ANAEROBIC DIGESTION OF HIGH STRENGTH WASTEWATER IN HIGH RATE ANAEROBIC BIOREACTOR SYSTEMS: CASE OF POULTRY SLAUGHTERHOUSE WASTEWATER (PSW)

by

MAHOMET NJOYA

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Supervisors

A.Prof. Seteno Karabo Obed Ntwampe (EngD; HDHET*)
Associate Professor and Head of Bioresource Engineering Research Group (*BioERG*)
Head of Department: Chemical Engineering
Faculty of Engineering & the Built Environment
Cape Peninsula University of Technology
Bellville campus

Dr. Moses Basitere (Doctor of Engineering: Chemical Engineering)
Senior Lecturer: Chemical Engineering Department
Department of Chemical Engineering
Faculty of Engineering & the Built Environment
Cape Peninsula University of Technology
Bellville campus

I. DECLARATION

I, **MAHOMET NJOYA**, declare that all the work in this thesis, save for the appropriately acknowledged, is my own unaided work, both in concept and execution, apart from the normal guidance of my supervisors. This thesis represents my own opinions and not necessarily those of the Cape Peninsula University of Technology and its sponsors.

Moreover, the thesis has not been submitted for any degree or examination in any other university.

Mahomet Njoya — October 1st, 2019

Signed — **Date**

II. ABSTRACT

Considering global and local challenges such as water scarcity, the pollution of surface water, the proliferation of water-borne diseases, and levies imposed by municipal Councils to industries for the discharge of untreated wastewater, it becomes essential for industries to select and implement enhanced wastewater treatment strategies geared towards reducing the concentration of contaminants and benefiting from the organic content of their effluent, if applicable and depending on the selected treatment process. An interesting option for the treatment of organic-laden wastewater, such as poultry slaughterhouse wastewater (PSW), is high rate anaerobic digestion. The latter has become popular since the development of configurations aimed at promoting a long solid retention time (SRT) for short hydraulic retention times.

This thesis elaborates on the treatment of poultry slaughterhouses effluent with three high rate anaerobic bioreactors systems (HRABS), including the Down-flow Expanded Granular Bed Reactor (DEGBR), the Expanded Granular Sludge Bed (EGSB) reactor, and the Static Granular Bed Reactor (SGBR). Moreover, it motivates the selection of HRABS for the treatment of PSW, after discussing different processing options for the conversion of different poultry slaughterhouses solid wastes into marketable by-products. Before selecting these HRABS, the PSW was analyzed, characterized and specific key water quality assessment parameters (tCOD, BOD_5, and FOG) were correlated towards the reduction of cost, time, and chemical waste generated from these analyses. Subsequently, to ensure conducive operation in down-flow HRABS (DEGBR and SGBR) relying on the support of an underdrain system to enable the retention of the required anaerobic biomass and the steady circulation of the HRABS' effluent, some packing materials (white pebbles, pea gravel, small-sized pumice stones, Ceramic marbles, and medium-sized pumice stones) were selected and evaluated. These were initially selected based on their inertness, affordability, and availability. Additionally, further suitability assessment parameters were defined and used for the selection of the most suitable packing material for the underdrain system. These parameters included their porosity, their permeability, their anaerobic sludge retention capacity, and their induced pressure loss. The medium-sized pumice stones showed the best suitability for the underdrain system with the

lowest induced pressure loss, and the highest permeability, porosity and anaerobic sludge retention capacity.

The selection of the most suitable packing material led to the operation and the assessment of the DEGBR, which showed a good performance for the treatment of PSW, with tCOD, BOD_5, and TSS average removal percentages >95%, and a FOG average removal percentage of 93.67 ± 4.51%, for an organic loading rate varying between 1.1 to 38.9 gCOD/L.day. Subsequently, the performance of the SGBR and EGSB was also investigated for the treatment of PSW. The EGSB also provided good results with 99.1%, 99.5%, and 97%, for the removal of tCOD, BOD_5 and FOG, respectively. At last, the SGBR achieved tCOD, BOD_5 and FOG percentage removal of 97.6%, 99.2%, and 97.7%, respectively. This good performance of down-flow HRABS led to recourse to the modified Stover-Kincannon and the Grau Second-order kinetic models for the prediction of the DEGBR and the SGBR, as well as their plant footprint. This study provided the best prediction of the performance of the down-flow HRABS with the modified Stover-Kincannon model.

III. DEDICATION

With love and gratitude to my parents:

My mother, *Amina Hendji Njoya*

&

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My late father, *Yacouba Njoya.*

IV. LIST OF OUTPUT

The following outputs reflect the contributions by the candidate to scientific literacy and progress during his doctoral candidacy (2018 to 2019):

The following DHET-accredited research articles, book chapters and conference proceedings were published from the studies reported in this thesis

Njoya, M., Basitere, M. and Ntwampe, S.K.O., 2019. Treatment of poultry slaughterhouse wastewater using a down-flow expanded granular bed reactor. *Water Practice and Technology*. https://doi.org/10.2166/wpt.2019.039.

Njoya, M., Williams, Y., Rinquest, Z., Basitere, M., and Ntwampe, S.K.O., 2019. Design of a Down-Flow Expanded Granular Bed Reactor (DEGBR) for High Strength Wastewater Treatment. *Nano and Bio-Based Technologies for Wastewater Treatment: Prediction and Control Tools for the Dispersion of Pollutants in the Environment*, pp.339-372. **doi:10.1002/9781119577119.ch10**.

Njoya, M., Basitere, M. and Ntwampe, S.K.O. 2019. High Rate Anaerobic Treatment of Poultry Slaughterhouse Wastewater (PSW). *New Horizons in Wastewaters Management: Emerging Monitoring and Remediation Strategies*. ISBN: 978-53615-659-1.

Njoya, M., Basitere, M. and Ntwampe, S.K.O., 2019. Analysis of the characteristics of poultry slaughterhouse wastewater (PSW) and its treatability. Water Practice & Technology, 14(4), pp.959-970. https://doi.org/10.2166/wpt.2019.077

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

Basitere, M., **Njoya, M.**, Rinquest, Z., Ntwampe, S.K.O. and Sheldon, M.S., 2019. Performance evaluation and kinetic parameter analysis for static granular bed reactor (SGBR) for treating poultry slaughterhouse wastewater at mesophilic condition. *Water Practice and Technology*, *14*(2), pp.259-268.

Rinquest, Z., Basitere, M., Ntwampe, S.K.O. and **Njoya, M.**, 2019. Poultry slaughterhouse wastewater treatment using a static granular bed reactor coupled with single stage nitrification-denitrification and ultrafiltration systems. Journal of water processing Engineering https://doi.org/10.1016/j.jwpe.2019.02.018.

Williams, Y., Ngongang, M.M., **Njoya, M.**, Basitere, M. and Ntwampe, S.K.O., 2018. Optimization of COD Removal from Poultry Slaughterhouse Wastewater Using Response Surface Methodology for an EGSB. Water Practice and Technology. https://doi.org/10.2166/wpt.2019.032.

Manuscript Submitted for Publication still under Review

Njoya, M., Basitere, M. and Ntwampe, S.K.O. 2019. Overview and Mitigation of the Effects of Poultry Slaughterhouse Waste: Case of Solid Waste. (Still needs to be submitted).

Njoya, M., Williams, Y., Rinquest, Z., Basitere, M. and Ntwampe, S.K.O. 2019. Performance Comparison of Three High Rate Anaerobic Bioreactors (EGSB, DEGBR & DEGBR) for the Treatment of Poultry Slaughterhouse Wastewater. **Submitted to Journal of Environmental health Science and Engineering (Manuscript ID:** JEHS-D-19-00341).

Njoya, M., Rinquest, Z., Basitere, M. and Ntwampe, S.K.O. 2019. Performance Evaluation and Kinetic Modelling of Down-flow High Rate Anaerobic Bioreactors for Poultry Slaughterhouse Wastewater Treatment. (Still needs to be submitted).

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

Njoya, M., Williams, Y., Rinquest, Z., Basitere, M. and Ntwampe, S.K.O. 2019. Performance Comparison of Three High Rate Anaerobic Bioreactors (EGSB, DEGBR & DEGBR) for the Treatment of Poultry Slaughterhouse Wastewater.11th Eastern Europe Young Water Professional, Czech Republic, Prague, 1-5 October 2019.

Njoya, M, Basitere, M, Ntwampe, S.K.O. Analysis of the characteristics of poultry slaughterhouse wastewater. Accepted for presentation at the 16th Specialized Conference on Small water and wastewater System, 1-5 December 2019.

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Mahomet Njoya

October 2019

VI. PREFACE

The structure of this thesis is provided in Figure vi.1.

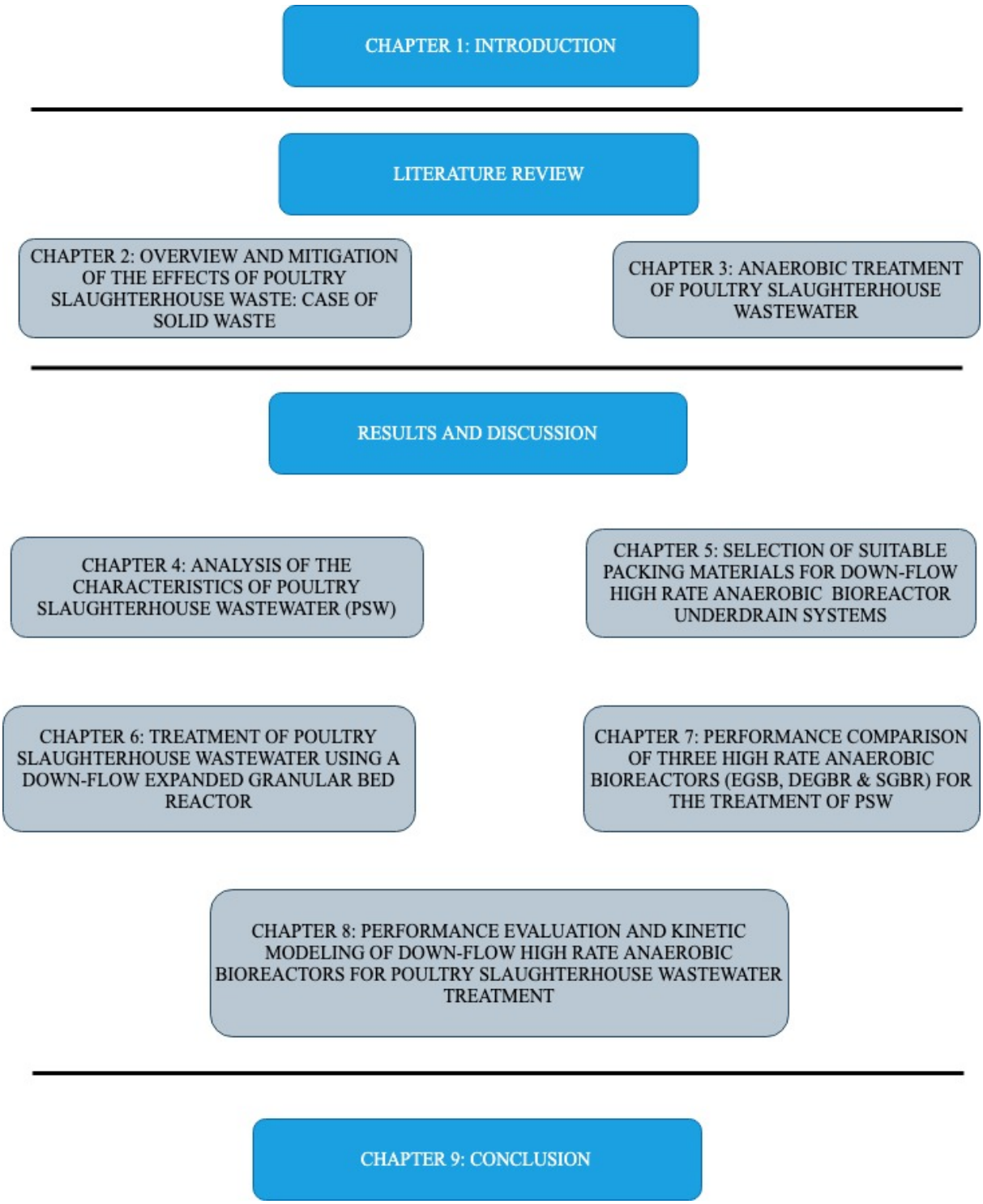

Figure vi.1: Thesis road map

This thesis elaborates on the requirement of treating liquid and solid wastes from poultry slaughterhouses, lists and describes treatment options for selected wastes, and reflects on treatment of poultry slaughterhouse wastewater using high rate anaerobic bioreactor systems. The high rate anaerobic bioreactors selected and evaluated in this study included the Expanded Granular Sludge Blanket (EGSB), the Down-flow Expanded Granular Bed (DEGBR), and the Static Granular Bed Reactor (SGBR). The operating conditions, operational challenges, performance, performance comparison, kinetic modelling and treatment plant footprint of these bioreactors are discussed in different sections and chapters constituting this thesis.

Chapter 1 provides a background to the research problem, motivates this study, elaborates on the hypotheses of its outcome, gives the aims and objectives of this study, provides its relevance, and delineates its scope.

Chapter 2 explains the requirement of improved management of poultry slaughterhouse waste to address global and local challenges such as the pollution of the fauna, flora and quality of water surfaces; water scarcity; and the exposure of poultry slaughterhouse neighbouring population to the effects of the release of untreated solid, and liquid wastes to the environment. Subsequently, this chapter lists the treatment options available for the processing of poultry slaughterhouses solid wastes, which represent a raw material to various industries. Additionally, poultry slaughterhouse wastewater (PSW) treatment is developed in Chapter 3.

Chapter 3 provides an overview of the high rate anaerobic treatment of poultry slaughterhouse wastewater. This section starts by describing poultry slaughterhouse wastewater and then explains why high rate anaerobic treatment the most suitable treatment option for this type of wastewater is. Therefore, the operational conditions, challenges, and advantages of this treatment option are listed. The first step towards an effective treatment of PSW is to characterize it. This objective is satisfied in Chapter 4.

Chapter 4 is the first chapter of the Results and Discussion section. It deals with the evaluation of the biodegradability of poultry slaughterhouse wastewater, its local characterization, and uses linear regressions to correlate PSW water quality parameters, including COD, BOD_5 and FOG. This approach aims at minimizing the time required to analyse parameters such as the

BOD_5, that takes up to five days, and FOG; reducing the quantity of chemical waste generated from these analyses; and limiting the cost of these analyses. The characterization of PSW leads to the conceptualisation of a good treatment option, and therefore a convenient High Rate Bioreactor System. The one suggested in this study is the Down-flow Expanded Granular Bed Reactor (DEGBR), which is operated in a down-flow configuration, hence requires the selection of the most suitable underdrain system for a conducive operation. This requirement is addressed in Chapter 5.

Chapter 5 addresses the requirement of selecting suitable packing materials for the underdrain system of down-flow high rate anaerobic bioreactors. The pressure loss through such packing arrangement often leads to the clogging of the underdrain system and therefore a perturbation of the process of anaerobic digestion materialised by the accumulation of the wastewater inside such bioreactors. In this chapter, five packing materials were selected, including white pebbles, medium-sized pumice stones, Ceramic marbles, small-sized pumices and pea gravel. The first conditions of selection of these materials included their density, their affordability and their inertness. However, for effective evaluation of such packing materials, other analysis parameters were introduced in this study (porosity, permeability, sludge retention capacity and induced pressure loss). The analysis of these other selection parameters culminated in the selection of the most suitable packing material for the SGBR and the DEGBR. Due to its novelty, the first down-flow high rate anaerobic bioreactor investigated was the DEGBR. Its performance is discussed in Chapter 6.

Chapter 6 introduces and evaluates the performance of a new high rate anaerobic bioreactor system (the DEGBR), designed to address the shortcomings of high rate anaerobic bioreactors used for the treatment of poultry slaughterhouse. These challenges include the washout of solids, the difficulty associated with the operation of a three-phase separator, the drainage of the biogas in the effluent and the energy requirement for bioreactors adopting an up-flow configuration. For bioreactors operated in a down-flow configuration, the challenges of channelling, short-circuiting, and clogging are often cited. The configuration of the DEGBR is geared towards addressing these challenges and thus its performance was evaluated and compared to other technologies used for the treatment of poultry slaughterhouse wastewater.

Subsequently, in Chapter 7, the treatment of poultry slaughterhouse wastewater is also evaluated using two other high rate anaerobic bioreactors (EGSB and SGBR), and the

performance of those is compared to the one of the DEGBR to determine which option is the most attractive. The good performance of these bioreactors, and, particularly, down-flow high rate anaerobic bioreactors motivated the development of kinetic models to predict its performance with respect to the quality of the feed. This kinetic modelling is discussed in Chapter 8.

Chapter 8 aims at predicting the performance and the footprint of the SGBR and the DEGBR using the modified Stover-Kincannon and the Grau second-order multicomponent substrate models. The kinetic parameters of these two models were determined and used to predict the substrate concentration in the effluent from the two bioreactors and were used to formulate a correlation that can be used to determine the volume of the bioreactors for each investigated model, as per targeted performance. These kinetic parameters were also compared to the ones provided by previous similar studies.

Chapter 9 wraps up this thesis with a conclusion and an overall discussion on the aim, objectives and outcomes of this thesis.

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X. ABBREVIATIONS

ABBREVIATION	DESCRIPTION
ASRB	Acetic acid oxidizing sulphate reducing bacteria
ATP	Adenosine triphosphate
BOD_5	Biochemical oxygen demand
CCT	City of Cape Town
COD	Chemical oxygen demand
DEA	Department of Environmental Affairs
DEGBR	Down-flow Expanded Granular Bed Reactor
EGSB	Expanded Granular Sludge bed
EPA	Environment Protection Agency
EPS	Extracellular polymeric substance
FASRB	Fatty acids sulphate reducing bacteria
FOG	Fats, oil and grease
HRABS	High rate anaerobic bioreactor systems
HRT	Hydraulic retention time
HSRB	Hydrogen oxidizing sulphate reducing bacteria
HUASB	Hybrid up-flow anaerobic sludge blanket
LRABs	Low rate anaerobic bioreactors systems
MBBR	Anaerobic moving bed biofilm reactor
MFB	Methane-forming bacteria
OECD	Organisation for Economic Co-operation and Development
OHPB	Obligate hydrogen producing bacteria

OLR	Organic loading rate
P	Phosphorus
PAHO	Pan American Health Organization
pH	Potential of hydrogen
PSW	Poultry slaughterhouse wastewater
RMSE	Root -mean-square deviation
RTC	Ready to cook
SBR	Sequencing batch reactor
sCOD	Soluble chemical oxygen demand
SGBR	Static Granular Bed Reactor
Sp	Species
Spp	Several species
SRB	Sulphate reducing bacteria
SRT	Solids retention time
tCOD	Total chemical oxygen demand
TDS	Total dissolved solids
TOC	Total organic carbon
TSS	Total suspended solids
UASB	Up-flow Anaerobic Sludge Blanket
VFAs	Volatile fatty acids
VSS	Volatile suspended solids
WWTP	Wastewater treatment plant

XI. LIST OF SYMBOLS

Symbol	Explanation	Unit
A	Area	m^2
C	Solid fraction	-
ΔP	Change in pressure	Pa
d	Diameter of particle	m
D	Equivalent diameter	m
e	Voidage	-
f	Ratio of wet to the dry mass of granules	-
f_B	Blake friction factor	-
g	Gravitational acceleration	$m.s^{-2}$
k	Permeability	m^2
K	Boltzman constant	-
K_B	Saturation value constant	
L	Length	m
r	Radius	m
Re	Reynolds number	-
s	Surface	m^2
S	Substrate Concentration	
t	Time	S
T	Tons	T
U	Removal constant	-
v	Interstitial velocity	m/s
v_o	Superficial velocity	m/s
V	Volume	m^3
W_d	Dry mass of the granules	Kg

Greek symbols

Symbol	Explanation	Unit
ε	Porosity	-
ρ	Density	Kg/m^3
σ	Surface tension	N/m
μ	Viscosity	Pa.s
$\emptyset$	Sphericity	-

Subscripts

Symbol	Explanation
B	Blake
0	Inlet
bd	Biodegradable
BC	Bacterial cells
F	Fluid
Max	Maximum
P	Particle
rec	Non-biodegradable
S	Surface equivalent
SV	Surface-volume equivalent
V	Volume equivalent

Chemical formulae

Element/Compound	Description
CH_3COOH	Acetic acid
CH_4	Methane
CO_2	Carbon dioxide
FE^{3+}	Ferric ion
H_2	Hydrogen
H_2CO_3	Carbonic acid
H_2O	Water
H_3O^-	Hydronium
HS^-	Bisulfide
H_2S	Hydrogen sulphide
NH_3	Ammonia
NH_4^+	Ammonium
NO_3^-	Nitrate
PO_4^{3-}	Phosphate
S^{2-}	Sulphide
SO_3^{2-}	Sulphite
SO_4^{2-}	Sulphate

XII. GLOSSARY

Aerobic treatment	Biodegradation and stabilization of organic matter by aerobes or facultative aerobes microorganisms in the presence of dissolved oxygen (Gerardi, 2003).
Anaerobic treatment	Bio-degradation and stabilization of organic matter through suitable microorganisms in an environment devoid of oxygen, with the production of biogas (Pol *et al*., 2004)
Anoxic	An environment in which bacteria use nitrate or nitrite ions (Metcalf & Eddy *et al*., 2003).
Biochemical reaction	a chemical reaction taking place inside a living cell
Biodegradable matter	organic matter that can be decomposed into basic molecules through biological processes carried out by a wide range of microorganisms. (Metcalf & Eddy *et al*., 2003)
Biogas	a mixture of gases consisting of methane, carbon dioxide, and hydrogen sulfide, amongst others, generated from anaerobic digestion (Gerardi, 2003).
Biomass	The quantity of all microorganisms within a biological treatment process.
Characteristics	General physical, biochemical and biological classes of wastewater elements.
Consortium	Grouping of various microorganisms for a common interest.
Constituents	Specific elements, components or biological entities, which serve to specify the quality of wastewater.

Contaminants	Elements added to the water by use
Digester	a hermetically enclosed vessel, in which anaerobic digestion of organic matter takes place.
High rate anaerobic bioreactors systems	Continuous biological anaerobic reactors developed to operate under reduced hydraulic retention time while improving the sludge retention for better performance (Henze *et al*., 2008).
Mesophilic	Temperature range (32 to 38^{o}C) at which microbial processes can take place.
Methanogens	Anaerobic microorganisms producing methane as by-product of a series of biodegradation of organic matter and transformation of by-products initiated by other microorganisms and enzymes (Gerardi, 2003).
Obligate	Required
Nutrient	Essential element required for the growth of plants and animals, often found in the form of nitrogen and phosphorus in wastewater.
Sludge granulation	The process of anaerobic granular sludge formation from the retention of the anaerobic biomass under suitable conditions in an environment devoid of dissolved oxygen (Pol *et al*., 2004).
Sludge retention	Retention of anaerobic biomass within a bioreactor (Henze *et al*., 2008).

Underdrain system	Physical system developed to ensure the retention of the anaerobic biomass in tubular anaerobic digesters while allowing the permeation of the effluent/wastewater treated (Metcalf & Eddy *et al.,* 2003).

CHAPTER 1

INTRODUCTION

Chapter 1: INTRODUCTION

1.1 Background of the research problem

The discharge of untreated high strength industrial wastewater (HSIW), such as poultry slaughterhouse wastewater (PSW), into surface water culminates in environmental and health stresses marked by eutrophication, the exposure of neighbouring population to pathogenic agents, environmental pollution and the alteration of the exposed fauna (Henze *et al.,* 2008). To prevent such incidences to the environment and people, local governments and administrations have enforced regulations that provide industrial wastewater discharge standards that should be respected to avoid being charged by relevant City Councils (Basitere *et al*., 2016). These discharge standards list the prescribed discharge concentration of industrial wastewater as per relevant water quality assessment parameters, such as the BOD_5, COD, FOG, TSS, pH, Alkalinity, heavy metals, amongst others. To abide by these regulations and avoid huge financial charges on levies, industries have leaned towards treating their effluent to meet the standards before discharge (Basitere *et al*., 2017). Consequently, various researchers have approached the treatment of medium to high strength wastewater using different technologies involving physical, chemical or biological processes (Henze et al., 2008; Avula *et al*. 2009; Chernicharo, 2007). These studies were geared towards finding the most cost-effective, environmental-friendly and efficient way of treating such wastewaters. As a result, several technologies have been evaluated and implemented for the treatment of (HSIW) concerning their composition (Ellis and Evans, 2008; Del Pozo *et al*., 2000). For high strength wastewater predominantly laden with organic matter such as PSW, anaerobic treatment is acknowledged as a very efficient and cost-effective treatment option (Henze *et al*., 2008). However, the operation of such systems presents some challenges that will be listed in this study.

1.2 Motivation for the research study

The poultry industry represents the largest segment of the South African agricultural sector. To sustain their activities and address the increasing demand for poultry products, this industry uses large quantities of potable water in their slaughterhouses, which relates to the production of significant quantities of poultry slaughterhouse wastewater (PSW). This wastewater is laden with organic and, to a less extent, an inorganic matter that can lead to harmful effects on the environment as well as the health of people exposed to it. City Councils control industrial effluents discharged into municipal water channels and enforce regulations governing the practices and quality of the wastewater of such industries to protect the environment and health of people exposed to such wastewater and the pathogens it contains. Furthermore, the extensive use of potable water for the processing of birds in poultry slaughterhouses is associated with costly water bills and contributes to the intensification of the water crisis in South Africa. However, the quantity of potable water used for the processing of poultry products can't be reduced, as it relates to hygienic standards that must be respected to ensure the safety of these products. Hence, the pressure on the poultry industry to adopt enhanced PSW treatment options to abide by the discharge standards and to contribute to the preservation of the environment. This approach contributes to limit the damages induced by poultry slaughterhouses operations to the environment.

1.3 Statement of the research problem

The operation of high rate anaerobic bioreactors systems (HRABS) present some challenges, including the difficulty of operating the three-phase separator for bioreactors in up-flow configurations (UASB, EGSB), the poor collection of biogas, head losses, biogas entrapment, pressure drop through the underdrain system for bio-digesters in down-flow configuration as well as limited distribution of the organic influent to the anaerobic biomass (Alphenaar, 1994; Basitere *et al*., 2016; Basitere *et al*., 2017; Aziz *et al*. 2018; Njoya *et al*., 2019). A new bioreactor design can address these challenges by providing new features developed based on previous shortcomings and the understanding of their effects on the anaerobic system. Therefore, the design of a new bioreactor to alleviate HRABS listed challenges is the first purpose of this study, which also aims at considering other aspects of PSW treatment.

An important step towards the treatment of PSW is its characterization. Several authors have provided PSW characteristics as per location and poultry slaughterhouse throughput (Avula *et al*., 2009; Kiepper, 2003). However, one challenge often faced by researchers is the time required to run some analyses such as the BOD_5 that requires a minimum of 5 days. This can be circumvented by developing a correlation aimed at determining the quality assessment parameter of PSW based on the ones that require less time to analyze. Apart from significantly reducing the amount of time required for analysis, this approach can also provide a good insight into the organic composition of PSW to facilitate the design of improved PSW treatment processes.

The validation of the design of a new bioreactor first imposes a good operation throughout the experiment, then a good performance assessed by the removal or reduction of the concentration of contaminants in the treated wastewater. This performance can be compared with well-established bioreactors used for the same purpose, to confirm the bioreactor's efficacy.

Furthermore, after the validation of the new design, the performance of the new bioreactor can be predicted for the modifications brought to the new design. Various modelling techniques can be used to reach this purpose, including Anaerobic Digestion Model No.1 (ADM1) (Balstone *et al*., 2002), the Monod or Stover-Kincannon kinetic models (Yu *et al*., 1998), the Grau second-order model (Debik and Coskun, 2009), Multiple Linear Regression from collected data or Artificial Neural Network (ANN) (Nasr *et al*., 2012). The development of a reliable model allows the prediction of the outcome of the treatment of a specific type of wastewater using an assessed HRABS and, therefore, facilitates the process of decision making when dealing with the requirement of selecting the most suitable treatment option for a given plant.

1.3.1 Overview: research rationale

The direct discharge of untreated PSW into water channels and the water surface would culminate in the pollution of the environment through eutrophication and affect the health of the people exposed to it. Furthermore, the high concentration in organic matter of such wastewater requires a significant amount of dissolved oxygen in water bodies, which culminates in the reduction of the availability of dissolved oxygen for the fauna that becomes endangered. To address the various issues related to the discharge of untreated industrial wastewaters, City Councils have enforced discharge standards and regulations that are geared

towards imposing the treatment of such wastewaters before its discharge. Therefore, industries such as poultry slaughterhouses have to implement efficient and cost-effective treatment processes that would prevent them to pay heavy levies and take advantage of the organic matter present in their wastewater through anaerobic digestion that enables the production of methane, remains an efficient treatment option for a wastewater laden with organic matter, and the generation of savings from the selection of a cheap wastewater treatment option as opposed to aerobic or chemical treatments. Hence the development of the DEGBR, which is geared towards achieving a good treatment performance through a good removal of contaminants to meet the discharge standards, while providing low operational costs.

1.3.2 Research questions

Can HRABS operated in a down-flow configuration to solve the challenges associated with the three-phase separator?

What model is the most suitable to represent the operation of the DEGBR and the prediction of its performance for the wastewater treated?

What is the most suitable underdrain system for down-flow HRABS?

What is the best way to minimize head losses in HRABS?

What analysis parameter could best relate to BOD_5 and FOGs to reduce PSW analysis time?

What is the best way to improve the production of bio-methane from HRABS? Could this product be improved through a new HRABS design?

1.4 Hypothesis

The design of a new HRABS would address the challenges experienced by these types of bioreactor during the treatment of PSW or medium to high strength wastewater. Furthermore, the development of an accurate model would allow the prediction of the performance of high rate anaerobic bioreactors evaluated in this study. Moreover, the development of a correlation between water quality assessment parameters of PSW would limit the number of required analysis and cut down the amount of time required to perform an analysis such as BOD_5. The hypothesis behind the selection of a suitable underdrain system lies in the fact that it can induce a good sludge retention capacity and the minimization of head losses.

1.5 Research aims and objectives

This aims at highlighting the requirement to treat solid and liquid wastes from poultry slaughterhouses, and addressing the challenges associated with the treatment of PSW in these facilities to prevent the expenditures associated with levies imposed by City Councils when the industrial wastewater does not meet the discharge standards when released into water channels or surface water. As such, the treatment of PSW before its discharge also contributes to preventing environmental hazards as well as the endangerment of the health of the population that may be exposed to the untreated discharged PSW. To reach this aim, the following objectives should be achieved:

1. The explanation of the requirement to treat solid and liquid waste from poultry slaughterhouses as well as the provision of available treatment options,
2. A description of the operations, associated challenges, physical and biological requirements of the anaerobic treatment of PSW,
3. The characterization of PSW, evaluation of its biodegradability, and the development of correlations between its water quality assessment parameters,
4. The evaluation of the porosity, sludge retention capacity, induced pressure loss and permeability of selected solid packing materials towards the selection of the most suitable packing material for the underdrain system of down-flow high HRABS,
5. The evaluation and comparison of the performance of the DEGBR to the one of the technologies assessed for the treatment of PSW in previous studies,
6. The comparison of the performance of three HRABS, including the DEGBR, SGBR, and EGSB, for the treatment of PSW, and
7. The prediction of the performance and footprint of two down-flow HRABS (SGBR and DEGBR), and the evaluation of their kinetic parameters using modified Stover-Kincannon as well as Grau second-order model for the treatment of PSW.

1.6 Significance of the research

Several points justify the significance of this study, namely:

- The requirement of limiting the number of analyses required to assess the performance of bioreactor as well as the minimization of the time required to perform such analysis;

- The alleviation of the challenges encountered during the operation of HRABS; and
- The prediction of the performance of the DEGBR.

These points contribute to reducing the time required to analyze PSW in Poultry slaughterhouses, the protection of the environment as well as the health of people exposed to areas where untreated PSW is discharged and the improvement of efficiency of HRABS.

1.7 Delineation of the Study

This study does not cover the followings:

- The pre-treatment of PSW;
- The post-treatment of PSW and the produced biogas;
- The economic evaluation of the process;
- The biochemical interactions of the microbial agents involved in the anaerobic digestion process; and
- The mass transfer operation intervening inside the DEGBR or other assessed HRABS.

1.8 Summary

Poultry slaughterhouse waste represents an environmental hazard that require enhanced management and treatment to limit its effects on the environment and the health of people exposed to it. This thesis seeks to provide a solution to these challenges through the investigation of high rate anaerobic treatment using different high rate anaerobic bioreactors, including the EGSB, the DEGBR, and the SGBR. However, prior to the performance of such treatment, it is essential to characterize the wastewater to be treated, to identify the challenges of related treatment processes and address them for conducive treatment operations. Subsequently, the performance of these HRABS can be evaluated and compared to determine which option is the most suitable. This option can then be upscaled and predicted using kinetic modelling.

However, poultry slaughterhouses do not only produce liquid waste, but also solid wastes, which represent a raw material to various conversion processes. Therefore, after a brief introduction of the effects of poultry slaughterhouse waste mismanagement, Chapter 2 aims

at providing at providing a list of treatment options usable to process poultry slaughterhouse solid wastes, and associated byproducts.

CHAPTER 2

OVERVIEW AND MITIGATION OF THE EFFECTS OF POULTRY SLAUGHTERHOUSE WASTES: CASE OF SOLID WASTE

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Chapter 2 : OVERVIEW AND MITIGATION OF THE EFFECTS OF POULTRY SLAUGHTERHOUSE WASTE: CASE OF SOLID WASTE

2.1 Introduction

Water scarcity is a global challenge. Northcutt *et al.* (2009) reported that potable water will only be available for domestic usage by 2025 if the current water usage is maintained. Unlike fossil fuels, there is no alternative to water yet found. Water is an essential substance required for a wide range of domestic, commercial, public and industrial activities (Metcalf & Eddy, 2003). This potable water requirement highlights its importance and the need to develop novel techniques to prevent its wastage through the development and implementation of enhanced wastewater treatment options geared towards providing a means of recycling used water or modifying non-efficient processing techniques that have a high potable water requirement.

In South Africa, the poultry industry is one of the industries that require huge quantities of potable water to sustain their activities (Hendricks, 2014). Barbut (2005) reported that the processing of a single bird usually requires approximately 26L of potable water, which gets laden of organic and inorganic contaminants during poultry processing operations and results in the formation of poultry slaughterhouse wastewater (PSW). The discharge of untreated PSW into water channels actions serious environmental concerns such as the eutrophication of water surfaces, elimination of the aquatic fauna and ultimately the pollution of the environment that affects the health and comfort of people living in the surroundings of exposed areas (EPA, 1997; Fuchs *et al*., 2003).

2.1.1 Chapter's objective

Considering the increase in the demand for poultry products with population growth, the production and discharge of untreated PSW into water channels will remain a major concern to the environment as well as the availability of potable water. Therefore, this section aims at

providing a description of the poultry slaughterhouse activities requiring huge quantities of potable water and resulting in the production of PSW and solid wastes, of which options of transformation to usable products will be discussed subsequently after a brief description of the natural cycle of water bodies and the state of water scarcity globally, and in South Africa in particular.

2.2 The natural cycle of water bodies

Water covers around 70% of the earth's surface (Roux *et al.*, 2014). The water cycle (see Figure 2.1) can be defined as a series of processes that culminates in the cyclic migration of water between land, sea, and clouds, thus allowing the supply of freshwater to streams, rivers, lakes and groundwater (World Bank Publications, 2014). The main processes contributing to this migration are evaporation, transpiration, condensation, and precipitation. However, this cycle is currently affected by the effects of climate change on precipitation, evaporation, as well as extreme droughts and floods (World Bank Publications, 2014).

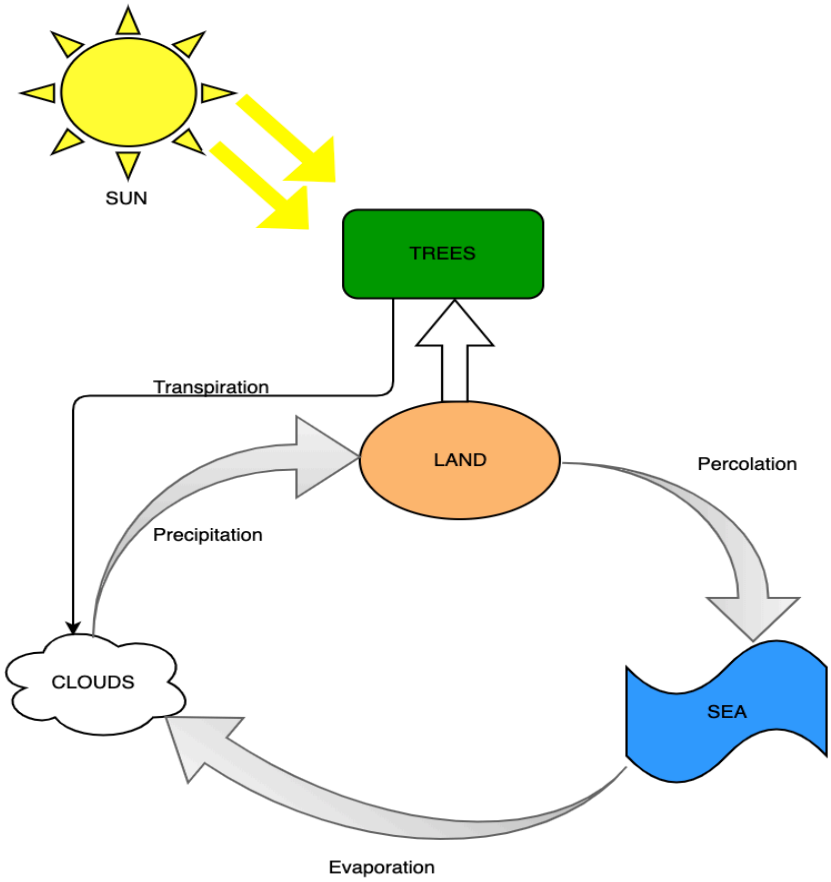

Figure 2.1: Natural cycle of water bodies

The water cycle differs from the natural life cycle of water bodies (See Figure 2.2), as the latter starts from the use of the sun's energy by various aquatic plants such as algae to produce oxygen and transform smaller inorganic molecules into larger organic ones (Falkenmark, 1997). Subsequently, waterborne animals utilize the dissolved oxygen and produced organic molecules to generate and supply energy to muscle tissue (Falkenmark, 1997; Oki and Kanae, 2006). When they die, these water-borne animals and plants are transformed back into inorganic matter which gets incorporated into bottom sediments of water bodies through anaerobic respiration and fermentation (Oki and Kanae, 2006). These newly formed inorganic molecules are then used by new aquatic plants and then begin the cycle again, as depicted in Fig.2 (Oki and Kanae, 2006).

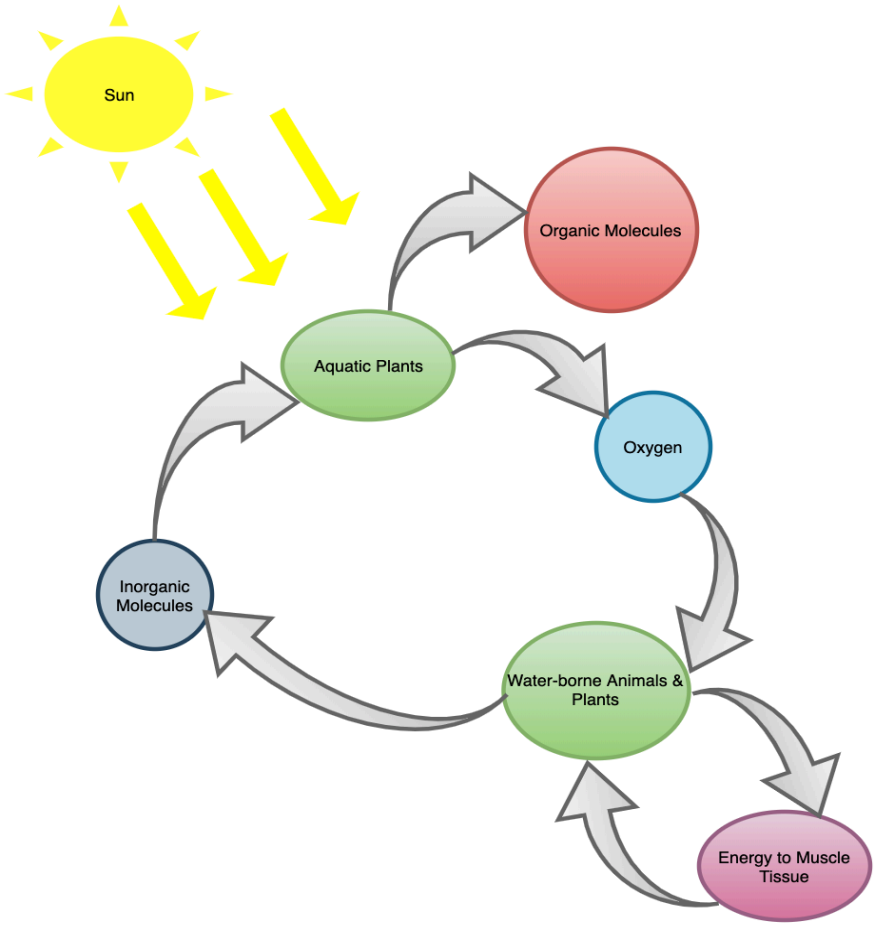

Figure 2.2: The natural water life cycle

This natural life cycle is altered when additional substances are added to natural waters, culminating in the supply of undesirable nutrients, such as phosphorus or nitrogen that can

increase the population of algae that contributes to alter water transparency and accelerate eutrophication (Lee *et al*., 1978).

Eutrophication can be explained as the water body's natural process of aging, resulting from its enrichment from the reception of a big supply of nutrients or organic matter (Lee *et al*. 1978; Falkenmark, 1997; Ritter *et al*., 2002)). This process is considered as irreversible and experienced by all water bodies, as it is not considered as pollution until the human activities culminating in an increased supply of nutrients and organic matter result to the acceleration of the process (Falkenmark, 1997). Therefore, this accelerated process is termed cultural eutrophication, which differs from the natural eutrophication by the addition of nutrients that might be contained in the wastewater discharged from various industries without an appropriate treatment (Lee *et al*., 1978; Falkenmark, 1997; Daniel *et al*., 1994). Thus, pollution occurs when the natural equilibrium of aquatic ecosystems is jeopardized by anthropogenic influences.

Nevertheless, some studies (Conley *et al*., 2009; Daniel *et al*., 1994; Ritter *et al*., 2002) have found that cultural eutrophication can be reversed through the reduction of nutrients and organic matter input. However, the significant reduction of nutrients and organic matter to the water body doesn't necessarily translate to an immediate response from the aquatic plants, like the tropic levels and the chlorophyll concentrations usually take more time to restore to normal levels (Daniel *et al*., 1994; Lee *et al*., 1978; Oki and Kanae, 2006). Furthermore, the issue of cultural eutrophication can also be addressed by various methods including the oxygenation of sediments and deep-water layers, chemical precipitation of phosphorus, harvesting of macrophytes, dredging of nutrient-rich sediment, as well as flushing of nutrients with dilute waters. However, prevention has always been better than cure; hence the implementation of preventive measures such as the control of the disposition of organic matter and nutrients borne materials into water surface from various sources, which include industrial activities, sewage treatment plants, combined overflows, agricultural activities, atmospheric deposition, construction or habitat modification (Ritter *et al*., 2002; Conley *et al*., 2009).

2.3 Water crisis in South Africa

Figure 2.3: Effects of Climate Change

Considering the current potable water usage trends, South Africa may be exposed to a water deficit of 17% in 2030 and a physical water scarcity by 2025 (Western Cape Government, 2015; Hedden and Cilliers, 2014). This water shortage prospect will be exacerbated by climate change, which is a current global challenge that is responsible for floods, drought, the shifting of animal and plant, and shrinking of glaciers, as illustrated in Figure 2.3 (Viljoen, 2015; Oki and Kanae, 2006).

Drought is one of the main factors affecting the water welfare of South Africa, with perceived harm on the agricultural sector that has further decreased its maize exports in recent years, resulting in the loss of 35 000 jobs in the fourth quarter of 2015 (Western Cape Government, 2015; Hendricks, 2014). This led to an additional 50 000 people moving below the poverty line and an increase of consumer inflation stimulated by rising food prices (Western Cape Government, 2015). The water demand in South Africa is significantly increasing, as a result of the demand of three major sectors that include the agricultural (63% of the demand); municipal and industrial sectors (26 and 11% of the demand, respectively) (Midgley *et al*., 2016; Hendricks, 2014).

Water scarcity corresponds to a higher level of total water demand than available supply, which differs from water shortage or water stress (Dinar *et al*., 2012). Water shortages may result from the lack of available supply, faults in infrastructure, environmental changes or simply deteriorating water quality; whereas water stresses represent the symptoms of water scarcity or water shortages. Weak and unpredictable supply, combined with high and growing demand and inadequate use of existing water resources, qualify South Africa as a water-constrained country, which is characterized by a low and fluctuating annual rainfall combined with high natural evaporation levels that place South Africa as the 30th driest country in the world (Dinar *et al*., 2012; Western Cape Government, 2015; Roux *et al*., 2014). This weak annual rainfall can be explained by an annual average rainfall of only 495 mm in South Africa, while the world average is 1033 mm, which represents a difference of 538 mm per year that is higher than the actual annual average rainfall in this country (Greencape, 2016). Furthermore, the evaporation losses are often three times higher than rainfall, which can average less than 100 mm of rain annually in some regions of South Africa (Greencape, 2016).

The National Development Plan of South Africa is a long-term perspective that was developed to ensure that all South Africans should have access to clean running water in their homes, the country should reach a food trade surplus, and produce sufficient energy to improve the economy in order to alleviate poverty and reduce inequality by 2030 (National Planning Commission, 2013). This plan is addressed to different sectors of society and abides by ideas developed by the National Water Resource Strategy of 2004 (NWRS1) that was released as a blueprint for water resource management as well as one of the requirements of the 1998 National Water Act of South Africa. A report entitled Parched Prospects: The Emerging Water crisis in South Africa (Hedden and Cilliers, 2014), stated that the increases in the water supply cannot meet the expected increase in demand without supplementary and far-reaching interventions. The report further explained that, besides engineering, demand management in terms of both efficiency and allocation will have to intervene in the efforts to close the water demand-supply gap in South Africa (Hedden and Cilliers, 2014). Therefore, about the key messages laid out in the National Water Resource Strategy of 2013 (NWRS2), it was agreed not to allow the waste of water, anywhere, anymore (Hedden and Cilliers, 2014). These key messages included (Hedden and Cilliers, 2014):

- The importance of the use of groundwater, which is under-valued and under-used, especially in rural farming areas,

- The potential to increase the reuse of municipal and industrial water at the coast and inland systems,
- The limitation of the opportunity for more dams or transfer schemes, which remain inevitable in certain areas,
- The storage of water in aquifers to be considered as part of future policy as well as the research of alternative means to reduce the problem of high levels of evaporation and transpiration in South Africa,
- The consideration of desalination projects as a way to increase the water supply, especially in coastal areas with limited alternative sources of supply, despite the high operating cost associated with such projects,
- The required incentives should be implemented for the transition to a recycling economy, in which water of different price and quality will be used for different purposes, and
- The evaluation and monitoring of the water sector, which is required to set and achieve the reduction of the water demand-supply gap in South Africa.

Thus, the NWRS2 observed that water scarcity may shortly emerge as one serious constraint on the country's human-development prospects (Hedden and Cilliers, 2014; Hendricks, 2014). Therefore, it is a duty for industries using a significant quantity of water to develop methods to reduce water consumption to address the current challenges of the country (Saldias *et al*., 2016). One of these industries is the poultry industry that utilizes huge quantities of potable water to sustain its activities.

2.4 Overview of the poultry industry

The growth and intensification of the poultry industry over the past decades have paved the way for an increase in environmental concerns. Efforts to accommodate lower production costs and higher poultry slaughterhouses throughput have intensified efficient operations through the improvement of integrated facilities and the recourse to animal genetics, enhanced nutrition, and innovative production techniques.

The recourse to larger facilities and the intensification of the poultry industry operations have resulted in increased environmental concerns at regional and global scales. These challenges can be regrouped in challenges at the level of production and processing sites, and those related to watershed-level pollution and poultry waste mismanagement.

Typical local negative amenities in the surroundings of poultry slaughterhouses include landscape degradation and local disturbances such as flies, rodents, and odor. Furthermore, soil and water pollution with heavy metals, nutrients, and pathogens usually results from the poor management of stored manure. However, the release of manure into the environment rarely occurs and is therefore not an environmental concern, as local councils enforce regulations geared towards protecting the environment and protecting hazardous practices from the poultry industry. The environment protection enforcement further motivates the sale or processing of manure to products such as fertilizers or animal feed.

The most concerning environmental challenge associated with poultry slaughterhouses operations is the discharge of untreated PSW into the environment. Poultry slaughterhouse facilities require significant quantities of potable water to sustain their activities and provide clean poultry products. During poultry processing operations, the used potable water collects organic and inorganic matters, which culminates in the generation of hazardous wastewater presenting a serious risk to the environment and the surrounding population.

Worldwide, the poultry meat production is dominated by the US, China, Brazil and the European Union, with production rates of 20959 KT-RTC, 18632 KT-RTC, 14312 KT-RTC, and 13393 KT-RTC, respectively. This production rate is driven by local and international demands characterized by high imports and exports, respectively. From 2015 to 2017, Brazil dominated global poultry meat exports with an annual rate of 4251 KT-RTC of poultry meat exports, while the US followed with an export with an annual rate of 3238 KT-RTC, the European Union with 1454 KT-RTC and Thailand with KT-RTC. The high export rate of Brazil stemmed from a production rate higher than the local demand, which was at 9146 KT-RTC in the same period. This low demand was also highlighted by a low import rate of 4 KT-RTC. Similarly, within the same period, the US exported more than it imported poultry meat into the country, with an import rate of approximately 77 KT-RTC per annum in the period of 2015 to 2017. Figure 2.4 illustrates the annual production of poultry meat in various countries of the world from the period of 2015 to 2017. As illustrated in Figure 2.5, this product will increase in the highlighted countries by the year 2027. Several parameters motivate this increase in poultry meat, namely:

- The increase of the world population;
- The development of enhance production and feed techniques;
- Scientific advancements towards the maintenance of the health of the broilers during their development;

- The enhancement of agricultural throughput for the increased supply of food to the broilers; and
- The enthusiasm of people towards white meat, which provides dietary features than red meat.

Figures 2.6 and 2.7 further illustrate the inclination of the increase of poultry meat demand with time, as per the projection of poultry meat demand in various countries by 2027. When compared to Figures 2.4 and 2.5, Figures 2.6 and 2.7 also illustrate that the poultry meat demand relates to the production rate of poultry meat in these countries. Furthermore, other factors such as the industrialization of these countries influence the production of poultry meat, which requires intensive and specialized operations. This statement can also be supported by Figures 2.8 and 2.9, which highlights the demand for poultry meat per capita in various countries. It can be observed that developed and developing countries produce and consume more poultry meat than third world countries.

South Africa is one of those countries with an annual poultry meat demand per capita of 47 Kg-RTC in 2017, which was higher than the one of Brazil, Canada, Russia and the European Union, with 42 Kg-RTC/Capita, 40 Kg-RTC/Capita, 36.44 Kg/Capita and 27 Kg/Capita, respectively. Seemingly, as per Fig 9, this trend will be maintained in future years and consequently, require enhanced poultry wastes processing techniques to respond to a higher production rate and eventual environmental and health challenges.

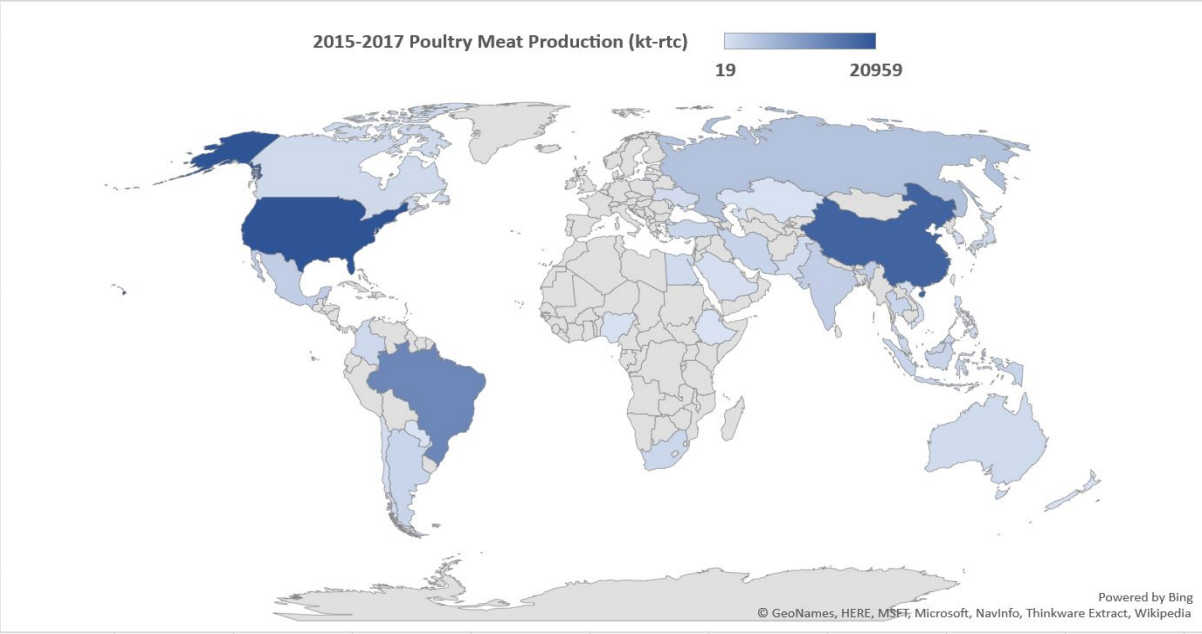

Figure 2.4: 2015-2017 Worldwide poultry meat production (KT-RTC) (Adapted from OECD, 2018)

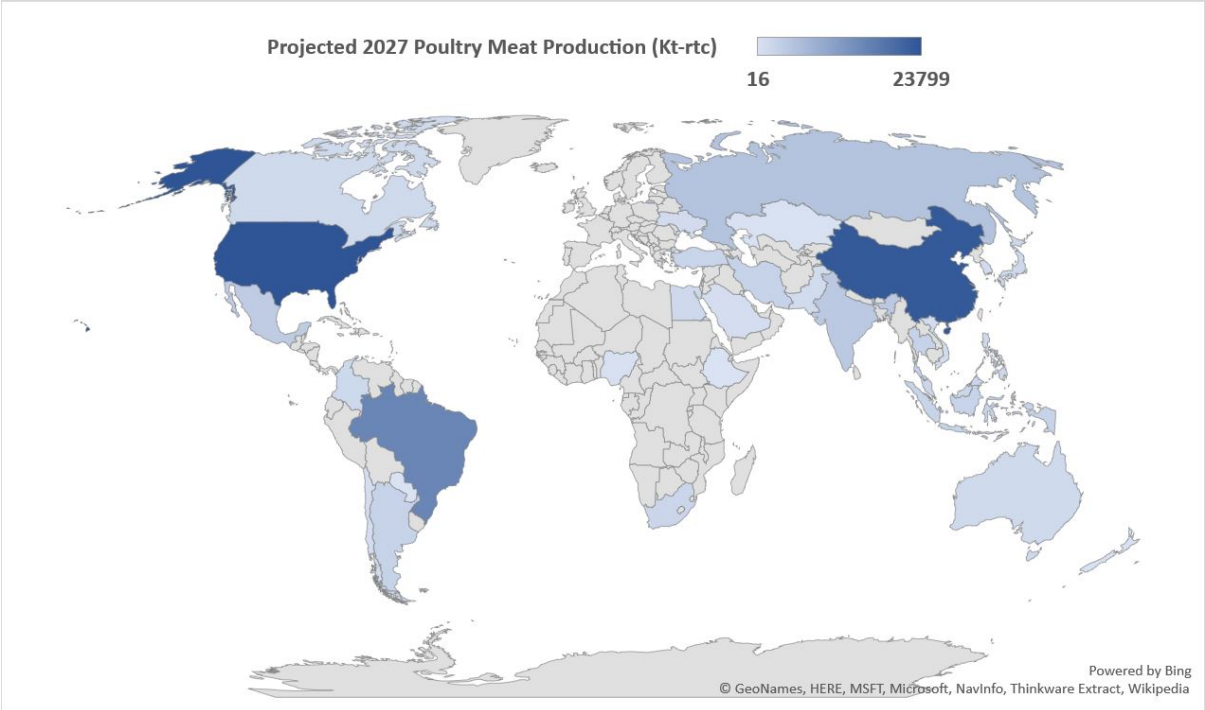

Figure 2.5: 2027 Projected Worldwide Poultry Meat Production (KT-RTC) (Adapted from OECD, 2018)

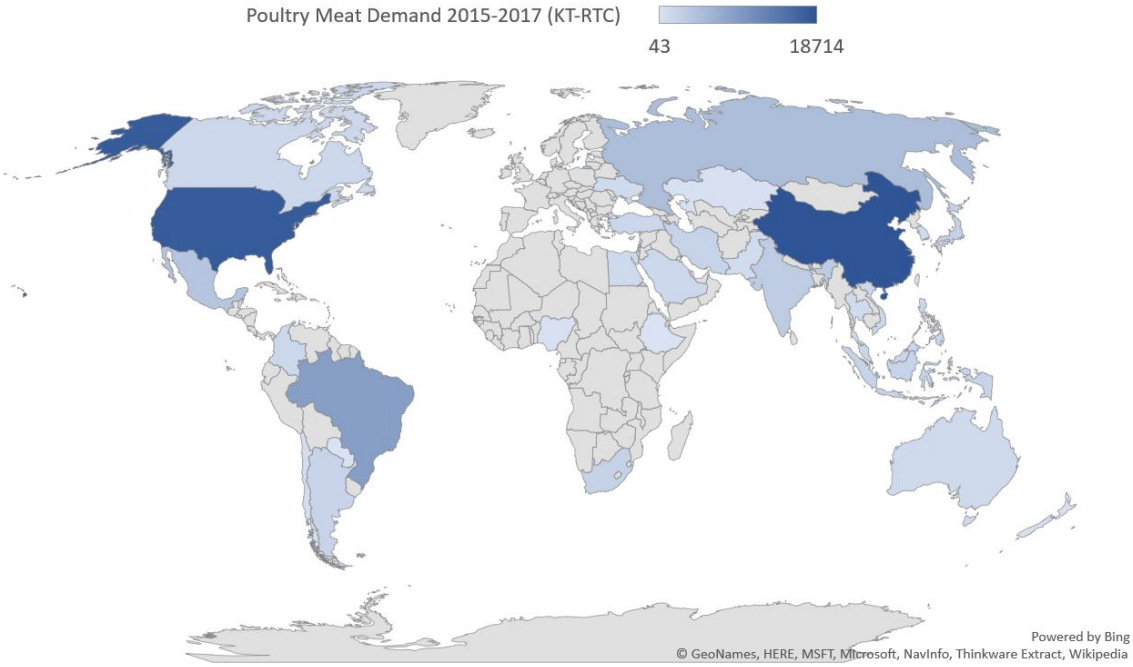

Figure 2.6: Worldwide Poultry Meat Demand 2015-2017 (KT-RTC) (Adapted from OECD, 2018)

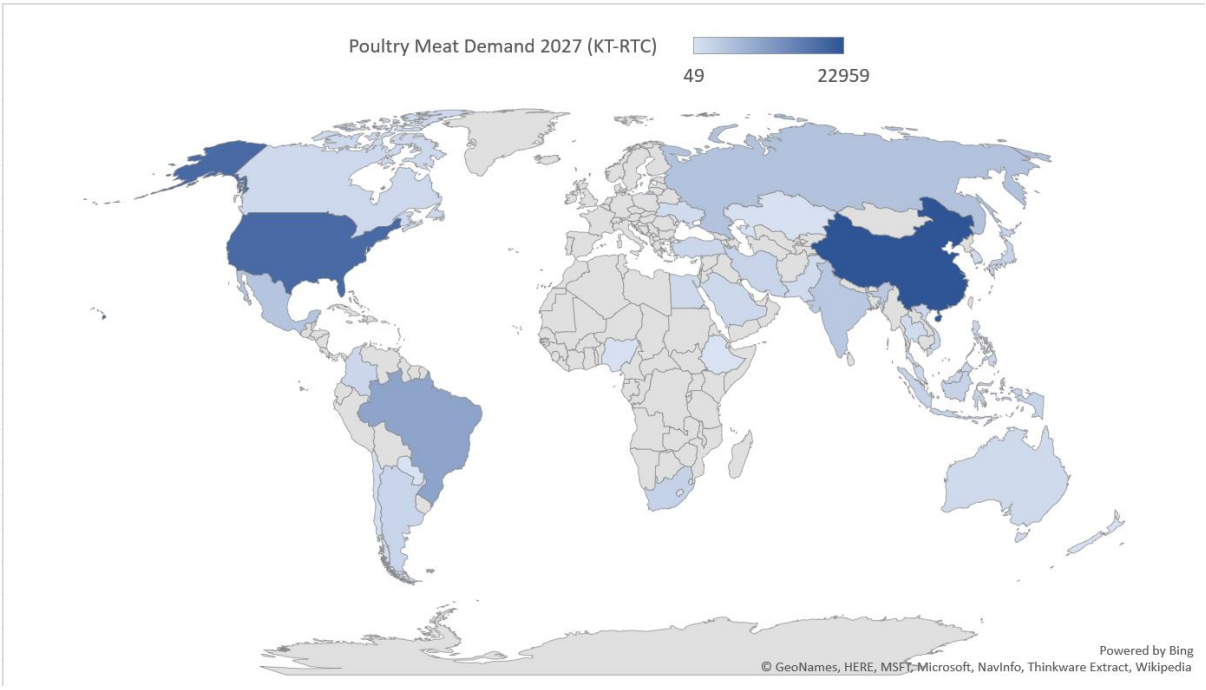

Figure 2.7: 2027 Projected Poultry Meat Demand in various countries (KT-RTC) (Adapted from OECD, 2018)

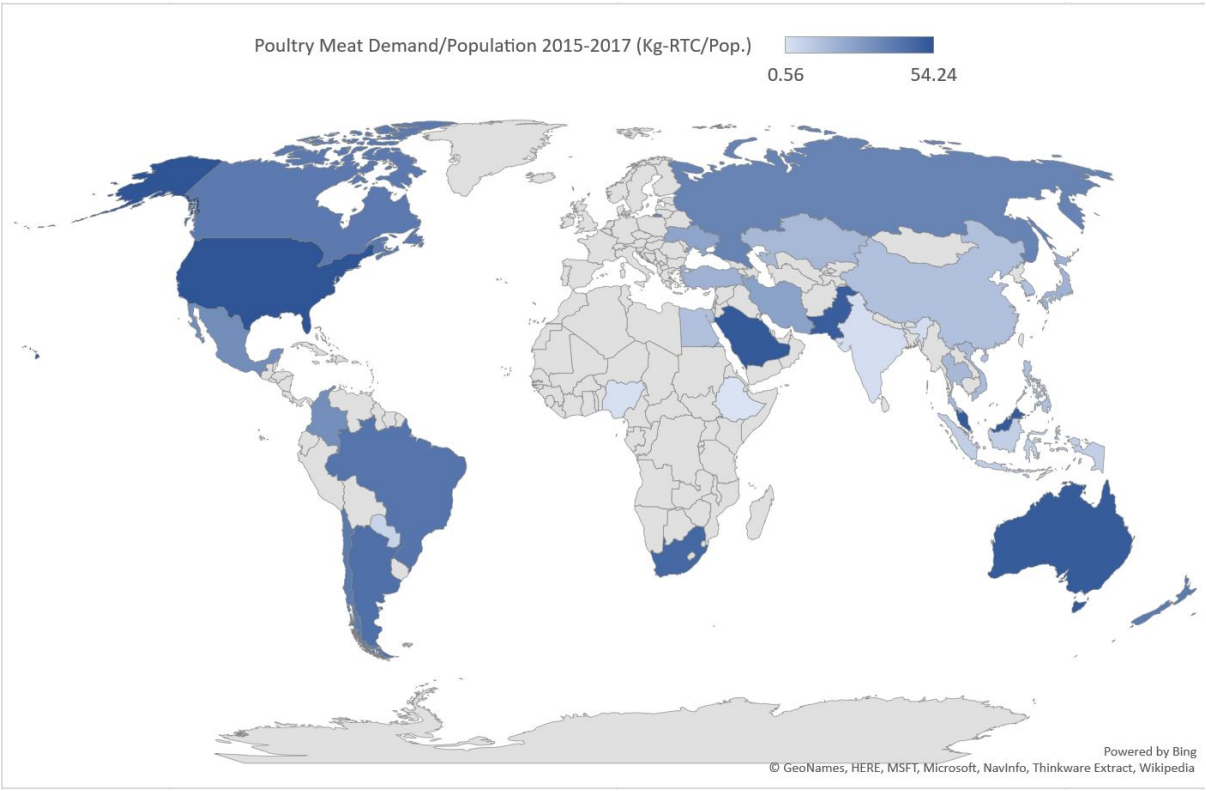

Figure 2.8: 2015-20178 Poultry Mead Demand/Population in various countries (Kg-RTC/Capita) (Adapted from OECD, 2018)

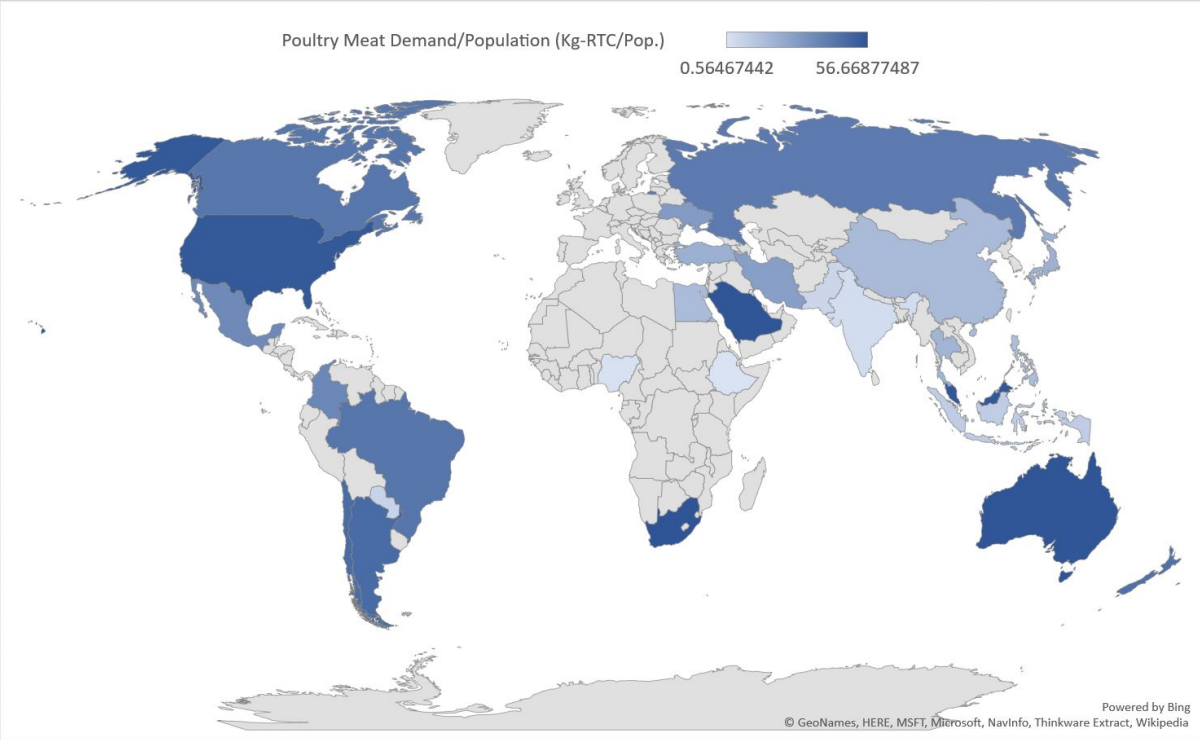

Figure 2.9: Projected 2027 Poultry Meat Demand/Population in various countries (OECD, 2018)

Agriculture is the most important segment of the South African economy and thus requires special attention for its important contribution to the country's gross domestic product (GDP), social welfare, food security, ecotourism and job creation (Hendricks, 2014). One important segment of the agricultural sector, with a contribution of 16% to the GDP, is the poultry industry (Hendricks, 2014). This industry consists of economically raising selected birds for their meat, eggs or show. Selected birds include chickens, quail, turkeys, ducks, guinea, and geese (Western Cape Government, 2015).

Several interlinked segments constitute the poultry industry. These segments are usually owned by the same company that applies vertical integration, which is a business management method that enables the maximum control of the products through a management method elaborated in a way that one segment depends on others in a structured hierarchy that results in efficient operations and products of good quality (Barbut, 2016; Henry and Rothwelle, 1995). Generally, this hierarchy consists of 8 levels as illustrated in Figure 2.10.

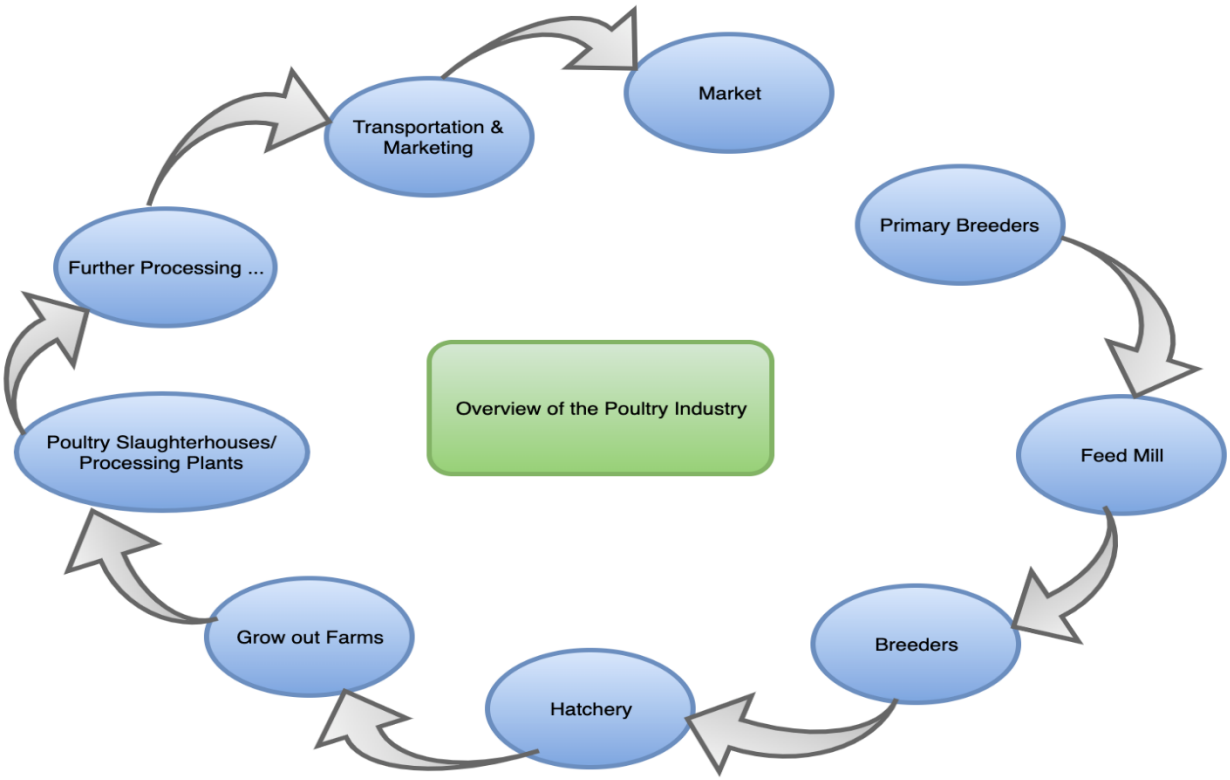

Figure 2.10: Segments of the Poultry Industry

These levels are briefly described subsequently.

2.4.1 Primary breeders

The first stage of the poultry industry hierarchy relies on primary breeders to develop and reproduce strains of chicken that meet the poultry industry's hygienic requirements. These requirements include efficient feed conversion and abundant white meat production (Barbut, 2016; Henry and Rothwelle, 1995).

2.4.2 Feed mill

The nutrition of the selected strains of chicken is very important in the poultry industry; therefore, chicken companies normally own feed mills, where raw materials are converted into finished products, following specific formulas developed by poultry nutritionists. These products usually differ according to the nutrition stage of the chickens (Barbut, 2016; Henry and Rothwelle, 1995).

2.4.3 Breeders

This segment of the poultry industry is usually controlled by contract growers who are in charge of raising the breeding hens and roosters under a highly secured biological environment in breeder farms to produce fertile hatching eggs. Furthermore, the brood of breeder parents will then be raised to become broilers for the industry (Barbut, 2016; Henry and Rothwelle, 1995; Godley and Williams, 2007).

2.4.4 Hatchery

This segment consists of a facility that serves to hatch fertile eggs from breeder farms. In this facility, fertile eggs are kept in incubators and maintained at a specific temperature and humidity to provide a suitable environment for the operation that is concluded when chicks hatch out of eggs, which are normally placed in hatching trays towards the end of the process (Barbut, 2016; Henry and Rothwelle, 1995).

2.4.5 Grow-out farms

From the hatchery, the chicks are displaced to grow-out farms where they are raised to market weight (~2 kg) that can be reached after six to seven weeks. This process is facilitated by the use of the poultry food coming from the feed mill, pharmaceuticals, water, barns, bedding, electricity and a good management skill (Barbut, 2016; Henry and Rothwelle, 1995).

2.4.6 Poultry slaughterhouses/processing plants

Once birds have reached market weight, they are harvested and inspected to control diseases and defects (Barbut, 2016). Those successfully passing this stage are then slaughtered and processed to collect carcasses that are then chilled to prevent bacterial growth. This is followed by cutting into parts, packaging and distribution to markets (Barbut, 2016; Henry and Rothwelle, 1995; Godley and Williams, 2007).

2.4.7 Further processing

Following the processing of birds, part of the products from the processing plants are further processed to specialized products. These processing techniques include cooking, breading or marinating and result in enhanced products with higher market value (Barbut, 2016; Godley and Williams, 2007).

2.4.8 Transportation and marketing

This represents the last segment of the poultry industry and deals with the transportation of poultry products in refrigerated trucks to further processing plants, and then distribution channels (Godley and Williams, 2007). It also deals with the marketing of these products to various markets to maintain or improve the sales of the company.

2.5 Poultry slaughterhouse operations and waste generation

Environmental challenges, high land field fees in urban areas and surcharges on wastewater laden with organic matter contribute to increasing the pressure on the food industry to implement methods aimed at reducing wastes and improving the recovery of by-products (Falkenmark, 1997; Western Cape Government, 2015; Hedden and Cilliers, 2014). Agricultural waste refers to residues that are produced from various agricultural activities like the planting and harvesting of field crops, operation of feedlots, and production of milk and animals for slaughter (Western Cape Government, 2015). In the poultry industry, carcasses or bird wastes that are not directed to consumption are considered as animal waste (Barbut, 2016). Furthermore, the processing of birds generates an important quantity of wastewater, that can be characterized in terms of organic or inorganic content (Basitere *et al*., 2017; Barbut, 2016). Overall, this type of wastewater is high in phosphorus, nitrogen, solids and BOD_5 levels (Barbut, 2016; Basitere *et al.*, 2017). Numerous methods can be used to quantify and express the organic matter content of the PSW. These include chemical oxygen demand (COD); biological oxygen demand (BOD_5); total suspended solids (TSS); total dissolved solids (TDS); or fats, oils, and grease (FOG) (Bustillo-Lecompte *et al*., 2016). However, the characterization of the wastewater generated in poultry slaughterhouses varies seasonably, daily, or even hourly due to factors such as the operation conducted or the quantity of potable water used

during the processing operations (Barbut, 2016; Henry and Rothwelle, 1995). These operations usually generate different types of wastes, as illustrated in Table 2.1. Ultimately, these various wastes, that differ in structure and composition, require different types of treatment, as discussed in subsequent sections.

As illustrated in Figure 2.11, the operations, around the processing of broilers, are concluded by the production of edible and inedible products (Henry and Rothwelle, 1995; Barbut, 2016; Godley and Williams, 2007). Generally, edible products can reach a percentage of 70% for poultry, differing from turkeys and ducks approaching percentages of 77 and 58 %, respectively (Barbut, 2016). This translates to a percentage of inedible products generated by the poultry industry varying between 23 and 42%, depending on the maturity of birds processed (Godley and Williams, 2007; Barbut, 2016). These inedible products also referred to as by-products, fall out of the human edible market and, therefore, are further processed for other markets or simply disposed of.

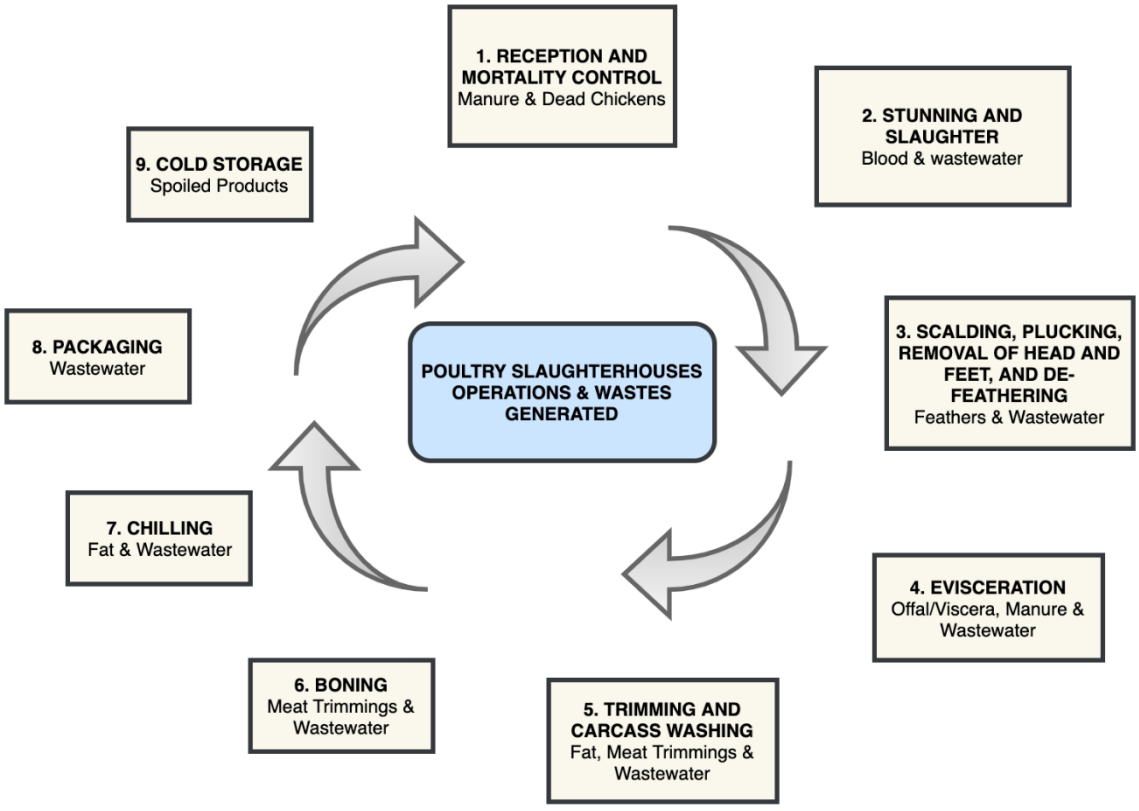

Figure 2.11: Poultry slaughterhouse operations and wastes generated (Adapted from Barbut,2016)

The term offal often refers to inedible poultry products, which include feathers, trimmings, bones, lungs, heads, intestinal tracts and their contents (Greencape, 2016). However, considering that liver, heart, gizzard and neck are poultry products consumable in some regions of the world, the term offal can be broken down into two sub-categories (see Figure 2.12), namely:

- Edible offal, and
- Inedible offal.

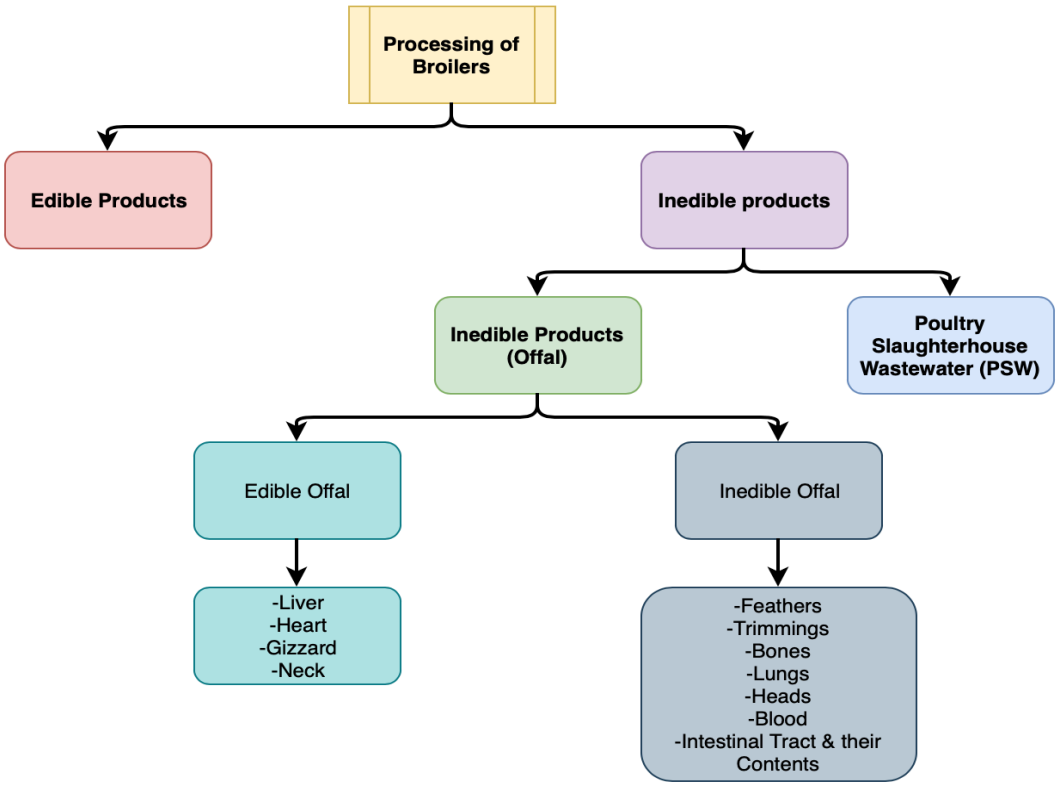

Figure 2.12: Products and by-products from the Processing of Broilers in Poultry Slaughterhouses

Generally, these by-products regrouped in the term offal served as raw materials for the rendering process, whereby they are transformed into feather meal, poultry meal, blood meal and fat in the form of oil and grease (Bonhotal *et al.*, 2008). The subsequent section deals with the scope of the poultry solid waste utilization.

2.6 Scope of poultry solid waste utilization

Various types of wastes are produced from the poultry industry. As illustrated in Figure 2.12, this includes poultry slaughterhouse wastewater, manure, dead chicken, blood, feathers,

offal/viscera, fat, meat trimmings, and even bones (Barbut, 2016). This sub-section deals with the processing pathways of these wastes excluding PSW, as blood can be considered as solid waste. Referring to poultry blood, it constitutes 10% of the bodyweight of broilers (Barbut, 2016). This blood is usually collected during the slaughtering process, then dried, ground and utilized in animal feed formulations (Kiepper, 2003; Barbut, 2016; Thyagarajan *et al*., 2013).

The solid wastes generated from the poultry industry presents interesting characteristics (Thyagarajan *et al*., 2013). Feathers are rich in keratin proteins and amino acids and thus can be turned into invaluable products such as bio-diesel, feather meal, bio-degradable plastic and fertilizer (Thyagarajan *et al*., 2013; Barbut, 2016). The nutrients content of poultry offal can also be utilized for methane production through anaerobic digestion or as dried fertilizer and manure (Plumber and Kiepper, 2011; Henry and Rothwelle, 1995). Thus, concerning treatment pathways, poultry solid waste can be split into three categories, namely:

- Poultry feather,
- Poultry inedible offal, and
- Poultry manure/litter.

It is important to dispose of or treat such wastes, to minimize the effect of eventual outbreaks of influenza or other diseases. This prevents disease spread and ensures biosecurity of other poultry houses and neighboring farms.

Among the various products that can be generated from the treatment of poultry solid waste, the light color and high palatability of poultry meal has motivated its use as a key ingredient in the pet food industry (Greencape, 2016). Poultry meal is produced from the rendering of raw offal (Barbut, 2016; Jayathilakan *et al*., 2012). The subsequent sub-sections describe the processes used for the transformation of these solid wastes into marketable products.

2.6.1 Poultry feather

Feathers from broilers processing operations can be used for various purposes, such as insulation, clothing, bedding, sporting equipment, decoration, fertilizer as well as feather meal from the rendering process (Freeman *et al*., 2009; Barbut, 2016; Godley and Williams, 2007). The production of feather meal starts from breaking down the complex protein keratin through hydrolysis to induce the digestibility of protein (Leeson and Summers, 2009). Therefore, these feathers are rendered separately from other offal constituents, and the

digestibility of the feather relies on two parameters, which are the time and cooking pressure (Barbut, 2016; Henry and Rothwelle, 1995). Thus, the improvement of the availability of amino acids as well as the biological value of feather meal can be reached through the intensification of the cooking process (Leeson and Summers, 2009; Barbut, 2016).

Generally, poultry feathers have a protein (keratin), lipids and water content of 91, 1 and 8%, respectively (Barbut, 2015; Barbut, 2016). This composition is also illustrated by an amino acid sequence similar to the ones of other birds' feathers (Barbut, 2015). Referring to amino acids, the most common ones in chicken feathers are Serine with an average content of 16%, other amino acids include Arginine, Aspartic acid, Glutamine, Threonine, Tyrosine, Leucine, Isoleucine, Valine, Cysteine, Alanine, Phenylalanine, Methionine, Proline and Aspargine (Barbut, 2015; Jayathilakan *et al*., 2012). These amino acids can be differentiated for their functional groups as illustrated in Figure 2.13 (Bertsch and Coello, 2005; Barbut, 2015). However, some amino acids such as histidine, glutamic acid, glycine, lysine, and tryptophan do not enter the composition of these feathers (Bertsch and Coello, 2005; Barbut, 2015).

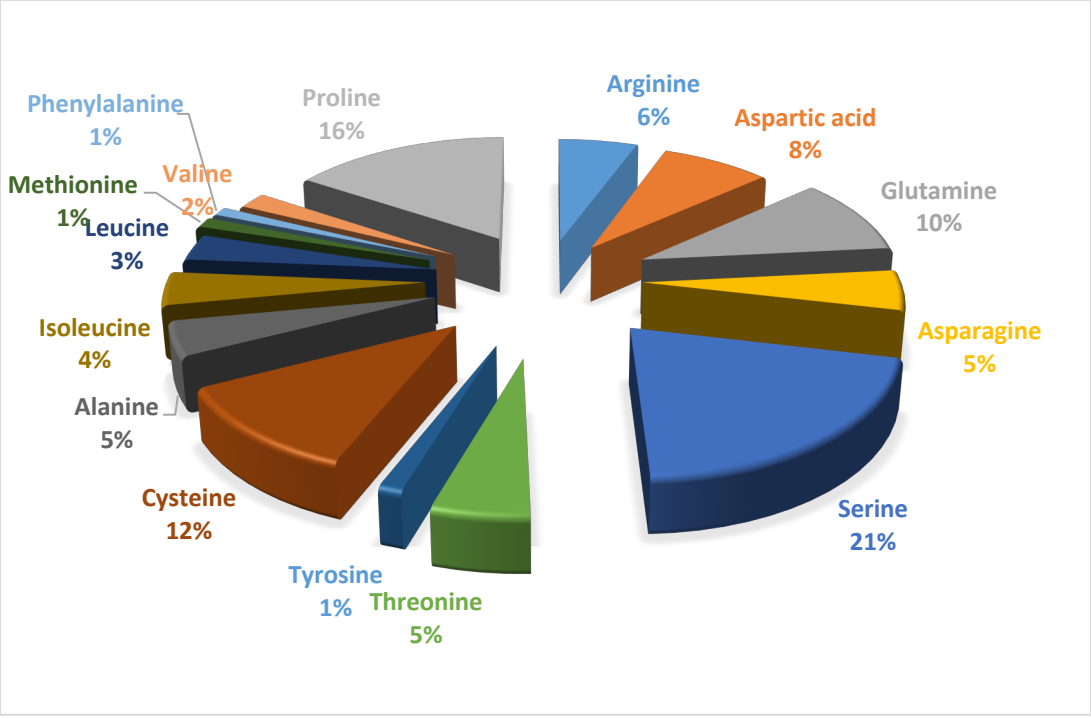

Figure 2.13: Amino-acids composition of chicken feathers

The structure of poultry feathers is particularly resistant to decomposition by the presence of keratin, which are insoluble proteins that belong to the scleroprotein groups (Jayathilakan *et*

al., 2012; Bertsch and Coello, 2005). These groups are known for being highly resistant to biological, chemical and physical actions (Jayathilakan *et al*., 2012; Bertsch and Coello, 2005). Thus, the mechanical stability, as well as high resistance to proteolytic degradation of keratin, is justified by the presence of hydrogen bonds, disulfide bonds, salt linkages as well as cross-linkages (Barbut, 2016; Bertsch and Coello, 2005).

2.6.1.1 Products from feathers processing

More than 5 million tons of chicken are generated globally every year (Barbut, 2016), representing a danger to the environment or simply an easily available raw material for various industries. The processing of chicken feathers is very important, as they represent a hazard to the natural environment due to their poor digestibility and their potential to be used as a source of microbial pathogens (Barbut, 2016; Bertsch and Coello, 2005). Thus, this hazardous characteristic of feathers can be addressed through the use of one of the following processing techniques, which lead to products listed in Figure 2.14.

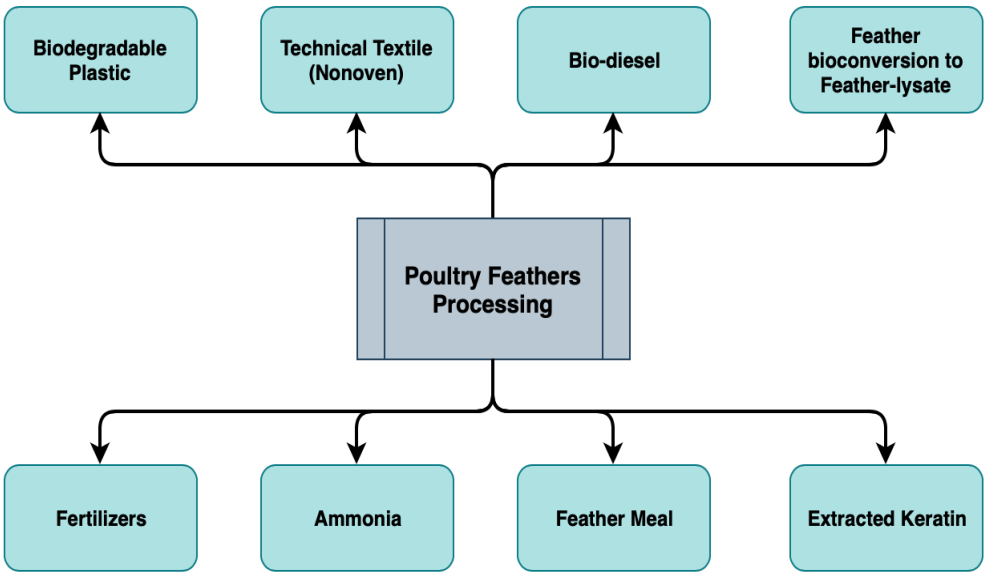

Figure 2.14: Products from Poultry Feathers Processing

2.6.1.1.1 Fertilizer production from chicken feathers

The transformation of chicken feathers into nitrogen fertilizer is made possible by the modification of its keratin fibers by steam hydrolysis for a period of 12 weeks to break disulfide

bonds, the enzymatic hydrolysis through the Bacillus licheniformis that break polypeptide bonds, and autoclaving or steam hydrolysis to improve mineralization, which is followed by cross-linking of protein by formaldehyde reaction in order to minimize excess mineralization (Bertsch and Coello, 2005).

2.6.1.1.2 Ammonia production from chicken feathers

It is reported that 2% of the world energy is utilized for the production of ammonia through the Haber process (Thyagarajan *et al*., 2013). This energy requirement can be reduced by using chicken feathers to produce ammonia. The process leading to the production of ammonia using chicken feathers starts from heating them at 600^{o}C for 3 hours in the presence of carbon dioxide (Thyagarajan *et al*., 2013). From this operation, two products can be formed. The first being ammonium bicarbonate, which can be further transformed into ammonia by supplementary heating at 60^{o}C; and the second being carbon micro-spheres, which can be utilized for a water-resistant coating or, using a catalyst, can be further transformed into carbon nanotubes, which have a broad range of uses including solar cells and biosensors, to name a few (Thyagarajan *et al*., 2013; Bertsch and Coello, 2005).

2.6.1.1.3 Feather meal production from chicken feathers

Feather meal is often used as animal feed, feed supplements, and organic fertilizers due to his high protein content (Barbut, 2016; Thyagarajan *et al*., 2013). The feather meal production is usually conducted through a hydrothermal process, whereby feathers are transformed under high pressure and temperature despite the fact that these conditions induce the destruction of important amino acids such as lysine, tyrosine, methionine and tryptophan, which contribute to its digestibility and low nutritional value (Thyagarajan *et al*., 2013).

2.6.1.1.4 Feather keratin extraction from feathers through chemical hydrolysis

Chicken feathers can also be treated at high temperatures coupled with treatment with calcium hydroxide (lime) to produce a substance rich in amino acid and polypeptides, which can be used as an animal feed supplement (Barbut, 2016; Thyagarajan *et al.*, 2013). The extraction of keratin using the aforementioned conditions depends on the temperature; the higher the temperature the less time required for effective extraction of keratin. Thus, it requires only 25

minutes to solubilize 80% of feather keratin at a temperature of 150°C, whereas it will require 5 hours for solubilizing the same quantity of feather keratin at 100°C (Thyagarajan *et al*., 2013). Optimally, 95% of feather keratin can be digested after 3 hours of hydrolysis at 150°C (Thyagarajan *et al*., 2013). One advantage of soluble keratin is the low production of ammonia from his digestion in rumen fluid when compared to urea, thus proving that the ammonia toxicity can be significantly counteracted by feeding cattle with such protein source (Thyagarajan *et al*., 2013; Barbut, 2015). These digestibility properties are further illustrated by cottonseed and soybean meals.

2.6.1.1.5 Feather bioconversion

The energy requirement for the hydrothermal treatment of feathers is high and therefore culminate in high operating costs (Bertsch and Coello, 2005; Thyagarajan *et al*., 2013). Therefore, a biodegradation approach was investigated, as some microorganisms alternatively contribute to increase the biological value of feathers (Bertsch and Coello, 2005). Such microorganisms include *Bacillus licheniformis*, which produces a feather-lysate that possesses nutritional features similar to soybean protein. However, some challenges such as the improvement of enzyme activities and yields should be addressed to implement these processes in the industry (Bertsch and Coello, 2005). To improve this biodegradation, feather-degrading bacteria (FDB) are isolated from poultry waste (Bertsch and Coello, 2005; Thyagarajan *et al*., 2013). These include three strains of *Bacillus subtilis, Bacillus cereus*, and *Bacillus pumilis,* which possess the potential to bio-degrade feathers for a production 142, 109 and 96 units of keratinolytic activities, respectively (Bertsch and Coello, 2005). The effectiveness of *Bacillus subtilis* can be justified by the fact that his culture can be operated in a broader pH range than *Bacillus pumilis* and *Bacillus cereus,* as the optimal conditions required for B (Bertsch and Coello, 2005; Thyagarajan *et al*., 2003). Subtilis prevail at a pH ranging between 5 and 9 and a temperature of 40°C, whereas *Bacillus pumilis* prevail at a ph ranging between 5 and 6 and a temperature of 40°C and *B. Cereus* at a pH of 7 at a temperature of 30°C (Bertsch and Coello, 2005).

2.6.1.1.6 Bio-diesel production from chicken feathers

The principal nutrient of interest in poultry waste is nitrogen, whose presence motivates the production of fertilizers from these wastes (Plumber and Kiepper, 2011). However, it is

reported that these wastes contain a significant portion of fat that can reach 12% (Kiepper, 2003). Therefore, this fat was extracted hydrothermally and transformed into biodiesel through transesterification, which is a chemical process that enables the conversion of fats into biodiesel through the use of selected catalysis (Bertsch and Coello, 2005). A comparison of the quality of the biodiesel produced from poultry fat to other common feedstocks, using ASTM analysis, confirmed the quality of this biodiesel (Thyagarajan *et al.*, 2013).

2.6.1.1.7 Biodegradable plastic from chicken feathers

The conversion of poultry feathers to biodegradable plastics is possible through the process of polymerization (Bertsch and Coello, 2005; Thyagarajan *et al*., 2013). The starting point of this process is the pulverization of feathers into fine dust (Thyagarajan *et al*., 2013). Then, chemicals that allow the formation of long polymer chains from the keratin molecules of feathers are used (Bertsch and Coello, 2005). The product from this polymerization can be molded into different shapes at a temperature of 170^{o}C to form final products such as furniture, plates and cups, which usually require raw materials from the petrochemical industry whose activities significantly affect the environment while the petroleum is depleting (Bertsch and Coello, 2005).

2.6.1.1.8 Technical textiles

One interesting textile material that can be produced from the processing of chicken feathers is nonwoven (Thyagarajan *et al*., 2013). This material is made from short or long fibers bonded together through mechanical, chemical, thermal and solvent treatment, and subsequently used in a wide range of industrial and consumer products with various properties including healthcare and surgical fabrics, apparel, absorbent hygiene products, home furnishing, filtration, construction, engineering, and wipes amongst others (Plumber and Kiepper, 2011; Thyagarajan *et al*., 2013).

2.6.2 Treatment pathways of poultry offal

During the broiler husbandry, they also accumulate various substances such as heavy metals, chemicals and veterinary drugs added in their feed for pharmaceutical or nutritional purposes (Barbut, 2016). Moreover, more than 100 different micro-organisms species have been

identified in contaminated feathers, intestinal contents, feet and processing equipment (Barbut, 2015; Thyagarajan *et al*., 2013). To this can be added harmful pathogens such as *Salmonella sp*., *Clostridium sp*., and *Staphylococcus sp* (Moore *et al*., 1995).

Different methods can be used for processing poultry offal, these include burial and control landfilling, composting, incineration, rendering as well as anaerobic digestion (See Figure 2.15) (Freeman *et al*., 2009).

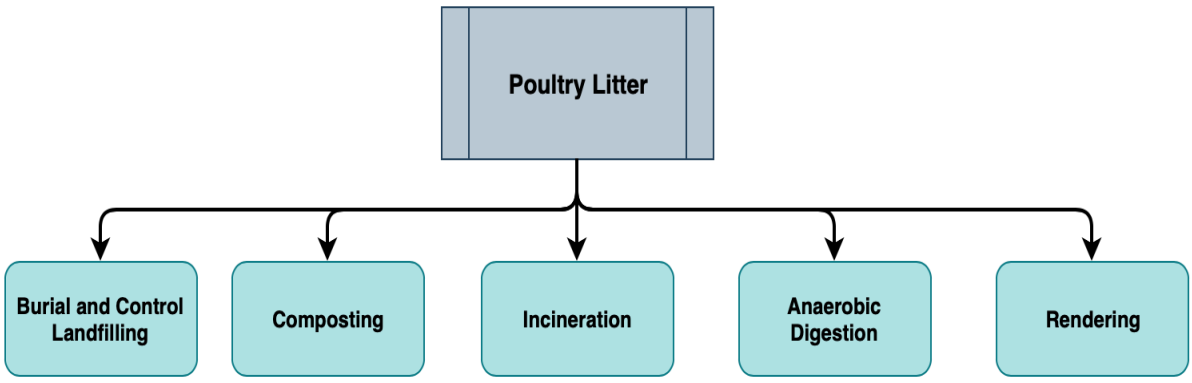

Figure 2.15: Processing methods of Poultry Litter

2.6.2.1 Burial and control landfilling

One way to get rid of poultry waste is through landfilling. However, it is important to strictly control the burial of dead birds to prevent adverse effects on the local environment through the pollution of groundwater, thus surface water, and also soil and air, through the production of gases such as carbon dioxide and methane from the digestion of the organic matter contained in the poultry waste (Barbut, 2015).

2.6.2.2 Composting

Another technique of treating poultry offal is composting, which is an aerobic process commonly used to process organic matter for the production of the compost that can be used as a soil conditioner or fertilizer (Bonhotal *et al*., 2008). This method is often used to treat poultry slaughterhouse organic wastes such as manure, litter, grease trap residues and feather to some extent. Although efficient, this method requires wastes possessing high fiber content,

as those with low fiber content and high moisture require higher quantities of moisture-sorbing and structural support to compost well, which increases the operating cost of this process (Bonhotal *et al*., 2008).

2.6.2.3 Incineration

Incineration is very effective for the extermination of potentially infectious agents. It is a processing technique that relies on the combustion of the wastes to be getting rid of but can also produce thermal energy during the process (Thyagarajan *et al.*,2013). It is reported that and air-dried poultry litter can be used as a combustible solid fuel that produces a gross calorific value of 13.5 GJ per ton, which is about half the calorific value of coal. However, this process must be strictly monitored for the control of air emission, solid and liquid residue as well as process conditions (Bonhotal *et al*., 2008).

2.6.2.4 Anaerobic digestion

The anaerobic digestion consists of the bioconversion of organic matter in an environment devoid of oxygen to produce biogas, which is a mixture of gases including, amongst others, methane that possesses a high calorific value and carbon dioxide, allowing this process to be an alternative source of energy that can be used to address the current challenges of petroleum depletion and the environment protection through clean energy production (Fountoulakis *et al*., 2008; Kiepper, 2003; Plumber and Kiepper, 2011). Thus, the organic matter content of poultry offal can be used as a feed to this process. Although the poultry offal requires more time than other poultry waste due to long-chain fatty acid inhibition, blood and bone meal produce methane rapidly. This potential for methane production is explained by their content in proteins and lipids, as well as the consortium of microorganisms contained in these substances (Barbut, 2015).

2.6.2.5 Rendering

Rendering is a process that consists of converting waste animal tissue into valuable products (Moore *et al*., 1995; Jayathilakan *et al*., 2012; Thyagarajan *et al.*, 2013). It can also be defined as

an evaporation process producing a condensate stream with a foul odor. The challenge of the odor can be minimized through (Jayathilakan *et al*., 2012; Thyagarajan *et al*., 2013):

- Pasteurizing the raw material before process it to stop biological processes that generate odor,
- Minimizing the stock of raw material that should be stored in a cold, closed and well-aerated place,
- Maintaining all working and storage area clean,
- Installing all equipment in closed spaces and operate under total or partial vacuum.

In the poultry industry, dead chicken and offal are turned into valuable products such as feather meal, poultry meal, blood meal as well as fat (Barbut, 2015). The rendering of poultry offal can also produce meat-bone-meal, which can be utilized as a fertilizer, in animal feed or further processed to produce biogas through anaerobic digestion or compost through composting (Bonhotal *et al.*, 2008).

During the rendering process, the cooking of raw offal results to the production of poultry oil that is extracted from these solids by making use of a screw press (Bonhotal *et al*., 2008; Thyagarajan *et al*., 2013). This oil has a high energetic value and significantly improves the palatability of pet food. Moreover, the rendering process also generates poultry grease, which, despite being useful as a by-product, often appears darker and poorer in grade than the fat usually recovered from the rendering of grease of other animals, such as pork and beef (Bonhotal *et al*., 2008).

2.6.3 Poultry manure

Poultry litter is usually composed of three types of waste, which includes the bedding material used for poultry housing, manure accumulated during poultry production and dead birds (Bonhotal *et al*., 2008). Poultry manure consists of approximately 150 g/kg of dry matter and regrouped chemical elements such as carbon, phosphorus, nitrogen, chlorine, calcium, chlorine, magnesium, manganese, sodium, iron, copper, zinc and arsenic as well as water. This richness can allow the direct utilization of manure as a fertilizer (Barbut, 2015; Freeman *et al*., 2009); however, this will result in the following effects (Thyagarajan *et al*., 2013):

- Release of odor attracting insects,
- Surface and ground pollution, and

- Over accumulation of manure and trace elements leading to reduced crop yields.

Therefore, the manure can be processed before use to prevent the aforementioned challenges. Poultry manure processing methods include composting, anaerobic digestion and combustion to produce compost, biogas and energy, respectively (Thyagarajan *et al.*, 2013). Another method that can be used for poultry manure processing is vermicomposting, which consists of combining poultry manure with cow dung (1:1, w/w) in the presence of P. ceylanensis for the production of a nutrient-rich compost (Barbut, 2015).

2.7 Summary

Huge quantities of potable water are being used in poultry slaughterhouse facilities to sustain their operations, which lead to the generation of PSW and solid wastes. In this study, the global and local water availability was assessed before browsing through to the natural circle of water bodies. Furthermore, attention was given to the solid wastes from the processing of birds in poultry slaughterhouse facilities as well as the processes that can be used to turn them into useful products. The following chapter will deal with the liquid waste from poultry slaughterhouses (PSW), which is generated from the processing of chicken and the cleaning of such a facility.

CHAPTER 3

ANAEROBIC TREATMENT OF POULTRY SLAUGHTERHOUSE WASTEWATER

Part of this chapter was published as

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Chapter 3: ANAEROBIC TREATMENT OF POULTRY SLAUGHTERHOUSE WASTEWATER

3.1 Introduction

As pointed out in Chapter 2, poultry slaughterhouses generate huge quantities of solid and liquid wastes from the processing of birds. The high production of PSW relates to the high consumption of potable water to meet hygienic standards and to ensure the supply of safe products to a growing market. From the various operations, ranging from receiving to packaging the poultry products, potable water collects organic and inorganic materials to form a reddish effluent, which can be classified as medium to high strength wastewater, depending on the prevailing operation in the poultry slaughterhouse. The release of this type of wastewater to water surfaces results in serious environment and health concerns, which can be prevented by a suitable treatment prior to the discharge. Various treatment options can be selected, but the preference goes to cost-effective, low energy intensity and environmentally friendly options, such as the anaerobic digestion.

3.1.1 Chapter's objective

This chapter aims at describing PSW and explaining its high rate anaerobic treatment, including the features and challenges of this treatment option.

3.2 Poultry slaughterhouse wastewater (PSW)

3.2.1 Parameters used for wastewater characterization

Wastewater in general and PSW, in particular, may be characterized by physical, biological or chemical constituents (Metcalf and Eddy, 2003). Table 3.1 lists important parameters

relevant to the assessment of the quality of various types of wastewater. The focus of this work is given to the secondary treatment of wastewater, with emphasis on anaerobic digestion. Generally, the secondary treatment of PSW entails the minimization of the concentration of contaminants contained in the PSW (Metcalf and Eddy, 2003; Barbut, 2015; Avula *et al*., 2009). Parameters used to monitor the removal of such contaminants include chemical oxygen demand (COD), biological oxygen demand (BOD_5), fats oil and greases (FOG), total suspended solids (TSS), total dissolved solids (TDS), or volatile suspended solids (VSS), amongst others (Barbut, 2015; Basitere *et al*., 2017; Avula *et al*., 2009). These parameters also reflect the quality of PSW and can be used to predict their effects when discharge untreated into surface water and water channels. The effects of the discharge of untreated PSW are highlighted in Figure 3.1.

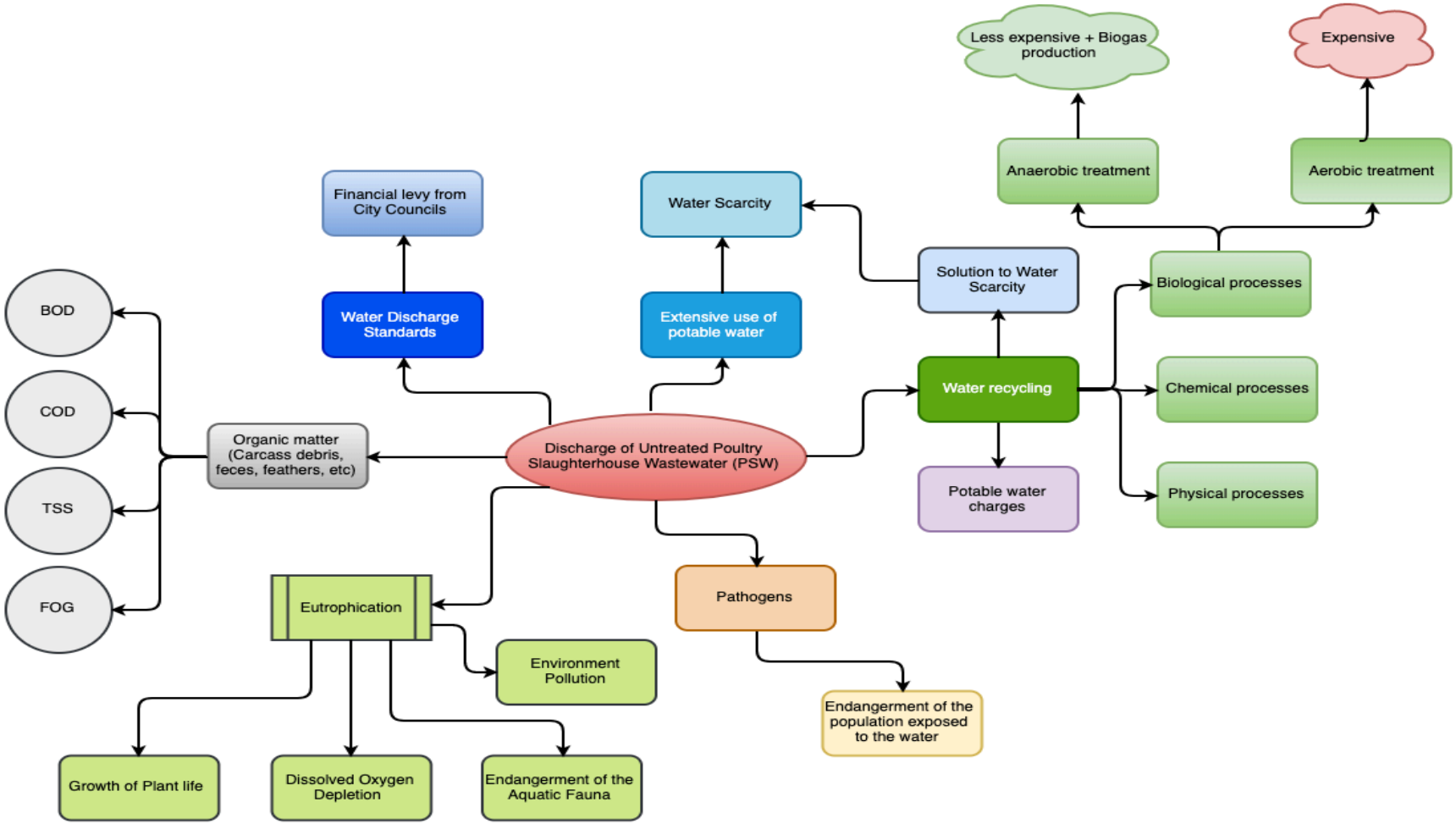

Figure 3.1: Effects of the discharge of untreated PSW to water channels and surface water

Table 3.1: Constituents of typical wastewaters (Adapted from Metcalf and Eddy, 2003)

Constituent	Relevance
Biodegradable matter	These essentially consist of proteins, carbohydrates, and fats. They are usually quantified in terms of BOD_5, COD or FOG. The release of wastewater, laden with a high concentration of such matters, into the environment will lead to the depletion of natural oxygen resources and development of septic conditions.
Nutrients	Along with carbon, both nitrogen and phosphorus are essential nutrients for the growth of plants and animals. These wastewater constituents can lead to groundwater pollution when discharged in excessive amounts, and induce the growth of undesirable aquatic life.
Suspended solids	These can lead to the development of anaerobic digestion and sludge deposits when not removed before being discharged in the aquatic environment.
Pathogens	Carriers of communicable diseases that can be transmitted to people exposed to the wastewater containing them
Priority pollutants	Inorganic and organic substances that can be found in wastewater, which are selected based on their acknowledged or suspected carcinogenicity, teratogenicity, mutagenicity, or high acute toxicity.
Heavy metals	These are generally added to wastewater from industrial or commercial activities and required removal from the wastewater before reuse or discharge to respect the industrial effluent discharge standards.
Refractory organics	These organic matters are usually not removable through conventional methods of wastewater treatment and thus require a special treatment method.
Dissolved inorganics	These include elements such as sodium, calcium, and sulfate. They need to be removed from the wastewater, as constituents such as sulfate can alter the production of methane through the competition imposed by sulfate-reducing bacteria.

3.3 Importance of the characterization of wastewater

The understanding of the nature and quality of wastewater is required for the design of operations related to its collection, treatment and recycling (Borja *et al*., 1998; Chernicharo, 2007; Henze *et al*., 2008). The quality of the wastewater discharged from various industries changes over time due to miscellaneous reasons such as the change of industrial processing methods or the use of newly developed chemical products utilized for enhanced results (Metcalf and Eddy, 2003; Rajakumar *et al*., 2012; Barbut, 2016). This leads to the change of industrial wastewater characteristics and subsequently the imposition of stricter limits on wastewater discharge standards (Basitere *et al*., 2017; Bustillo-Lecompte *et al*., 2016). To respect these limits, industries need to characterize their effluent and develop suitable technologies to treat their liquid waste. Furthermore, as process modeling is largely used in the design and optimization of biological treatment processes, wastewater characterization is highly required for such operations (Bustillo-Lecompte et al., 2016; Henze et al., 2008; Debik and Coskun, 2009). Process modeling usually requires experimental assessment of kinetic and stoichiometric constants. Thus, the fractionation of parameters such as COD, TSS or total organic carbon into particulate and soluble elements can be utilized to optimize the performance of both old and novel biological treatment systems conceptualized to improve the removal of nutrients in such industrial effluents (Chernicharo, 2007).

3.4 Generation, collection and characterization of poultry slaughterhouse wastewater (PSW)

Avula *et al.* (2009) reported that poultry slaughterhouses consume an average of 26 liters/bird during primary and secondary processing, as stricter microbiological standards have led to increased water requirements in this industry. During these operations, cleaning accounts for 30 to 50% of the daily potable water consumption (Barbut, 2015; Kiepper *et al*., 2008). Thus, it is important to implement efficient water management to reach the required cleanliness and hygienic standards without waste, as cost of both freshwater and wastewater disposal is steadily increasing globally (Dinar *et al*., 2012). The minimization of water consumption in such facilities can be achieved through the implementation of the following measures (Barbut, 2015):

- Usage of taps with automatic shutoff, using high water pressure, and improving the process layout,

- Usage of flat spray nozzles instead of showers,
- Usage of air instead of water chilling,
- Elimination of the wet transport of wastes, such as feathers and intestines,
- Prevention of any solid wastes or concentrated liquids from entering the wastewater stream to reduce the liquid waste load,
- Usage of the Aero-scalder that uses steam rather than water,
- Implementation of dry cleaning of the equipment and production areas before wet cleaning,
- Equipment of outlets of wastewater channels with screens and fat traps to recover and reduce the concentration of coarse material and fat in the combined wastewater stream,
- Cover collection channels in the production facility with grids to reduce the amount of solids entering the wastewater,
- Separation of cooling water from wastewater and process water, to recirculate cooling water,
- Optimization of the use of detergents and disinfectants in washing water, and
- Removal of manure from the intestine processing and stockyard in solid form.

However, another important approach to the minimization of the water consumption would be the treatment of the PSW produced that can be recycled and used in certain operations such as the cleaning of the live haul area as well as certain equipment and vehicles.

Efficient treatment of PSW starts with the determination of its characteristics in terms of organic and inorganic matter content (Henze *et al*., 2008; Chernicharo, 2007). Several methods have been developed for providing standard characteristics of various types of wastewater (Metcalf and Eddy, 2003).

To determine the average composition of PSW, Kiepper *et al.* (2008) investigated the PSW collected from three different broiler slaughterhouses located in the Southeast United States. In these slaughterhouses, offal and wastewater were generated in similar areas. Thus, the produced PSW contained un-collected blood, feathers, viscera, and the water collected from the cleaning of the live haul area. Table 3.2 summarizes the processing and PSW treatment operations at the three facilities. All these poultry slaughterhouses processed young broilers that averaged a live weight of 2.0 kg.

Table 3.2: Processing and PSW treatment operations in poultry slaughterhouses (Adapted from Kiepper et al., 2008)

Item	Plant A	Plant B	Plant C
Broiler slaughtered per day	340 000	245 000	140 000
Other processing operations			
Cut-up	X	X	X
Deboning	X	X	
Marinating		X	
Wastewater treatment physical systems			
Screen types	**IR**	**IR**	**IR-DD**
Number of screens	3	3	2
Feather screen gap size (μm)	1 588	3 175	1500
Viscera screen gap size (μm)	3 175	4 763	1 500
Secondary screen gap size (μm)	508	508	508

IR: Internally fed rotary; DD: double drum screen

The analysis of PSW samples of particulate matter for moisture, fat, ash and fiber resulted in the tabulation of Table 3.2 that provides the composition of the particulate matter of the PSW from the three different slaughterhouses.

Table 3.3: Composition of particulate matter in PSW (Adapted from Kiepper et al., 2008)

	Plant (Mean percentage dry matter ± SEM)			
Fraction	**Plant A**	**Plant B**	**Plant C**	**Mean**
Fat	59.4 ± 4.3	49.7 ± 2.6	56.7 ± 3.9	55.3
Protein	22.8 ± 2.2	33.1 ± 2.0	25.4 ± 1.9	27.1
Ash	4.5 ± 0.8	8.2 ± 0.5	5.7 ± 1.0	6.1
Fiber	4.9 ± 0.9	3.0 ± 0.4	4.5 ± 0.6	4.1

Table 3.4: Mean concentration of 15 mineral in PSW (Adapted from Kiepper et al., 2008)

Item (mg/L)	Plant A	Plant B	Plant C
Sodium	126.1 ± 2.5	144.2 ± 7.4	89.3 ± 2.7
Potassium	53.3 ± 0.7	41.3 ± 0.2	88.3 ± 3.1
Phosphorus	33.9 ± 0.8	34.8 ± 1.5	31.9 ± 1.1
Calcium	17.1 ± 0.6	14.6 ± 0.4	47.1 ± 2.0
Silicon	7.3 ± 0.3	18.1 ± 0.7	16.4 ± 3.1
Magnesium	5.3 ± 0.1	4.5 ± 0.1	17.0 ± 3.1
Iron	1.2 ± 0.1	2.1 ± 0.2	2.1 ± 0.1
Aluminum	0.3 ± 0.02	0.85 ± 0.07	0.75 ± 0.08
Zinc	0.23 ± 0.01	0.24 ± 0.04	0.37 ± 0.03
Copper	0.26 ± 0.01	0.03 ± 0.00	0.19 ± 0.03
Manganese	0.086 ± 0.003	0.072 ± 0.004	0.147 ± 0.012
Boron	0.031 ± 0.002	0.028 ± 0.002	0.048 ± 0.003
Molybdenum	0.019 ± 0.005	0.021 ± 0.006	0.015 ± 0.003
Nickel	0.016 ± 0.003	0.015 ± 0.003	0.018 ± 0.001
Chromium	0.011 ± 0.001	0.009 ± 0.001	0.019 ± 0.002

A good understanding of the composition of PSW particulate matter is important for developing efficient physical separation systems in the poultry processing industry (Barbut, 2015; Henze *et al*., 2008). Particulate matter refers to any suspended or dissolved matter in a sample of wastewater. As illustrated in Table 3.3, fat content dominates the composition of PSW particulate matter and thus requires effective removal for efficient processing of this type of wastewater (Kiepper *et al*., 2008). Furthermore, the concentration means for 15 minerals in PSW from these 3 facilities were investigated and tabulated in Table 3.4

Various parameters can be used to monitor the treatment of PSW. In anaerobic treatment, it is required to control parameters such as the BOD_5, COD, TSS, or FOG. Barbut (2015) investigated the characteristics of poultry slaughterhouse wastewater in terms of COD, BOD_5, TSS, and VSS and tabulated the results as illustrated in Table 3.5.

Table 3.5: Characteristics of PSW (Adapted from Barbut, 2015)

Source	COD (mg/L)	BOD_5 (mg/L)	TSS (mg/L)	VSS (mg/L)
First study	2000-6200	1300-2300	850-6300	660-5250
Second study	5800	2200-9800	2400-9400	nd
Third study	4000	1730	2580	1960
Fourth study	3980-7120	2030-4200	285-2660	nd

nd: not determined

As illustrated in Table 3.3, 3.4, and 3.5, the PSW can be characterized in different ways with respect to its organic or inorganic content. For this study, the PSW was collected from a poultry slaughterhouse located in the Western Cape, South Africa. This facility processes on average a million birds a week. Considering that one bird requires 26.5 L of potable water for its processing, it is estimated that 26 500 m^3 of water is used every week only for the processing of broilers, excluding the wastewater generated from the cleaning of equipment and the facility or auxiliary tasks. This represents a significant quantity of water that relates to huge expenses associated with the billing of potable water used and municipal financial penalties on the industrial wastewater effluent.

One way to avoid such expenses is to reduce the potable water intake through recycling the red water produced from the slaughterhouse activities after appropriate treatment. Therefore, the ultimate role of the PSW treatment should be to reach the potable water standards to meet the hygienic standards imposed on such facilities. The other advantage of the treatment of PSW before its discharge into the municipal sewage system or reuse is the industrial effluent rebates defined in the paragraph 11.16 of the City of Cape Town Tariff policies 2016/2017, which grants rebates for industries improving the quality of their wastewater. Moreover, in the beginning of 2018, the City of Cape Town implemented the level 6 water restrictions that requires all agricultural users to reduce their potable water consumption to 65% from the corresponding period in 2015; which may affect the production rate of such facilities if alternative solutions were not found (City of Cape Town, 2018).

3.5 Requirement for treating PSW

Various reasons can motivate the requirement to treat PSW, the first being the protection of the environment, as the discharge of such wastewater to surface water can lead to serious

environmental damages such as cultural eutrophication, as well as the alteration of the aquatic fauna and flora (Dinar *et al*., 2012; Hedden and Cilliers, 2014; Kiepper, 2003). Further reasons, as mentioned above, include the reduction of the charges associated with the billing of potable water and the legislative requirement to use potable water sparingly and treat such wastewater before discharge to comply with the administrative regulations (DEA & DP, 2015; Viljoen, 2015). These regulations differ among countries due to different environmental or water resource conditions (Bustillo-Lecompte *et al*., 2016). In the case of South Africa, the Department of Environment Affairs (DEA) enforces these regulations through city/municipal councils (DEA & DP, 2015). The one of concern in this study is the Council of the City of Cape Town that enforces the policies developed by the DEA (City of Cape Town, 2018). Therefore, the tasks of the Council include the evaluation of the quality of the wastewater generated, issuance of discharge permits and the imposition of penalties to industries not complying with the standards imposed (DEA & DP, 2015). The industrial effluent standards are presented in Figure 3.2 and provide the guidelines to industries in terms of quantifiable parameters.

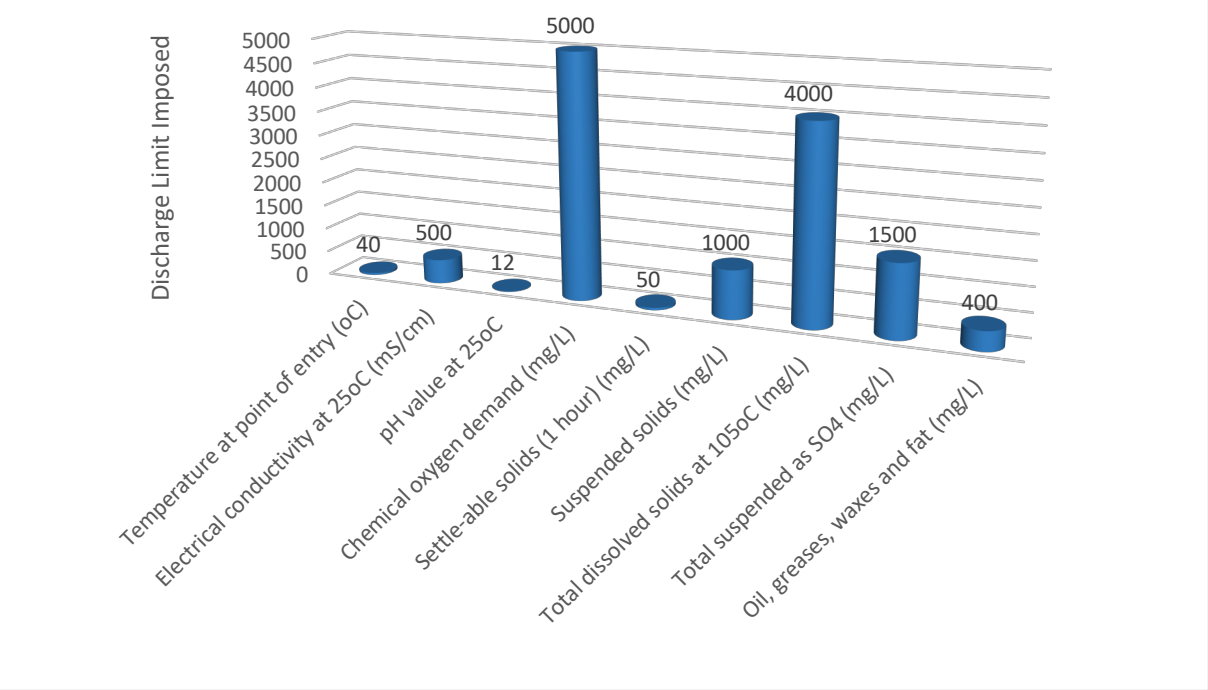

Figure 3.2: City of Cape Town Industrial Effluent Discharge Standards (Adapted from DEA & DP, 2015)

A comparison of Table 3.5 to Figure 3.2 reveals the requirement to treat PSW, as values of COD and TSS tend to exceed the discharge limit (Bustillo-Lecompte *et al*., 2016; Kiepper, 2003).

Generally, these values relate more or less to other organic measuring parameters, as they provide information about the organic load of such wastewater. It should be noted in this regard that the quality of the PSW may vary from one slaughterhouse to another. Hygienic standards impose the usage of huge quantities of water, but the current water shortage challenge in Cape Town encourages and enforces the reduction of the consumption of potable water, which could translate to higher concentration of organic matter in such effluents and therefore an increase of the gap between the industrial effluent discharge standards and the actual concentration of the enlisted contaminants in the industrial effluents, suggesting the need to close this gap.

Once again, the water shortage can be listed as an important factor motivating the need to find efficient ways to reduce water usage in facilities such as slaughterhouses. This issue is not only local, as it does affect other parts of the world to the extent that it has been reported by Northcutt and Jones (2004) that the availability of water will be reduced to solely domestic usage by 2025, suggesting the necessity of adopting innovative solutions to tackle this challenge. In the case of the wastewater generated by the poultry industry, efficient and innovative treatment options should be adopted, as discussed in the subsequent sections.

3.6 PSW treatment options

The first step towards selecting the appropriate treatment option for any type of wastewater is to characterize it. PSW is reddish water laden with organic matter, with a high concentration of fat and subsequently protein, ash and fiber. Thus, different treatment options can be applied for the treatment of such wastewater, with a preference for the most efficient and cost-effective method (Avula *et al*., 2009; Barbut, 2015). These treatment options can be regrouped into three main categories (physical, chemical and biological) and intervene at different stages of the wastewater treatment. According to Barbut (2015), the most common steps of PSW treatment include:

- Preliminary operations (screening of meat pieces and feathers),
- Primary sedimentation,
- Secondary treatment,
- Secondary sedimentation,
- Tertiary treatment,
- Disinfection, and

- Sludge dewatering.

One important step in this series of steps is the secondary treatment, which preferably consists of the biological conversion of the organic matter contained in the PSW, as inorganic materials are present in low concentrations. This treatment option is developed in the subsequent section.

3.6.1 Biological treatment of PSW

Naturally occurring microorganisms serve as engines to wastewater treatment (Gerardi, 2003; Henze *et al*., 2008). They consist of fungi, protozoa, bacteria, and rotifers, amongst others, and grow on specific compounds contained in the wastewater in which they are identified. The secondary treatment of wastewater usually involves highly engineered bioreactors systems developed to provide optimum conditions to these microorganisms for the biodegradation of organic matter contained in the wastewater and thus its renovation (Chernicharo, 2007; Henze *et al*., 2008). However, these engineered biological systems may differ by the availability of dissolved oxygen (Gerardi, 2003; Henze *et al*., 2008), leading to the separation of biological systems into two sub-groups:

- The aerobic treatment, and
- The anaerobic treatment.

The anaerobic treatment can be firstly differentiated from the aerobic treatment by the presence or absence of oxygen (Gerardi, 2003). Thus, the anaerobic treatment normally takes place in an environment devoid of oxygen, while aerobic treatment requires a continuous supply of dissolved oxygen (Gerardi, 2003; Buchanan and Seabloom, 2004). This continuous supply of oxygen is usually implemented through sparging, which is associated with high energy consumption and therefore high operating cost; while, comparatively, the operating cost of the anaerobic treatment is very low (Buchanan and Seabloom, 2004). However, before the development of high rate anaerobic bioreactors, the performance of anaerobic digesters was ineffective, as the solid retention time (SRT) was not dissociated from the hydraulic retention (HRT), culminating in the washout of the required biomass, weak organic content removal, and low production of biogas (Henze *et al*., 2008; Chernicharo, 2007). The separation of the SRT to the HRT, through a configuration that allows long SRT through the retention of the required biomass for short HRT (SRT>>HRT) was reached through the development of high rate anaerobic bioreactors, which have reached widespread acceptance from the early

1980s by the success of the Up-flow anaerobic sludge blanket (UASB) reactors developed by Lettinga and coworkers (Dendooven and Escamilla-Silva, 2005; Del Nery *et al.,* 2001; Henze *et al*., 2008; Pol *et al*., 2004). However, this great acceptance of the anaerobic treatment resulted to the development of innovative concepts and implementation of treatment with serious conceptual problems (Alphenaar, 1994; Pol *et al*., 2004; Baddour *et al*., 2006; Bhatti, 1995).

3.6.1.1 Aerobic treatment of PSW

Aerobic treatment is a biological process occurring in the presence of dissolved oxygen (Gerardi, 2003). This process takes place faster than the anaerobic digestion, as the microorganisms involved in this process prevail and dominate biological systems when dissolved oxygen is available (Henze *et al*., 2008). Comparatively to anaerobes, aerobes possess a faster reproduction cycle and induce exothermic reactions (Botheju and Bakke, 2011; Buchanan and Seabloom, 2004). Aerobic wastewater systems allow the growth of naturally-occurring aerobic microorganisms to biologically implement the renovation of wastewater (Buchanan and Seabloom, 2004). In a bioreactor, aerobic conditions are usually created through the mechanical addition of dissolved oxygen, thus allowing aerobic and facultative microbes to rapidly oxidize soluble biodegradable organic and nitrogenous compounds (Gerardi, 2003; Botheju and Bakke, 2011; Buchanan and Seabloom, 2004). Generally, the oxidation of 1 kgCOD requires 1kWh of aeration energy when the aerobic treatment is selected for wastewater treatment (Henze *et al*., 2008). Oxygen is slightly soluble in water; therefore, the supply of dissolved oxygen must be maintained through an engineered system conceptualized to distribute dissolved oxygen in such systems, as the transfer of oxygen from the gas phase to the liquid phase, which is called absorption, is driven by the concentration gradient between the atmosphere and the bulk liquid (Henze *et al*., 2008; Buchanan and Seabloom, 2004). This aeration requirement comes along with the space required to provide a large surface for efficient oxidation of the organic matter contained in the wastewater; which increases the costs associated with the implementation of such technology (Henze *et al*., 2008). Unlike anaerobic digestion, aerobic treatment is often associated with high operational costs. The other drawback of the technology is the high production of excess sludge, which requires further treatment as it is a waste. Furthermore, there is no production of energy that can be captured and transformed, but a loss of heat (Chernicharo, 2007; Henze *et al*., 2008).

3.6.1.2 Anaerobic digestion of PSW

Anaerobic digestion is a biological process that consists of the biodegradation of organic matter through fermentation in an environment devoid of dissolved oxygen, which results to the production of biogas (Geradi, 2003; Debik and Coskun, 2009; Hadin, 2016; EPA, 1997). The biogas has the virtue of containing methane, which is a gas with high calorific value (10.5 kWh/m^3) (Chernicharo, 2007). As a rule of thumb, 1 m^3 of biogas produces 6 kWh of energy, which corresponds to 21.6 MJ (Chernicharo, 2007; Henze *et al*., 2008). Table 3.6 provides the typical composition of biogas from biological waste processing.

Table 3.6: Biogas composition (Adapted from Gerardi, 2003)

Gaseous elements	Chemical symbol	Volumetric concentration
Methane	CH_4	55% - 70%
Carbon dioxide	CO_2	35% - 40%
Hydrogen sulfide	H_2S	20-20 000 ppm
Water	H_2O	2% (20°C)- 7% (40°C)
Oxygen	O_2	<2%
Nitrogen	N_2	<2%
Hydrogen	H_2	<1%
Ammonia	NH_3	<0.05%

Thus, anaerobic treatment also represents an alternative source of energy. Further features of the anaerobic digestion are listed as follows (Gerardi, 2003; Henze *et al*., 2008):

- High organic loading rates;
- The possibility of the rapid start-up of the digestion by using granular anaerobic sludge as seed biomass;
- The market value of sludge when the anaerobic granules are produced inside the digester;
- Significant reduction in excess sludge production;
- Rapid influent treatment through an improved selected biomass retention system;
- Simple operation;
- Less energy requirement;
- Pathogen reduction in the sludge;

- Minimal requirements for additives;
- Reduced plant footprint; and
- Simplified conservation of anaerobic granular sludge, which can remain unfed for a long period (Gerardi, 2003; Henze *et al*., 2008).

However, these advantages compete with some disadvantages such as (Chernicharo, 2007; Gerardi, 2003; Henze *et al*., 2008):

- The susceptibility of the process to inhibition by a large number of compounds;
- Weak removal of pathogens, phosphorus, and nitrogen;
- The advent of the generation of effluent with unpleasant aspect;
- Slow start-up of the process in the absence of the required anaerobic granular sludge;
- Requirement of further treatment of the effluent to comply with the standards;
- The complexity of the microbiology and biochemistry of the anaerobic digestion; and
- Toxicity and unpleasant odor of hydrogen sulfide contained in the biogas generated from such processes.

In anaerobic treatment, depending on the efficiency of the design used, most of the biodegradable organic matter introduced into the biodigester is transformed into biogas. A small portion of the fed organic matter is transformed into microbial biomass that enters in the composition of the excess sludge produced from the bio-digestion process (Henze *et a*l., 2008). This excess sludge appears more concentrated than the sludge produced from the aerobic treatment and presents enhanced dewatering characteristics, with a consortium of microorganisms that can be used as biomass seed to another anaerobic bioreactor, hence its commercial value (Chernicharo, 2007; Henze *et al*; 2008).

The oxidation process under anaerobic conditions usually requires inorganic electron acceptors such as nitrate, sulfate or carbon dioxide. When all these electron acceptors are present during the anaerobic digestion, nitrate will be used at first, due to the low energy requirement of the operation and its affinity to most microorganisms (Gerardi, 2003). Subsequently, the second choice is sulfate and then carbon dioxide (Gerardi, 2003). However, methanogenesis is affected in environments where dissolved oxygen, sulfate and nitrate are readily available, as the process of ammonia and hydrogen sulfide production will lead to methanogenesis, which is a very energetic sensitive process (Gerardi, 2003; Henze *et al*., 2008). Methanogenesis may take place in various natural environments. These include soil, lakes, seas, river sediments, swamps, and in even the digestive organs of ruminants, in which the

redox potential approximates –300 mV (Chantrasakdakul *et al*., 2015; Fountoulakis *et al*., 2008; Gavala *et al*., 2003)

3.7 Oxygen removal before anaerobic digestion initiation

One critical parameter for a good performance of anaerobic treatment is the lack of oxygen. This is usually determined through the redox potential that should remain <-50 mV for anaerobic digestion and <-300 mV for a good methanogenic activity (Gerardi, 2003). For a digester hermetically closed, there is usually no need to attempt to remove the oxygen present, as the BOD in the wastewater consumes the oxygen present rapidly since aerobes and facultative aerobes normally use 100 mg/L of dissolved oxygen to degrade 100 mg/L of BOD (Henze *et al*., 2008). Furthermore, for lab studies and industrial scales, oxygen removal must be implemented through nitrogen purging, which includes three main methods (Gerardi, 2003), namely:

- Displacement purging,
- Pressurizing purging, and
- Dilution purging.

Purging consists of the replacement of one gas by another one in an enclosed chamber or space. In the case of anaerobic digestion, it consists of the removal of the oxygen contained in the biodigester by applying one of the aforementioned methods using nitrogen gas.

In some instances, the anaerobic digestion process can be separated into two components, with the first one consisting of the addition of a carbon source in the wastewater, which is introduced in a pre-column wherein the aerobes and facultative aerobes scrub out the oxygen prior to the transfer of wastewater into a methanogenic column (Chernicharo, 2007).

3.8 Microbiology of anaerobic treatment

The anaerobic digestion involves a consortium of microorganisms, which work interactively in the transformation of complex organic matter into new bacterial cell as well as products such as water, hydrogen sulfide, methane, carbon dioxide, and ammonia, amongst others (Barbut, 2003; Gerardi, 2003; Henze *et al*., 2008). This process is complex and consists of series and parallel reactions. Although anaerobic digestion can be considered as a two-stage process consisting firstly of the conversion of complex organic compounds into simpler carbonic

matters through the action of a group of facultative and anaerobic bacteria (Gerardi, 2003); and a second stage whereby hydrogen and organic acids are converted into biogas, which includes a variety of gases (Pol *et al*., 2004; Vidal *et al*., 2000; Zheng *et al*., 2006). These reactions can be further regrouped into four successive stages (Gerardi, 2003), which are:

- Hydrolysis,
- Acidogenesis,
- Acetogenesis/dehydrogenation, and
- Methanogenesis.

These stages are controlled by five types of bacteria (Gerardi, 2003), namely:

- Fermentative bacteria,
- Hydrogen-producing acetogenic bacteria,
- Hydrogen-consuming acetogenic bacteria,
- Carbon dioxide-reducing methanogens, and
- Aceticlastic methanogens.

These microorganisms interact in series of stages that constitute the anaerobic digestion, as discussed subsequently.

3.8.1 Hydrolysis/solubilization

Complex particulate matter present in the PSW, also referred to as polymers, can't usually be assimilated by the microorganisms intervening in the conversion of the organic matter present in the wastewater (Barbut, 2015; Bustillo-Lecompte *et al*., 2016). Thus, these matters require to be converted into a simpler dissolved matter that can penetrate through the cell membranes of the anaerobic bacteria. This conversion consists of the transformation of proteins to amino acids; lipids to long chains fatty acids (LCFA); and polysaccharide to simple sugars (Gerardi, 2003). This process of polymers degradation takes place through hydrolysis, in which exoenzymes secreted by hydrolytic fermentative bacteria enable such conversion under anaerobic conditions (Henze *et al.*, 2008). Table 3.7 lists these exoenzymes and their substrates.

Table 3.7: Hydrolysis exoenzymes and corresponding substrates (Adapted from Gerardi, 2003)

Substrate available	Required exoenzyme	Example	Bacterium	Product
Proteins	Proteolytic	Protease	*Bacillus*	Amino acids
Polysaccharides	Saccharolytic	Cellulase	*Cellulomonas*	Simple sugar
Lipids	Lipolytic	Lipase	*Mycobacterium*	Fatty acids

Despite the lack of oxygen, other factors may impact the hydrolysis (Geradi, 2003; Rajakumar *et al.*, 2012), namely:

- Composition of the substrate,
- The residence time of the wastewater in the bioreactor,
- Operational temperature of the bioreactor,
- pH of the medium,
- Particles size,
- NH_4^+-N concentration, and
- Concentration of products from hydrolysis.

These factors contribute to increase the sensitivity of this process. Due to the sensitivity of this process and its slowness, it is considered as a rate-limiting stage that can influence the entire process of anaerobic digestion (Henze *et al*., 2008; Gerardi, 2003). The other stage that can also be considered as rate-limiting in anaerobic digestion is the methanogenesis, which is an energy-sensitive process also affected by the competition of the substrate consumption by sulfate-reducing bacteria (SRB) (Chernicharo, 2007; Gerardi, 2003).

Once converted into soluble products, the substrate is metabolized inside the cells of the fermentative bacteria for conversion into simpler compounds, which are then released by the cells (Gerardi, 2003). These simpler compounds are alcohols, volatile fatty acids (VFA), lactic acid, carbon dioxide, hydrogen, ammonia, hydrogen sulfide as well as new bacterial cells. While some products from hydrolysis such as acetate and hydrogen can be used by methanogens in the methanogenesis, a significant part of these compounds must be further converted through other phases of the anaerobic to smaller molecules such as acetic acid, which ultimately will be used in the methanogenesis (Gerardi, 2003; Henze *et al*., 2008).

3.8.2 Acidogenesis

Acidogenesis is also referred to as the acid-forming step. It is the most rapid conversion step in anaerobic digestion and is conducted by a diverse and wide group of fermentative bacteria, which usually belong to the clostridia group (Chantrasakdakul *et al*., 2015; Gerardi, 2003; Henze *et al*., 2008; Schoen, 2010). This step consists of the conversion of the organic matter from hydrolysis by acid-forming bacteria into organic products such as butyric acid, propionic acid, acetic acid, carbon dioxide and hydrogen (Gerardi, 2003). Subsequently, these products are used by acetogenic bacteria in the following stage (Henze *et al*., 2008). However, some products from acidogenesis such as hydrogen and acetate can be directly utilized by methanogenic microorganisms for the production of methane (Chernicharo, 2007; Henze *et al*., 2008).

Acidogenesis can also be considered as a sensitive step in the anaerobic digestion, as the type of end products generated from it depends on the reactor medium conditions (Gerardi, 2003). In this way, the concentration of hydrogen in the reactor medium influences the type of products generated; as the removal of H_2, by H_2 scavenging organisms such as methanogenic microorganisms, leads to the production of acetate as the main end product. Similarly, a delay in methanogenesis, resulting in the accumulation of acetate, favors the production propionate and butyrate or even more reduced compounds such as alcohols or lactate (Gerardi, 2003; Henze *et al*., 2008).

Due to the rapidity of acidogenesis, anaerobic digesters are often subjected to a sudden pH drop (souring), as a result of the consumption of the alkalinity by the produced acids, which eventually leads to severe inhibition of the methanogens (Chernicharo, 2007). This souring of the bioreactor medium may get more acute through even quicker accumulation of VFAs and a further drop of the pH, boosted by the fact that acidifiers are more active at low pH (Henze *et al*., 2008). However, the Stickland reaction usually governs the acidogenic conversion of amino acids, in which amino acids are de-ammonified by anaerobic digestion, thus generating VFAs and H_2, along with reductive ammonification of other amino acids consuming the generated H_2 (Gerardi, 2003). In these two reactions, NH_3 is released and acts as a proton acceptor, thus contributing to increase the pH and preventing a pH drop (Gerardi, 2003).

3.8.3 Acetogenesis/dehydrogenation

This step serves to oxidize the products generated from acidogenesis into a substrate more digestible by the methanogens. The products generated from acetogenesis include hydrogen, acetic acid and carbon dioxide (Gerardi, 2003; Henze *et al*., 2008).

The formation of propionic acids and acetic acids usually results in the generation of a large quantity of hydrogen, which causes a decrease of the medium pH (Gerardi, 2003). Thus, for the stability of the anaerobic digestion, this modification of pH is prevented by its consumption that usually occurs through two ways (Gerardi, 2003; Henze *et al*., 2008)), namely:

- Formation of butyric and propionic acids through a reaction involving acetic acid, hydrogen, and hydrogen, and
- Consumption of hydrogen and carbon dioxide by methanogens to produce methane.

It is reported that at least 50% of biodegradable COD is transformed into butyric and propionic acids, which provides a significant load to acetogenic microorganisms that induce the formation of acetic acid and hydrogen (Henze *et al*., 2008). The most important substrates to this phase are propionate and butyrate, which are critical intermediates in the anaerobic treatment process.

The concentration of hydrogen in this step of the anaerobic digestion process is very important, as, although the acetogenic bacteria are obligate hydrogen producers, their metabolism is inhibited by hydrogen (Henze *et al*., 2008). Investigation on acetogenic conversion have illustrated the required associations between the H_2-producing acetogenic bacteria and the H_2-consuming methanogens, thereby controlling the H_2 level in such an environment, which is very critical as these reactions are thermodynamically unsuitable (Chernicharo, 2007; Henze *et al*., 2008). However, when the anaerobic digestion process is kept under stable conditions, the hydrogen partial pressure remains at an extremely low level, as a result of its effective uptake of the hydrogen by the methanogenic microorganisms and the sulfate-reducing bacteria (Fuchs *et al*., 2003; Gerardi, 2003).

3.8.4 Methanogenesis

This represents the last stage of the anaerobic digestion, which leads to the formation of methane and carbon dioxide as a result of the action of the methanogenic archaea (Gerardi,

2003). It is only in this stage that occurs the transformation of the influent COD into a gaseous form (Chernicharo, 2007). The microorganisms involved in this stage use selected substrates, including acetic acid, hydrogen/carbon dioxide, methanol, formic acid, carbon monoxide and methylamines (Gerardi, 2003; Henze *et al.*, 2008). Considering the extent of methane production and their affinity for substrate, methanogens can be subdivided into two principal groups, namely (Gerardi, 2003):

- Acetate-using microorganisms, and
- Hydrogen-using microorganisms.

Table 3.8 further regroups these species and provides the list of their substrates.

Table 3.8: List of methanogens and their corresponding substrates (Adapted from Gerardi, 2003)

Species	Type of substrate
Methanobacterium formicium	Hydrogen, carbon dioxide, formate
Methanobacterium thermoantotrophicum	Hydrogen, carbon dioxide, carbon monoxide
Methanococcus frisius	Hydrogen, methanol, methylamine
Methanococcus mazei	Acetate, methanol, methylamine
Methanosarcina bakerii	Acetate, carbon dioxide, hydrogen, methanol, methylamine

Although acetate-using microorganisms, also referred to as aceticlastic methanogens, prevail in anaerobic digestion, few can form methane from acetate (Gerardi, 2003; Metcalf and Eddy, 2003). These methanogens are responsible for about 70% of all the methane generated from such a process, leaving the rest of the methanogenic substrate intake to hydrogen and carbon dioxide (Chernicharo, 2007). The growth rate of these acetate-using microorganisms is extremely low, leading to doubling times of several days (Gerardi, 2003). This low growth rate explains the long start-up period requirement of anaerobic digesters when the adapted seed material is not used (Henze *et al*., 2008).

Two genera use acetate as the substrate to produce methane, these include *Methanosarcina* and *Methanosaeta*. *Methanosarcinas* are characterized by a coccoid shape and can also use other substrates such as H_2/CO_2, methanol, formate, and methylamines for methane production (Gerardi, 2003; Pol *et al*., 2004). Furthermore, they prevail above an acetate concentration of 10^{-3} M and have a relatively low substrate affinity (Gerardi, 2003; Metcalf and Eddy, 2003). Unlike

Methanosarcinas, *Methanosaetas* are developed in the form of filaments, and look like large spaghetti conglomerates, which can only use acetate as substrate and possess a very high substrate affinity (Henze *et al*., 2008). As compared to *Methanosarcinas*, *Methanosaetas* may be more sensitive to pH fluctuations and have lower yields. Furthermore, while *Methanosaetas* require longer SRT, *Methanosarcinas* differ by a greater growth rate (Gerardi, 2003). *Methanosaetas* are the most common acetate-using microorganisms in anaerobic systems with high SRT, as wastewater treatment systems are designed to remove as much organic matter from the wastewater as possible, and therefore reduce the concentration of the substrate in such systems to minimal concentrations, thus providing a competitive advantage to *Methanosaetas* over *Methanosarcinas* (Gerardi, 2003). Generally, the domination of the *Methanosaeta* in anaerobic digestion enables very effective wastewater treatment, illustrated by very low effluent acetate concentrations (Gerardi, 2003; Henze *et al*., 2008). This is further explained by the inferior kinetic properties of *Methanosarcinas* at low substrate concentrations and their poor adherence properties. These characteristics suggest the maintenance of effluent acetate concentrations at a very low level during the start-up of an anaerobic digester not incubated with the adapted seed material (Chernicharo, 2007; Metcalf and Eddy, 2003).

Unlike aceticlastic methanogens, hydrogen-using microorganisms also referred to as hydrogenotrophic methanogens, possess a much higher maximum growth rate, with doubling times varying between 4 and 12 hours (Henze *et al*., 2008). This short growth rate provides a remarkable stability to high rate anaerobic bioreactor systems under various conditions despite the delicacy of the acetate-using bacteria (Gerardi, 2003). However, both acetogenic bacteria are very important in the finalization of the anaerobic digestion, as they intervene in the conversion of the hydrogen generated from previous phases; therefore, the lowering of the H_2 partial pressure in the medium, which allows the acidogenic and acetogenic reactions (Gerardi, 2003; Henze *et al*., 2008; Metcalf and Eddy, 2003).

3.8.5 Action of alternative electron acceptors in anaerobic treatment

3.8.5.1 Bacterial competition in organic medium

Besides the methanogenic bacterial consortium, other microbial communities exist in anaerobic digesters (Wang *et al*., 2014). These microorganisms can compete with methanogenic microorganisms to consume the available methanogenic substrates. These microorganisms

present different microbial respiration systems and may use different electron acceptors, including oxygen, by facultative anaerobes, sulfate, and sulfite by sulfate-reducing bacteria, nitrate by denitrifiers, and iron by iron reducers (Gerardi, 2003).

3.8.5.2 Competition of sulfate-reducing bacteria (SRB)

The presence of compounds such as sulfite, sulfate, and thiosulfate induce the ability of sulfate-reducing bacteria (SRB) to use different intermediates from the anaerobic mineralization process (Gerardi, 2003; Metcalf and Eddy, 2003). These bacteria possess a wider substrate spectrum than methanogenic bacteria and can convert sulfate into hydrogen sulfide (Gerardi, 2003). This substrate spectrum includes methanogenic substrates such as formate, hydrogen, acetate pyruvate and methanol, as well as other organic substrates including butyrate, propionate, ethanol and higher alcohols, lactate, succinate, higher and branched fatty acids, malate, fumarate, aromatic compounds (Gerardi, 2003; Henze *et al*., 2008). Thus, intermediary products from the anaerobic digestion such as hydrogen and acetic acid can be utilized by SRB, methane-producing bacteria (MPB), and/or obligate hydrogen-producing bacteria (OHPB), as these three microorganisms operate under the same conditions (Gerardi, 2003). This results in a competition between the aforementioned microorganisms for the use of the available substrate in the anaerobic medium.

The presence of sulfate in an anaerobic medium does not necessarily reduce the rate of degradation of organic substrates but reduce the quantity of substrate available for methanogens and result in the production of hydrogen sulfide (Henze *et al.,* 2008; Metcalf and Eddy, 2003). The latter presents the disadvantage of being more soluble in water than methane (Gerardi, 2003). This competition on the available methanogenic substrate results in the reduction of the removal of COD through this process, as only methanogens can convert the COD into another product, which is methane and carbon dioxide (Chernicharo, 2007; Gerardi, 2003). Furthermore, the production of hydrogen sulfide can result in the occurrence of the following challenges in anaerobic treatment (Gerardi, 2003):

- The bad smell of hydrogen sulfide and the corrosion problems this gas induces, culminating in the increase of costs associated with the maintenance of the installation and requirement of extra investments to prevent such inconveniences;
- The toxicity of hydrogen sulfide to MPB, acetogenic bacteria and SRB; and

- The presence of part of the sulfide in the liquid output of the anaerobic digester, resulting in lower overall treatment efficiency of the anaerobic treatment system.

Concerning the preference of substrate, SRB can be regrouped into three categories (Gerardi, 2003):

- Acetic acid oxidizing (ASRB),
- Hydrogen oxidizing SRB (HSRB), and
- Fatty acids oxidizing SRB (FASRB).

Another interesting fact from SRB is that, although considered as strict anaerobes, some of them were found to be able to operate under aerobic conditions, which is a very intriguing fact that could be used in some bioengineering development (Henze *et al*., 2008; Metcalf and Eddy, 2003). Furthermore, excluding the reduction of sulfate, SRB also reduces sulfite and thiosulfate. This by *Desulfovibrio* strains, which have been reported to be able to reduce di-, tri-, and tetra-thionate; similarly, *Desulfovibrio dimutans* and *Desulfobacter curvatus* dismutate sulfate or thiosulfate (Geradi, 2003).

3.8.5.3 Possibility of denitrification occurrence in anaerobic systems

Generally, denitrification does not occur in environments devoid of oxygen, unless the wastewater fed to the anaerobic digester contains nitrate (Gerardi, 2003). This process is facilitated by denitrifying microorganisms such as chemohetereotrophic bacteria which possess the ability to oxidize biodegradable matter in the presence of nitrate, resulting in the conversion of the latter, via nitrite and nitrogen oxide, to nitrogen gas. Because of the energy yield of oxygen, this molecule is preferred by denitrifiers as electron acceptors (Metcalf and Eddy, 2003). However, to cope with organic load in the aerobic purification processes, denitrifiers prevail when the oxygen gets depleted (Gerardi, 2003). Similarly, in the activated-sludge plant, denitrification will occur under the provision that dissolved oxygen concentration is lower or equal to 1 mg/L (Gerardi, 2003).

3.9 Essential operational parameters for the control of anaerobic digestion

Biological processes heavily rely on the growth and bio-preservation of the required microorganisms (Chernicharo, 2007). This is made possible by the identification and control of essential operational parameters such as the temperature, pH, organic loading rate, carbon to

nitrogen ratio, inoculation and start-up of the biodigester, mixing, and inhibition factors. The efficient control of these parameters enables the improvement of the microbial activity inside the biodigester for an enhanced anaerobic digestion process.

3.9.1 The control of the concentration of oxygen in anaerobic systems

The majority of strict anaerobes are scavengers and prevail in environments devoid of oxygen. These microorganisms grow and effectively degrade organic matter when the oxidation-reduction potential (ORP) of their surroundings vary between -200 and -400 mV (millivolts). The ORP is raised by any amount of dissolved oxygen in the anaerobic system and result in the alteration of the anaerobic digestion process through the phases of hydrolysis, acetogenesis, and methanogenesis (Botheju and Bakke, 2011; Gerardi, 2003; Metcalf and Eddy, 2003). Thus, the ORP serves to measure the relative quantity of oxidized materials, such as nitrate ions (NO_3^-), ammonium ions (NH_4^+), as well as sulfate ions (SO_4^{2-}) (Gerardi, 2003). ORP values greater than +50 mV translates to the availability of free molecular oxygen in the wastewater or sludge, contributing to create oxic conditions in a bioreactor and the inhibition of anaerobic microorganisms (Gerardi, 2003). The cellular activity concerning the ORP is illustrated in Table 3.9.

Table 3.9: Cellular activity of microorganisms as per ORP (Adapted from Gerardi, 2003)

ORP (mV)	Carrier molecule for the degradation of organic matter	Environmental condition	Prevalent respiration method
>+50	O_2	Oxic	Aerobic
+50 to -50	NO_3^- or NO_2^-	Anaerobic	Anoxic
<-50	SO_4^{2-}	Anaerobic	Fermentation, sulfate reduction
<-100	Organic compound	Anaerobic	Fermentation, mixed acid production
<-300	CO_2	Anaerobic	Fermentation, methane production

3.9.2 The importance of the control of the temperature in anaerobic digestion

Although anaerobic digestion can take place at various thermal conditions, such a process produces less methane at temperatures below 15°C (Chernicharo, 2007; Metcalf and Eddy, 2003). This shortcoming is observed in regions where cyclic cold climatic conditions prevail, requiring the installation of heating or insulation systems to enhance or maintain a stable anaerobic digestion. Thus, in cold regions, the installation of such systems requires additional investment costs, which consequently reduces the attraction towards such processes. Generally, such technologies are recommended in developing countries due to their tropical climate that contributes to significantly reduce the investment and operational costs required, while providing an alternative source of energy (Rajakumar *et al*., 2011; Moreira *et al*., 2009). However, it should be noted that day and night variation of temperature in these regions can be detrimental for anaerobic digestion, as maintaining such a system under a specific temperature range is very important to ensure stable operations translated to constant biogas production and organic matter transformation.

Anaerobic digesters can be operated under different temperature ranges, including (Metcalf and Eddy, 2003):

- Psychrophilic (0-15°C),
- Mesophilic (20-40°C),
- Thermophilic (45-60°C), and
- Hyper-thermophilic (>65°C).

Of these four temperature ranges, mesophilic and thermophilic microorganisms provide a better performance in terms of organic matter conversion and therefore biogas production (Borja et al., 1998; Henze *et al*., 2008). However, the mesophilic range appears to be the most stable range to operate on, as the mesophilic microorganisms can adapt the best to fluctuating environmental conditions and require less energy (Chernicharo, 2007). Furthermore, as compared to thermophilic conditions, the inhibition by ammonium is less dominant in mesophilic conditions due to the lower concentration of free ammonia at lower temperatures (Chernicharo, 2007; Gerardi, 2003). However, mesophilic microorganisms induce a slower methanogenic activity and therefore require a longer HRT to process the organic matter influent as compared to thermophilic microorganisms that possess the ability to degrade

organic matter faster for an improved substrate distribution; but more energy translates to more operating costs.

3.9.3 The importance of the control of the pH in anaerobic digestion

One essential parameter in anaerobic digestion is the pH, as the required anaerobic biomass can only perform effectively at a specific pH range. The prescribed range is often given at 6.5 – 8, with anaerobic digestion stages such as hydrolysis and acetogenesis more inclined to occur at pH varying between 5.5 and 6.5, while the methanogenic stage generally occurs at a pH varying between 6.5 and 8.2 (Gerardi, 2003; Metcalf and Eddy, 2003). Figure 3.3 lists the average pH required for the optimum growth of some methane-forming bacteria (Gerardi, 2003).

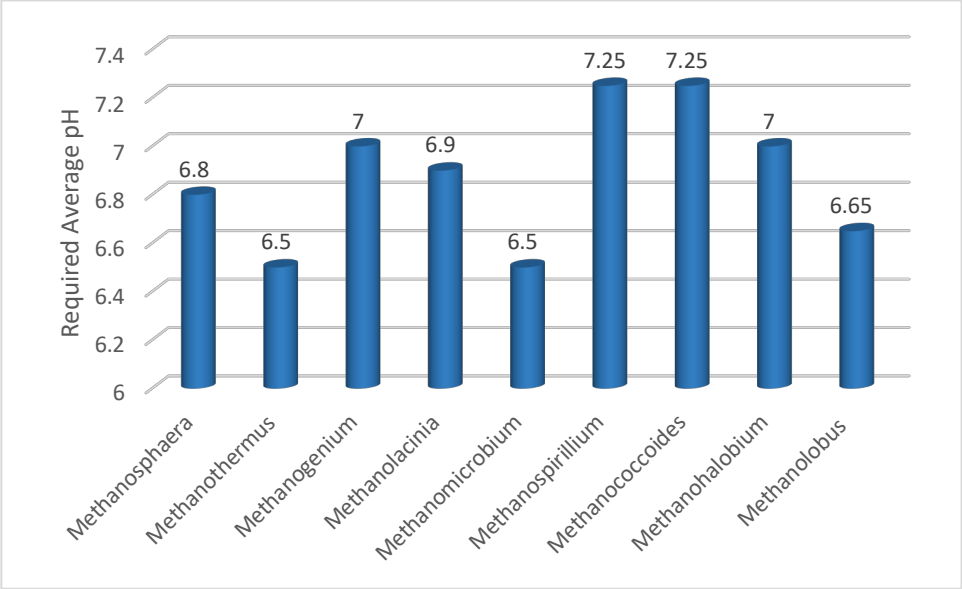

Figure 3.3: Required pH for the growth of various methanogens (Adapted from Metcalf and Eddy, 2003)

Thus, these anaerobic environmental conditions also require the availability of an alkalinity level around 3000 mg/L throughout the anaerobic digestion process (Gerardi, 2003). For this to be implemented, the chemicals listed in Table 3.10 can be used to modify the pH when acidic conditions prevail in anaerobic bioreactors (Gerardi, 2003).

Table 3.10: List of chemicals that may be used to modify the pH in anaerobic medium (Gerardi, 2003)

Chemical	Chemical formula	Buffering cation
Potassium bicarbonate	$KHCO_3$	K^+
Sodium bicarbonate	$NaHCO_3$	Na^+
Sodium carbonate (Soda ash)	Na_2CO_3	Na^+
Potassium carbonate	K_2CO_3	K^+
Calcium hydroxide (quick lime)	$Ca(OH)_2$	Ca^{2+}
Calcium carbonate (lime)	$CaCO_3$	Ca^{2+}
Sodium nitrate	$NaNO_3$	Na^+
Anhydrous ammonia (gas)	NH_3	NH^{4+}

3.9.4 Role of nutrients in anaerobic digestion

The microbial cells of the microorganisms involved in anaerobic digestion possess a chemical composition that influences their nutritional needs. This composition is not precisely known, but nutrient requirements are estimated from the empirical composition of the microbial cells, based on the fact that all living cells present fairly similar chemical composition (Gerardi, 2003; Henze *et al*., 2008). Thus, the chemical composition of the methanogens is provided in Figure 3.4.

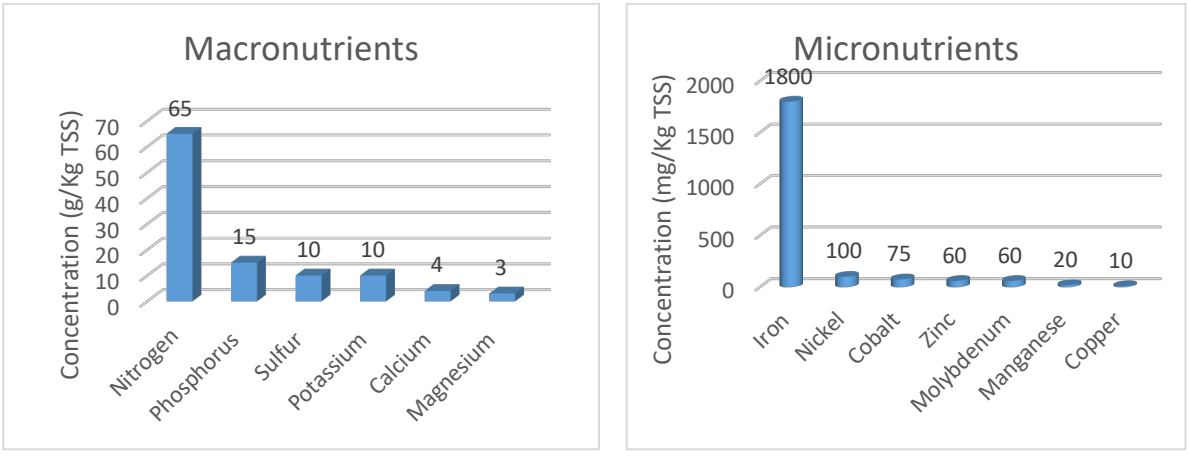

Figure 3.4: Chemical composition of methanogens (Adapted from Metcalf and Eddy, 2003)

Therefore, for efficient biological treatment processes, required inorganic nutrients should be supplied to promote the growth of the required microorganisms. The nutrients requirement

relates to the chemical composition of methanogenic microorganisms, as provided in Figure 3.4.

3.9.5 Importance of the control of carbon to nitrogen ratio in anaerobic digestion

C:N ratio represents the relationship between the quantity of carbon and nitrogen in the organic matter (Metcalf and Eddy, 2003). This ratio serves to estimate the nutrient deficiency and ammonia inhibition, as a high C/N translates to a rapid consumption of nitrogen by methanogenic microorganisms for a lower biogas production, and a low C:N ratio reveals an accumulation of ammonia for a pH level that may exceed 8.5 and therefore be detrimental to the methanogenic activity (Henze *et al*., 2008). Thus, an optimal C:N ratio in anaerobic systems lies between 16 and 25 (Gerardi, 2003). This optimal condition can be maintained by supplying an influent that presents ideal C:N ratio levels.

3.9.6 The importance of the monitoring of hydraulic retention time in anaerobic treatment

The supply of organic matter to anaerobic digesters and the control of the residence time of these matter in such systems is very important for the maintenance of stable methanogenic activity. The hydraulic retention (HRT) provides the retention time of liquid influents in anaerobic digesters and is determined by the ratio of the reactor working volume to the influent flow rate. Initial anaerobic digesters required long HRT to reach an appreciable performance and were usually linked to the solids retention time (SRT), as there was a mechanism that allowed the separation of both parameters before (Basitere *et al*., 2017). However, the development of high rate anaerobic bioreactor systems allowed the separation of these two parameters through the implementation of techniques of solid retention, which enabled short HRTs for similar or improved performance because the required biomass was conserved within the bioreactor for much longer. This separation between SRT and HRT is mostly reached for liquid influents, as the separation of solid influents from the biomass remains difficult to achieve (Chernicharo, 2007).

3.9.7 Importance of the control of organic loading rate in the anaerobic digestion

The organic loading rate (OLR) represents the rate of supply of organic matter to a biodigester and is usually determined from the ratio of the influent COD to the hydraulic retention time of the system. The control of this parameter is very important in high rate anaerobic systems due to the advent of overloading that may lead to a jump in the concentration of volatile fatty acids, which can induce the acidification and failure of the system (Henze *et al*., 2008). Therefore, for stirred and non-stirred anaerobic digesters, an organic loading rate below 2 kg VS/m^3/Day is recommended, as opposed to a prescribed OLR in the range of 4-8 kg VS/m^3 for enhanced anaerobic digesters (Chernicharo, 2007). Other important parameters for the control and the investigation of anaerobic digestion are provided in Table 3.11.

Table 3.11: Parameters used to control anaerobic digestion (Chernicharo, 2007; Henze *et al*., 2008)

Parameter	Formula	Description
Hydraulic retention time (HRT) (days)	V/Q	V: Reactor volume Q: Flow rate
Organic loading rate (OLR) (kg substrate	Q*S/V	Q: Substrate flow rate S: Substrate concentration in the influent V: Reactor volume
Gas production rate (GPR)	Q_{biogas}/V	Q_{biogas}: Biogas flow rate V: Reactor volume
Specific gas production (SGP)	Q_{biogas}/Q*S or GRP/OLR	Q_{biogas}: biogas flow rate Q: Inlet flow rate S: Substrate concentration

3.9.8 Control of the inoculation and start-up in anaerobic treatment

Anaerobic treatment requires a suitable bacteria consortium to enable the digestion of organic matter (Alphenaar, 1994; Pol *et al*., 2004). This is usually implemented through the inoculation of anaerobic digesters with the required biomass at the start of the process, with the inoculum collected from another anaerobic digester such as anaerobic granular sludge from high rate

anaerobic bioreactor systems treating liquid influents or diluted cow dung for solid influents (Alphenaar, 1994; Pol *et al.*, 2004). This inoculation can be assisted by the supply of a carbon source such as diluted milk to ensure the growth of the bacteria and may require a period of acclimation to adapt the inoculated biomass to the feedstock (Debik and Coskun, 2009; Basitere *et al*., 2007). This adaptation can be implemented through a gradual and controlled increase of the influent flow rate, following the observation of the adaption of the system to the influent through the change of COD removal percentage for instance (Basitere *et al*., 2007; Debik and Coskun, 2009). Furthermore, other parameters relevant to the stability of the anaerobic process such as the temperature, pH, and accumulation of toxic substances should be strictly monitored during this phase to allow smooth acclimatization of the anaerobic biomass (Chernicharo, 2007; Fuchs *et al*., 2003).

Generally, the gas produced during the start-up of anaerobic digestion systems is mostly composed of CO_2, and therefore is not flammable and thus can be released to the atmosphere (Henze *et al*., 2008). The methane content in the biogas increases as the COD removal of the system increases as a result of a stable methanogenic activity (Chernicharo, 2007). This methane content in the gas outlet can be verified by its flammability.

3.9.9 Importance of mixing in anaerobic treatment

In anaerobic digestion systems, mixing serves to enhance the contact between the biomass and organic matter, for improved organic matter conversion and biogas production (Chernicharo, 2007; Fuchs *et al*., 2003). Furthermore, such operation presents several other advantages, namely (Chernicharo, 2007):

- The elimination or reduction of scum;
- The elimination of thermal stratification of localized zones of depressed temperature;
- The dispersion of metabolic wastes generated during organic matter digestion;
- Maintenance of the bioreactor physical and chemical uniformity throughout the vessel;
- The dispersion of toxic materials entering the digester; and
- The prevention of grit deposition.

The importance of the good distribution of substrate in anaerobic systems is also highlighted by an increase in the growth of filamentous bacteria as compared to flocculating bacteria in zones of low substrate concentrations, which limits the performance of such systems (Gerardi, 2003). Thus, the growth of flocculating bacteria is preferred. The formation of scum in such

bioreactors should also be prevented to avoid the blockage of gas pipe and its development into foaming over the bioreactor, which can result in the washout of solids or the displacement of slurry into pipes and process equipment, culminating in corrosion or malfunction (Chernicharo, 2007; Metcalf and Eddy, 2003).

However, the choice of the mixing device or technique should be done carefully, as mechanical mixing has been reported to be detrimental for anaerobic granular sludge, and therefore pneumatic mixing is often recommended for systems requiring such inoculum (Chernicharo, 2007). In this regard, most high rate anaerobic bioreactor systems rely on biogas elevation, recycle streams, and the effluent up-flow velocity for mixing the digester content; but anaerobic digesters treating solid wastes may require mechanical mixing at gentle velocities to facilitate the contact between the organic matter and biomass (Gerardi, 2003; Caixeta *et al*., 2002; Freeman *et al*., 2009).

Another important aspect relevant to mixing in anaerobic systems is the dispersion of toxic substances that contribute to reduce their concentrations in a specific zone of the digester bed, thus preventing the increase of their concentration and eventually the inhibition of the methanogenic biomass (Gerardi, 2003).

3.9.10 Prevention of inhibition in anaerobic digestion

A close look at factors that may lead to the inhibition of the anaerobic process is as important as the control of critical parameters such as the temperature and pH. Generally, inhibition depends on the concentration of the inhibiting substances, the composition of the substrate and adaption of the biomass to these substances (Henze *et al*., 2008; Yu *et al*., 2014). In anaerobic digestion, common inhibitors include the organic and inorganic substances listed in Figure 3.5.

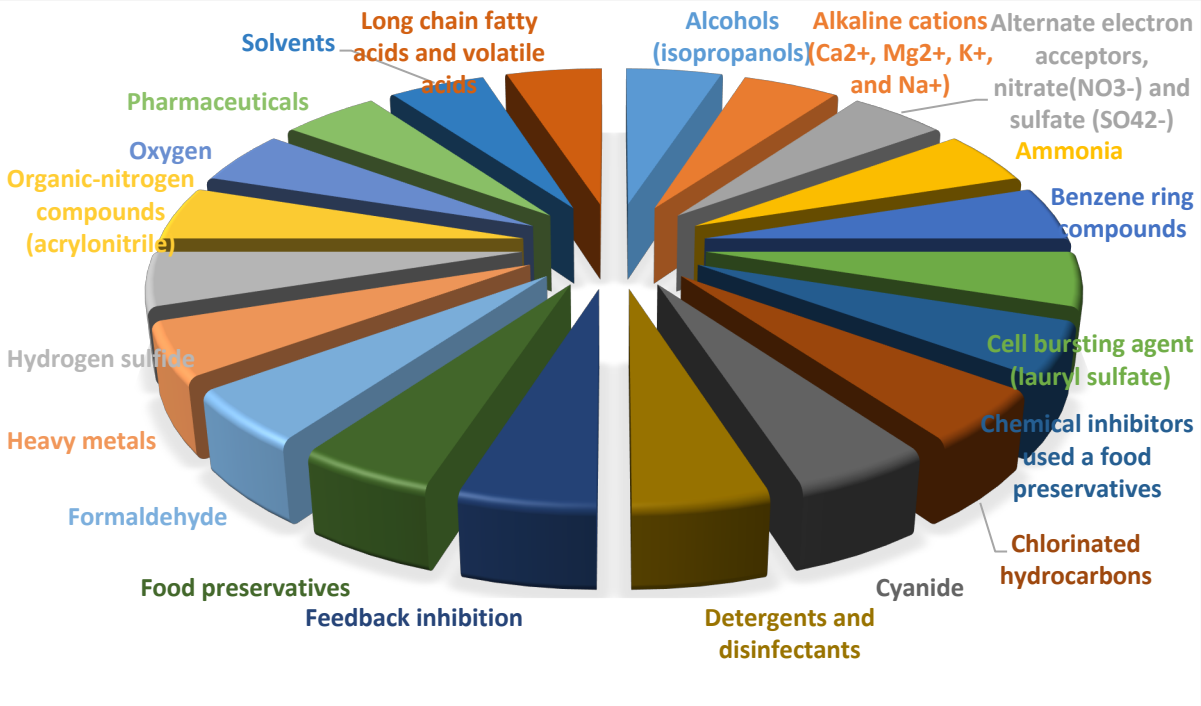

Figure 3.5: List of common inhibitors in anaerobic digestion (Adapted from Henze *et al.*, 2008; Yu *et al.*, 2014)

Of these inhibitors, ammonia nitrogen represents a real source of concern, as ammonia inhibition may occur in a wide range of concentrations i.e. between 1400 and 17 000 mg N/L of total inorganic nitrogen (Gerardi, 2003). In anaerobic digesters, ammonia and the protonised form of ammonium mainly constitute the total inorganic nitrogen, with the biggest share of the latter being in the form of ammonium for normal pH ranges and ammonia for increased pH and temperature. This inhibition induces an imbalance and accumulation of anaerobic digestion by-products such as VFAs, which may lead to the acidification of the anaerobic bioreactor (Alphenaar, 1994; Pol *et al.*, 2004).

Overall, the operational parameters essential to anaerobic digestion are summarized in Table 3.12.

Table 3.12: Requirements for good anaerobic digestion process (Adapted from Gerardi, 2003; Henze et al., 2008)

Parameter	Unit	Optimum range
pH	-	6.5 – 8
Temperature		
Psychrophiles	^{o}C	0 - 15
Mesophiles	^{o}C	20 - 40
Thermophiles	^{o}C	45 - 60
Hyper-thermophiles	^{o}C	>65
C/N	-	16 - 25
Alkalinity as $CaCO_3$	mg/L	1500 - 3000
VFA/Alkalinity	-	<0.3
NH_3-N concentration	mg/L	1500 - 3000
VFAs as acetic acid	mg/L	50 - 500
Ammonia	mg/L	<1500
Gas composition		
The volume percentage of methane	%	65 – 70
The volume percentage of carbon dioxide	%	30 - 35
Oxidation-reduction potential	mV	-200 to -400

3.10 Features of high rate anaerobic treatment systems

High rate anaerobic bioreactor systems differ from low rate anaerobic bioreactor systems by an SRT >> HRT, promoted through the development of systems of suitable bacteria immobilization within the anaerobic digester, as this biomass usually has a low growth rate (Chernicharo, 2007). This lack of independent control of the SRT and HRT has been the disadvantage of low rate anaerobic bioreactor systems for a long period of time (Metcalf and Eddy, 2003), as the microorganisms required for maintaining stable anaerobic operations were discarded from the anaerobic digester with the effluent and thus required a new start-up of the system (Chernicharo, 2007). Thus, the retention of highly active anaerobes in high rate anaerobic bioreactor systems relies on a series of factors and mechanisms, described in the following sub-sections:

3.10.1 Biomass retention by attachment

Several factors such as the availability of nutrients, pH, temperature as well as stratification contribute to the survival and growth of anaerobic microorganisms within aqueous systems such as anaerobic digesters (Henze *et al*., 2008). Furthermore, to overcome the instability of such environments, these microorganisms rely on immobilization through attachment on fixed or moving surfaces, which usually culminates in the formation of a biofilm attached to a support medium (Kobayashi *et al*., 2015). The growth of such biofilm results from a complex process entailing the transport of inorganic and organic molecules and microbial cells to a surface, which are then adsorbed to the surface for an irreversible attachment facilitated by the production of extracellular polymeric substances (EPS) (Kobayashi *et al*., 2015). EPS consists of a complex mixture of biopolymers such as polysaccharides, as well as nucleic acids, proteins, humic substances, and lipids. The roles of EPS include the facilitation of the initial attachment of cells to different substrata as well as the protection against dehydration and environmental stress (Kobayashi *et al*., 2015; Chernicharo, 2007).

3.10.2 Biomass retention by flocculation

The mechanism of flocculation is very complex and still poorly understood (Chernicharo, 2007; Metcalf and Eddy, 2003). The sludge flocculability plays an important role in solid/liquid separation in sewage treatment, as poor flocculability culminates in the increase of the effluent turbidity (Chernicharo, 2007). The importance of the process of flocculation is demonstrated in up-flow anaerobic sludge blanket (UASB) reactors and two-stages processes (Pol *et al*., 2004; Rajakumar *et al*., 2011).

3.10.3 Biomass retention by interstitial retention

Interstitial retention occurs through the retention of anaerobic microorganisms in the interstices of fixed solid surfaces serving as fixed beds for anaerobic digesters (Chernicharo, 2007). Despite the interstices in which the microorganisms grow dispersedly, the roughness of the surface of the packing materials used in such systems provide more surface area for the retention of these microorganisms (Chernicharo, 2007).

3.10.4 Biomass retention by granulation

Anaerobic granules can be defined as biomass including all bacterial species required for the processing of organic wastes under anaerobic conditions (Alphenaar, 1994; Pol *et al*., 2004). These granules present the qualities of high sedimentation velocity and high methanogenic activity (Henze *et al*., 2008). Unlike biofilms, anaerobic granules do not require a support media for their formation and are often generated from the operation of UASB reactors or similar ones (Rajakumar *et al*., 2011; Pol *et al*., 2004). Morphologically, these granules possess a regular shape as well as a well-defined surface with a diameter > 0.5 mm (Meier *et al*., 2011; Mu *et al*., 2006; Alphenaar, 1994). The formation of granules within anaerobic digesters, also referred to as granulation, occurs in specific anaerobic digesters such as the UASB and its variants as well as anaerobic filters, to a lesser extent (Chernicharo, 2007). This phenomenon usually occurs during the treatment of wastewater rich in volatile acids and carbohydrates and was not elucidated 25 years ago (Alphenaar, 1994). However, it is believed that the mechanisms that govern the selection and formation of granules relate the following physical, chemical and biological factors (Pol *et al*., 2004):

- The superficial rate of biogas liberation and gravitational compression of the sludge granules;
- The up-flow velocity of the liquid through the sludge bed;
- Immobilization of the required biomass;
- SRT>>HRT;
- Presence of finely dispersed matter;
- Presence of support material and/or specific growth nuclei;
- The quality of the substrate; and
- Stable appropriate operational conditions for the growth of the methanogenic archaea.

Of these factors, the up-flow velocity of the liquid is particularly important, as it induces a consistent selective pressure on the anaerobes that enable their adherence to each other and subsequently culminate in the formation of granules (Henze *et al*., 2008). This up-flow velocity is usually assisted by the elevation of produced biogas for a combination of upward forces that enable the washout of light particles and the retention of bigger microorganism conglomerates, which ultimately result in the formation of granules (Yoochatchaval *et al*., 2008). This granulation improves the settle-ability of the biomass.

From an engineering point of view, the granular configuration presents some advantages, namely (Chernicharo, 2007; Henze et al., 2008):

- The settle-ability of the granules;
- The aggregation of microorganisms that contributes to increase the density of the granules;
- The non-activity of anaerobic granules on inert medium allows the maximum use of the anaerobic digester working volume; and
- The maximum microorganism/volume ratio promoted by the spherical shape of the granules.

The aggregation of microorganisms follows a specific arrangement described in Figure 3.6, from which it can be observed that the bacterial consortia appear to be selectively arranged in layers on top of each other (Henze *et al.*, 2008). Generally, *Methanosaeta spp.* are found in the center of the mature granules, and are often hypothesized to be serving as nuclei for granulation and growth support for other microorganisms required for the anaerobic digestion (Henze *et al.*,2008; Alphenaar, 1994).

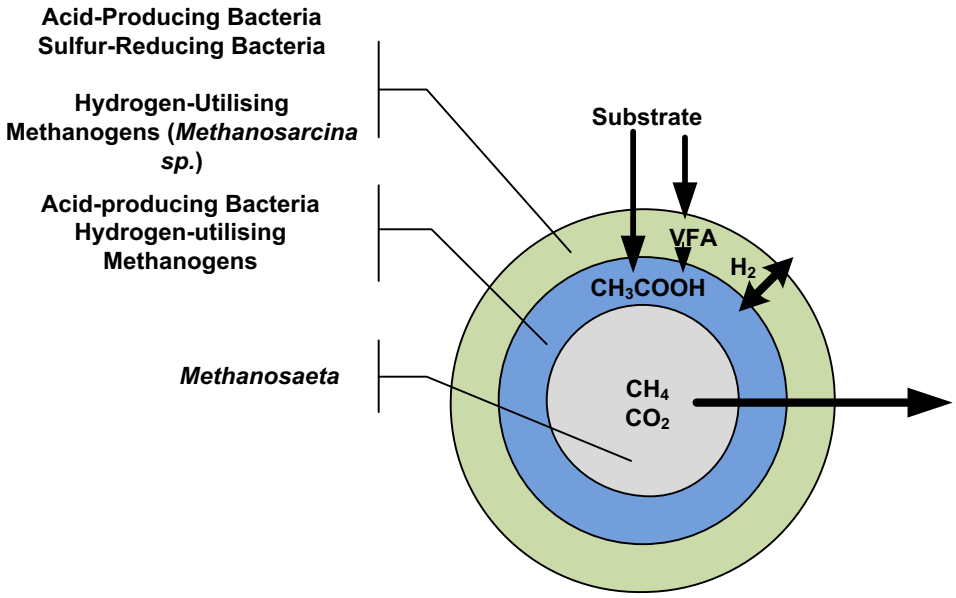

Figure 3.6: Aggregation of methanogens in anaerobic granule (Adapted from Henze et al., 2008)

3.11 Granule-based reactor technologies used for the treatment of PSW in previous studies

The development of HRABS has led the way to the increase of the popularity of granule-based technologies, which are widely acclaimed as a cost-effective way of treating wastewater laden

with organic matter (Basitere *et al*., 2007; Basitere *et al*., 2016; Evans, 2004; Oh, 2012; Pol *et al*., 2004; Zheng *et al*., 2006). Several advantages of the anaerobic treatment over the aerobic treatment were listed above, with the most important ones being the low energy requirement, significant reduction of the plant footprint, low levels of sludge generation and the production of biogas (Del Nery *et al*., 2007). Amongst these anaerobic treatment technologies, granule-based technologies are the most popular nowadays (Chernicharo, 2007; Del Nery *et al*., 2001). They differ from other anaerobic technologies by the occurrence of granulation, which results in the formation of anaerobic granules that significantly contribute to increasing the efficiency of such processes (Lettinga and Pol, 1991). The first of the kind was the UASB, which was developed in the 1970s, in the Netherlands, by Lettinga and co-workers and represents the most widely used HRABS for industrial as well as domestic wastewater treatment (Letting and Pol, 1991; Pol *et al*., 2004). The design of the UASB was later improved to another variant, which is called the expanded granular sludge blanket (EGSB) reactor that differs from the UASB by the extension of the height of the reactor and the use of the a recycle stream to improve the mixing inside the reactor (Pol *et al*., 2004; Chernicharo, 2007). Furthermore, other creative HRABS designs and modes of operation have contributed to optimize these processes under conditions that were previously considered sub-optimal and apply to a wide range of wastewater; thereby expanding the applicability and scope of wastewater anaerobic treatment (Oh, 2012; Pol *et al*., 2004; Henze *et al*., 2008; Chernicharo, 2007; Evans, 2004; Ellis and Evans, 2008; Lim, 2009). The following sub-sections described selected HRABS.

3.11.1 Treatment of PSW using a UASB

The mode of operation of the UASB attracts by its simplicity, as it consists of an anaerobic digester containing immobilized anaerobic granules, through which the wastewater influent flows upward from an inlet located at the bottom of the AD to an outlet stream connected to a three-phase separator, which is a mechanism located at the upper part of the AD and that serves to separate the biogas produced from the anaerobic digestion process and the UASB's effluent from the solid content of the bioreactor (Lettinga and Pol, 1991). Through the upward motion of the liquid influent, this system enables the contact between the substrate contained in the wastewater and the anaerobic microorganisms, which culminates in the degradation of the organic matter contained in such wastewater and consequently the production of biogas (Lim, 2009; Dendooven and Escamilla-Silva, 2005; Caixeta *et al*., 2002; Bhatti, 1995). Despite

being an alternative source of energy through its methane content, the biogas produced in this system, combined with the upward flow of the influent, serves to mix the granular bed for a better contact between the substrate and the biomass, the dispersion of toxic substrate, and alleviation of temperature stratification, amongst other benefits of mixing in such systems (Chernicharo, 2007; Lim, 2009).

The three-phase separator is an important component of this bioreactor, as it was designed to reduce the washout of the anaerobic granules and therefore the maintenance of the performance of this system, which heavily relies on the presence of this required biomass (Lettinga and Pol, 1991). Furthermore, the three-phase separator maximizes the collection of biogas from the system by minimizing the advent of a gas collection in the effluent, which, deprived of biogas and solids, will be of better quality (Chernicharo, 2007; lIM, 2009).

The UASB has been proven to be effective in the treatment of medium- to high-strength wastewater, such as PSW, but also toxic, recalcitrant and low-strength wastes (Pol et al., 2004). This is usually achieved through highly concentrated biomass.

3.11.2 Treatment of PSW using EGSB

The EGSB is a variant of the UASB, thus presents similar characteristics such as the reliance to the immobilized biomass for the treatment of various types of effluent, the up-flow configuration, and the use of a three-phase separator on top of the bioreactor (Chernicharo, 2007; Lim, 2009; Basitere *et al*., 2006). It was developed to improve the contact between the substrate and biomass through mixing, which is implemented by the increase of hydraulic up-flow velocity, which can exceed 6 m/h, and the height of the bioreactor to accommodate the bioreactor with the elevation of settled granular particles, and prevent their washout from the action of upward forces (Buoyancy and drag forces) overcoming their weight in order to retain the required biomass within the bioreactor (Lim, 2009; Yoochatchaval *et al*., 2008). This increase of the up-flow velocity is achieved either by the change of the influent flowrate or the use of a recycle stream, and results in the intensification of the hydraulic mixing within the EGSB (Chernicharo, 2007). The other objective behind the design of the EGSB was to improve the rate of treatment of various ranges of influent, through suitable contact between the organic matter fed into the system and anaerobic granular sludge (Henze *et al*., 2008). Therefore, EGSB reactors achieve extreme OLRs exceeding 30 to 40 kgCOD/m^3.day. Furthermore, the EGSB has been proven more efficient in the treatment of specific types of wastewaters, whose

appropriate treatment could have not been implemented using a standard UASB (Pol *et al*., 2004). According to Henze *et al.* (2008) these types of wastewater include:

- Wastewaters laden with biodegradable compounds, such as methanol formaldehyde;
- Wastewaters comprising long-chain fatty acids, which tend to absorb the sludge and form inaccessible clumps at low up-flow velocities of the UASB;
- Dilute and cold wastewaters, which have a low production of biogas and therefore a weak hydraulic mixing potential. In this case, high up-flow velocities of the EGSB allows the improvement of mixing in such medium; and
- Wastewaters culminating in foaming challenges in UASB systems.

3.11.3 Internal circulation (IC) reactor

The success of the EGSB contributed to increasing its popularity amongst HRABS (Pol *et al*., 2004; Lim, 2009). Thus, to further improve the performance of such systems, the internal circulation (IC) reactor, which is a variant of the EGSB, was developed (Henze *et al*., 2008). This bioreactor differs from the EGSB by an internal circulation system, in which the produced biogas is separated halfway the bioreactor by a gas-liquid separator device and transported upwards through a pipe to an expansion device or degasifier unit (Chernicharo, 2007; Henze *et al*., 2008). At this level, the collected biogas is taken out of the system, while the mixture of sludge and water returns to the bed of the bioreactor through another pipe. In this system, the recirculation of the liquid and granular sludge occurs over the lower part of the bioreactor and is conducted through the lifting forces of the collected biogas (Henze *et al*., 2008). This recirculation depends on the extent of biogas production from the system and culminates in improved contact between the biomass and the wastewater, and thus increased organic loads in such a system (Henze *et al*., 2008).

Further HRABS and their performance for the treatment of PSW will be discussed in subsequent sections.

3.12 Effects of static head losses on HRABS

The stability of high rate anaerobic bioreactor systems (HRABS) is usually determined through the maintenance within prescribed range of critical operational parameters such as the temperature, pH, lack of oxygen, feed composition, organic loading rate (OLR),

VFA/Alkalinity ratio or inhibitory concentration (Geradi, 2003; Henze *et al*., 2008). However, the operation of such systems heavily relies on the continuous flow of the feed through the system. This circulation may be jeopardized by head losses through the anaerobic granular sludge and/or underdrain systems when they are required mainly for down-flow configurations (Oh, 2012; Ellis and Evans, 2008). The anaerobic granular sludge is composed of aggregated cells which are usually spherical and therefore can be visualized as a packed bed of small spherical particles ranging from 0.06 to 0.50 cm (Mu *et al*., 2006). Anaerobic systems are airtight systems devoid of atmospheric pressure, thus the head losses experienced by the wastewater as it flows through the granules and underdrain systems result in the alteration of the fluid velocity along the height of the packed structure, which may culminate in the limitation of the distribution of the organic influent to the anaerobic biomass (Oh, 2012; Basitere *et al*., 2019). The importance of the good distribution of substrate in anaerobic systems is also highlighted by an increase in the growth of filamentous bacteria as compared to flocculating bacteria in zones of low substrate concentrations, which limits the performance of such systems (Gerardi, 2003). This limitation could further worsen the operation of HRABS by an accumulation of the feed in the system, the minimization of the process throughput and related challenges depicted in Figure 3.7. Therefore, it appears critical to account for head losses when monitoring HRABS.

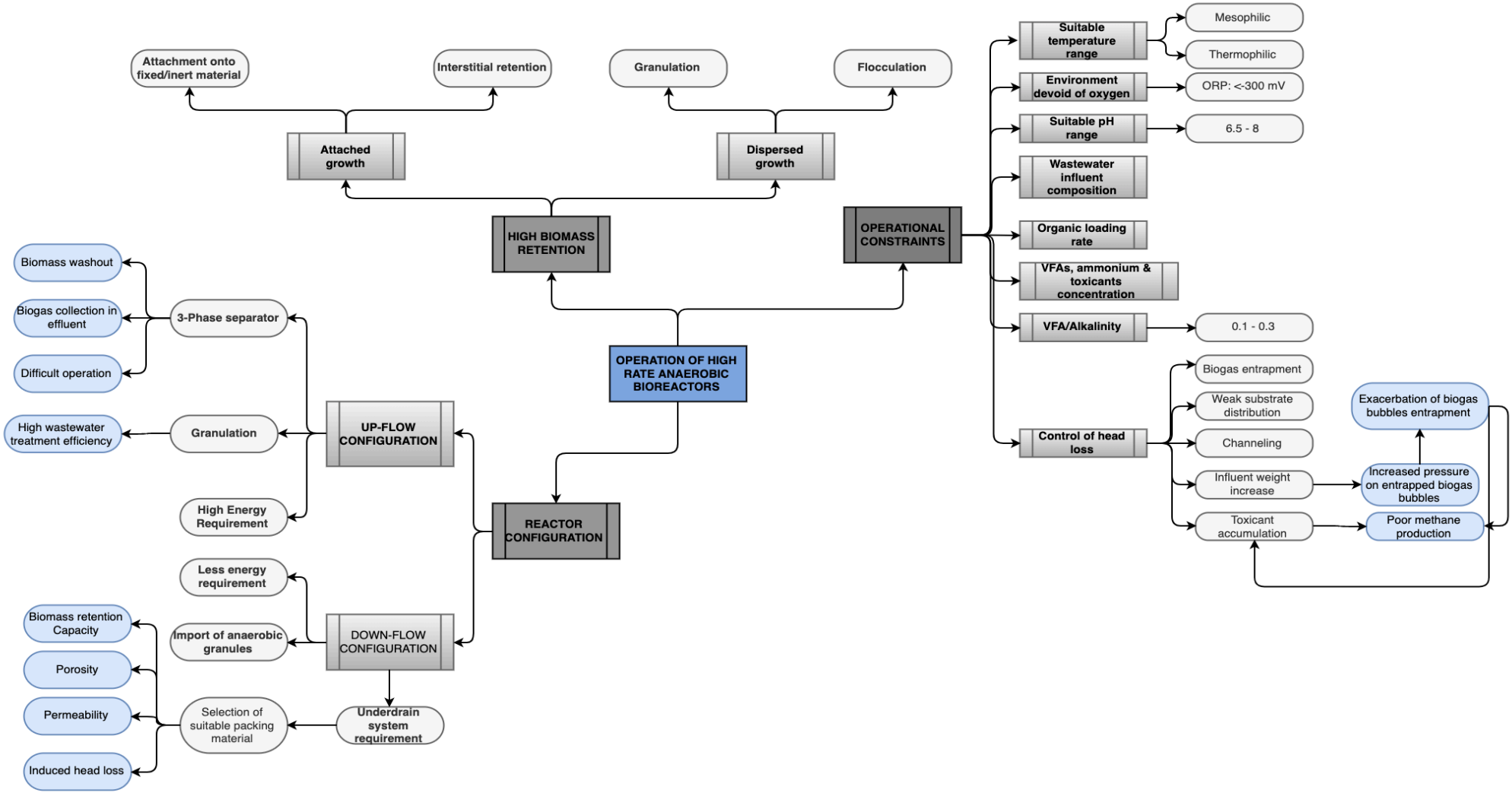

Figure 3.7: Parameters affecting the operation of high rate anaerobic bioreactors (Adapted from Gerardi, 2003; Metcalf and Eddy, 2003)

3.12.1 Evaluation of head losses in HRABS

In wastewater engineering, fixed bed or packed bed bioreactors are commonly used with attached biofilms. They are widely used with immobilized cells, which are fed with nutrients either from the top or the bottom of the bioreactor (Metcalf and Eddy, 2003; Henze *et al*., 2008). The disadvantages of such bioreactors include the changed flow characteristics stemming from the alteration of the bed porosity during the operation; the bed compaction occurring during fermentation culminates in high-pressure drop across the bed; and channeling, which may occur as a result of turbulence in the packed bed (Chernicharo, 2007; Henze *et al*., 2008; Metcalf and Eddy, 2003). Additionally, such a system may be affected by back mixing, which might change the characteristics of fermentation (Metcalf and Eddy, 2003).

The evaluation of the pressure drop across packed systems has been approached by several researchers including Darcy (1856), Forchheimer (1901), Brinkman, Blake (1922), Carman (1937), Kozeny (1927) and Ergun (1952). Darcy (1856) expressed the pressure gradient to the superficial velocity, given by the ratio of the viscosity to permeability. However, his approach lacked to account for the turbulent regimes developed as a result of superficial velocity increase. Forchheimer (1901) attempted to correct this shortcoming by including a second-order velocity term to the Darcy model to consider the inertial effects caused by the acceleration of flow through packed beds. Brinkman further developed the Forchheimer model by introducing a macroscopic shearing parameter between the pore walls and the walls. This by including a second-order derivative to the Darcy model to describe the velocity profile. However, this model is not highly acclaimed due to the negligible change of velocity profile across pores within the porous media. Blake (1922) further investigated the pressure drop across packed beds by suggesting a modified dimensionless group that includes the voidage (ε) of the packed bed and the use of the interstitial velocity instead of the superficial velocity. Kozeny (1927) and Carman (1937) also investigated the behaviour of various fluids through different packed materials and adopted a pipe flow analogy, which envisions a packed bed as a group of parallel and identical channels. This approach led to the development of a correlation that is limited to the creeping flow range, which requires empirical models for cases not considered in the evaluated range. This limitation was considered by Ergun (1952), who developed a semi-empirical correlation widely used. The Ergun correlation can be used to determine the pressure drop across a packed bed composed of both regular and irregularly-

shaped packing materials under any type of flow (laminar or turbulent). Ergun used the Carman-Kozeny correlation for laminar flow conditions and complemented it with the Burke-Plummer correlation for fully-turbulent flow conditions to come up with a model that accounts for various flow regimes and different shapes of packing materials. The Ergun correlation is provided by Equation 3.1. The only limitation of this correlation is its inability to predict the pressure drop after the incipient of fluidization, as a result of packed bed voidage changes.

$$\frac{\Delta P}{L} = \frac{150\mu v_0}{\emptyset^2 d_P^2}\frac{(1-\varepsilon)^2}{\varepsilon^3} + \frac{1.75\rho_F v_0^2}{\emptyset d_P}\frac{(1-\varepsilon)}{\varepsilon^3} \quad (3.1)$$

Where $\emptyset$ is the sphericity, ΔP is the pressure drop (Pa), L the height of the packed bed (m), μ the fluid viscosity ($Pa.s$), d_p particle diameter (m), ε packed bed voidage, ρ_F fluid density (kg/m^3), v_0 superficial velocity (m/s).

The Ergun equation can be integrated into the mechanical energy balance, which provides a means to correlate the pressure drop across such systems to the velocity of the flowing substrate as well as other parameters involved in the transport of the fluid. The mechanical energy balance provides a clear interpretation of how the energy is conserved in a system, despite its transfer or distribution from one point to another within the system in question (Felder and Rousseau, 2005). It follows the same reasoning as the material balance, which is governed by the principle of conservation of mass, or the momentum balance in fluid flow related cases (Felder and Rousseau, 2005).

The mechanical energy balance is given by Equation 3.2.

$$W_p = \frac{(P_b - P_a)}{\rho} + g(Z_b - Z_a) + 1/2 \propto \left(V_b^2 - V_a^2\right) + h_f \quad (3.2)$$

From the left to the right, the first term represents the shaft work done by the pump, the first right term is the pressure difference between the end and source point, the second term is the hydrostatic pressure resulting from the height difference between the end and the source points, the third term is the pressure change resulting from velocity change, and the last term relates to the friction losses within the system, which can be calculated with the Ergun equation for frictions generated by a packed bed such as the anaerobic granular bed.

Equation 3.2 can be used provided that there is a single input, single output, no phase change, no variation in temperature and no reaction. Furthermore, relates to the type of flow regime. It is equivalent to 1 for turbulent flows and 0.5 for laminar flows (Felder and Rousseau, 2015).

3.12.2 Effects of head losses on anaerobic granule-based reactors

As per Equation 3.2, the first inconvenient of head losses in HRABS is the incidence of the energy required to induce the flow of PSW across the granular bed. This allows the contact between the substrate and the biomass and thus facilitates the maintenance and growth of the anaerobic biomass within the bioreactor, and the treatment of the organic influent. Good methanogenic activity in HRABS results in the production of biogas. However, there were instances where a good removal of the substrate from the influent, which usually translates to a good COD or BOD_5 removal percentage, didn't align with consequent production of biogas (Basitere *et al*., 2016; Basitere *et al*., 2017). This shortcoming may be justified by several factors including the entrapment of the of biogas within the anaerobic granular bed as a result of loss in kinetic energy due to frictions losses, a weak connected porosity of the anaerobic granular bed or high surface tensions weakening the emergence of biogas bubbles (Meier *et al*., 2011; Mu *et al*., 2006; Basitere *et al.* 2017).

The exploration of the phenomena related to the emergence of biogas bubbles from a submerged granular bed has led to the finding that the emergence of gas from a submerged granular bed follows two different modes (Meier *et al*., 2011), namely:

- The percolation of small bubbles through the interstices of the granular bed (mode 1); and
- The cumulative growth of a larger bubble that emerges after overcoming the surface tension effects by its buoyancy (mode 2).

This emergence, particularly for the second mode of emergence, contributes to the mixing of the granular bed, and therefore improves the contact between the biomass and substrate and facilitates the dispersion of toxic substances that may be detrimental to anaerobic digestion (Henze *et al*., 2008; Chernicharo, 2007). The emergence of gas bubbles from a granular bed is usually accompanied by their shrinking as they face the effects of surface tension in such medium, but this effect is alleviated as they reach near the surface of the granular bed; this alleviation normally translates to the expansion of these bubbles (Mu *et al.*, 2006).

The mode of emergence of gas bubbles from the granular bed depends on factors such as the diameter of the granular grains, size of the bubbles, density of the granular bed, porosity of the granular bed, which also depends on the arrangement, shape and size of the grains (Hulschoff Pol *et al*., 2004; Meier *et al*., 2011). Thus, for a given bubble flow rate, mode 1 would prevail for larger grain sizes, whereas the mode 2 would occur for small grain sizes. Mode 2 would also be prevalent for increased gas flow rates. This was demonstrated by the experiment

conducted by Meier *et al*. (2011) on the study on bubbles emerging from a submerged granular bed, whereby the two modes of bubbles emergence were observed from varying the airflow rate inside a granular bed and the size of the glass beads composing the granular bed. Furthermore, this study also examined the effect of the particle density through using two distinct 3 mm glass beads (hollow and solid), and it was found that the bubbles flowing through the solid beads (2500 kg/m^3) were more inclined to display the percolation of small bubbles through the interstices of the glass beads, while the ones flowing through the hollow beads (1400 kg/m^3) predominantly demonstrated the mode 2 of bubbles emergence.

The difference between these two modes of bubbles emergence can be explained by the size and arrangement of grains, as the arrangement of large grains often provide large interstices that allow small bubbles to percolate through, while a similar arrangement of smaller grains would result in smaller interstices not large enough to facilitate the percolation of the gas bubbles, but to allow the collection of these bubbles until the agglomerated bubble becomes large enough for its buoyancy to overcome the surface tension forces exerted on them (Mu *et al*., 2006; Hulschoff Pol *et al*., 2004; Meier *et al*., 2011). Thus, including parameters such as the density of the liquid in which the granules are immersed, surface tension, and the diameter of the grains, the bubble diameter required for matching the surface tension and buoyancy can be calculated as prescribed by Equation 3.3 (Meier *et al*., 2011).

$$D_B = \frac{6S}{\rho g d} \tag{3.3}$$

Where g is the acceleration of gravity.

From Equation 3.3, it can be observed that the value of the surface tension, which represents the main resistance to the bubble's emergence, is proportional to the density of liquid, as well as the diameters of the grains and bubble, suggesting that the denser the grains the less is the likelihood of having the bubbles emerging according to the mode 2, but alternatively through the mode 1. However, the mode 1 can be altered by the connected porosity or the height of the granular bed, as the elapsed distance is a critical parameter affecting the pressure drop, and thus the kinetic velocity of fluids, in such systems.

The connected porosity may be defined as the ratio of the connected pore volume to the total volume of a packing arrangement (Holdich, 2002). The connected porosity differs from the normal porosity because the latter lacks to provide information regarding the pore size, their degree of connectivity and distribution (Holdich, 2002, Meier *et al*., 2011). In this regard, a packing arrangement of miscellaneous packing materials may have a similar porosity but

different permeabilities, as the flow of the fluid might not be continuous throughout the height of the packing arrangement. Generally, the initial porosity also referred to as the pre-diagenesis, is influenced by four microstructural parameters including the grain packing, particle shape, grain size, and distribution of grain sizes (Meier et al., 2011; Gavala et al., 2003). In reality, the ordered packing lattices are not randomly formed due to the energetic instability of an ordered arrangement, which favours a random distribution of grains, which may not provide the best value of connected porosity (Mu et al., 2006; Meier *et al.*,2011). The arrangement of particles presenting irregular shapes usually offers larger gaps in their interstices, thus increasing the porosity of such solid particle arrangement (Leva *et al.*, 1951; Metcalf and Eddy, 2003; Holdich, 2002).

3.13 Summary

This Chapter provided the characteristics of PSW and elaborated on the treatment options that can be used to approach its treatment. From the options suggested, high rate anaerobic treatment of PSW appeared as the most attractive treatment option due to smaller plant footprint requirement, high efficiency, the production of biogas, and the generation of a manageable quantity of sludge that can be used for the inoculation of another HRABS. Furthermore, the anaerobic treatment was thoroughly described, and challenges associated with its use were discussed. To provide good descriptions, some HRABS were selected and described in further detail. Other HRABS will be presented in subsequent sections. There were essentially developed to address the limitations of listed HRABS, which will also be discussed. However, it is essential to gain a good insight into the wastewater to be treated before approaching its treatment. This analysis and characterization are provided in Chapter 4.

CHAPTER 4

ANALYSIS OF THE CHARACTERISTICS OF POULTRY SLAUGHTERHOUSE WASTEWATER (PSW)

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Chapter 4: ANALYSIS OF THE CHARACTERISTICS OF POULTRY SLAUGHTERHOUSE WASTEWATER (PSW)

4.1 Introduction

4.1.1 Background

The increase of the global population aligns with the growing demand for food, including meat products. The United States Department of Agriculture (USDA) (2019) compared the total production of beef and veal, pork, and chicken meats in selected countries of the world (See Figure 4.1), including South Africa, from which it was observed that chicken meat is highly sought after (USDA, 2019). This demand in poultry meat comes before beef and veal meat and shortly after pork meat. This trend has remained consistent over the last 4 years and is predicted to remain as such for the next years (USDA, 2019).

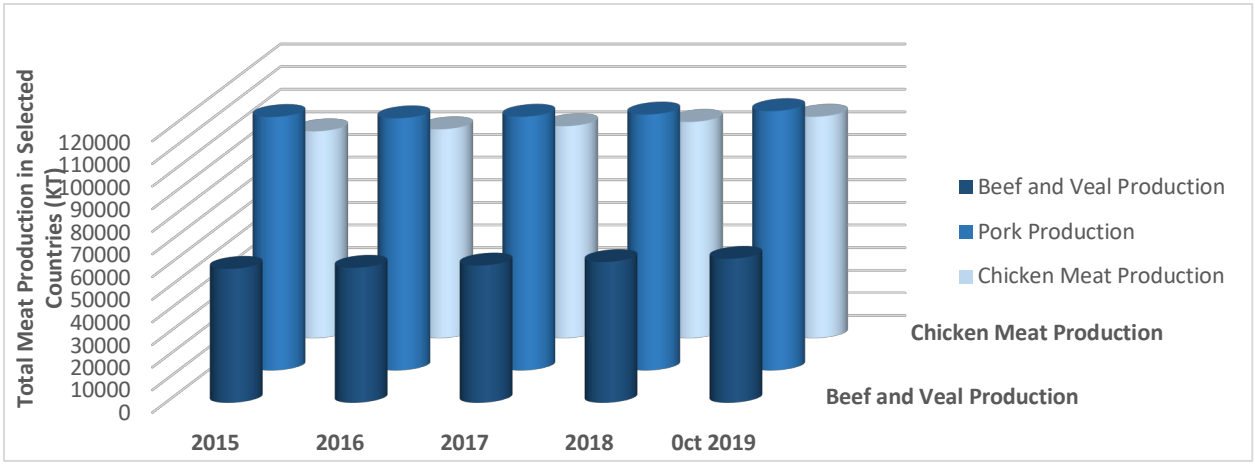


Figure 4.1: Comparison of consumption of beef and veal, pork and chicken meat worldwide (Adapted from USDA, 2019)

The global production and consumption of poultry meat are further highlighted in Figure 4.2 and 4.3, respectively; from which the domination of United States in the production of poultry meat can be noticed, while South Africa fails to be ranked among the highest producers of poultry meat (USDA, 2019). However, this trend changes when it relates to the consumption of poultry meat (see Figure 4.3) with a noticeable consumption of poultry meat from South Africa, which has remained high over the last 4 years and is projected to increase in the future. Apart from the increase of the population, this demand of poultry meat is also driven by other factors, including its palatability and affordability, which is driven by an increased supply due to technological advances and the increase of the number of poultry farms in the country (Barbut, 2015; Bolton, 2015). This increase in the production of poultry meat is associated with an increased generation of PSW, which results from an abundant use of potable water (approximately 26.5 L/Bird) for the processing of birds.

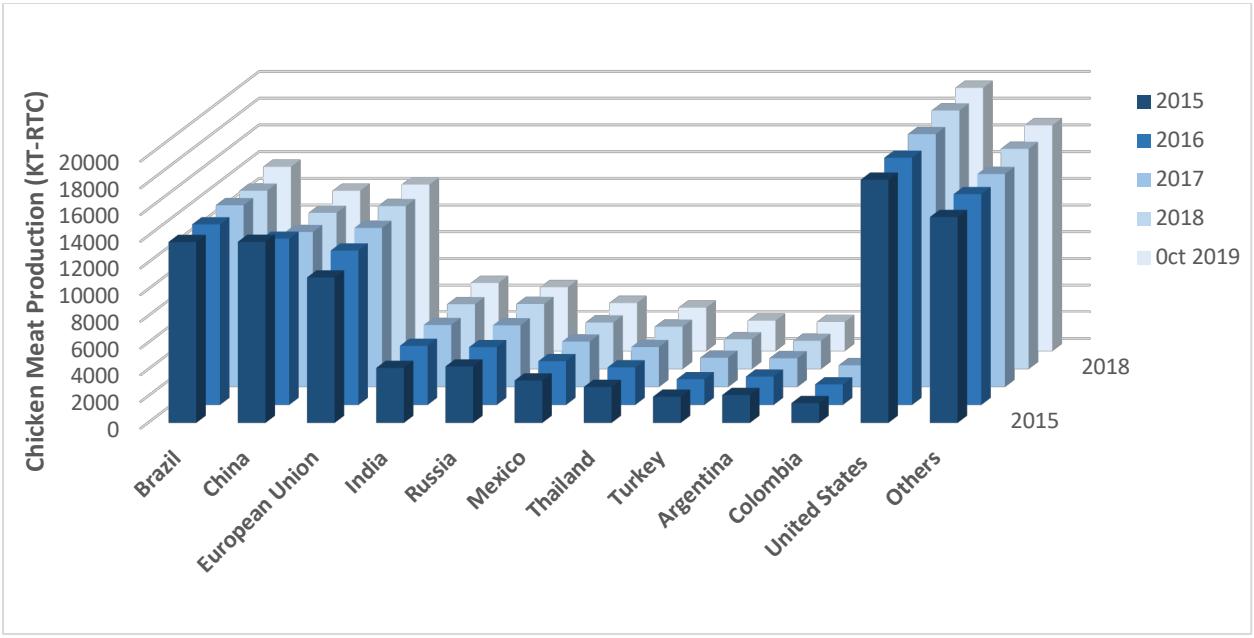


Figure 4.2: Comparison of the production of poultry meat in selected countries (Adapted from USDA, 2019)

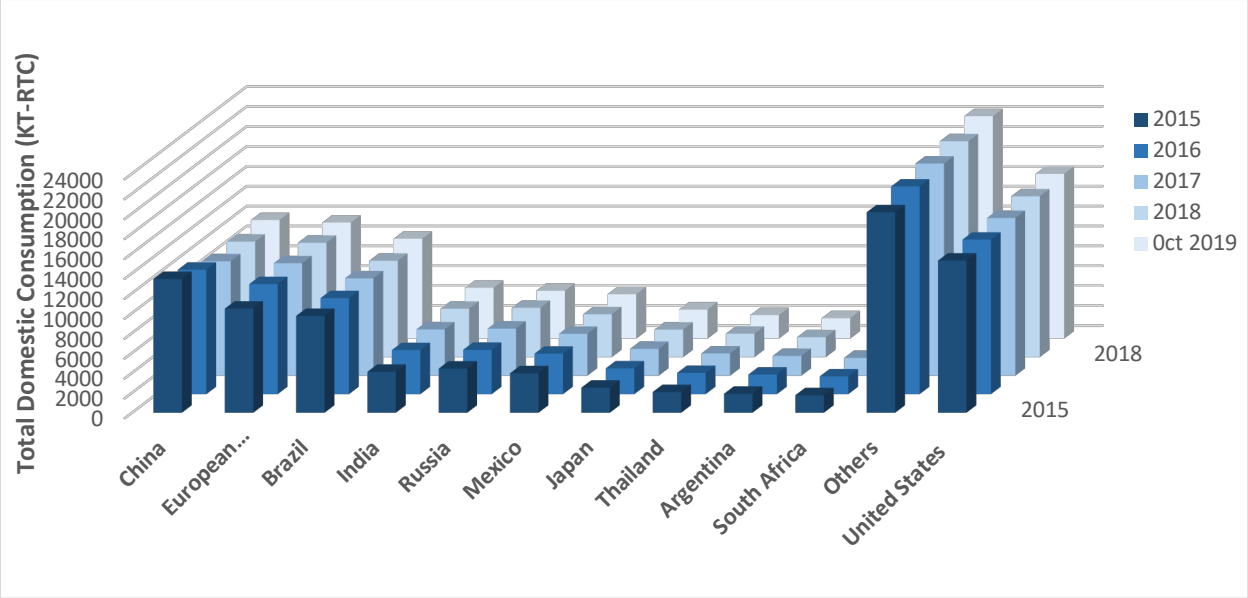


Figure 4.3: Comparison of the consumption of poultry meat in selected countries (Adapted from USDA, 2019)

Eutrophication and spreading of water-borne diseases are listed among the effects of the discharge of untreated poultry slaughterhouse wastewater (PSW) into water channels and surface water (Basitere *et al.*, 2017; Njoya *et al.*, 2019). These effects may lead to serious environmental and health concerns that could be prevented by the treatment of PSW before its discharge. Moreover, the global challenge of water scarcity could be approached by promoting the treatment and recycling of the wastewater generated from various industries, including the poultry industry. In 2014, the average annual consumption of potable water by the poultry industry in South Africa was estimated at 32 564 576 m^3 (DEA & DP, 2015). Certainly, this consumption is higher now when considering an annual growth of 7% in this sector. In poultry slaughterhouse facilities, potable water is usually required for various operations (stunning and slaughtering; de-feathering; evisceration; trimming and carcass washing; de-boning; chilling; cleaning and waste disposal, amongst others), which culminate in the generation of a reddish wastewater laden with nutrients, fats, oil and greases, faeces, carcass debris, blood, feathers and traces of heavy metals. The characterization of PSW is deemed important for the design of an efficient and cost-effective process for its treatment to address the challenges listed above.

Therefore, various researchers have investigated PSW characteristics (Bustillo-Lecompte and Mehrvar, 2017; Yaakobb *et al*., 2018; Barbut, 2015), which are provided in Table 4.1.

Table 4.1: Characteristics of PSW from previous studies (Adapted from Yaakob et al., 2018; Bustillo-Lecompte et al., 2016)

	Location	Parit Raja, Malaysia		Ontario, Canada	
Parameter	Unit	Range	Average	Range	Average
pH	-	7.3 - 8.6	8.2 ± 0.42	4.9 - 8.1	6.95
BOD_5	mg/L	1341 - 1821	1602 ± 243	610 - 4635	1209
tCOD	mg/L	3154 - 7719	5422 ± 2282	1250 - 15900	4221
TSS	mg/L	377 - 5462	3438 ± 2696	300 - 2800	1164
TN	mg/L	162.6 - 564	361 ± 215	50 - 841	427
TOC	mg/L	195 - 651	419 ± 222	100 - 1200	546
PO_4^{3-}	mg/L	7 - 17.1	12.3 ± 4.25	n.a.	n.a.
NO3	mg/L	1.64 - 3.3	2.24 ± 0.58	n.a.	n.a.

n.a.: Not applicable

From Table 4.1, it is noticeable that the parameters investigated by the researchers, i.e. Bustillo-Lecompte et al. (2016) in Ontario, Canada, and Yaakob et al. (2018) in Parit Raja, Malaysia, differ from one study to another. This could be related to various factors including the prevailing slaughterhouse operation during the wastewater sampling, the quantity of potable water used for processing a single bird, or the difference in the nutritional quality of the birds slaughtered (Avula *et al*., 2009). These factors could be driven by various socio-economic factors related to the location of the poultry slaughterhouse in the world (Barbut, 2015); Yaakob *et al*., 2018; Njoya *et al*., 2019). Therefore, it appears legitimate to provide a local characterization of PSW. Moreover, the development of correlations between relatable parameters of PSW can also serve to circumvent the requirement of running all the analyses to characterize PSW. One of these tests is the BOD_5 that takes up to 5 days to provide results while the tCOD could be tested in a couple of hours (3 to 4 hours).

4.1.2 Objectives

This study aims at characterizing PSW and providing a solution towards the reduction of number analysis tests performed to assess the quality of such an effluent. To this end, the following objectives should be achieved:

- Provide a local characterization of PSW to enable the selection or design of suitable treatment processes,
- Correlate the concentration of COD, BOD_5, and FOG using linear regressions to further characterize PSW and minimize the number of required analysis tests,
- Use the correlation equations to provide a means to reduce PSW analysis cost, and the minimization of chemical wastes generated from these analyses, and
- Determine PSW biodegradability to assess its inclination towards environmental pollution when discharged untreated, and its biological treatability.

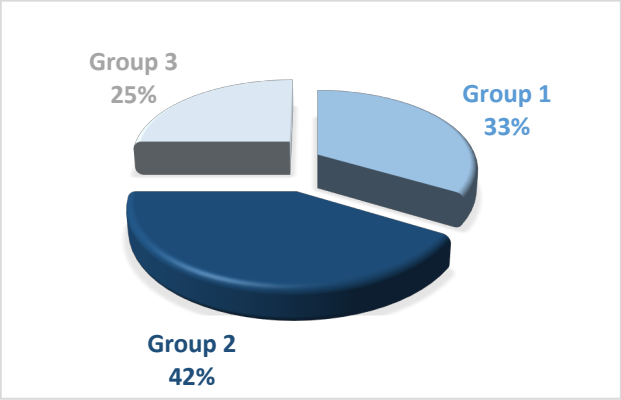
4.2 Materials and Methods

4.2.1 Background

The PSW used in this study was collected from a poultry slaughterhouse located in the Western Cape, South Africa, at different stages of the poultry processing process. To factor in prevailing operations at various stages of the poultry processing, the samples were collected at different periods of the day. Samples from group 1 were collected in the morning, while samples of group 2 were collected in the afternoon, and the samples from the third group were collected around noon. The distribution of the samples is provided in Figure 4.4. The poultry slaughterhouse from which samples were collected had a weekly throughput of a million birds averaging a weight of 2.2 kgs/bird. The samples were collected from a wastewater stream to a clarification tank with a 1 L bucket that was used to fill up a 20L polystyrene container during the processing of chickens. The 20 L container was stored in the refrigerator at a temperature below or equal to 4^{o}C after getting a representative sample from agitating the container's content before sampling in the laboratory.

Number of samples		
Group 1	Group 2	Group 3
20	25	15

(a)



(b)

Figure 4.4: Grouping of samples

4.2.2 PSW analysis

After collection, the samples were analyzed every week to determine the concentration of water quality assessment parameters, including the total suspended solids (TSS), total chemical oxygen demand (tCOD), biological oxygen demand (BOD_5), volatile fatty acids (VFA), alkalinity, and fats, oil and grease (FOG), as per the methods illustrated in Table 4.2. Other tests, including pH, total dissolved solids, salinity, temperature, and turbidity, were performed daily. All analyses were performed in triplicate. As per Figure 4, a total of 60 samples regrouped into three groups were analyzed.

Table 4.2: PSW analysis methods

Parameter	Method
pH	EPA Method 9040C
Total dissolved solids (TDS)	EPA method 160.1
Salinity	EPA method 320
Temperature	EPA Method 9040C
Turbidity	EPA method 180.1
Total suspended solids (TSS)	EPA method 160.2
Total chemical oxygen demand (TCOD)	EPA method 410.4
Biological oxygen demand (BOD5)	EPA method 5210 B
Volatile fatty acids (VFAs) as acetic acid	Potentiometric titration
Alkalinity as $CaCO_3$	Titration method 2320 B
Fats, oils, and grease (FOG)	EPA method 10056

The analysis methods of FOG, tCOD, and BOD_5 are described subsequently.

4.2.2.1 FOG Analysis (EPA method 10056)

FOG analysis consisted of determining the concentration of fats, oils, waxes and other related constituents found in the PSW. The release of untreated wastewater with a high FOG concentration to surface waters can interfere with biological life in such an environment and promote the creation of hideous films at its surface.

In this study, the analysis of FOG was done externally in the City of Cape Town Wastewater Treatment Plant Laboratory, as per the EPA method 10056. The EPA method 10056 relies on N-hexane for the extraction of animal fats, waxes, greases, soaps, non-volatile hydrocarbons and related lipids (Down and Lehr, 2005). FOGs are hydrophobic compounds; therefore, their analysis requires glassware that is pre-rinsed with the sample of PSW before collection (Down and Lehr, 2005; Kaur, 2007). To avoid the contamination of the sample before the analysis, it was conserved at a temperature below $4^{o}C$ for a maximum of 28 days. Additionally, in the beginning, the sample was conserved at pH <2 using sulfuric (H_2SO_4) or hydrochloric (HCl) acid. The sample was then transferred to a separatory funnel, 20 mL of N-hexane was added to it, the content of the funnel was agitated energetically, and then allowed to settle to enable the separation of the FOG from the solution. The denser layer (aqueous solution) was drained into a different container to separate it from the hexane layer, which was then transferred to a funnel containing anhydrous sodium sulfate to minimize the concentration of water from the extract. The extract was kept in a pre-weighed flask. This procedure was repeated three times to ensure the collection of all grease and oil compounds from the sample. Thereafter, the solvent was evaporated, and the pre-weighed flask was weighed once again. The difference in mass provided the concentration of fats, oil, and grease in the sample.

4.2.2.2 Total chemical Oxygen demand (tCOD) analysis (EPA method 410.4)

The total chemical oxygen demand serves as a method to determine the quantity of oxygen that would be used by a body of receiving water to process the nutrients contained in the wastewater. This differs from soluble chemical oxygen demand (sCOD), which is deprived of suspended

solids before the analysis and therefore has a lower oxygen requirement (Kaur, 2007). The tCOD reflects the energy content of a feedstock by indicating the concentration of total oxidizable material in a sample of wastewater (Kaur, 2007; Down and Lehr, 2007). The results of the tCOD test are provided in mg/L COD. This translates to milligrams of oxygen-depleted per liter of sample. The tCOD analysis consisted of heating a sample of PSW at 148°C for 2 hours with sulfuric acid (H_2SO_4) and a strong oxidizing agent such as potassium dichromate ($K_2Cr_2O_7$). For improved oxidation performance, a silver sulfate catalyst can be added to the solution. While being heated, the oxidizable organic compounds reduced the yellow dichromate ion ($Cr_2O_7^{2-}$) to green chromic ion (Cr^{3+}) during the reaction. It should be noted that 1 mol of $K_2Cr_2O_7$ is equivalent to 1.5 mol of O_2. The reduction of dichromate ions was quantified spectrophotometrically at 445 nm and directly related to the mass of oxygen consumed per liter of solution (mg/l COD is proportional to mg/L of oxygen).

The tCOD of PSW can be evaluated at different ranges (low and high range), as illustrated in Table 4.3 and 4.4. These tables provide the volume of reagents and samples mixed and shacked in glass cell tubes, before being heated at 148°C for two hours in a thermo-reactor. After two hours, cell tubes were removed and allowed to cool down in cell tube trays. Half an hour later, the content of the glass cell tube was mixed on a shaker, and then the concentration of the COD concentration in each sample was measured using a NOVA 60 photometer, which was calibrated as per the range investigated.

Table 4.3: Parameters of the measurement of COD in a high range

HIGH RANGE		Range (mg/L) 500 - 10000
	Volume (mL)	Content
Solution A	2.2	Sulfuric acid, Mercury (III) Sulphate
Solution B	1.8	Sulfuric acid, Potassium dichromate
Sample	1	PSW
Total	5	

Table 4.4: Parameters of the measurement of COD in low range

LOW RANGE	Range (mg/L) 100 - 1500	
	Volume (mL)	Content
Solution A	0.3	Mercury (II) Sulphate
Solution B	2.3	Sulfuric acid, Potassium dichromate
Sample	3	PSW
Total	5.6	

4.2.2.3 BOD_5 analysis (EPA method 5210 B)

The BOD_5 and tCOD both measure the quantity of organic matter in a sample of wastewater. However, the BOD_5 differs from the tCOD because it quantifies biologically oxidized organic matter, while the tCOD quantifies materials that can be chemically oxidized.

The EPA method 5210 B is based on the determination of the quantity of dissolved oxygen consumed by a sample of PSW in 5 days. To this end, an airtight 300 mL incubation bottle was filled with PSW sample and incubated at 20°C for five days. It was important to maintain the incubator dark to prevent the formation of dissolved oxygen in the sample through photosynthesis. In this analysis, the concentration of dissolved oxygen in the samples was measured before and after the incubation. The dissolved oxygen concentration in the samples was measured using a dissolved oxygen sensor. Before the measurement, the sample was vigorously manually agitated to promote the accuracy of the measurement through the dispersion of floatable and settleable solids, and the homogeneity of the sample.

4.3. Results and Discussion

4.3.1 Characteristics of PSW

Table 5 provides a summary of the results of the PSW analysis. The PSW of the three sampling groups analyzed present fairly similar results, with tCOD average concentrations of 4981 ± 1832, 5216 ± 2534, 5354 ± 1810, for groups 1, 2 and 3, respectively. This similarity is further illustrated for the concentration of TSS, BOD_5, salinity, conductivity, total dissolved solids (TDS), alkalinity

and VFA in the three groups. The turbidity level of each group appears to be following the same trend, with average turbidity values falling into the same range (~730 NTU). For the FOG, the concentration in the second group (5216 ± 2534) is significantly higher than the ones of groups 1 and 3 (795 ± 367 and 738 ± 374). This difference reflects a higher concentration of FOG in the wastewater during the sample collection period of group 2, which might be related to nutrition and/or size of the birds during that period. The FOG concentration of such wastewaters usually originates from a fatty carcass that gets collected in the PSW during operations such as evisceration or carcass washing. This high FOG content in the second group reflects also a challenge associated with the treatment of PSW, which is known for its high FOG concentration (Kiepper, 2003, Avula et al., 2009), and suggests the requirement of a pre-treatment unit such as filtration to reduce the FOG concentration prior to a biological treatment like anaerobic digestion (Basitere et al. 2017; Williams et al., 2018).

The tCOD, BOD_5, and TSS values determined from this study (Table 4.5) were compared to the ones provided by previous studies (Table 4.1) (Bustillo-Lecompte *et al*., 2016; Yaakob *et al*., 2018). While the tCOD and TSS of this study concentrations values are close to the one of previous studies (Table 4.1), the BOD_5 of this study looks higher than the one of previous studies. This suggests a difference of the characteristics of PSW with the location, as a result of different operational requirements and techniques.

Table 4.6 provides the discharge standards of industrial effluent to water bodies in different parts of the world (Bustillo-Lecompte and Mehrvar, 2017; DWAF, 1998). A comparison of the results of this study to these discharge standards stresses the requirement of treatment of PSW, as it has a BOD_5 concentration at least 50 times higher than the limit imposed by regulations. Excess BOD_5 may lead to the depletion of dissolved oxygen of receiving water bodies, culminating in the death of the aquatic fauna and anaerobiosis (Abdel-Raouf *et al*., 2012). Low levels of dissolved oxygen can be detrimental to aquatic life, while high levels can induce the corrosion of metal pipes. This excess concentration is also noticed for the tCOD and TSS, which should be significantly reduced before discharge.

Table 4.5: Characteristics of PSW in this study

Parameters		Group 1		Group 2		Group 3	
	Unit	Range	Average (±SD)	Range	Average (±SD)	Range	Average (±SD)
pH	-	6 - 8	-	6.13 - 7.24	-	6.29 - 7.13	-
Conductivity	µs/cm	798 - 2360	1479 ± 412	973 - 2405	1604 ±414	899 - 2450	1769 ± 425.96
TDS	ppm	567 - 2145	1059 ± 303	691 - 1693	1138 ±294	639 - 1740	1250 ± 302.09
Salinity	ppm	390 - 926	772 ± 178	529 - 1413	916 ±179	451 - 1240	880 ± 189.80
Turbidity	NTU	99 - 1847	749 ± 342	237 - 997	719 ±201	328.5 - 864.5	758 ± 158.50
tCOD	mg/L	1423 - 11068	4981 ± 1832	2517 - 12490	5216 ±2534	2280 - 11425	5354.50 ± 1810
TSS	mg/L	60 - 5165	1399 ± 1213	313 - 8200	1654 ±1695	291 - 5044	1750.16 ± 1125
FOG	mg/L	312 - 1542	795 ± 367	2517 - 12490	5216 ±2534	280 - 1668	738.00 ± 374
BOD_5	mg/L	850 - 6125	3090 ± 1453	925 - 5000	2477 ±1347	850 - 4250	3000 ± 958
VFA	mg/L	71 - 721	383 ± 230	105 - 898	375 ±213	74 - 548	350 ± 167.64
Alkalinity	mg/L	415 - 1022	520.8 ± 145	322 - 923	499 ±158	360 - 926	602 ± 208.68

Table 4.6: Regulations and discharge limits in different areas of the world (Adapted from Bustillo-Lecompte and Mehrvar, 2017; DWAF, 1998)

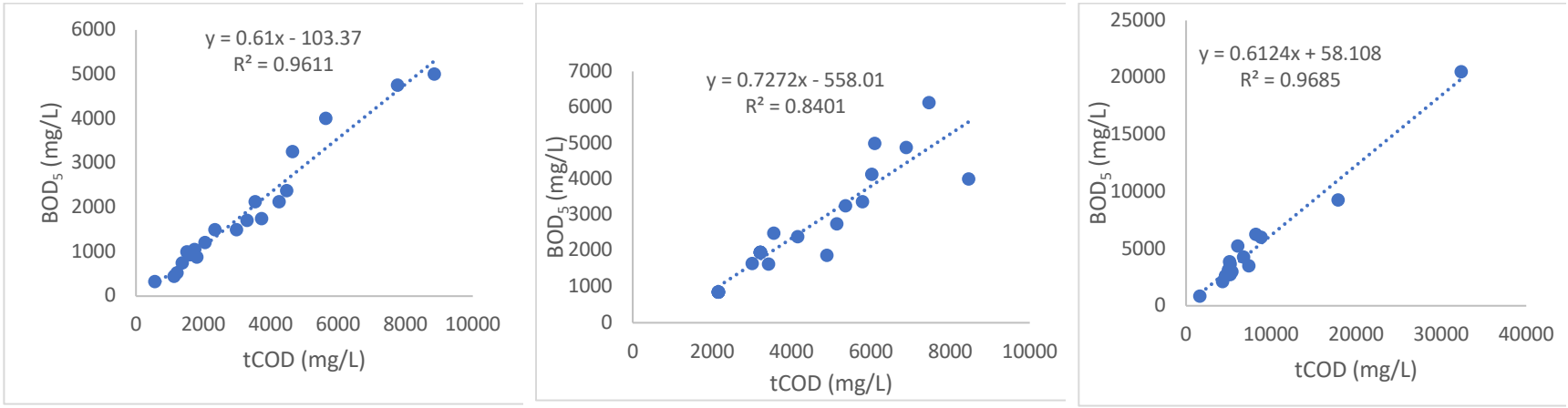
Parameter	Unit	World Bank	EU	USA	Australia	Colombia	South Africa	China	India	Canada
BOD_5	mg/L	30	25	16 - 26	5 -20	50	-	20 -100	30 -100	5 - 30
tCOD	mg/L	125	125	-	40	150	75	100 - 300	250	-
TN	mg/L	10	10 -15	4 - 8	10 - 20	10	15	15 - 20	10 -50	1.25
TOC	mg/L	-	-	-	10	-	-	20 - 60	-	-
TP	mg/L	2	1 - 2	-	2	-	10	0.1 - 1	5	1
TSS	mg/L	5	35 - 60	20 - 30	5 - 20	50	25	20 - 30	100	5 - 30
pH	-	6 - 9	-	6 - 9	5 - 9	6 - 9	5.5 - 9.5	6 - 9	5.5 - 9	6 - 9
Temperature	°C	-	-	-	<2	-	25	-	<5	<1

4.3.2 Biodegradability index of PSW and correlation between FOG and tCOD

The study of the biodegradability index of various types of wastewater has been approached by different researchers (Abdalla and Hammam, 2014; Papadoupolos *et al.*, 2001; Esener *et al*., 1981) to improve the design and operations of wastewater treatment systems. The biodegradability index of wastewater translates to its ability to get biologically decomposed (Abdalla and Hammam, 2014). Furthermore, this correlation (BOD_5/COD) can be used to determine the BOD_5 of PSW without running a test, which requires 5 days. The tCOD of analyzed samples of the three groups was plotted against their corresponding BOD_5, as illustrated in Fig 4.5.a, b, and c. It can be noticed that the correlation coefficients R^2 are 0.96 for groups 1 and 3, which translates to a BOD_5 concentration equivalent to 0.61 times the tCOD concentration during normal operational hours (morning and noon, respectively). This ratio also suggests a good biodegradability potential of PSW. The evaluation of the biodegradability of PSW provides a means to assess the degree of potential pollution it may induced when discharged untreated and determine the efficacy of biological treatment to minimize the endangerment of the environment. tCOD measures the concentration of organic matter in wastewater, while BOD_5 provides the concentration of biodegradable matter in the same sample (Vollersten and Hvitved-Jacobsen, 2002). Therefore, a slope 0.61 from a linear regression between BOD_5 and tCOD suggests that roughly more than 60% of the organic matter represented by tCOD could be biologically processed.

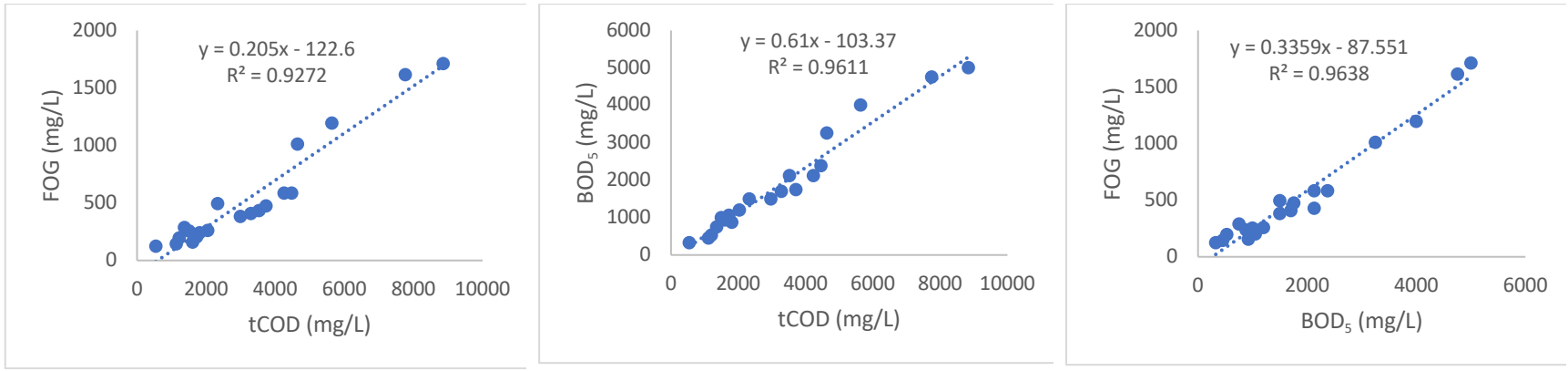
Furthermore, this slope also provides a means to relate the concentration of the tCOD to the one of BOD_5 for PSW. A higher slope (0.73 with an R^2 of 0.84) was noticed from the second, which was collected in the afternoon when the poultry slaughterhouse operations were winding down in the poultry slaughterhouse. This higher slope from the ratio between tCOD and BOD_5 in samples of group 2 suggests the presence of even more biodegradable organic matter in the PSW collected during that time of the day, as illustrated by Figure 9.a and 9.b, from which it can be noticed than the average concentration of BOD_5 is slightly higher than the average concentration of BOD_5 in samples from the group 1 and 3, as illustrated by Figure 4.7.a, 4.7.b, 4.11.a and 4.11.b. This difference between the slope of the linear regression of samples of group 2 to samples of

group 1 and 3 may be attributed to the prevailing operation during that time of the day in the poultry slaughterhouse operated routinely, when operations cease and the equipment is cleaned to maintain hygienic standards in facilities. Products collected during the cleaning of the slaughtering equipment may include feces, carcass debris, feathers, and blood from the carcass broilers, which get collected in the effluent and contribute to increasing the organic content of PSW, as demonstrated by the slope of BOD_5 to tCOD for the sample collected during this period of the day.



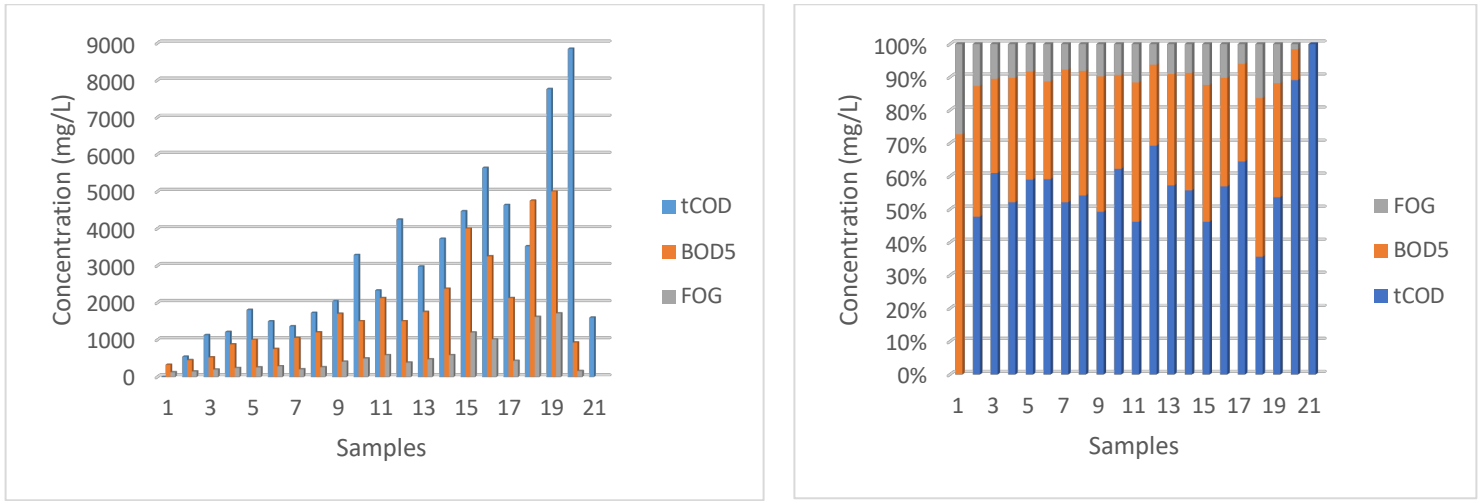
(a) (b) (c)

Figure 4.5: Biodegradability of the samples of group1 (a), group 2 (b), and group 3 (c)



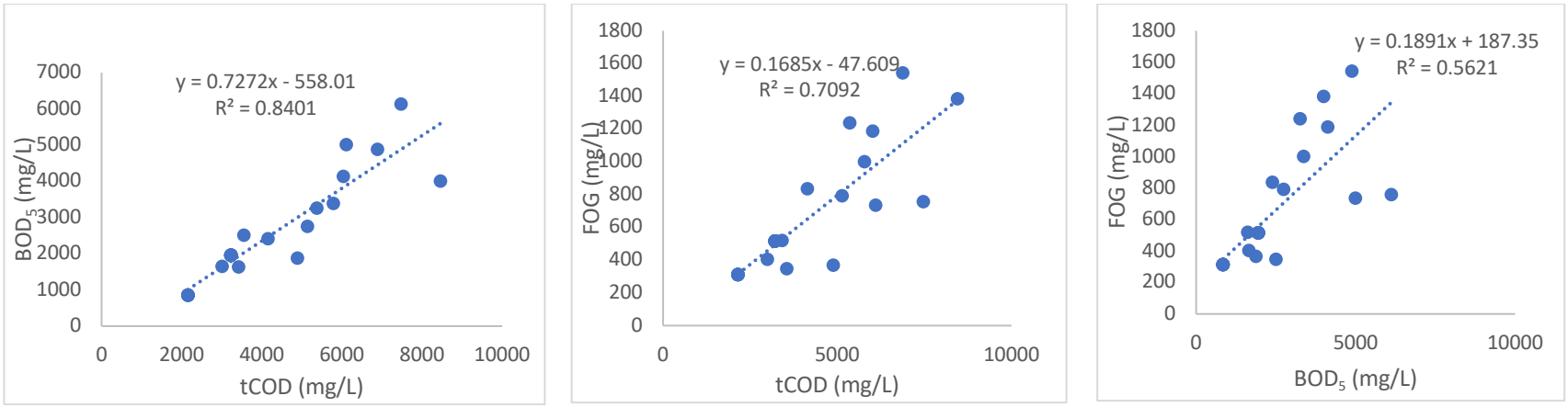
(a) (b) (c)

Figure 4.6: (a) FOG/tCOD, (b) BOD_5/tCOD, and (c) FOG/BOD_5 in group 1



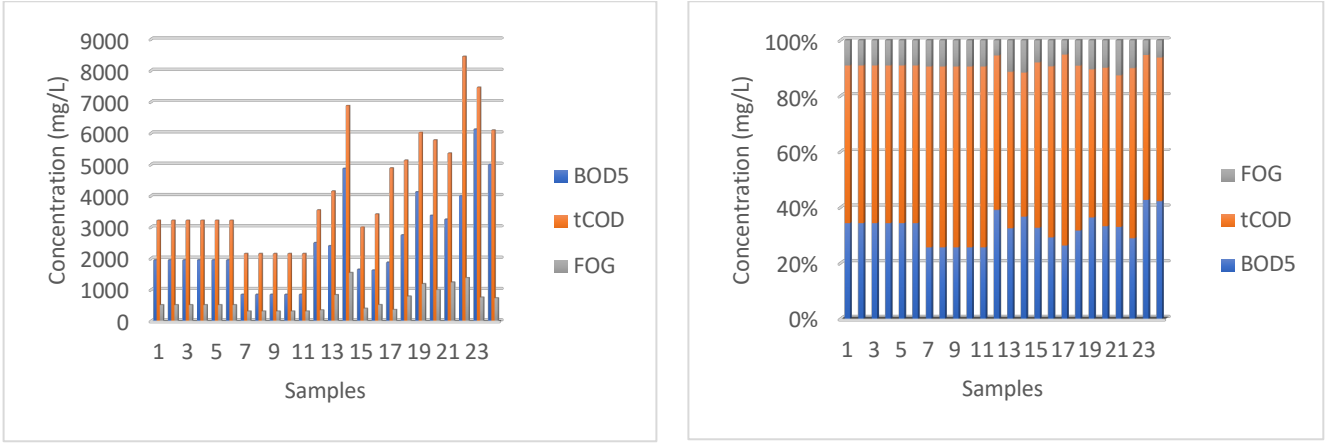
(a) (b)

Figure 4.7: (a) Concentration of tCOD, FOG and BOD_5 in PSW, and (b) Concentration percentage of BOD_5, FOG, and tCOD in group 1



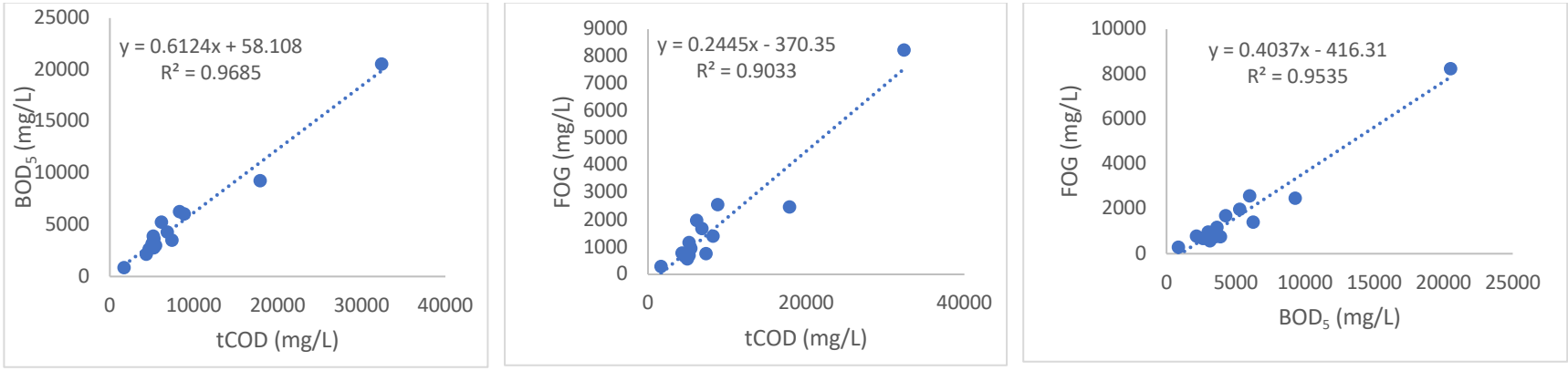
(a) (b) (c)

Figure 4.8: (a) BOD_5/tCOD, (b) FOG/tCOD, and (C) FOG/BOD_5 in group 2



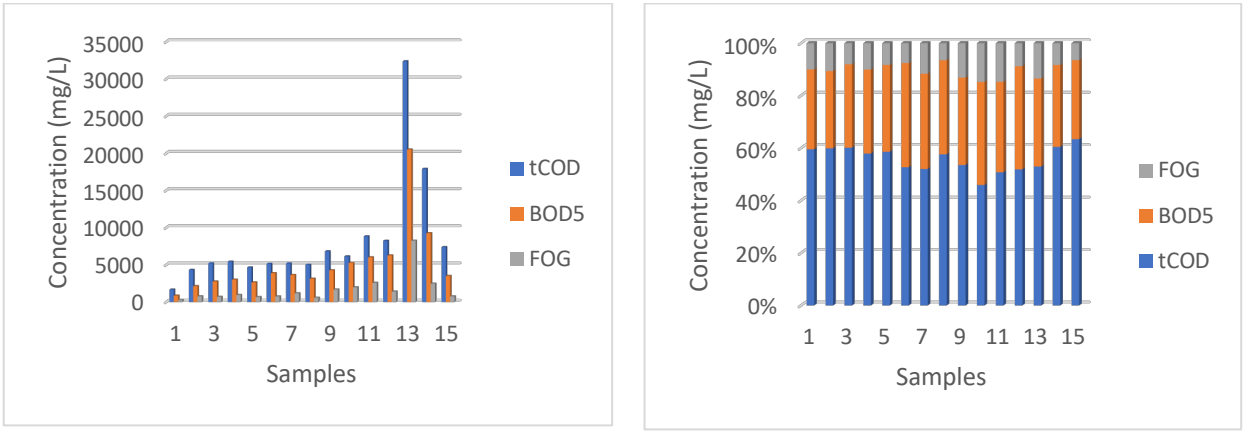
(a) (b)

Figure 4.9: (a) Concentration of tCOD, FOG and BOD_5 in PSW, and (b) Concentration percentage of BOD_5, FOG, and tCOD in group 2



(a) (b) (c)

Figure 4.10: (a) BOD_5/tCOD, (b) FOG/tCOD, and (C) FOG/BOD_5 in group 3



(a) (b)

Figure 4.11: (a) Concentration of tCOD, FOG and BOD_5 in PSW, and (b) Concentration percentage of BOD_5, FOG, and tCOD in group 3

In the Figures 4.7.a, 4.7.b, 4.9.a, 4.9.b, 4.11.a, and 4.11.b, despite some fluctuations probably related to the density of organic matter in the PSW, the tCOD concentration consistently remains higher than the BOD_5 and FOG concentrations in the samples of the three groups. Then follows the concentration of BOD_5, which also consistently remains higher than the one of FOG for all the samples analyzed. This consolidation of results comforts the uniformity of PSW and leads the path towards the development of correlations that could be used to determine the other characterization parameters, such as the FOG.

4.3.3. Linear regressions between tCOD, FOG, and BOD_5, in the three groups of samples

Figures 4.6, 4.8, and 4.10 provide the linear regressions between FOG, BOD_5, and tCOD for each group of samples. These results are summarized in Table 4.7. The correlations provide good R^2 values for the first and second group (see Table 4.7), but a lower R^2 value for the second group. This could be explained by the deviation of the FOG concentration of the second group of samples noticed in Table 4.5, when compared to the ones of the first and third group of samples. This suggests that the concentration of FOG is significantly higher in the PSW collected from the treatment of equipment; probably related to the fats and meat trimmings from the chicken carcasses. This high concentration of FOG in this sample leads to the slope between FOG/BOD_5, as opposed to the ones of groups 1 and 3. This difference is further illustrated by a low coefficient of determination (R^2) of 0.56 for the linear regression between FOG and BOD_5. This suggests that the average FOG concentration of the second group does not perfectly reflect the characteristics of PSW. This difference in FOG concentration of samples from group 2 is further illustrated in the correlation between tCOD/FOG in group, with an R^2 of 0.71. However, in this case, the value of slope (0.17) is close to those of group 1 (0.21) and group 3 (0.24).

Table 4.7: Summary of the linear regressions results

	Group 1		**Group 2**		**Group 3**	
	Slope	R^2	Slope	R^2	Slope	R^2
tCOD/BOD_5	0.61	0.96	0.73	0.84	0.61	0.96
tCOD/FOG	0.21	0.93	0.17	0.71	0.24	0.90
FOG/BOD_5	0.34	0.96	0.19	0.56	0.4	0.93

Overall, with $R^2 > 0.75$, good correlations were found to relate BOD_5, tCOD, and FOG, which can allow the determination of one of these parameters from a single water quality assessment parameter. This can enable the reduce the time to perform the analysis of BOD_5 that takes up to five days, or the determination of the tCOD from FOG, interchangeably. This approach also generates savings from the reduction of analysis tests, which represent an essential step in wastewater processing.

4.4. Conclusion

The characterization of PSW provides better clarity on the operations required for its treatment and therefore contributes to the design of appropriate treatment systems. PSW fails to be characterized universally, as its characteristics depend on several factors, such as the hygienic standards imposed to poultry slaughterhouses, the nutrition of the broilers or the prevailing operation during the collection of the sample. This was demonstrated in this study, with a noted variation of the characteristics and the slope of linear regressions correlating the FOG, BOD_5, and tCOD of the samples analyzed. It was also found that PSW has a good biodegradability, which also varies with the prevailing operation in the poultry slaughterhouse. This good biodegradability translates to a potential risk for the environment if the PSW is discharged untreated, and the suitability of biological processes for the treatment of such wastewater. The development of such correlations between key water quality parameters can reduce the costs associated with the analysis of PSW, and reduce the time required to gain a good insight into the characteristics of the effluent. Furthermore, the limitation of analysis may contribute to reducing the quantity of chemical waste generated from the accumulation of analysis waste.

Following the method used in this study, the correlation between the water quality assessment parameters of other types of wastewater could be investigated to reduce the number of analyses required to characterize PSW or a different type of wastewater.

4.5 Summary

The first step towards selecting a suitable treatment option for poultry slaughterhouse treatment is to characterize it. Various parameters such as the pH, tCOD, BOD_5, TSS, FOG, turbidity, salinity or conductivity were analyzed in this study and provided values

significantly higher than discharge limits imposed by various countries. Furthermore, the biodegradability index (BOD_5/COD) of PSW was determined and averaged value of 0.61 for the samples collected during normal processing operations in the slaughterhouse and. 0.72 for samples collected during the cleaning of equipment after broiler processing operations. This good biodegradability translated to a good biological decomposition potential, which can be a risk for the environment as a result of untreated effluent, and also highlights the suitability of biological treatment processes for the treatment of such wastewater. Moreover, to reduce the production of chemical waste from the toxic reagents used for some analyses, and to alleviate the cost and the time required for these analyses, linear regressions were used to correlate three water quality parameters (tCOD, BOD_5, and FOG), interchangeably. These linear regressions provided a good relationship between these parameters, with R^2>0.9 for the samples collected during normal operation periods; and weaker correlation for the FOG/BOD_5 and FOG/tCOD of samples collected during the cleaning of processing equipment. This was attributed to the high concentration of FOG (5216 ±2534) in the samples collected during this period, from the collection of carcass debris and fats left on the equipment during the slaughtering.

The following step, in Chapter 5, entails the selection of suitable packing materials for preventing operational challenges associated with a less effective underdrain system for HRABS adopting a down-flow configuration.

CHAPTER 5

SELECTION OF SUITABLE PACKING MATERIALS FOR DOWN-FLOW HIGH RATE ANAEROBIC BIOREACTOR UNDERDRAIN SYSTEMS

Part of this chapter was published as

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Chapter 5: SELECTION OF SUITABLE PACKING MATERIALS FOR DOWN-FLOW HIGH RATE ANAEROBIC BIOREACTOR UNDERDRAIN SYSTEMS

5.1. Introduction

High rate anaerobic bioreactor systems (HRABS) have demonstrated good performance for the treatment of medium to high strength wastewaters (Basitere *et al*., 2016; Basitere *et al*., 2017; Debik and Coskun, 2009; Njoya *et al*., 2019). The most popular HRABS include the Up-flow Anaerobic Sludge Blanket (UASB), the Expanded Granular Sludge Bed (EGSB), and the Internal Circulation (IC) reactor, which are widely used worldwide and have been providing a good alternative to aerobic treatment options when biological treatments were required (Henze *et al*., 2008; Lettinga and Hulshoff Pol, 1991; Karthikeyan and Kandasamy, 2009). In wastewater treatment processes, biological treatments are often used in the secondary phase of the overall treatment process, after a primary phase that often consists of the removal of solid contaminants from the wastewater (Metcalf and Eddy, 2003). Figure 5.1 provides an insight into the anaerobic treatment process by listing the inputs and output of the HRAB.

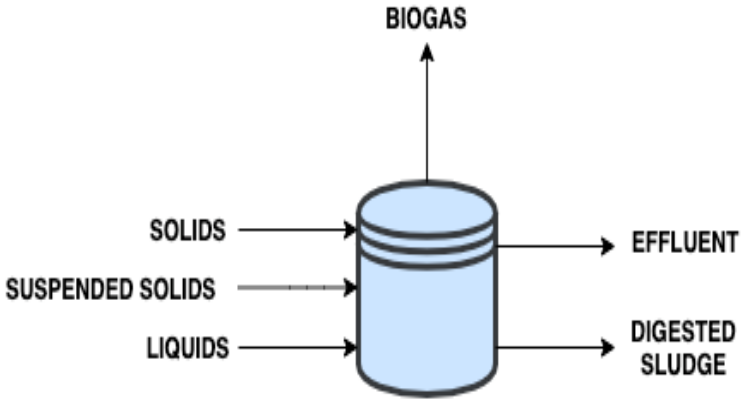


Figure 5.1: Typical inputs and outputs of an HRABS

Most HRABS adopt an up-flow configuration, which is believed to be essential for the granulation of the anaerobic biomass (Lettinga and Hulshoff Pol, 1991; Alphenaar, 1994). Anaerobic granules are aggregates of facultative and anaerobic micro-organisms that contribute to the anaerobic digestion (Alphenaar, 1994; Henze *et al*., 2008; Lettinga and Hulshoff Pol, 1991). Their agglomeration improves the efficiency of such treatment processes and leads to the adoption of the up-flow configuration in most HRABS (Henze et al., 2008; Lettinga and Hulshoff Pol, 1991; Chernicharo, 2007). However, this up-flow configuration requires the use of a three-phase separator to segregate solids, the water effluent, and the gas from the top outlet of the bioreactor (Alphenaar, 1994; Lettinga and Hulshoff Pol, 1991).

Figure 5.2 provides the distribution of Anaerobic treatment plants installed by 4 international companies (ADI, Biothane, Enviroasia, and Paques) in sub-tropical regions of the world. This survey was conducted in 2001 and highlighted the domination of countries such as Brazil and India on the adoption of anaerobic treatment technologies (Karthikeyan and Kandasamy, 2009).

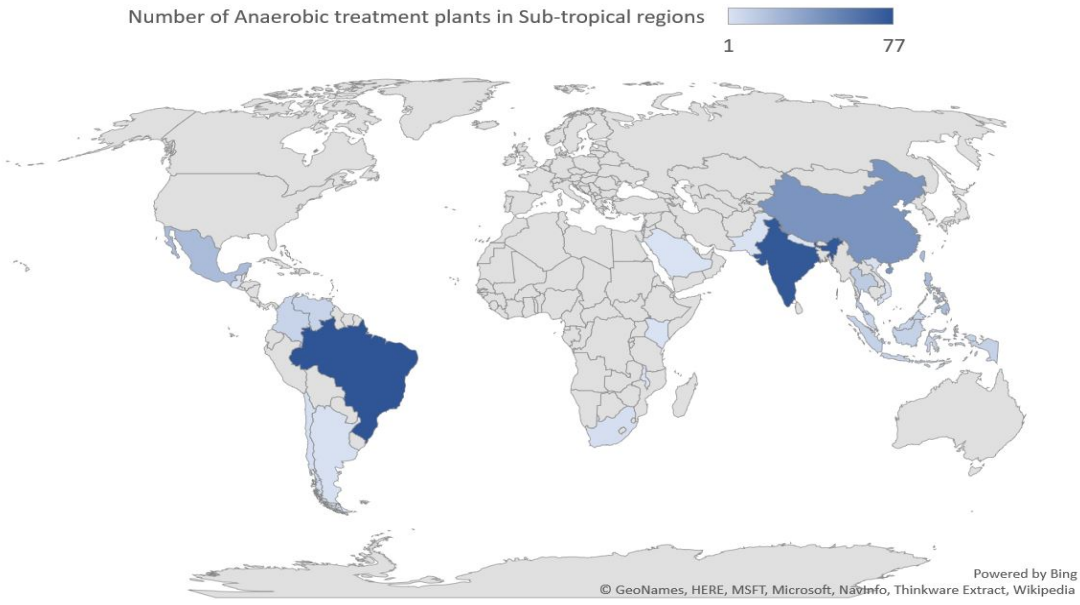


Figure 5.2: Distribution of Anaerobic Treatment Plants installed by four leading companies in Sub-tropical Countries (Adapted Karthikeyan and Kandasamy, 2009)

Hulshoff Pol *et al.* (1998) also conducted a comprehensive survey of full-scale anaerobic plants in the world, which results in a total of 1229 full-scale plants distributed as illustrated in Figure 5.3.

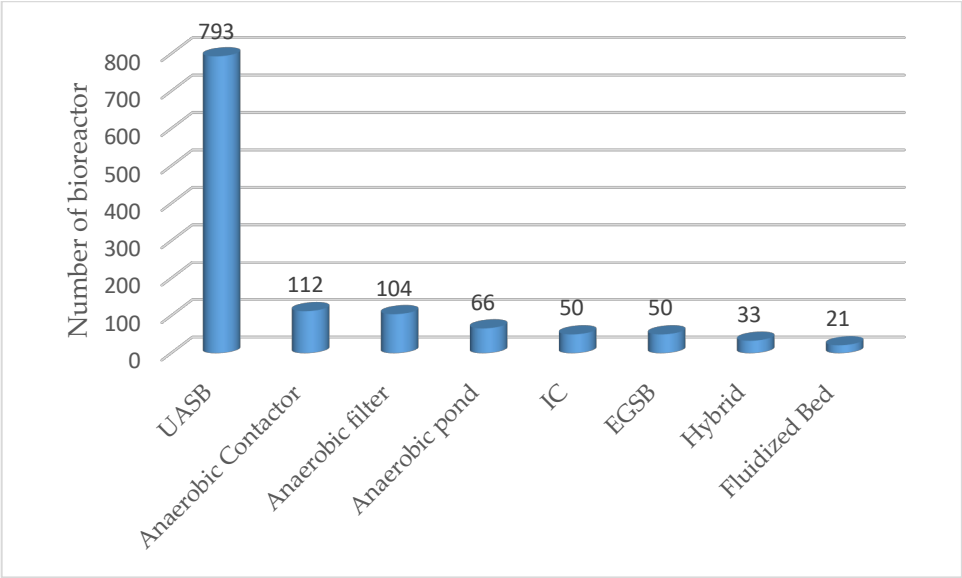


Figure 5.3: Survey of full-sclae anaerobic plants in the world (Adapted from Hulshoff Pol *et al*., 1998)

5.1.1 The advantages of adopting a down-flow configuration for HRABS

As opposed to a down-flow configuration that takes advantage of gravity forces that contribute to the circulation of the wastewater through the anaerobic granular bed and towards the outlet stream, up-flow HRABS require more energy to overcome the resistance to the up-flow of the treated wastewater in HRABS (Basitere *et al*., 2016; Henze *et al*., 2008; Njoya *et al*., 2019). This higher power requirement relates to pressure drop and frictions losses through the anaerobic granular bed (Chernicharo, 2007; Njoya *et al*., 2019). HRABS are usually hermetically closed, therefore the atmospheric pressure effects on the uprising wastewater in up-flow HRABS are not accounted for.

To facilitate the separation of gas (biogas), the effluent and solids i.e. anaerobic biomass on top of up-flow HRABS, these bioreactors (UASB, EGSB) require a three-phase separator (Chernicharo, 2007; Alphenaar, 1994). However, these three-phase-separators do not work as efficiently as planned due to challenges such as the washout of solids i.e. anaerobic biomass, or the drainage of biogas in the effluent stream (Basitere *et al*., 2016; Brooks *et al*., 1999; Del Nery *et al*., 2007; Ellis and Evans, 2008; Evans, 2004). This challenge can be addressed by the adoption of a down-flow configuration whereby the biogas could be collected up-stream whereas the effluent could be collected down-stream with the assistance of a suitable underdrain system to retain the anaerobic biomass, which is very essential for HRABS that rely on a solid retention time (SRT) significantly higher than the hydraulic retention time

(HRT) (Evans, 2004; Ellis and Evans, 2008; Rinquest *et al*., 2019). Hence the importance of a good underdrain system in such configurations. One challenge with down-flow configurations is the granulation of the anaerobic biomass, which is readily observed in up-flow HRABS (Lettinga and Hulshoff Pol, 1991; Alphenaar, 1944; Chernicharo, 2007). However, this challenge can be circumvented by importing the granular biomass from an existing HRABS such as the UASB treating wastewater similar to the wastewater to be treated. This technique has been used in several studies (Henze *et al*., 2008; Basitere *et al*., 2017; Oh, 2012).

5.1.2 Head loss through the underdrain system

The use of a down-flow configuration in high rate anaerobic bioreactor systems enables the retention of the anaerobic biomass within the bioreactor and subsequently the treatment of wastewater with such configuration (Henze *et al*., 2008; Chernicharo, 2007). Such an underdrain system may require the occupation of a defined volume to accommodate the arrangement of solids used for the underdrain system (Chernicharo, 2007; Njoya *et al*., 2019). This volume may vary with the size of the bioreactor. Although this arrangement of solid particles allows the retention of the anaerobic biomass while facilitating the collection of the effluent, the percolation of the effluent might be affected by the head loss induced by such arrangement (Leva *et al*., 1951; Evans, 2004). Therefore, the selection of packing materials used for such purpose should be done carefully to avoid inconveniences such as the clogging of the underdrain system, the accumulation of the wastewater inside the bioreactor, which may be detrimental for the entire anaerobic treatment process (Njoya *et al.*, 2009). Therefore, before deciding on which packing material is to be used as an underdrain system, their porosity, permeability as well as sludge retention capacity should be evaluated.

Depending on their ability to retain anaerobic biomass, their porosity as well as permeability, various solid particles can be used for the underdrain system of bio-digesters. Holdich (2002) suggested the following conditions when selecting packing materials to be used for various purposes including the formation of an underdrain system:

- The affordability of the material,
- The availability of the material, and
- The inertness of the material to mechanical/ pneumatic mixing and microbial attack.

However, other parameters should motivate the selection of packing materials. These include:

- The density of solid particles,

- The structure, and
- The settle-ability when immersed in water.

Packing materials may be available in different sizes and shapes. But the first step towards evaluating their suitability for an underdrain system is to characterize them.

5.1.3 Characterization of solid particles used for underdrain systems

Particle size can be characterized by one or several linear dimensions, depending on its shape (Holdich, 2002). Due to its unique shape, spherical particles can be characterized by a single dimension that provides its maximum length i.e. diameter. However, not all solid particles that can be selected for an underdrain system come in a spherical shape, as they can be angular, cubic or flaky. Therefore, to uniformize the criteria of selection of such solid particles, it is advisable to relate the dimensional properties of non-spherical particles to a single linear dimension, the equivalent diameter, that could provide a means of comparison to a sphere (Pabst and Gregora, 2007; Gibilaro, 2001). This equivalent diameter can be determined using different approaches listed I the following sub-sections.

5.1.3.1 The surface-equivalent sphere diameter

The surface-equivalent is one of the methods used to determine the equivalent diameter of a solid particle. It is given by Equation 5.1 (Yang, 2013).

$$D_{surf} = \left(\frac{6}{\pi} S_P\right)^{\frac{1}{2}} \quad (5.1)$$

Where D_{surf} relates to the diameter of a sphere with a similar surface as a given particle and S_p the surface of the same particle

5.1.3.2 The volume-equivalent sphere diameter

The volume-equivalent sphere diameter of a solid particle is given by Equation 5.2 (Pabst and Gregora, 2007).

$$D_{Vol} = \left(\frac{6}{\pi} V_P\right)^{\frac{1}{3}} \quad (5.2)$$

Where D_{vol} relates to the diameter of a sphere with similar volume as a particle of volume V_P

5.1.3.3 The hydrodynamic equivalent diameter

The hydrodynamic equivalent diameter is a correlation that provides the diameter of a sphere having the same translational diffusion coefficient as a non-spherical particle in the same fluid and under the same conditions (Pabst and Gregora, 2007). It can be calculated using the Stokes-Einstein relation (Equation 5.3) (Pabst and Gregora, 2007):

$$D_H = \frac{KT}{3\pi\mu D_{translation}} \tag{5.3}$$

Where T relates to the absolute temperature, K the Boltzman constant, and μ to the viscosity of the liquid medium

5.1.3.4 The sieve diameter

The sieve diameter represents the diameter of the sphere passing through the same opening of a sieve of defined mesh (Yang, 2013).

5.1.3 5 The Stokes diameter

The Stokes diameter relates to the diameter of a sphere presenting the same settling velocity as a particle settling in the same fluid under laminar conditions (Pabst and Gregora, 2007). This diameter can be determined from the Stokes relation (Equation 5.4) (Pabst and Gregora, 2007):

$$D_S = \sqrt{\frac{18\mu v}{(\rho_s - \rho_L)g}} \tag{5.4}$$

Where v relates to the final settling velocity, μ to the viscosity of the liquid, g to the gravitational acceleration, ρ_s to the density of the solid particle and ρ_L to the density of the pure liquid medium that should contains particles.

5.1.3.6 The laser diffraction equivalent diameter

The laser diffraction equivalent diameter relates to the diameter of a sphere producing the same electronic response from an optical signal (diffraction pattern) when the geometrical aspect of the solid particle is detected (Yang, 2013; Pabst and Gregora, 2007).

5.1.3.7 The volume surface diameter

The volume surface diameter, also referred to as the Sauter diameter, is calculated from the ratio of the cube of the volume-equivalent diameter to the square of the surface-equivalent diameter as expressed by Equation 5.5 (Pabst and Gregora, 2007):

$$D_{SV} = \frac{D_V^3}{D_S^2} \tag{5.5}$$

Opinions diverge on the characterization of solid particles using different particle characterization approaches. Each approach determines the equivalent diameter using a distinct physical principle. Thus, a light scattering device will tend to provide an average value for the particles flowing randomly through the light beam, generating a size distribution that would range from the smallest to the largest dimensions; while, for a sieve, the particle will tend to direct themselves towards their smallest dimension to pass through the openings of the sieve, affecting the reliability of the determination of the equivalent-diameter (Yang, 2013; Horiba Scientific, 2012).

5.1.4 Determination of pressure through an underdrain system using the Ergun correlation

The flexibility of the Ergun correlation across different types of low has made it the most utilized semi-empirical correlation for determining the pressure drop through a packed bed consisting of solid particles of regular or irregular shapes (Yang, 2013, Ergun, 1952). This correlation can be used for any flow type and condition (laminar or turbulent) (Ergun, 1952). It was derived from the addition of the Carman-Kozeny equation for laminar flow to the Burke-Plummer equation developed for fully-turbulent flows (Yang, 2013; Gibilaro, 2001). Therefore, the Ergun equation can be used for various fluids and packing materials. However, the Ergun equation fails to predict the pressure drop after the incipient point of fluidization due to the changes of packed bed voidage that results from the bed expansion (Yang, 2013). This Ergun equation is provided by Equation 5.6 (Yang, 2013; Gibilaro, 2001):

$$\frac{\Delta P}{L} \cdot \frac{g d_P \emptyset}{2\rho_F {v_0}^2} \cdot \frac{\varepsilon^3}{(1-\varepsilon)} = 75\frac{(1-\varepsilon)}{\emptyset Re_P} + 0.875 \tag{5.6}$$

Where Ø relates to the Sphericity factor that can be replaced by the particle diameter when it can be determined using one of the equivalent-diameter approaches explained in previous sections. The introduction of sphericity parameters into the Ergun Equation results to Equation 5.7 (Yang, 2013, Gibilaro, 2001):

$$\frac{\Delta P}{L} = \frac{150\mu v_0}{d_P^2}\frac{(1-\varepsilon)^2}{\varepsilon^3} + \frac{1.75\rho_F v_0^2}{d_P}\frac{(1-\varepsilon)}{\varepsilon^3} \quad (5.7)$$

The Ergun equation is widely used and relates to the Blake-Kozeny-Carman equation at low Reynolds numbers, and to the Burke-Plummer equation under turbulent regimes (Yang, 2013).

5.1.5 Permeability of an underdrain system

To prevent the creation of detrimental conditions to the anaerobic biomass, wastewater is usually treated in HRABS under laminar conditions, therefore low Reynolds numbers (Hulshoff Pol *et al*., 2004). The permeability of a packed bed is an essential parameter for the determination of the pressure drop across a packed bed. It serves also to evaluate the quality of the fluid flow through such an arrangement. The permeability can be derived from the Kozeny-Carman equation, which results to Equation 5.8 (Holdich, 2002):

$$\frac{r^2}{k} = \frac{75(1-\varepsilon)^2}{2\varepsilon^3} \quad (5.8)$$

Where *k* is the permeability and *r* the radius of particles

Equation 8 allows a reliable estimation of the permeability, provided that the porosity remains between 0.26 and 0.80 (Holdich, 2002).

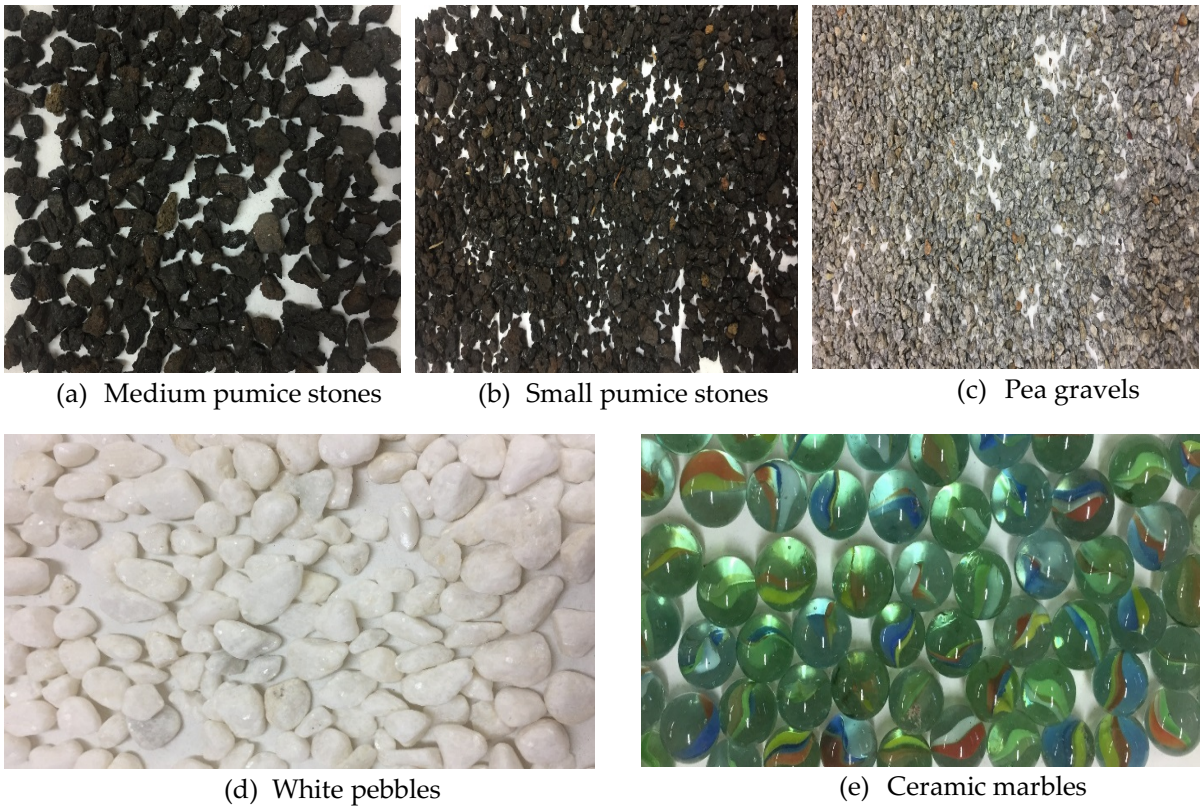
5.1.6 Chapter's objective

This chapter addresses the challenge of selecting suitable packing materials for the underdrain system of down-flow high rate anaerobic bioreactors and aims to determine which packing material is the most suitable for down-flow high rate anaerobic bioreactor underdrain system. The pressure loss through such packing materials arrangement often leads to the clogging of the underdrain system and therefore a perturbation of the process of anaerobic digestion through the accumulation of the wastewater inside such bioreactors. Therefore, five packing materials were selected, including white pebbles, medium-sized pumice stones, cerami marbles, small-sized pumices and pea gravel, based on their density, affordability and inertness. However, for effective evaluation of such packing materials, other analysis parameters were introduced (porosity, permeability, sludge retention capacity and induced pressure loss). The analysis of these other selection parameters will allow the selection of the most suitable packing material for the SGBR and the DEGBR.

5.2. Materials and Methods

Five materials were selected towards the determination of the most suitable material for a down-flow HRABS underdrain system. These include the following materials:

- Pea gravel (Figure 5.4.c),
- Medium pumice stones (Figure 5.4.a),
- Small pumice stones (Figure 5.4.b),
- White pebbles (Figure 5.4.d) and
- Ceramic marbles (Figure 5.4.e).



(a) Medium pumice stones (b) Small pumice stones (c) Pea gravels

(d) White pebbles (e) Ceramic marbles

Figure 5.4: Packing materials selected for the underdrain system

These materials were pre-selected as per their availability, affordability, inertness as well as ability to conduct the bioreactor's effluent.

5.2.1 Determination of the porosity of the packing materials

A simple apparatus was used to determine the porosity of various packing materials. The apparatus consisted of two pieces of PVC materials, including a cylindrical PVC tube

terminated at one end by a 2mm mesh strainer, and a PVC lid that could be used to close that end of cylindrical PVC tube as illustrated in Fig 5 block the circulation of the liquid used during the experiment. The porosity was quantified by using the volume between the water herein referred to as the total volume (V_T), and the volume of packing material (V_P), which resulted in the determination of the void volume (V_V). Then, the porosity of each packing material investigated was determined from the ratio of the void volume to the total volume. This procedure was repeated in duplicate for all selected packing materials to get a representative sample of data.

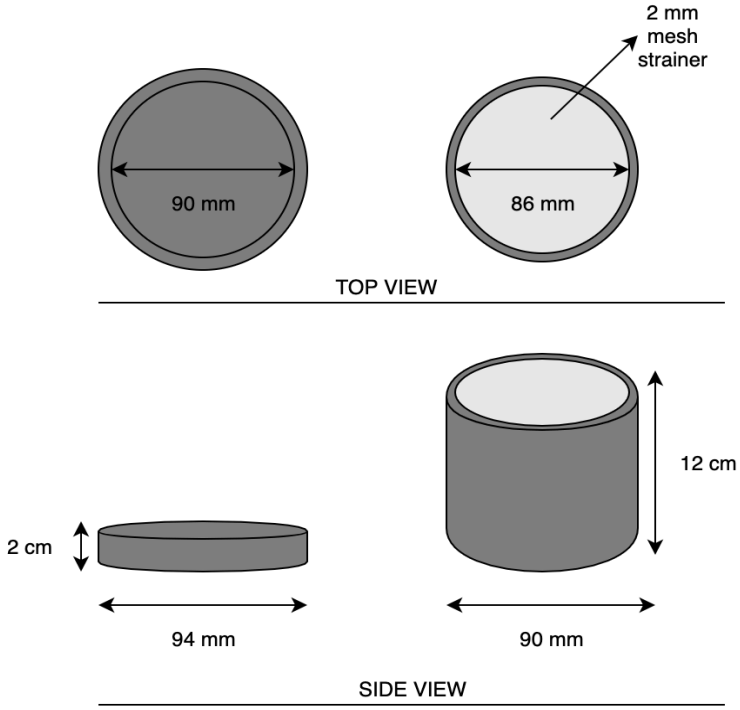


Figure 5.5: PVC cylindrical apparatus used for determining the porosity and sludge retention capacity of packing materials

5.2.2 Packing/underdrain material, retention capacity for granular sludge

Another important parameter aimed at selecting a good packing material for an underdrain system is the sludge retention capacity, which assesses the ability of packing material to retain the anaerobic biomass within the HRABS. Due to the down-flow configuration of the system, the anaerobic biomass is imported from an existing HRABS and therefore comes fully grown inside the bioreactor; thus, with a spherical structure that has gained in diameter from the aggregation of the micro-organisms. Consequently, they can be better retained than an immature sludge. The assessment undertaken consisted of using a given volume of a specified

quality of anaerobic granular sludge whose mass was known. For each packing material investigated, the packing materials were placed in the PVC cylindrical apparatus described in the previous section. The PVC lid was removed from the bottom of the cylindrical apparatus to allow the flow of the sludge when poured on a specific volume of packing material retained by the metallic screen (See Figure 5.5). Before being poured on the packing materials, the mass of the anaerobic granular sludge was measured and recorded. Thereafter, the mass of the unretained sludge collected at the other end of the cylindrical apparatus was collected and measured as well. Based on the principle of conservation of mass, the mass of anaerobic granules retained by the packing material was determined using the difference between the initial mass of anaerobic sludge poured onto the packing material arrangement and the mass which was washed-out. Thereafter, the granular retention capacity was calculated from the ratio of the mass of the anaerobic granular sludge washed out from the column to the initial mass used. The same procedure was repeated in duplicate for all packing materials investigated using a constant volume of packing materials.

5.2.3 Measurement of the size of the packing materials

The size of the packing material was measured with the assistance of Vernier caliper. For non-spherical particles, the length, width, and breadth were measured to estimate an equivalent diameter. This was done by averaging the values measured after multiplying the average by the corresponding sphericity determined by the visual inspection of the shape of various particles composing the packed bed. For small-sized particles, only two sizes perpendicular to the centre of the particle were considered and measured.

5.2.4 Wadell sphericity coefficients

The packing materials investigated towards the selection of a suitable HRAB underdrain system presented the following shapes:

- Ceramic marbles (spherical)
- Small-sized pumice stones (angular)
- Medium-sized pumice stones (angular)
- The pea gravel (angular)
- White pebbles (rounded)

Holdich (2002) listed the Wadell sphericity of common particles as depicted in Figure 5.6. These are used to describe the shape of the selected material for the underdrain.

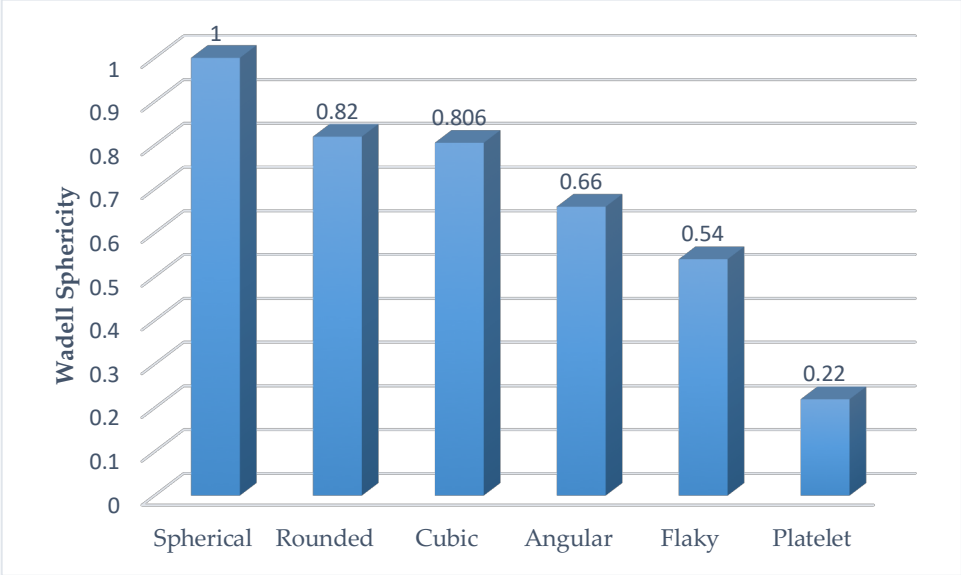


Figure 5.6: Wadell sphericity for different particle shapes

The Wadell sphericity provides a coefficient that corrects the irregularity of a non-spherical particle towards enabling its characterization and determining and comparing it to other solid particles presenting a different shape (Holdich, 2002; Zeng and Grigg, 2006). This Wadell sphericity can also be used towards calculating the pressure drop induced by the arrangement of solid particles in an HRABS (Holdich, 2002). Therefore, it serves as a useful tool to quantifying the effects of solid particles of various shapes and allow their comparison if required. As a result, the first step towards determining the Wadell sphericity of various packing material was to determine its shape that is relatable to a coefficient as depicted by Fig 6 Subsequently, the Wadell sphericity can be multiplied by a corresponding representative diameter to determine the equivalent diameter of packing material.

5.3. Results and discussion

5.3.1 Comparison of the porosity of the selected packing materials

The porosity of the selected packing materials was determined from the ratio of the void volume to the total volume. Figure 5.7 provides a comparison between the porosity of the packing materials selected towards the evaluation of their suitability to an HRABS underdrain system. It is observed that the medium-sized pumice stones possess the highest porosity (0.66),

followed by the small-sized pumice stones (0.57), the Ceramic marbles (0.4) and the pea gravel (0.36). The packing materials with the lowest porosity were the white pebbles with a porosity of 0.34.

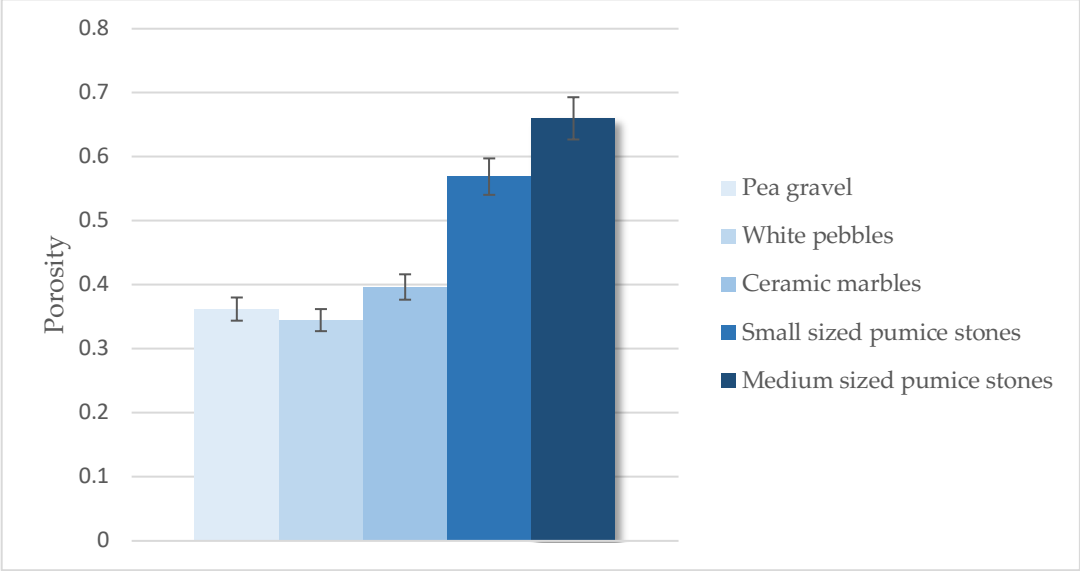
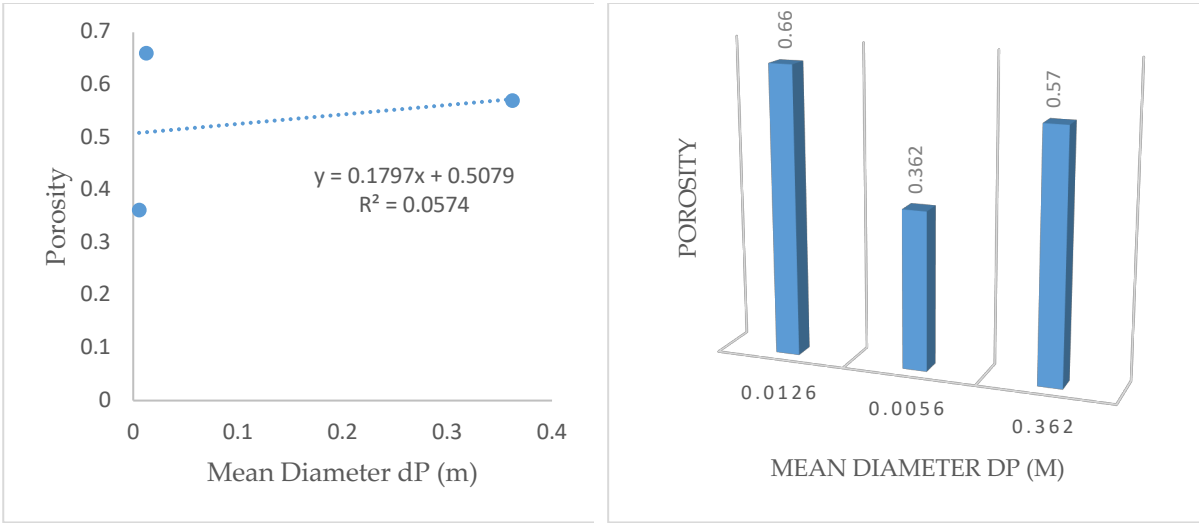


Figure 5.7: Porosity of the selected packing materials

Medium and small-sized pumice stones are the same materials but differ with sizes. Pumice stones are volcanic rocks composed of highly vesicular rough textured glass pyroclastic with very thin and translucent bubble walls of extrusive igneous rocks (Sepehr *et al*., 2013; Pietsch, 1990). Although they have the same structure, medium-sized pumice stones were on average 1.8 larger than the small sized pumice stones. Table 1 provides the shapes and Wadell sphericity of various packing materials, from which the similarity between the medium and small-sized pumice stones is once again highlighted. It can also be noticed that the pea gravels share the similarity of shape with the pumice stones but fail to possess a porosity close to them. Furthermore, no solid correlation was found between them by plotting their mean diameter against their porosity, as illustrated by Figure 5.8.a and 5.8.b from which a weak coefficient of determination ($R^2 = 0.06$) served to demonstrate a weak correlation between the three types of packing materials possessing the same shape. One explanation of this difference can be related to the difference in the structure and physical properties of two types of materials (pumice stones and white pebbles). Furthermore, the good porosity of some materials can be related to their arrangement in the packed bed, which depends on the size and structure of the solid particles.

Table 5.1: Characteristics of selected packing materials

Parameters	Medium-sized pumice stone	Pea Gravel	Ceramic marbles	Small-sized pumice stone	White pebbles
Wadell sphericity	0.66	0.66	1	0.66	0.82
Shape	Angular	Angular	Spherical	Angular	Rounded
Mean diameter dP (m)	0.0126	0.0056	0.0157	0.0070	0.0147



(a) (b)

Figure 5.8: Linear regression on the mean diameter and porosity of the granular particles

5.3.2 Comparison of the head losses generated by selected packing materials

Pressure loss across an arrangement of solid particles is an important parameter in the selection of packing materials for an underdrain system to ensure conducive anaerobic digestion and the mitigation of challenges such as clogging and thus the accumulation of the effluent inside the HRABS. The determination of head losses across the packed-bed was experimentally performed using the Ergun model (Equation 5.7) for superficial velocities varying between 3.855×10^{-6} and 5×10^{-5} m/s that corresponded to an HRT ranging from 37 to 3 hours according to the bioreactor scale and set-up. The pressure drop of each group of packing materials was evaluated across a same volume of packed-bed randomly arranged. Figure 5.9 compares the variation of the pressure drop of selected packing materials at different superficial velocities. Pea gravels stand out as the packing materials producing higher pressure losses than other solid particles under the same superficial velocity, with a pressure drop of 0.17 Pa at a superficial velocity of $5X10^{-5}$ m/s.

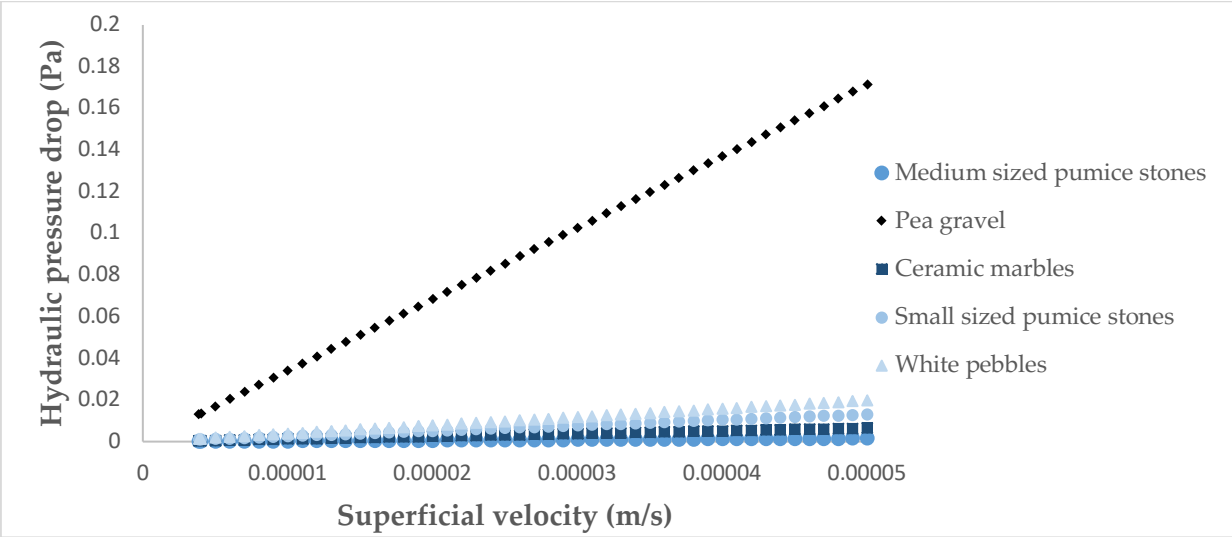


Figure 5.9: Variation of Hydraulic pressure drop of the selected packing materials with different superficial velocities

Further comparison of the head loss generated by other packing materials, except that observed for the pea gravel, is illustrated in Figure 5.10, from which it can be observed that the white pebbles came second in the list of selected solid particles generating high-pressure loss. They were followed by small-sized pumice stones and Ceramic marbles. Therefore, the medium-sized pumice stones provided the best results in terms of induction of hydraulic pressure loss across a packed bed when compared to other packing materials, including pea gravel, small-sized pumice stones, white pebbles and Ceramic marbles.

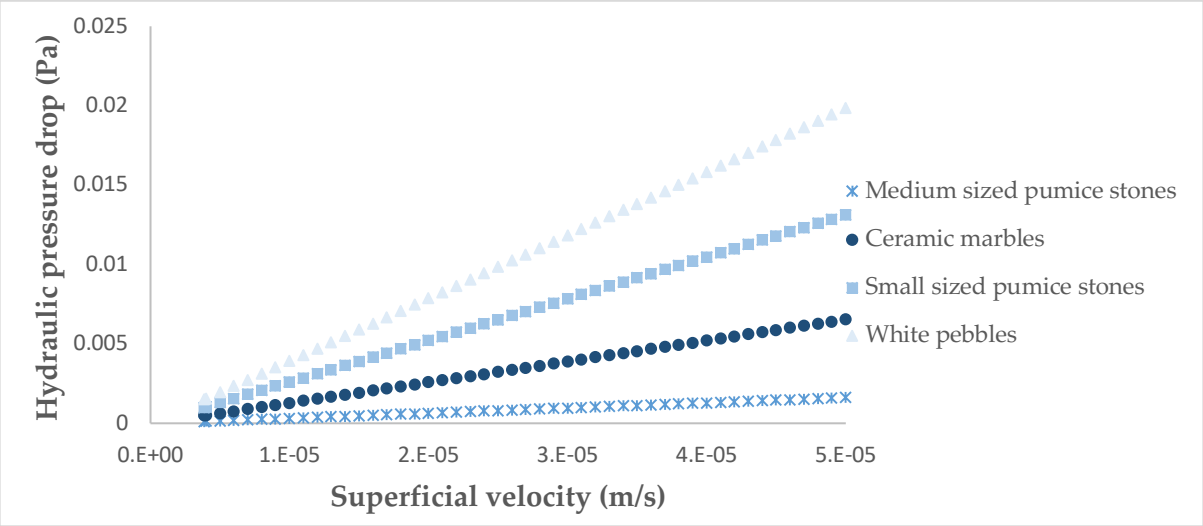


Figure 5.10: Comparison of the head losses induced by selected packing materials at different superficial velocities

5.3.3 Comparison of the permeability of selected packing materials

An in-depth analysis of the behaviour of the listed packing materials (See Table A.2) towards the facilitation of wastewater permeation of the bioreactor, was also evaluated as depicted in Figure 5.11.

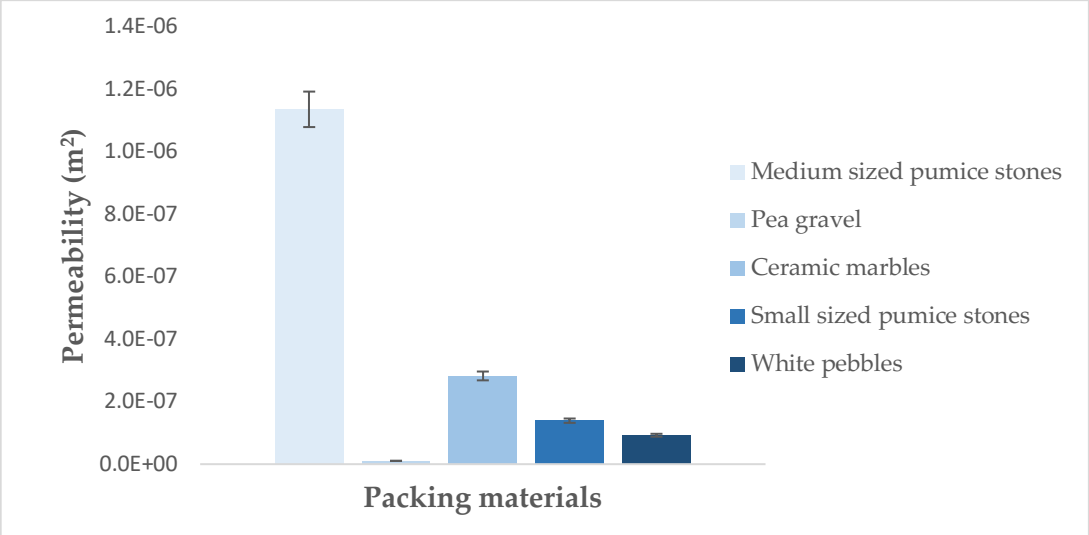


Figure 5.11: Comparison of the permeability of assessed packing materials

Similar to the trend provided for the pressure drop evaluation, the medium-sized pumice stones were observed to more permeable than other selected packing materials, with the pea gravels being the less permeable material. This weak permeability of pea gravels was also highlighted in some studies evaluating the treatment of medium to high strength wastewater using a down-flow HRABS (Basitere *et al.*, 2017; Evans, 2004). In these studies, the underdrain system was getting clogged after a long period of operation. However, the clogging of the underdrain system may also be related to another factor such as the development of a biofilm as a result of extracellular polymeric substances (EPS) production.

5.3.3.1 Reduction of the permeability of the packed bed by the formation of extracellular polymeric substances

The colonization of solid surfaces by microorganisms relates to the production of EPS. EPS biosynthesis is favoured by optimal environment conditions enabling the exchange of genetic material between the cells (Laspidou and Rittman, 2002). This exchange culminates in the attachment and aggregation process of EPS, which contribute to the formation of a microbial

biofilm on abiotic surfaces, such as bioreactor underdrain systems' packing materials (Czaczyk and Myszka, 2007; Laspidou and Rittman, 2002). These EPS play an important role in various biological areas, including biodeterioration, biotechnology, biofouling, and even immunology. They contribute to enabling the initial attachment of cells to abiotic surfaces; the formation and maintenance of microcolony; the maturation of biofilms; and the enhancement of the biofilm resistance to environmental stress and disinfectants. Moreover, EPS may facilitate the capture of nutrients by the bacteria (Czaczyk and Myszka, 2007).

The EPS matrix usually varies between 0.2 μm to 30 nm. They are composed of macromolecules such as glycoproteins, nucleic acids, polysaccharides, phospholipids and proteins (Laspidou and Rittman, 2002). This matrix is marked by the presence of polypeptides, which relate to very few Gram-positive bacteria cells. However, the most common components of the EPS layer are proteins and polysaccharides (Czaczyk and Myszka, 2007).

The microbial biofilm formation is mostly explained by the EPS molecules, which promote a more developed stage of cell attachment processes also called specific adhesion stage or irreversible adhesion stage. EPS is extensively produced during this phase. These molecules are deemed important because they consolidate the interactions between the microorganisms and subsequently facilitate the aggregation of cells on solid particles (Czaczyk and Myszka, 2007; Laspidou and Rittman, 2002)

5.3.3.2 Added advantage of permeable packed beds: filtration

Water filtration can be defined as a physical or mechanical process that enables the separation of suspended and colloidal particles from fluids through a medium that only permeates the fluid. This medium is usually a granular material, which doesn't affect the physical or chemical composition of the permeate and retentate. The use of a suitable underdrain system in down-flow HRABS also enables the filtration of the treated wastewater and may contribute to the overall efficiency of the treatment system, depending on the porosity and the permeability of the granular bed and subsequently the underdrain system.

5.3.4 Comparison of the sludge retention capacity of the assessed packing materials

The most important requirement of high rate anaerobic digestion is the retention of the active anaerobic biomass within the HRABS. In down-flow HRABS, this requirement is met by the use of a suitable underdrain system. Therefore, an important parameter towards the

evaluation of this suitability is the sludge retention capacity that contributes to determining which packing material retains the most the anaerobic biomass within the HRABS. Therefore, the sludge retention capacity of assessed packing materials was assessed and compared, as illustrated in Figure 5.12. From the same figure, it was noticed that angular packing materials provided the best retention capacities with 0.87, 0.87 and 0.86 (Table A.4), for medium-sized pumice stones, small-sized pumice stones and white pebbles, respectively. The white pebbles and Ceramic marbles displayed a weak sludge retention capacity with values of 0.32 and 0.13, respectively. These low retention capacities can be attributed to their size, structure, and shape. This poor retention capacity translates to a weak ability to effectively maintain conducive anaerobic digestion in HRABS.

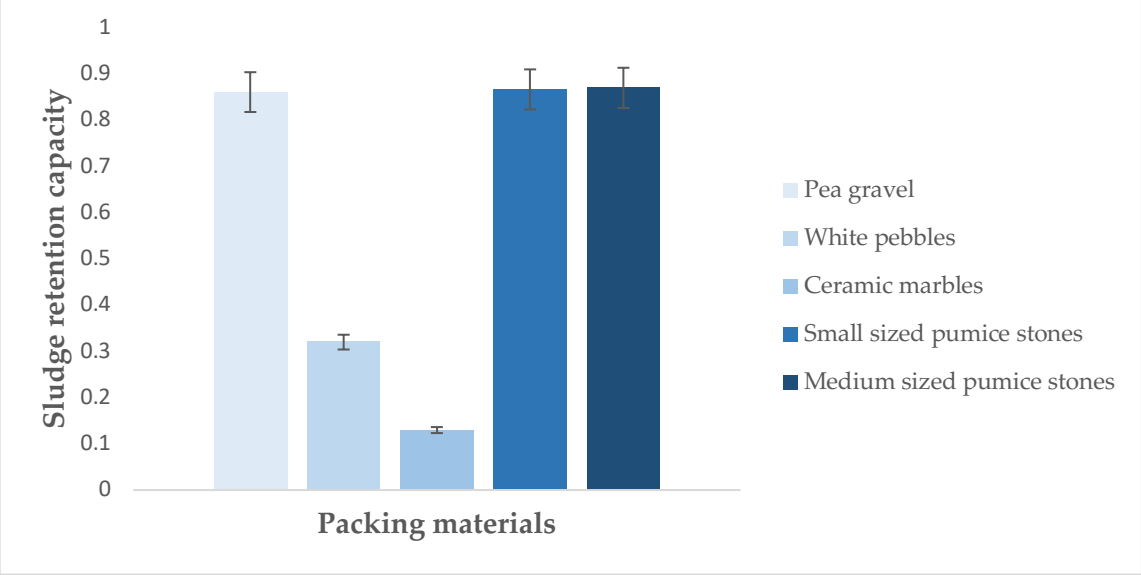
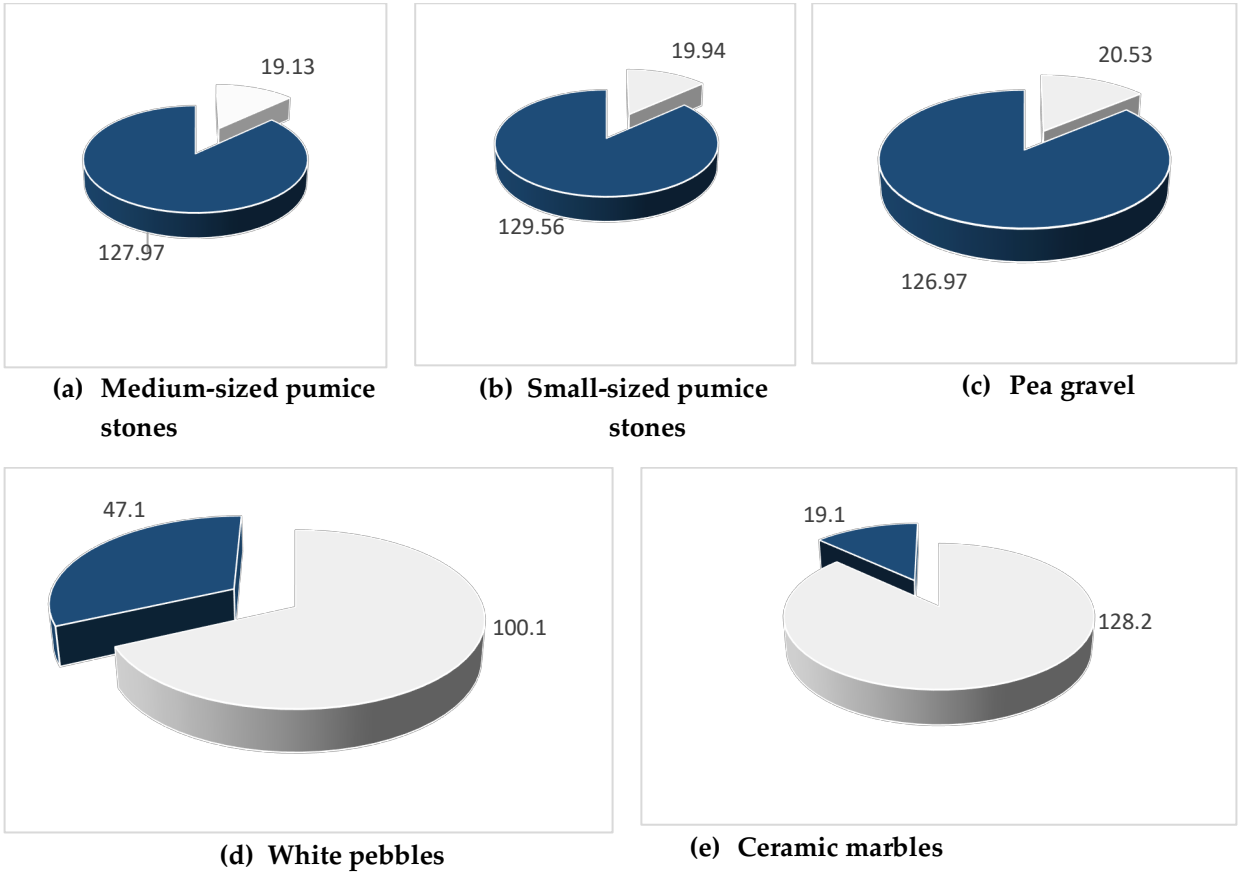


Figure 5.12: Comparison of the sludge retention capacity of the selected packing materials



(a) Medium-sized pumice stones

(b) Small-sized pumice stones

(c) Pea gravel

(d) White pebbles

(e) Ceramic marbles

Figure 5.13: Comparison of the quantity of sludge retained by of the selected packing materials

The coarse surface of pumice stones represents another factor that can justify their success in retaining the granular sludge as opposed to Ceramic marbles and white pebbles. This rough surface provides more surface area for the retention of the granular biomass. This is further illustrated by the poor retention capacity of Ceramic marbles that have a poor surface that poorly retains the anaerobic granular sludge by failing to provide resistance to their flow. The good retention of pumice stones is further illustrated by Figure 5.13 a, 5.13b, 5.13c, 5.13d and 5.13e, which provides a representation of the quantity of sludge retained (dark section of the pie) and the sludge washed out (light section of the pie).

5.4. Conclusion

The retention of anaerobic digestion in down-flow HRABS while maintaining a conducive anaerobic digestion process relies heavily on the selection of a good underdrain system. In this study, five materials were selected for the evaluation of their suitability for the underdrain system of a down-flow HRABS. These packing materials included white pebbles, medium-

sized pumice stones, pea gravel, small-sized pumice stones, and Ceramic marbles. From an evaluation using different selection methods, including porosity, permeability, induced pressure loss, and sludge retention capacity; it was found that medium-sized pumice stones represented the most suitable packing material for such an underdrain system. Furthermore, the added advantage of filtration of such packing material arrangement may contribute to further improve the quality of the effluent from such a process. However, the challenge of biofilm formation through the growth of EPS was also mentioned as a factor that may alter the effectiveness of such an underdrain system, when not properly monitored.

5.5 Summary

A critical requirement for effective high rate anaerobic treatment of wastewater is a good and long retention of the required anaerobic biomass within the bioreactor. This usually translates to a solid retention time significantly higher than the hydraulic retention time. The good retention of the required biomass is satisfied in down-flow anaerobic bioreactor systems using a good underdrain system ideally composed of suitable packing materials. This study evaluates the suitability of five packing materials, including medium-sized pumice stones, white pebbles, Ceramic marbles, small-sized pumice stones, and pea gravels, through the comparison of their porosity, induced pressure loss, permeability, and sludge retention capacity. It was found that medium-sized pumice stones represented the most suitable packing with the best porosity, permeability, sludge retention capacity and the least induced pressure loss determined by the Ergun Equation. Consequently, the packing material was used in to set up the DEGBR used for the treatment of PSW, as described and discussed in Chapter 6.

CHAPTER 6

TREATMENT OF POULTRY SLAUGHTERHOUSE WASTEWATER USING A DOWN-FLOW EXPANDED GRANULAR BED REACTOR

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Chapter 6: TREATMENT OF POULTRY SLAUGHTERHOUSE WASTEWATER USING A DOWN-FLOW EXPANDED GRANULAR BED REACTOR

6.1. Introduction

The efficacy of anaerobic digestion for the secondary treatment of low to high strength wastewater has been highly acclaimed since the development of high rate anaerobic bioreactor systems (HRABS) (Chernicharo, 2007; Henze *et al*., 2008. Metcalf & Eddy, 2003). High rate anaerobic bioreactor systems (HRABS) heavily rely on the development of anaerobic granular sludge and improved biomass retention (Hulschoff Pol *et al*., 2004), which culminates in an effective solid retention time (SRT) and suitable hydraulic retention time (HRT) (Henze *et al*., 2008; Alphenaar, 1994). This results in enhanced wastewater treatment performance in terms of effluent quality and processing time. In comparison to the aerobic treatment of wastewater, the anaerobic treatment has numerous advantages including a reduced plant footprint (Henze *et al*., 2008; Debik and Coskun, 2009); less energy requirement, which is usually associated with the supply of dissolved oxygen in aerobic systems (Chernicharo, 2007; Henze *et al*., 2008); low initial and operating costs (Debik and Coskun, 2009); less sludge generation, which does not require further treatment but can be used for inoculating another biodigester and therefore reduce the start-up time (Henze *et a*l., 2008); and biogas production, whose methane content represents an alternative source of energy (Chavez *et al*., 2005). Following the success of the up-flow anaerobic sludge blanket (UASB) (Hulschoff Pol *et al*., 2004; Lettinga and Hulschoff Pol, 1991), various HRABS, such as the expanded granular sludge bed (EGSB) reactor (Kato *et al*., 1994; Basitere *et al*., 2016), the internal circulation (IC) reactor (Driessen *et al*., 1999), up-flow anaerobic filter (UAF) (Yilmaz *et al*., 2008), static granular bed reactor (SGBR) (Basitere *et al*., 2017; Ellis & Evans, 2008)

or the anaerobic baffled reactor (ABR) (Bachmann *et al*., 1985), among others, have been developed for the biological treatment of low to high strength wastewater. However, some challenges were encountered during the operation of such bioreactors, including the washout of solids and the difficulty associated with the operation of the three-phase separator for bioreactors operating under an up-flow configuration (Basitere *et al*., 2016; Ellis and Evans, 2008; Henze *et al*., 2008); the weakened distribution of substrate to the anaerobic biomass and weak dispersion of toxicants within the system due to the pressure loss affecting the mobility of these substances within the anaerobic bed (Basitere *et al*., 2017; Gerardi, 2003; Ellis and Evans, 2008); and the energy requirement associated with the pumping and recycling lines in reactors such as UASB, EGSB, UAF, and IC, which were addressed through the development of the SGBR that offers a down-flow configuration that reduced the overall energy requirements of the system and eliminated the need for a three-phase separator (Ellis and Evans, 2008). However, this configuration also came with some challenges related to head losses (Basitere *et al*., 2017), which translated to the loss of the fluid and gas kinetic energy, as the drivers of the limitation of substrate distribution to the biomass, gas entrapment and subsequently the accumulation of toxic substances such as ammonia and hydrogen sulphide within the anaerobic granular bed of such system (Meier *et al*., 2011; Yamamoto *et al*., 2009; Gerardi, 2003).

Thus, this study aimed at addressing these shortcomings through the development of the down-flow expanded granular bed reactor (DEGBR) that was designed to alleviate the aforementioned challenges for an enhanced performance of HRABS in the treatment of medium to high strength wastewater. In this study, the performance of the DEGBR was assessed by using poultry slaughterhouse wastewater (PSW), whose discharge to water surface represents a threat to human health and the environment, as it contains biological contaminants, pathogens and is being produced in significant quantities (Barbut, 2015; Borja *et al*., 1998). However, it should be noted that attention was not given to the removal of pathogens in this study.

The poultry industry represents the largest segment of the South African agriculture industry (Bolton, 2015). The processing of birds in poultry slaughterhouses is associated with significant consumption of potable water. Northcutt (2004) reported that the processing of a single bird in a poultry slaughterhouse usually requires an average of 26.5 L/Bird; thus, depending on the

throughput of a poultry slaughterhouse, which is highly influenced by the demand of poultry products, availability of broilers and processing capacity, huge volumes of potable water is usually used in such facilities (Barbut, 2015), despite water scarcity challenges. This high consumption of potable water originates from the requirements imposed by high hygienic standards to which poultry industries should abide to ensure the supply of safe products (Bustillo-Lecompte *et al*., 2016). While significantly contributing to the processing of birds, the potable water is then contaminated with blood, fats, faeces, bones, meat trimmings as well as other pollutants, to form poultry slaughterhouse wastewater (PSW), which is characterised by a high concentration of chemical oxygen demand (COD), fats, oil and greases (FOG) or biological oxygen demand (BOD_5), and thus culminates in a wastewater that can be harmful to the public health and environment, while being a source of financial penalties to the producing industry if discharge standards were not to be respected (Bustillo-Lecompte *et al*., 2016; Barbut, 2015; Debik and Coskun, 2009). The extent of the treatment of such effluent is prescribed by the legislation of relevant countries. However, to reduce the potable water intake in such facilities, the option of water recycling may be adopted and therefore stringent treatment methods are required to avoid the contamination of poultry products being processed.

The treatment of PSW has been attempted by various researchers. Basitere *et al.* (2017) used a Static Granular Bed Reactor (SGBR) and achieved a COD, TSS and FOG removals of 93%, 95% and 90%, respectively. The SGBR was also used by the Debik and Coskun (2009), which resulted in an average COD removal >95% for an organic loading rate (OLR) varying between 0.25 and 5 gCOD/L.day. Furthermore, Basitere *et al*. (2016) also evaluated the treatment of PSW using an expanded granular sludge bed (EGSB) reactor for a COD removal of 57 % with the highest OLR being 1 gCOD/L.day. In this study, the washout of solids, facilitated by the attachment of the biomass to the FOG, the up-flow configuration and the limitations of the three-phase separator, were highlighted as factors significantly contributing to the limitations of the performance of the EGSB for the treatment of PSW. The EGSB is a variant of the UASB, which was used by Del Nery (2001) for the treatment of PSW in a full-scale operation that resulted in tCOD and sCOD removals of 65 and 85%, respectively, for an average OLR of 1.64 $kgCOD/m^3.day$. Another variant of the UASB, the hybrid up-flow anaerobic sludge blanket (HUASB), was also assessed for the treatment

of PSW under mesophilic conditions by Rajakumar *et al*. (2012), which culminated in the removal of tCOD and sCOD varying between 70 to 80%, and 80 to 92%, respectively, for an OLR of 19 kgCOD/m^3, which resulted in a methane gas concentration of 72% at a rate of 1.1 and 5.2 m^3/m^3.day. Rajukumar *et al.* (2011) also evaluated the treatment of PSW using a UAF under low up-flow velocity that resulted in a tCOD and sCOD removals of 70 and 79%, respectively, using a non-granular sludge as inoculum, with anaerobic granules varying between 1 and 2 mm. Sindhu and Meera (2012) also evaluated a bioreactor presenting similar features as the UAF, the up-flow anaerobic packed bed reactor (APBR), which was randomly packed with PVC pipe pieces as packing material and achieved a COD, TDS, and suspended solids removals of 88, 15 and 85-98%, respectively, for an ammonia nitrogen reduction of 30% with an OLR varying between 4 to 5 kgCOD/m^3.day.

The performance of HRABS highly depends on the maintenance of suitable environmental conditions to induce the growth of the required anaerobic biomass, which may agglomerate in granules when required conditions prevail. Opinions diverge on these conditions, but, besides the standard anaerobic operation conditions, the ones often listed include the up-flow distribution of the effluent, and presence of inert carriers as well as suitable organisms (Hulschoff Pol *et al.*, 2004; Henze *et al*., 2008). These anaerobic granules are characterized by good settling velocities and high specific methanogenic activity (Henze *et al*., 2008); therefore, these characteristics of anaerobic granules result to the success of bioreactors such as the SGBR, IC or EGSB (Ellis and Evans, 2008; Basitere *et al*., 2016; Basitere *et al*., 2017; Henze *et al*., 2008). Anaerobic granules are represented by an arrangement of spherical and well-defined surface conglomerates of anaerobic micro-organisms. This arrangement can be visualized as a packed bed of granules, whose diameter typically varies between 0.15 and 4 mm (Henze *et al.*, 2008). The resistance to fluid flow generated by these anaerobic granules may culminate in PSW pressure loss inside the bio-reactor, which may result to a limitation of substrate distribution and ultimately promote biogas entrapment, poor effluent collection in down-flow configurations, and/or weakened dispersion of toxic substances such as ammonia and hydrogen sulphide contained in the entrapped biogas. As a result, this toxicity may reduce methanogenic activity. Therefore, it is

deemed necessary to develop an innovative configuration, such as the one provided by the DEGBR, to address the aforementioned challenges.

6.1.1 Chapter's objective

This chapter introduces and evaluates the performance of DEGBR, which was designed to address the shortcomings of high rate anaerobic bioreactors used for the treatment of poultry slaughterhouse. These challenges include the washout of solids, the difficulty associated with the operation of a three-phase separator, the drainage of the biogas and the energy requirement for bioreactors adopting an up-flow configuration. For bioreactors operated in a down-flow configuration, the challenges of channelling, short-circuiting, and clogging are often cited. The configuration of the DEGBR is geared towards addressing these challenges and thus its performance (PSW contaminants removal) is evaluated and compared to other technologies used for the treatment of poultry slaughterhouse wastewater.

6.2. Materials and Methods

6.2.1 Experimental set-up

The PSW used in this study was collected from a poultry slaughterhouse processing an average of a million birds a week and located in the Western Cape, South Africa. The experimental set-up was composed of three different stages including pre-treatment, bio-digestion and gas processing, as illustrated in Figure 6.1.

The first stage of the experimental set-up consisted of the removal of coarse solids, feathers, and part of the FOG content from the PSW through filtration using a 9.51 mm aperture size metallic sieve.

This phase was followed by the storage of the DEGBR feed in a feed holding tank, before feeding the bioreactor. The DEGBR consisted of an 86 mm ID PVC cylinder (2 mm wall thickness) that had a total height of 69 cm, when including the top (4 cm) and bottom (5 cm) cones that served to collect the biogas and effluent, respectively, from the DEGBR. The latter was surrounded along

with its height by a coiled water jacket connected to a water bath, whose temperature control system served to monitor the intended operating temperature range, mesophilic (30-35°C). Inside the DEGBR, a sieve of 25.4 mm mesh size was placed at the base of the bottom inverted cone to carry the packing material that served to retain the biomass within the reactor. The flow in and out of the DEGBR was controlled by a peristaltic pump. Three factors were taken into consideration when selecting packing materials, including the affordability of the material, the inertness of the material to mechanical and/or pneumatic mixing and microbial attack, and the availability of the material. However, to select the most suitable packing material from a pre-selection consisting of pea gravels, white pebbles, Ceramic marbles, and pumice stones, other parameters were considered, namely:

- The porosity,
- The head loss induced by the selected materials,
- Its sludge retention capacity, and
- The permeability of the packing material.

From these series of tests, the pumice stones demonstrated the ability to be the most suitable packing material, as it induced less pressure drop, had better retention of the anaerobic sludge and provided the best permeability. The packing materials were placed at the bottom of the reactor and occupied height of 5 cm. The pumice stones are volcanic rocks characterized by a rough vesicular texture, which provides a greater surface area than a typical rock of the same diameter. Their sizes vary, but the ones used in this study had an equivalent diameter varying between 6 to 14 mm and a sphericity of 0.66. The DEGBR was inoculated with an anaerobic granular sludge collected from an operating UASB reactor treating a local brewery wastewater (SABMiller, Newlands, South Africa), and conserved at 35°C before the inoculation. During the inoculation, 3 L of the anaerobic granular sludge was poured into the DEGBR. To acclimatize the inoculum to PSW, 1 L of PSW and 50 mL of a 20% dry milk solution were also poured into the DEGBR, which was hermetically closed and maintained at mesophilic conditions for two days under a batch condition before running the system under a continuous flow.

The third stage of the experimental set-up served to treat and collect the biogas produced from the system. The first step of this stage entailed the minimization of hydrogen sulphide from the

biogas using a scrubber, which consisted of a 15 cm long and 2.5 cm ID transparent PVC cylindrical tube filled with uncoated iron oxide mesh (steel wool). Mogomnang and Villanueva (2015) assessed this technology for H_2S removal and reported an efficiency >95%. Following the gas scrubber, a water displacement set composed of a 2 L glass container filled with a 5% w/v barrier solution of potassium hydroxide (KOH), and 100 mL measuring cylindrico-conical cylinder connected at its end to a valve that controlled the flow of the gas to a 500 mL Tedlar bag.

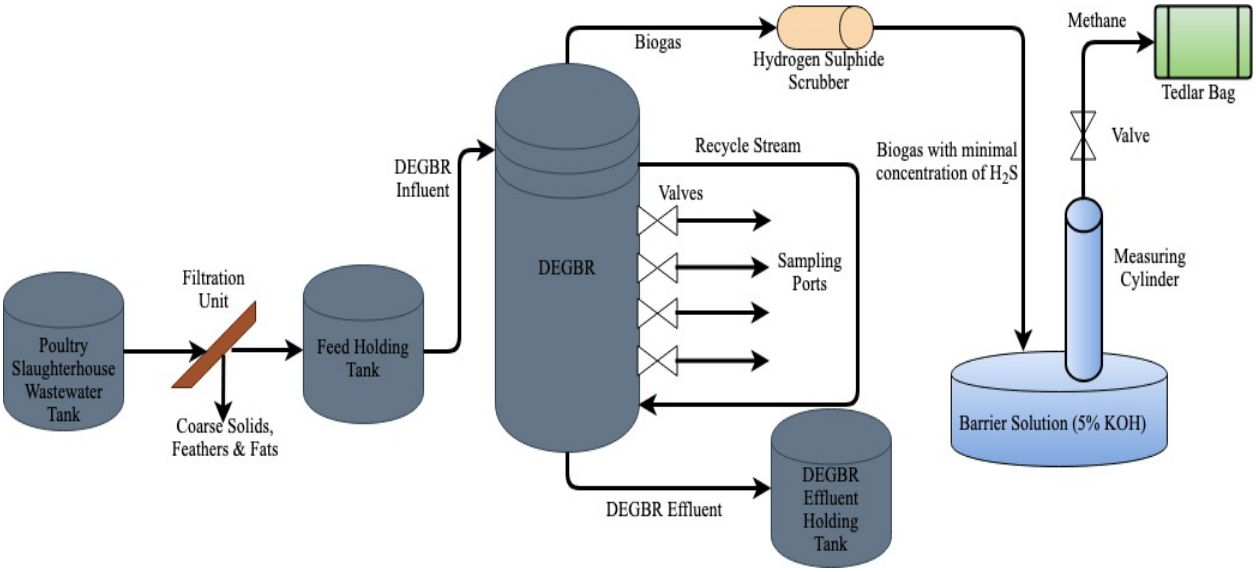

Figure 6.1: Experimental set-up

6.2.2 Operating conditions

During the first two weeks of the DEGBR operation, its feed was diluted with an equivalent amount of potable water to reduce the concentration of COD and thus facilitate the acclimation of the biomass to this new type of wastewater. As illustrated in Figure 6.1, the DEGBR was operated in a down-flow configuration and possessed a recycle stream that contributed to improving the distribution of the influent to the anaerobic biomass, and development a counter-current flow inside the bioreactor for enhanced mixing of its content. The distribution of the influent was improved by the provision of another inlet of PSW to the anaerobic granular bed by the recycle stream. This secondary inlet was located at the bottom of the granular bed to circumvent the head loss induced by the latter. The circulation of influent in bioreactors operating

in down-flow configurations is hindered by the pressure loss as it flows down the bioreactor. Therefore, to address this challenge, the DEGBR aims at improving the circulation of influent in such bioreactor configuration by providing another inlet to the bioreactor located under the level of the main inlet. This improves the contact between and organic influent and the biomass, which culminates in good methanogenesis at every level of the anaerobic biomass and the release of biogas. The elevation of the latter creates some channels that can be used by the organic influent to stream down the bioreactor.

Furthermore, this recycle stream, which was collecting PSW from the top of the DEGBR to feed its bottom part, was controlled by a separate pump that alternated the flow rate of the recycle stream according to operational requirements. In the event of channeling or accumulation of the influent inside the DEGBR, intermittent fluidization was implemented through a significant increase of the recycle stream flow rate, which could generate an up-flow velocity as high as 10 m/hr.

Provided smooth operation, the recycle stream flow rate was similar to the one of the influent PSW, which varied with the change of the system HRT. The latter varied between 15 and 40 hours, for an OLR varying between 1.1 to 38.9 gCOD/L.day. The DEGBR was operated under mesophilic conditions (30-35^{o}C). To prevent shock loading, the system was initially operated at an HRT of 35 hours, which corresponded to an OLR varying between 1.1 to 4.5 gCOD/L.day. This HRT was increased to 40 hours after 4 weeks of operation due to a periodic temperature upset that led to the alteration of the DEGBR performance. However, after a regain of stability, the HRT was step-wisely decreased to 24, 20 and then 15 hours for increased OLRs. Despite being used for providing an up-flow circulation of the influent within the granular bed at low up-flow velocities (varying between 0.12 to 0.8 m/hr), the recycle stream was also used for intermittent fluidization (10 to 15 mins) of the granular bed, when the need was required, to alleviate the pressure effects on the granular bed and thus to allow the dispersion of toxic substances, emergence of the biogas and improvement of substrate distribution. In this study, the intermittent fluidization was performed twice, at days 23 and 35, after the occurrence of a temperature anomaly and the clogging of the granular bed, illustrated by an accumulation of the influent inside the DEGBR. To tackle this challenge, intermittent fluidization was implemented through the increase of the recycle stream

flow rate to achieve the minimum fluidizing velocity of the granular bed. The fluidization re-opened the path for the circulation of PSW and allowed the dispersion of toxicants entrapped in the bottom part of the anaerobic granular bed. The sampling ports placed along the height of the DEGBR served to collect samples from the reactor and could be connected to the recycle stream when the influent needed to be distributed at a certain height of the granular bed. However, the location of the recycle stream was not moved throughout the course of this study, but this alternative can be used in other experiments should the requirement arise.

6.2.3 Analytical methods

The performance of the DEGBR was monitored using the tCOD, BOD_5, FOG, volatile fatty acids (VFAs), total suspended solids (TSS), turbidity, total dissolved solids (TDS) and alkalinity. These analyses were performed according to the APHA Standard Methods (APHA, 2005). The pH and the temperature were measured every day; whereas, the TDS and turbidity were measured every two days; and other parameters, such as the tCOD, BOD_5, FOG, VFA, and alkalinity were measured every week, as values tended to be similar over the week. Furthermore, the BOD_5 analysis took up to 5 days per sample. The biogas production was determined daily using the water displacement set, and the biogas sample collected in the Tedlar bag was analysed using a Geotech Biogas 5000 portable gas analyser for determining its composition.

6.3. Results and Discussion

The characteristics of the DEGBR influent and effluent were tabulated in Table 6.1. The efficiency of the DEGBR on PSW treatment was noticed by the decrease of contaminant concentration noticed in the effluent. The parameters used to quantify these contaminants include the turbidity, FOG, TSS, tCOD, and BOD_5. Only the concentration of the TDS increased in the effluent, suggesting an increase in the conductivity of the effluent as compared to the influent. The improved appearance of the effluent as compared to the influent was illustrated by a lower average turbidity in the effluent, as well as its TSS and FOG concentrations. The performance of

the DEGBR in the treatment of PSW under mesophilic conditions is further discussed in subsequent sub-sections.

Table 6.1: DEGBR influent and effluent characteristics

PSW	Influent		Effluent	
Parameter (mg/L)	**Range**	**Average**	**Range**	**Average**
TDS (mg/L)	639 - 1740	1250 ± 302	836 - 2670	1410 ± 350
Turbidity (NTU)	328.5 – 864.5	758 ± 158	11.46 – 286.5	33.65 ± 45.17
TSS (mg/L)	291 - 5044	1750 ± 1124	4.25 – 231.46	51.64 ± 45
tCOD (mg/L)	1664 – 32 375	8284 ± 7309	125 - 449	222 ± 97
BOD_5 (mg/L)	850 – 20 500	5132 ± 4549	25 - 225	77.33 ± 56.83
FOG (mg/L)	280 - 8228	1655 ± 1880	34 -116	57.47 ± 22.37

6.3.1 Stability of the DEGBR

Throughout this study, the stability of the DEGBR during the treatment of PSW was highlighted by a pH remaining in a range of 6.5 and 8, as well as the VFA/Alkalinity ratio remaining under 0.3, as illustrated in Figure 6.2, translating to a suitable biodegradability of the influent organic matter. This stability was also deduced by the concentration of VFA as acetic acid in the effluent maintained <500 mg/L, as depicted in Figure 6.2. To maintain stable methanogenic activities, it was essential to monitor the concentration of VFA, Alkalinity, and pH throughout the study. Furthermore, the ratio VFA/Alkalinity of the DEGBR's effluent was used to assess the stability of the process.

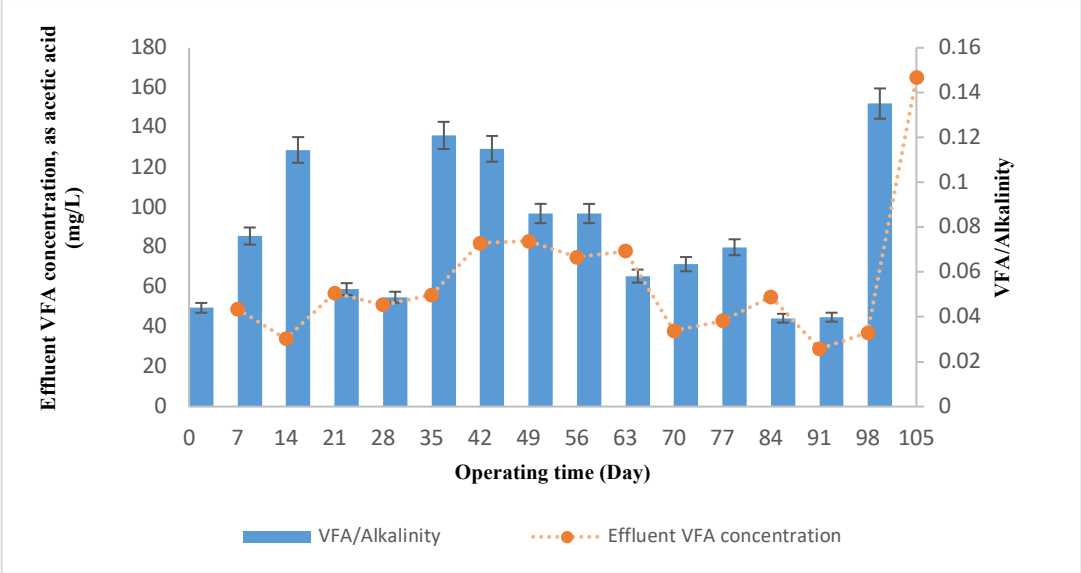

Figure 6.2: DEGBR effluent VFA concentration and VFA/Alkalinity ratio

6.3.2 Total COD removal

The control of the tCOD removal is essential to wastewater treatment. At the beginning of this study, the tCOD removal was lower (73% after a week of operation) than the consistent trend that followed. This can be explained by the acclimation of the anaerobic biomass to the new type of wastewater, as the previous one (brewery wastewater) presented different characteristics in terms of the quality of the organic matter content present in the wastewater. In this study, the highest tCOD removal achieved was 99.61%, as illustrated in Figure 6.3. This high removal percentage was related to the high influent tCOD concentration (32 375 mg/L), which was higher than the average tCOD concentration (8284 mg/L), for an effluent presenting similar characteristics. This high tCOD concentration in the sampled PSW could be explained by the prevailing poultry slaughterhouse operation at the time when the PSW was collected from the poultry slaughterhouse. Another reason may be the quantity of potable water that was used for the processing of birds before the PSW collection, as the quantity of used potable water affects the dilution of contaminants in the PSW. However, the tCOD removal percentage was maintained above 90% from the second week of operation, despite a slight depreciation of the DEGBR performance from week 4 to 5, as a result of a temperature anomaly due to a failure of the water

bath control system. The correction of the anomaly led to the stabilization of the tCOD removal around 95%, despite further decreases of the HRT that translated to increases of the OLR. In anaerobic treatment, tCOD removal corresponds to its conversion into biogas during the methanogenesis or its accumulation within the bioreactor in the form recalcitrant or, to some extent, biodegradable solids, partly due to a poor efficiency of the hydrolysis, which, similarly to the methanogenesis, is a limiting phase in the anaerobic digestion (Henze *et al*., 2008; Gerardi, 2003). However, the configuration of the DEGBR allowed a better distribution of the organic matter contained in the PSW to the anaerobic biomass, which culminated in improved degradation and subsequent conversion of the organic matter. Unlike the EGSB and the UASB that have an up-flow configuration (Henze *et al*., 2008; Del Nery *et al*., 2001), the DEGBR takes advantage of gravity as a supplementary force to improve the transport of PSW through the granular bed. The up-flow configuration of bio-reactors, such as the EGSB and UASB, has a higher energy requirement first to overcome the gravitational forces and then to compensate for the friction losses through the granular bed. Furthermore, the DEGBR has an added advantage over the SGBR (Basitere *et al*., 2017; Debik and Coskun, 2009; Ellis and Evans, 2008) by the fact that it uses a recycle stream that adds another PSW distribution port at a different location of the bio-reactor to improve the PSW distribution. Moreover, the down-flow configuration of DEGBR eliminates the requirement of the three-phase separator as the effluent was collected at the bottom of the reactor, while the gas was collected on top of the reactor and the biomass was retained inside the DEGBR by a selected underdrain system. As demonstrated by previous studies (Basitere *et al*., 2016, Henze *et al*., 2008), the three-phase separator did not guarantee complete retention of the anaerobic biomass raised to the top of up-flow anaerobic reactors, such as the EGSB and the UASB, from the emergence of biogas bubbles. In this study, the anaerobic sludge retention capacity was evaluated in various packing materials (pea gravels, white pebbles, Ceramic marbles and pumice stones), which culminates to the selection of pumice stones as packing materials for its good sludge retention capacity, reduction of heat loss and a good permeability. Furthermore, throughout the study, the good quality of the effluent, demonstrated by the concentration of tCOD, VSS, TSS, TDS or BOD_5, confirmed good retention of anaerobic

granules, which constitutes the required biomass for the anaerobic digestion and the methanogenic activity.

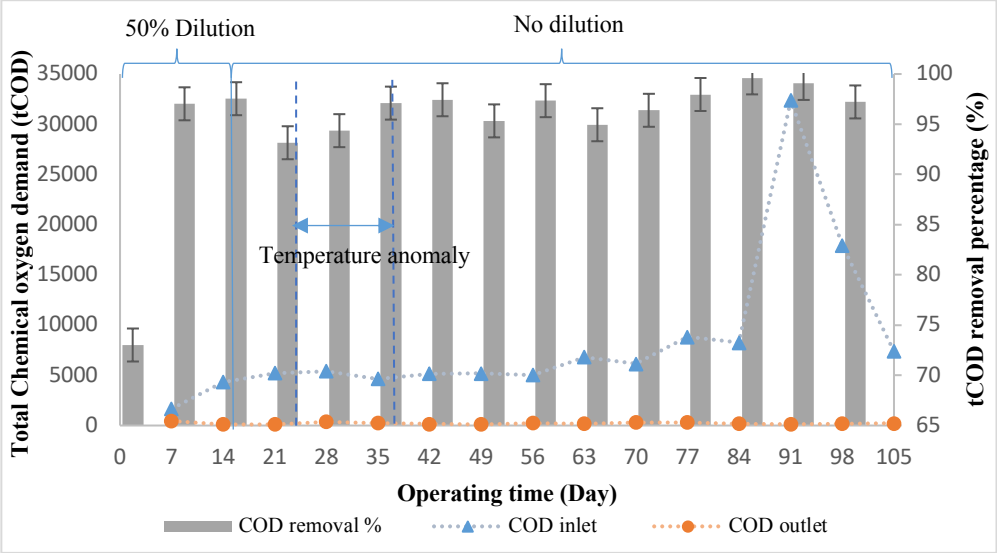

Figure 6.3: Variation of the DEGBR influent and effluent tCOD during the study

6.3.3 Further evaluation of the DEGBR performance

Further evaluation of the DEGBR performance was done by monitoring the tCOD, FOG and BOD_5 removals for the variation of the OLR throughout the study (See Figure 6.4). It resulted from this approach that the trends of the FOG and BOD_5 were quite similar to the one of the tCOD. A jump in the removal percentage of the three parameters evaluated was noticed after a period of acclimation facilitated by the dilution of the DEGBR feed with an equivalent quantity of tap water. After this period, it was also noticed that the percentage removal of the BOD_5 was the highest among the three, with values ranging between 94.7 to 99.8%, suggesting a very good conversion of the organic matter within the DEGBR despite the variation of the OLR. However, the FOG trend showed some deviations from the other trends on day 56 and 105, where the values of the percentage removal were under 90%. This was explained by a lower concentration of the FOG in the influent for a similar effluent quality. But, here also, the variation of the OLR didn't affect the performance of the DEGBR, despite the effects of temperature anomaly, continuously

happening between the day 15 and 37, as demonstrated by the alteration of the DEGBR performance during that phase.

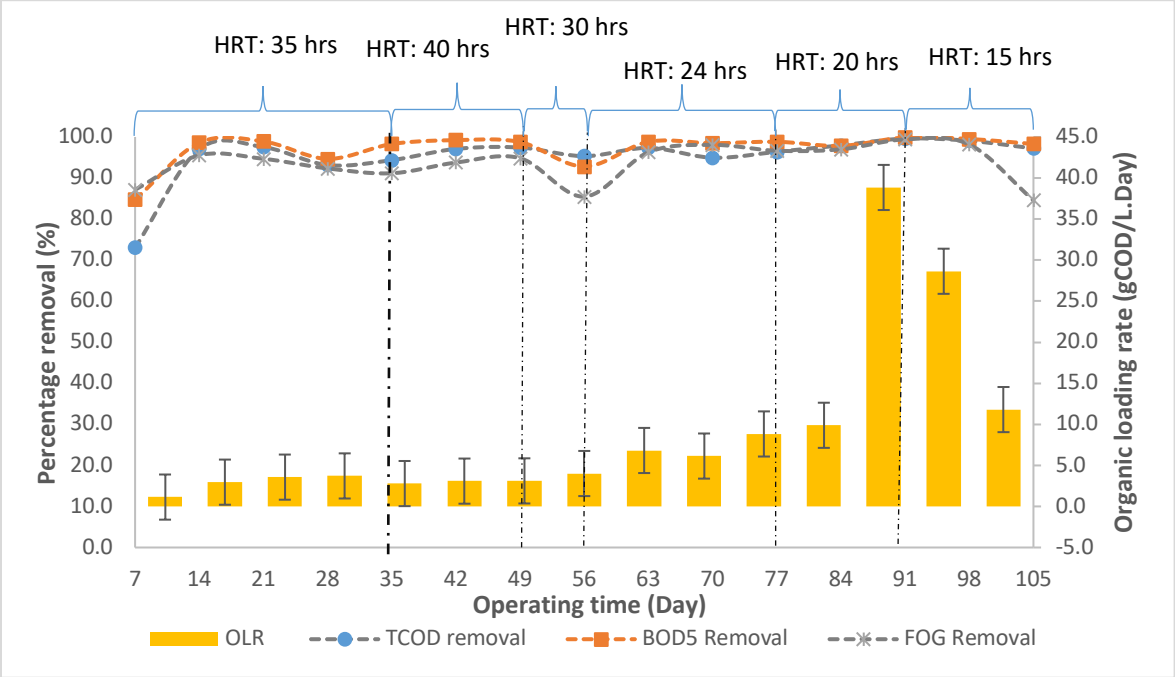

Figure 6.4: Evaluation of the DEGBR performance VS the OLR variation

This performance of the DEGBR could be compared to one of anaerobic bio-reactors used in previous studies for PSW treatment. Basitere *et al.* (2016) assessed the treatment of PSW using an EGSB and achieved a tCOD removal of 65% at an OLR of 1 gCOD/L.day. Then, Basitere *et al.*, (2017) evaluated the anaerobic treatment of PSW using an SGBR and achieved an improved performance characterized by a tCOD, TSS and FOG removals of 93%, 95%, and 90%, respectively, at an OLR varying between 1.01 and 3.14 gCOD/L.day. Debik and Coskun (2009) also used the SGBR for PSW treatment and achieved a TCOD removal of 95%. At last, Del Nery *et al.* (2007) evaluated the treatment of PSW using a UASB and achieved 85% soluble COD removal and 67% tCOD removal, at an OLR of 1.6 ± 0.4 kgCOD/m^3.day and an up-flow velocity of 0.3 ± 0.1 m/h. Table 6.2 summarizes and compares the results of the DEBGR to the ones of previous studies where the treatment of PSW was approached using similar technologies. From

this comparison, the DEGBR displayed the best results and can be considered as a good option for the treatment of PSW.

Table 6.2: Comparison of the DEGBR's results to the ones of similar bioreactors used for the treatment of PSW

Reference	Technology used	Parameters	Results
Del Nery *et al.* (2007)	UASB	OLR: 1.6 ± 0.4 KgCOD/m^3.day; up-flow velocity: 0.3 ± 0.1 m/h	85% soluble COD removal; 67% total COD removal
Basitere *et al.* (2015)	EGSB	OLR: 1 gCOD/L.day, Operational temperature: 30 - 35°C	65% total COD removal
Basitere *et al.* (2018)	SGBR	OLR: 1.01 to 3.14 gCOD/L.day, Operational temperature: 30 - 35°C	93% COD, 95% TSS, and 90% FOG
Rajakumar *et al.* (2011)	Up-flow anaerobic filter	Low up-flow velocity: 1.38 m/day; mesophilic temperature (29-35 °C), Inoculation with non-granular sludge; 147 days to complete the start-up	70% total COD removal; 79% soluble COD; Methane yield at maximum removal efficiency: 0.24 m3CH_4/KgCODremoved.day
Chavez *et al.* (2005)	UASB	OLR: 32 KgBOD_5/m^3.day; Operational temperature: 25 - 39°C	95% BOD_5 removal
This Study	Downflow Expanded Granular Bed Reactor (DEGBR)	Temperature: (30-35°C), OLR varying between: 1.1 to 38.9 gCOD/L.day	99.6 % COD Removal, 99.8% BOD_5 removal; 93.7 % FOG removal

6.3.4 Biogas production

The continuous production and collection of biogas was a requirement that motivated the design of the DEGBR, as the entrapment of biogas within the granular bed or poor production of biogas are challenges that often affect the collection of biogas from some anaerobic systems (Basitere *et al.*, 2017; Yamamoto *et al.*, 2009). The continuous production and collection of biogas were accomplished as illustrated in Figure 6.5. The collected biogas showed a composition of 80.8 % of CH_4, 3.6% of CO_2, 12,1% of O_2, 0.5% of H_2, 0% of H_2S and traces of other gases. The lack of H_2S in the biogas composition attested to the efficiency of the uncoated iron oxide scrubber on the

removal of H_2S. Furthermore, the low concentration of CO_2 proved the efficacy of the barrier solution (5% w/v KOH) in the dissolution of CO_2, as a higher concentration is normally expected from anaerobic digestion. However, the high concentration of O_2 was also noticed from this analysis. This could result from the penetration of air through a unit of the biogas treatment and collection set such as the Tedlar bag. Overall, the temperature anomaly highly influenced the biogas production, as the DEGBR was no longer operating under mesophilic conditions between day 24 and 37. To facilitate the re-adaptation of the system after the correction of the temperature anomaly, the HRT was increased to reduce the OLR until the system showed a return to a consistent biogas production on day 51. This return to appreciable biogas production rate was followed by step-wise reductions of the HRT for increased OLRs. Ultimately, this increase in OLR contributed to increase in the production rate of biogas.

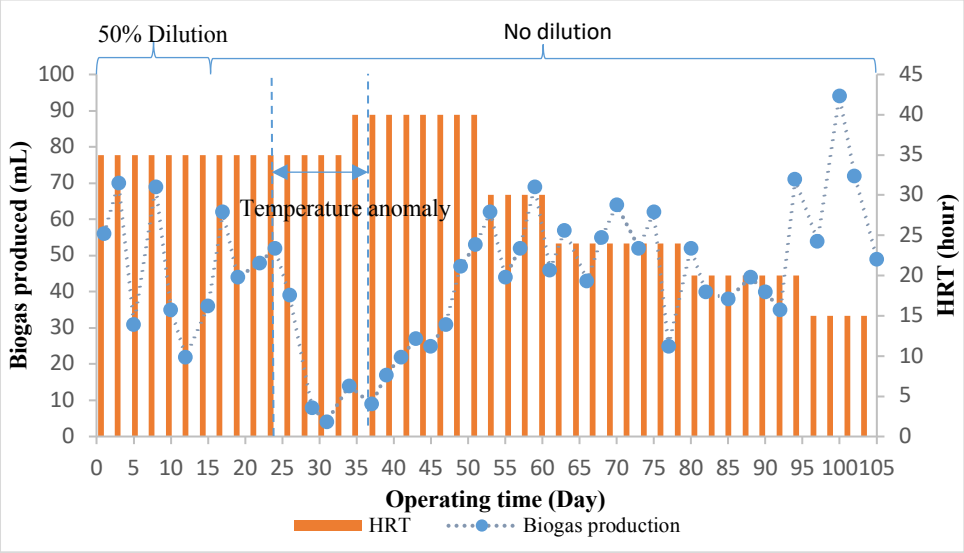

Figure 6.5: Biogas production VS HRT variation

6.4. Conclusion

Overall, the bench-scale DEGBR showed a good performance in terms of contaminants removal and biogas production, while addressing the challenges usually encountered in HRABS. These challenges included the difficulty associated with the operation of the three-phase separator, the washout of the biomass, head losses, biogas entrapment, limitation in the distribution of the

organic matter to the biomass, and poor dispersion of toxic substances. They were addressed through the configuration and features of the DEGBR. The latter was operated in a down-flow configuration to avoid the use of a three-phase separator and prevent the washout of the anaerobic biomass; with a recycle stream to improve the organic matter distribution to the biomass and subsequently its conversion, as well as the implementation of granular bed fluidization when required to improve the collection of biogas as well as the contact between the biomass and substrate, mixing of the granular bed, and dispersion of toxic substances.

6.5 Summary

This study evaluated the performance of the Down-flow Expanded Granular Bed Reactor (DEGBR) for the treatment of poultry slaughterhouse wastewater. This system consisted of a granule-based technology operated in a down-flow configuration, with the assistance of medium-sized pumice stones used as packing materials for the retention of the anaerobic granules, to avoid challenges associated with the use of the three-phase separator of up-flow systems and the washout of the anaerobic biomass. Furthermore, a recycle stream was applied to the system to improve the mixing inside the DEGBR, the influent distribution to the granular biomass, and the implementation of intermittent fluidization when required to alleviate the effects of pressure drop in such systems. The DEGBR was operated under mesophilic conditions (30-35^{o}C) and achieved tCOD, BOD_5, and TSS average removal percentages >95%, and a FOG average removal percentage of 93.67% ± 4.51, for an organic loading rate varying between 1.1 to 38.9 gCOD/L.day.

Overall, the DEGBR provided good results. To further compare this performance to existing configurations, the treatment of PSW was assessed using the SGBR and the DEGBR, which were run concurrently using the same methodology. The comparison of their performance is discussed in Chapter 7.

CHAPTER 7

PERFORMANCE COMPARISON OF THREE HIGH RATE ANAEROBIC BIOREACTORS (EGSB, DEGBR & SGBR) FOR THE TREATMENT OF PSW

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Chapter 7: PERFORMANCE COMPARISON OF THREE HIGH RATE ANAEROBIC BIOREACTORS (EGSB, DEGBR & SGBR) FOR THE TREATMENT OF PSW

7.1. Introduction

The processing of birds in poultry slaughterhouses requires huge quantities of potable water to meet the hygienic requirements imposed by regulatory bodies and deliver safe products to a growing clientele. Rajakumar *et al.* (2011) reported that it requires 18.9 to 38 L of potable water, for an average of 26 L/bird, to process a single bird in a poultry slaughterhouse. The blood, faeces, carcass debris, suspended solids and floating materials collected by the used potable water results in the formation of poultry slaughterhouse wastewater (PSW). This waste is essentially organic and has a higher strength than domestic sewage (Baddour *et al*., 2016; Rajakumar *et al*., 2011; Njoya *et al*., 2019). Therefore, the discharge of untreated PSW into sewage systems and water surface could be harmful to the environment and the health of people exposed to it (Avula *et al*., 2009). Various treatment options, including physical, chemical or biological processes, can be selected for the treatment of PSW. However, efficiency and cost-effectiveness remain the main drivers behind the selection of a suitable treatment option (Debik and Coskun, 2009).

Various studies, illustrated in Table 7.1, investigated the treatment of PSW. These studies resulted in a significant reduction of the concentration of wastewater characterization parameters such as the total chemical oxygen demand (tCOD), soluble COD, biological oxygen demand (BOD_5), fats, oil and grease (FOG), or total suspended solids (TSS), amongst others (see Figure 7.1). This was achieved through the use of various technologies, with the majority being high rate anaerobic bioreactor systems (HRABS). HRABS differ from other anaerobic bioreactors systems by their ability to retain the anaerobic biomass for a long period of time, which culminates in the growth

and efficiency of an anaerobic biomass that will minimize the retention time of PSW for high purification percentages (Del Nery *et al*., 2007; Baddour *et al*., 2016; Aziz *et al*., 2018). This high speed and quality of wastewater treatment complements other advantages such as small plant footprint requirement, low operational cost, reduction of excess sludge production, high organic loading rates, minimal requirement for additives and the production of methane, which is an alternative source of energy (Gerardi, 2003; Henze *et al*., 2008; Basitere *et al*., 2017; Njoya, 2017).

The most successful HRABS is the UASB, which is operational in more than 1000 wastewater treatment plants. Its success has been attributed to its up-flow configuration, which enables the formation of anaerobic granules (Pol *et al*., 2004; Sindhu and Meera, 2012). Anaerobic granules are aggregates of anaerobic microorganisms intervening in the anaerobic digestion (Alphenaar, 1994). The factors driving their formation are still obscure, despite different explanations provided by various researchers (Pol *et al*., 2004; Alphenaar, 1994).

The expanded granular sludge bed (EGSB) reactor is a variant of the UASB (Basitere *et al*., 2016). It differs from the UASB by the use of recycle stream and an expanded reactor height useful to accommodate higher up-flow velocities for the treatment of wastewater at higher organic loading rates (OLR). This bioreactor has been successfully used for the treatment of various types of wastewater, including PSW (Basitere *et al.,* 2016). The static granular bed reactor (SGBR) (Ellis and Evans, 2008) and down-flow expanded granular bed reactor (DEGBR) are relatively new bioreactors. The former was developed by a group of researchers at Iowa University, the United States and the latter was configured at the Cape Peninsula University of Technology, South Africa. These two bioreactors differ from the EGSB and UASB by a down-flow configuration promoted by the use of an underdrain system to retain the anaerobic biomass import from an operational UASB or EGSB reactor. Following the development of the EGSB from the UASB, the DEGBR also differs from the SGBR by the use of the recycle stream, which improves the distribution of the organic matter to the anaerobic biomass. Due to their down-flow configuration that eliminates the requirement of a three-phase separator and prevents the washout of the biomass. These two recent bioreactors have lower operational cost stemming from lower pressure requirements (Ellis and Evans, 2008; Basitere *et al*., 2017). The import of the granular anaerobic sludge from an operational UASB or EGSB treating wastewater of similar characteristics

significantly minimizes the start-up of the SGBR and DEGBR, to provide good results rapidly (Chernicharo, 2007).

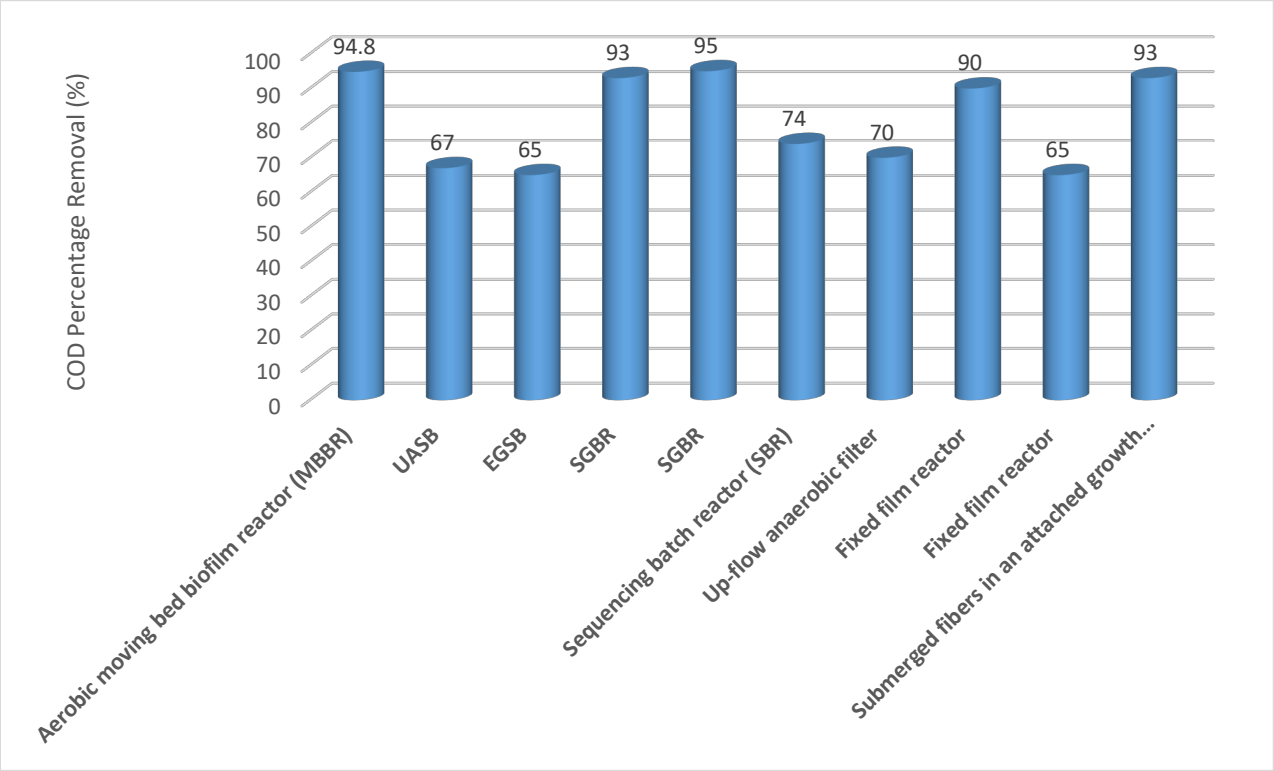

Figure 7.1: Performance of selected bioreactors in the treatment of PSW

7.1.1 Chapter's objective

This study aims to compare the performance of the EGSB, SGBR and DEGBR for the treatment of PSW at a mesophilic temperature range, to promote the treatment of medium to high strength wastewater at lower costs. Furthermore, due to their difference in configuration, this study will compare the performance of the up-flow and down-flow configurations for the treatment of PSW under similar operating conditions.

Table 7.1: Summary of previous PSW treatment studies

Reference	Technology used	Parameters	Results
Sindhu & Meera (2012)	Up-flow Anaerobic Packed bed Reactor (APBR)	OLR: 4 - 5 kg COD/m^3/day HRT: 24 h	88% COD removal; 85-98% TSS removal; 15% TDS removal
Baddour *et al.* (2016)	Aerobic moving bed biofilm reactor (MBBR)	38 days of detention time	61.7% TDS removal; 94.8% COD removal
Del Nery *et al.* (2007)	UASB	OLR: 1.6 ± 0.4 KgCOD/m^3.day; up-flow velocity: 0.3 ± 0.1 m/h	85% soluble COD removal; 67% total COD removal
Basitere *et al.* (2015)	EGSB	OLR: 1 gCOD/L.day	65% total COD removal
Basitere et al. (2018)	SGBR	OLR: 1.01 to 3.14 gCOD/L.day	93% COD, 95% TSS, and 90% FOG
Debik & Coskun (2009)	SGBR		95% COD removal
Moreira *et al.* (2002)	Sequencing batch reactor (SBR)		74% COD removal
Rajakumar *et al.* (2011)	Up-flow anaerobic filter	Low up-flow velocity: 1.38 m/day; mesophilic temperature (29-35 ^oC), Inoculation with non-granular sludge; 147 days to complete the start-up	70% total COD removal; 79% soluble COD; Methane yield at maximum removal efficiency: 0.24 m3CH_4/KgCODremoved.day
Del Pozo *et al.* (2000)	Fixed film reactor	OLR: 8 Kg/m^3.day	85 - 95% COD removal
Del Pozo *et al.* (2000)	Fixed film reactor	OLR: 35 Kg/m^3.day	55-75% COD removal
Chavez *et al.* (2018)	UASB	OLR: 32 KgBOD_5/m^3.day; Operational temperature (25 - 39^oC)	95% BOD_5 removal
Aziz *et al.* (2018)	Submerged fibres in an attached growth sequential batch reactor		96% BOD_5 removal; 93% COD removal

7.2. Materials and methods

7.2.1 PSW sampling

The PSW used in this experiment was sampled during the processing operations in a 20L polystyrene container from a poultry slaughterhouse facility located in the Western Cape, South Africa. After collection, the sample was conserved in a refrigerator whose temperature was maintained below 4°C to prevent PSW acidification.

7.2.2 Granular anaerobic inoculum collection and storage

The inoculum to three bioreactors (EGSB, SGBR, and DEGBR) used in this study was collected from a UASB operated from the treatment brewery wastewater in a local brewery, SAB Miller, Newlands, Cape Town. The UASB was continuously operated at a mesophilic temperature range (29 – 36°C). The inoculum was stored in a 20L polystyrene container and then conserved at 32°C before being used to inoculate the three bioreactors.

7.2.3 Experiment set-up

The three bioreactors were set-up as illustrated in Figure 7.2, 7.3 and 7.4. In each set-up, three 5L polystyrene containers were used to hold non-filtered PSW, filtered PSW (feed), and the product of each bioreactor. The filtration unit consisted of a series of two similar stainless-steel sieves (2 mm aperture size). These sieves were used to filter big flocs of fats as well as floating materials from the PSW to prevent operational inconveniences such as clogging of pipe and limitation of substrate distribution and biogas collection (Basitere *et al*., 2016). A water jacket, connected to a water bath circulating water at 36°C, surrounded each bioreactor to maintain mesophilic conditions (29 – 35°C) inside the bioreactors. The biogas produced from each bioreactor was passed through a packed bed of iron oxide mesh to minimize its hydrogen sulphide concentration (Magomnang and Villanueva, 2015; Al Mamun and Torii, 2015). The outlet stream of the hydrogen sulfide scrubber was connected to a barrier solution (5% v/w KOH), which was used to prevent the biogas to escape to the atmosphere while minimizing its concentration of CO_2, and remaining traces of H_2S (Abdel-Hadi, 2008). The outlet of the stream was placed inside a glass measuring cylinder that served to measure the volume of the

produced methane from a difference between the final and initial volume. After the measurement of the methane volume, a valve connected at the end of the measuring cylinder was open to allow the collection of methane in 500 mL Tedlar bag.

The SGBR and DEGBR both adopt a down-flow configuration, which prevents the use of a three-phase separator for the collection of biogas and control of biomass washout. Basitere *et al*. (2016) reported that serious difficulties associated with the operation of the three-phase separator (EGSB), which culminated in the washout of biomass during the emergence of biogas bubbles and/or at high up-flow velocities, as well as the collection of the biogas in the effluent stream. The anaerobic granular biomass was retained inside these two reactors by an underdrain system consisting of pumice stones (diameter varying between 3 to 20 mm). The SGBR was made of glass and had an inner diameter (ID) of 0.065 m and a height of 0.62 m, for a working volume of 2L. while the DEGBR was made of PVC and had an ID of 0.086 m and a height of 0.6 m for a working volume of 2,6 L. Both reactors had sampling ports along their height, as illustrated in Figure 7.2 and 7.3, but the DEGBR differed from the SGBR by a recycle stream that enabled the upward distribution of the PSW to the bottom of the DEGBR, therefore creating a counter-current distribution system inside the bioreactor, as illustrated in Figure 7.3.

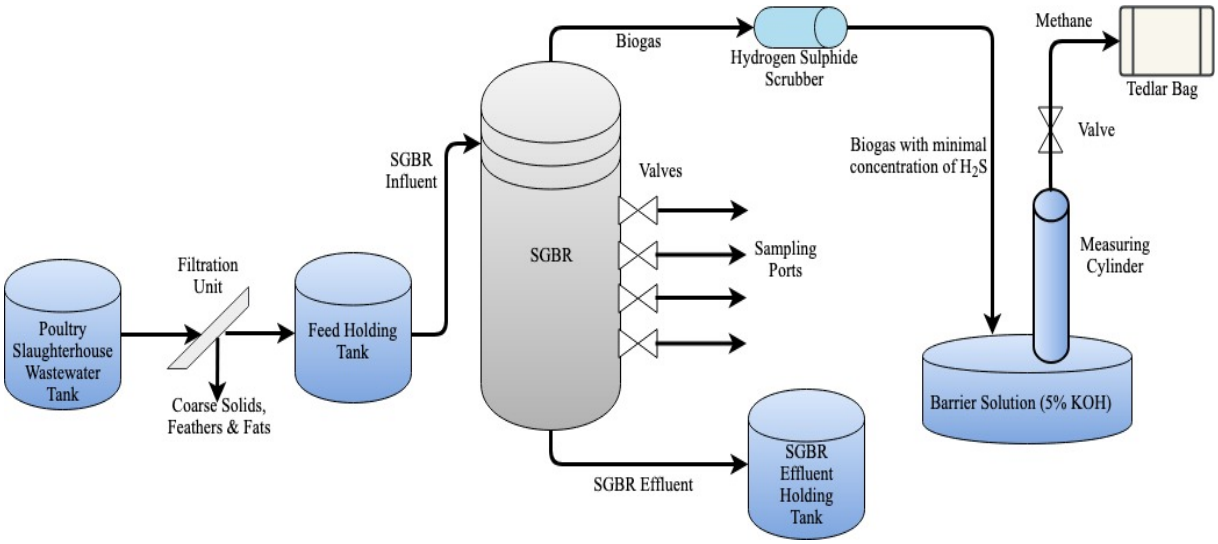

Figure 7.2: Static granular bed reactor experimental set-up

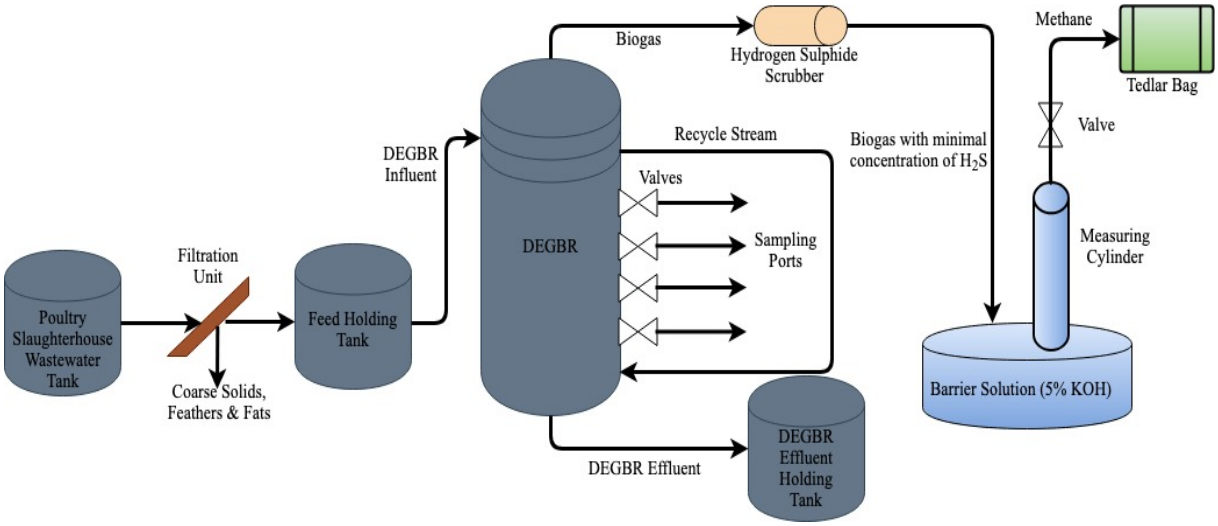

Figure 7.3: Down-flow expanded granular bed reactor experimental set-up

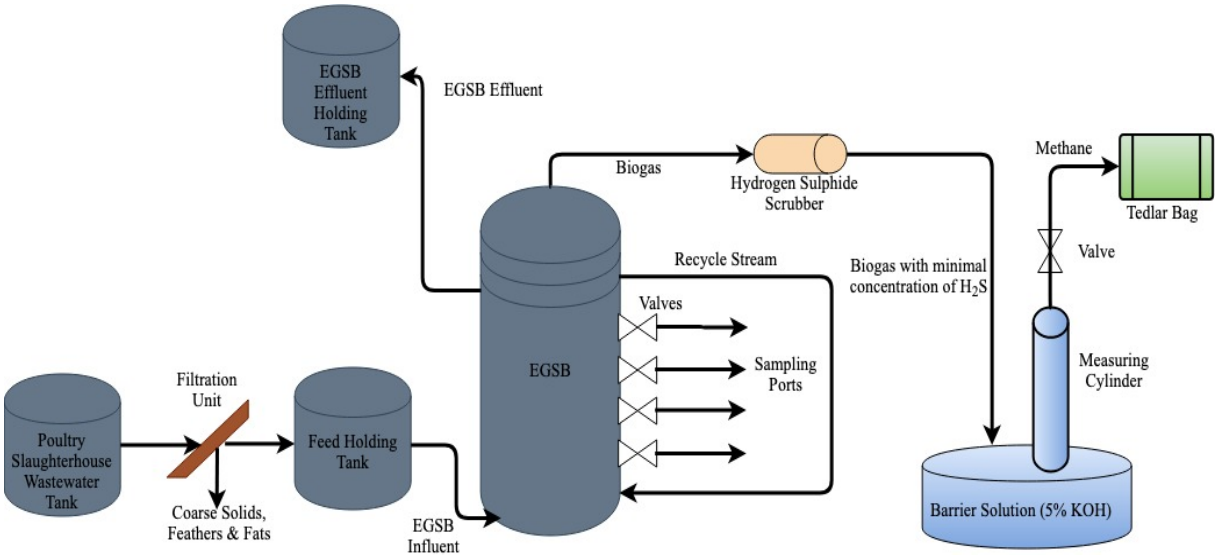

Figure 7.4: Expanded granular sludge bed reactor experimental set-up

Unlike the DEGBR and SGBR, the EGSB (Figure 7.4) consisted of an up-flow configuration that made use of a three-phase separator on top of the reactor to separate the effluent, anaerobic biomass, and biogas during the operation. The bioreactor was made of glass and had an ID of 0.065 m, a height of 0.872 m and operated at a working volume of 2.7 L. Ceramic marbles of a diameter of 0.0157 m were placed at the bottom of the reactor to minimize the clogging of the feed inlet stream by the anaerobic granular sludge. The EGSB also possessed a recycle stream than enabled the higher up-flow velocities and improved mixing of the bioreactor content.

7.2.4 Inoculation of the bioreactors

The three bioreactors used in this study were inoculated with different quantities of anaerobic granular inoculum collected from an operational UASB. The bioreactors were inoculated with volumes of 0.66 L, 0.99 L, and 0.86 L for the SGBR, EGSB and DEGBR, respectively. This difference in inoculum volume was motivated by the difference of the working volume of each bioreactor. To acclimate the inoculum to the PSW, 1,32 L, 1,8 L, and 1,72 L of PSW were also introduced in the SGBR, EGSB, and DEGBR, respectively. To promote the growth of the anaerobic biomass, a nutrient source of 15 mL of a 50% v/w solution of dry milk was also introduced into each all three bioreactors, which were then hermetically closed for a period 2 days before the start of the PSW treatment operation (Gerardi, 2003).

7.2.5 Operating conditions of the bioreactors

The three bioreactors were operated at mesophilic conditions (29 – 35^{o}C) with a step-wise increase of the hydraulic retention time (HRT) after as the response of the anaerobic granular biomass improved through appreciable organic matter reduction from the PSW. As observed from previous studies (Debik and Coskun, 2009; Basitere *et al*, 2017), the change of HRT has a direct influence on the organic loading rate (OLR). Thus, to prevent shock loading at the beginning of the treatment process, low HRTs were used for each bioreactor.

7.2.6 Samples analyses

Samples of feed and product of each bioreactor were collected every day and analysed for the pH, conductivity, TDS, salinity, and turbidity. Furthermore, every week, samples of feed and product were analysed to determine the concentration of tCOD, BOD_5, alkalinity, VFA, and FOG. All these analyses were performed as per the methods provided in Table 7.2.

Table 7.2: Sample analysis methods

Parameter	Method
pH	EPA method 9040C
Total dissolved solids (TDS)	EPA method 160.1
Salinity	EPA method 320
Temperature	EPA method 9040C
Turbidity	EPA method 180.1
Total suspended solids (TSS)	EPA method 160.2
Volatile suspended solids (VSS)	EPA method 1684
Total chemical oxygen demand (tCOD)	EPA method 410.4
Alkalinity as $CaCO_3$	Titration method 2320 B
Biological oxygen demand (BOD_5)	EPA method 5210 B
Volatile fatty acids (VFAs), as acetic acid	Potentiometric titration
Fats, oils and grease (FOG)	EPA method 10056

7.3. Results and discussion

7.3.1 Comparison of the performance of the SGBR, EGSB, and DEGBR

A comparison of the characteristics of the feed (Table 7.3) to the product (Table 7.4) of the three bioreactors (SGBR, DEGBR, and EGSB) provides an insight into the performance of these units for the treatment of poultry of PSW. A significant decrease in the average concentration of parameters such as the tCOD, TSS, FOG, BOD_5, turbidity was noticed for the three bioreactors. The significance of turbidity in the product of the three bioreactors translated to an effluent clearer, with a lighter coloration, as highlighted by the decrease of the concentration of TSS. This clarity of the effluent was also accompanied by the reduction of the smell of products as compared to the feeds, but the parameter was not quantified. From the three bioreactors, the DEGBR product presented the lowest concentration of TSS, tCOD, and BOD_5, with average concentrations of 51.64 ± 44.98, 264 ± 187.99, 45 ± 67.25, respectively. However, the lowest concentrations of FOG and turbidity were observed in the SGBR product, with average values of 51 ±22 and 14.7±24.6, respectively.

Table 7.3: Characteristics of the bioreactors' feed

Parameters		FEED					
		EGSB		SGBR		DEGBR	
	Unit	Range	Average (±SD)	Range	Average (±SD)	Range	Average (±SD)
pH		6 - 8	-	6.13 - 7.24	-	6.29 - 7.13	-
Conductivity	µs/cm	798 - 2360	1479 ± 412	973 - 2405	1604 ±414	899 - 2450	1769 ± 425.96
TDS	ppm	567 - 2145	1059 ± 303	691 - 1693	1138 ±294	639 - 1740	1250 ± 302.09
Salinity	ppm	390 - 926	772 ± 178	529 - 1413	916 ±179	451 - 1240	880 ± 189.80
Turbidity	NTU	99 - 1847	749 ± 342	237 - 997	719 ±201	328.5 - 864.5	758 ± 158.50
tCOD	mg/L	1423 - 11068	4981 ± 1832	2517 - 12490	5216 ±2534	2280 - 11425	5354.50 ± 1809.74
TSS	mg/L	60 - 5165	1399 ± 1213	313 - 8200	1654 ±1695	291 - 5044	1750.16 ± 1124.91
FOG	mg/L	312 - 1542	795 ± 367	2517 - 12490	5216 ±2534	280 - 1668	738.00 ± 373.84
BOD_5	mg/L	850 - 6125	3090 ± 1453	925 - 5000	2477 ±1347	850 - 4250	3000 ± 957.94
VFA	mg/L	71 - 721	383 ± 230	105 - 898	375 ±213	74 - 548	350 ± 167.64
Alkalinity	mg/L	415 - 1022	520.8 ± 145	322 - 923	499 ±158	360 - 926	602 ± 208.68

Table 7.4: Characteristics of the bioreactors' product

Parameters		PRODUCT					
		EGSB		SGBR		DEGBR	
	Unit	Range	Average (±SD)	Range	Average (±SD)	Range	Average (±SD)
pH		6 - 8.6	-	6.29 - 8.59	-	7.33 - 8.29	-
Conductivity	µs/cm	524 - 3495	1515 ± 205	1021 - 2323	1608 ±328	1173 - 3770	1992 ± 496.58
TDS	ppm	372 -2470	1073 ± 420	725 - 1643	1142 ±232	836 - 2670	1410 ± 350.40
Salinity	ppm	238 -1790	718 ± 278	529 - 1187	882 ±134	622 - 1970	957 ± 263.01
Turbidity	NTU	4 - 487	48 ± 43	3.57 - 234	14.7±24.6	11.47 - 286.5	33.65 ± 45.17
tCOD	mg/L	550 - 2798	1359 ± 108	482 - 974	729 ±98	127 - 1154	264 ± 187.99
TSS	mg/L	10 - 520	173 ± 83	13 - 160	63 ±38	4.25 - 231.46	51.64 ± 44.98
FOG	mg/L	30 - 189	60 ± 31	24 - 100	51 ±22	34 - 82	58.00 ± 15.99
BOD_5	mg/L	10 - 275	112 ± 82	10 -175	95 ±62	30 - 225	45 ± 67.25
VFA	mg/L	27 - 837	243 ± 298	21 - 402	124±118	34 - 83	57 ± 16.31
Alkalinity	mg/L	243 - 891	499 ± 186	347 - 933	588 ±163	447 - 1148	871 ± 235.55

A look at the pH of products, which is also the pH of bioreactor's content, reveals a slight increase in the pH ranges as compared to the ones of the feeds. This may be explained by a good conversion of the acids produced during the anaerobic digestion, as illustrated by low VFA concentrations in the product. The stability of such a treatment system can be assessed by the VFA/Alkalinity ratio of the samples from bioreactors, which should be maintained under a ratio of 0.4 for stable operations.

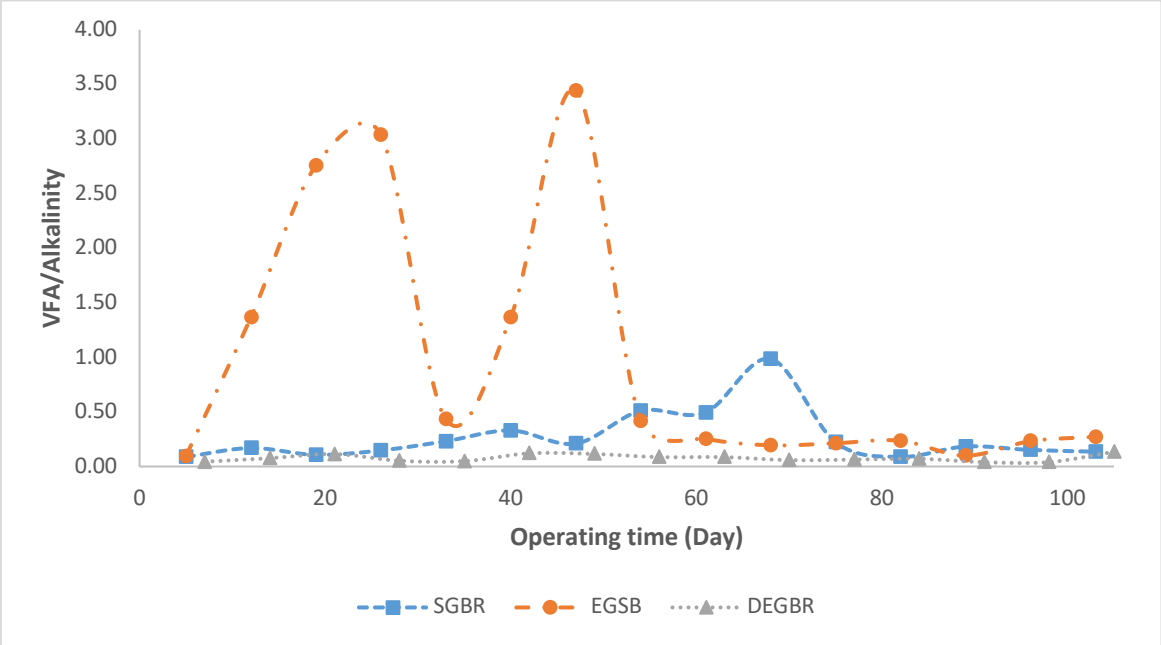

Figure 7.5: VFA/Alkalinity ratio of the SGBR, EGSB, and DEGBR

Figure 7.5 reveals that the DEGBR was the most stable bioreactors among the three bioreactors throughout the treatment of PSW; followed by the SGBR, while the EGSB was the least stable, mainly at the beginning of the process. This instability led to a longer operation time for the EGSB as illustrated by Figure 7.6, 7.7 and 7.8, to allow the system to stabilize. This also motivated less change of operating conditions (HRT, OLR) to prevent an upset of the anaerobic biomass. Due to slight operation instability, the SGBR was also operated for a longer time as compared to the DEGBR. Moreover, a slow improvement of the key parameters (tCOD, BOD_5, FOG, TSS) at the beginning of the process motivated a decrease of the OLR through an increase of the HRT at the beginning of the process, as illustrated in Figure 7.7. Thereafter, higher removal percentages motivated the decrease of the HRT for higher OLRs.

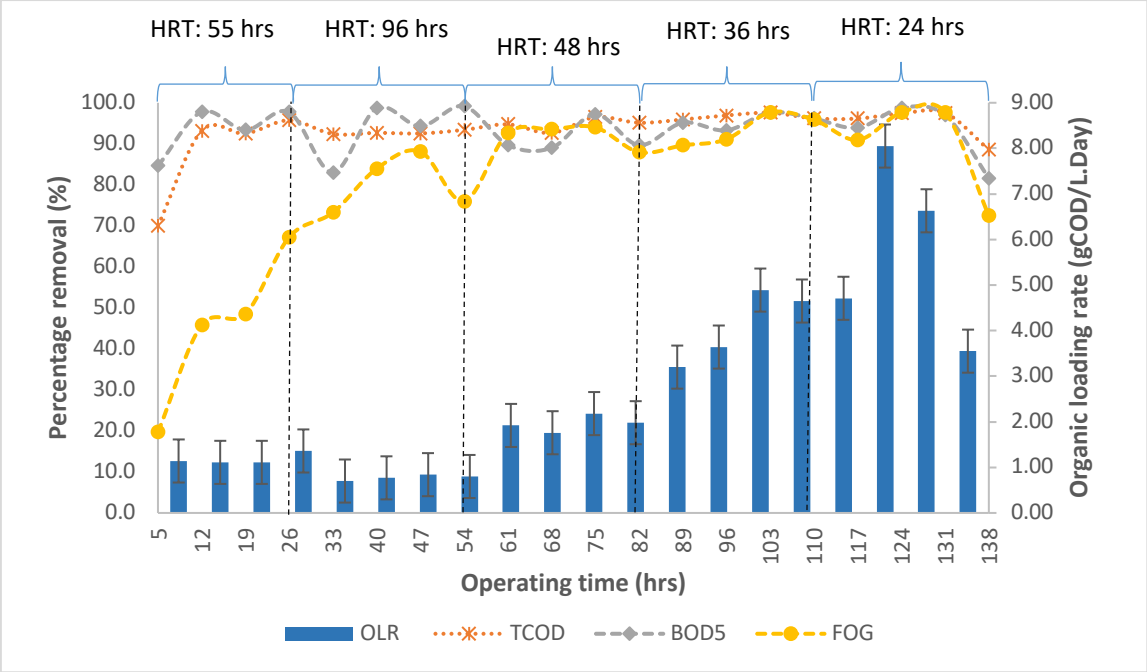

Figure 7.6: SGBR performance evaluated in terms of tCOD, BOD_5, and FOG removal

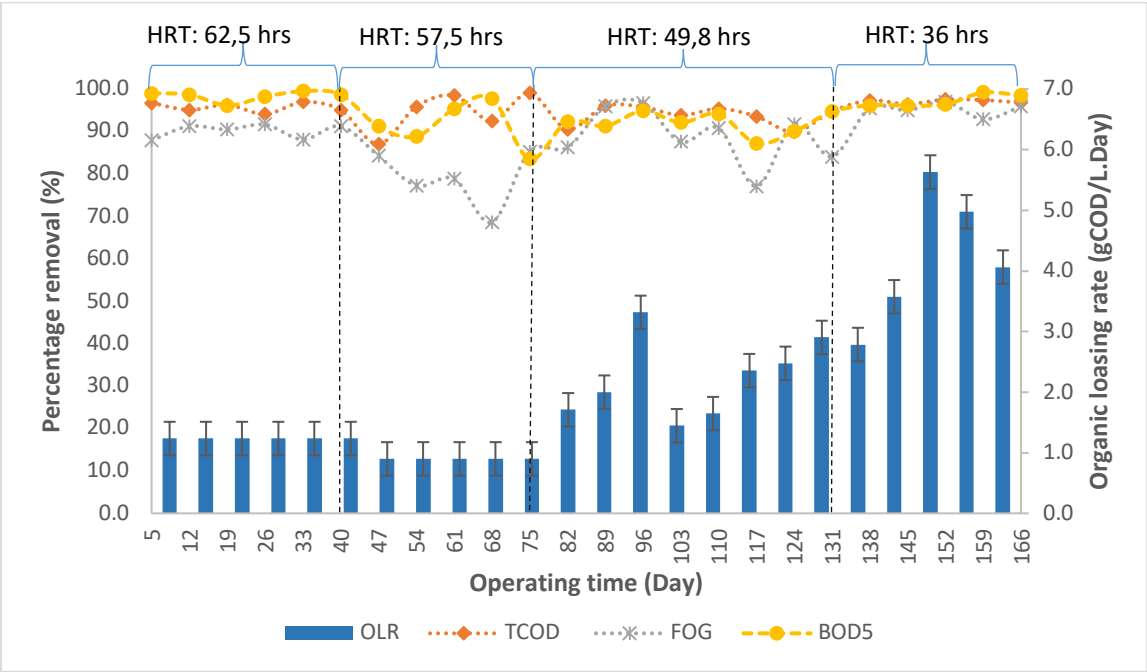

Figure 7.7: EGSB performance evaluated in terms of tCOD, BOD_5, and FOG removal

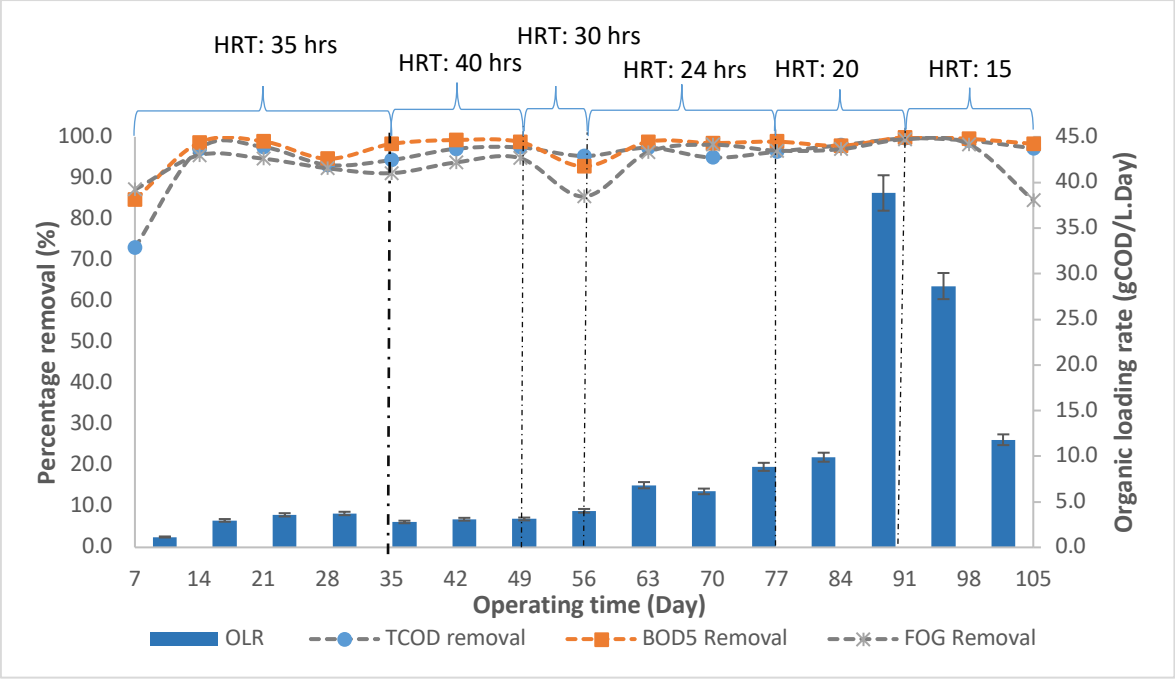

Figure 7.8: DEGBR performance evaluated in terms of tCOD, BOD_5, and FOG removal

A progressive increase of the OLR was also implemented in the DEGBR and EGSB, as illustrated in Figure 7.7 and 7.8. Overall, the three bioreactors responded well to these increases of OLR after 45 days of operation, with removal percentages maintained above 80% for each parameter. However, the DEGBR presented the best results with maximum removal percentages of 99.6%, 99.9%, and 99.4%, for the code, BOD_5, and FOG, respectively; at a maximum OLR of 38.9 gCOD/L.day. While the EGSB performed at maximum removal percentages of 99.1%, 99.5%, and 97%, for the tCOD, BOD_5, and FOG, respectively; at a maximum OLR of 5.6 gCOD/L.day. At last, the SGBR achieved maximum removal percentages of 97.6%, 99.2%, and 97.7% for the tCOD, BOD_5, and FOG, respectively; at a maximum OLR of 8.1 gCOD/L.day. These are appreciable results, when compared to the results provided by previous studies summarized in Table 7.1.

7.3.2 Methane production

Despite the good results of the SGBR and EGSB, they had minimal production of biogas, thus methane. Only the DEGBR presented a consistent production of methane, which was correlated to the removal of total COD, as illustrated in Figure 7.9. From this correlation, marked by a correlation factor of 0.92, it can be noticed that an mL of methane produced required approximately 6 gCOD/L.

The very weak production of biogas from the SGBR and EGSB could be related to several factors such as the instability of the system at the beginning of the process, the entrapment of the biogas bubbles in the anaerobic granular sludge or the collection of the biogas in the effluent for the EGSB. Further factors may be related to poor methanogenesis that relates to the weak stability of both EGSB and SGBR at the beginning of the process, as illustrated in Figure 7.5. The methanogenesis is a very sensitive stage of the anaerobic digestion, whereby a shift in environmental conditions may favour the prevalence of sulphate-reducing bacteria over methanogens (Gerardi, 2003). Moreover, a poor biogas collection system such as the three-phase separator may justify a weak biogas collection, which does not necessarily relate to biogas production.

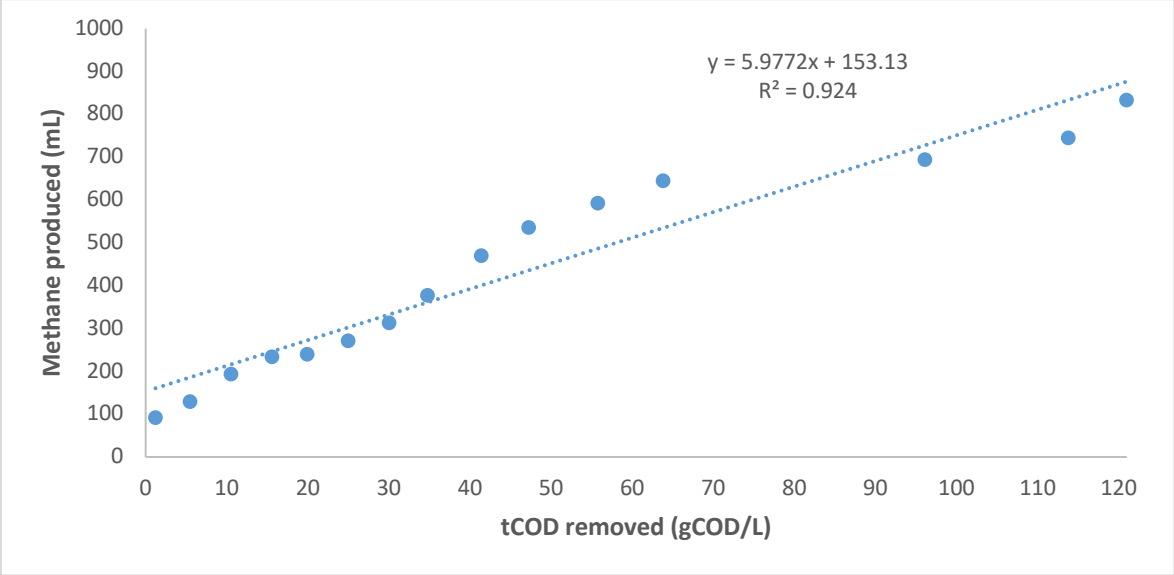

Figure 7.9: Correlation between the removal of tCOD and methane production

In comparison to the SGBR, the configuration of the DEGBR improves the distribution of the organic matter to the anaerobic biomass and provides additional up-flow forces to facilitate the emergence of biogas bubbles. Also, the DEGBR down-flow configuration eliminates the requirement of a three-phase separator for a simplified biogas collection system whereby only the biogas is collected on top of the reactor but requires the selection of a good suitable underdrain system to prevent the challenges of clogging or weak effluent collection.

7.4. Conclusion

The performance of three bioreactors (EGSB, SGBR, and DEGBR) was evaluated for the treatment of PSW under mesophilic conditions. Despite good results achieved by the SGBR and the EGSB, the DEGBR provided the best results in terms of organic matter removal, methane production and process stability. The process instability of the EGSB was more pronounced at the beginning of the process and culminated in a longer operating period for the assessment of its performance. Overall, the down-flow bioreactors (DEGBR and SGBR) displayed good performance despite changes of the OLR, suggesting that the import of the anaerobic granular sludge from an operational can significantly reduce the start-up of such bioreactors and reduces the operational costs required by up-flow configurations.

7.5 Summary

This study consisted of comparing the performance of the Expanded Granular Sludge Bed (EGSB) bioreactor, Static Granular Bed Reactor (SGBR) and Down-flow Expanded Granular Bed Reactor (DEGBR) for the treatment of poultry slaughterhouse wastewater (PSW). Three bioreactors were operated under mesophilic conditions and a similar operational methodology, which consisted of increasing the organic loading rate (OLR), as the bioreactor responded positively; this to improve their throughput and as well as their performance. However, the three bioreactors differed in their configuration, which is described in this chapter. Of the three bioreactors, the DEGBR provided the best results with tCOD, BOD_5 and FOG maximum removal percentages of 99.6%, 99.9% and 99.4%, for a maximum OLR of 38.9 gCOD/L.day. While the EGSB also provided appreciable results with 99.1%, 99.5% and 97%, for the removal of tCOD, BOD_5 and FOG, respectively. At last, the SGBR achieved tCOD, BOD_5 and FOG percentage removal of 97.6%, 99.2%, and 97.7%, respectively.

The good performance of the DEGBR demonstrated that down-flow HRABS can be used as a good alternative to the traditional up-flow HRABS. Therefore, Chapter 8 aims at predicting the performance of HRABS in such a configuration and providing correlations to determine their plant footprint.

CHAPTER 8

PERFORMANCE EVALUATION AND KINETIC MODELING OF DOWN-FLOW HIGH RATE ANAEROBIC BIOREACTOR SYSTEMS FOR POULTRY SLAUGHTERHOUSE WASTEWATER TREATMENT

To be submitted for Publication as

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Chapter 8: PERFORMANCE EVALUATION AND KINETIC MODELING OF DOWN-FLOW HIGH RATE ANAEROBIC BIOREACTORS FOR POULTRY SLAUGHTERHOUSE WASTEWATER TREATMENT

8.1. Introduction

The poultry industry requires enhanced wastewater treatment options for the treatment of PSW before its discharge into municipal water channels or freshwater sources (Bustillo-Lecompte and Mehrvar, 2017; Basitere *et. al;* 2017; Njoya *et al*., 2019). This contributes to protecting the environment and prevents poultry slaughterhouses from significant expenditures on municipal levies/charges for discharging untreated wastewater to the environment (Avula *et al*., 2009; Njoya *et al*., 2019; Basitere *et al*., 2019). This measure appears as an incentive from local governments, through City Councils, to drive better industrial wastewater treatment before discharge. Moreover, this measure is geared towards preventing the pollution of the flora, the alteration of the fauna and the mitigation of the health endangerment of people exposed to such wastewater (Avula *et a*l., 2009; Baddour *et al*., 2016). Various technologies have been investigated for the treatment of PSW, including physical, chemical and biological processes (Barbut, 2016; Bustillo-Lecompte *et al*., 2016; Debik and Coskun, 2009; Njoya *et al*., 2019). Of these alternatives, the anaerobic treatment of PSW has been highly praised due to a smaller plant footprint requirement, less sludge production, low energy intensity, and the generation of biogas that contains methane, which has a high calorific value. Anaerobic treatment has regained a huge interest during the last decades after the development of high rate anaerobic bioreactor systems (HRABS), which promote long SRT to maintain and ensure the growth of the anaerobic biomass within the HRABS

for shorter HRT and therefore increased wastewater treatment plant (WWTP) throughput (Henze *et al*., 2008; Chernicharo, 2007; Del Nery *et al*., 2001; Basitere *et al*., 2017). However, the good outcome of these studies should be mathematically represented by a model that correlates their drivers. Thus, kinetic modeling appears as an essential tool that can be used to predict HRABS efficiency and footprint based on experimental studies. Various kinetic models can be utilized to achieve this purpose, including the Graef and Andrews model (Andrew and Graef, 1970; Andrews, 1974), the Michaelis-Menten model (Tzafriri, 2003), the Monod model (Monod *et al*., 1965), the McCarthy and Young Model (McCarthy and Mosey, 1991; Shete and Shinkar, 2014), the Contois model (Shete and Shinkar, 2014; Basitere *et al*., 2019; Njoya *et al*., 2019), the Borja substrate balance model (Borja *et al.,* 2006), the Grau second-order multi-component substrate removal model (Grau *et al*., 1975), the Haldane model, the modified Stover-Kincannon model (Stover and Kincannon, 1982; Abtahi *et al*., 2011) or the anaerobic digestion model no.1 (Balstone *et al*., 2002). These models differ from each other by the adoption of a different approach to describe and predict the operation and efficiency of HRABS, but converge through the highlight of the importance of determining kinetic model parameters for the design of new or existing HRABS.

8.1.1 Kinetic modelling of down-flow HRABS

Most HRABS adopt an up-flow configuration as a result of the success of the UASB (Lettinga and Hulshoff Pol, 1991; Lettinga et al., 1980; Lim, 2009, Del Nery *et al*., 2001). Despite this success, this configuration presents some challenges associated with the difficulty of operating the three-phases separator, the washout of the biomass and the biogas, and the high energy required to overcome the friction losses induced by the anaerobic granules, which create a resistance to the upwards displacement of the influent (Evans, 2004; Lim, 2009; Njoya *et al*., 2019). A solution proposed to mitigate these challenges is the adoption of a down-flow configuration, which takes advantage of the gravity for the circulation of the substrate through the granular bed (Evans, 2004; Oh, 2012; Basitere *et al*., 2017; Njoya *et al*., 2019). This configuration can be effectively adopted by exporting the required anaerobic biomass from an operational HRABS, and thus bypassing the requirement of sludge granulation that requires a long operational time and which is not yet properly understood, but often associated with up-flow HRABS (Lettinga and Hulshoff

Pol, 1991). Thus, this sludge granulation is believed to occur high rate anaerobic reactors adopting an up-flow configuration such as the UASB or EGSB, and enables the aggregation of methanogenic bacteria for improved anaerobic digestion efficiency (Henze *et al*., 2008). Additionally, the down-flow configuration requires the selection of a suitable underdrain system to enable a steady-state operation, exemplified by the continuous collection of the effluent downstream, and to retain the required anaerobic biomass within the HRABS (Chernicharo, 2007; Njoya *et al*., 2019). The good retention of the anaerobic biomass within the bioreactor, illustrated by a long SRT, promotes a good bacterial growth, which is assisted by a good and continuous supply of nutrients provided through a short HRT of medium to high strength PSW (Lettinga and Hulshoff Pol, 1980; Balstone *et al*., 2002). One of these HRABS is the Static Granular Bed Reactor (SGBR) developed by a group of researchers from Iowa State University (Evans, 2004; Ellis and Evans, 2008). This HRABS has demonstrated good results in the treatment of PSW with COD removal percentages exceeding 95% (Basitere *et al*., 2017; Rinquest *et al*., 2019; Debik and Coskun, 2009). Another recent down-flow HRABS is the Down-flow Expanded Granular Bed Reactor (DEGBR), which differs from the SGBR by the use of a recycle stream (Njoya *et al*., 2019). This recycles stream improves the distribution of the influent at different locations of the bioreactor and enables the implementation of intermittent fluidization, when required, to maintain steady-state operations and address the challenge of granular bed clogging (Njoya *et al*., 2019). The DEGBR has also shown good results demonstrated by a good removal percentage (>95%) of BOD_5, COD, FOG and TSS (Njoya *et al*., 2019). This good performance of down-flow HRABS for the treatment of PSW motivates the requirement of modelling them to predict their performance and define their design parameters should an up-scale be required.

8.1.2 Kinetic modelling of HRABS

Kinetic modelling is a useful tool for industrial anaerobic reactor design (Kalyuzhnyi *et al*., 1998; Verma *et al*., 2015; Borja *et al*., 2006). It enables the understanding of anaerobic reactor design, operation, and conversion (Balstone *et al*., 2002). This modelling approach is widely used and defines parameters essential to the anaerobic system performance (Andrews, 1974; Monod *et al*., 1965; McCarthy and Mosey, 1991). Furthermore, kinetic modelling can be used to predict and

control the WWTP operation performance and to optimize the plant from the results of pilot studies (Verma *et al*., 2015).

Kinetic modelling can be divided into unstructured and structured models (Shete and Shinkar, 2014). Structured models relate to intracellular products while unstructured models apply to extracellular products generated through the action of enzymes (Shete and Shinkar, 2014). Kinetics provides an insight into the reaction rate describing how a microbial population converts the organic matter present in the wastewater into biogas (Balstone *et al*., 2002). Microbial fermentation differs from enzyme fermentation because the first relates to the conversion of substrate into biogas by microorganisms that increase in number and size during the process, while enzyme fermentation relies on an enzyme to induce the degradation of the enzyme (Shete and Shinkar, 2014). Thus, the kinetics can be split into four phases, including the lag phase, the log phase, the stationary and the death phase (Andrews and Graef, 1970; Grau *et al*., 1975; Verma *et al*., 2015).

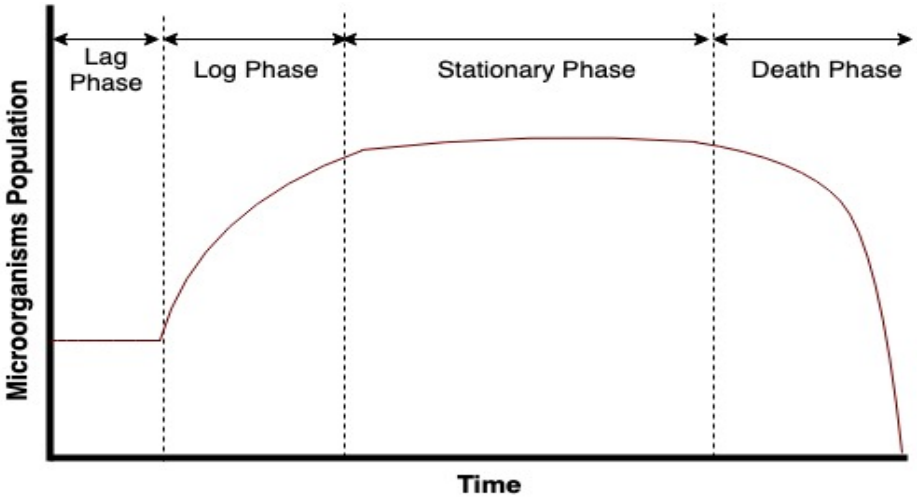

Figure 8.1: Phases of micro-organism growth

The lag phase, also known as the adaptation phase, refers to the period of acclimatization of the microbial population to a new environment and substrate (Metcalf, 2003; Gerardi, 2003)). The duration of this phase is driven by the microorganisms' physical and environmental conditions during this phase (Gerardi, 2003; Metcalf, 2003). These conditions include the prevailing pH and temperature, the age of the cell, the concentration of inhibitors and the quality of the substrate

(Gerardi, 2003). This phase is usually followed by the log phase, where exponential growth of the microorganism population is noticed as depicted in Figure 8.1 (Gerardi, 2003; Metcalf, 2003). Similar to the lag phase, prevailing conditions in the medium may affect the growth rate and critical parameters such as the temperature, pH, and the influent quality should be maintained within suitable ranges to ensure good growth (Henze *et al*., 2008; Gerardi, 2003). Subsequently, this phase is tailed by the stationary phase marked by the interruption of the exponential growth of microorganism to a steady-state process, whereby the death rate of microbial population is balanced by the microbial population growth (Henze *et al*., 2008; Gerardi, 2003; Abtahi *et al*., 2011). Here, the death rate may be promoted by the lack of nutrients, adverse environmental conditions, the generation of inhibitors or poor respiration (Henze *et al*., 2008; Gerardi, 2003; Abtahi *et al*., 2011). The prevalence of these factors leads to the last phase referred to as the death phase, where the death rate is significantly and consistently higher than the growth rate (Henze *et al*., 2008; Gerardi, 2003; Abtahi *et al*., 2011).

8.1.3 Chapter's objectives

This chapter aims at predicting the performance and the footprint of the SGBR and the DEGBR using the modified Stover-Kincannon model and the Grau second-order multicomponent substrate model. The kinetic parameters of these two models were determined and used to predict the substrate concentration in the effluent from the two bioreactors and were used to formulate a correlation that can be used to determine the volume of the bioreactors for each investigated model and as per targeted performance. These kinetic parameters were also compared with the ones provided by similar studies.

8.2. Materials and methods

The following steps describe the materials and methods used in this study.

8.2.1 PSW sample collection and storage

The PSW used in this study was sampled during peak operational time, in a 20 L container, from a poultry slaughterhouse located in the Western Cape, South Africa. This poultry slaughterhouse has a throughput of a million birds a week, suggesting that the sample collected from this facility provided a good representation of the wastewater investigated (PSW). The collected PSW was then stored in a refrigerator set at 4^{o}C and thereafter collected in batches of 1.5 L to prevent the influent acidification during the treatment.

8.2.2 Granular biomass export and conservation

The inoculum used in this study was collected from an operational UASB reactor used for the treatment of brewery wastewater at SAB Miller, a brewery located in Newlands, Cape Town. The collected inoculum was then stored in an incubator set at 35^{0}C, to mimic the temperature of the UASB, and then transferred to the SGBR and the DEGBR during the inoculation process.

8.2.3 Experimental set-up

The experimental set-ups used in this study are displayed in Figures 8.2 and 8.3. For each bioreactor, they consisted of a pre-treatment unit whereby coarse solids, feathers, and fats were removed by filtration through a 2-mesh sieve; a secondary treatment unit consisting of the SGBR in Figure2 and the DEGBR in Figure 8.3; and a tertiary treatment phase, or biogas treatment phase, consisting of an arrangement of hydrogen sulphide scrubber containing iron oxide mesh aims at reacting with the incoming biogas to minimize its concentration of hydrogen sulphide (Al Mamum and Torii, 2015), a tank containing a barrier solution of potassium hydroxide wherein was immersed the open part of a measuring cylinder terminated on the other end by a valve opening to a Tedlar bag (Abdel-Hadi, 2008), as illustrated in Figure 8.2 and 8.3. This biogas treatment process was geared towards minimizing the contaminants contained in the biogas to ensure the collection of methane in the Tedlar bag.

The SGBR used in the secondary stage of PSW treatment consisted of a sealed cylindrical glass (inner diameter of 0.065 m and a height of 0.62 m for a working volume of 2 L) surrounded by a

hot water jacket that contributed to maintaining the HRABS content within mesophilic conditions (29 – 35^{0}C). Its underdrain system was composed of pumice stones with a diameter varying between 3 to 20 mm. While the DEGBR consisted of a PVC cylindrical PVC tank (inner diameter of 0.086 m and a height of 0.6 m for a working volume of 2.6 L) surrounded by a coiled jacket of hot water insulated by a 2 mm thick layer of asbestos attached to the coiled jacket by a tape. The DEGBR underdrain system was also constituted of pumice stones and both reactors were terminated at both ends by cones, which facilitate the collection of the biogas and the effluent, on top and the bottom of the HRABS, respectively.

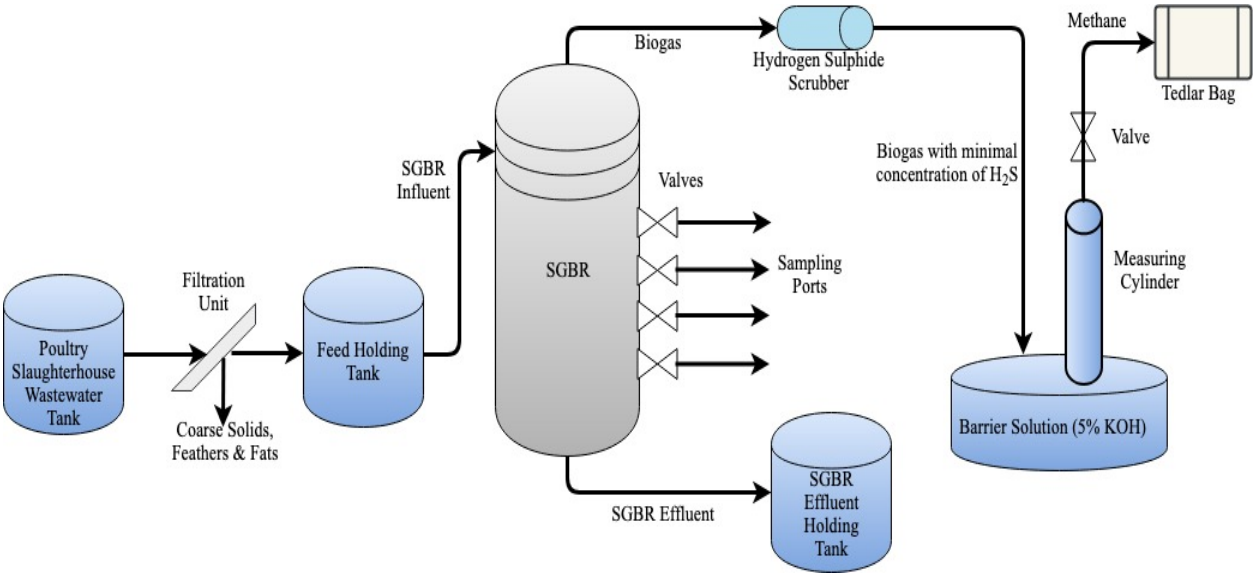

Figure 8.2: Experimental set-up of the SGBR

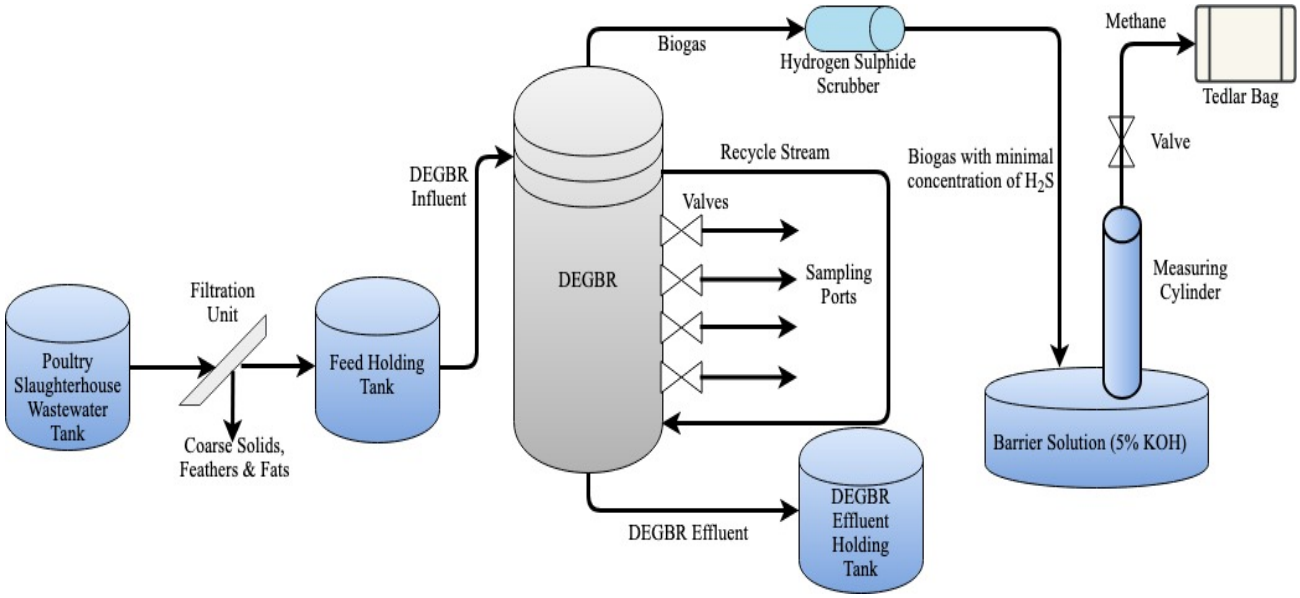

Figure 8.3: Experimental set-up of the DEGBR

The SGBR and DEGBR were inoculated with 0.66L and 0.86L of granular sludge, respectively. To promote the growth and the adaptation of the biomass to the new substrate, 15 mL of a 50% v/w of a solution of dry milk was added to each bioreactor, along with 1.32 L and 1.72L of PSW in the SGBR and DEGBR, respectively. After the addition of all these substances into each bioreactor, they were sealed and left un-operational for two days. Thereafter, both bioreactors were set to operate at steady-state with high HRT at the beginning of the process, which was decreased step-wisely as the HRABS responded well to the treatment of PSW through high contaminant removal percentages. This procedure also enables the control of the OLR to the two bioreactors.

8.2.4 SGBR and DEGBR influent and effluent analysis

Samples of the feed and product of each bioreactor were collected three times a week and were analysed to quantify the concentration of the total COD and volatile suspended solids (VSS). Furthermore, the pH, the temperature, alkalinity, and volatile fatty acids were assessed daily to ensure conducive anaerobic digestion throughout the treatment process. The analyses performed followed the methods provided in Table 8.1.

Table 8.1: SGBR and DEGBR samples analysis methods

Parameter	Method
pH	EPA method 9040C
Temperature	EPA method 9040C
Turbidity	EPA method 180.1
Total suspended solids (TSS)	EPA method 160.2
Volatile suspended solids (VSS)	EPA method 1684
Total chemical oxygen demand (tCOD)	EPA method 410.4
Alkalinity as $CaCO_3$	Titration method 2320 B
Biological oxygen demand (BOD_5)	EPA method 5210 B
Volatile fatty acids (VFAs), as acetic acid	Potentiometric titration
Fats, oils and grease (FOG)	EPA method 10056

8.3. Results and Discussion

8.3.1 Performance evaluation

The performance of the DEGBR and the SGBR was evaluated in terms of the removal of organic matter contained in the influent under different HRTs and therefore OLRs. A decrease of the HRT relates to an increase of the OLR, which quantifies the charge of the organic matter transferred into a bioreactor, as more substrate is made available to the biomass within the bioreactor through a short residence time of the influent PSW. This short residence time or HRT translates to a higher load of the substrate to the anaerobic digestion. From the analysis of the effluent from each bioreactor at an early stage of the treatment process, it was found that the DEGBR and the SGBR provided good results in terms of the removal of the COD and BOD_5 from the influent PSW with removal percentage remaining above 85% after two weeks of operation, as illustrated by Figure 8.4 and 8.5. However, the SGBR did not perform as good as the DEGBR for the removal of the FOG. It took more than 50 days for the SGBR to reach a high removal percentage of this wastewater contaminant, as opposed to the DEGBR that displayed good results after the period of acclimatization of the biomass to the PSW. The two HRABS were suggested to the same environmental conditions (mesophilic temperature and same substrate and inoculum), and were

therefore expected to produce similar results. However, the configuration of the DEGBR, which includes a recycle stream that improves the distribution of the influent to the anaerobic biomass, may explain this difference in performance. Additionally, hydrolysis is often listed as a limiting step in anaerobic digestion, as poor hydrolysis i.e. degradation of macromolecular organic matter into simpler compounds much easier to process, maybe a big contributing factor to the limited performances in anaerobic digestion (Gerardi, 2003; Henze *et al*., 2008).

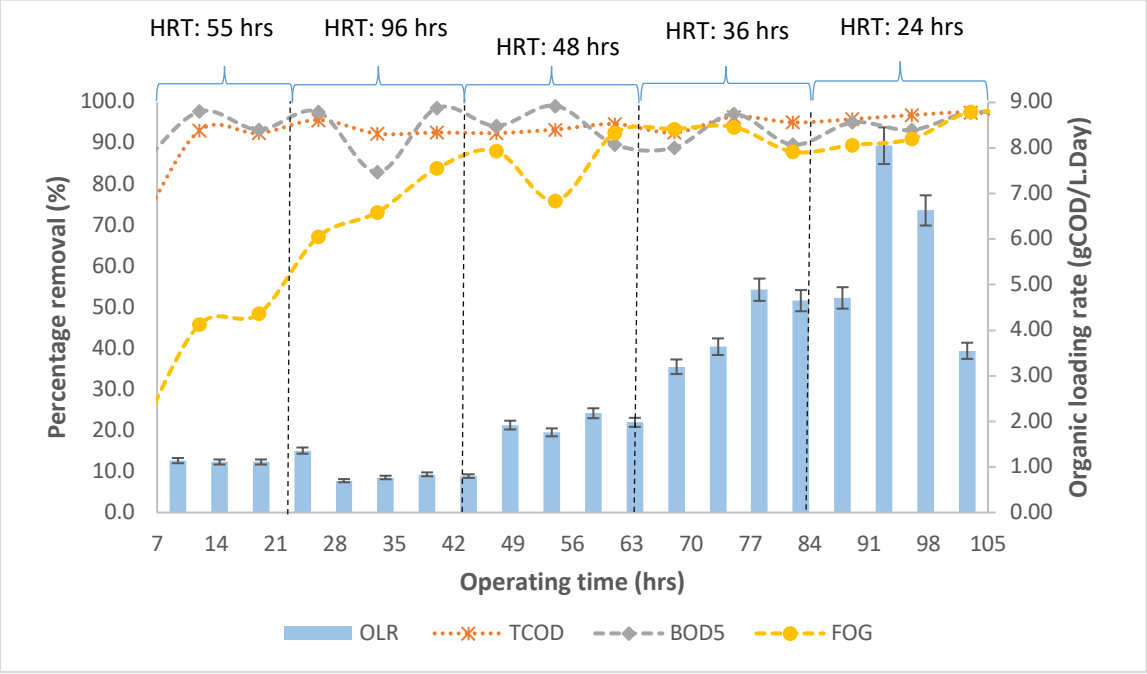

Figure 8.4: Performance of the SGBR for the treatment of PSW

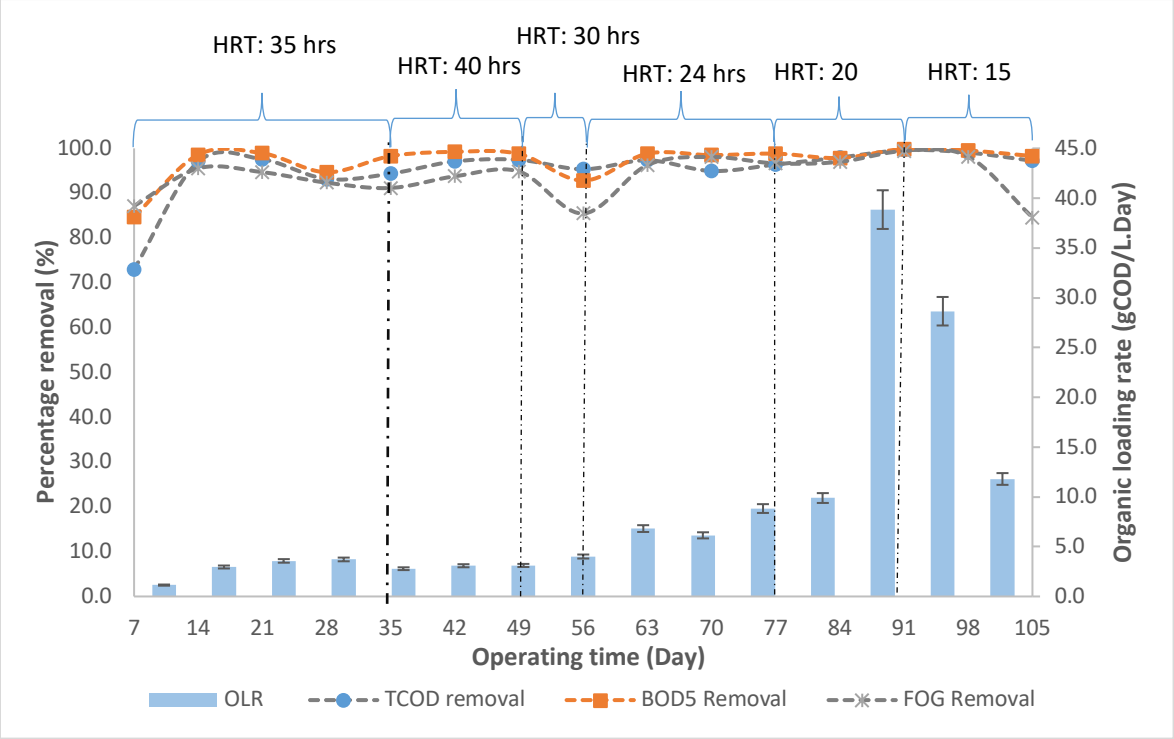

Figure.8.5: Performance of the DEGBR for the treatment of PSW

However, overall, the two HRABS provided good results with the percentage removal of the BOD_5 reaching 99%, and the tCOD achieving 98% for both bioreactors. This motivates the selection of such HRABS for the treatment of PSW and the determination of their kinetic parameters towards their design and the prediction of their performance.

8.3.2 Modified Stover-Kincannon model

Many studies have been conducted towards the formulation of kinetic models describing and predicting effluent substrate concentration and substrate transformation in anaerobic systems (Grau *et al*., 1975; Abtahi *et al*., 2011; Stover and Kincannon, 1982; Borja *et al*., 2006; Andrews and Graef, 1970). In the early 1980s, Stover and Kincannon suggested a kinetic model for biofilm reactor, in which the substrate utilization rate related the organic loading rate used in monomolecular kinetics for biofilm bioreactors (Stover and Kincannon, 1982). The original Stover-Kincannon model was initially suggested for rotating biological contactor (RBC) systems and was expressed by Equation 8.1 (Stover and Kincannon, 1982).

$$\frac{dS}{dt} = \frac{U_{max}(\frac{qS_O}{A})}{K_B + (\frac{qS_0}{A})} \quad (8.1)$$

Where A relates to the disc surface area serving of support to the active biomass; S_o provides the initial substrate concentration (gCOD/L), S indicates the substrate concentration in the bioreactor a time (t), q relates to the flow rate, U_{max} gives the maximum removal rate constant and K_B translates to the saturation value constant.

Equation 8.1 was amended by substituting the substrate utilisation rate to the organic loading rate as expressed by Equation 8.2.

$$\frac{dS}{dt} = \frac{U_{max}(\frac{qS_O}{V})}{K_B + (\frac{qS_0}{V})} \quad (8.2)$$

Where V relates to the volume of the anaerobic bioreactor.

Under steady-state conditions marked by no accumulation of the organic matter within the bioreactor, dS/dt could be linearized as expressed by Equation 8.3.

$$\frac{dS}{dt} = \frac{q(S_O - S)}{V} \quad (8.3)$$

The linearization of Equation 8.2 using Equation 3 results in Equation 8.4.

$$\frac{V}{q(S_0 - S)} = \frac{K_B V}{U_{max} q S_0} + \frac{1}{U_{max}} \quad (8.4)$$

At a steady-state, the hydraulic retention time is provided by Equation 8.5.

$$HRT = \frac{V}{q} \quad (8.5)$$

Equation 8.5 can be used to simplify Equation 4 and results in Equation 8.6.

$$\frac{HRT}{(S_0 - S)} = \frac{K_B HRT}{U_{max} S_O} + \frac{1}{U_{max}} \quad (8.6)$$

According to Equation 8.6, a plot of $HRT/(S_o\text{-}S)$ versus HRT/S_o should provide a straight line with intercept $1/U_{max}$ and slope equivalent to K_B/U_{max}, as illustrated in Figure 8.6 and 8.7, which provide the Stover-Kincannon kinetic parameters of the SGBR and DEGBR, respectively, determined from the treatment of PSW using the listed HRABS. The high coefficient of performance (R^2 = 0.992 for the SGBR with a RMSE of 0, and R^2 = 0.9889 for the DEGBR with a RMSE of 0.004) reflects high

confidence in the accuracy of the Stover-Kincannon kinetic parameters, which can be calculated from the intercept and the slope of the straight line. These parameters can be used to predict the concentration of substrate in the effluent as well as the performance of investigated HRABS. This can be achieved by simplifying Equation 8.6, which results in Equation 8.7 and 8.8. Equation 8.8 provides a correlation that enables the determination of the substrate concentration in the effluent for each bioreactor.

$$\frac{1}{(S_0-S)} = \frac{K_B}{U_{max}S_O} + \frac{1}{HRT.U_{max}} \tag{8.7}$$

$$S = S_0 - \frac{U_{max}}{\frac{K_B}{S_0}+\frac{1}{HRT}} \tag{8.8}$$

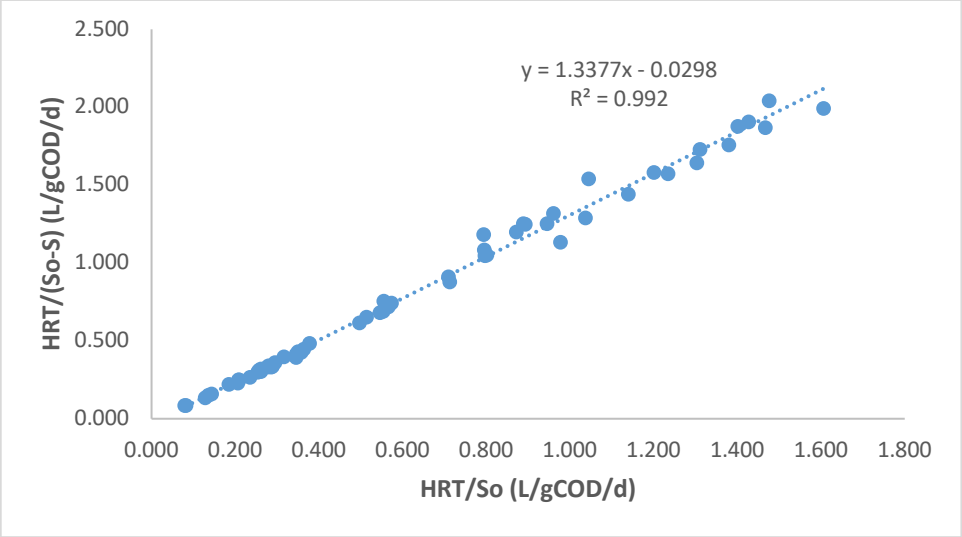

Figure 8.6: Evaluation of the Stover-Kincannon kinetic parameters on the SGBR

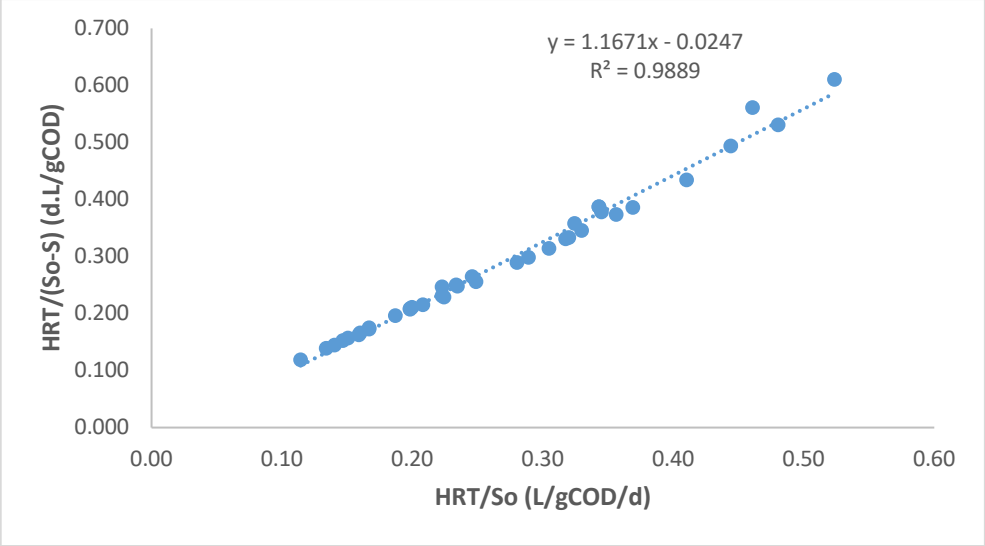

Figure 8.7: Evaluation of the Stover-Kincannon kinetic parameters on the DEGBR

Figures 8.8 and 8.9 compare the experimental to the predicted COD concentration in the SGBR and the DEGBR, respectively. It can be noticed from them that the effluent COD concentration of both bioreactors is well predicted, with the DEGBR showing a more consistent trend throughout the experiment than the SGBR. Furthermore, it can be noticed than the DEGBR provided a better performance in terms of COD removal demonstrated by the concentration of the substrate in the effluent mostly remaining under 0.5 g/L throughout the anaerobic treatment process using the DEGBR, while the concentration of the substrate in the effluent from the SGBR remained above 0.5 g/L for both experimental and the predicted effluent COD. Furthermore, the prediction from the DEGBR matches more the actual COD concentration from the DEGBR effluent than the SGBR effluent. This difference can be related to the ratio of VFA/Alkalinity, which provides the stability of both bioreactors during the treatment of PSW (Metcalf, 2003; Henze *et al*., 2008). This stability is illustrated by VFA/Alkalinity ratio ranging between 0.1 and 0.3 during the anaerobic treatment process. A concentration higher than 0.3 suggests the acidification of the HRABS content, which might inhibit the methanogenesis and lead to adverse outcomes for the anaerobic digestion.

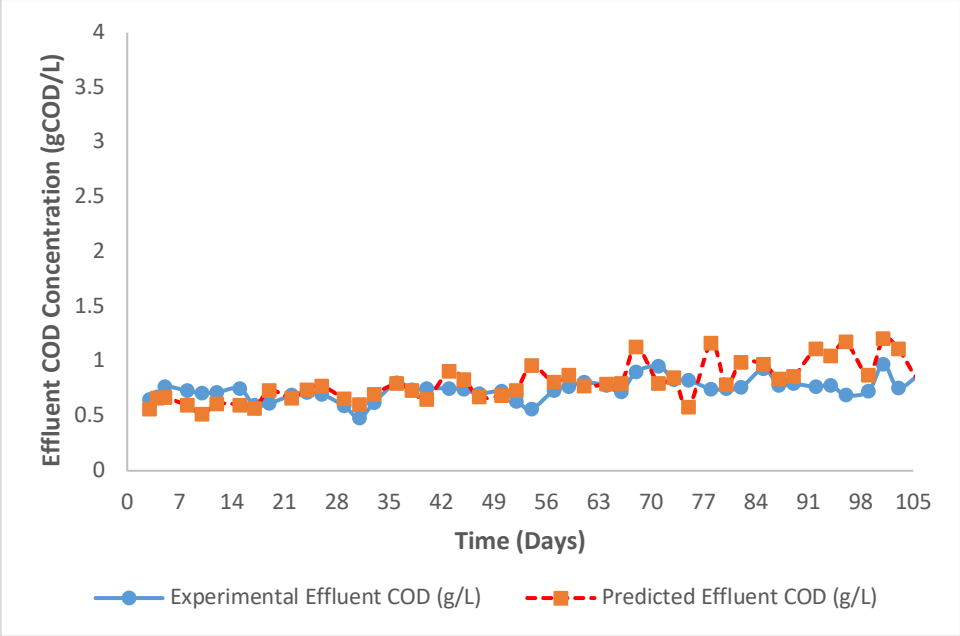

Figure 8.8: Comparison between the actual and predicted SGBR effluent COD concentration (Modified Stover-Kincannon Model)

This stability of both HRABS during the anaerobic treatment of PSW is demonstrated by Figure 8.10, which provides the VFA/Alkalinity ratio of both bioreactors. This ratio remained under 0.3 for the DEGBR throughout the treatment process, while the SGBR surpassed the stability indicator between days 53 and 77, as illustrated by Figure 8.10. This suggests that the DEGBR was more stable than the DEGBR for the treatment of PSW.

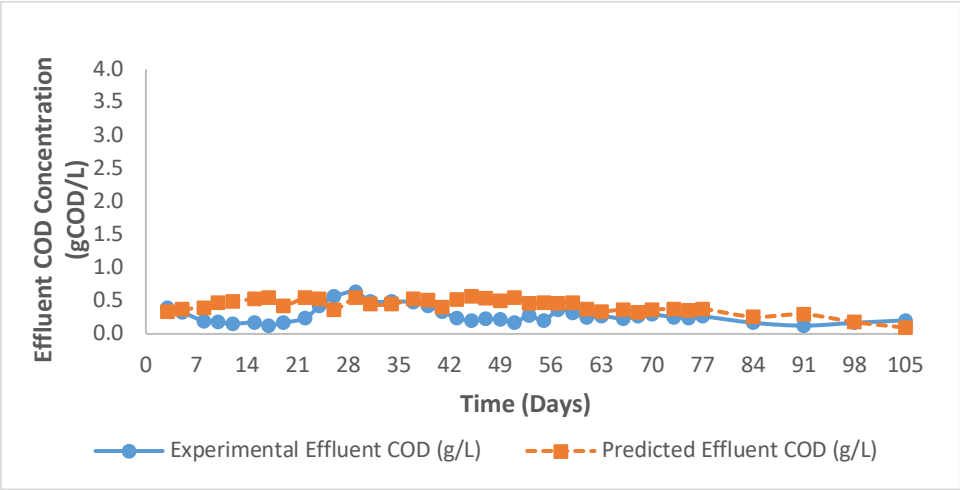

Figure 8.9: Comparison between the actual and predicted DEGBR effluent COD concentration (Modified Stover-Kincannon Model)

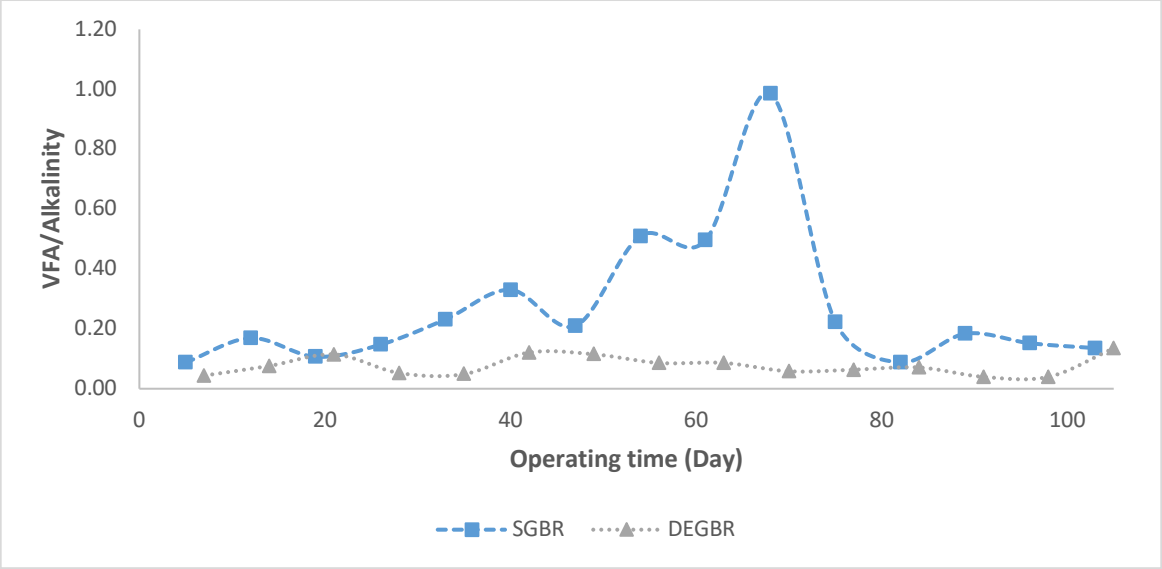

Figure 8.10: VFA/Alkalinity ratio of the DEGBR and SGBR

The other advantage of the modified Stover-Kincannon model is the provision of the volume of the HRABS as per the model kinetic parameters as well as the bioreactor performance, as illustrated by Equation 8.9.

$$V = \frac{q}{\frac{U_{max}}{S_0 - S} - \frac{K_B}{S_0}} \tag{8.9}$$

Where U_{max} and K_B can be derived from the straight-line equation provided by Figure 8.6 and 8.7 for the SGBR and the DEGBR, respectively.

8.3.3 Grau second-order multi-component substrate removal model

Grau *et al.* (1975) developed a linear multi-component substrate model to describe and predict substrate concentration. This model consists of a second-order chemical reaction kinetics combined with the Monod model (Monod *et al.*, 1965), whose influent substrate concentration is independent of effluent substrate concentration. The general equation of the Grau second-order kinetic model is provided by Equation 8.10.

$$-\frac{dS}{dt} = K_s.X.\left(\frac{s}{S_0}\right)^2 \quad (8.10)$$

The integration and linearization of Equation 8.10 results to Equation 8.11, which provides a correlation that can be used to determine Grau second-order multi-component substrate removal model parameters required for predicting effluent substrate concentration.

$$\frac{HRT.S_0}{S_0-S} = HRT - \frac{S_0}{K_S X} \quad (8.11)$$

Equation 11 can be further simplified to Equation 8.12.

$$\frac{HRT}{E} = a + b.HRT \quad (8.12)$$

Where a represents the substrate kinetics ($\frac{S_O}{K_S.X}$), E the substrate removal efficiency given by S_0 - S/S_0, and b the coefficient of the HRT, which relates to a value close to zero, translating to the impossibility of reaching a total removal of the substrate from PSW.

Figure 8.11 and 8.12 provide the outcome of the evaluation of the kinetic parameters of the Grau second-order multi-component substrate model of the SGBR and the DEGBR, with coefficients of determination reaching 0.98 (RMSE = 0) and 0.97 (RMSE = 0.02), respectively. This high R^2 value suggests a good determination of the required kinetic parameters, which are then used to predict the substrate concentration in the effluent from the SGBR and the DEGBR from Equation 8.13.

$$S = S_0\left(1 - \frac{HRT}{a+b.HRT}\right) \quad (8.13)$$

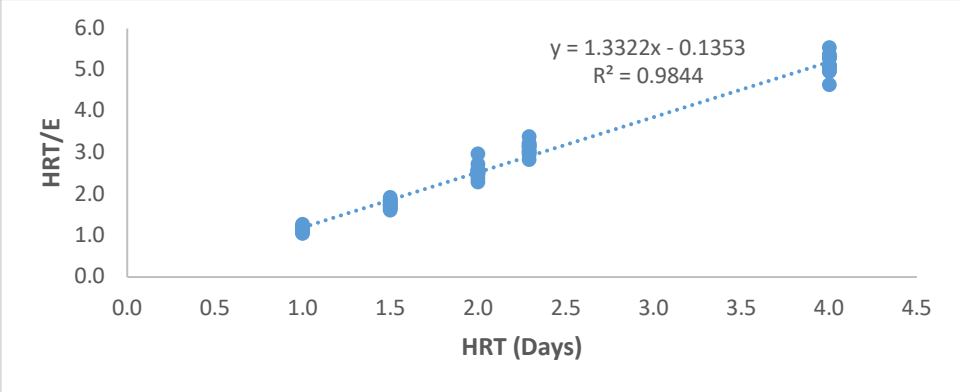

Figure 8.11: Evaluation of the SGBR Grau second-order kinetic parameters

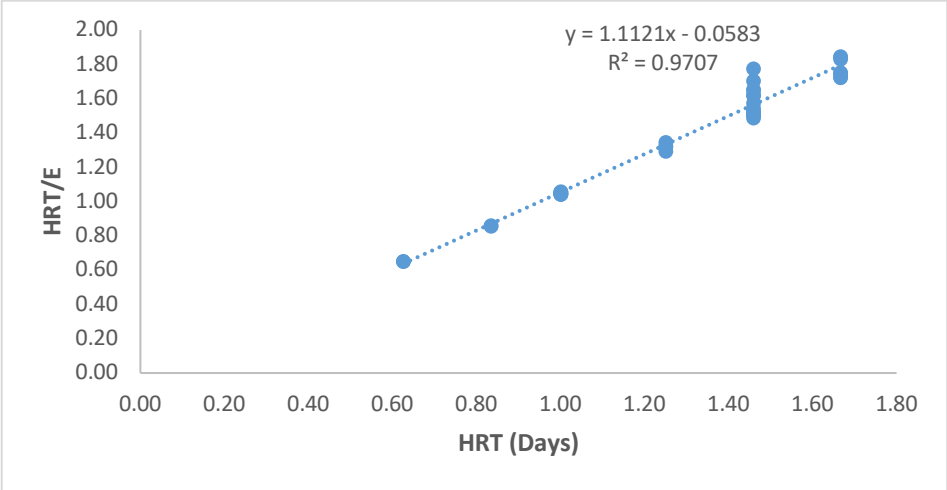

Figure 8.12: Evaluation of the DEGBR Grau second-order kinetic parameters

A comparison between the predicted and experimental substrate concentration in the effluent of the SGBR is illustrated in Figure 8.13, from which consistency is noticed from the beginning of the anaerobic treatment process till the 65th day of operation. From that day, which is also marked by an increase of the acidity of the bioreactor, as illustrated in Figure 8.10, a fluctuating deviation from the experimental substrate concentration in the effluent was noticed. This may be explained by several factors, including the role of filtration in such down-flow systems, which is not accounted for by the Grau second-order multicomponent substrate removal model. Thus, the packing materials that serve as underdrain system in such configuration do not only retain the anaerobic granules inside the bioreactor but may also retain organic matter within the bioreactor. This can be referred to as filtration, which is more of a physical treatment process that cannot be well explained by a biological kinetic model. Another factor that can explain this difference between the experimental and the predicted concentration of the substrate in the effluent, in parallel with the effects of the filtration of the underdrain system, is the effects of low HRT on the prediction of such treatment process marked with a significant step-wise decrease of the HRT from the beginning to the end. As per Equation 8.13, this may lead to an overestimation of the concentration of the substrate in the effluent under low HRT, which translates to high OLR and therefore the high load of organic matter to the anaerobic biomass. Thus, the model may assume

a loss of the performance of the process when exposed to such changes within a relatively short operating time. This last argument can be grouped with the first one to justify the performance of the down-flow HRABS under such conditions.

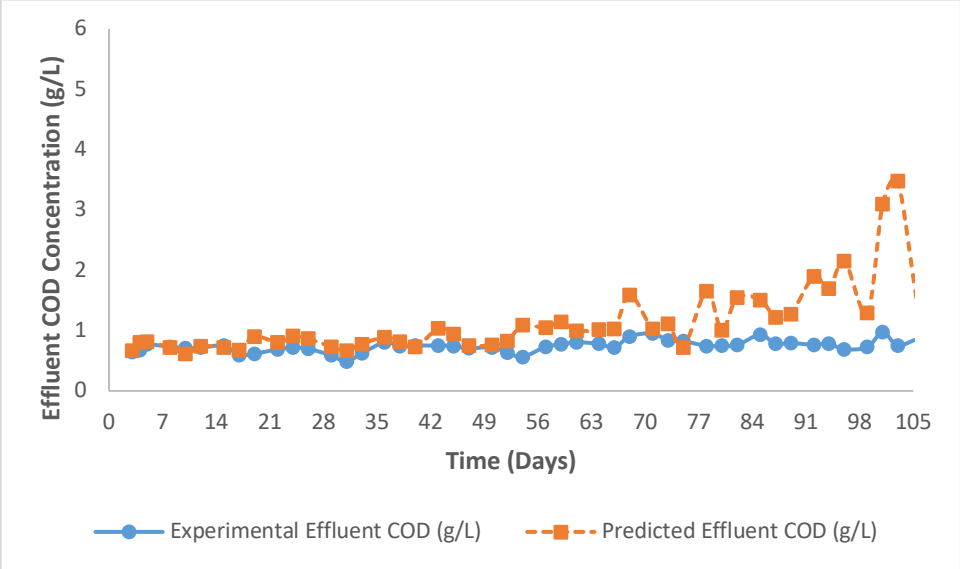

Figure 8.13: Comparison between the actual and predicted SGBR effluent COD concentration using the Grau second-order multi-component substrate

The same comparison was done with the DEGBR predicted and experimental substrate concentration in the effluent, as illustrated in Figure 8.14, from which a less pronounced deviation of the predicted concentration of substrate in the effluent from the experimental one was noticed. This deviation can also be justified by the points listed above for this kinetic model. However, that the inlet COD concentration in PSW varies between 4 to 9 g/L, the comparison between the experimental and predicted COD percentage removal will provide approximately the same result throughout the operation of the DEGBR. This illustrates the consistency of the DEGBR performance for both the experimental and predicted substrate removal, while a comparison between the quality of the experimental and predicted quality of the effluent from the two HRABS (see Figure 8.13 and 8.14) demonstrates that the DEGBR provides better results for the treatment of PSW.

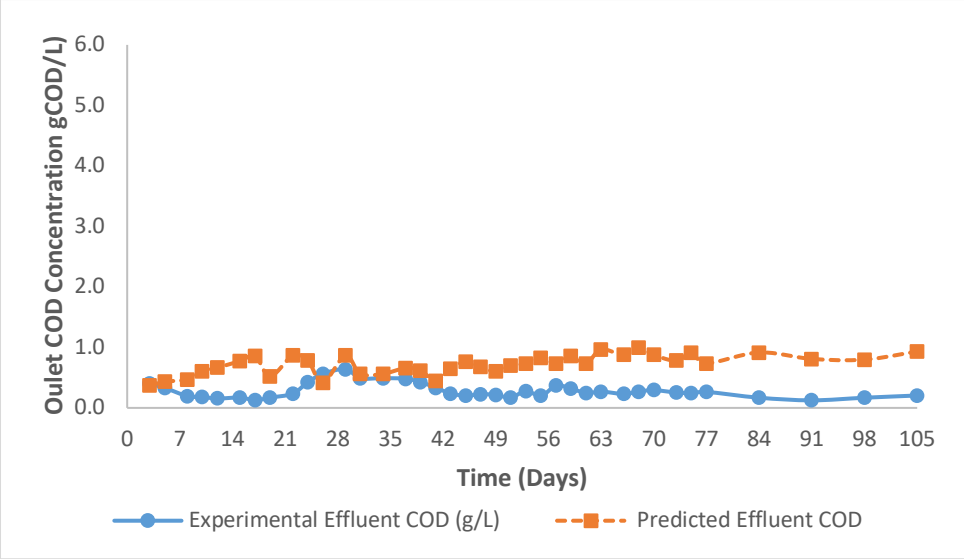

Figure 8.14: Comparison between the actual and predicted DEGBR effluent COD concentration using the Grau second-order multi-component substrate

From Equation 8.13, based on the performance of the HRABS and the Grau second-order kinetic parameters, the volume of each bioreactor can be calculated as provided by Equation 8.14.

$$V = \frac{a.Q}{S_0 - b.S_0 - b.S} \qquad (8.14)$$

Where *a =0.135 and b= 1.33*, and *a=0.058* and *b= 1.112*, for the SGBR and the DEGBR, respectively. Additional Grau-second and modified Stover-Kincannon kinetic parameters determined from previous studies on the treatment of poultry and meat slaughterhouse wastewater using down-flow HRABS are provided in Table 8.2, which can also be used to compare those kinetic parameters as per the model selected.

Table 8.2: Comparison of the kinetic parameters of the Grau second-order and the Stover-Kincannon models from different studies

Model	Reactor type	Type of wastewater	Kinetic Parameters		R^2	Reference
			Umax (gCOD/L.day)	**KB (gCOD/L.day)**		
Modified Stover-Kincannon	SGBR	Poultry slaughterhouse	12.7	18.2	0.95	Basitere *et al.* (2019)
	SGBR	Meat Slaughterhouse	192.3	206	0.99	Oh (2012)
	SGBR	Poultry slaughterhouse	164.5	177.2	0.99	Debik & Coskun (2009)
	SGBR	Poultry slaughterhouse	33.6	44.9	0.99	This study
	DEGBR	Poultry slaughterhouse	40.5	47.3	0.99	This study
			a	**b**		
Grau second-order	SGBR	Poultry slaughterhouse	0.062	1.32	0.95	Basitere *et al.* (2019)
	SGBR	Meat Slaughterhouse	0.017	1.05	0.99	Oh (2012)
	SGBR	Poultry slaughterhouse	0.173	1.155	0.95	Debik & Coskun (2009)
	SGBR	Poultry slaughterhouse	0.135	1.33	0.98	This study
	DEGBR	Poultry slaughterhouse	0.058	1.112	0.97	This study

8.4. Conclusion

This study aimed at evaluating the performance of the DEGBR and SGBR for the anaerobic treatment of PSW, and describing as well as predicting the substrate composition from the effluent of the two down-flow HRABS. The two bioreactors demonstrated a solid performance for the treatment of PSW and motivated the requirement of performing their kinetic analysis. Consequently, the modified Stover-Kincannon and the Grau second-order multicomponent substrate models were used. Their kinetic parameters were determined from straight-line equations which produced a R^2 value above 97% for each linear regression. Subsequently, the kinetic parameters were used to predict the substrate concentration from the effluent of the bioreactors investigated. Of the two kinetic models, the modified Stover-Kincannon provided the best predictions. Furthermore, the kinetic parameters were used to determine to estimate the bioreactors footprint as per targeted performance.

8.5 Summary

The success of up-flow high rate anaerobic bioreactor systems (HRABS) has consolidated the selection of this configuration for the treatment of different types of wastewater. However, this up-flow configuration is associated with operational challenges, including the difficulty of operating the three-phase separator, the biomass washout, and the energy required to overcome the gravity and friction losses across the granular bed; which can be circumvented using a down-flow configuration. In this study, the treatment of poultry slaughterhouse wastewater (PSW) was evaluated using two down-flow HRABS, including the Down-flow Expanded Granular Bed Reactor (DEGBR) and the Static Granular Bed Reactor (SGBR). These two bioreactors demonstrated good performance with removal percentages of the BOD5, COD, and FOG exceeding 95% during peak performance days. Hence, this study approached the kinetic analysis of these two HRABS using the modified Stover-Kincannon and the Grau second-order multi-component substrate models. Of these two models, the modified Stover-Kincannon provided the best prediction for the concentration of the substrate in the effluent from the two HRABS. This analysis led to the determination of the kinetic parameters of the two models that can be used for the design of the two HRABS and the prediction of the performance of the SGBR and DEGBR.

This chapter meets the last objective of this thesis and leads to a conclusion and overall discussion in chapter 9.

CHAPTER 9

CONCLUSIONS AND RECOMMENDATIONS

Chapter 9: CONCLUSIONS AND RECOMMENDATIONS

9.1 Benefit of the treatment of poultry slaughterhouses solid and liquid wastes

The processing of birds in poultry slaughterhouses is a water-intensive process that results in the generation of PSW and solid wastes (Barbut, 2015; Bustillo-Lecomte *et al*., 2016). Global and local challenges such as the pollution of the aquatic fauna and flora, water scarcity, municipal levies imposed on poultry slaughterhouses for the discharge of PSW not meeting the industrial wastewater discharge standards, and the endangerment of the health of the population exposed to micro-organisms attached to such wastes, were listed among the reasons why such wastes should be treated within poultry slaughterhouses instead of being released untreated into the environment (Basitere *et al*., 2017; Barbut, 2015; Avula *et al.,* 2009). Furthermore, such wastes serve as a raw material to various solid and liquid waste treatment and conversion processes, using various processes described in this thesis. However, in this study, the focus was given to the high rate anaerobic treatment of PSW, which represents an attractive option for the treatment of such wastewater, as it requires a small plant footprint, produces a high calorific gas (methane) contained in the generated biogas, is less energy-intensive than the aerobic treatment, presents minimal carcinogenic effects as compared to chemical treatment options, is more efficient than physical treatment, has a high substrate removal rate that can enable further processing of the effluent towards the production of a recyclable water, which can address the challenge of water scarcity and reduces the cost associated with water bills in such facilities (Chernicharo, 2007; Henze *et al*., 2008). As such, the high anaerobic digestion was thoroughly described. This description included its principle, the required operating conditions, biochemical interactions during the treatment process, challenges that may jeopardize the treatment process and solutions to address them.

9.2 Design and performance evaluation of the DEGBR for the treatment of PSW

9.2.1 Motivation of the design of the DEGBR

One solution to various challenges listed in this thesis was the design of a new HRABS, which is the DEGBR. The DEGBR suggested and adopted a configuration aimed at addressing operational challenges such as the washout of biomass from the bioreactor, the drainage of the biogas in the effluent during operation, the energy requirement of up-flow HRABS, the clogging of underdrain system, channelling and short-circuiting in the granular bed, which tends to limit the distribution of substrate to part of the anaerobic biomass in a granular bed. The configuration proposed by the DEGBR consisted of a down-flow configuration, assisted by a recycle stream for improved distribution of the influent, and an underdrain system composed of selected packing materials aimed at limiting the intensity of head losses across the packed bed, which results to the clogging of the underdrain system. The common effect of the clogging of the underdrain system is the accumulation of the influent and effluent in the bioreactor, which may induce the loss of biogas due to increased pressure on the biogas bubbles, which may lead to the inhibition of the anaerobic biomass as a result of the increased concentration of hydrogen sulphide (anaerobic digestion inhibitor) contained in the biogas. The control of such operation is very essential to a steady operation of HRABS, and particularly down-flow HRABS, which raises the importance of the selection of a good underdrain system, which represents another contribution of this thesis.

9.2.2 Selection of suitable packing materials for the underdrain system of down-flow HRABS

The selection of packing materials for down-flow underdrain systems requires analyses that extend beyond essential pre-selective requirements such as their inertness, their availability and their affordability. Further selection drivers include the evaluation of their porosity, their permeability, the intensity of pressure loss they induce at operational hydraulic retention times, and their sludge retention capacity. These parameters are deemed relevant to the selection of the most suitable packing materials because they evaluate the occurrence of operational challenges and, thus are geared towards limiting adverse operational conditions to ensure a conducive anaerobic treatment process. Packing materials pre-selected in this study included pea gravels, white pebbles, medium-sized pumice stones, Ceramic marbles,

and small-sized pumices. The medium-sized pumice stones displayed the best suitability with less induced pressure loss, and the best sludge retention capacity, porosity, and permeability. Therefore, this packing material was selected for down-flow HRABS (SGBR and DEGBR) used and investigated in this study.

9.2.3 Performance of the DEGBR for PSW treatment

Generally, the performance of HRABS is evaluated through operational consistency during the treatment and the removal water contaminants quantify by water quality assessment parameters such as the COD, the BOD_5, the TSS, and the FOG.in this regard, the DEGBR displayed a good consistency in the treatment of PSW after a short acclimatization period. This consistency was displayed by an average total COD, BOD_5, TSS and FOG percentage removal remaining above 95%. This performance exceeded one of the selected technologies used for the treatment of PSW in previous studies and, consequently, demonstrated the suitability of such an alternative for the treatment of PSW.

9.3 Comparison of the performance of the EGSB. DEGBR and SGBR for the treatment of PSW

The performance of the DEGBR led to another study geared towards evaluating and comparing the performance of the latter to the one similar HRABS and thus the re-assessment of the suitability of HRABS for the treatment of PSW. The three bioreactors displayed a solid performance, with the DEGBR providing the best performance despite an OLR varying between 1.1 to 38.9 gCOD/L.day. This good performance of the DEGBR was attributed to its stability throughout the anaerobic treatment process and its configuration that was designed to prevent operational encountered in most HRABS and that enabled an improved distribution of the substrate to anaerobic biomass and the minimization of the inhibitors in the anaerobic granular bed.

9.4 Kinetic modelling of down-flow HRABS (DEGBR and SGBR) and prediction of their performance and bioreactor footprint

Two kinetic modelling approaches were used in this study for the prediction of substrate concentration in the effluent and the design of selected HRABS, namely the modified Stover-

Kincannon and the Grau second-order substrate multi-component model. These kinetic modelling options were selected from the efficacy in the prediction of the performance of anaerobic bioreactors. In this study, the focus was given to down-flow HRABS, as this thesis recommends as an alternative to up-flow HRABS for the treatment of PSW and similar types of wastewater. Both kinetic modelling approaches provided good correlations for the determination of their kinetic parameters (R^2>0.97); however, the modified Stover-Kincannon model predicted the best the concentration of the substrate in the effluent from both HRABS. Subsequently, using the kinetic parameters provided by the two kinetic models, a correlation was derived for the determination of the volume of the DEGBR and the SGBR as per targeted performance.

9.5 Summary and recommendations

Overall, this thesis provided an insight into the global and local hygienic, environmental and water stresses induced by the poor management of poultry slaughterhouse solid and liquid wastes. As a result, solid wastes and PSW treatment options were suggested and described. Subsequently, the focus was given to high rate anaerobic digestion of PSW, which was thoroughly described and investigated through the use of three different HRABS for the treatment of PSW. The comparison of the performance of these HRABS suggested that down-flow HRABS, such as the DEGBR proposed in this work and the SGBR, represents a good alternative to up-flow HRABS that are usually associated with operational challenges listed in this thesis. Furthermore, the prediction of the performance of down-flow HRABS (SGBR and DEGBR) was approached using the modified Stover-Kincannon model and the Grau second-order multicomponent substrate model. A good prediction resulted from the modified Stover-Kincannon kinetic parameters that can be used for bioreactor design purposes.

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APPENDICES

Appendix A: SELECTION OF PACKING MATERIALS FOR THE UNDERDRAIN SYSTEM

Table A.1: Determination of the porosity of the packing materials

Packing material	Pea gravel	White pebbles	Ceramic marbles	Small pumice stones	Medium pumice stones
Total volume (mL)	290	290	290	290	290
Void volume (mL)	105	100	115	165	191.4
Porosity	**0.362068966**	**0.344827586**	**0.396551724**	**0.568965517**	**0.66**

Table A.2: Determination of the permeability of the packing materials

Parameters	Medium pumice stone	Pea Gravel	Ceramic marbles	Small pumice stone	White pebbles
Volume (ml)	80	80	80	80	80
Mass (g)	52.5	123.9	111.64	64.67	139.41
Bulk density (g/ml)	0.65625	1.54875	1.3955	0.808375	1.742625
Bulk density (kg/m^3)	656.25	1548.75	1395.5	808.375	1742.625
Sphericity	0.66	0.66	1	0.66	0.82
Mean particle diameter (m)	0.01255	0.0056	0.01574	0.006958333	0.014716667
Equivalent diameter (m)	0.008283	0.003696	0.01574	0.0045925	0.012067667
Porosity	0.66	0.362068966	0.396551724	0.568965517	0.344827586
Permeability (m^2)	**1.13752E-06**	**1.06218E-08**	**2.82838E-07**	**1.39393E-07**	**9.27364E-08**

Table A.3: Further parameters of the system

Parameter	Value
Water viscosity at 35°C (Pa.s)	0.000726
Water density at 35°C ($kg.m^3$)	993.95
Bed Height (m)	0.05

Table A.4: Determination of the sludge retention capacity of the packing materials

Packing material	Pea gravels	White pebbles	Ceramic marbles	Small pumice stones	Medium pumice stones
Volume of sludge (mL)	150	150	150	150	150
Mass of sludge (g)	147.5	147.2	147.3	149.5	147.1
Mass of sludge washed out (g)	20.53	100.1	128.2	19.94	19.13
Mass of sludge retained (g)	126.97	47.1	19.1	129.56	127.97
Sludge retention capacity	**0.860813559**	**0.319972826**	**0.129667346**	**0.866622074**	**0.869952413**

Table A.5: Determination of the head losses across each packing material

Superficial velocity (m/s)	Medium PS head loss (Pa)	Pea gravels head loss (Pa)	Ceramic marbles head loss (Pa)	Small PS head loss (Pa)	White pebbles head loss (Pa)
0.000003855	0.000123119	0.013170059	0.000495213	0.001003866	0.001509643
0.000004	0.000127757	0.013665614	0.00051387	0.001041651	0.001566493
0.000005	0.000159759	0.017083599	0.000642605	0.001302285	0.001958692
0.000006	0.000191785	0.020502216	0.000771447	0.001563008	0.002351121
0.000007	0.000223836	0.023921466	0.000900396	0.00182382	0.002743781
0.000008	0.000255912	0.027341348	0.001029451	0.00208472	0.003136671
0.000009	0.000288012	0.030761863	0.001158614	0.002345709	0.003529791
0.00001	0.000320138	0.03418301	0.001287884	0.002606787	0.003923141
0.000011	0.000352288	0.03760479	0.00141726	0.002867953	0.004316722
0.000012	0.000384464	0.041027202	0.001546744	0.003129208	0.004710534
0.000013	0.000416664	0.044450247	0.001676334	0.003390551	0.005104575
0.000014	0.000448889	0.047873924	0.001806031	0.003651983	0.005498847
0.000015	0.000481138	0.051298234	0.001935836	0.003913504	0.005893349
0.000016	0.000513413	0.054723177	0.002065747	0.004175113	0.006288082
0.000017	0.000545712	0.058148752	0.002195765	0.004436811	0.006683044
0.000018	0.000578036	0.061574959	0.00232589	0.004698598	0.007078238
0.000019	0.000610386	0.065001799	0.002456122	0.004960473	0.007473661
0.00002	0.000642759	0.068429272	0.002586461	0.005222437	0.007869315
0.000021	0.000675158	0.071857377	0.002716907	0.00548449	0.008265199
0.000022	0.000707582	0.075286114	0.00284746	0.005746631	0.008661313
0.000023	0.00074003	0.078715484	0.00297812	0.006008861	0.009057658
0.000024	0.000772503	0.082145487	0.003108887	0.006271179	0.009454233
0.000025	0.000805001	0.085576122	0.00323976	0.006533586	0.009851038
0.000026	0.000837524	0.089007389	0.003370741	0.006796082	0.010248074
0.000027	0.000870072	0.092439289	0.003501829	0.007058666	0.01064534
0.000028	0.000902645	0.095871822	0.003633023	0.007321339	0.011042836

Superficial velocity (m/s)	Medium PS head loss (Pa)	Pea gravels head loss (Pa)	Ceramic marbles head loss (Pa)	Small PS head loss (Pa)	White pebbles head loss (Pa)
0.000029	0.000935242	0.099304987	0.003764324	0.007584101	0.011440563
0.00003	0.000967864	0.102738784	0.003895733	0.007846951	0.01183852
0.000031	0.001000511	0.106173215	0.004027248	0.00810989	0.012236707
0.000032	0.001033183	0.109608277	0.00415887	0.008372917	0.012635125
0.000033	0.00106588	0.113043972	0.0042906	0.008636034	0.013033773
0.000034	0.001098602	0.1164803	0.004422436	0.008899238	0.013432651
0.000035	0.001131348	0.11991726	0.004554379	0.009162532	0.013831759
0.000036	0.001164119	0.123354853	0.004686429	0.009425914	0.014231098
0.000037	0.001196916	0.126793078	0.004818586	0.009689385	0.014630667
0.000038	0.001229736	0.130231936	0.00495085	0.009952944	0.015030467
0.000039	0.001262582	0.133671426	0.005083221	0.010216592	0.015430497
0.00004	0.001295453	0.137111549	0.005215698	0.010480328	0.015830757
0.000041	0.001328348	0.140552304	0.005348283	0.010744154	0.016231247
0.000042	0.001361269	0.143993692	0.005480975	0.011008068	0.016631968
0.000043	0.001394214	0.147435712	0.005613773	0.01127207	0.017032919
0.000044	0.001427184	0.150878365	0.005746679	0.011536161	0.017434101
0.000045	0.001460178	0.15432165	0.005879691	0.011800341	0.017835512
0.000046	0.001493198	0.157765568	0.006012811	0.012064609	0.018237154
0.000047	0.001526242	0.161210119	0.006146037	0.012328966	0.018639027
0.000048	0.001559312	0.164655301	0.00627937	0.012593412	0.019041129
0.000049	0.001592406	0.168101117	0.006412811	0.012857946	0.019443462
0.00005	0.001625525	0.171547565	0.006546358	0.013122569	0.019846026

Appendix B: AUXILIARY PARAMETERS USED FOR THE DEGBR OPERATION

B.1 Preparation of the barrier solution

The barrier solution used for the minimisation of contaminants in the water displacement set was prepared every 5 days. It consisted of a 5% (W/V) solution of potassium hydroxide (KOH) and the percentage weight per volume was calculated as per Equation B.1.

$$\%\frac{W}{V} = \frac{Mass\ of\ the\ solute\ (g)}{Volume\ of\ the\ solution\ (mL)} X100 \quad \text{(B.1)}$$

Two litres of the solution was required every 5 days, therefore the mass of the solute required was determined by Equation B.2 that was derived from Equation B.1.

$$Mass\ of\ solute\ = \frac{2000\ X\ 5}{100} = 100\ g \quad \text{(B.2)}$$

Thus, 100 g of KOH was added to 2 L of distilled water and gently mixed with a magnetic stirrer for complete dissolution prior to use.

B.2 Determination of the hydraulic retention time (HRT)

The hydraulic retention time, which provides the time a given volume of substrate is retained in a bioreactor is given by Equation B.3.

$$HRT\ (hrs) = \frac{Working\ volume\ of\ the\ bioreactor\ (m^3)}{Influent\ flow\ rate\ \left(^{m^3}/_{hrs}\right)} \quad \text{(B.3)}$$

B.3 Determination of the organic loading rate (OLR)

The organic loading rate can b calculated by Equation B.4.

$$OLR\ \left(COD\frac{mg}{L}.hrs\right) = \frac{Influent\ COD\ \left(\frac{mg}{L}\right)}{HRT\ (hrs)} \quad \text{(B.4)}$$

B.4 Determination of the percentage removal of organic matters

In this study, the performance of the bioreactor was evaluated from the deduction of the percentage removal of some parameters such as the TSS, the COD and the BOD_5. The correlation that served for the determination of this removal percentage is provided by Equation B.5.

$$Removal\ percentage\ (\%) = \frac{Inlet\ value - Outlet\ value}{Inlet\ value} X\ 100 \quad \text{(B.5)}$$

B.5 Determination of the recycle stream up-flow velocity

The recycle stream and inlet up-flow velocity was determined by Equation B.6 and B.7.

$$V_{up} = \frac{Q}{A} \quad \text{(B.6)}$$

Or,

$$V_{up} = \frac{V}{HRT\ X\ A} \quad \text{(B.7)}$$