 3-27 shows average values of alkalinity, which ranged between 1048 to 702 mg/L, whereby the average VFA of 157.97 mg/L, and the alkalinity of 888.12 mg/L, culminated in the VFA/Alkalinity ratio of 0.2. Such a VFA/Alkalinity ratio was satisfactory, albeit slightly low for a high-performance reactor as compared to other similar processes used for anaerobic digestion. This was the case for the DEGBR, as illustrated in Figure 3-27, that provides a variation of the VFA/Alkalinity ratio, including the variation of the alkalinity and VFA concentration, throughout the experiment.

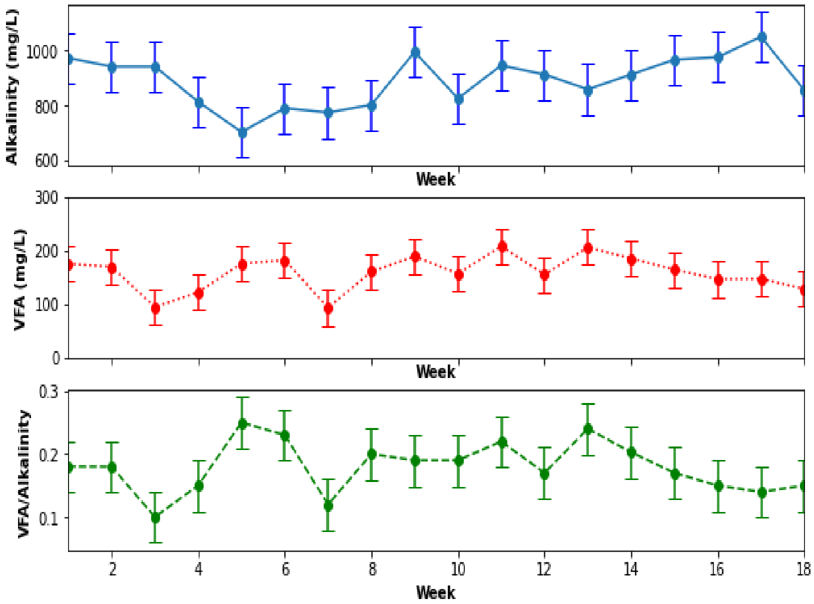


Figure 3-27: Variation of the VFA, Alkalinity, and VFA/Alkalinity in the DEGBR

3.4.3 Biogas production

Biomethane gas production rate is calculated according to Equation (3.2) (Meng *et al*., 2017):

$$K = \frac{(\sum_{t-0}^{n} \mathrm{Dt})}{n} \qquad (3.2)$$

Where *k* is the biomethane production rate (mL/d), t is the digestion time (d), Dt being the daily methane gas production (mL), and *n* refers to the day when the daily biomethane production is less than 5% of the maximum daily biomethane production for five days.

Biogas production happens in a process called methanogenesis. However, before methanogenesis, the bacteria in the reactor converts fatty acids and alcohols into acetic acid, hydrogen, and carbon dioxide (see Figure 2-9). Methanogenesis is the process whereby acetic acid, hydrogen and carbon dioxide products are degraded to produce methane and carbon dioxide (Sarker *et al.*, 2019; Zehnder & Gujer, 1983). Ordinarily, a suitable OLR and HRT can result in the production of methane gas (Mao *et al*., 2015). Furthermore, it has been established that presence of ammonium-nitrogen (NH_4N) in the system can inhibit methane gas production (Majd *et al*., 2017). It was reported in another study whereby a static granular bed reactor (SGBR) was used for the treatment of industrial wastewater that the biogas production increased with the increase in the organic loading rate (Park *et al*., 2009). For this research project, it was found that biogas production was reduced from 0.3 L CH4/g.COD (71%) degraded to being non-existent (0%) after 30 days from the initial start-up period, as shown in Figure 3-28. This was attributed to the continuous supply of facultative bacteria present in the bioremediation agent (Eco-flush^{TM}) which was hypothesized to have had an effect of sup-pressing the methanogenesis process in the DEGBR.

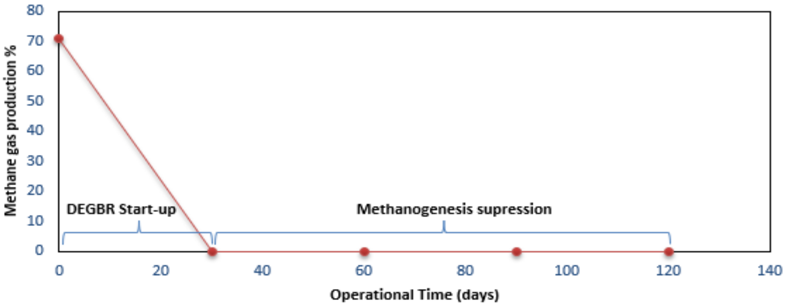


Figure 3-28: Biogas production during the operation of the DEGBR

3.5 Overall performance of the DEGBR

Figure 3-29 provides an overall performance of the complete system, for the removal of the COD, TSS, and FOG during the experiment, despite a slow start for the removal of COD during the first three weeks. However, the average removal efficiency was 97±0.8% for the FOG and, subsequently, improved to 92±6.3% for the COD and 97±1.2% for the TSS. The robustness of the entire system is demonstrated by the low standard deviation of the TSS and FOG removal efficiencies, as also illustrated in Figure 3-29. This also demonstrates that such an arrangement can provide similar overall results for the treatment of PSW.

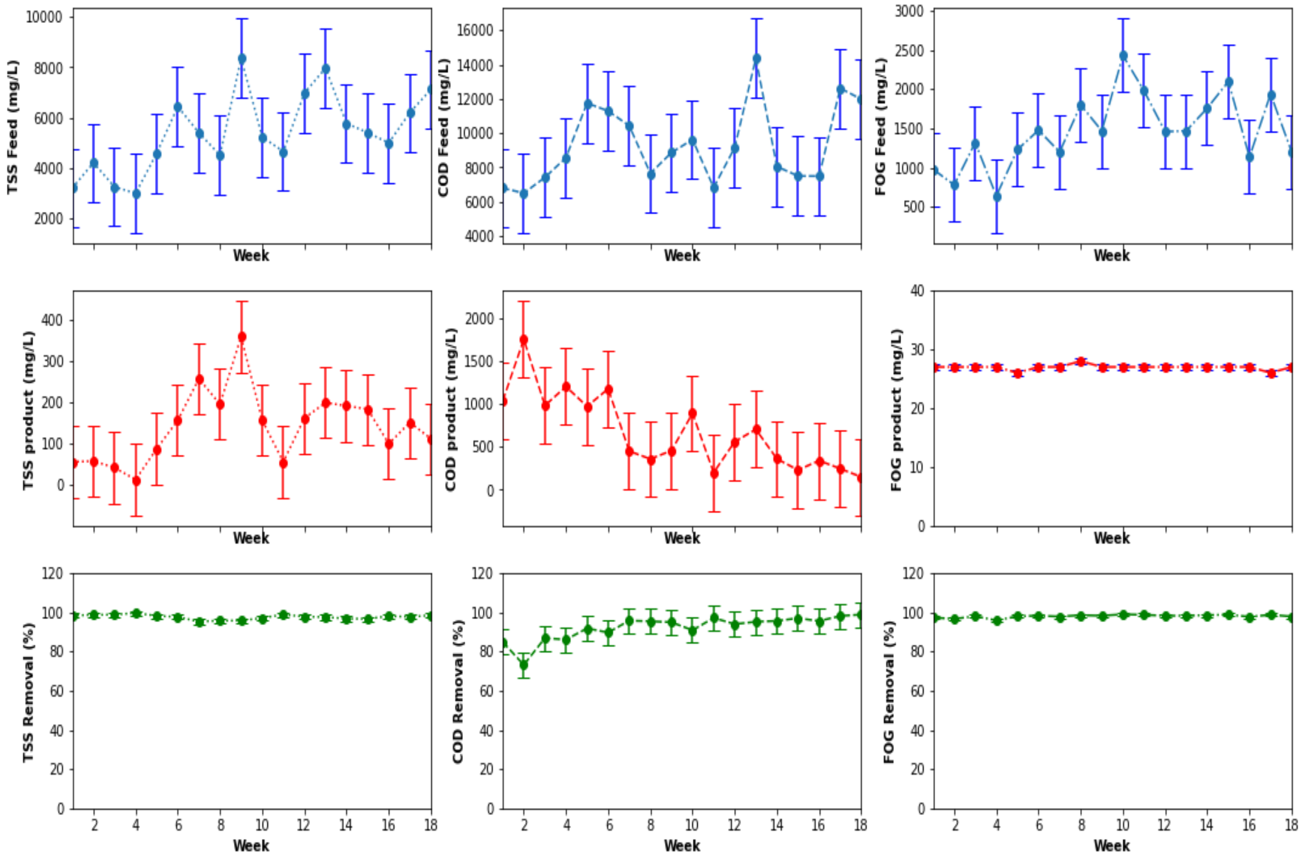


Figure 3-29: Overall performance of the complete system

Table 3 below illustrates the overall performance of the biological pre-treatment of PSW coupled with the DEGBR. For overall removal efficiency results of the system, the average feed of the pre-treatment and the DEGBR product was used for the calculations. When comparing the overall performance in terms of COD removal, of the UASB and the DEGBR, the results indicated a better performance for the DEGBR in comparison with another similar study in which the treatment of PSW was undertaken, albeit the used UASB was operated for 1228 days, yielding a removal efficiency of up to 85% for COD (Del Nery *et al*., 2008). Additionally, the overall biological pre-treatment of PSW coupled with DEGBR of this study, performed better when compared to another similar study which ran concurrently with this study under similar conditions using a similar pre-treatment strategy for the PSW (i.e., using Eco-flush^{TM} in the pre-treatment unit). Although, in the other study, an EGSB was used whereby it was reported that low FOG, COD and TSS removal efficiencies of about 80%, 60% and 80% were achieved (Meyo *et al*., 2021).

Table 3: Overall performance of the biological pre-treatment coupled with DEGBR

Parameter	Inlet Feed	DEGBR product	Efficiency *(η)*
COD	5896 mg/L	669 mg/L	92±6.3%
TSS	5399 mg/L	185 mg/L	97±1.2%
FOG	1765 mg/L	28 mg/L	97±0.8%

3.5.1 An in-depth analysis of pre-treatment unit-DEGBR performance parameter

3.5.2 COD Removal

Most PSW treatment technologies normally comprise of the dissolved air floatation (DAF) system coupled with the up-flow anaerobic sludge blanket (UASB) as a primary treatment for PSW (Del Nery *et al*., 2016). However, in most cases the latter type of anaerobic reactor usually fails to meet discharge standards because of the effluent produced from the UASB that contains high content of ammonia nitrogen and a significant amount of residual COD (Barana *et al*., 2013). Another study of PSW treatment plant for high strength effluent, the DAF pre-treatment system reported FOG and COD removal efficiency of 28.0 ± 5.6% and 38.7 ± 8.0%, respectively (Del Nery *et al*., 2016). The second step of biological treatment using the UASB yielded FOG and COD removal efficiencies of about 34.3 ± 12.5% and 69.1 ± 6.9%, due to the insoluble nature of lipids in the PSW, with some being purported to absorbed onto sludge granules. Since, in this study, the COD removal efficiency was a crucial parameter that was used to evaluate alternative technologies either as single units or in combination, determination of the average COD removal efficiency needed to be much higher (92±6.3%) than that reported elsewhere. As such, this study proves that the designed lab-scale plant had a significant effect of reducing COD. This could be attributed by a variety of factors ranging from the use of the Eco-flush^{TM} in the pre-treatment stage, the use of sieves/screens which eliminated larger particles in the pre-treatment units, as well as the use of a DEGBR reactor which further promoted biodegradation of organic matter, thus reducing pollutants in the wastewater. A performance attribute of 92±6.3% COD removal for this study was slightly higher in comparison with other similar studies whereby the reported COD was 85% (Del Nery *et al*., 2008), refer to Figure 3-29 and Table 3. Furthermore, it was observed that neither an increase in ORL nor a decrease in HRT had an adverse effect on the process (Debik & Coskun, 2009).

3.5.3 TSS Removal

The TSS removal efficiency was another parameter used in this study to measure the performance of the system used. In another study of the biological treatment of an actual slaughterhouse wastewater (SWW) treated in an anaerobic baffled reactor (ABR), followed by an aerobic activated sludge (AS) reactor in continuous mode at laboratory scale, it was indicated that a gradual growth in the biomass led to a more stabilized TSS removal (Bustillo-lecompte & Mehrvar, 2016), as the anaerobic bed acted as a biofilter. Moreover, it was also established that an optimum minimum TSS residual is achieved when both the influent total organic carbon (TOC) concentration and feed flow rate are minimum. For this study, having an average TSS removal efficiency of the entire process being 97 ± 1.2%*,* at an operational HRT of 106 h over 18 weeks (126 days), was commendable. This means that the longer the operational time and the lower the volumetric flow rates, the better the TSS removal efficiencies due to an improved biomass growth resulted in an acclimation process which was adequate. Moreover, increased organic loading rates (OLRs) and constant high hydraulic retention times (HRTs) resulted in a well-developed granular sludge bed in the DEGBR, which also led to enhanced TSS removal efficiencies. The high TSS removal efficiency was further attributed to the used inoculum from a brewery-activated sludge system, which had fully acclimatized and resulted in a well-developed biomass that promoted the filtering of TSS present in the PSW (Dlangamandla *et al*., 2018).

3.5.4 FOG Removal

Further results obtained from this study showed that, by using the Eco-flush^{TM}, solubilization of some FOG components resulted in the decoupling of TSSs, which resulted in the further filtering efficiency of the PSW in the pre-treatment stage, subsequently yielding better performance in FOG removal in the DEGBR. Comparatively, another study used for long-term PSW treatment, whereby the DAF contributed to the reduction of FOG, COD and TSS prior to the application of an UASB (Del Nery *et al*., 2007), only FOG, COD and TSS removal efficiencies of 63%, 67%, and 61%, respectively, were achieved. In another study, PSW treatment using a pilot SGBR reactor, with organic loading rates between 0.63 to 9.72 kg/m^3/d and a hydraulic retention time (HRT) of 9 to 48 h, only reported removal efficiencies of COD above 90% and a TSS above 80%, respectively (Park *et al*., 2015), results which were comparatively similar to the ones reported herein using a DEGBR.

Therefore, by using Eco-flush$^{\text{TM}}$ as a solubilization agent, it was indicated that the microorganisms present in it aided in the biological hydrolysis of some pollutants present in the PSW. Since the raw PSW was first aerated to invigorate the microorganisms in the Eco-flush$^{\text{TM}}$, the proliferation of bacteria in the agent resulted, which contributed to the initial degradation of organic material and pollutants. Overall, the microbes partially consumed the organic contaminants and further bound less soluble matter found in the raw PSW, which also reduced the odor of the wastewater.

3.5.5 VFA/Alkalinity ratio in the DEGBR

Since the DEGBR ran at a stable VFA/Alkalinity ratio of 0.2, which is below 0.3, it means that there was adequate organic matter digestion in the reactor (Debik & Coskun, 2009). It has been established that an acidic digester or reactor is one which is above a 0.8 VFA/Alkalinity ratio; therefore, the consequence of an acidic reactor is that there could be a microbial inhibition of methane gas production (Park *et al*., 2009). However, it was found that the methanogenetic activity in the DEGBR decreased only after the addition of Ecoflush$^{\text{TM}}$ in the biological pre-treatment stage (i.e., as a preceding step before the DEGBR process), whereby methanogenesis was expected to ensue. This means that the supplementation of solubilizing agents in pre-treatment units for PSW might inhibit methane gas production. Overall, the DEGBR proved that there was no need of backwashing, as the recycle stream incorporated in the DEGBR design enhanced the system's performance and consistency by preventing clogging problems (Dlamini *et al*., 2019). A comparison of the results of this study is shown in Table 3 further illustrates the performance of other systems similar to the DEGBR.

Table 4: Comparison of DEGBR's results to other similar wastewater treatment studies.

Technology Used	Type of wastewater	Results	Reference
SGBR	PSW	93% COD, 95% TSS, and 90% FOG	(Basitere *et al.*, 2019)
Up-flow anaerobic filter	PSW	70% tCOD, VFA/alkalinity ratio was in the range of 0.12-0.34. The average methane content varied between 46 and 56 %	(Rajakumar *et al.*, 2011)
EGSB	Cooking wastewater	72.9% COD	(Dong *et al.*, 2013)
UASB	High fat wastewater	91.2% COD and 98.5% FOG	(Damasceno *et al.*, 2018)

3.6 A comparison of product parameters with the City of Cape Town Specifications

Generally, the DEGBR product samples obtained in this study were within the limit prescribed by the City of Cape Town by-laws (2013). This study reported average dis-charge parameters for COD, TSS and FOG of 699, 185 and 28 mg/L, respectively, in comparison with the City of Cape Town's prescribed compliance limits for COD, TSS and FOG of 5000, 1000 and 400 mg/L, respectively. Table 5 also shows that the DEGBR product samples reported an average pH and temperature values of 7.3 and 37.5 °C, which are within the prescribed discharge standards (i.e., pH range of 5.5–12 and a temperature of 0–40 °C). However, this study's' treated PSW did not meet the minimum required for environmental, health and safety (EHS) guidelines values. The EHS guidelines are technical reference standards with general and industry specific examples of good international industry practice (GIIP). The product samples from this study (i.e., post the pre-treatment and DEGBR) are shown in Figure 3-30. Moreover, even though the water requires further processing in order for it to reach reusable standards, treated water could be suitable to be used for other purposes such as irrigation (Sheldon & Erdogan, 2016).

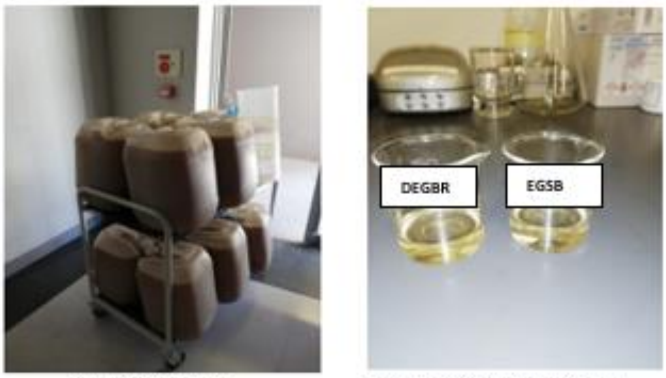
Table 5: DEGBR's product in comparison to City of Cape Town Specifications and others

Parameter	CCT* by-law limits	EHS*	DEGBR product
COD	5000 mg/L	250 mg/L	669 mg/L
TSS	1000 mg/L	50 mg/L	185 mg/L
FOG	400 mg/L	10 mg/L	28 mg/L
pH	5.5 -12	6 – 9	7.3
Temperature	0- 40 ^{0}C	<3^b	37.5 ^{0}C
VFA	-	-	157.97 mg/L
Alkalinity	-	-	888.12 mg/L

CCT* City of Cape Town: wastewater and industrial effluent by-law, 2013.

EHS* - Environmental, Health, and Safety Guidelines poultry production, world bank group, 2007.

[b] At the edge of a scientifically established mixing zone which takes into account ambient water quality, receiving water use, potential receptors and assimilative capacity.



Raw PSW effluent *DEGBR Product PSW effluent*

Figure 3-30: A comparison of Raw PSW effluent and DEGBR product sample

3.7 Summary

The study of the lab-scale plant of the biological pre-treatment of the PSW coupled with the DEGBR was successfully employed. At the pre-treatment stage, the FOG, COD and TSS removal efficiencies that were obtained were 80±6.3%, 38±8.4%, and 56±7.2%, respectively. Similarly, the DEGBR achieved average FOG, COD and TSS removal efficiencies of 89±2.8%, 87±9.5% COD, and 94±3.7%, even at high OLR of up to 8.3538 g COD/L. day with an HRT of 106 hr. The DEGBR system was operating under stabilized conditions since the pH was within the optimal range of 6.6–8 and the VFA/alkalinity ratio was consistently around 0.2, albeit slightly lower than the recommended 0.3 throughout the experimental period. The average methane gas production reported during the start-up period was 0.3 L CH_4/g. COD, which reduced as the operation ensued. This was attributed to the application of Eco-flush^{TM} containing pre-treated wastewater feed to the DEGBR. A full-scale application of the DEGBR to treat similar high-strength wastewater treatment would provide benefits to the slaughterhouse wastewater treatment plants, due to its simple design and operational advantages over conventional high rate anaerobic systems. Lastly, the designed lab-scale plant can be used in small and large poultry processing industries.

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CHAPTER FOUR

CONCLUSION & RECOMMENDATIONS

CHAPTER FOUR

4. CONCLUSION AND RECOMMENDATIONS

4.1 Conclusion

The biological pre-treatment system coupled with DEGBR process was found to be capable and efficient to reduce FOG, COD and TSS of poultry slaughterhouse wastewater over a period of 18 weeks (126 days). In this study, Eco-flush^{TM} was used as a biological degradation agent in the pre-treatment process. The biological pre-treatment process managed to yield FOG, COD and TSS removal efficiencies of 80±6.3%, 38±8.4% and 56±7.2%. Therefore, with the high removal efficiencies of FOG, COD and TSS obtained, it can be concluded that the addition of Eco-flush^{TM} had a positive effect on the pre-treatment of raw poultry slaughterhouse wastewater (PSW) in terms of its biological performance evaluation. Moreover, the subsequent process of the DEGBR, operated at a hydraulic retention time (HRT) of 106 hrs and at an average organic loading rate (OLR) of 3.53 g COD/L.day, respectively. However, it is important to maintain a proper HRT and OLR in order to achieve a dense biomass or granulation. In this study, it was observed that the DEGBR was able to consistently reduce the organic matter, suspended solids and fats content of the pre-treated PSW throughout its 126 days of operation, with FOG, COD and TSS removal efficiencies 89±2.8%, 87±9.5%, and 94±3.7% on average. In addition, it was found that once the microbial biomass developed and stabilizes, the VFA/Alkalinity ratio becomes lower than 0.3 which is recommended from literature (Debik & Coskun, 2009) and the pH also stabilises. This study also reported an average VFA/Alkalinity ratio of about 0.2, which meant that the DEGBR process was stable during operation. Biogas was also produced during the start-up of the reactor, with a content of 0.3 L CH4/g.COD, however, it was reduced from 71 % to zero 0% after the DEGBR reactor was fed with pre-treated poultry slaughterhouse wastewater (PSW) containing Eco-flush^{TM} bioremediation agent.

In overall, the biological pre-treatment system coupled with DEGBR bioreactor performed satisfactorily with regard to the removal efficiencies of FOG, COD and TSS in the treatment of PSW effluent. The study reported an overall performance of removal efficiencies of COD, FOG, and TSS of 97±0.8%, 92±6.3% and 97±1.2%. Lastly, the pilot plant scale of this study can be upscaled and used in the treatment of PSW for large or smaller poultry processing factories.

4.2 Recommendations

- It is recommended that in the future, the process be operated continuously at different HRTs and ORLs which are monitored to check the performance and efficiency of the system.
- It is recommended that the in the future, the DEGBR is operated at different feed flowrates which is proportional to an increase in COD in order to see the effect of producing an increased/decreased amount of biogas.
- It also recommended that the process would be operated by an automated process control unit to avoid clogging of the reactor when the process is unmonitored.
- Since, this study is novel, it is highly recommended that local poultry slaughterhouse wastewater treatment plants use Eco-flush^{TM} in their pre-treatment stages to avoid or mitigate any foul smell produced by effluent ponds.
- Lastly, it would be recommended that the proposed process of the biological pre-treatment system coupled with the DEGBR is designed as a pilot-scale plant to be used in the treatment for local poultry slaughterhouses.

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APPENDICES

APPENDIX A: ANALYTICAL METHODS

APPENDIX A1: Methods used to determine the pH, TDS using the PCSTestr 35 multiparameter meter

Calibration procedure of the PCSTestr 35 multiparameter meter:

1. Switch on the PCSTestr 35 meter.
2. Place the PCSTestr 35 probe into 100 ml of distilled water for 2 minutes.
3. Take the PCSTestr 35 probe out of the distilled water and pat dry using tissue paper.

Do not rub in order to avoid damaging the probe.

pH calibration:

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

TDS calibration:

1. Press the MODE key on the PCSTestr 35 meter until the TDS screen is reached.
2. Place the PCSTestr 35 probe in the 300 ppm TDS buffer solution and press the CAL key.
3. Press the HOLD key to increase the value in the top digital display screen and the CAL key to decrease the value in the top digital display screen until the value is set to the known concentration of the buffer i.e. 300 ppm.
4. Once the desired value is reached, press the CAL key to confirm the calibration and exit the TDS calibration screen.
5. Rinse the probe with distilled water and pat dry.

pH, and TDS measuring procedure:

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

Total Suspended Solids (TSS) determination:

1. Prepare a glass fibre filter disk by weighing it before placing it into a Buchner 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

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

Where A is the weight of the filtered disc and sample residue (mg)
Where B is the weight of the filter disc (mg)
Where C is the volume of sample filtered (ml).

Method to determine chemical oxygen demand (COD):

1. Switch on the Spectroquant thermoreactor TR420 and select the pre-set setting of 148 °C for 2 hours. It will take approximately 10 minutes for the thermoreactor TR420

to heat up to 148 °C.

2. For the 500 to 10 000 mg/L measuring range (using Merck COD Solution A, Cat. No.1.14679.0495 and Merck COD Solution B, Cat. No. 14680.0495):

- Pipette 2.2 ml of COD solution A into an empty test tube.
- Pipette 1.8 ml of COD solution B into the test tube.
- Pipette 1 ml of the sample into the test tube.
- Close the test tube with the screw cap and mix using a vortex mixer.
- Heat the test tubes in the Spectroquant thermoreactor TR420 at 148 °C for 2 hours.
- Remove the test tubes after 2 hours and place in a test tube rack to cool down for 10 minutes.
- Mix the test tube contents using a vortex mixer.
- Allow the test tubes to cool down to room temperature for 30 minutes.
- Place the test tubes in the Nova 60 Spectroquant and enter the code **024 (**500 to 10 000 mg/L measuring range).
- Record the measurement displayed on the screen.

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

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

APPENDIX B: FORMULAS USED IN THE CALCULATION OF PARAMETERS

T: HRT CALCULATION:

$$T = \frac{\text{working volume of reactor (m}^3\text{)}}{\text{influent flow rate (m}^3\text{/hr)}} \quad \frac{V}{Q}$$

O: OLR CALCULATION:

$$O = \text{influent COD} * 1/\ \text{HRT}$$

Q: FLOW RATE:

$$Q = \text{Volume (m}^3\text{)} / \text{Time (hr)} \quad V/T$$

η: REMOVAL EFFICIENCY:

$$\eta = \frac{\text{influent (mg/L)} - \text{effluent (mg/L)}}{\text{influent (mg/L)}} * 100\%$$

APPENDIX C: TABLES OF DATA USED TO PLOT THE GRAPHS

Table 6: Pre-treatment data parameters

WEEKS	OLR	COD removal	TSS removal	FOG removal	FOG outlet	FOG inlet	COD outlet	COD inlet	TSS outlet	TSS inlet
1	8	27	63	67	320	980	4968,25	6800	1200	3200
2	6	28	53	71	230	780	4700	6500	1985	4200
3	9	33	55	84	210	1320	4968,25	7415	1450	3245
4	7	42	60	69	198	640	4968,25	8566	1180	2985
5	8	43	54	80	245	1230	6700	11754	2100	4580
6	9	50	48	75	370	1478	5645	11290	3345	6433
7	8	23	43	80	245	1200	8030	10429	3100	5395
8	9	35	74	85	265	1800	4975	7654	1173	4500
9	7	53	62	92	312	3840	9850	21000	3200	8363
10	8	33	49	87	320	2430	6450	9627	2650	5227
11	8	36	54	83	345	1980	4370	6828	2140	4650
12	9	44	55	80	298	1460	5130	9161	3120	6969
13	9	56	64	93	320	4500	6320	14364	2850	7964
14	9	42	57	85	265	1760	4670	8052	2460	5767
15	8	36	48	88	242	2100	4800	7500	2785	5395
16	8	29	57	81	210	1135	5320	7493	2145	4993
17	8	42	52	89	210	1940	7320	12621	2980	6194
18	6	42	55	81	232	1200	6945	11974	3200	7117

Table 7: DEGBR data parameters

Weeks	VFA/Alkalinity	Alkalinity	VFA	pH	Temperature	COD inlet	COD outlet	ORL	TSS outlet	TSS inlet	FOG inlet	FOG outlet	COD removal	TSS removal	FOG removal
1	0,18	971	175	7,03	37,3	4200	1031	9,0018	56	1200	320	43	75	95	87
2	0,18	940	169,2	6,9	37,6	4560	1750	7,83	58	1985	230	27	62	97	88
3	0,10	940	94	6,6	37,3	4800	980	4,7574	42	1450	210	25	80	97	88
4	0,15	812	122	6,73	37,4	5300	1200	9,4716	13	1180	198	11	77	99	94
5	0,25	702	175,5	6,59	37,6	6700	970	19,035	88	2100	245	26	86	96	89
6	0,23	789	181,47	6,6	37,6	5645	1174	26,7084	700	3345	370	32	79	79	91
7	0,12	773	92,76	6,87	37,9	8030	450	16,7184	258	3100	245	18	94	92	93
8	0,20	801	160,2	6,64	37,6	4975	358	12,879	196	1173	265	28	93	83	89
9	0,19	993	188,67	6,81	37,4	9850	456	12,5496	360	3200	312	42	95	89	87
10	0,19	824	156,56	6,78	37,8	6450	890	10,881	402	2650	320	23	86	85	93
11	0,22	944	207,68	7,01	37,7	4370	200	16,632	56	2140	345	27	95	97	92
12	0,17	911	154,87	6,98	37,4	5130	560	19,5102	162	3120	298	12	89	95	96
13	0,24	857	205,68	6,98	37,4	6320	703	9,9522	200	2850	320	32	89	93	90
14	0,20	911	184,865	6,825	37,65	4670	360	8,2917	192	2460	265	37	92	92	86
15	0,17	965	164,05	6,67	37,9	4800	230	6,6312	184	2785	242	42	95	93	83
16	0,15	974	146,1	6,84	37,7	5320	340	10,0872	100	2145	210	29	94	95	86
17	0,14	1048	146,72	7,02	37,8	7320	246	9,639	152	2980	210	26	97	95	88
18	0,15	854	128,1	7,04	37,8	6945	149	7,9434	112	3200	232	27	98	97	88