REACTOR RECONFIGURATION FOR ENHANCED PERFORMANCE OF A DOWN-FLOW EXPANDED GRANULAR BED REACTOR (DEGBR) FOR POULTRY SLAUGHTERHOUSE TREATMENT

By

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DECLARATION

I, **MAHOMET NJOYA**, declare that all the work in this thesis, save for that which is 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.

October 1st, 2017

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ABSTRACT

The poultry industry is one of the largest industries in the South African agricultural sector. To sustain their various operations, this industry utilises a large quantity of potable water to process slaughtered birds in order to satisfy hygiene and sanitation requirements in processing facilities. Thus, the consumption of potable water during poultry slaughterhouse operations results in the production of high-strength poultry slaughterhouse wastewater (PSW), which is laden with a variety of pollutants, including fats, oil and grease (FOG), carcass debris, feathers and organic matter, including proteins, that should be removed from the wastewater, or at least reduced in concentration, prior to the PSW being discharged into the environment. This is to avoid and/or minimise levies and non-compliance penalties from monitoring institutions in charge of controlling the quality of effluents in the area from which the PSW was collected for this study. Furthermore, the option of treating and recycling the PSW to address the current issue of water scarcity in the Western Cape (South Africa), and to minimise possible harmful effects on the environment, will reduce the overreliance on slaughterhouses in the region on potable/drinking water, thus also lessening running costs associated with water procurement for operations.

Various technologies, involving physical, chemical or biological processes, have been evaluated for the treatment of PSW, with this study focusing on anaerobic treatment (part of the biological treatment) of PSW, using a high-rate anaerobic bioreactor system (HRABs), which provides for low production of sludge, the production of biogas as a source of energy and the provision of high performance in terms of organic matter removal. Moreover, HRABs are cheaper, when compared to other aerobic treatment technologies. However, numerous potential challenges were encountered when using HRABs, such as low production of biogas due to gas entrapment, head losses across the granular bed, sludge washout in upflow HRABs, uneven wastewater distribution, and thus poor dispersion of the organic matter, which impacts on the adequacy of treatment, poor release of toxic substances contained in the entrapped biogas (NH_3 or H_2S), clogging of the underdrain system for down-flow HRABs, or the formation of dead zones within the granular bed, resulting in short-circuiting.

To alleviate these problems, this study proposes the design of a novel HRABs, i.e. the down-flow expanded granular bed reactor (DEGBR), which consists of a down-flow configuration to prevent the washout of anaerobic granules and incorporates the implementation of an underdrain system consisting of solid particles, evaluated using a series of assessment methods developed to address the clogging of the underdrain system. Furthermore, a recycle stream was included in the design of the DEGBR to improve the substrate distribution across

the anaerobic granules and counteract head losses, which was thoroughly studied and discussed. Moreover, intermittent bed expansion, via a water distribution placed above the underdrain system which faced upwards, was an added feature of the DEGBR to enable both the re-stratification of the granular bed and the release of the biogas entrapment in the granular bed, and therefore the release of toxic substances from the granular bed.

The first stage of this study consisted of selecting solid particles to be evaluated for the underdrain system of the DEGBR. These were pea gravel, medium- and small-sized pumice stones, ceramic marbles and white pebbles. The selection of suitable packing material for the underdrain system led to the second phase, which was the operation of a bench-scale PVC DEGBR characterised by an inner diameter of 8.6 cm, a wall thickness of 2 mm and a total height of 61 cm. After placing the selected packing material at the bottom of the bioreactor, the DEGBR was inoculated with 3 L of anaerobic sludge, 1 L of PSW and 50 mL of a 20% v/w of a solution of dry milk. Subsequently, an acclimatisation period of two days followed, prior to the operation of the DEGBR for a period of 77 days at HRT of 35, 40, 30 and 24 hours. The operation of the DEGBR was complemented by the PSW feed tank to feed the bioreactor, a PSW product tank to collect the product, a hydrogen sulphide scrubber to treat the hydrogen sulphide content of the biogas, and a water displacement system consisting of a 2 L volumetric glass beaker, a 100 mL volumetric cylinder, a stand as well as a Tedlar bag, which was used to collect and measure the biogas. All these experimental setup components were connected by 10 cm inner diameter silicone tubes. The samples collected in duplicate from the bioreactor, as well as the feed and product tanks, were analysed according to EPA methods and EPHA methods, developed for the analysis of pH, conductivity, salinity, TDS, TSS, BOD_5, FOG, VFA, sCOD, tCOD and alkalinity.

Results obtained from the study of suitable packing materials for the underdrain system indicated that that the medium-sized pumice stones were the most appropriate to use for the underdrain system; and the minimisation of the underdrain system clogging confirmed this conclusion. Furthermore, the performance of the DEGBR for the treatment of PSW was evaluated, with the result that the DEGBR reached average percentage removal of tCOD, sCOD, BOD_5, FOG, and TSS of 95.68 ± 3.63%, 88.75 ± 5.12%, 98.59 ± 4.54%, 93.77 ± 3.57%, and 97.44 ± 5 %, respectively. This effective organic matter removal culminated in an average production of biogas of 44 ± 18.55 mL/day for an average OLR of 148.69 ± 83 mg/L.hr for a bench-scale DEGBR.

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October, 2017

DEDICATION

.

To my parents:

My mother, Amina Hendji Njoya

&

My late father, Yacouba Njoya

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ABBREVIATIONS

ABBREVIATION	DESCRIPTION
ASRB	Acetic acid oxidising 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 oxidising 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
OHPB	Obligate hydrogen producing bacteria
OLR	Organic loading rate
P	Phosphorus
PAHO	Pan American Health Organization
pH	Potential of hydrogen

PSW	Poultry slaughterhouse wastewater
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

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 dry mass of granules	-
f_B	Blake friction factor	-
g	Gravitational acceleration	$m.s^{-2}$
k	Permeability	m^2
K	Boltzman constant	-
L	Length	m
r	Radius	m
Re	Reynolds number	-
S	Surface	m^2
t	Time	s
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
Ø	Sphericity	-

Subscripts

Symbol	Explanation
B	Blake
bd	Biodegradable
BC	Bacterial cells
F	Fluid
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

GLOSSARY

Aerobic wastewater treatment	Biological treatment of wastewater, in systems whereby microorganisms' growth and activity is promoted by sparging dissolved oxygen (Metcalf & Eddy *et al*., 2003).
Anaerobic granules	Aggregates of anaerobic microorganisms attached together in a slime or extracellular polymeric matrix generated by microorganisms (Pol *et al*., 2004)
Anaerobic wastewater treatment	Biological treatment of wastewater in an environment devoid of dissolved oxygen to ensure the growth and the activity of anaerobic microorganisms (Metcalf & Eddy *et al*., 2003).
Barrier solution	A solution used to scrub biogas for methane recovery (Parajuli, 2011).
Biodegradation	Biochemical process whereby materials are dissolved by microorganisms (Metcalf & Eddy *et al*., 2003)
Biogas	A mixture of gases (mostly CH_4 and CO_2) produced during anaerobic organic matter biodegradation (Gerardi, 2003).
High-rate anaerobic bioreactors systems	Continuous biological anaerobic reactors developed to operate under reduced hydraulic retention time while improving sludge retention for better performance (Henze *et al*., 2008).
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).

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

In comparison to the aerobic treatment of wastewater, anaerobic treatment is seen as a convenient and economical way of treating different types of wastewater, as it generates minute quantities of sludge, and produces biogas that contains methane, which has a high calorific value (Henze *et al*., 2008). Moreover, anaerobic digestion requires less space, produces sludge with a high market value and has low operating costs (Henze *et al*., 2008). Different types of high-rate anaerobic reactors (HRABs) have been developed since the 1970s for the biological treatment of different types of wastewater. The focus for this research project is on poultry slaughterhouse wastewater (PSW), which has specific characteristics, such as high concentrations of suspended solids: fats, oil and greases (FOG), as well as proteins (Bustillo-Lecompte *et al*., 2016). PSW anaerobic treatment has been implemented in previous studies using various HRABs, such as the Up-flow Anaerobic Sludge Blanket (UASB), the Expanded Granular Bed Reactor (EGSB) and, most recently, the Static Granular Bed Reactor (SGBR); all of which resulted in high removal of organic matter expressed in terms of critical parameters such as chemical oxygen demand (COD), biochemical oxygen demand (BOD_5) or total suspended solids (TSS) (Chavez *et al*., 2005; Del Nery *et al*., 2008; Basitere *et al*., 2016; Basitere *et al*., 2017; Evans, 2004). Furthermore, the development of such anaerobic treatment systems is required to reach a good performance in terms of organic matter removal.

1.2 Motivation for the research study

Since the poultry industry is one of the largest industries in the South African agricultural sector and uses a large quantity of potable water to sustain its various operations (Bolton, 2015; Bustillo-Lecompte *et al*., 2016), a large quantity of PSW is produced by these operations, with the PSW containing a high concentration of organic matter. The discharge of PSW into sources of fresh water, such as rivers, results in eutrophication that leads to severe aquatic pollution (Bustillo-Lecompte *et al*., 2016), largely due to the constituents of this type of wastewater, i.e. blood, faeces, feathers or carcass debris. To prevent pollution and environmental health challenges and ecological degradation, municipal authorities in most countries impose standards that must be adhered to prior to the discharge of the wastewater into the municipal sewage system or rivers (City of Cape Town, 2015; DEA, 2014). These standards are painstakingly enforced to help ensure water security and thus protect the human population from the effects of contamination by untreated wastewater.

Globally, water usage will be restricted to domestic use by 2025 (Avula *et al*., 2009). Periodic drought has affected most parts of South Africa during the summer seasons to date. This has severe effects on industries requiring a large quantity of potable water to sustain their operations. Hence the need to develop solutions to circumvent challenges posed by the lack of potable water on these industries. Wastewater treatment for recycling would contribute significantly to the reduction in usage of potable water by these industries. The other advantage of recycling of treated wastewater generated by the poultry industry is the use of the organic matter present in the wastewater to generate biogas that can, in turn, be used for electricity generation or simply for commercial purposes, i.e. to sell to the local population as a source of clean energy. The feasibility of such as undertaking is dependent on the use of HRABs treatment systems for PSW that can contribute to the conversion of COD content in the wastewater into biogas, and the production of recycled water with characteristics close to discharge standards. Generally, further treatment is usually required thereafter to purify the water to drinking standards. PSW treatment using HRABs has the advantages of a short hydraulic retention time (HRT) for a long solids retention time (SRT), for an optimised contact time between the wastewater and organic matter biodegradation, maintenance of anaerobic conditions, suitable pH and temperature for the maintenance of appropriate environmental conditions, and minimisation of residual sulphates and nitrates in the wastewater (Henze *et al*., 2008). Anaerobic technology is preferred over aerobic, chemical or physical treatment processes because it has low operating costs, produces biogas that contains methane, and does not require synthetic chemicals that can culminate in the need for subsequent treatment stages and cause human/environmental health problems; furthermore, since the PSW has high organic loading, anaerobic treatment is preferable over aerobic treatment (Henze *et al*., 2008; Chernicharo, 2007).

1.3 Statement of the research problem

Some problems were encountered during the treatment of PSW with the technologies mentioned in section 1.1, such as the washout of granules and solids from the reactors with an up-flow configuration, such as the UASB and the EGSB, or head losses due to the accumulation of solids caused by the down-flow configuration offered by the SGBR (Basitere *et al.*, 2016; Basitere *et al*., 2017). Furthermore, with the exception of the EGSB, which has a recirculation system that contributes to the expansion of the granular bed by increasing the up-flow velocity, these anaerobic reactors' only source of mixing was provided by the elevation of biogas (CH_4, CO_2, H_2S…) produced as a result of methanogenic activity of anaerobic granules used (Evans, 2004; Del Nery *et al*., 2008). Moreover, the entrapment of the biogas by the granular bed is observed when using the UASB (Bhatti, 1995) as well as the SGBR, resulting

in reduction in biogas production, as well as the increase in the acidification of the system due to CO_2 solubility in the PSW within the system, culminating in operational deviation of the system from an operating pH range that lies between 6 and 8 (Evans, 2004; Henze *et al*., 2008). Furthermore, the stagnation of anaerobic granules results in reduced dispersion of toxicants (H_2S, NH_3 etc.), which ultimately leads to the inhibition of methanogens and the accumulation of volatile fatty acids (VFAs) within the bioreactor, which effectively renders the system treatment efficiency redundant (Gerardi, 2003; Chernicharo, 2007). Thus, this research project proposes the development of a novel HRAB that helps alleviate the aforementioned challenges by using a down-flow configuration with a semi-porous underdrain system and mixing promoted by a recycling system to improve the substrate (organic matter) biodegradation, thus improving the distribution of the organic matter to the biomass. This reduces dead zones, thus short-circuiting the reduction of pressure exerted on the biogas bubbles generated due to the compact static bed, and improves the anaerobic activity of the granular sludge used.

1.3.1 Overview: research rationale

The direct discharge of PSW into municipal sewage systems and into surface water sources, i.e. rivers, streams, etc. would result in severe pollution, contributing to environmental degradation. The treatment of wastewater can allow for greater ease in compliance with environmental legislation by various governments, although current methods suffer from high input costs. HRABs provide a means to treat PSW with a decrease in the number of treatment stages required in a wastewater treatment plant (WWTP), while also producing less sludge and biogas; hence the development of the Down-flow Expanded Granular Bed reactor (DEGBR) that would also contribute to producing an effluent which meets discharge standards and lessens pollution. Furthermore, the treatment of PSW may result in the production of a treated water that can be reused within the slaughterhouse facilities after a series of treatment operations and thus reduce the intake of potable water.

1.3.2 Research questions

The following questions revolved around the development of the DEGBR to circumvent the challenges encountered while treating PSW with previous HRABs.

- Does the configuration of the DEGBR provide good performance in terms of organic matter removal?
- Can the DEGBR perform better than similar technologies?
- Can the DEGBR improve the production of biogas?

- Can the use of a recycle stream provide an effective mitigation strategy associated with ineffective distribution of organic matter to the anaerobic biomass using a down-flow configuration?

1.4 Hypothesis

The design of an anaerobic reactor with a down-flow configuration, a recycle stream, and a semi-porous underdrain system would contribute to an increase in the contact between the organic matter and the reactor's anaerobic biomass, thus reducing biogas bubble entrapment and eliminating dead zones, and therefore in turn short-circuiting within the bioreactor through an increase in the permeability within the system. Furthermore, a good distribution of organic matter throughout the bioreactor would contribute to increasing the size of the granules, promoting anaerobic activity while preventing clogging of the underdrain system. This improved distribution would also contribute to enhanced treatment of PSW and thus an improvement in the production of biogas.

1.5 Research aims and objectives

The aim of this study is to design and evaluate the performance of a Down-flow Expanded Granular Bed Reactor (DEGBR), through the mitigation of the previously observed challenges such as biogas entrapment within the granular bed, the improvement of the distribution of the organic matter to the biomass, and the elimination of short-circuiting, thus dead zones as well as the accumulation of toxicants in the treatment of PSW.
Therefore, the objectives of this research project are:

- To determine the performance of the DEGBR in terms of organic matter removal;
- To compare this performance with that observed in bioreactors used in previous studies;
- To facilitate and evaluate the production of biogas;
- To determine head loss effects within the DEGBR and suggest a solution to circumvent these operational shortcomings;
- To establish the effect of the use of a recycle stream on the distribution of the organic matter to the anaerobic biomass, and on the performance of the DEGBR; and
- To determine a suitable packing material for the bioreactor's underdrain system.

1.6 Significance of the research

Various technologies have been used for the treatment of PSW. Anaerobic treatment is usually preferred for this type of wastewater, due to its high organic matter content and the advantages

related to the utilisation of anaerobic digesters, such as the reduction of number of stages and therefore plant footprint, as well as the production of biogas and the reduction of sludge produced. Most HRABs used for the treatment of PSW have some disadvantages, such as the difficult operation of the three-phase separator for wastewater-solid-gas separation, the washout of the sludge and the biogas entrapment, which are challenges that the configuration of the DEGBR would address to culminate in a HRAB with better/optimised performance. The success of such a novel design would translate to the improvement of biomass retention and microbial growth, and therefore methanogenesis for increased biogas production and an effluent that can be further treated for reuse, and/or disposed of (discharged) without incurring penalties and/or levies associated with such wastewater disposal.

1.7 Delineation of the Study

This study did not focus on the following:

- The assessment of techniques associated with biogas treatment and methane collection;
- The evaluation of packing material size distribution;
- The economic evaluation of the process, either on a pilot plant and/or industrial scale;
- The evaluation of the physical characteristics of anaerobic granules; and
- The utilisation of post-treatment systems for further effluent treatment from the DEGBR.

CHAPTER 2

LITERATURE REVIEW

Chapter 2 : LITERATURE REVIEW

2.1 Introduction

Though often misused, water is a critical resource required in virtually every industry and household (World Bank, 2014). Water availability is more important than the availability of crude oil, as there are no alternatives to it, and it is a fundamental constituent for the building-blocks of life (World Bank, 2014). One of the effects of climate change is drought, which is currently affecting many parts of the world, such as Australia, Asia, Africa and North America; resulting in acute water shortages. This is obviously detrimental to humans living in these areas, as water sustains our food chain and facilitates agricultural development (Greencape, 2016). Currently, water scarcity is a global challenge that needs to be addressed through different methods, ranging from the development of new technologies that will allow the efficient use of water, harvesting of groundwater, desalination of sea water and the re-use of treated wastewater (Western Cape Government, 2015; Assessment; Zwane and Montmasson-Clair, 2016).

The average annual consumption of water for the poultry industry in South Africa in 2014 was 32 564 576 m^3. If one allows for an annual growth of 7% in this sector, water consumption has increased proportionately to date. The poultry industry with its contributions of 17.5% (2013) and 15.5% (2014), is a leader in terms of contribution to total gross agricultural production in South Africa (Western Cape Government, 2015). For the province of the Western Cape, agriculture contributes to 23% of the national agricultural value (Western Cape Government, 2015), with 14 registered poultry/chicken abattoirs been identified in this province. These facilities are all subject to legislation governing effluent discharge standards for the protection of the environment, a strong motivation for treating the wastewater produced in these facilities, as it contains organic matter collected during various operations necessary to provide a product meeting hygienic standards (Greencape, 2016). The discharge of poultry slaughterhouse wastewater (PSW) into surface water resources without treatment culminates in eutrophication, which eventually leads to environmental pollution (Saldias *et al*., 2016).

Water scarcity and compliance to discharge standards can be addressed through the treatment of PSW for reuse or for discharge. Depending on the stage in the wastewater treatment plant,

several technologies can be used for this endeavour, but the focus in this research project is on anaerobic treatment as the second stage and/or sequential step after the pre-treatment of the wastewater treatment. This chapter focuses on insights into PSW anaerobic treatment, with the characteristics of the PSW being highlighted, including information relevant to its generation.

2.2 Poultry slaughterhouse processing units, potable water consumption and wastewater generated

The poultry industry is one of the largest industries in the South African agricultural sector, with a large contribution (16%) to the gross domestic product for this sector (Bolton, 2015). This industry generates a high quantity of wastewater, which is generated from continuously rinsing the meat while it is cut and packaged (Avula *et al*., 2009; Plumber and Kiepper, 2011). Thus, PSW contains high concentrations of suspended solids, fat, oil and grease (FOG), nitrogen and phosphorus. The PSW generated varies in quantity and quality from plant to plant, indicating process dependency for the quantity of water utilised per bird slaughtered (Del Nery *et al*., 2007). Furthermore, Avula *et al*. (2009) indicate that PSW is composed of other constituents, among which proteins, carbohydrates, blood, skin and feathers were determined to be the most common. Similarly, wastewater resulting from miscellaneous operations is also polluted, with a reasonable quantity of grit and other organic matter. Northcutt and Jones (2004) further report an average potable water usage of 26 L/bird (US), for scalding, chilling, bird debris washing and plant sanitation. Similarly, Avula *et al.* (2009) report an average water consumption of 26.5 L/bird, of which most of the potable water is used during the primary and secondary processing of poultry products.

According to Nelson (2009), the processing of birds can be separated into three main parts:

- Primary processing units,
- Secondary processing units, and
- Tertiary processing units.

The primary processing units consist of bird slaughtering, de-feathering (as well as the evisceration of carcasses), with the secondary processing units being dedicated to the cutting of the carcass into a number of parts, and de-boning (Nelson, 2009, Bustillo-Lecompte, 2016), while tertiary processes are used for convenient value-added products for the consumer, e.g. flavouring, marinating, cooking and/or breading (Nelson, 2009, Bustillo-Lecompte, 2016).

At each stage, the quality characteristics of the PSW generated varies; although, the primary and secondary units contribute to 80% of the organic matter in the PSW in the form of particulate matter with a mean size of 75 to 100 μm, culminating in an average BOD_5 of 2500 mg/L (Nelson, 2009).

Table 2.1: Poultry industry operations and by-products as well as products generated (adapted from Barbut, 2015)

Operations in order of occurrence	By-products or waste generated
Delivery and holding of birds	Manure, mortalities
Stunning and slaughtering	Blood, wastewater
De-feathering	Feathers, wastewater
Evisceration	Offal/viscera, manure, wastewater
Trimming and carcass washing	FOG + meat trimmings, wastewater
Deboning	Meat trimming, wastewater
Chilling	FOG, wastewater
Packaging	Wastewater
Cold storage	Spoiled products

Table 2.2: Characteristics of poultry slaughterhouse wastewater from different studies (n=4) (adapted from Barbut, 2015)

Source (Turkey)	COD (mg/L)	BOD_5 (mg/L)	TSS (mg/L)	VSS (mg/L)	Total P (mg/L)
1st study (2002)	2000-6200	1300-2300	850-6300	660-5250	15-40
2nd study (2003)	5800	2200-9800	2400-9400	nd	nd
3rd study (2003)	4000	1730	2580	1960	171
4th Study (2005)	3980-7120	2030-4200	285-2660	nd	54-92

nd: not determined

There are numerous methods of determining and expressing organic matter content in PSW. These methods include biological oxygen demand (BOD_5); chemical oxygen demand (COD); total dissolved solids (TDS); total suspended solids (TSS); and FOG quantification (Barbut, 2005). As such, the improper disposal of PSW, with its high concentration of nitrogen, phosphorus, solids and BOD_5, can ultimately lead to environmental and public health problems, if such wastewater is disposed of into receiving water bodies, like rivers, as many people use river water for irrigation and drinking purposes (Barbut, 2005). A further analysis culminated in the regrouping of the

poultry slaughterhouse operations, to identify by-products as well as wastes produced, as outlined in Table 2.1.

Nelson (2006) further quantified PSW generation, highlighting potable water usage in individual processes, as illustrated in Fig 2.1. When characterising PSW quality of different poultry slaughterhouses, using analytical water quality parameters identified, it becomes obvious that the quality of the wastewater varies for each slaughterhouse, as illustrated in Table 2.2 (Barbut, 2015).

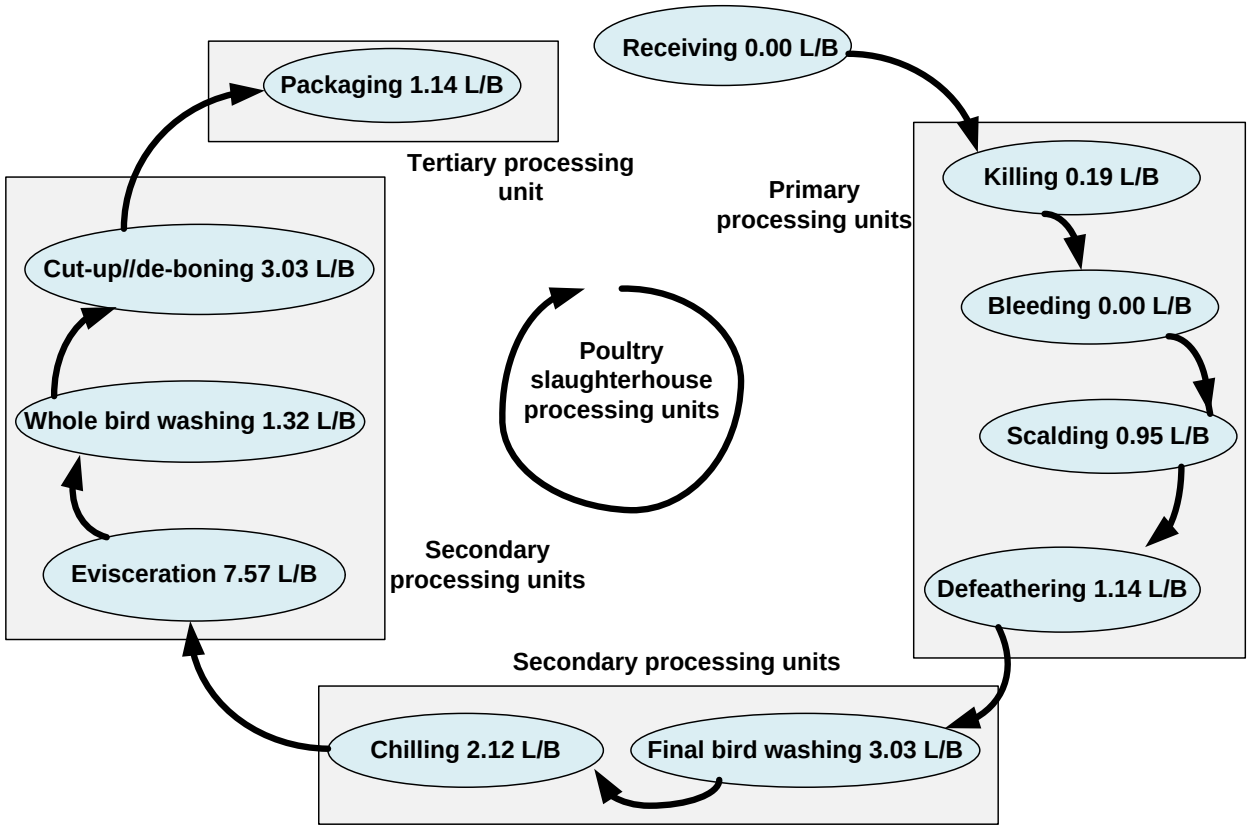


Figure 2.1: Operations in a poultry slaughterhouse associated with water consumption, quantified in litres per bird slaughtered (adapted from Nelson, 2006)

2.3 Importance of the treatment of poultry slaughterhouse wastewater

Numerous arguments can be used to advocate for the need to treat PSW, among which is the protection of the environment. Additionally, prior to discharge of the PSW from the slaughterhouses, it is a legislative requirement to treat such wastewater in order to comply with

environmental regulations (Bustillo-Lecompte *et al*., 2016; Kiepper *et al*., 2008). These regulations vary among countries. In South Africa for instance, the Department of Environmental Affairs (DEA) enforces the regulations, with monitoring being conducted by city/municipal councils throughout the country (DEA, 2014). For Cape Town, which is the focus of this study, the Council of the City of Cape Town is tasked with the enforcement of the policies developed by the DEA (City of Cape Town, 2015). Thus, the Council evaluates the quality of the wastewater generated from a variety of industries, issues permits for discharge or imposes penalties if the discharge standards imposed are not met. These discharge standards, as listed in Table 2.3, provide guidelines for industrial effluent standards, highlighting key quantifiable parameters.

Table 2.3: CCT Industrial wastewater discharge standards (adapted from City of Cape Town, 2015)

Parameter	Not to exceed
Temperature at point of entry (°C)	40
Electrical conductivity at 25°C (mS/cm)	500
pH value at 25°C	12
Chemical oxygen demand (mg/L)	5000
Settle-able solids (60 minutes) (mg/L)	50
Suspended solids (mg/L)	1000
Total dissolved solids at 105°C (mg/L)	4000
Total sulphate as SO_4 (mg/L)	1500
Oils, greases, waxes and fat (mg/L)	400

The other influential factor which can be used as one of the reasons to motivate for the treatment of PSW is the availability of potable water. Northcutt and Jones (2004) stated that several agencies, such as the Pan American Health Organization (PAHO), have reported that the availability of potable water globally would be reduced to only domestic usage by 2025, taking into account that the global population would reach 8.9 billion by this date. Water scarcity is currently being experienced in South Africa, with drought in parts of the country, including the Western Cape (Western Cape Government, 2015), a phenomenon attributed to global warming and the attendant changing weather patterns (World Bank, 2014). During the summer, the imposition of water usage restrictions has been implemented in the Western Cape.

Therefore, to aid in addressing these issues, various technologies have been evaluated for the treatment of PSW, which ultimately can culminate in the treated water being recycled to reduce

water usage, particularly in the poultry industry, in order to address operational inconveniences related to the lack of potable water (Bustillo-Lecompte *et al*., 2016). Of these miscellaneous technologies, anaerobic wastewater treatment appears to be a suitable mitigation option, particularly when considering secondary treatment stages (i.e. for the treatment of PSW) due to low operating costs, production of relatively small quantities of sludge and production of biogas, which can be harnessed for energy generation (Yoochatval *et al*., 2008, Caixeta *et al*., 2002; Chernicharo, 2007).

2.4 Anaerobic wastewater treatment

2.4.1 The efficacy of anaerobic wastewater treatment technology

Different techniques are currently being used for the treatment of different types of industrial wastewater, utilising physical, chemical or biological methods. The biological treatment of wastewater using aerobic systems has been tried, but it culminates in high operating costs associated with sparging, with costs increasing as the organic matter loading rates increase (EPA, 1997; Henze *et al*., 2008). Therefore, following the development of high-rate anaerobic treatment systems, anaerobic treatment of wastewater with a high organic matter concentration has gained interest from researchers (Alphenaar, 1994; Henze *et al*., 2008).

Anaerobic treatment is a process in which organic matter undergoes fermentation, in an environment devoid of dissolved oxygen, to produce biogas. Anaerobic treatment has been proven to be effective in the removal of biodegradable compounds in wastewater, resulting in residual by-products such as NH_4^+, PO_4^{3-} and S^{2-} (Chernicharo, 2007). Furthermore, this process results in the production of relatively small quantities of stabilised sludge, which may itself have economic value (Henze *et al*., 2008). These features differentiate anaerobic treatment from aerobic treatment technology, with the latter generating a large quantity of sludge, requiring further treatment (Fuchs, 2003; Henze *et al*., 2008). In the context of cost-effective wastewater treatment, Chernicharo (2007) stated that the selection of anaerobic wastewater treatment over aerobic wastewater treatment can be advantageous taking into consideration the following:

- The market value of excess sludge;
- The rapid start-up of the treatment system through the use of granular anaerobic sludge as a seed biomass;
- High organic loading rates;

- Significant reduction in excess sludge production;
- Reduced plant footprint;
- Production of biogas (methane);
- Simplified and implementable technology, which can be operated by an unskilled labour force (such as South Africa's), providing high treatment efficiencies;
- Rapid influent treatment through the application of high-rate systems;
- Possibility of storing anaerobic sludge unfed for a long period of time, and;
- Minimal requirements for additives, which can culminate in toxicant residue in sludge.

Another advantageous feature of anaerobic wastewater treatment is the compact nature of the systems, as demonstrated in full-scale operations; in which a daily input of 25 tons of COD can be treated using an anaerobic reactor that has the following configurations, a height of 25 m and a diameter of 6 m. This reactor configuration results in the daily production of less than 1 ton of sludge, which itself has economic value, to mitigate against input/operational costs and which can also be used as a seeding sludge for other bioreactors (Henze *et al*., 2008).

Additionally, due to ever-increasing energy prices and concerns associated with global warming, energy source harvesting from anaerobic wastewater treatment can be achieved by the beneficiation of the biogas produced (Avula *et al*., 2009). It was demonstrated that a daily input of 25 tons of COD of agro-industrial wastewater can generate 7000 m^3 of methane, which translates to an energy equivalence of 250 GJ/d (Henze *et al*., 2008). Furthermore, carbon credits can be gained by producing a renewable energy source from this wastewater treatment technology, defined as an environmentally benign technology (Chernicharo, 2007), since a natural-gas-driven energy generating plant produces half the quantity of CO_2 emissions when compared to a coal-driven power plant (Chernicharo, 2007).

However, anaerobic wastewater treatment does have some disadvantages, such as (Gerardi, 2003):

- Longer start-up time to develop and acclimatise the seeding biomass;
- May require periodic alkalinity adjustments;
- The effluent from the system may require further treatment to reduce by-products formed, in order to meet discharge standards;
- Minimal biological nitrogen and phosphorus removal;
- Systems sensitivity to adverse environmental effects such as lower temperatures, which can reduce reaction rates;

- Susceptibility to toxicant concentrations in the influent, and
- The production of odour and corrosive gases, e.g. H_2S and other gases.

2.4.2 The microbiology of anaerobic wastewater treatment systems

Anaerobic digestion can be described as an environment where a group of bacteria interacts for the transformation of complex polymers into end products, such as methane, carbon dioxide, hydrogen sulphide, water and ammonia and the generation of new bacterial cells (Gerardi, 2003; Vidal *et al*., 2000; Pol *et al*., 2004). Several microbial groups contribute to various metabolic processes that can be classified into four metabolic mechanisms (Gerardi, 2003), namely:

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

Furthermore, the bacteria prevalent in anaerobic digestion, which facilitate biological reactions, can be grouped into the following categories (Henze *et al*., 2008):

- Fermentative bacteria,
- Hydrogen-producing acetogenic bacteria,
- Hydrogen-consuming acetogenic bacteria,
- Carbon dioxide-reducing bacteria methanogens (or hydrogen-using methanogens, as they utilise both carbon dioxide and hydrogen), and
- Aceticlastic methanogens.

Table 2.4: Anaerobic bacteria grouping with respect to their oxygen tolerance (adapted from Gerardi, 2003)

Group	Species	Implication
Oxygen tolerant	*Desulfovibrio sp.*	Reduction of SO_4^{2-} to H_2S
	Desulfomarculum sp.	Reduction of SO_4^{2-} to H_2S
Oxygen intolerant	*Methanobacterium formicium*	Production of CH_4
	Methanobacterium propionicum	Production of CH_4

Anaerobic bacteria may also be separated with respect to their oxygen tolerance, as illustrated in Table 2.4.

2.4.2.1 Hydrolysis for depolymerisation of organic matter

The organic matter available in wastewater is usually polymeric in structure, and thus needs to be broken down into monomeric constituents (Chernicharo, 2007; Evans, 2004). However, most microorganisms responsible for anaerobic digestion are unable to assimilate these polymers (Henze *et al*., 2008; Chernicharo, 2007). Hence, the initiation of anaerobic digestion requires the hydrolysis of polymeric organic matter into simpler monomeric dissolvable organic matter, which can infiltrate the cell membrane of fermentative bacteria (Gerardi, 2003). Thus, fermentative bacteria excrete enzymes i.e. cellulase, protease and lipase, that transform complex and undissolved organic matter, i.e. polysaccharides, proteins and lipids, into less complex, assimilative organic matter (Gerardi, 2003; Henze *et al*., 2008). This essential step in anaerobic digestion takes place slowly under anaerobic conditions and it is influenced by several factors, namely (Henze *et al*., 2008):

- The bioreactor's operational temperature;
- The pH of the wastewater;
- The composition of the organic matter;
- The metabolic activity and size of the granules;
- The hydraulic residence time of the wastewater in the bioreactor;
- The concentration of NH_4^+ - N, which is highly influential and can inhibit some processes in anaerobic digesters; and
- The concentration of both the dissolved materials produced, e.g. volatile fatty acids, and toxicants in the wastewater.

Thus, hydrolysis can be a rate-limiting step for the overall anaerobic digestion process and therefore the design of anaerobic reactors is usually grounded on the hydrolysis stage (Chernicharo, 2007). Moreover, it is usually recommended that the feed/influent to the anaerobic reactor undergoes a preparatory stage, whereby physico-chemical pre-treatment may be applied to facilitate hydrolysis, such as the pre-acidification of the bioreactor influent or the physical degradation of big particles herein contained (Alphenaar, 1994).

2.4.2.2 Monomer conversion through acidogenesis

Since the breakdown of polymeric organic matter, through hydrolysis, results in the generation of fermentable monomeric substances such as monosaccharides, amino acids, fatty acids and alcohols (Evans, 2004), acidogenesis subsequently ensues, whereby metabolisable constituents

are transformed by fermentative bacteria for cellular proliferation and maintenance (Gerardi, 2003), culminating in the extracellular production of volatile fatty acids (VFAs), lactic acids, alcohols, carbon dioxide, hydrogen gas, hydrogen sulphide, and ammonia, as well as bacterial cells (Chernicharo, 2007); a bioprocess facilitated by a broad and diverse group of fermentative bacteria. This process occurs rapidly due to conversion of fermentable monomeric substances in the anaerobic bioreactor (Henze *et al*., 2008). Consequently, the pH of anaerobic bioreactors at this stage may suddenly drop due to acidification of the wastewater as a result of an accumulation of organic acids, reducing the waters' alkalinity and creating a higher concentration of non-dissociated VFAs, which can result in severe inhibition of methanogens (Gerardi, 2003).

2.4.2.3 Acetogenesis as a precursory metabolic process for methanogenesis

Subsequent to acidogenesis, a process in which organic acids are produced, oxidation of these organic acids by acetogenic bacteria through acetogenesis ensues, generating by-products which can conveniently be used by methanogenic bacteria (Gerardi, 2003; Henze *et al*., 2008). These by-products are acetic acid, hydrogen gas and carbon dioxide (Gerardi, 2003; Henze *et al*., 2008), with propionate and butyrate being among the prevalent organic compounds produced at this stage, along with lactate, methanol, ethanol, hydrogen gas and carbon dioxide. These by-products are important intermediates in the anaerobic digestion process (Kobayashi *et al*., 2015), with the formation of a high quantity of hydrogen gas, from acetic and propionic acids, inducing the pH of the wastewater to decrease (Henze *et al*., 2008). Additionally, the consumption of hydrogen gas in the wastewater can take place via two biocatalytic routes (Chernicharo, 2007):

- Utilisation by methanogenic bacteria to yield methane, and
- Additional production of organic acids, e.g. propionic and butyric acids, as a consequence of a reaction involving hydrogen gas, carbon dioxide and acetic acid.

2.4.2.4 Dependence of methanogenesis on hydrolysis, acidogenesis and acetogenesis

Methanogenesis constitutes the last stage of the anaerobic digestion biocatalytic process, and it is highly dependent on preceding processes, i.e. hydrolysis, acidogenesis and acetogenesis. This penultimate biological reaction is essential and results in the influent's COD transformation into biogas, which can be used for energy requirements (Henze *et al*., 2008); a process facilitated by methanogens, which primarily utilise specific substrates, such as acetic acid, hydrogen gas, carbon dioxide, formic acid, carbon monoxide, methylamines and methanol (Gerardi, 2003). Due to the complexity of by-products produced in preceding biocatalytic processes, the quantity and

quality of preferential by-products influences the rate of production of methane by methanogens, which can be categorised into two main groups, namely (Chernicharo, 2007):

- Aceticlastic methanogens, i.e. acetate-using microorganisms, and
- Hydrogenotrophic methanogens, i.e. hydrogen-using microorganisms.

Table 2.5: Main processes and sub-processes of anaerobic treatment (adapted from Gerardi, 2003)

Process	Sub-processes	Required biomass	Indicators
Hydrolysis of biopolymers	Hydrolysis of proteins Hydrolysis of polysaccharides Hydrolysis of fats	Fermentative bacteria	Amino acids, sugars. Fatty acids and alcohols
Acidogenesis/fermentation	Anaerobic oxidation of amino acids and sugars Anaerobic oxidation of higher fatty acids and alcohols	Fermentative bacteria	Intermediate products i.e. propionate, butyrate, etc.
Acetogenesis	Formation of acetic acid and H_2 from intermediary products (principally VFAs) Formation of acetic acid from H_2 and CO_2	-Hydrogen-producing Acetogenic bacteria. -Hydrogen-consuming Acetogenic bacteria.	Acetate, hydrogen, carbon dioxide
Methanogenesis	Methane generation from acetic acid Methane generation from hydrogen and carbon dioxide	-Carbon dioxide-reducing methanogens. -Aceticlastic methanogens.	Methane, carbon dioxide

Although aceticlastic methanogens prevail in anaerobic digestions, only a few methanogens illustrate the capacity of methane generation from acetate (Gerardi, 2003; Henze *et al*., 2008), with a contributory scale of between 60% to 70% to methane generation, with two genera, i.e. *Methanosarcina,* prevailing at acetate concentration higher than 10^{-3} M, and *Methanosaeta* that prevail at concentration lower than 10^{-3} M (Pol *et al*., 2004). Table 2.5 illustrates (sub) processes in anaerobic treatment.

Methanosarcina are less sensitive to pH fluctuations than *Methanosaeta,* with higher methane yields than *Methanosaeta* (Chernicharo, 2007). The former has better growth rates and is unaffected by long solids retention time (SRT) when compared to *Methanosaeta*, which can

proliferate at lower acetate concentrations (Chernicharo, 2007). *Methanosarcina* can be identified by their coccoid shape and the ability to utilise a variety of substrates, such as acetate, H_2/CO_2, methanol, methylamines, and formate, while *Methanosaeta* are filamentous conglomerates that can only convert acetate (Pol *et al*., 2004). As such, *Methanosaetas* are the most common acetotrophic methanogens in high-rate anaerobic systems, which have high SRT (Pol *et al*., 2004), due to low acetate availability as a consequence of low organic matter availability and/or conversion to acetate, through hydrolysis, acidogenesis and acetogenesis, which culminates in wastewater acetate concentrations inside biofilms, including sludge granules, being minute when the acetate concentration in the bulk liquid is low (Henze *et al*., 2008). The sludge granules' domination by *Methanosaeta* results in an effective wastewater treatment system, leading to extremely low anaerobic reactor effluent acetate concentrations (Alphenaar, 1994; Pol *et al*., 2004).

Unlike aceticlastic methanogens, almost all of the hydrogenotrophic methanogens are able to generate methane from carbon dioxide and hydrogen gas, with the most common isolated genera in anaerobic reactors being *Methanobacterium sp.*, *Methanobrevibacter sp.* and *Methanosperillum sp.* (Gerardi, 2003). Since these species facilitate the consumption of hydrogen gas generated from previous phases, both the aceticlastic as well as hydronetrophic methanogens are required for pressure reduction in the anaerobic reactors used, to allow sequential production of other biological by-products by acidogens and acetogens (Chernicharo, 2007).

As a result, the main processes governing anaerobic digesters can be summarised and classified into sub-processes, as depicted in Table 2.5. However, the production of methane is not always the ultimate end-product of anaerobic digestion, as reversible reactions may occur, due to the formation of a higher quantity of volatile fatty acids and alcohols from acetate and propionate (Gerardi, 2003; Chernicharo, 2007). These back reactions may result from the malfunctioning or perturbation of the anaerobic reactor, or when a specific reaction is purposefully favoured by altering bioreactor environmental conditions. Moreover, the presence of alternative electron acceptors, such as NO_3^- and SO_4^{2-}, may result in different bacterial groups prevailing in the anaerobic reactor (Gerardi, 2003; Chernicharo, 2007) i.e. denitrifiers and sulphate-reducers.

2.4.2.5 Effects of alternative electron acceptors in anaerobic digesters

Mixed microbial communities are commonly found in anaerobic reactors; depending on bioreactor conditions and competitive inhibition among bacteria due to substrate limitation (Henze *et al*.,

2008; Evans, 2004). Furthermore, these bacteria possess different microbial respiration systems, and thus can utilise different electron acceptors such as dissolved oxygen by facultative aerobic bacteria, nitrate (NO_3^-) by denitrifiers, sulphate (SO_4^{2-}) or sulphite (SO_3^{2-}) by sulphate-reducing bacteria and iron (Fe^{3+}) by iron-reducers (Henze *et al*., 2008). The role of each group of microorganisms is further assessed in subsequent subsections.

2.4.2.6 Facultative anaerobic bacteria

The growth of facultative bacteria is not affected by the presence or absence of dissolved oxygen; although they prefer respiration over fermentation if dissolved oxygen is available, as respiration generates more energy (ATP yield) than fermentation (Gerardi, 2003; Henze et al., 2008). Facultative anaerobes play an important role in the degradation of organic matter in biological systems. They constitute approximately 80% of the biomass in aerobic systems, and are the most popular microorganisms within suspended growth processes and fixed-film processes (Gerardi, 2003). From the biodegradation of organic matter in anaerobic processes, facultative anaerobes such as *Enterobacter spp*. generate a variety of alcohols and acids, as well as carbon dioxide and hydrogen gas (Gerardi, 2003). Furthermore, other facultative anaerobes such as *Escherichia coli* generate malodorous substances such as skatole and indole (Henze *et al*., 2008).

2.4.2.7 Sulphate reducing bacteria (SRB)

Sulphate reducing bacteria (SRB) transform sulphate into hydrogen sulphide gas due to the presence of sulphates, sulphites and/or thiosulsulphates in anaerobic reactors' influent, which may lead to the consumption of several intermediates generated in the anaerobic mineralisation process, as SRB possess a broader substrate spectrum range (Henze *et al*., 2008; Gerardi, 2003). This substrate spectrum range goes beyond substrates produced during anaerobic digestion, such as acetate, formate, methanol, molecular hydrogen and pyruvate, propionate, lactate, and butyrate, as well as branched fatty acids, fumarate, succinate, malate and other aromatic compounds (Henze *et al*., 2008). SRB, methanogens and obligate hydrogen producing bacteria (OHPB) normally proliferate under similar environmental conditions, and thus compete for similar substrates, which are formed as main intermediary by-products of organic matter bio-decomposition during the anaerobic digestion process (Chernicharo, 2007). Therefore, when such substrate competition exists, it results in the formation of two primary products, i.e. H_2S, which is a toxicant when produced in large quantities, and methane, which can dissolve in the wastewater, as illustrated in Fig. 2.2 (Gerardi, 2003), from the following processes:

- Methane from methanogenesis, and
- Sulphide from sulphate reduction bacteria.

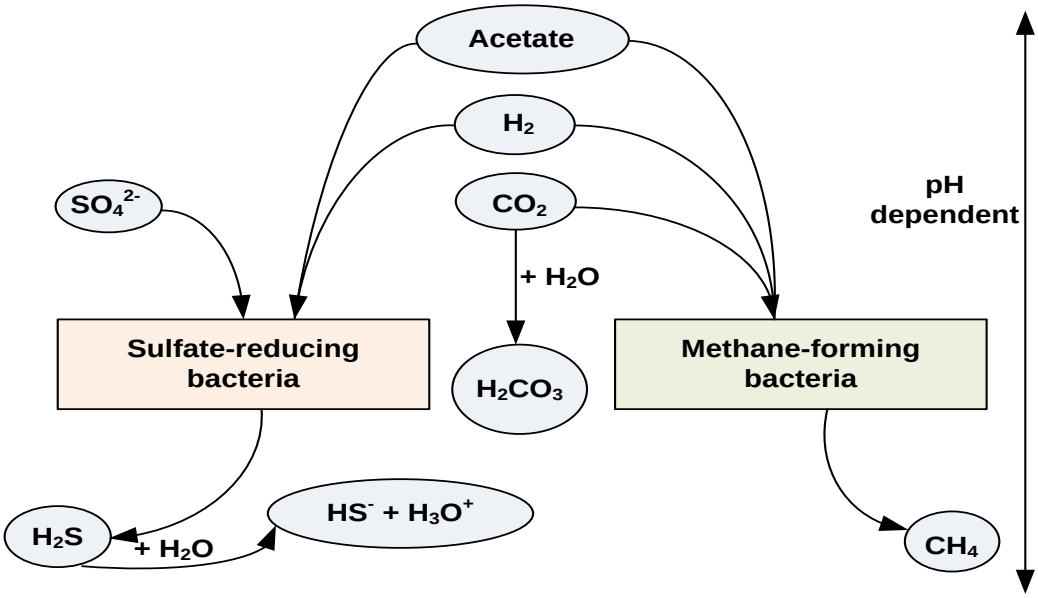


Figure 2.2: Competition between SRB and MFB (adapted from Gerardi, 2003)

Aspects governing these processes are notably bioreactor pH and the COD/SO_4^{2-} ratio in wastewater being treated (Henze *et al*., 2008; Alphenaar, 1994). As a result, several problems arise from excessive production of sulphides during the anaerobic treatment of wastewater, namely (Chernicharo, 2007):

- The formation of H_2S, which is an inhibiting compound for the methanogenesis. In fact, the methanogenic bacteria become more inhibited when the COD/SO_4^{2-} ratio is below 7. At COD/SO_4^{2-} ratio > 10, a significant quantity of the H_2S is formed, and thus is removed from the wastewater with biogas, thereby decreasing its inhibiting effect, although biogas scrubbing is required.
- The formation of sulphide has an effect on the metabolic processes, due to an increase in dissolved oxygen demand in the effluent, and leads to odour generation, requiring an additional post-treatment operation.

- The consumption of by-products available in the wastewater by the SRB decreases the quantity of organic matter available for methanogenesis, culminating in a decrease in methane production. Thus, a reduction of 1.5 g of SO_4^{2-} translates into the use of 1 g of COD; therefore, minimal COD is available for transformation into methane gas.

Based on their substrate consumption preference, SRB can be classified into (Chernicharo, 2007):

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

2.4.2.8 Rarity of denitrification in anaerobic digester

Generally, there is minimal denitrification occurring during anaerobic digestion (Chernicharo, 2007), with ammonium being produced from organically bound nitrogenous compounds. The process of denitrification may only occur if the influent contains nitrates (Chernicharo, 2007), with the feasibility of denitrification by denitrifying microorganisms, i.e. chemoheterotrophic, being capacitated by the oxidation of organic matter containing nitrates (Chernicharo, 2007), with its sequential conversion via the nitrite transformation pathway into nitrogen oxide and N_2 gas. The denitrification process ideally occurs in aerobic environments, as denitrifying microorganisms favour dissolved oxygen for which concentrations > 0.5 g/m^3 molecular oxygen act as an electron acceptor, culminating in excessive metabolic heat generation (Henze *et al*., 2008).

Ammonia in an anaerobic reactor may exist in the form of ammonium ions or dissolved ammonia gas (Gerardi, 2003; Henze *et a*l., 2008), forming an equilibrated availability in the wastewater, due to the dependence on fluctuating pH in the anaerobic reactor. A pH ≤ 7.2 would favour the presence of ammonium ions, while a pH > 7.2 would facilitate the availability of dissolved ammonia (Gerardi, 2003), which is toxic to methanogens, with high alkalinity resulting in the formation of scum and thus proliferation of scum-forming organisms (Gerardi, 2003) which may render the anaerobic digestion process ineffective. A pH between 6.8 and 7.2 normally reduces dissolution of ammonia, which reduces its toxicity to the methanogens (Gerardi, 2003).

2.5 Anaerobic biomass formation in anaerobic digesters

The ideal anaerobic treatment process allows for long SRT or for low HRTs (Evans, 2004; Oh, 2012; Alphenaar, 1994), as observed in several studies that eventually led to the development of high-rate anaerobic bioreactors (HRABs) (Pol *et al*., 2004). HRABs resolved the challenges associated with long retention times of the biomass within anaerobic reactors while allowing for short HRTs, which subsequently made high organic loading rates possible (Pol *et al*., 2004; Alphenaar, 1994).

Microbial cells are encountered in a broad range of sizes, structural forms and growth phases; either individually or as conglomerates in several microstructures (Lettinga *et al*., 1980; Gerardi, 2003). These structural arrangements play a vital role in anaerobic digestion, as the form of the biomass has a direct influence on the survival and growth of anaerobic microorganisms, and ultimately the efficiency of the anaerobic treatment process (Pol *et al*., 2004). It is understood that the long SRTs, i.e. retention of microorganisms within the anaerobic reactor, are crucial for anaerobic digester functionality. For long SRTs, biomass immobilisation can be done using inert support material mounted onto fixed matrices, as in anaerobic filters, which can operate either in an up-flow or down-flow mode (Lettinga *et al*., 1980; Chernicharo, 2007). These matrices can also be free-floating, such as those used in fluidised-bed systems or moving-bed bioreactors. In cases where there is minimal, or no support material utilised, the auto-immobilisation of bacteria on to themselves to form bacteria conglomerates and thus biofilms may occur (Chernicharo, 2007). These bacterial conglomerates mature, and develop in round shape granular sludge (Alphenaar, 1994; Pol *et al*., 2004). Such retention of biomass can be classified into four common retention methods (Chernicharo, 2007):

- Retention by attachment onto a fixed/inert material,
- Retention by flocculation,
- Retention by granulation, and
- Interstitial retention.

To better understand these biomass retention mechanisms, see subsections 2.5.1 to 2.5.4.

2.5.1 The retention of biomass by attachment

Miscellaneous factors, such as pH, temperature, nutrient availability and stratification, affect the survival and growth of anaerobic microorganisms within an anaerobic environment (Gerardi,

2003; Baddour *et al*., 2016). One way of overcoming the fluctuations in environmental conditions, is to attach the biomass to an inert surface (Pol *et al*., 2004). This attachment can be harnessed such that the microorganisms in question can withstand shearing forces, while shedding deactivated biomass (Chernicharo, 2007). This form of immobilisation can be achieved on fixed- or moving-bed surfaces, whereby individual cells adhere to an inert surface (Fig. 2.3) with the assistance of EPS, a glutinous extracellular substance produced by the microorganisms.

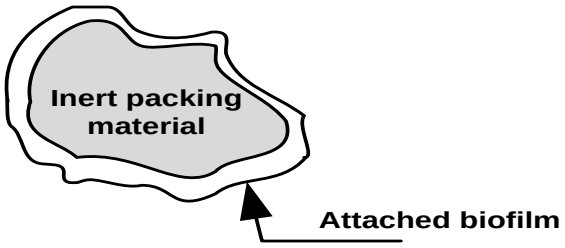


Figure 2.3: Attached biofilm on a packing material

2.5.2 The retention of biomass by flocculation

This type of immobilisation is often encountered in sewage treatment, as sedimentation allows the flocculating microstructures to be separated from the liquid phase (Chernicharo, 2007). This phenomenon can be observed in up-flow anaerobic sludge blanket (UASB) processes, as well as other two-stage anaerobic bioprocesses (Lettinga *et al*., 1980; Chernicharo, 2007).

2.5.3 The retention of biomass by granulation

Granulation is a natural process occurring in systems where some basic conditions exist, such as the sufficient availability of soluble substrates, and up-flow operational mode facilitated by pneumatic eddies with SRTs significantly higher than HRTs (Pol *et al*., 2004). However, sludge granulation was also observed in reverse flow Dorr Oliver Clarigesters operated in South Africa (1950), which suggests that sludge granulation is independent of regime operational modes (Lettinga *et al*., 1980; Henze *et al*., 2008). Furthermore, sludge granulation can be observed even under psychrophilic, mesophilic or thermophilic conditions (Alphenaar, 1994), although such a retention mechanism, i.e. granulation, is usually linked to the treatment of wastewaters rich in volatile acids and carbohydrates (Alphenaar, 1994).

One important parameter is often listed when referring to sludge granulation: the up-flow velocity (Henze *et al*., 2008; Rajakumar *et al*., 2011). As a result of the constant selective pressure that

an up-flow velocity sustains, the microorganisms start attaching to each other, leading to the formation of granules that show good settleability (Pol *et al*., 2004). From an engineering perspective, the granular configuration provides numerous advantages, namely:

- Good aggregation of the microorganisms,
- Effective use of the reactor's operational volume through the non-use of inert support materials,
- Good settleability of the granules, and
- Sphericity of the granules, which allows for maximised microorganism/bioreactor volume ratio (Chernicharo, 2007).

An advantageous feature of anaerobic granules is the arrangement of the biomass structure, with different bacterial sub-groups selectively gathering in layers on top of each other, as depicted in Figure 2.4.

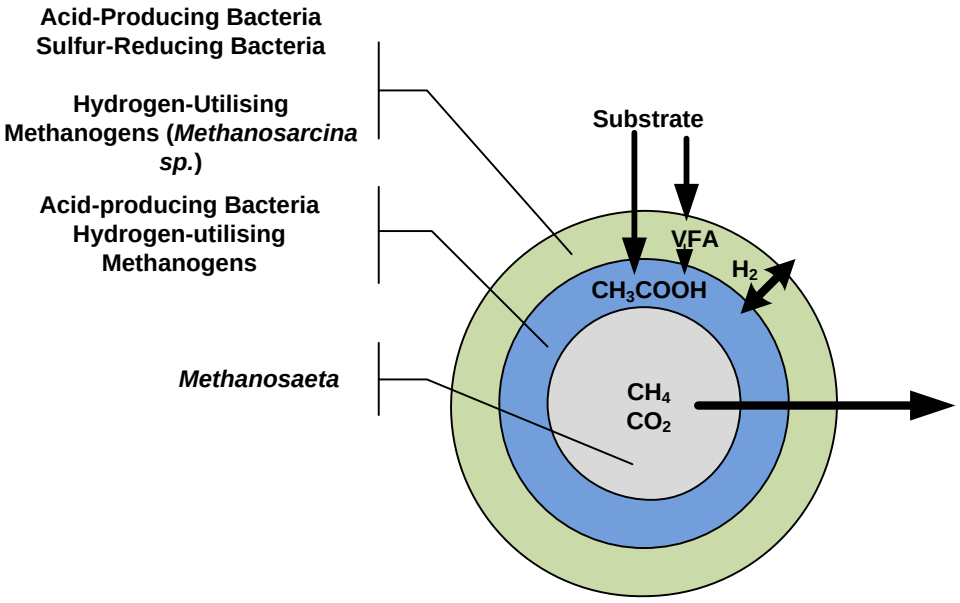


Figure 2.4: Structure of anaerobic granules (adapted from Chernicharo, 2007)

Table 2.6: Characteristics of a granular sludge of good quality (adapted from Alphenaar, 1994)

Metabolic activity	Specific methanogenic activity range of granular sludge	– 2.0 kgCOD/kgVSS.d
	Typical values for industrial wastewater	0.5 – 1.0 kgCOD-CH_4/kgVSS.d
Settle-ability and other physical properties	Settling velocities	2-100 m/h, typically: 15-50 m/h
	Density	1.0-1.05 g/L
	Diameter	0.1-8 mm, typically: 0.15-4 mm
	Shape	Spherically-formed and well-defined surface
	Colour	Black/ grey/ white

This arrangement contributes to the improvement of the anaerobic treatment, through a direct transfer of various substrates across the thickness of the granulated sludge, which ensures that different substrates are consumed, with the by-products being used by a subsequent layer of microorganisms. Such an arrangement will also mitigate against high toxicant loads, and thus the protection of methanogens which are located at the centre of the granules, an area which is totally devoid of dissolved oxygen. Table 2.6 characterises the morphology (structure) of granulated sludge.

2.5.4 Interstitial retention of anaerobic sludge

This type of biomass immobilisation is often encountered in the interstices of stationary support materials, such as in fixed-bed anaerobic reactors or static granular bed reactors (SGBRs) (Evans, 2004). For such retention, the surface of the stationary materials provides a support for attachment of bacteria, with empty space existing between the bacteria being colonised by other microorganisms (Pol *et al*., 2004) as illustrated in Figure 2.5.

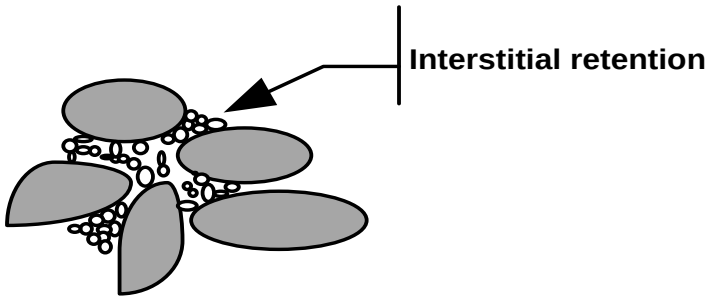


Figure 2.5: Interstitial retention

This retention strategy is largely influenced by the treatment technology used for organic matter in wastewater decomposition, as discussed in subsequent sections.

2.6 Anaerobic treatment technologies: Microbial perspective and classification

The 19th century witnessed the development of anaerobic bioreactors with the conception of the automated scavenger and the septic tank to reduce the sludge in sewerage systems (Chernicharo, 2007). In 1905, Karl Imhoff conceived the first anaerobic bioreactor, named the Imhoff tank, which enabled the stabilisation of solid sediments within a single tank (Henze *et al*., 2008). Throughout the years, other bioreactors have been developed, incorporating new features and improved efficiency. Until recently, anaerobic processes failed to provide profitable and easy-to-operate configurations (Chernicharo, 2007). However, the development of HRABs resolved some operational issues that were encountered before, promoting long SRTs while maintaining low HRTs for increased treatment rates and better methane yield (Debik & Coskun, 2009; Oh, 2012; Del Nery *et al*., 2007). Fig. 2.6 illustrates two main groups that can be described to demonstrate these developments.

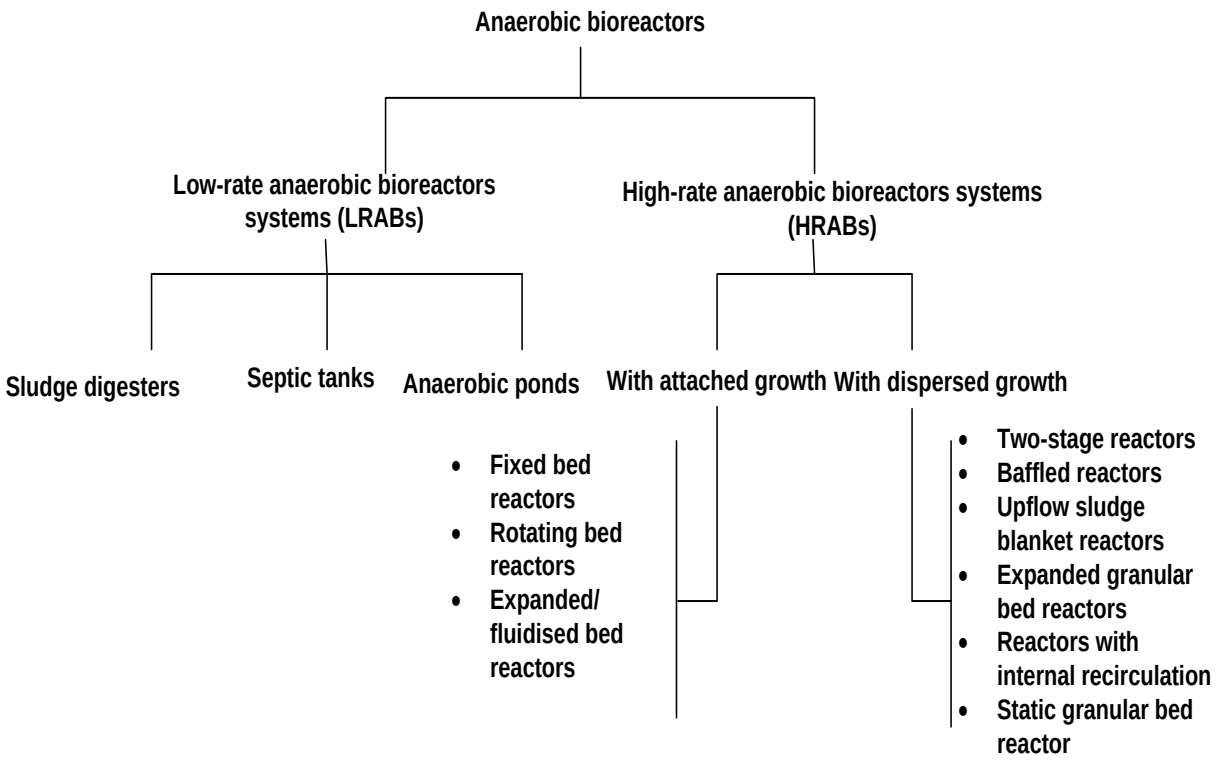


Figure 2.6: Anaerobic systems (adapted from Chernicharo, 2007)

2.6.1 Low-rate anaerobic bioreactors systems

LRABs refer to anaerobic bioreactors operating with low volumetric organic loads, since they do not possess the required sludge retention system for large quantities of high-activity biomass (Henze *et al*., 2008). These types of bioreactors can be differentiated from high-rate anaerobic systems as they (Chernicharo, 2007):

- lack sludge retention mechanisms in the bioreactor,
- have HRTs associated to the SRTs, and
- have low effluent production rates, i.e. low wastewater throughput rates.

These issues combined to make the use of anaerobic treatment processes problematic before the development of high-rate anaerobic systems.

2.6.2 High-rate anaerobic bioreactor systems

The HRABs differ from most conventional systems, due to their application of biomass retention mechanisms which enable biomass proliferation within the systems for low HRTs (Pol *et al*., 2004), i.e. with dispersed bacterial growth, including free bacterial granules or flocs within the anaerobic bioreactor. Variations in their design would include bacteria aggregation on an inert support material, which contributes to the formation of biofilms (Pol *et al*., 2004; Kobayashi *et al*., 2015). Unlike aerobic systems, in an anaerobic or anoxic system, the maximum allowed load is not dictated by the maximum rate at which a required reactant, such as dissolved oxygen, can be supplied, but by the quantity of active biomass that is in complete contact with the wastewaters' organic matter (Henze *et al.*, 2008). As a result of high biomass concentrations, high COD_{bd} loading rates may be applied, thus facilitating high organic loading during the treatment of a specific type of wastewater, provided the following conditions are adhered to (Chernicharo, 2007):

- High retention of active biomass within the bioreactor, irrespective of environmental operating conditions;
- Appropriate contact between the activated sludge biomass and the wastewater (in the event of the deprivation of the substrate to parts of the reactor, the active bacterial biomass contained in the sludge would be of no importance);
- The active biomass adapts or acclimatises to the type of wastewater being treated; and
- The existence of suitable environmental conditions for microorganisms, constituting sludge granules.

The first generation of HRABs for medium strength wastewaters failed to provide attractive outcomes (Del Nery *et a*l., 2008; Alphenaar, 1994), as the separation of the sludge from the wastewater could not be achieved. Furthermore, the use of high-intensity agitation in the anaerobic reactor, although considered important at the time, directly culminated in detrimental effects, due to mechanical shearing of the sludge biomass, and therefore reduced efficiency (Alphenaar, 1994). This is consistent with opinions of Chernicharo (2007) that mechanical mixing devices should not be used in new HRABs with dispersed growth, as they have a negative impact on the aggregation of the sludge and consequently, the formation of granules. Nowadays, with the impact on the development of granules, a more gentle and intermittent mode of mixing must be utilised, with inducement of mixing being rather by pneumatic means or wastewater eddies for an effective distribution of both sludge and COD_{bd}. Current technologies, such as the UASB and the EGSB, utilise such operational designs (Chavez *et al*., 2005; Del Nery *et al*., 2008). As such,

it is prudent to evaluate anaerobic digesters currently available on the market, and adopt and/or modify their designs, for treatment of medium to high strength wastewater, such as PSW from poultry slaughterhouses.

2.6.3 Evaluation of technologies used for poultry slaughterhouse wastewater (PSW) treatment

Various technologies have been assessed for the treatment of PSW. Table 2.7 lists these technologies as well as their performance, as reported in numerous studies. From these studies, it is apparent anaerobic technologies that provided good performance at low operating costs are the SGBR, the EGSB as well as the UASB. These bioreactors are all systems with dispersed bacterial growth, i.e. they rely significantly on the ability of the biomass to form flocs and settle. Their design, functionality and efficiency are discussed in subsequent subsections.

2.6.3.1 Up-flow anaerobic sludge blanket (UASB)

Initially utilised in the Netherlands, and illustrated in Figure 2.7, the UASB reactor was invented by Lettinga and co-workers, (Lettinga *et al*., 1980; Alphenaar, 1994). It consists of a process that allows wastewater to be pumped upwards from the bottom of the bioreactor, passing through a sludge bed containing an active biomass. The bed profile of the UASB usually consists of a sludge bed that is constituted by dense and granular sludge with good settle-ability, thus congregating at the bottom of the reactor, with a dispersed sludge blanket made of lighter sludge debris dispersed at the top of the bioreactor, i.e. a free-floating sludge blanket (Pol *et al*., 2004). The conversion of organic matter in this system takes place throughout the bioreactor, and its contents are mixed by the upward flow of the wastewater and rising gas bubbles. Two fundamental principles govern the operation of the UASB. The first is its capacity to grow high-activity biomass through a monitored start-up process to promote suitable conditions for the growth of required active biomass. The second principle is the presence of a gas-liquid-solid separation system, located at the top of the bioreactor, which separates the solids from the effluent in order to facilitate the collection of the biogas (Henze *et al*., 2008). The typical up-flow velocity in these systems varies between 0.5 and 1 m/hr, and the height-to-depth ratio is normally between 0.2 and 0.5. An elongated period is usually required for bioreactor stabilisation and for the growth of the required biomass- a procedure which can be overcome by the inoculation of sludge granules from a similar bioreactor (Del Nery *et al*., 2008). Thus, the healthier the inoculant used, the greater the quantity of organic loading rates that can be fed to the bioreactor. Modifications of this bioreactor resulted

in technologies such as the UASB filter system, the hybrid anaerobic reactor and the hybrid up-flow anaerobic sludge blanket (HUASB) (Rajakumar *et al*., 2012).

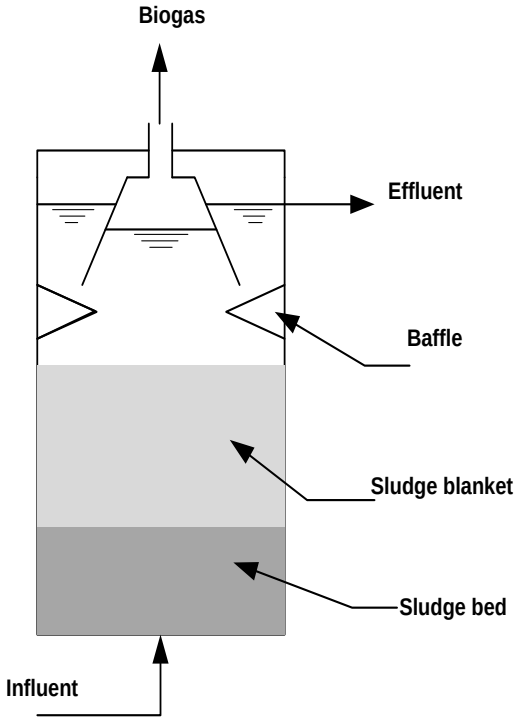


Figure 2.7: Up-flow anaerobic sludge blanket (adapted from Chavez *et al*., 2005)

Table 2.7: Technologies previously used for the treatment of PSW

Technology used	Performance	Comments	Reference
Electrocoagulation	COD removal: 93% Oil & Grease: 98%		Kobya *et al.,* (2006)
Static granular bed reactor (SGBR)	COD removal: 93% TSS removal: 95 % FOG removal: 90%	The UF post-treatment yielded COD removal: 64% TSS removal: 88% FOG removal: 29%	Basitere *et al*., (2017)
Expanded granular sludge reactor (EGSB)	COD removal: 57% Highest OLR: 1 gCOD/L.day	The COD removal of the overall system (EGSB-anoxic/aerobic) was 65%	Basitere *et al*., (2016)
Combination of DAF and UASB	Oil & Grease removal: 51 +/- 16% TSS removal: 37 +/- 16% (for each stage)	Up-flow velocities of 0.3 +/- 0.1 m/h	Del Nery *et al*., (2007)
Aerobic reactors	COD removal > 90%		Rusten *et al.* (1998)
Stabilisation pond system	COD removal > 90%		Del Nery *et al*. (2005)
UASB	BOD_5 removal: 95%	Operated at ambient temperature Maximum HRT of 4h	Chavez *et al*. (2005)
UASB	tCOD removal: 65% sCOD removal: 85%	Full scale operation Average OLR: 1.64 kgCOD/m^3.day	Del Nery *et al*. (2008)
Sequencing batch reactor (SBR)	COD removal: 74%	Based on a fill and draw activated sludge system	Moreira *et al*. (2002)
Static granular bed reactor	COD removal > 95%		Debik and Coskun (2009)
Hybrid up-flow anaerobic sludge blanket (HUASB)	OLR: 19 kgCOD/m^3 tCOD removal: 70 to 86% sCOD removal: 80 to 92% Biogas 1.1 to 5.2 m^3/m^3.d	Operated under mesophilic conditions Methane content in the biogas equal to 72%	Rajakumar *et al.* (2012)
Aerobic moving bed biofilm reactor (MBBR)	COD removal: 94.77% TSS removal: 61.43%		Baddour *et al.* (2016)
Continuous stirred tank reactor coupled with a membrane filtration unit	COD removal > 90%		Fuchs *et al.* (2003)

2.6.3.2 Expanded granular sludge bed (EGSB)

The EGSB reactor belongs to the family of UASB bioreactors, with a design similar to that of the UASB, although it differs from the UASB in the expansion of its sludge bed and the sludge type used (Henze *et al*., 2008). For the EGSB, the expansion of the bed intensifies hydraulic mixing within the system, which improves the contact between the sludge bed and the wastewater fed to the bioreactor (Henze *et al*., 2008; Chernicharo, 2007). Unlike the UASB, in which the sludge remains somewhat static, the application of high-effluent recirculation rates associated with the EGSB configuration induces high surface velocities of the liquid within the bioreactor, ranging from 5 to 10 m/hr (Henze *et al.,* 2008). Thus, the EGSB is suitable for the treatment of high-strength wastewater with organic loading rates up to 30 kg/m^3.d (Chernicharo, 2007). Furthermore, treatment of FOG-laden wastewater using the EGSB, reveals that the bioreactor has a filtration effect that can be controlled by the recycle ratio, as most of the FOG in the influent was absorbed or entrapped by the granules, which progressively disintegrated into the tanks of the recirculation system (Chernicharo, 2007). The same recirculation system also improves the up-flow velocity, and can be used in proportion to the required COD removal rates (Henze *et al*., 2008). However, when the EGSB was used for the treatment of some influents, sludge washout was also reported (Basitere *et al.*, 2016). The design of the EGSB is illustrated in Fig. 2.8.

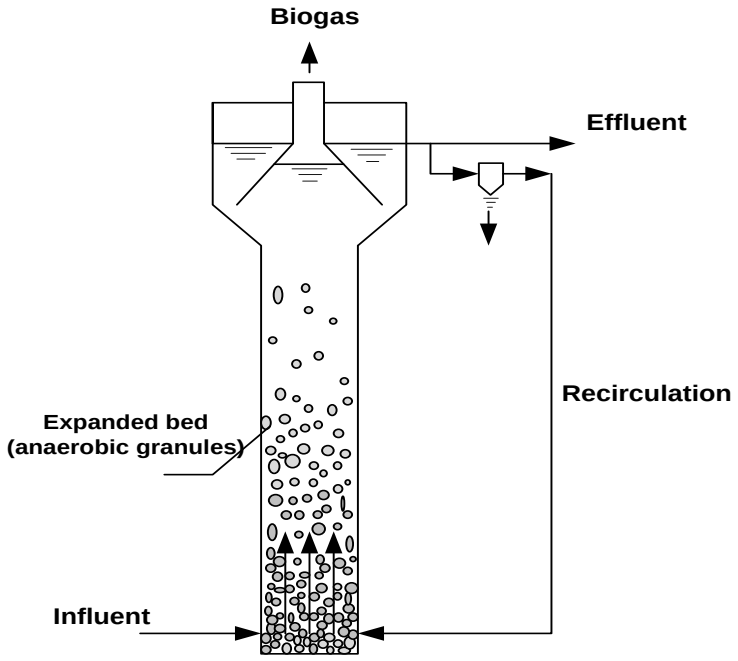


Figure 2.8: Expanded Granular Sludge Bed (adapted from Henze *et al*., 2008)

2.6.3.3 Static granular bed reactor (SGBR)

The static granular bed reactor was developed by a group of researchers from Iowa State University (Ellis and Evans, 2008; Debik & Coskun, 2009). It differs from the UASB and the EGSB, as it primarily uses a down-flow configuration mode that contributes to reduced operating costs (Debik & Coskun, 2009). This bioreactor has been proven to be efficient for the treatment of different types of wastewater (Debik & Coskun, 2009; Oh, 2012; Evans, 2004). Its configuration is simplified, as it doesn't require additional equipment or a mixing system (Evans, 2004). The flow of the wastewater in the SGBR is not well understood; however, as it was hypothesised that wastewater mixing occurred by means of the circulating movement through the static granular bed, which was also assumed to be facilitated by the gas bubble movement. Other researchers report the observation of some movement of the granules, but not to the extent seen in a homogenously mixed sludge bed (Evans, 2004). Head losses were also reported during the operation of the SGBR (Basitere *et al*., 2017). These were explained by higher organic loading rates, accumulation of fine particles in the bioreactor and the entrapment of biogas within the bioreactor (Evans, 2004; Basitere *et al*., 2017). These operational problems can be resolved by backwashing operations that consist of reversing the flow configuration of the bioreactor to unclog the underdrain system. Clogging occurs as a result of smaller size granule entrapment in the underdrain, a process facilitated by the EPS, which frequently occurs during longer operating times, as the sludge granules increase in size, producing various by-products including excessive EPS, thus facilitating head losses (Evans, 2004). The other feature of the SGBR is its ability to retain its biomass for much longer than the EGSB or the UASB, due to a configuration that minimises biomass washout (Oh, 2012; Ellis & Evans, 2008, Basitere *et al*., 2017).

In summary, these three technologies have been proven to sustain required performances, but possess some weaknesses. The UASB and the EGSB have high operating costs associated with their up-flow velocities and recycling streams used, and this is particularly true for the EGSB. Both systems require a gas/liquid/solids separator; and they are more prone to solid washout than the SGBR. Furthermore, the SGBR's limitations are directly associated with higher loading rates, which induce clogging through the accumulation of solids on top of the granular bed. This causes wastewater accumulation, dead-zones, short-circuiting, gas entrapment, and to some extent, the accumulation of sulphides that have a detrimental effect on methanogens. The inhibition of methanogens usually is as a result of the accumulation of volatile fatty acids and consequently the reduction in the pH that also has a detrimental effect on the methanogens' metabolic activity.

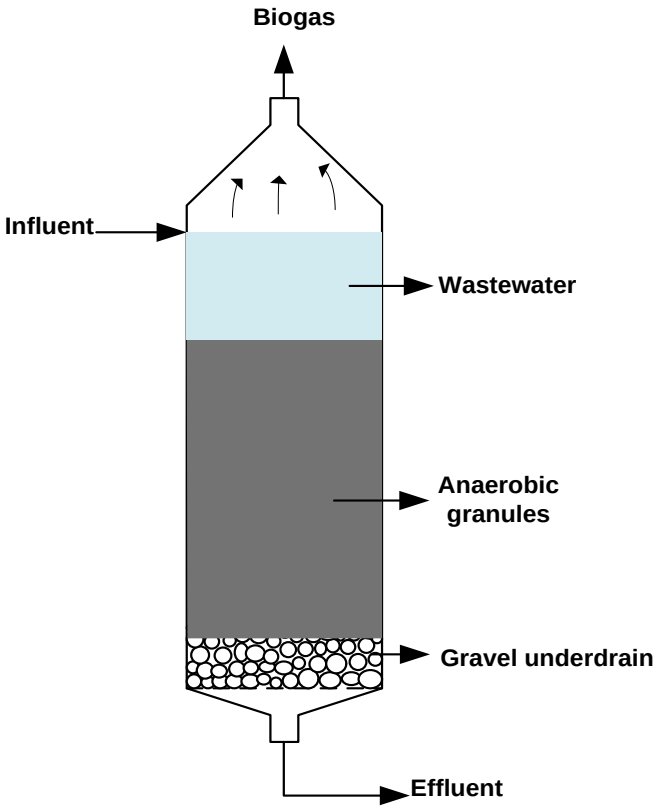


Figure 2.9: Static Granular Bed Reactor (adapted from Basitere *et al.,* 2017)

Furthermore, Kobayashi *et al.* (2015) report that anaerobic granular sludge releases considerable quantities of extracellular polymeric substance (EPS) in the presence of sulphides and methanethiol, as a protection mechanism against these toxicants. This leads to a rapid increase in turbidity and reduction of the size of the anaerobic granules, due to sulphide concentration accumulation in the digester (Kobayashi *et al*., 2015). From this study, it was concluded that the presence of sulphides within the ecosystem of the anaerobic digester reduces methane production, COD_{bd} removal rate and biomass retention. Although backwashing has been reported to be used to address these problems, it results in associated sludge washout, reducing the productivity of the system.

2.7 Operational control of anaerobic digesters

2.7.1 Control of key anaerobic environmental parameters

Apart hydrolysis of particulate matter, the other major rate-limiting bio-reaction step in anaerobic wastewater treatment is the conversion of volatile fatty acids to methane, as methanogens derive minute quantities of energy from the conversion of volatile fatty acids (Chernicharo, 2007). This energy is mostly used for methane generation i.e. by-product

formation, restricting the proliferation of methanogens, which results in the need to maintain optimum operational environmental conditions within the anaerobic bioreactor (Gerardi, 2003). The conservation of methanogens is dependent upon these conditions, which include an environment devoid of dissolved oxygen, suitable pH, temperature and alkalinity (Gerardi, 2003). The ratio of VFA to alkalinity enables assessment of the process stability, as a ratio <0.3 indicates stable operating conditions, while a ratio ranging between 0.3 and 0.4 reflects an instability of the system that requires corrective action to reduce the ratio to <0.3 (Oh, 2012). However, an increase of the VFA/alkalinity to a value equivalent to, or higher than, 0.8, translates to an acidification of the bioreactor and the inhibition of methanogens as a result of VFA accumulation (Oh, 2012). Similarly, one has to keep the effluent ammonia concentration < 200 mg/L, as high free ammonia concentration may be detrimental to the methanogenic activity (Gerardi, 2003; Oh, 2012). Consequently, it is important to control these environmental parameters to ensure optimum operational conditions for the anaerobic system. Additionally, other environmental parameters, such as the oxidation-reduction potential and the concentration of volatile fatty acids, can contribute to the monitoring of anaerobic activity (Gerardi, 2003; Henze *et al.,* 2008). Table 2.8 and 2.9 provide operational conditions required for adequate methanogenic activity and the subsequent production of biogas.

Table 2.8: Operational conditions to be followed for a good anaerobic activity (adapted from Gerardi, 2003; Oh, 2012)

Condition	Units	Optimum range
Alkalinity as $CaCO_3$	mg/L	1500-3000
VFA/Alkalinity	-	<0.3
Gas composition		
Volume percentage of methane	%	65-70
Volume percentage of carbon dioxide	%	30-35
pH		6.5-8
Temperature		
Psychrophiles	^oC	5-25
Mesophiles	^oC	30-35
Thermophiles	^oC	50-56
Hyperthermophiles	^oC	>65
NH_3-N concentration	mg/L	1500-3000
VFAs as acetic acid	mg/L	50-500
Ammonia	mg/L	<200

Table 2.9: Oxidation-reduction and cellular activity (adapted from Gerardi, 2003)

Approximate ORP (mV)	Compound used for the biodegradation of substrate	State	Prevalent operation
>+50	O_2	Oxic	Aerobic
+50 to -50	NO_3^- or NO_2^-	Anaerobic	Anoxic
<-50	SO_4^{2-}	Anaerobic	Fermentation, sulphate production
<-100	Organic compound	Anaerobic	Fermentation, mixed acid production
<-300	CO_2	Anaerobic	Fermentation, methane production

2.7.2 Importance of agitation for the maintenance of methanogenic activity

Gerardi (2003) recommends that mixing of the content of anaerobic bioreactors be considered, as mixing allows for the even distribution of bacteria and nutrients throughout the bioreactor. Furthermore, mixing reduces differentiation in the temperature profile inside the bioreactor and contributes to efficient hydrolysis of substrates, their conversion to organic acids and alcohols by aceto- and acidogens. The advantages of mixing listed by Gerardi (2003) are:

- The elimination or reduction of scum build-up;
- The elimination of thermal stratification and localised pockets of depressed temperatures;
- The rapid dispersion of metabolic products generated during substrate decomposition;
- The dispersion of toxic materials entering the tank; and
- The prevention of grit deposition.

However, not all types of mixing should be applied to anaerobic bioreactors, as mechanical mixing was reported to have a detrimental effect on the biomass of anaerobic reactors (Chernicharo, 2007). Therefore, slow and gentle mixing is usually recommended, as it doesn't negatively impact either biomass formation or disintegration in anaerobic bioreactors. These types of mixing may be induced by (Henze *et al*., 2008):

- The recirculation of biogas produced;
- The recirculation of the bioreactor supernatant as applied in the EGSB; and
- The upward flow of the influent.

It should be noted that the recirculation of biogas produced from anaerobic activity might reintroduce hydrogen sulphide back into the system, thereby increasing its toxicity inside the bioreactor, i.e. souring the digester.

2.8 Prediction of methane production potential in anaerobic digesters

Generally, there is a variety of organic compounds available in wastewater, which makes it difficult to identify and thus quantify the concentration of these compounds individually. Therefore, for quantification purposes, the ability of oxidising agents to oxidise these organic compounds is utilised (Ellis and Evans, 2008), preferably using two standard tests, i.e. BOD and COD (Northcutt and Jones, 2004), which are based on the determination of dissolved oxygen required to oxidise the organic compounds. The BOD_5 is related to the biochemical quantity of dissolved oxygen necessary for aerobic organisms to oxidise the organic matter, which obviously determines biodegradability under aerobic conditions (Borja *et al*., 2008). This BOD_5 value is usually noticeably lower than the COD value, as not all organic compounds are biodegradable with the COD test method relying on chemical oxidation of all organic compounds (Baddour *et al*., 2016). Although the BOD and COD are commonly utilised to determine gross quantities of organic matter in wastewater, total organic carbon (TOC) is sometimes preferred (Evans, 2004), a method based on the evolution of CO_2 due to the incineration of organic matter available in the sample of wastewater (Chernicharo, 2007). It should be noted that TOC is less useful than the BOD_5 and COD.

The organic compounds available in the wastewater can be ranked as biodegradable, partially-degradable, or non-degradable. Biodegradable compounds are easily fermented by anaerobic microorganisms (Chernicharo, 2007), while partially degradable organic compounds, alternatively named polymeric substrates, require a substantial exposure to specialised biomass or by-products of such biomass, in order to be fermentable (Chernicharo, 2007). Lastly, non-biodegradable organic compounds are viewed as inert to microbial action and are thus not degradable, even in anaerobic conditions (Chernicharo, 2007). Therefore, the COD contributing organic matter can be separated into two sub-groups that can be further divided into other sub-groups, as illustrated in Fig. 2.10.

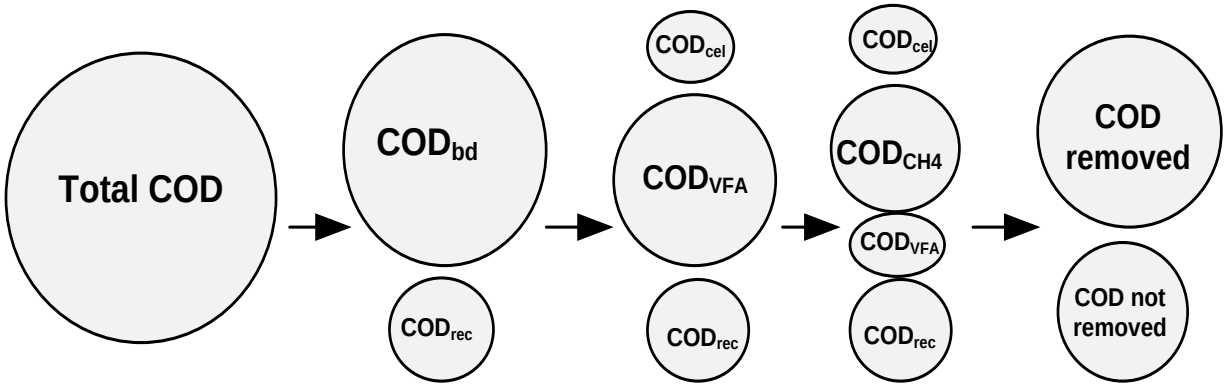


Figure 2.10: Description of the repartition of the total COD in an anaerobic treatment (adapted from Chernicharo, 2007)

From Figure 2.10, the biodegradable COD (COD_{bd}) component represents a way of characterising wastewater treatability, i.e. a definitive component of COD, which can be biologically degraded under anaerobic conditions (Chernicharo, 2007; Gerardi, 2003), with COD_{VFA} representing the fraction of volatile fatty acids obtained from the biodegradable COD, which is partly converted to COD_{CH4}, i.e. a fraction of the influent converted into CH_4. Lastly, the COD_{rec} refers to the non-biodegradable COD, which is a component of organic matter that cannot be degraded by anaerobic microorganisms (Chernicharo, 2007). In the case of a biodegradable compound, $C_nH_aO_bN_d$, its total conversion into methane, carbon dioxide and ammonia by anaerobic organisms can be stoichiometrically defined by the Buswell equation, Eq. 2.1 (Chernicharo, 2007).

$$C_nH_aO_bN_d + \left(n - \frac{a}{4} - \frac{b}{2} + \frac{3d}{4}\right)H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3d}{8}\right)CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4} + \frac{3d}{8}\right)CO_2 + dNH_3 \quad (2.1)$$

In Eq. 2.1, the production of methane is assumed to be the maximum which can be stoichiometrically achieved. Thus, the use of organic matter by other routes of transformation, such as for the production of bacterial biomass, are not taken into account. The COD represents an accurate means of determining the oxidation state of wastewater, and consequently, the quantity of methane that can be generated from it (Henze *et al*., 2008; Alphenaar, 1994). Therefore, a technique of determining the production of methane can be estimated using COD biodegradation in anaerobic reactors, namely the concentration of COD removed from the wastewater, as shown in Equation 2.2 (Chernicharo, 2007).

$$V_{CH_4} = \frac{COD_{CH_4}}{K(t)} \quad (2.2)$$

Where:

V_{CH4} is the volume of methane expressed in litres,
COD_{CH4} is the load of COD removed and turned into CH_4 expressed in gCOD, and
K (t) is the correction factor related to the operational temperature of the reactor expressed in gCOD/L.

This correction factor can be estimated using Eq. 2.3 (Chernicharo, 2007):

$$K\,(t) = \frac{P\,.K}{R\,.T} \quad (2.3)$$

Where:

P is the atmospheric pressure, which is equivalent to 1 atm,
K is the quantity of COD equivalent to one mole of methane (64 gCOD/mol),
R is the universal gas constant equivalent to 0.08206 atm.L/mol.K, and

T is the operational bioreactor temperature expressed in Kelvin (K).

2.9 Biogas treatment and methane collection

2.9.1 Removal of hydrogen sulphide from biogas using uncoated iron oxide mesh

Hydrogen sulphide is a corrosive, flammable, poisonous, and pollutant gas found in biogas. It is produced as a result of the degradation of proteins and sulphur-containing compounds during anaerobic organic matter digestion. It is responsible for the bad odour associated with anaerobic reactors and has a detrimental effect on equipment it may flow through. Thus, it is necessary to scrub this biogas constituent, because it can cause harm to plant personnel, the environment and equipment. Various technologies have been proposed for its removal from biogas, such as water scrubbing, adsorption with zero-valent iron, amine-facilitated processes or simply the use of iron oxide mesh (Al Mamun and Torii, 2015; Magomnang and Villanueva, 2015; Abdel-Hadi, 2008). The latter appears to be a cost-effective process, as reported by Magomnang and Villanueva (2015), with scrubbing efficiency of 95% with regard to hydrogen sulphide removal. This process consists of the adsorption of H_2S from a mixture of gases contained in the biogas generated by a fixed-bed of iron oxide. This fixed-bed adsorbs and oxidises hydrogen sulphide to elemental sulphur, which can be collected for other uses.

2.9.2 Methane collection from a mixture of carbon dioxide and methane

Subsequent to H_2S scrubbing from biogas generated from an anaerobic system, miscellaneous techniques, such as lubricated syringes, volume displacement devices, manometer assisted syringes, pressure manometers or transducers, or low-pressure switch meters can be used to quantify the biogas produced (Parajuli, 2011). Gas chromatography, an essential analytical instrument used for the determination of the composition of biogas; can be used to analyse biogas concentration, thus its constituents due to its high resolution, high sensitivity and good quantitative results it presents (Parajuli, 2011). However, it can be costly to operate than the liquid displacement method, which consists of an arrangement of a tank and a measuring cylinder as illustrated in Fig. 2.11. The mixture of biogas entering the system will be passed through a barrier solution, i.e. a solution of potassium hydroxide or sodium hydroxide, which extracts carbon dioxide from the gaseous phase allowing methane to pass through, as it is not soluble in the barrier solution, thus get collected at the end of the measuring cylinder (Parajuli, 2011). The collection of methane results in the displacement of the barrier solution due to the volume that the biogas occupies; culminating in the periodic estimation of the quantity of methane generated.

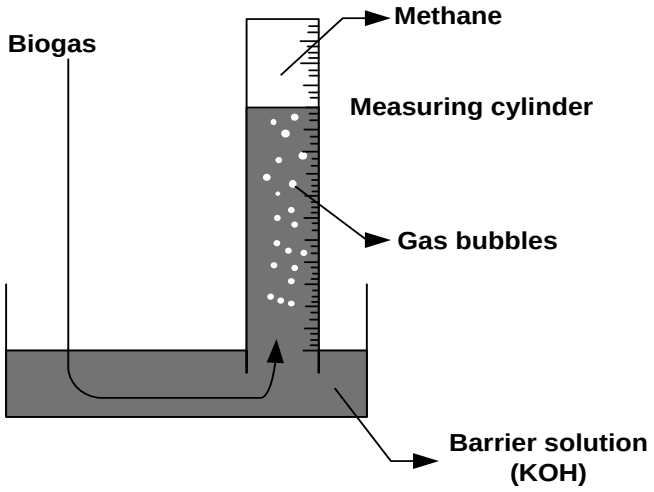


Figure 2.11: Liquid displacement apparatus (adapted from Parajuli, 2011)

2.10 Development and utilisation of a COD balance for anaerobic digesters

In contrast to aerobic systems, there is minimal destruction of elemental COD_{bd} in anaerobic processes, it is simply converted into other forms based on the principle of the conservation of mass (Henze *et al*., 2008). In anaerobic treatment processes, polymeric substances are transformed into simple intermediates which are ultimately transformed into methane, carbon dioxide and other by-products (Oh, 2012; Gerardi, 2003). All the COD entering an anaerobic system is either converted into methane or used in the generation of new bacterial cells with residual COD being released with the effluent (Hulshoff Pol *et al*., 2004). Thus, the COD_{bd} is usually considered as a controllable input variable which can be used to control anaerobic systems; therefore, mass balance generally used in process engineering can be accomplished by utilising COD as an input parameter. There is minimal doubt that the COD_{bd} characterises the important parameters for the evaluation of concentration for a number of by-products formed from wastewater, and as such it can be used to monitor and control the operation of an anaerobic system (Rajakumar *et al*., 2012; Kobya *et al*., 2006; Chernicharo, 2007). For this to be achievable, Eq. 2.4 can be used.

$$COD_{in} = COD_{out} \tag{2.4}$$

For process control purposes, it is assumed that COD_{bd} in an anaerobic system is converted into gaseous, liquid and solid by-products, as shown in Fig. 2.12, which depicts the fate of COD_{bd} in an anaerobic system.

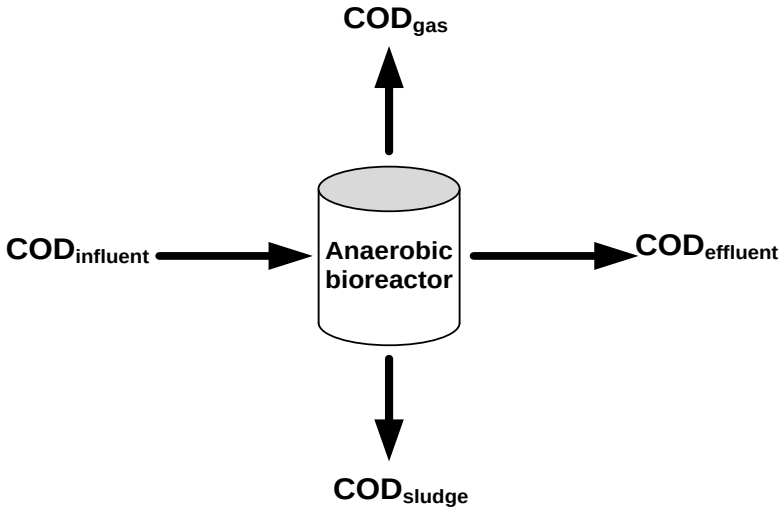


Figure 2.12: Balance of COD in an anaerobic system (adapted from Chernicharo, 2007)

The only by-product, whose COD_{bd} could not be directly accounted for, is the gaseous one. However, by using flow rate, COD_{bd} concentrations, and additional information on the biodegradability of the COD_{bd}, the rate of production of methane can be easily estimated. The combustion of methane yields carbon dioxide, which signifies that, at standard temperature and pressure, 22.4 m^3 of CH_4 requires 2 moles of O_2 (COD_{bd}), which is equivalent to 64 kg of COD_{bd} (Henze *et al*., 2008; Chernicharo, 2007). This means that 1 kg COD_{bd} can theoretically be turned into 0.35 m^3 of methane (Henze *et al*., 2008; Chernicharo, 2007). Moreover, with an approximate composition of $C_5H_7O_2N$, the theoretical COD_{bd} corresponding to 1 kg bacterial VSS can be calculated as 1.42 kgCOD/kgVSS (Chernicharo, 2007). Therefore, the COD_{bd} equivalent of the methane produced, as well as the newly grown bacteria, can be determined. A mass balance can thus be performed to determine whether the influent COD_{bd} corresponds to that in the effluents and by-product streams from anaerobic digesters.

It may transpire that the COD_{bd} balance does not result in stoichiometrically equivalent constituents when comparing between the influent and effluent streams. This can be explained by the loss of electrons during the anaerobic treatment process, whereby losses of oxidizable anions such as SO_4^{2-} and NO_3^- occur (Henze *et al*., 2008). In such cases, either the concentration of electron acceptors should be quantified, and/or all reduced gases should be known. Furthermore, differentiation in COD_{bd} balance might be attributed to entrapment or accumulation of the COD_{bd} in the sludge bed (Chernicharo, 2007). This is common during the biological treatment of wastewater containing fat-or long chain fatty acids, such as PSW (Chernicharo, 2007). However, it is difficult to mathematically estimate such a phenomenon, an area of research which can be explored in further studies.

2.11 Summary

This section provides information relevant to the importance of treating PSW and an overview of its characteristics. Anaerobic digestion is demonstrated as a suitable option for the treatment of PSW. The process offers low cost, along with the advantages of being a process that reduces the plant footprint requirement, the quantity of sludge produced, and even allowing for the production of biogas that contains methane, an energy source. Furthermore, an insight into anaerobic treatment is highlighted through a description of various stages and the parameters to be monitored to ensure a smooth operation. An elaboration on the mechanisms required to implement HRABs and thus an overview of the most popular HRABs and their advantages, as well as challenges associated with the operation of bioreactor systems, was carried out. It is highlighted that HRABs can be further regrouped into up-flow and down-flow configurations, which respectively present advantages as well as disadvantages that are briefly discussed. Finally, the last section suggests two processes pertaining to biogas treatment, through the reduction of hydrogen sulphide and carbon dioxide, for the recovery of methane**.**

CHAPTER 3

PARAMETERS OF THE DESIGN OF THE DEGBR, A NOVEL HIGH-RATE ANAEROBIC BIOREACTOR

Chapter 3 : PARAMETERS OF THE DESIGN OF THE DEGBR, A NOVEL HIGH-RATE ANAEROBIC BIOREACTOR

3.1 Introduction

Various mechanisms have been developed for biomass retention in anaerobic reactors, culminating in the development of HRABs (Henze *et al*., 2008), which provide suitable features in terms of wastewater treatment as well as biogas production. However, some challenges were noticed while using some HRABs in previous studies for the treatment of PSW (Henze *et al*., 2008; Chernicharo, 2007; Evans, 2004; Gerardi, 2003; Basitere *et al*.,2017), these were:

- Requirements associated with the operation of the three-phase separator for HRABs using an up-flow configuration;
- Entrapment of biogas within the granular bed;
- Head loss in HRABs using a down-flow configuration;
- Sludge washout; and
- The weak dispersion of organic matter and toxicants in static beds.

Therefore, this chapter is devoted to the provision of information on a novel high-rate anaerobic digestion configuration design, which addresses the aforementioned challenges by identifying the key parameters associated with such operational inadequacies and using these to inform a redesign with the aim of circumventing the challenges identified.

3.2 Design considerations

The design of the DEGBR was intended to alleviate the challenges mentioned in section 3.1 and to facilitate a smooth operation during the treatment of PSW for improved efficiency of HRABs. Thus, the following subsections address these challenges and their mitigation.

3.2.1 Bioreactor configuration

The most popular HRABs are the UASB and the EGSB (Lim, 2009; Henze *et al*., 2008), which consist of an up-flow configuration whereby the feed is introduced at the bottom of the bioreactor and the product is collected at the top through a three-phase separator that enables the separation of the gas, liquid and solids (Henze *et al*., 2008; Chernicharo, 2007). The primary purpose of the three-phase separator is to facilitate the collection of the biogas and

the bioreactor's effluent while retaining the solids (anaerobic biomass) within the bioreactor (Chernicharo, 2007). However, this system tends to be difficult to operate, as it requires a pressure build-up from the collection of biogas on top of the bioreactor to operate efficiently, contributing, unfortunately, to the drainage of a portion of the biogas in the effluent (Basitere *et al*., 2016; Chernicharo, 2007). Furthermore, the expansion of the granular bed due to the biogas elevation culminates in the washout of solids, via flotation to the top of the bioreactor, resulting in a sludge washout (Henze *et al*., 2008). To circumvent this, a down-flow configuration can be used, as suggested by the group of researchers from Iowa State University who designed the SGBR (Evans, 2004). In a down-flow configuration, the wastewater is fed from the top, the effluent is collected at the bottom, and the biogas is collected at the top of the bioreactor through a different stream. This configuration does not require the use of a three-phase separator and improves the retention of the biomass within the system through the use of an appropriate underdrain system whereby only the effluent may exit the system (Evans, 2004; Oh, 2012). Fig. 3.1 illustrates, from left to right, the UASB, the EGSB and the SGBR. It can be deduced that the down-flow configuration provided for by the SGBR design allows for a simple operation that does not require a three-phase separator to separate the liquid, solid and biogas, with a simplified underdrain system that enables long SRTs while permitting permeation of the bioreactor effluent. However, the underdrain system reduces the hydraulic pressure (as does the head loss through the static granular bed) at the bottom of the reactor (Evans, 2004). Furthermore, challenges related to clogging of the underdrain system due to interstitial retention of sloughed-off anaerobic granules between the solid materials constituting the underdrain system, were also reported (Evans, 2004; Oh, 2012).

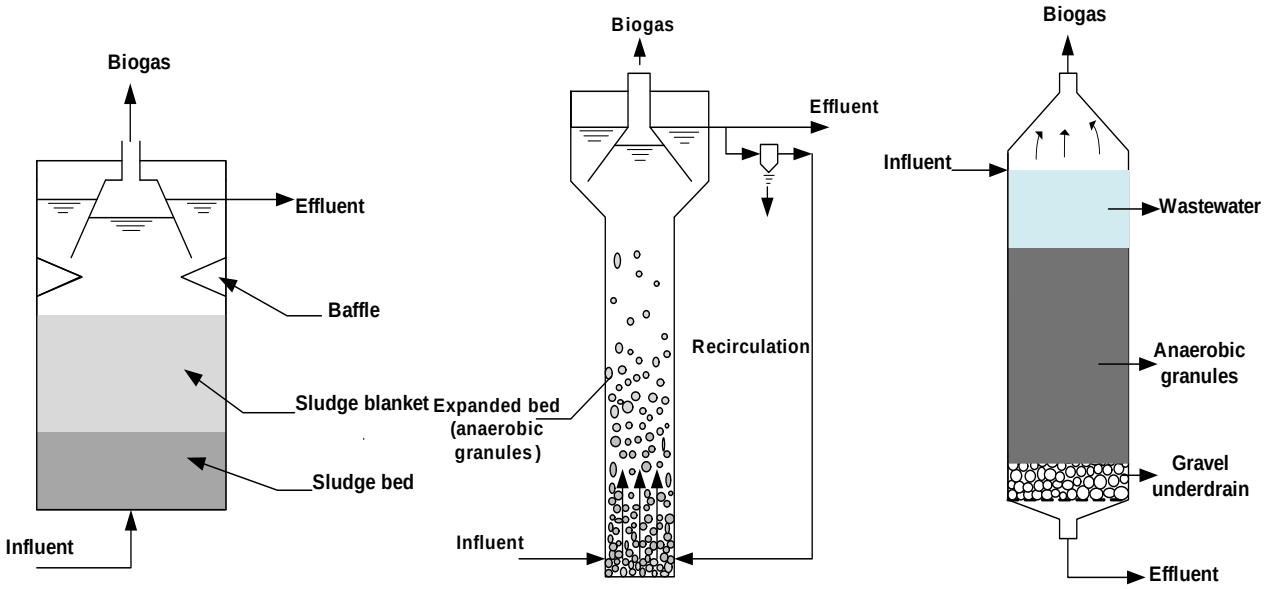


Figure 3.1: From left to right, the UASB, the EGSB and the SGBR

3.2.2 Evaluation of head loss across different sections of the bioreactor

The down-flow configuration offers probable outcomes associated with the three-phase separator (Evans, 2004). However, the use of an underdrain system to retain the anaerobic biomass within the bioreactor induces hydraulic head loss in the system (Evans, 2004; Oh, 2012). Fig. 3.2 depicts the variation of pressure and thus the head loss within a down-flow anaerobic bioreactor configuration (the SGBR) incorporating a static bed. The system is not open to the atmosphere and does not allow dissolution of oxygen into the system. Therefore, there is minimal atmospheric pressure acting on the system. It can be observed that there would be a build-up of pressure from the top to the bottom of the bioreactor (as the pressure of the biogas collected on top of the bioreactor can be negligible). However, this pressure is firstly lost through the granular bed and further through the underdrain system, culminating in the reduction of the head loss of wastewater within the system. This variation and the accompanying reduction in pressure within the bioreactor is illustrated in Fig. 3.2:

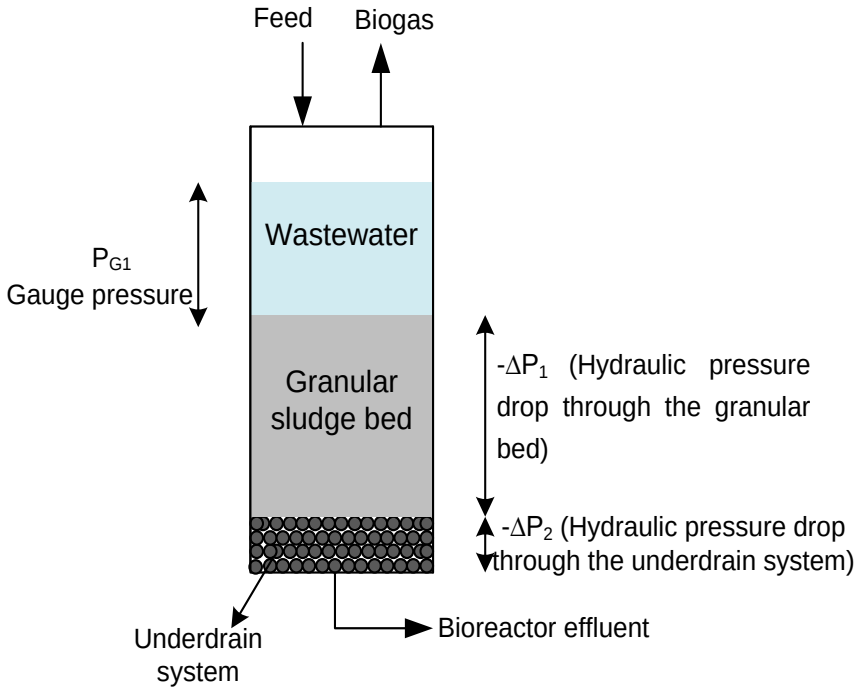


Figure 3.2: **Hydraulic head variation in a SGBR**

3.2.2.1 Head loss through the granular bed

The evaluation of the pressure drop across a system begins with the characterisation of the solid particles or the solid matrix that induces this drop. Furthermore, the pressure drop across a packed bed is assumed to be related to the bed height, the fluid velocity, the packing diameter, the wastewater's viscosity and density, as well as the sphericity and porosity of anaerobic granules (Holdich, 2002). It has been reported (Evans, 2004; Mu *et al*., 2006) that

the anaerobic granules have a spherical shape, with a diameter ranging from 0.06 to 0.50 cm, and can thus be separated into three groups:

- Large granules with a size varying from 0.35 to 0.50 cm,
- Medium granules with a size varying from 0.17 to 0.35 cm, and
- Small (fine) granules with a diameter varying from 0.06 to 0.17 cm.

Various correlations have been developed to evaluate the pressure drop across spherical or non-spherical granular sludge (Yang, 2013; Holdich, 2002; Gibilaro; 2001; Leva *et al*., 1951). Darcy's law (1856) provides a semi-empirical correlation that describes the wastewater flow in packed bed for a single-phase transportation under laminar flow conditions, and it is expressed by Eq. 3.1 (Holdich, 2002).

$$\frac{\Delta P}{L} = \frac{\mu}{k} . \frac{dV}{dt} . \frac{1}{A} \tag{3.1}$$

Where L is the height of the packing material, ΔP the change in pressure, μ the viscosity of the liquid, k the permeability of the packed bed, V the volume flowing during the time t, and A the cross-sectional area.

The superficial velocity (v_o) may be introduced in Eq. 3.1, as it is given by the ratio of the flow rate of the liquid (wastewater) through the empty volume that the packing material will occupy (Holdich, 2002; Yang, 2013), see Eq. 3.2.

$$v_o = \frac{Q}{A} = \frac{dV}{dt} . \frac{1}{A} \tag{3.2}$$

Thus, Eq. 3.1 can be transformed into Eq. 3.3.

$$\frac{\Delta P}{L} = \frac{\mu}{k} . v_o \tag{3.3}$$

Whereby the permeability is a property of the packed bed and with the Darcy unit being equivalent to 9.87 x 10^{-9} cm^2 (Yang, 2013). Thus, as permeability is an important parameter, it has to be quantitatively determined for the pressure drop through the granular bed. The Darcy model signifies that the pressure gradient ($\Delta P/L$) is proportional to the superficial velocity of the fluid if the resistance, expressed as the ratio of the viscosity to the permeability, remains constant (Orudu *et al*., 2012). However, it is hypothesised that, when the flow becomes turbulent as a result of an increase of the superficial velocity, the pressure drop will increase at a higher rate than the superficial velocity (Zeng and Grigg, 2006). Thus, there would be a deviation from the linear expression deduced by Darcy's model at high flow rates, which is also referred to as non-Darcy flow, inertial flow or turbulent flow (Orudu *et al*., 2012). There have been numerous attempts to amend Darcy's law. For example, Forchheimer (1901) included a

second order velocity term to the Darcy model to express the microscopic inertial effects caused by the acceleration and the deceleration of flow through the packed-beds (Orudu *et al*., 2012; Zeng & Grigg, 2006). This model is known as the Forchheimer equation and it is given by Eq. 3.4.

$$\frac{\Delta P}{L} = \frac{\mu v_0}{k} + \beta \rho_F v_0^2 \tag{3.4}$$

Where β is the non-Darcy coefficient and ρ_F is the density of the fluid.

The Forchheimer model was further developed by Brinkman, who included a macroscopic shearing parameter between the pore walls and the fluid by modifying the model to include a second-order derivative to describe the velocity profile to the original Darcy model (Zeng and Grigg, 2006). The Brinkman model is given by Eq. 3.5:

$$\frac{\Delta P}{L} = \frac{\mu v_0}{k} - \mu \left(\frac{\partial^2 v_0}{\partial Y^2} + \frac{\partial^2 v_0}{\partial Z^2} \right) \tag{3.5}$$

Where *X*, *Y* and *Z* are perpendicular directions

However, the Brinkman model is not widely used due to the negligible change of velocity profile across pores within the porous media, as the pore diameter is often negligible in most porous media used for the underdrain in anaerobic digesters (Zeng & Grigg, 2006).

Subsequently, numerous correlations have been developed to quantitatively determine the pressure loss across a packed bed. The other correlations developed thereafter were the Blake, the Carman and Kozeny correlations and the Ergun correlation.

3.2.2.1.1 Blake's correlation

Blake (1922) suggested the use of a modified dimensionless group that includes the voidage (*e*) of a packed bed and the interstitial velocity instead of the superficial velocity (Yang, 2013). The interstitial velocity is given by Eq. 3.6:

$$v = \frac{v_0}{e} \tag{3.6}$$

Where *e* is the voidage, which, in a porous medium, can be deduced by the difference between the solid fraction of a packed bed from unity (Holdich, 2002). Furthermore, the porosity of a packed bed of solid materials can be determined using Eq. 3.7 (Yang, 2013).

$$\epsilon = \frac{Total\ volume - Volume\ of\ solid}{Total\ volume} \tag{3.7}$$

However, according to Mu *et al*. (2006), the porosity of an active granular bed can be determined by Eq. 3.8.

$$\varepsilon = 1 - \frac{6fW_d}{\pi\rho_{BC}d^3} \tag{3.8}$$

Where *f* is the ratio of the wet mass to the dry mass of the granules, W_d is the dry mass of the granules, *d* is the granule diameter and ρ_{BC} is the density of the bacterial cells.

The Reynolds number and friction factor suggested by Blake are given by Eq. 3.9 and Equation 3.10, respectively (Yang, 2013).

$$(Re)_B = \frac{v.d_P.\rho_F}{\mu(1-\varepsilon)} \tag{3.9}$$

$$f_B = \frac{\Delta P}{L}.\frac{d_P}{\rho_F {v_0}^2 g}.\frac{\varepsilon^3}{(1-\varepsilon)} \tag{3.10}$$

Where d_P is the diameter of the particles of the packing material, ρ_F the density of the fluid, μ the viscosity of the fluid, *g* the gravitational coefficient and *L* the height occupied by the packing constituents of the bed.

These models and correlations resulted in the development of Eq. 3.11, that may be used to determine the pressure drop (Yang, 2013).

$$\frac{\Delta P}{L} = \frac{2f_B\rho_F {v_0}^2}{gd_P} \tag{3.11}$$

3.2.2.1.2 The Carman and Kozeny correlations

Carman (1937) and Kozeny (1927) also studied the behaviour of different fluids through various packing materials and found that the friction losses of fluids can be determined using Eq. 3.12 for porosities of 0.26 - 0.89 (Yang, 2013; Gibilaro, 2001).

$$f = \frac{90(1-\varepsilon)^2}{\varepsilon^3 Re_P} \tag{3.12}$$

Where R_{eP} is the Reynolds number determined from the particle size, as given by Eq. 3.13 (Yang, 2013; Gibilaro, 2001; Holdich, 2002).

$$Re_P = \frac{v_o.d_P.\rho_F}{\mu(1-\varepsilon)} \tag{3.13}$$

Eq. 3.13 can thus be applied to different packing material shapes, provided that their surface can be easily determined, such that the particle diameter can be determined from Eq. 3.14 (Yang, 2013).

$$d_P = \frac{6V_P}{S_P} \tag{3.14}$$

Where V_P is the particle volume and S_P is the surface of the particle.

Using the pipe flow analogy, which entails that a packed bed is similar to a group of parallel and identical channels, Kozeny derived Eq. 3.15 that can be used to determine the pressure drop across a granular bed (Yang, 2013).

$$\frac{\Delta P}{L} = \frac{180(1-\varepsilon)^2 \mu v_0}{g\varepsilon^3 d_P^2} = \frac{5(1-\varepsilon)^2 \mu v_o}{g\varepsilon^3 (\frac{V_P}{S_P})^2} \quad (3.15)$$

However, the Kozeny approach is limited to the creeping flow range, as beyond this range, empirical models must be used, such as the one suggested by Ergun (Yang, 2013; Gibilaro, 2001).

3.2.2.1.3 Ergun correlation

Ergun correlation (1952) is widely used as a semi-empirical correlation for determining the pressure drop through a packed bed consisting of both regular and irregularly-shaped packing materials (Yang, 2013). The Ergun correlation can be applied to any flow type, i.e. laminar to turbulent flow regimes. It was developed by modifying the Carman-Kozeny correlation for laminar flow, and supplementing it with the Burke-Plummer correlation developed for fully-turbulent flows (Yang, 2013). Thus, the Ergun correlation can be used for various fluids and packing materials. However, it has limitations, as it doesn't predict the pressure drop after the incipient point of fluidisation, as the bed expansion results in changes of packed bed voidage (Yang, 2013). The Ergun correlation is expressed by Eq. 3.16 (Yang, 2013; Gibilaro, 2001).

$$\frac{\Delta P}{L} \cdot \frac{g d_P \emptyset}{2\rho_F {v_0}^2} \cdot \frac{\varepsilon^3}{(1-\varepsilon)} = 75\frac{(1-\varepsilon)}{\emptyset Re_P} + 0.875 \quad (3.16)$$

Where Ø is the sphericity factor that can be substituted with the particle diameter whereby the diameter of such solid particles can be estimated or quantified using numerous equivalent-diameter quantification techniques, such that Eq. 3.17 can be used to assess a pressure drop across a packed bed (Yang, 2013; Gibilaro, 2001).

$$\frac{\Delta P}{L} = \frac{150\mu v_0}{d_P^2}\frac{(1-\varepsilon)^2}{\varepsilon^3} + \frac{1.75\rho_F v_0^2}{d_P}\frac{(1-\varepsilon)}{\varepsilon^3} \quad (3.17)$$

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

3.2.2.2 Permeability of a packed-bed

Wastewater is usually treated in anaerobic reactors under laminar flow conditions; as such, the flow is at low Reynolds numbers (laminar flow) in order to adverse the effects thus

detrimental conditions for the anaerobic biomass within a bioreactor (Pol *et al*., 2004). From the previous sub-sections (3.2.2.1.1 to 3.2.2.1.3), it was observed that the permeability of a packed bed is an important parameter used for the pressure drop assessment, or to evaluate the flow regime of the wastewater through the packed bed. The permeability can thus be determined by rearranging the Kozeny-Carman correlation to derive Eq. 3.18 (Holdich, 2002).

$$\frac{r^2}{k} = \frac{75(1-\varepsilon)^2}{2\varepsilon^3} \tag{3.18}$$

Where *k* is the permeability and *r* the radius of particles within the packed-bed.

Eq. 3.18 enables reliable estimation of the permeability, provided that the porosity is 0.26 to 0.80 (Holdich, 2002).

3.2.2.3 Recourse associated with granular bed expansion to circumvent head losses through the granular bed of an anaerobic digester

The head loss through a static granular bed is a challenge in down-flow anaerobic bioreactors, as granules are assumed to consist of small spherical particles that are denser than the wastewater usually fed to the bioreactors (Evans, 2004). They therefore settle and aggregate at the bottom of the bioreactor because of gravity, reducing the pressure exerted by the wastewater flowing through it, culminating in the creation of dead-zones or short-circuiting in the granular bed. One way to circumvent such a challenge is to fluidise the granular bed, so as to alleviate the pressure drop by increasing the porosity of the granular bed (Lim, 2009), increasing the surface contact area between the anaerobic biomass and the organic matter in order to facilitate the reduction in the biogas entrapment and thus biogas release from the granular bed.

Fluidisation consists of pumping the wastewater or biogas upwards through a packed bed at a rate that is sufficient to counteract the pressure in the bed in question, so as to impart high porosity characteristics (Yang, 2013; Gibilaro, 2001; Holdich, 2002). The determination of the minimum fluidising velocity, which is the minimum velocity required to fluidise a packed bed, is governed by the principle that the pressure drop across the granular bed must be equivalent to the effective weight per unit area of the biomass at the point of emerging fluidisation (Holdich, 2002; Yang, 2013). This can be expressed by Eq. 3.19 (Holdich, 2002).

$$\Delta P = (\rho_P - \rho_F)(1-\varepsilon)gL \tag{3.19}$$

By using the Ergun correlation to quantitatively determine the pressure drop across the packed bed, the minimum fluidising velocity can be quantified using Eq. 3.20 (Yang, 2013; Gibilaro, 2001).

$$\frac{\Delta P}{L} = \frac{150\mu v_{mf}}{d_P^2}.\frac{(1-\varepsilon)}{\varepsilon^3} + \frac{1.75\rho_F v_{mf}^2}{d_P}.\frac{1}{\varepsilon^3} = g(\rho_P - \rho_F) \quad (3.20)$$

Where v_{mf} is the minimum fluidising velocity required.

The fluidisation process can be described as shown in Fig. 3.3, whereby it can be observed that the bed remains fluidised after reaching the minimum fluidising velocity (Gibilaro, 2001), provided such a velocity is maintained. This superficial velocity can thus be increased further to accentuate the fluidisation while maintaining the same pressure drop. The other characteristic (see Figure 3.3) is the unrecoverable pressure loss that causes a deviation from the idealised relationship between the pressure drop and the superficial velocity. This deviation can be estimated by Equation 3.21 (Gibilaro, 2001).

$$\Delta P = \Delta p - \rho_F Lg = (\rho_P - \rho_F)(1-\varepsilon)Lg \quad (3.21)$$

Where Δ_p is the total pressure drop across the bioreactor length L, while the product $\rho_F Lg$ represents the energy irrevocably dissipated from the momentum of the wastewater, dissipated as heat, due to the frictional interaction between the wastewater and the biomass (Gibilaro, 2001; Holdich, 2002).

For effective bioreactor designs, the anaerobic granular bed is supported by a denser underdrain system that contributes to the collection of bioreactor effluent free of biomass, while allowing long SRTs within the system. Due to its densification, this underdrain system normally generates higher pressure drops than the one experienced across the granular biomass bed; therefore, culminating in the requirement for a higher energy or velocity to fluidise the anaerobic granular bed through the underdrain, particularly when excessive EPS is embedded in the pores of the underdrain material. To circumvent this eventual loss of energy, a wastewater distribution system consisting of small pores oriented upwards, placed on top of the underdrain system, can facilitate the fluidisation of the anaerobic granular bed without pumping the wastewater through the underdrain system, as illustrated in Fig. 3.4. Thus, this wastewater distribution system can be connected to a recycle stream that will collect the wastewater from the upper part of the bioreactor and redistribute it back to the bottom of the bioreactor counter-currently, thus resulting in an enhanced contact between the organic matter and the biomass, de-clogging the system underdrain. However, it should be noted that the wastewater distribution system cannot cover the whole surface area of the granular bed if one is to allow the effluent from the bioreactor to exit from the anaerobic system, as illustrated in Fig. 3.4. Thus, the term “expansion” is preferred to describe the operation conducted inside the bioreactor, as some parts of the granular bed will not be fluidised, but will be moved from their previous position to create a mixing pattern within a portion of the granular bed.

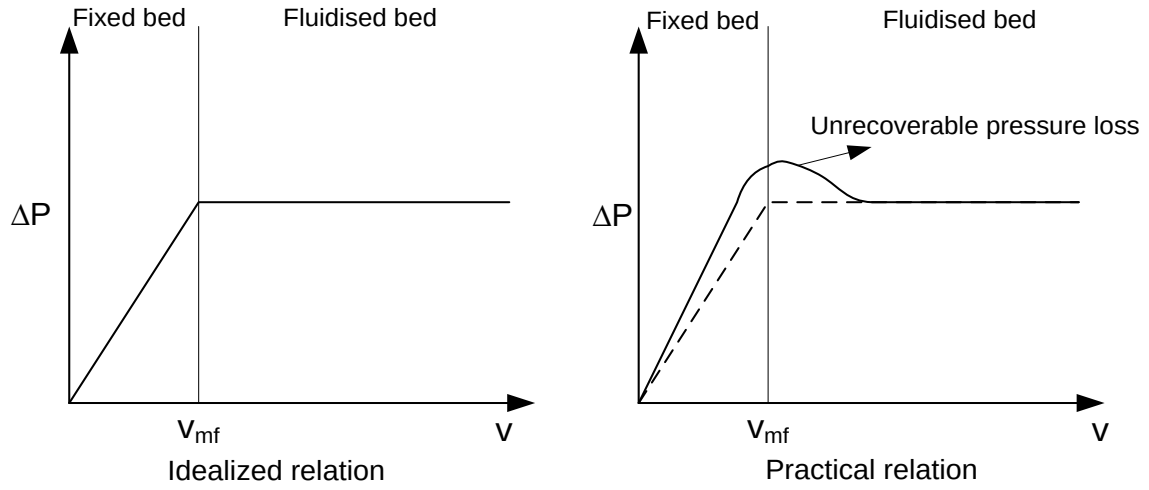


Figure 3.3: Diagrams of the variation of the pressure drop with the superficial velocity across a packed bed (adapted from Gibilaro, 2001)

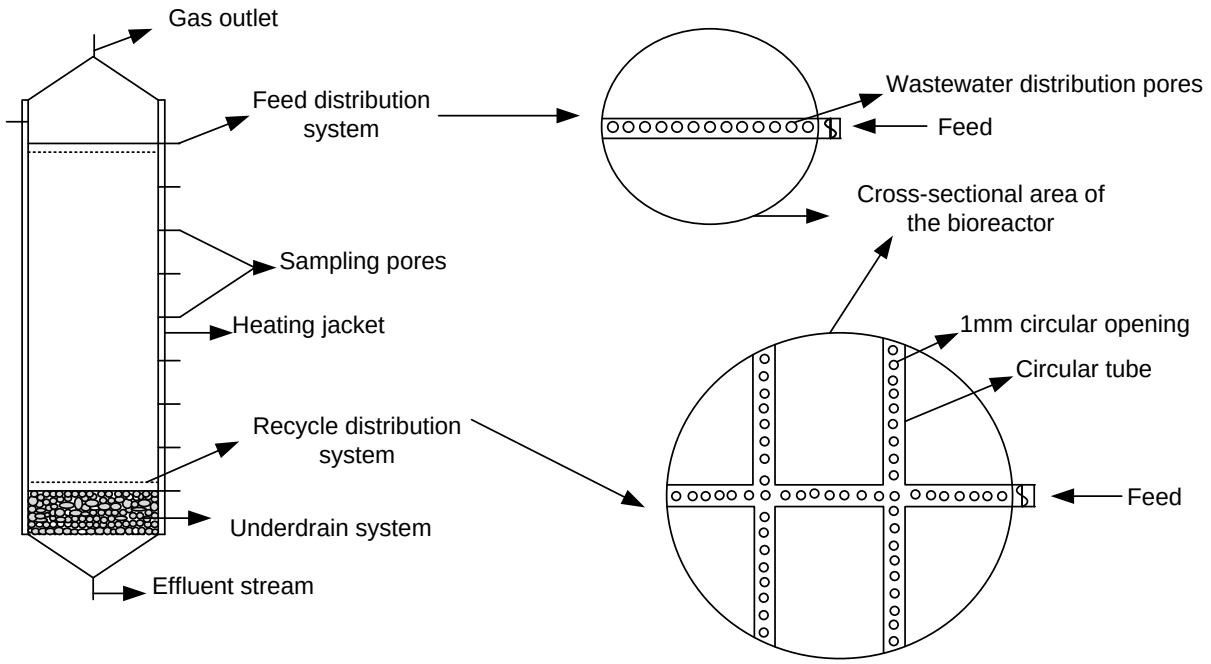


Figure 3.4: Configuration of the DEGBR

3.2.2.4 Recourse to bed expansion to alleviate gas entrapment in the granular bed

Biogas is generated in a bioreactor from the biomass slurry, consisting of anaerobic granules (Henze *et al*., 2008; Evans, 2004). The anaerobic granules consist of the biomass required for digesting the organic matter fed into the bioreactor, culminating in the production of biogas at the end of a series of metabolic processes (Henze *et al*., 2008). The production of biogas bubbles depends on various factors, such as a good distribution of the organic matter to the

anaerobic granules, and the required environmental conditions (pH, temperature, absence of toxicants, etc.). The evolution is seen when excess pressure inside a newly formed bubble overcomes the external pressure acting on it, and it then rises through the granular bed (Gerardi, 2003, Parajuli, 2011). There are two main external forces acting on a biogas bubble (Jiang *et al*., 2016; Parajuli, 2011):

- The external pressure resulting from the gauge pressure (P_G) at a given height, and
- The excess pressure resulting from surface tension, which can be estimated by the Young-Laplace equation.

The Young-Laplace equation can also be used to determine the exerted compression pressure on an anaerobic granule, as determined by Eq. 3.22 (Jiang *et al*., 2016).

$$P_{exc} = \sigma\left(\frac{1}{R_1} + \frac{1}{R_2}\right) \tag{3.22}$$

Where P_{exc} is the excess pressure or the disjunction pressure across the bubble interface, R_1 and R_2 are principal radii of curvature and σ is the surface tension. By assuming that biogas bubbles have a spherical shape, Eq. 3.22 can be simplified to Eq. 3.23 (Jiang *et al*., 2016; Parajuli, 2011).

$$P_{exc} = \frac{2\sigma}{R} \tag{3.23}$$

Where R is the radius of the spherical bubble.

The other external pressures exerted on biogas bubbles at a given height in the bioreactor can be estimated by Eq. 3.24 (Parajuli, 2011).

$$P_{ex} = P_{atm} + \rho g h \tag{3.24}$$

However, under anaerobic conditions, there is minimal atmospheric pressure acting on the system, and only a portion of the biogas produced can accumulate at the top of the bioreactor. Thus, the total external pressure acting on the biogas bubble is given by Eq. 3.25.

$$P_{T,ex} = P_{exc} + \rho g h \tag{3.25}$$

Consequently, a bubble can only be released from the bioreactor bed when the pressure inside the bubble exceeds the total external pressure, usually because of the formation of a crater through the static granular bed (Jiang *et al*., 2016). Eq. 3.25 also shows that the pressure exerted on the biogas bubbles increases with depth, including the packed granular bed height, which generates a resistance to the elevation of the biogas bubbles through an unrecoverable pressure drop across the granular bed, resulting in biogas entrapment, as illustrated in SGBRs in Figure 3.5. This entrapment of biogas can also be alleviated by fluidising the bed through a

fluidisation process, as described in sub-section 3.2.2.1.5 (Bhatti, 1995). Thus, the expansion of the granular bed not only provides the advantages of improving the distribution of organic matter, reducing head losses, and dispersing toxicants, but contributes to the improvement of the recovery or processed biogas from the bioreactor.

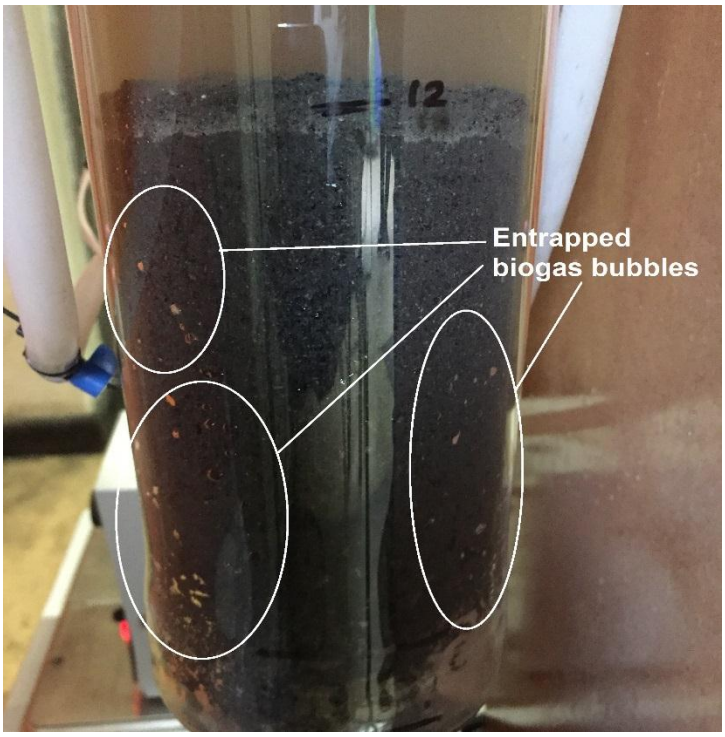


Figure 3.5: Entrapped biogas bubbles in a static granular bed

It appears that the entrapment of biogas within the granular bed is mostly related to the effect of external pressure acting on the biogas bubbles. Thus, the fluidisation of the anaerobic granules, therefore the granular bed can accentuate this entrapment effect through the increase of pressure exerted on the biogas bubbles and the decrease of the porosity of the granular bed. The decrease of the porosity of the granular bed, combined with the increase of the size of the anaerobic granules, was reported by Mu *et al.* (2006) who evaluated their permeability as the CH_4-producing granules were changing in size when the bioreactor was operated for a prolonged period. This change in anaerobic granule size can be induced by an improved organic matter distribution through the bed expansion; requiring a consequent increase of the up-flow velocity to accommodate the change, allowing for effective biogas collection. However, a very high up-flow velocity can be detrimental to anaerobic granules, culminating in sloughing. The highest up-flow velocity reported for an EGSB reactor was 6 m/h (Lim, 2009), and 2 m/h for the UASB reactor (Pol *et al.*, 2003). Taking into consideration that the fluidisation process in the case of the DEGBR designed and used for this study doesn't directly affect the biomass of the entire anaerobic granular bed, higher velocities can be assessed.

3.2.2.5 Head loss through the underdrain system: influence of configuration

In HRABs arranged in a down-flow configuration, made possible by the use of an underdrain system that significantly contributes to retention of anaerobic granules within the bioreactor, and exacerbates hydraulic pressure lost through the underdrain system, the result is kinetic energy losses due to shock losses through the packing materials. Thus, prior to deciding on which packing material to use for the underdrain system, the pressure drop through such systems should first be evaluated to determine their permeability.

Various packing materials could be used for retaining the anaerobic biomass within the bioreactor. These should be denser than the anaerobic granules and possess adequate porosity and a permeability that would allow suitable flow of the effluent while retaining the anaerobic biomass within the bioreactor, while allowing for a long SRT, as discussed in sections 2.6.1 to 2.6.4. The packing materials may come in different sizes and shapes that should be considered prior to determining their permeability, thus allowing for quantification of the pressure drop that they can induce for a given height of a packed bed (Yang, 2013). Thus, the first step prior to the determination and selection of suitable packing material, is to characterise them (see Chapter 5).

3.2.2.5.1 Characterisation of the particles constituting the underdrain system

Particle size can be understood as a linear dimension that characterises a particle (Holdich, 2002). However, only spheres can be characterised by a single dimension, which is their diameter, as particles with other shapes may require more than one dimension for characterisation (Yang, 2013). Furthermore, correlations developed for determining the pressure drop and the permeability of packing beds were developed under the assumption that particles have a spherical shape (Yang, 2013; Holdich, 2002). Thus, it is important to relate the dimensional properties of these non-spherical particles to a single linear dimension, i.e. their equivalent diameter, which can be used as the equivalent diameter of a sphere, which can be a representative of the particle, as they may have the same volume. The equivalent diameter can be determined using different methods such as the surface-equivalent sphere diameter, the volume-equivalent sphere diameter, the hydrodynamic equivalent diameter, the Stokes diameter, the sieve diameter, the projected area diameter, the laser diffraction diameter or the volume-surface diameter correlations (Pabst & Gregorova, 2007).

3.2.2.5.1.1 The surface-equivalent sphere diameter

The surface-equivalent sphere diameter is given by Eq. 3.26 (Yang, 2013).

$$D_{surf} = \left(\frac{6}{\pi} S_P\right)^{\frac{1}{2}} \quad (3.26)$$

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

3.2.2.5.1.2 The volume-equivalent sphere diameter

Equation 3.27 gives the correlation that can be used to determine the volume-equivalent sphere diameter (Pabst & Gregorova, 2007).

$$D_{Vol} = \left(\frac{6}{\pi} V_P\right)^{\frac{1}{3}} \quad (3.27)$$

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

3.2.2.5.1.3 The hydrodynamic equivalent diameter

The hydrodynamic equivalent diameter represents 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 & Gregorova, 2007). It can be determined by the Stokes-Einstein relation (see Eq. 3.28) (Pabst & Gregorova, 2007).

$$D_H = \frac{KT}{3\pi\mu D_{translation}} \quad (3.28)$$

Where T is the absolute temperature, K the Boltzman constant and μ the viscosity of the liquid medium.

3.2.2.5.1.4 The Stokes diameter

The Stokes diameter corresponds to the diameter of a sphere possessing the same settling velocity as a particle settling in the same fluid under laminar conditions (Pabst and Gregorova, 2007). This diameter can be determined from the Stokes relation and is given by Eq. 3.29 (Pabst and Gregorova, 2007).

$$D_S = \sqrt{\frac{18\mu v}{(\rho_s - \rho_L)g}} \quad (3.29)$$

Where v is the final settling velocity, μ the viscosity of the liquid, g the gravitational acceleration, ρ_s the density of the solid particle and ρ_L the density of the pure liquid medium in which the particle settles.

3.2.2.5.1.5 The sieve diameter

The sieve diameter corresponds to the diameter of a spherical particle passing through the same opening of a sieve of defined mesh (Yang, 2013).

3.2.2.5.1.6 The laser diffraction equivalent diameter

This diameter corresponds to the diameter of a sphere producing the same electronic response from an optical signal (diffraction pattern) when the particle geometry is determined (Yang, 2013; Pabst & Gregorova, 2007).

3.2.2.5.1.7 The volume surface diameter

The volume surface diameter, also called the Sauter diameter, is determined from the ratio of the cube of the volume-equivalent diameter to the square of the surface-equivalent diameter as expressed by Eq. 3.30 (Pabst & Gregorova, 2007).

$$D_{SV} = \frac{D_V^3}{D_S^2} \tag{3.30}$$

However, some disagreements arise when characterising particles using different particle characterisation methods. Each method determines the equivalent diameter using a distinct physical principle. Furthermore, 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 orient themselves towards their smallest dimension to pass through the openings of the sieve, affecting the reliability of the determination of the equivalent-diameter (Horiba Scientific, 2016).

3.2.2.5.2 Parameters of the selection of the support materials for the underdrain system

The particle characterisation is usually followed by the determination of the permeability using various correlations, such as the ones provided from section 3.2.2.1 to 3.2.2.2. The evaluation of the porosity and the permeability enables the selection of the most suitable packing material to be used as the underdrain system of the bioreactor. However, apart from the intensity of the pressure drop across an aggregate of packing materials, other parameters also play an important role in the selection of support materials (Holdich, 2002), such as:

- The affordability of the material,
- The availability of the material, and
- The inertness of the material to mechanical shearing and microbial attack.

3.3 DEGBR development

The total volume of the DEGBR depends on various parameters, such as the quantity of anaerobic granules inoculated into the system, the headspace reserved for accommodating the elevation of biogas from the anaerobic system, the volume occupied by the PSW, as well

as the hydraulic retention required. However, the DEGBR system was conceptualised to be operating at steady state i.e. with minimal variation in hydraulic accumulation within the system, which differs from the HRT, as the same quantity of wastewater that will be pumped into the system will also be collected at the bottom, making the HRT a parameter directly proportional to the working volume of the system and indirectly proportional to the influent flow rate. Furthermore, the expansion of the granular bed requires a convenient height to accommodate the operation desired. A similar expansion procedure and/or assessment as used for the EGSB, whereby a height-to-width ratio of 4 to 5 of the bioreactor was used to accommodate the granular expansion, with a down-flow configuration, was implemented for the DEGBR, as highlighted in section 3.2. This was to minimise the washout of anaerobic granules through the three-phase separator. For a down-flow configuration, it was reported by Lim (2009) that the SGBR provided a better performance at a height-to-width ratio of 7 than at a height-to-width ratio of 2 for the same working volume; a statement that reiterates the advantage of operating with a bioreactor having a height-to-width ratio above 4. Taking into consideration the requirement of having a suitable height-to-width ratio for accommodating the granular bed expansion, the height-to-width ratio of 7 was selected, which included the height occupied by the packing material.

Similar to numerous HRABs, the DEGBR was designed to have a reduced footprint when compared with other anaerobic treatment systems, while facilitating the treatment of wastewater with high OLRs. It consists of a cylindrical bioreactor, as illustrated in Fig. 3.4, in which openings across its length allow for the sampling of the bioreactor's content at different heights, as well as creating a recycle stream, and the collection of small granules when implementing a backwash operation when the need is required (de-clogging of the underdrain system). The conical component at the bottom of the bioreactor not only serves for collecting the effluent but also allows for suitable permeability of the system through the observation of the level of effluent resting on that part of the bioreactor, as the system was always maintained under steady-state conditions. A heating jacket surrounded the surface area of the bioreactor to maintain the system under a selected suitable temperature. Two hydraulic distribution systems were installed in the bioreactor, as illustrated in Fig. 3.4. The top cone distributes the feed downward across the bioreactor width to prevent channelling, while the bottom one distributes the wastewater from the recycle stream upwards to induce the expansion of the granular bed in order to improve the distribution of the wastewater, and thus the organic matter, across the system. Lastly, the opening at the top of the bioreactor serves to collect the biogas produced from the anaerobic activity of the granular sludge within the bioreactor.

3.4 Summary

The retention of the granular biomass for a long SRT remains a critical operational requirement for the development of HRABs. Various challenges during the operation of some HRABs have been reported in several studies (Evans, 2004; Oh, 2012; Lim, 2009; Henze *et al*., 2008). These challenges were adequately addressed and discussed in this section by the development of a new configuration for HRABs, to enable long SRTs while improving the wastewater distribution, toxicant dispersion and alleviation of biogas entrapment within the bioreactor. It is demonstrated in this section that hydraulic head losses across the anaerobic granular bed and the underdrain system induce additional challenges, such as sub-optimal collection of the effluent, the creation of dead zones and short-circuiting, as well as weak distribution of wastewater being treated. This also demonstrates that static beds are inconvenient for HRABs operation, and cannot be recommended for the treatment of influents with a high OLR.

CHAPTER 4

MATERIALS AND METHODS

Chapter 4 : MATERIALS AND METHODS

4.1 Introduction

The PSW used in this study was generated from a poultry slaughterhouse located in the Western Cape, South Africa. The poultry slaughterhouse processes about one million birds per week, generating a large quantity of PSW, considering that an average of 26.5 L of potable water per bird is used (Avula *et al*., 2009). The PSW results from various operations listed in Fig. 2.1 and was partially treated on-site, along with other wastes generated through the processing of culled birds, to meet the discharge standards. The technology used for this purpose is rudimentary and incurs high operational expenses. The output (production) of PSW generated, was the motivation for its selection, and thus the rationale behind collection of PSW samples from this slaughterhouse, which is a fair representative of the type of wastewater generated by the poultry product industry in South Africa.

This section highlights the processes used for the treatment of PSW, sampling regime, experimental setup and the analyses used to quantify the quality characteristics of the PSW (raw/treated), including biogas analysis. Further details are provided in the subsequent sections.

4.2 Poultry slaughterhouse wastewater (PSW) sampling

The slaughtering and processing of birds in the selected poultry slaughterhouse takes place from early morning to afternoon. The PSW was collected during this time in clean 25 L polyethylene containers, which were then stored in a refrigerator (4 ^{o}C), prior to the PSW being used in the experiments.

The PSW was sampled from a draining system connecting the slaughterhouse to a holding tank, which was the first unit of the PSW treatment system of the poultry slaughterhouse selected.

4.3 Anaerobic granular inoculum and collection

The anaerobic granular sludge used for inoculating the DEGBR was collected from a full-scale UASB utilised for treating brewery effluent from a local brewery (SABMiller, Newlands, South

Africa), located in Cape Town. The inoculum was collected in clean 5 L polypropylene containers and stored (37 ^{o}C) prior to being inoculated into the DEGBR.

4.4 Experimental set-up; DEGBR

The experimental set-up (Fig. 4.1and 4.2) consisted of a four-stage process composed of a pre-treatment unit (filtration), the DEGBR (anaerobic digester), the iron oxide mesh hydrogen sulphide scrubber and the water displacement set, consisting of a 2 L glass barrier solution container and a 100 mL calibrated glass cylinder connected to a Tedlar bag, for biogas collection.

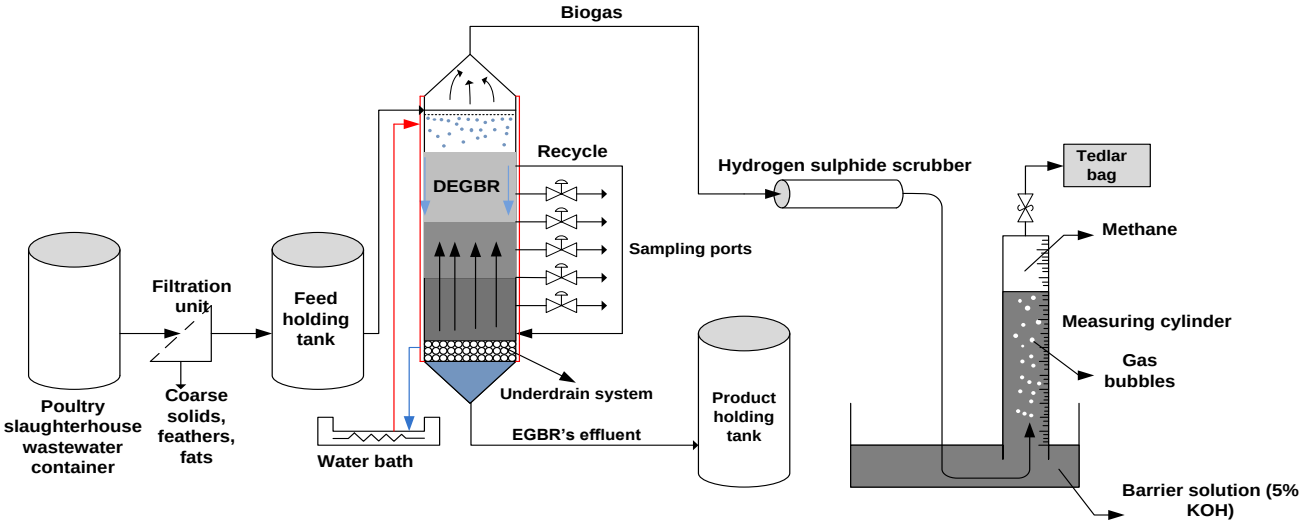


Figure 4.1: Process flow diagram of the experimental set-up

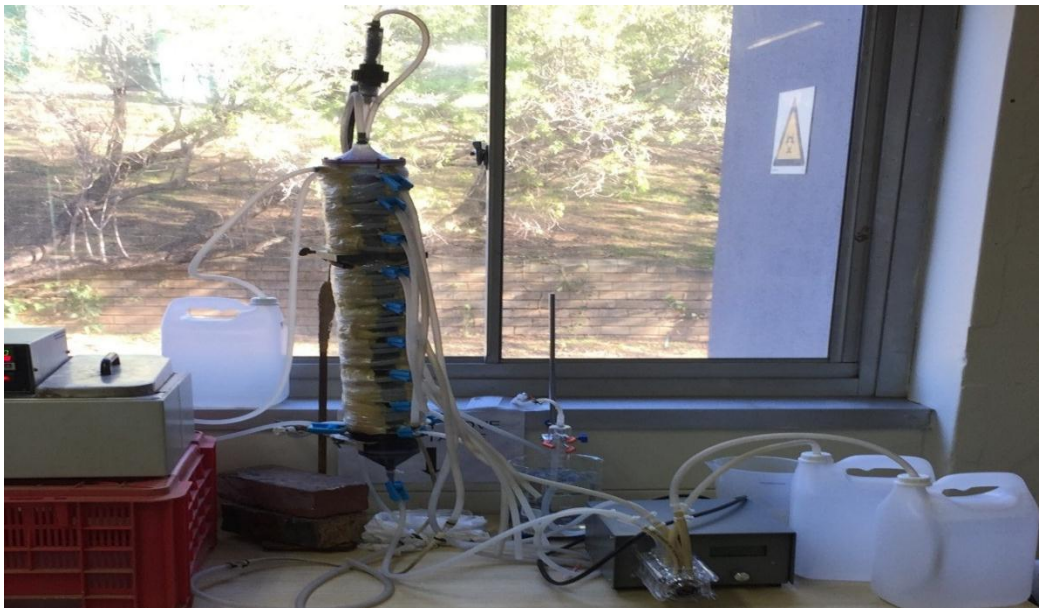


Figure 4.2: Photographic illustration of the experimental set-up (bench-scale)

4.4.1 Pre-treatment operation

The pre-treatment, i.e. filtration of the PSW was conducted prior to the influent being fed to the DEGBR, to remove coarse solids, feathers and FOG conglomerates to minimise clogging in the process lines and within the DEGBR. The filtration unit consisted of a metallic sieve (9.51 mm aperture size). The filtration operation consisted of sieves (n=2) in series to ensure the adequate retention of suspended particulate matter. Subsequently, the pre-treatment process permeate was stored in a 5 L polypropylene container, which was used as a holding tank subsequent to feeding wastewater into the DEGBR.

4.4.2 The down-flow expanded granular bed reactor (DEGBR) set-up

The DEGBR is a novel high-rate anaerobic bioreactor developed to address the shortcomings and/or challenges encountered with HRABs used in previous studies (Basitere *et al*., 2016; Basitere *et al*., 2017). The following sub-sections provide information relevant to its design and set-up for this study.

4.4.2.1 DEGBR underdrain system

The first stage of the DEGBR set-up consisted of a selection of the most suitable and convenient packing materials. The following materials were selected and assessed to determine their suitability, with the primary focus being minimal resistance to hydraulic flow:

- Pea gravels (Figure 4.3.b),
- Medium pumice stones (Figure 4.3.a),
- Small pumice stones (Figure 4.4.b),
- White pebbles (Figure 4.6.d) and
- Glass marbles (Figure 4.7.e).

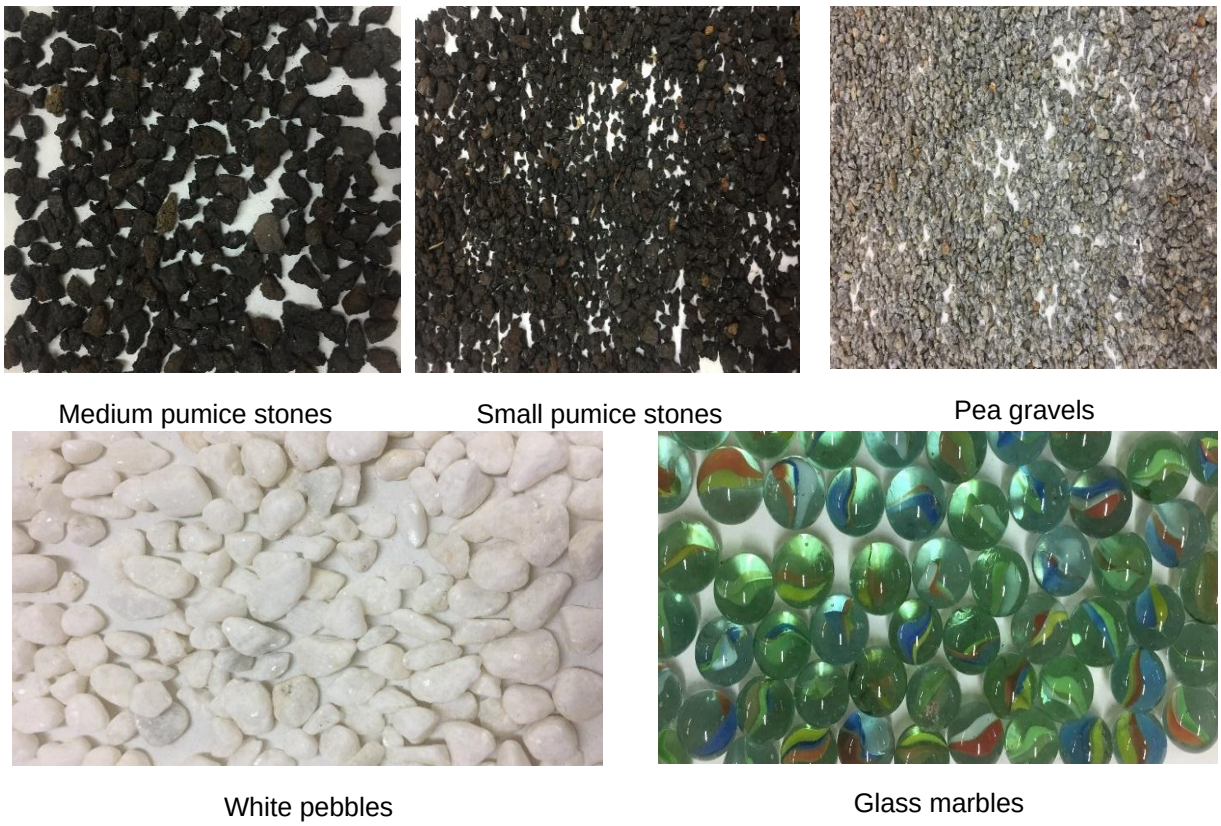


Figure 4.3: **Packing materials selected for the underdrain system**

The porosity, granular retention capacity and size of the packing materials were determined.

4.4.2.1.1 Determination of the porosity of the packing materials

The porosity of the packing material was determined using a PVC cylinder (Fig. 4.4) that had a similar inner diameter (86 mm) to that of the bench-scale DEGBR. The porosity was quantified by using the volume of the water, referred to as the total volume (V_T), and the volume of packing material (V_p), which culminates in the void volume (V_V). Subsequently, the porosity was determined from the ratio of the void volume to the total volume. A similar procedure was repeated in duplicate for all selected packing materials.

4.4.2.1.2 Packing/underdrain material, retention capacity for granular sludge

Since the packing materials selected for evaluation for an underdrain system can have an altered porosity when anaerobic granules are used instead of water, granular sludge retention capacity can be used to assess or predict the biomass washout capacity, and can also be a means to determine material suitability for the underdrain system. The assessment undertaken consisted of using a given volume of a specified quality of anaerobic granules whose mass was known. By using a similar method to that reported in the preceding sub-sections, the granular sludge was poured into a known volume of the packing materials, using a PVC cylinder (86 mm inner diameter) retained by a screen (25.4 mm aperture size) that facilitated

the outflow of the unrestrained anaerobic sludge granules (Figure 4.4). Furthermore, the outflow of the non-retained anaerobic granules was collected periodically (10 min intervals), with the bulk sludge mass being determined using a weighing scale. 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 into the packing column and the mass which was washed out. Thereafter, the granular retention capacity was determined from the ratio of the mass of the anaerobic granules washed out from the column to the initial mass used. The same procedure was repeated in duplicate for all packing materials, using a constant volume of the packing materials assessed.

Figure 4.4: PVC cylindrical apparatus used for determining the porosity and sludge retention capacity of packing materials

4.4.2.1.3 Measurement of the size of the packing materials

A Vernier calliper was used to measure the size of the packing materials. For non-spherical particles, the length, width and breadth were measured in order to determine an equivalent diameter, by averaging the values measured subsequent to multiplying the average by the corresponding sphericity determined by the visual inspection of the shape of various particles constituting the packed bed. For small-sized particles, only two sizes perpendicular to the centre of the particle were measured.

4.4.2.2 Description of the DEGBR

A bench scale PVC DEGBR was designed and commissioned as per process engineering requirements; thereafter, it was determined suitable for use in this study. Its configuration (see Figure 3.4), was similar to that used for other down-flow high-rate anaerobic bioreactors, with the influent being fed from the top through a perforated cylindrical distribution system, while the effluent was collected through an outlet port, at the bottom. A counter-current flow configuration was also incorporated through the use of a recycle stream attached to the PSW distribution system, with its flow being from the bottom of the reactor upwards to distribute the

wastewater collected from the top part of the bioreactor to the biomass located at the bottom of the bioreactor. Furthermore, the bottom PSW distribution system, resting on top of the underdrain system i.e. packing material, was also used for intermittent fluidisation operation when required. It consisted of an arrangement of perforated cylindrical tubes described in Fig. 3.4. The DEGBR had a height-to-width ratio of 7, with an inner diameter of 86 mm (cylinder wall thickness of 2 mm). The underdrain system consisted of a screen (sieve mesh size of 25.4 mm) on top of which a 5 cm layer of packing materials rested. Both ends of the DEGBR had a conical shape; with the bottom having a height of 5 cm, with its function being primarily to collect the effluent from the DEGBR into the outlet stream and to signal system clogging of the underdrain system, which was assessed through effluent accumulation; while the conical top of 4 cm height was used for biogas collection into the iron oxide mesh scrubber. Several 8 mm inner diameter sampling ports (controlled by valves) were located along the length of the DEGBR to collect samples from the bioreactor at different heights, and to enable the backwashing of floating smaller granules from the top of the top back to the bottom (sludge bed), when required.

4.4.2.3 Inoculation of the DEGBR and start-up procedure

The DEGBR was inoculated with anaerobic granular sludge collected from a full scale UASB operated for the treatment of brewery wastewater generated at a brewery in Cape Town, South Africa. A volume (3 L) of the anaerobic granular sludge was fed into the reactor, along with 1 L of PSW and 50 mL of a 20% solution of dry milk, to enable the acclimatisation of the granular biomass to PSW while providing a sufficient quantity of nutrients to induce anaerobic granular biomass growth; using a fed-batch technique such that the contents within the DEGBR were kept and maintained within the bioreactor under batch conditions for 48 hours to allow for anaerobic conditions to develop and to minimise shock loading through the continuous supply of PSW from the start of the process. A heated coil jacket, connected to a water bath around the DEGBR, ensured that mesophilic conditions are maintained (30-35 ^{o}C). A 5 cm layer of an asbestos material was attached to the outer surface of the warm water jacket, and covered with a transparent, polypropylene based, pressure-sensitive tape to minimise heat loss from the water jacket. During the acclimatisation period, the biogas collection port remained open to collect the produced biogas.

4.4.2.4 Operation of the DEGBR

After an acclimatisation period of 48 hours, the feed, product and recycle stream valves were opened and the DEGBR was kept at a steady-state by pumping (Gilson peristaltic pump) the feed and collecting the product at the same rate. The monitoring and control of the pumping rate used was to ascertain consistent operational HRTs. To ensure further acclimatisation and

growth of the anaerobic biomass, the initial HRT was 37 hours with minimisation of shock loading being ensured by diluting the PSW with a similar quantity of tap water (50% W/V dilution). After an observed improvement in organic matter removal rate, as deduced from the analyses of the feed and the effluent produced, the raw PSW (without dilution), was fed to the reactor at varying HRTs. The recycle stream was operated independently of the feed and product streams, with the objective of assessing the impact of different up-flow velocities provided for by the recycle stream, as well as the influence on the degradation of organic matter of these various velocities.

4.4.2.5 Backwashing operation of the DEGBR

Backwashing to unclog the underdrain system was implemented as a result of interstitial entrapment of small anaerobic granules in the underdrain system, and the formation of an inactive gelatinous matter at various points with the packed bed and/or the underdrain section of the DEGBR. The backwashing operation consisted of reversing the flow of the product stream using the product collected, while the feed stream was stopped by a valve. Subsequently, the sampling port at the top of the reactor was periodically opened to allow for the removal of flocculable/smaller granules, and inactivated biomass. This operation was implemented for a period of 15 minutes at a velocity that did not allow for the washout of the biomass.

4.4.3 Hydrogen sulphide scrubber

A 15 cm long and 2.5 cm inner diameter transparent PVC cylindrical tube, filled with uncoated iron oxide mesh (steel wool) was used to minimise the concentration of hydrogen sulphide (H_2S) from the biogas generated. Magomnang and Villanueva (2015) reported a H_2S removal efficiency higher than 95% using this technology. The transparency of the scrubber allowed the researcher to determine when the packing material needed to be changed, as a result of iron oxide oxidation by H_2S.

4.4.4 Water displacement system for biogas correction

The water displacement system was used primarily for biogas washing for CO_2 minimisation and quantification (of the volume of biogas produced), i.e. for biogas exiting the hydrogen sulphide scrubber, through its dissolution into the barrier solution that also prevented methane from escaping to the atmosphere. The water displacement system used constituted a 2 L glass volumetric beaker that was used to hold the barrier solution, which was a 5% w/v KOH (the preparation of which is described in section B.1 of Appendix B); a 100 mL inverted glass volumetric cylindro-conical cylinder with a 6 mm outer diameter tip to which a silicone tube was

attached, the other end of which was attached to a Tedlar bag for biogas collection via an air-tight metallic valve. In order to measure the biogas collected from the operation, the metallic valve was closed, and the cylindro-conical cylinder was filled with the barrier solution prior to being inserted into a similar barrier solution bath in the form of a beaker. The silicone tube from the hydrogen sulphide scrubber was immersed into the barrier solution and the end of the tube was orientated upwards and in alignment to the cylindro-conical cylinder to ensure for an adequate strategy for the collection of the biogas, while also allowing for biogas contact with the barrier solution to enable the carbon dioxide contained in the biogas to react with the potassium hydroxide present in the barrier solution, as illustrated in Figure 4.1. The collection of the biogas in the cylindro-conical cylinder was observed by the displacement of the barrier solution with respect to the volume of biogas generated, with the volume being read from the cylindro-conical cylinder. This method enabled the researcher to determine whether there was production of biogas. Subsequently, the metallic valve was periodically opened to allow the biogas to flow into the Tedlar bag, which had a capacity of 500 ml.

4.5 Sample collection and analyses

The samples collected in this study were regrouped into two categories:

- Liquid samples, and
- Biogas samples.

4.5.1 Liquid samples analyses

The liquid samples were collected from the feed holding tank and the product (effluent from the DEGBR) holding tank. Only the evaluation of the temperature of the DEGBR required the collection of a liquid sample directly from the DEGBR through the sampling ports. The content of the feed and product holding tanks were discarded into the appropriate waste containers after the samples were collected, to provide an accurate evolution of the process at different stages of experimentation. The liquid samples were collected daily (24 hours) and analysed as illustrated in Table 4.1, using methods listed in Table 4.2.

4.5.2 Biogas sample analysis

The biogas stored in the Tedlar bag was analysed using a Geotech Biogas 5000 portable gas analyser. The apparatus provided the percentage of the following gases per sample analysed:

- CH_4,
- CO_2,
- O_2,

- H_2, and
- H_2S.

This device lacked the ability to identify other gases present in the sample, but expressed their percentage as a balance.

Table 4.1: Analysis period and frequency

Parameter	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
pH	x	x	x	x	x	x	x
Total dissolved solids (TDS)	x	x	x	x	x	x	x
Salinity	x	x	x	x	x	x	x
Temperature	x	x	x	x	x	x	x
Turbidity	x	x	x	x	x	x	x
Total suspended solids (TSS)	x		x		x		
Volatile suspended solids (VSS)	x		x		x		
Total chemical oxygen demand (tCOD)	x		x		x		
Soluble chemical oxygen demand (sCOD)	x		x		x		
Biological oxygen demand (BOD_5)	x						
Volatile fatty acids (VFAs)	x						
Fats, oils and grease (FOG)	x						

Table 4.2: Analysis parameters and 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
Soluble chemical oxygen demand (SCOD)	APHA standard method 5220 D
Biological oxygen demand (BOD_5)	EPA method 5210 B
Volatile fatty acids (VFAs)	Potentiometric titration
Fats, oils and grease (FOG)	EPA method 10056

These analyses were completed in duplicate to obtain a representative average value of each parameter assessed.

4.6 Summary

The DEGBR was designed to resolve some challenges encountered during the treatment of PSW in previous and similar studies, which were the biogas entrapment, the pressure drop across the granular bed, the weak distribution of the wastewater as well as the poor distribution of toxicants entrapped with the biogas. Thus, the materials and methods described in this section would allow the researcher to adequately address and evaluate these problems and suggest suitable mitigation strategies that could be implemented to minimise the identified challenges.

Furthermore, this section introduced a novel method (the determination of the sludge retention capacity), which can be very useful for the selection of suitable underdrain systems. The challenges related to effluent percolation have been outlined in previous studies (Evans, 2004; Oh, 2012). Moreover, the series of methods used for determining the suitable packing material is also a contribution to strategies to minimise clogging of underdrain systems in anaerobic digesters, particularly HRABs.

CHAPTER 5

SELECTION OF PACKING MATERIAL FOR THE UNDERDRAIN SYSTEM AND EVALUATION OF THE HEAD LOSS EFFECTS INSIDE THE DEGBR

Chapter 5 : SELECTION OF THE PACKING MATERIAL FOR THE UNDERDRAIN SYSTEM

5.1 Introduction

Chapter 3 introduced the parameters analysed for the design of the DEGBR. These were the bioreactor configuration and the consideration of the head losses through the underdrain system. Attention was focused on the head loss across the granular bed and suggested strategies to circumvent these issues are covered in this chapter. The selection of a down-flow configuration was primarily motivated by improved retention of anaerobic granules, along with the added advantage of not requiring a three-phase separator for the collection of the biogas produced by the system. The head loss through the underdrain system can be minimised by the selection of a suitable packing material from amongst the materials suggested in section 4.4.2.1, while intermittent fluidisation was suggested as a means to minimise the formation of dead zones, short-circuiting, and gas entrapment, as well as the introduction of a recycle stream for the improvement of substrate distribution.

The start-up period of the HRABs is shortened by the direct inoculation of anaerobic granules collected from an industrial HRAB. Brewery HRABs are usually preferred, as they offer stable and sufficiently robust anaerobic granules (Henze *et al*., 2008). These HRABs are usually UASB reactors, as they are the most commonly used (Lim, 2009). Adequate retention of anaerobic granules is one of the important parameters used to evaluate the operation of HRABs (Henze *et al*., 2008; Chernicharo, 2007). In down-flow configurations, biomass retention is promoted by the use of an underdrain system that minimises the wash-out of anaerobic granules. However, the packing materials usually selected for this task induce pressure losses which culminate in substantial reduction of the hydraulic kinetic energy, resulting in operational difficulties associated with the collection of the effluent. Thus, it is important to select suitable packing materials to promote good retention of the anaerobic granular sludge and to minimise head losses through the underdrain system.

Furthermore, this chapter evaluates the effects of head losses across anaerobic granular sludge, by firstly establishing a relationship between the variation of the velocity of the substrate across the granular bed, and secondly discussing its effects, which include the uneven distribution of the substrate, the formation of dead zones, and moreover, biogas entrapment. Solutions to these challenges are suggested and discussed in subsequent sections.

5.2 Selection of the packing materials for the underdrain system

Holdich (2002) suggested the following factors for consideration in the selection of packing materials:

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

Three other relevant factors were:

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

The packing materials selected for this evaluation are illustrated in Figure 4.3 and included the following solids and their shape (description):

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

Holdich (2002) listed the Wadell's sphericity of common particles, as depicted in Table 5.1, which were used to describe the shape of the selected material for the underdrain.

Table 5.1: Wadell's sphericity of different solid materials (adapted from Holdich, 2002)

Material description	Wadell's sphericity
Spherical	1
Rounded	0.82
Cubic	0.806
Angular	0.66
Flaky	0.54
Platelet	0.22

Wadell's sphericity provides a means to determine a single dimension (equivalent diameter) of non-spherical particles for a correlation that is developed for non-spherical particles. Furthermore, the Ergun correlation (Equation 3.17) enables the determination of pressure loss across a packed bed of particles assumed to have identical spherical shapes. The particles selected for evaluation with regard to suitability for use as an underdrain system were neither

identical nor, with the exception of ceramic marbles, spherical. Thus, the first step towards the characterisation of these particles was to classify them according to their shape, corresponding to a specific Wadell's sphericity. Thereafter, an evaluation of their distribution was determined as a single average diameter that would be used with the corresponding sphericity to determine the required equivalent diameter. Moreover, some parameters must be known for the determination of the head loss induced by the selected packing material. These parameters include the porosity of the packed bed, the height of the packed bed, the dynamic viscosity of the fluid, the superficial velocity of the fluid, and the density of the fluid. PSW is mostly constituted of water, and therefore the properties of water at an average mesophilic temperature (35ºC), which is the operating temperature of the bioreactor, were used for this evaluation.

5.2.1 Evaluation of the porosity of the packing materials

The porosities of the different packing materials, quantitatively determined from the ratio of the void volume to the total volume as prescribed by Eq. 3.7, are given in Figure 5.1.

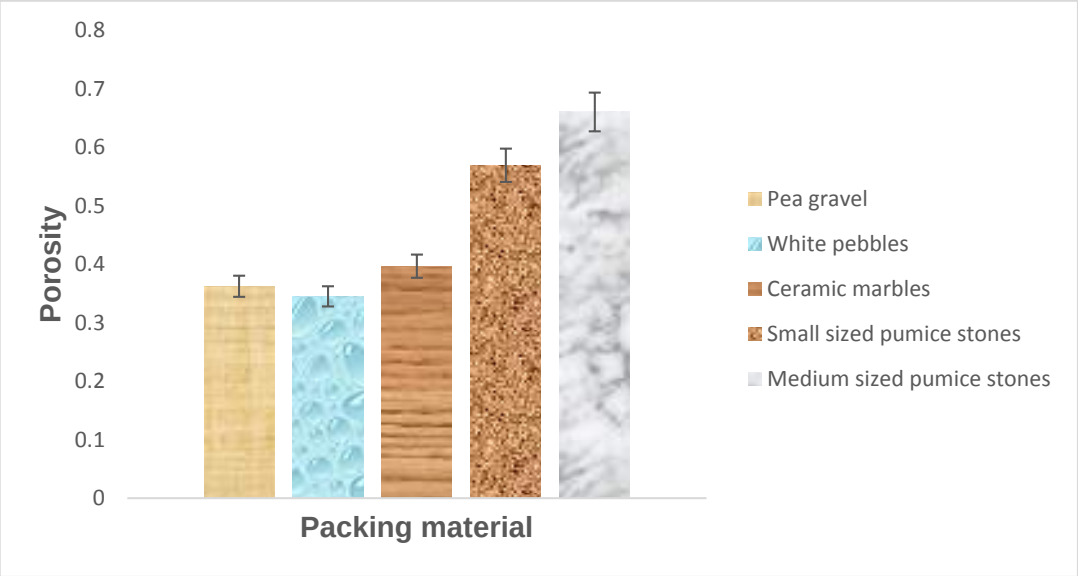


Figure 5.1: Porosity of the selected packing materials

As illustrated in Figure 5.1, the medium pumice evaluated had a more porous structure than other packing materials, with a porosity of 0.66; a higher porosity than the small pumice stones, which was determined to have a porosity of 0.57. These two packing materials have a similar structure and differ only in terms of size. Their characteristics are listed in Table 2 (Appendix A) from which it can be determined that the medium-sized pumice stones are 1.8 times larger than those classified as being miniaturised. This difference in porosity can be attributed to a more compact arrangement of particles of a smaller size that occupy a larger voidage area

than the medium pumice stones. This was noticed only for particles that were classified as being similar in shape (angular shape in this case). Pea gravel, which also had an angular structure, had an even lower porosity. The influence of the particle shape on the porosity in packed beds was further highlighted by the porosity of round white pebbles, which had a porosity of 0.34 for an average equivalent diameter higher than that of small and medium sized pumice, as well as pea gravels.

The effect of particle arrangement and their size on packing porosity occupying the same volume was further illustrated by the porosity of spherical ceramic marbles, with the porosity found to be 0.39 for the highest average equivalent diameter of 0.01574 m, which was observed to be the most porous, despite their size.

5.2.2 Evaluation of the head loss generated by the packing materials

Further parameters used for the calculation of the head loss across each packed bed are given in Tables 1 and 3 (Appendix A.1).

The evaluation of head losses across the packed bed was experimentally performed using the Ergun correlation (Eq. 3.16) for superficial velocities ranging from 3.855 x 10^{-6} to 5 x 10^{-5} m/s that corresponded to a HRT ranging from 37 to 3 hours, according to the bioreactor scale and set-up. The pressure drop across the packing material is depicted in Figure 5.2, and it was observed that, for the same surface and height of packed bed, the pea gravel used generated the highest head loss, determined to be significantly higher than the pressure loss induced by other packing materials used, that culminated in losses lower than 0.025 Pa for a superficial velocity of 5.10^{-5} m/s. Furthermore, it was also observed that the pressure drop increased with the superficial velocity, a trend that was more pronounced for pea gravels, reaching a pressure drop of 0.17 Pa at a superficial velocity of 5.10^{-5} m/s.

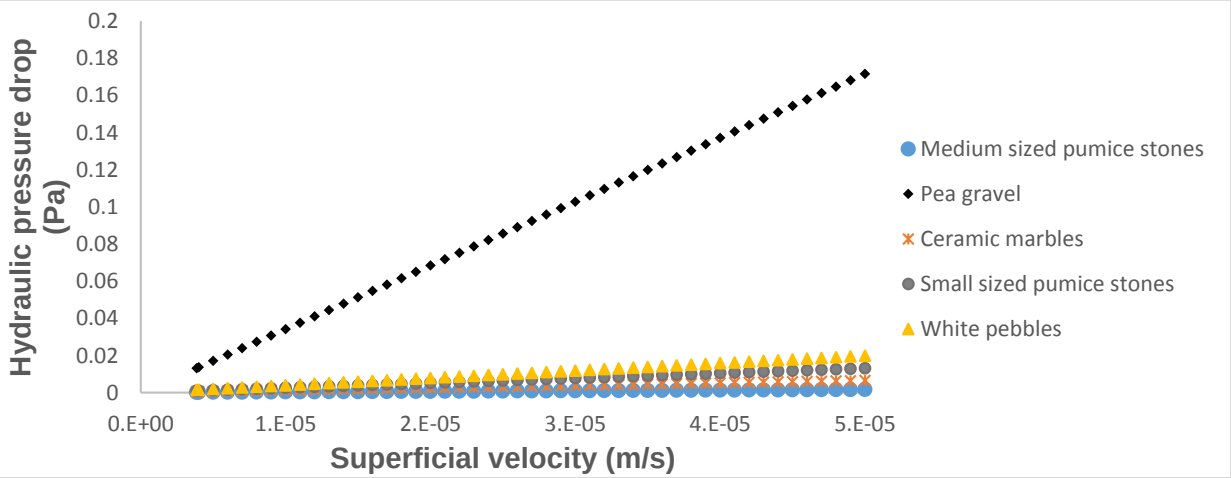


Figure 5.2: Variation of the hydraulic pressure drop of the selected packing materials with different superficial velocities

Further analyses of the head loss generated by other packing materials, except that observed for the pea gravel, as illustrated in Figure 5.3, revealed that white pebbles were one of the packing materials generating a high pressure drop, followed by small pumice stones, ceramic marbles and medium-sized pumice stones, respectively. It was observed that the trend in pressure drop for the listed packing materials differed slightly from the ranking when compared to their porosity, with the small pumice stones not directly being followed by the medium pumice stones in terms of observed pressure drop, although they were the second most porous packing material. Overall, it was observed that the medium pumice stones induced minimal head loss when used as an underdrain system for the DEGBR and therefore minimised the hydraulic kinetic energy losses through the underdrain system, which can culminate in better percolation within the bioreactor in comparison to when other packing materials are used.

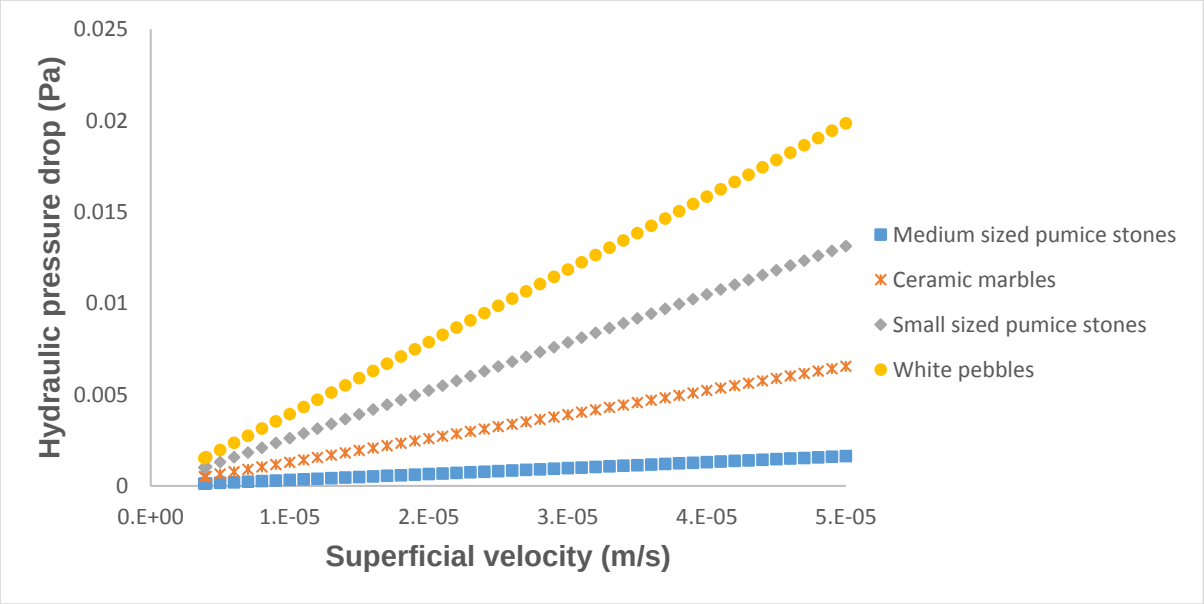


Figure 5.3: Variation of the head losses induced by selected packing materials at different superficial velocities

5.2.3 Evaluation of the permeability of the selected packed beds

An in-depth evaluation of the behaviour of the listed packing materials with regard to the facilitation of wastewater permeation of the bioreactor, was also undertaken, as illustrated in Figure 5.4.

Analogous to the trend observed for pressure drop assessments, the medium pumice stones were observed to be more permeable than other materials assessed, with the pea gravels exhibiting a weakened permeability in comparison to other packing materials. Some studies, where pea gravels were used as the underdrain system for SGBRs, point out clogging of the underdrain system after a certain period of operation as a problem (Basitere *et al*., 2017;

Evans, 2004). The solution used to circumvent this problem was the periodic backwash of the underdrain system, which could have been prevented by the selection of a suitable packing material.

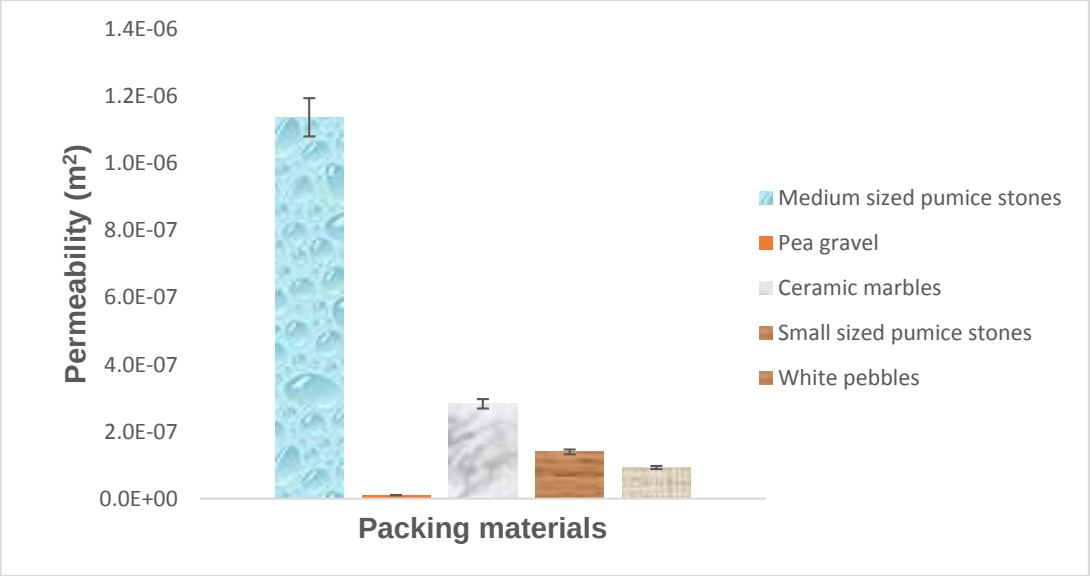


Figure 5.4: Permeability of the selected packing materials

5.2.4 The sludge retention capacity of the selected packing materials

The selection of the packing materials cannot only be motivated by the porosity of the packed bed, the pressure drop and/or the permeability of the underdrain system, as the retention of anaerobic granular sludge is also a critical parameter to consider.

Thus, the sludge retention capacity of each material in a packed bed was determined (see Table 4 of the Appendix A) as illustrated in Figure 5.5, from which it was deduced that angular materials (pumice stones and pea gravels) provided a suitable retention capacity for the anaerobic granular sludge, with a retention capacity > 0.86. Overall, the ceramic marbles provided the least sludge retention capacity (0.13), which was even lower than that observed when white pebbles were used. This reduced retention is further illustrated in Figure 5.6, where it can be observed that, for the anaerobic granular sludge with similar characteristics, the white pebbles and the ceramic marbles facilitated a higher quantity of sludge washout than other packing materials. From visual inspection, the filtrate from the pea gravels and the pumice stones packed beds, appeared to be less dark than the initial sludge used, which suggested that inactive and thus sloughed-off biomass was easily washed out from the underdrain being designed. Moreover, during the operation of the bioreactor (post-inoculation period), a minute quantity of sludge wash-out was observed using these materials.

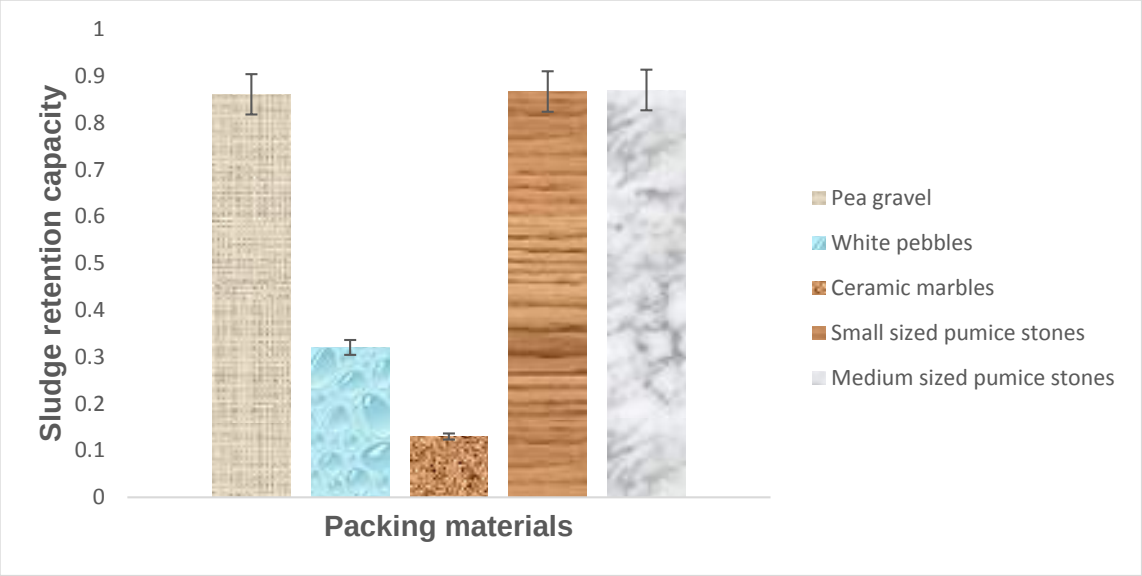
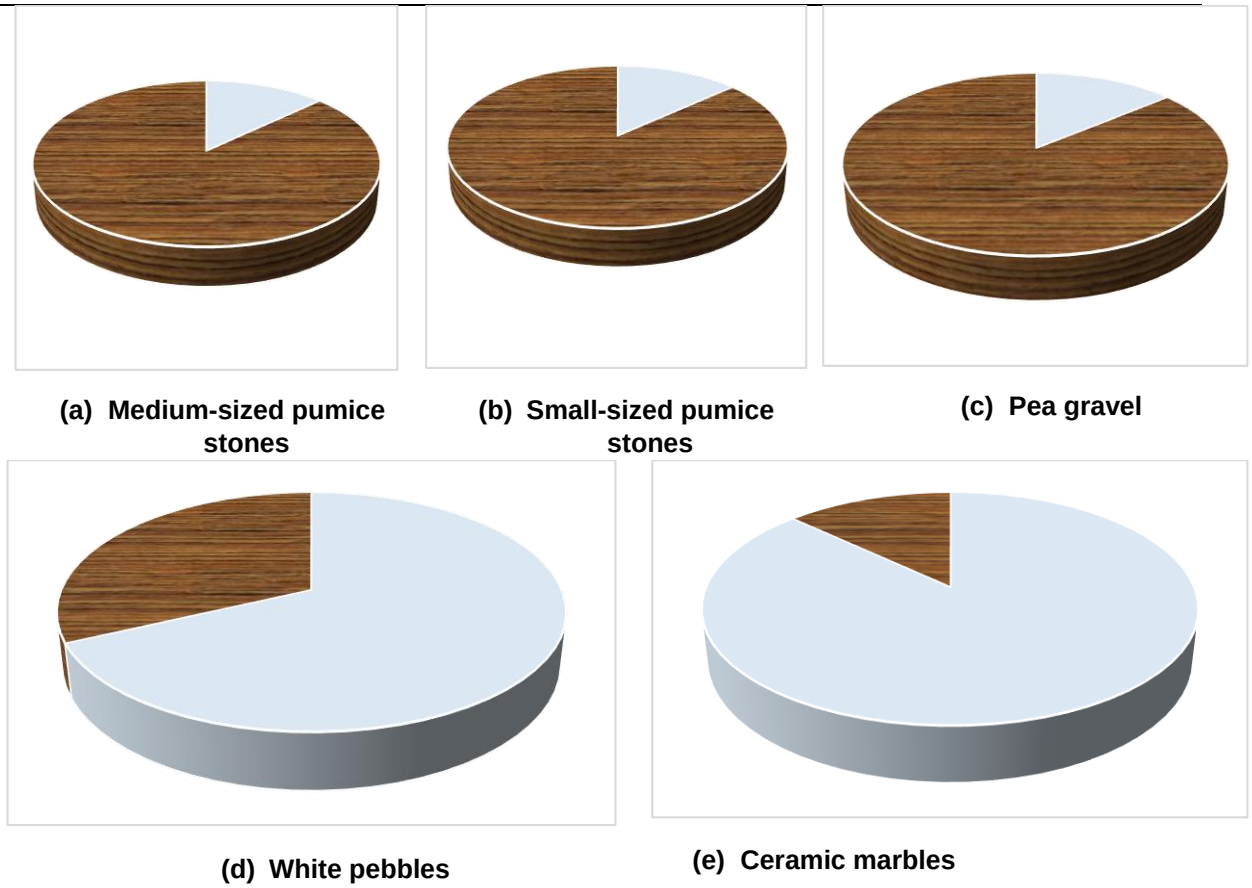


Figure 5.5: Sludge retention capacity of the selected packing materials

The suitable retention of sludge by angular-shaped materials (pea gravels and pumice stones) can be explained by their shape as well as their size. However, one parameter that contributes to the efficient retention of anaerobic granules is the coarse surface of the pumice stones that offer a suitable attachment surface area for the anaerobic granules when compared to the pea gravel, which has a smoother surface when compared to the pumice stones, whose size and shape enable a packing arrangement convenient for entrapping the anaerobic granules. Furthermore, the smooth surface of ceramic marbles (even softer than the pea gravels) contributes to the poor sludge retention capacity of these packing materials. Generally, the smoothness of these particles induces minimal friction losses to the flow of the anaerobic granules and this contributes to poor retention of biomass through the minimal loss of kinetic energy through the packed beds designed with these materials.

The ability of the medium-sized pumice stones to retain anaerobic sludge while possessing the most attractive permeability and porosity, including minimal pressure loss across the bed when compared with other selected packing materials, resulted in its selection to serve as the sole and suitable material for the underdrain system of the DEGBR. Using these characteristics, a design variation can be implemented using smaller pumice stones that also provide for a suitable retention of anaerobic granular sludge in addition to adequate permeability.



(a) Medium-sized pumice stones **(b) Small-sized pumice stones** **(c) Pea gravel**

(d) White pebbles **(e) Ceramic marbles**

Figure 5.6: Distribution of the sludge retained (dark section) and the sludge washed out (light section) for each packing material

5.3 Effects of head losses across the anaerobic granular bed

The study of the effects of head loss across the anaerobic granular bed of a bioreactor operated in a down-flow configuration, commenced with the evaluation of head losses across a static granular bed, as exemplified by the SGBR, as illustrated in Figure 5.7.

The SGBR is an open system with a single input and output port, with its operation being maintained at steady state. Assuming no phase changes, minimal variation in temperature and limited reactivity in the bioreactor, its hydraulic flow can be described by the mechanical energy balance equation (Eq. 5.1) (Felder & Rousseau, 2008).

$$\frac{\Delta P}{\rho} + \frac{\Delta(V^2)}{2} + g\Delta z + gh_L = W_s \qquad (5.1)$$

Where ΔP is the pressure difference between two points of the system, ρ is the density of the fluid contained in the bioreactor, ΔV is the change in velocity between two points, g is the gravitational acceleration, h_L the head loss and W_s the shaft work.

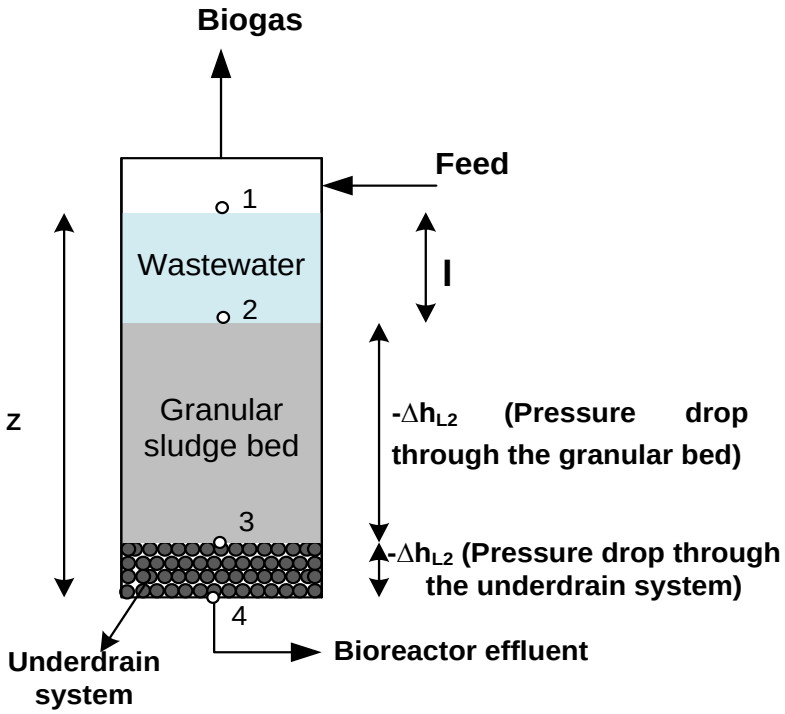


Figure 5.7: SGBR system

The SGBR can be separated into distinct sections, as illustrated in Figure 5.7 (1, 2, 3 and 4). The section of interest in this part of the study is the static granular bed, starting from the second boundary (2) and ending at the third boundary (3). Furthermore, due to the characteristics of the packed bed and the structure of the granular bed, the change in potential energy as well as the hydraulic pressure drop at the third point (P_3) can be assumed to be negligible in comparison to boundary (2). Consequently, Eq. 5.1 can be reduced to Eq. 5.2:

$$\frac{-P_2}{\rho} + \frac{\Delta(V^2)}{2} + gh_L = W_s \tag{5.2}$$

By neglecting the effect of the shaft work inputs, as pumps are mostly used for monitoring the collection of effluent in such systems, Eq. 5.2 can be further transformed into Eq. 5.3

$$\frac{V_3^2 - V_2^2}{2} = \frac{P_2}{\rho} - gh_L \tag{5.3}$$

$$V_3^2 - V_2^2 = 2\left(\frac{P_2}{\rho} - gh_L\right) \tag{5.4}$$

The velocity at the second boundary (2) of the system can also be estimated using a mechanical energy balance and results in V_2=0 m/s when wall friction losses are neglected. Considering that the system is not open to the atmosphere and devoid of dissolved oxygen, with minimal production of biogas during the start-up period, P_2 can be quantified- see Eq. 5.5.

$$P_2 = \rho g l \tag{5.5}$$

Where *l* is the length of the first section of the bioreactor. Therefore, Eq. 5.4 can be rewritten as Eq. 5.6.

$$V_3 = (2gl - 2gh_L)^{1/2} \tag{5.6}$$

Where h_L is given by the Ergun correlation (Eq. 3.17), which leads to Eq. 5.7

$$V_3 = \left[2gl - 2gL\left(\frac{150\mu v_0}{\rho d_P^2}\frac{(1-\varepsilon)^2}{\varepsilon^3} + \frac{1.75 v_0^2}{d_P}\frac{(1-\varepsilon)}{\varepsilon^3}\right)\right]^{1/2} \tag{5.7}$$

Where *L* is the length of the granular bed.

From Eq. 5.7, it can be deduced that the change in velocity will vary with the length of the granular bed, as other parameters are kept constant under steady-state conditions. Therefore, Eq. 5.7 can be re-written as Eq. 5.8:

$$V_X = \left[2gl - 2gL_X\left(\frac{150\mu v_0}{\rho d_P^2}\frac{(1-\varepsilon)^2}{\varepsilon^3} + \frac{1.75 v_0^2}{d_P}\frac{(1-\varepsilon)}{\varepsilon^3}\right)\right]^{1/2} \tag{5.8}$$

Where the subscript *x* represents a location in the granular bed. This translates to an uneven distribution of PSW across the granular bed, which can otherwise be explained by a progressive diminution of the PSW velocity in the distribution of the substrate (organic matter) to the biomass, as the PSW is flowing through the granular bed. This results in the stratification of the granular bed and eventually the formation of dead zones when the final velocity reaches 0 m/s. Moreover, the formation of dead zones leads to the development of short-circuiting that contributes to the uneven distribution of the substrate.

Mu *et al.* (2006) studied the permeability of anaerobic CH_4-producing granules and classified them into three categories (Table 5.2).

Table 5.2: Characteristics of three types of anaerobic granules (adapted from Mu *et al.*, 2006)

Anaerobic Granule	Diameter range (cm)	Specific gravity	Porosity
Type 1	0.06 - 0.17	1.028 ± 0.008	0.90
Type 2	0.17 – 0.35	1.050 ± 0.005	0.71
Type 3	0.35 – 0.50	1.075 ± 0.006	0.64

The studies by Mu *et al*. (2006), and Evans (2004), considered that the anaerobic granules have a spherical shape; a parameter that can be used to assess the variation of the pressure drop they induce through various porous materials; hence, when compared under similar superficial velocities, trends such as those depicted in Figure 5.8 can be observed, with type

2 granules inducing higher levels of pressure drop, followed by type 3 and type 1 biomass, respectively. It was also determined that, for each biomass type, the head loss increased with the superficial velocity, as illustrated in Figure 5.9, where it was noticed that the head loss increased with reduced HRTs, indicating head losses may accentuate at lower HRTs.

Generally, the size of the anaerobic granules is related to their maturation (growth), usually promoted by stabilised environmental factors and substrate availability. Therefore, poorly designed anaerobic bioreactors can cause in an increase in head loss across their granular bed, which will eventually affect the distribution of the substrate. In a down-flow configuration, one way to circumvent this shortcoming, i.e. negating poor substrate distribution, is the installation of a recycle stream for wastewater collection above the granular bed and redistribution to the bottom of the granular bed, as suggested by the design of the DEGBR (Figure 3.4). This can enhance the distribution of the substrate, allowing for an appropriate continuous operation and thus an increase in efficiency, for an improved design and suitable availability of the substrate for biodegradation that culminates in the production of an effluent with an organic content which is significantly reduced. For long term operations, the enhanced bioavailability of the substrate and its decomposition would culminate in the production of a sufficient quantity of biogas.

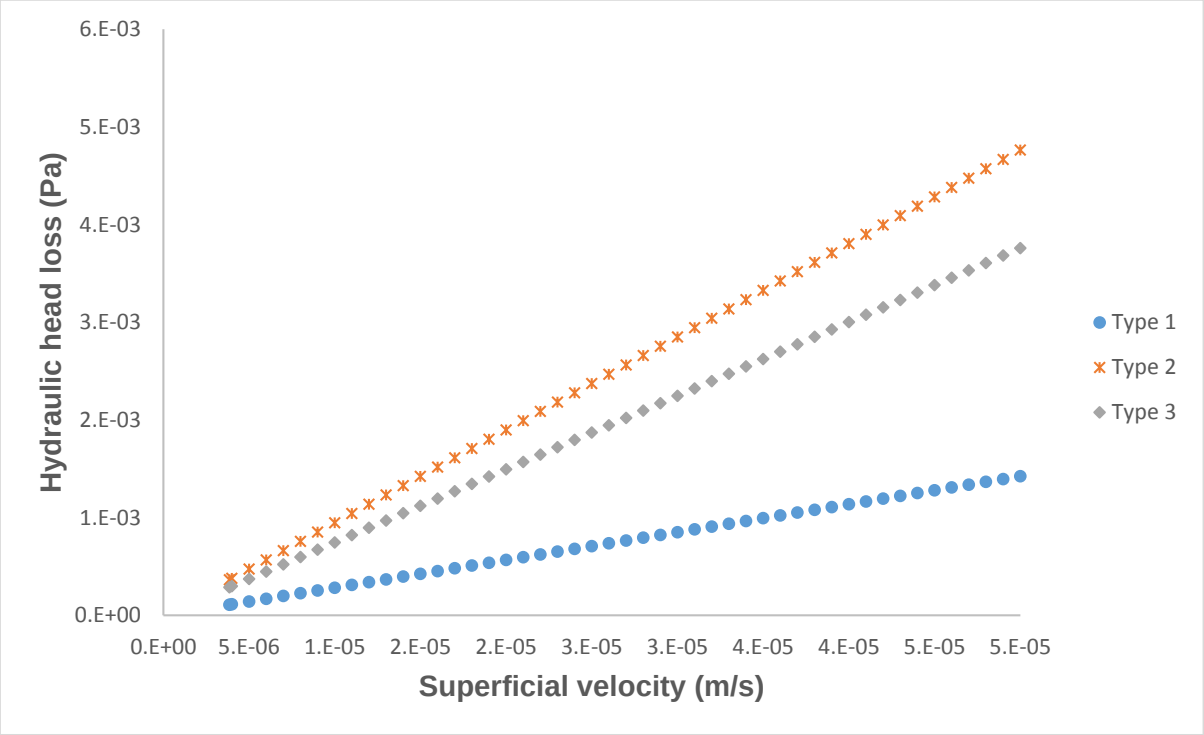


Figure 5.8: Variation of hydraulic head loss with superficial velocities across the granular bed

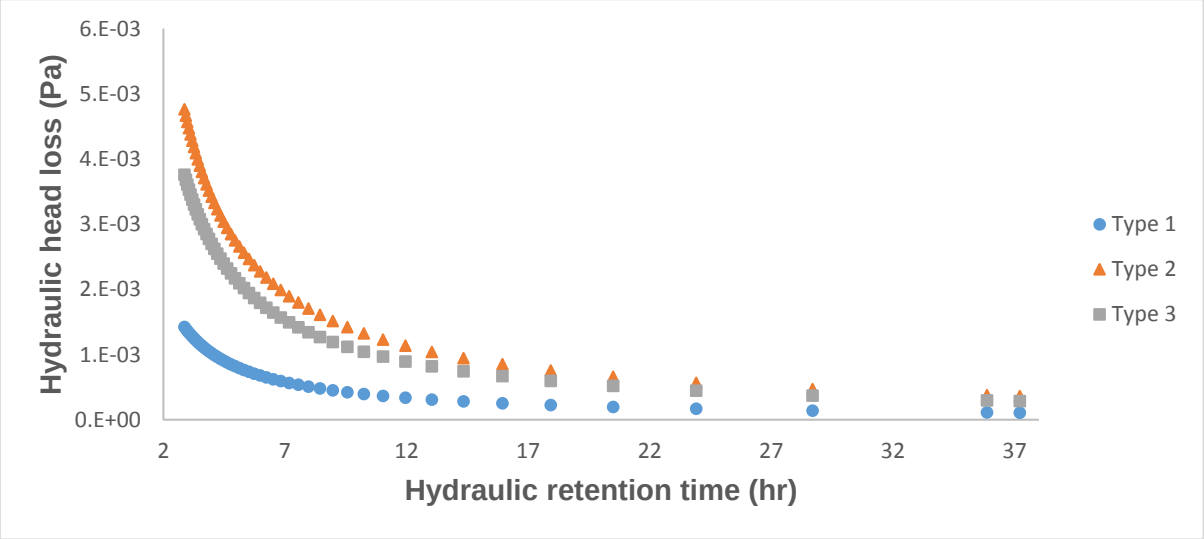


Figure 5.9: Variation of hydraulic head losses with the HRTs across the granular bed

For biogas emergence, Meier *et al.* (2011) reported two distinct bubble emergence methods; the first being through elevation of biogas bubbles through the liquid/biomass interfaces as a result of bubble growth, such that the surrounding forces are overcome by the buoyancy of the biogas bubbles; and the second being through the percolation of small bubbles through the interstices of the granular bed. The first mode of biogas bubble emergence is further discussed by Yamamoto *et al.* (2009) and Brooks *et al*. (1999), who point out that a channel flow is created when the buoyancy forces are large enough to overcome the capillary pressures described as the pressure difference across the biogas-liquid interface, as outlined by the Young-Laplace model (Eq. 3.22). Meier *et al.* (2011) further opine that the first mode of biogas emergence is likely to occur for a granular bed composed of small anaerobic granules, while the second would occur for larger-sized anaerobic granules.

This biogas percolation is subject to the effects of head losses across the granular bed that can culminate in the loss of kinetic energy and therefore, entrapment of biogas bubbles. This entrapment can further be exacerbated by the fact that the likelihood of the first mode of biogas emergence being reduced by the growth of anaerobic granules, increases with specific gravity (refer to Table 5.2). This increase in density of anaerobic biomass and production of EPS contributes further to biogas bubble entrapment at the bottom of the granular bed. To overcome the pressure exerted on such bubbles, as expressed in Eq. 3.25, the expansion of the surface granular bed has been recommended (Evans, 2004) and this as a result of less pressure exerted on the biogas bubbles at the surface of the granular bed that favours the first mode of biogas bubbles emergence.

The biogas consists of various gases, including CH_4, CO_2, H_2S, N_2, H_2, etc. The accumulation of the biogas within the anaerobic granular bed can be inhibitory for the anaerobic biomass, as gases such as H_2S or NH_3 are very toxic to this biomass and can also contribute to changes in the pH of the system. A pH outside of the prescribed range (6 to 8), will be detrimental to the anaerobic activity and enable sulphate-reducing bacteria to dominate methane-forming bacteria, for a lower production of methane and an increased formation of H_2S that will further contribute to the prevalence of sulphate-reducing bacteria. Thus, weak biogas emergence results in weak toxicant dispersion and an alteration of the methanogenic activity that is also illustrated by a weak transformation of CO_2, as sulphate-reducing bacteria do not transform CO_2, unlike methane-forming bacteria (Figure 2.2). Consequently, the accumulation of CO_2, soluble in PSW, will further modify the pH of the anaerobic system and produce a weak bioreactor performance.

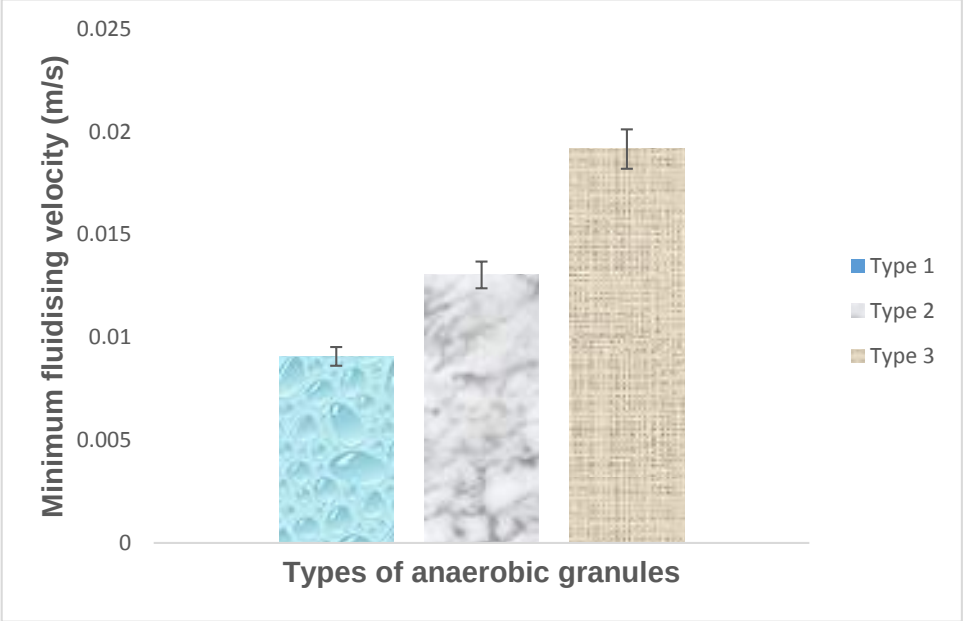


Figure 5.10: Minimum fluidising velocity for each type of anaerobic granules in the DEGBR system

The DEGBR designed addresses the problems associated with biogas entrapment, using intermittent fluidisation of the granular bed, which contributes to pressure loss alleviation through the granular bed, as described in section 3.2.2.4. For this to be implemented and for further minimisation of the energy losses, the minimum fluidisation for each type of anaerobic biomass was quantified for a granular bed height of 25 cm, which was the height of the granular bed used in the bench-scale DEGBR. Figure 5.10 provides the minimum fluidising velocity required for each type of anaerobic granular biomass using a bed height of 25 cm.

From Figure 5.10, the third biomass type requires the highest minimum fluidisation velocity, followed by the type 2 and 3 biomasses respectively, highlighting the impact of the density of the anaerobic granules, with denser granules requiring higher energy input when implementing the fluidisation strategy. The added advantage of fluidisation is that it facilitates the rearrangement of the anaerobic granules within the granular bed, as denser particles tend to settle faster than the less dense granules and therefore tend to occupy the bottom of the bioreactor bed, further contributing to the reduction in the head loss on top of the granular bed.

5.4 Summary

The evaluation of the porosity, permeability, induced head loss and the sludge retention capacity resulted in the selection of medium-sized pumice stones with a sphericity of 0.66 to be used as underdrain system. Furthermore, the effects of the head losses across the granular bed in a down-flow configuration are discussed through mathematical analyses providing an adequate explanation of strategies to overcome operational challenges. These factors influenced the design of the DEGBR, whose performance is evaluated and discussed in Chapter 6.

CHAPTER 6

RESULTS AND DISCUSSION: PERFORMANCE EVALUATION OF THE DEGBR IN THE TREATMENT OF PSW

Chapter 6 : RESULTS AND DISCUSSION: PERFORMANCE EVALUATION OF THE DEGBR IN THE TREATMENT OF PSW

6.1 Introduction

Generally, the performance of HRABs is evaluated through the removal or reduction of the concentrations of tCOD, sCOD, BOD_5, FOG or TSS. These parameters were evaluated over a selected operating time and at various HRTs and OLRs to assess the stability and efficacy of the system designed. The configuration provided by the DEGBR included a recycle stream that enabled the implementation of a counter-current flow inside the bioreactor through a recycle stream that collected the PSW from the top of the bioreactor and distributed it back to the bioreactor across the anaerobic granular bed from the bottom via a PSW distribution system placed on top of the underdrain system. The recycle stream operation was quantified by the up-flow velocity it induced, determined by the ratio of the recycle stream flow rate and the cross-sectional area of the bioreactor. Other parameters, such as the pH, temperature, alkalinity, concentration of VFAs or the turbidity, were assessed continuously to evaluate the performance of the DEGBR.

Wastewater discharge standards established by regulatory agencies vary from country to country and, in some cases, city to city. These standards provide limits for effluent quality characteristics, which must be adhered to by various industries prior to the discharge of the wastewater produced to receiving water bodies. Adherence to these discharge standards enables the discharging industries to mitigate against financial penalties, providing an added advantage as the treated wastewater can be recycled for reuse. In this section, the effluent of the DEGBR was also compared to CCT discharge standards, which govern the characteristics of the effluent to be discharged by the poultry slaughterhouse, from which PSW samples were collected.

Furthermore, the assessment of the effects of the bioreactor configuration on the treatment of PSW can be determined by comparing its performance to one of the existing technologies. Table 2.7 summarises the performance of such technologies. Another crucial parameter used for the evaluation of the performance of the DEGBR was the biogas production. In this research project, the biogas produced by the DEGBR was collected via a biogas collection set, illustrated in Fig. 4.1.

Overall, this chapter evaluated and discussed the performance of the DEGBR on the treatment of PSW under mesophilic conditions. Observed trends from analysis of critical parameters

were compared to gain an insight into the anaerobic digestion of PSW using the configuration offered by the DEGBR.

6.2 Overview of the DEGBR results

The results from the analyses of the DEGBR influent and effluent are reported in Table 6.1; with average values of the parameters, minimum and maximum values, indicating a superior performance of the DEGBR operation.

Table 6.1: Overall DEGBR results

Parameter	Minimum	Maximum	Average
Conductivity inlet (µS)	899.00	2450.00	1769 ± 425.96
Conductivity outlet (µS)	1173.00	3770.00	1992 ± 496.58
Biodegradability (BOD_5/COD)	0.49	0.75	0.57 ± 0.08
TDS inlet (mg/L)	639.00	1740.00	1250 ± 302.09
TDS outlet (mg/L)	836.00	2670.00	1410 ± 350.40
Salinity inlet (mg/L)	451.00	1240.00	880 ± 189.80
Salinity outlet (mg/L)	622.00	1970.00	957 ± 263.01
Turbidity inlet (NTU)	328.50	864.50	758 ± 158.50
Turbidity outlet (NTU)	11.47	286.50	33.65 ± 45.17
Turbidity reduction (%)	**17.20**	**98.63**	**94.67 ± 4.11**
TSS inlet (mg/L)	291.04	5044.02	1750.16 ± 1124.91
TSS outlet (mg/L)	4.25	231.46	51.64 ± 44.98
TSS removal (%)	**20.47**	**99.82**	**97.44 ± 5.04**
tCOD inlet (mg/L)	2280.00	11452.50	5354.50 ± 1809.74
tCOD outlet (mg/L)	127.00	1154.00	264 ± 187.99
tCOD removal (%)	**49.39**	**98.05**	**95.68 ± 3.63**
sCOD inlet (mg/L)	1002.50	2675.00	2050 ± 354.49
sCOD outlet (mg/L)	90.50	826.50	208.50 ± 138.29
sCOD removal (%)	**57.29**	**94.88**	**88.75 ± 5.13**
FOG inlet (mg/L)	280.00	1668.00	738.00 ± 373.84
FOG outlet (mg/L)	34.00	82.00	58.00 ± 15.99
FOG removal (%)	**85.46**	**96.28**	**93.77 ± 3.58**
VFA inlet (mg/L)	74.00	548.00	350 ± 167.64
VFA outlet (mg//L)	34.00	83.00	57 ± 16.31
BOD_5 inlet (mg/L)	850.00	4250.00	3000 ± 957.94
BOD_5 outlet (mg//L)	30.00	225.00	45 ± 67.25
BOD_5 removal (%)	**84.71**	**99.23**	**98.59 ± 4.54**
Alkalinity inlet (mg/L)	360.00	926.00	602 ± 208.68
Alkalinity outlet (mg/L)	447.00	1148.00	871 ± 235.55
OLR (mg/L.hr)	65.14	366.25	148.69 ± 83.68
Biogas produced (mL/d)	4.00	70.00	44 ± 18.56

For each parameter evaluated, a reduction of various pollutants in the influent was observed; illustrating the proficiency of the DEGBR on the treatment of PSW, with tCOD, sCOD, BOD_5, FOG, TSS, FOG and turbidity being parameters of interest. Thus, the median turbidity, TSS, tCOD, sCOD, FOG, BOD_5 reduction throughout the experiment being 95.5 %, 97.02%, 94.87%, and 87.21%, 93.77%, 98.58%; with standard deviations of 3.74, 5.31, 3.8, 5.28, 3.58, and 4.45, respectively. For a modified and newly designed anaerobic bioreactor, the DEGBR performance showed adequate and constant performance for further development at pilot scale.

6.2.1 Performance of the DEGBR on the removal of PSW's chemical oxygen demand

Generally, the aggregate organic matter concentration in wastewater can be quantified using three distinct laboratory methods, i.e. the BOD_5, COD and TOC (total organic carbon). In this section, the total and soluble COD of the influent and effluent from the DEGBR were evaluated, as well as the removal efficiency of the bioreactor operation.

The tCOD differs from the sCOD due to the totality of dissolved oxygen required to convert all organic matter within a sample, making tCOD concentration higher than that of sCOD, as observed in Figure 6.3. From the initiation of the DEGBR process, the tCOD concentration of the effluent from the system met the limit imposed by CCT, i.e. a discharge limit set at 5000 mg/L, with the influent far exceeding direct disposal standards into the receiving bodies of water. By using the DEGBR, it was demonstrated that the treatment of PSW can thus be achieved without additional process units if the primary aim is to discharge the treated water and if meeting CCT discharge standards was the sole objective of the treatment. However, strict hygienic requirements for the treated water are required in order to reuse the wastewater, which will then require further treatment to further minimise pollutant concentration levels.

Similarly, as illustrated in Figure 6.1, tCOD rates were deemed adequate, with removals higher than 80% from day 3 of the bioreactor operation. The highest removal reached was 98.05%, with a median value of 94.87%. Temperature fluctuation culminated in a decrease in tCOD removal at day 26; a performance restoration was observed after the rectification of the temperature anomaly and a subsequent increment in HRT to a slightly higher value, translating to an increased retention time for the PSW in the DEGBR. Similar behavioural changes were noticed, when TSS was considered as a comparative parameter, in percentage removal, (see Figure 6.4), which suggested a similar response to temperature changes. Consequently, sCOD would also exhibit a similar phase response, although, as depicted in Figure 6.3, sCOD can be defined as easily biodegradable organic matter, and was not adversely affected by the temperature instability, an indication of a visible biomass structure within the DEGBR.

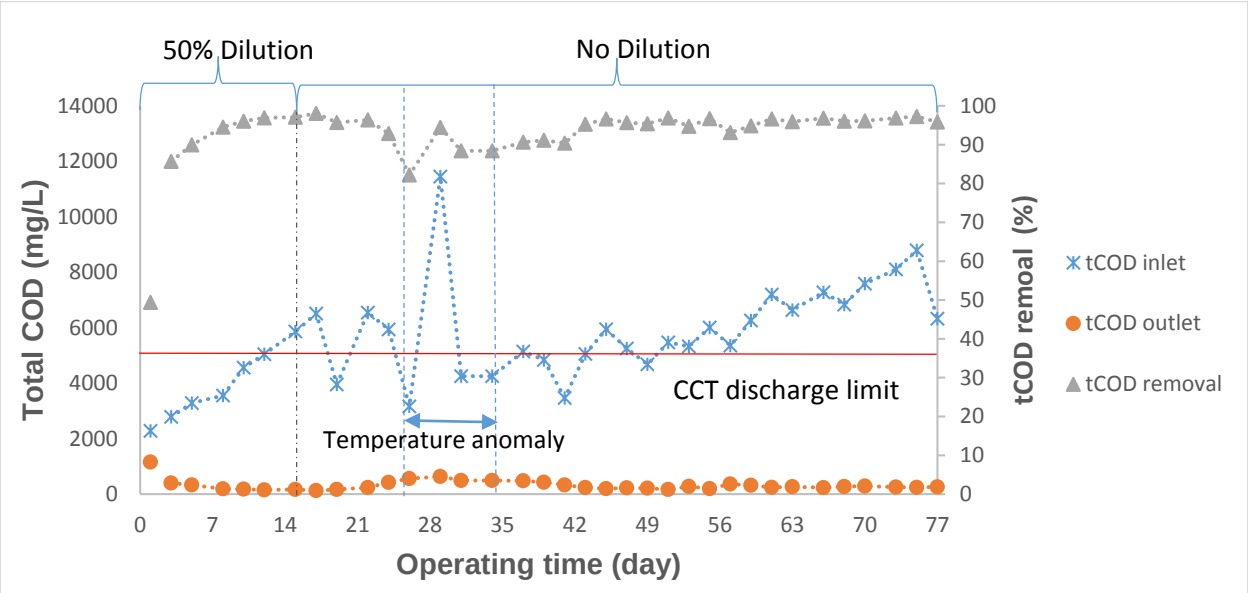


Figure 6.1: Variation of the tCOD concentration during the DEGBR operation

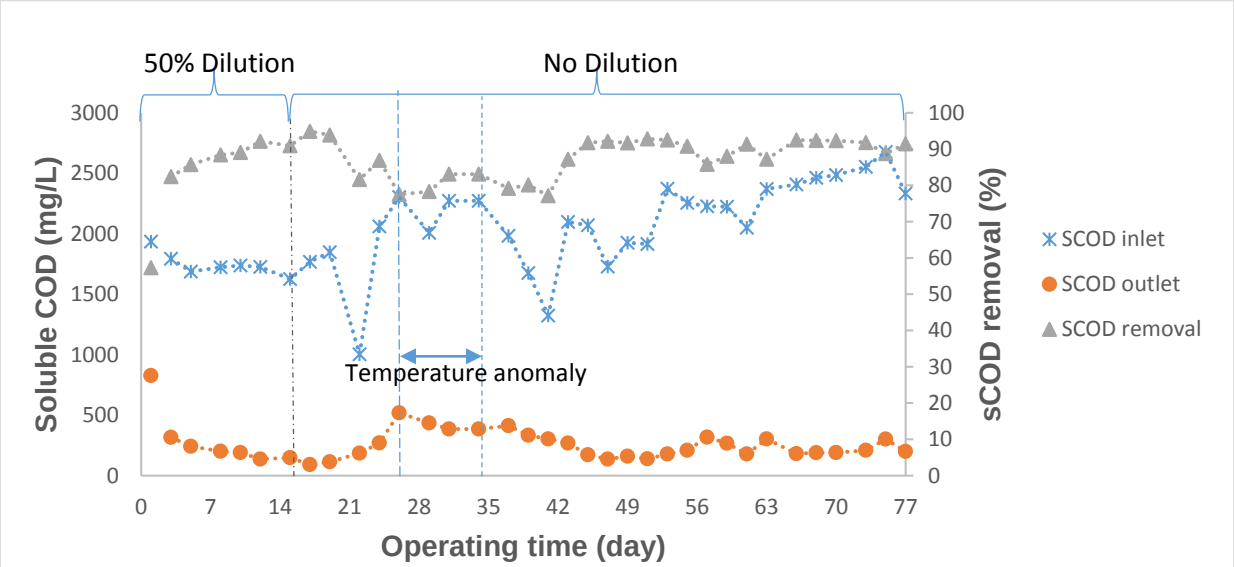


Figure 6.2: Variation of the sCOD concentration during the DEGBR operation

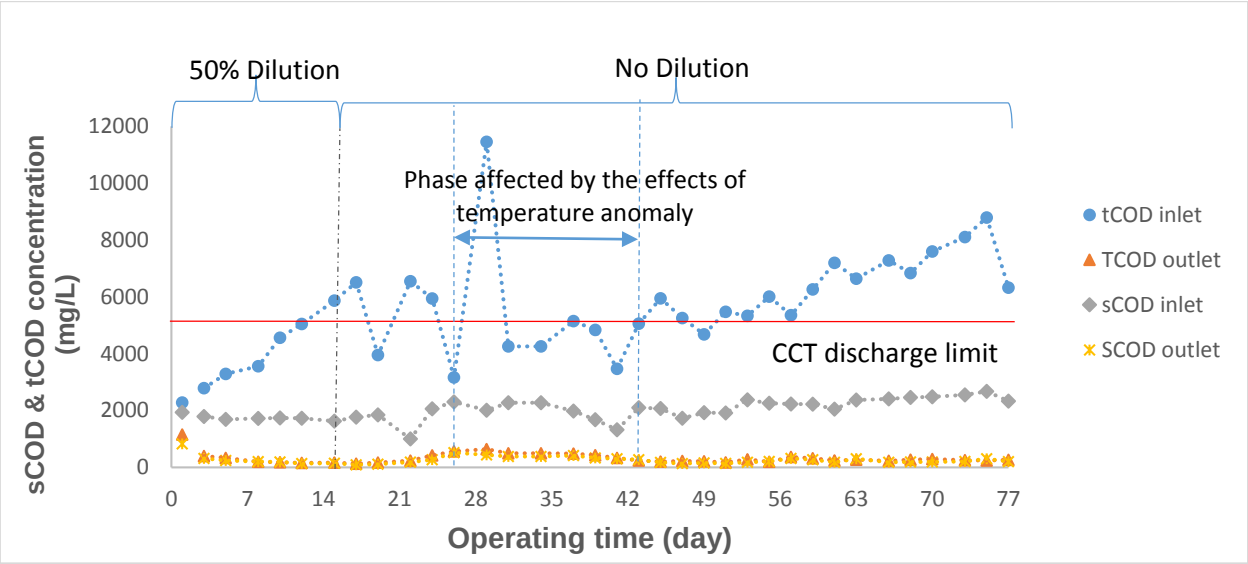


Figure 6.3: Comparison between the sCOD and tCOD percentage removal during the DEGBR operation

Upon further analysis of sCOD, and during a phase in which the effects of temperature changes were prolonged, i.e. day 26 to 43, as well as a reduction in the HRT and the implementation of a recycle stream, which effectively improved the organic matter redistribution for increased PSW/anaerobic biomass interaction, it was seen that these can be used as external mitigation and implementable strategies to minimise such a thermostatic variation.

Overall, the DEGBR achieved a diminished sCOD concentration (87.2%), even with the fluidisation of the anaerobic granular bed, which was assumed to have had a greater influence on organic matter conversion.

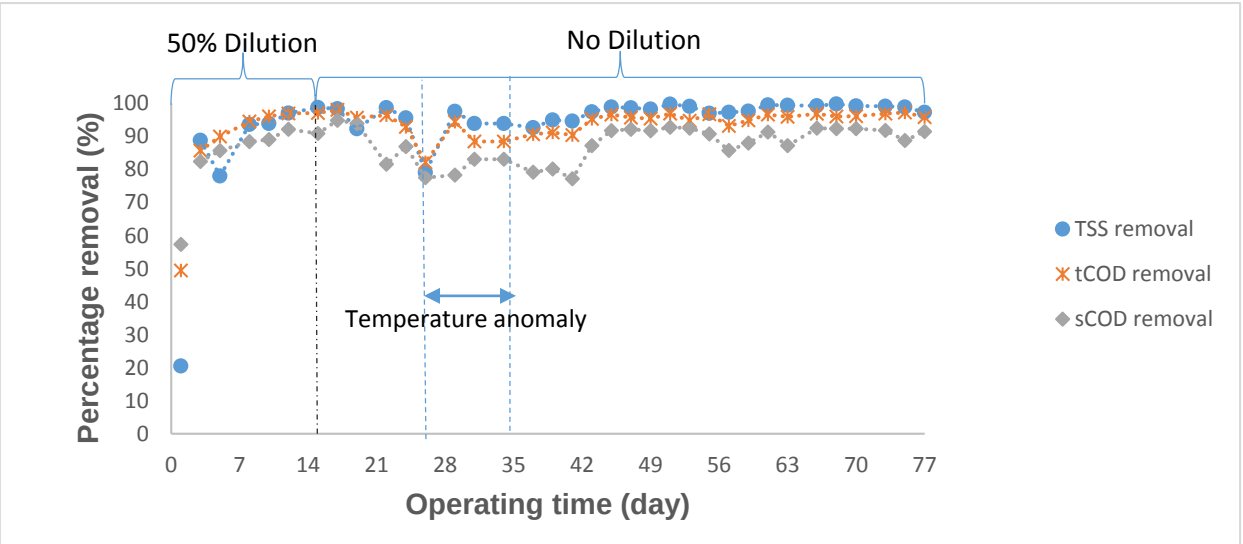


Figure 6.4: Comparison between the TSS, tCOD and sCOD percentage removal during the DEGBR operation

6.2.3 Performance of the DEGBR on the removal of PSW's BOD_5

The BOD_5 is a parameter which is as important as the tCOD, as it provides the quantity of biodegradable organic matter present in a wastewater. There are components quantifiable as BOD_5 which can be classified as non-soluble. BOD_5 was determined as the quantity of oxygen consumed by suitable microorganisms which are attuned to the biodegradation of such organic matter (Henze *et al*., 2008). Though naturally biodegradable, the excessive availability of nutrients in such large quantity in any environment could alter the natural habit and therefore balance, effectively modifying such an environment, thus unpropitiously changing the ecological balance. The availability of such dissolved organic materials to aquatic plants, and microbial species may lead to eutrophication of surface water or pathogenesis, culminating in the death of aquatic animals. Thus, the evaluation of the efficacy of BOD_5 removal from the PSW was crucial, as it is a rich source of nutrients. As shown in Figure 6.5, BOD_5 concentration in the effluent from the DEGBR was maintained at low values, despite variations in the inlet BOD_5, with effective removal efficiency between 84.7 and 99.2%, (98.58% as a median), results indicating decomposability of organic matter within the PSW supplied to the DEGBR, effectively illustrative of an improved proficiency in comparison to other HRABs, due to its novel configuration that promotes an improved substrate distribution.

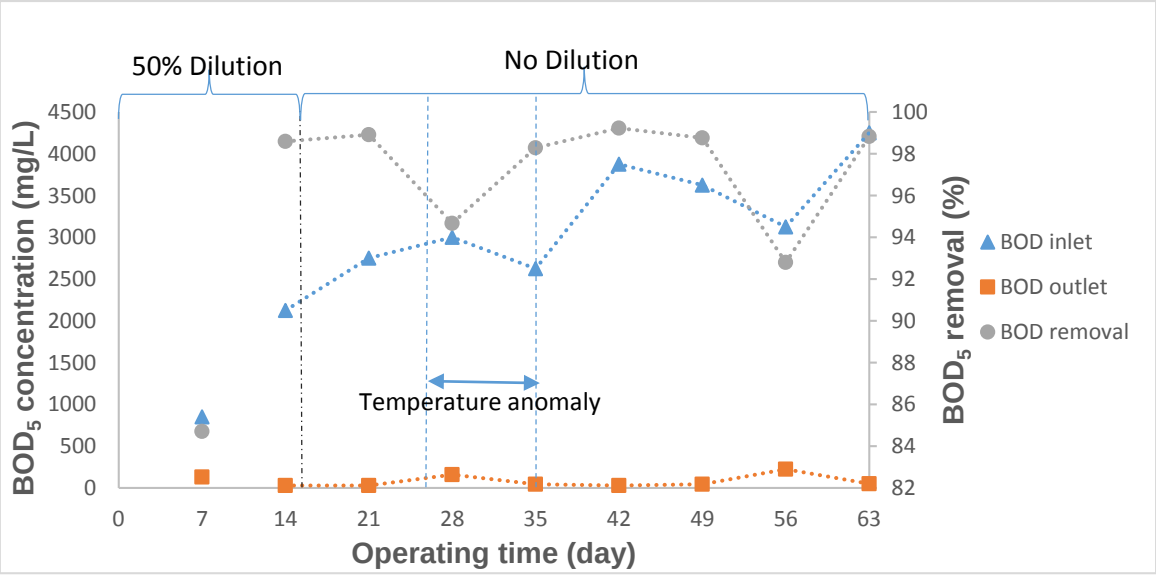


Figure 6.5: Variation of the BOD_5 concentration during the DEGBR operation

6.2.3 Assessment of the DEGBR performance through the evaluation of the effluent VFA concentration

The concentration of VFA has a direct influence on the biocatalytic activity of methanogens, and although such an influence might not adversely affect tCOD conversion, it was important to quantify. The accumulation of VFAs within a bioreactor can result in the souring of the

sludge, (i.e. acidification) and subsequently the inhibition of methanogens in the long term (Henze *et al*., 2008). As previously elucidated, this acidification of the bioreactor promotes the proliferation of sulphate-reducing bacteria, culminating in increases in H_2S production, a colourless, foul-smelling toxicant which has further accentuation abilities for methanogen inhibition. Thus, an anaerobic bioreactor effluent with VFAs concentration should, where possible, range between 50 and 500 mg/L, as a hypothetically or presumed measure or adequate methanogenic activity within an anaerobic system, with concentration exceeding 500 mg/L being associated with the accumulation of organic acids, including acetic acid. Figure 6.6 illustrated that the effluent from the DEGBR had low VFAs, with values being close to that of the limit of 50 mg/L, suggesting proficient transformation of VFAs during the DEGBR operation. Furthermore, it must be noted that VFAs concentration values lower than 50 mg/L associated with reduced fatty acid production during acidogenesis or a stuck hydrolysis phase with reduced polymeric conversion rates. Furthermore, Figure 6.6 shows an incremental trend whereby the VFAs concentration in the PSW influent suggest the pre-acidification of the PSW in the holding tank due to environmental conditions, such as ambient temperature. Although chilling units in the wastewater treatments would add to operational costs, it was decided that pre-acidification can benefit the DEGBR by facilitating the degradation of organic matter. Moreover, it was evident that further biodegradation of polymeric substances was within the DEGBR and not the holding tank, as VFAs concentration increases were observed inside the bioreactor. Overall, it was observed that biomass activity enabled the removal of most VFAs to minimal values, of 34 to 83 mg/L, for a pH maintained within the prescribed range for the proliferation of methane-forming bacteria over sulphate-reducing bacteria.

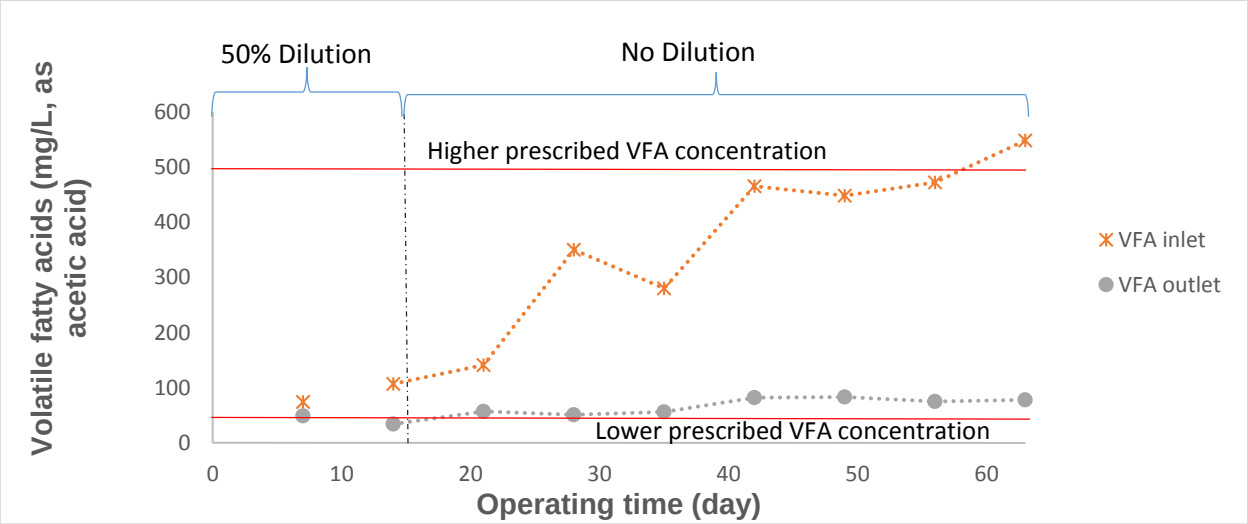


Figure 6.6: Variation of the VFAs concentration during the DEGBR operation

6.2.4 Performance of the DEGBR on the PSW FOG removal

PSW is often referred to as a wastewater with high FOG content (Barbut, 2015). Thus, the task of reducing the concentration of FOG from PSW is an important one for its treatment and the functionality of the anaerobic biomass. The difference in FOG concentration between the influent and the effluent of the DEGBR, (Figure 6.7) from which it was observed that the DEGBR effluent met the CCT discharge standards, as the limit of 400 mg/L was not exceeded, although the influent FOG concentration was consistently higher than this limit subsequent to the first week of operation. The system maintained a performance of 85.4 to 96.2% removal efficiency to 9 weeks, with an averaged performance of 93.77 ± 3.6%, with the treated PSW FOG concentration averaging a concentration of 58 ± 16 mg/L, despite fluctuations and treatment anomalies. It is postulated that future research must be undertaken in order to assess PSW treatment and the DEGBR's suitability for treatment of similar types of wastewater containing an even higher concentration of FOG, since the acclimatisation period was only limited to 7 days. The results, including performance evaluations, show that the DEGBR biomass did require an elongated period of adaption, even when FOG-laden PSW was reached without dilution, as the concentration of FOG was maintained at low levels from the beginning of the process.

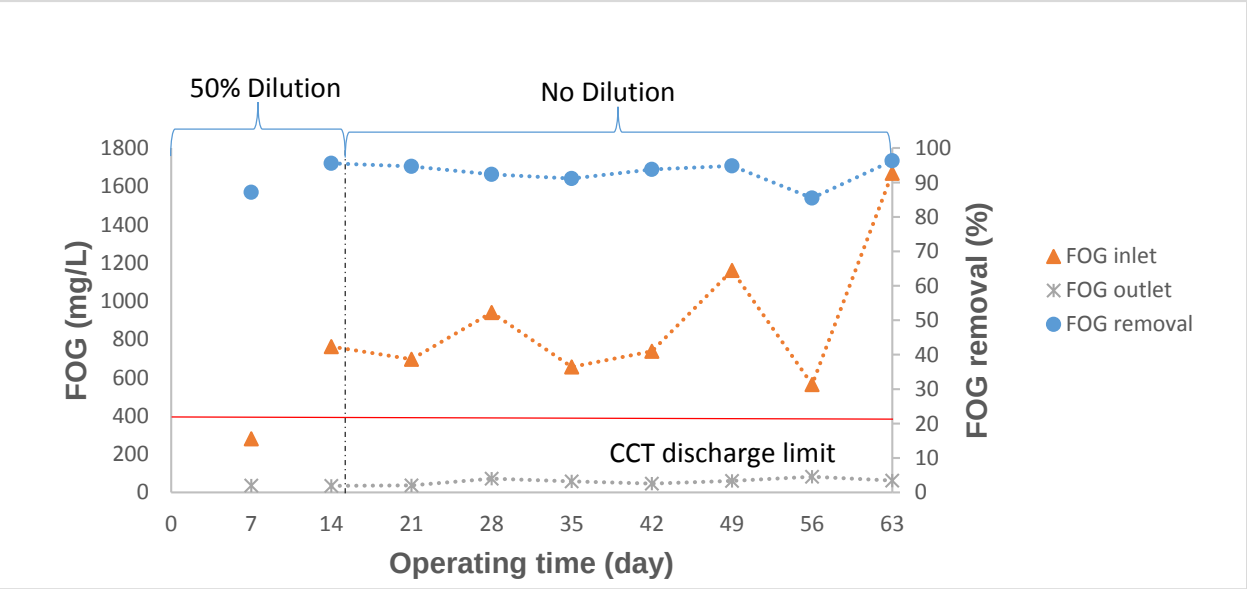


Figure 6.7: Variation of the FOG concentration and removal during the DEGBR operation

Another process that contributed to the overall reduction of FOG from the PSW was the filtration: a pre-treatment procedure was utilised, which is a standard practice even in industrial-scale treatment plants. Such pre-treatment of PSW with a filtration unit allowed for suspended solids reduction, FOG being reduced to avoid operational challenges such as the clogging of supply lines or the development of an inhibitory layer of FOG within of the DEGBR, which

would have culminated in the clogging of the recycle stream. This would have culminated in a weakened distribution of the organic matter and a weakened collection of the deemed biogas.

A slight decrease in the performance of the DEGBR was noticed at day 56, due to the reduction of the influent FOG concentration from a value of 1160 to 564 mg/L, which culminated in the FOG removal percentage dropping below 94.8 to 85.46%, a period identified to be associated to secondary and tertiary processes used in the slaughterhouse. However, the influent FOG concentration increased from 564 to 1668 mg/L at day 56 to 63, returning to the previously observed FOG removal efficiency of over 96 %.

Overall, the performance of the DEGBR on the removal of FOG was satisfactory.

6.3 Evaluation of the DEGBR stability during the PSW treatment

6.3.1 Evaluation of the pH variation during the DEGBR operation

In wastewater anaerobic treatment, the pH is used to assess and control the anaerobic activity inside a bioreactor. The recommended range is often at a pH of between 6 and 8, suggesting that the anaerobic activity could be jeopardised by a pH deviating from this range. In cases where a deviation is observed from this range, corrective strategies such as the addition of an alkaline or acidic solution can be used. Fig. 6.8 illustrates the pH values of the influent, as well as that of the effluent from the DEGBR throughout the PSW treatment operation. It was observed that the pH of the effluent from the DEGBR, which was assumed to be the pH inside the bioreactor, was maintained within CCT discharge limits, i.e. a pH range of 5.5 and 12. Furthermore, the effluent pH at the initiation of the operation slightly exceeded the prescribed anaerobic digestion range of 6 to 8; however, a reduction was observed within 7 days, an indication of the bioreactor stabilisation. This was without the supplementation of pH-rectifying additives, an illustration of a rapid stabilisation protocol for the DEGBR.

A slight deviation was observed from day 34 to 37, which corresponded to the period following a temperature offset due to a failure of the water bath that controlled the temperature inside the bioreactor. The incident happened at day 26 and persisted over a week. Thereafter, the water bath was changed and an increment of HRT from 35 to 40 hours was applied, to facilitate the system's adaptation as a result of the change in operating temperature. Such an anomaly can be said to mimic local conditions in South Africa, whereby intermittent electricity distributions are periodically observed and attributed to inadequacy of coal supplied to the sole electricity producer in the country. However, the change in HRT did not significantly affect the pH inside the bioreactor. An expected deviation in pH from this range would have resulted in the development of conditions detrimental to the anaerobic biomass required for an effective

anaerobic digestion. An example of the effect of the change in pH in the DEGBR is illustrated in Fig. 2.2, whereby pH-dependent competition between the sulphate-reducing bacteria and methane-forming bacteria is depicted, suggesting a domination of sulphate-reducing bacteria over methane-forming bacteria if the pH were out of the prescribed range, which subsequently would have led to an increase in the formation of hydrogen sulphide, an undesirable toxicant with the ability to inhibit the anaerobic biomass (Gerardi, 2003; Henze *et al*., 2008; Metcalf & Eddy *et al*., 2003). Moreover, the maintenance of the pH in the prescribed range illustrates that a low concentration of non-dissociated VFAs in the effluent or bioreactor content was present. In high concentrations, these can be inhibitory to methanogens. An important stage in anaerobic treatment of the PSW is hydrolysis, whereby polymeric organic matter is transformed into simple monomeric matter, a process bio-catalytically facilitated by fermentative bacteria. Therefore, the bioreactor's pH, along with other environmental parameters listed in sub-section 2.4.2.1, plays an important role in the inducement and therefore maintenance of this phase that influences subsequent biological reactions and/or phases of the anaerobic digestion, i.e. processes required for the transformation or the removal of organic matter from the PSW. As previously highlighted, hydrolysis can be a rate-limiting phase in the performance of overall anaerobic digestion; highlighting the importance of controlling and stabilising the pH.

Furthermore, it was observed that the alkalinity of the influent was lower than the effluent, during the operation of the DEGBR. The pre-acidification of the PSW inside the holding tank used to supply the feed could partially explain the stabilisation and consistent pH maintenance observed. However, during phase transitions of anaerobic digestion, the production and consumption of various compounds in the bioreactor contributed to an increase in pH, which was hypothesised to be an indication of the consistent maintenance of metabolic activity inside the DEGBR, despite changes in the OLR used.

A suitable pH range in anaerobic digestion is often reported to be 6.8 and 7.2 (Gerardi, 2003), with other studies indicating this range to be 6 to 8 (Henze *et al*., 2008; Oh, 2012). In this study, the pH was not rectified throughout the DEGBR operation. Since the pH was analysed at room temperature, i.e. at temperatures of 18.5 to 24^{o}C; the real pH values inside the DEGBR were most likely slightly lower than those recorded during analysis, as there is a correlation between pH and temperature (Metcalf & Eddy *et al*., 2003), suggesting that the actual pH range could fall into the narrowly prescribed range, as the DEGBR operating temperature was 35^{o}C.

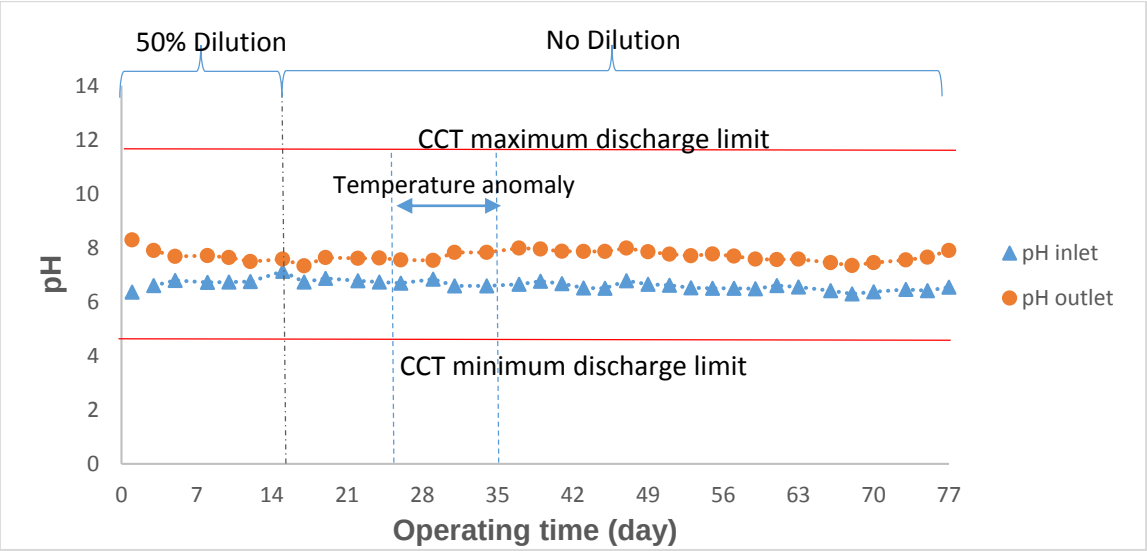


Figure 6.8: pH variation during the DEGBR operation

6.3.2 Further assessment of the DEGBR stability

Further parameters, such as the ratio of the concentration of VFAs to the alkalinity as well as the organic matter biodegradability efficiency, can be used to evaluate the stability and efficacy of an anaerobic system for the treatment of specific types of wastewater. Researchers prescribe that a VFA/alkalinity ratio lower than 0.3 is required for an adequate methanogenic activity (Gerardi, 2003; Oh, 2012). Thus, the VFA/alkalinity ratio observed for the DEGBR used, as illustrated in Fig. 6.9, was indicative of excellent methanogenic activity as the VFA/alkalinity ratio remained below 0.3, with values ranging between 0.044 and 0.12, even under a temperature instability phase, when the ratio reduced from >0.1 to 0.044. This indicates stable PSW digestion conditions.

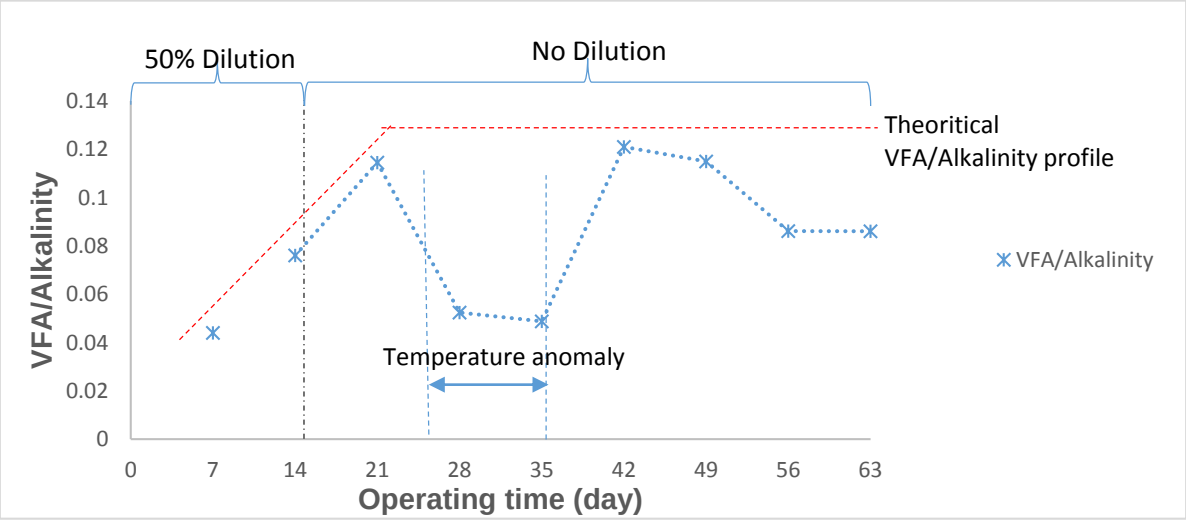


Figure 6.9: VFA/Alkalinity variation during the DEGBR operation

Similarly, the digestibility of the organic matter in the PSW was indicated by the ratio of the BOD_5 to the tCOD of the DEGBR influent (Metcalf & Eddy *et al.*, 2003), which serves to quantitatively assess the biodegradation capacity of a bioreactor (as depicted in Fig. 6.10). This varied during the first 9 weeks of operation; with this ratio ranging between 0.49 and 0.75. During bioreactor operation initiation, the lowest biodegradability values were recorded, although the feed was diluted to minimise shock loading.

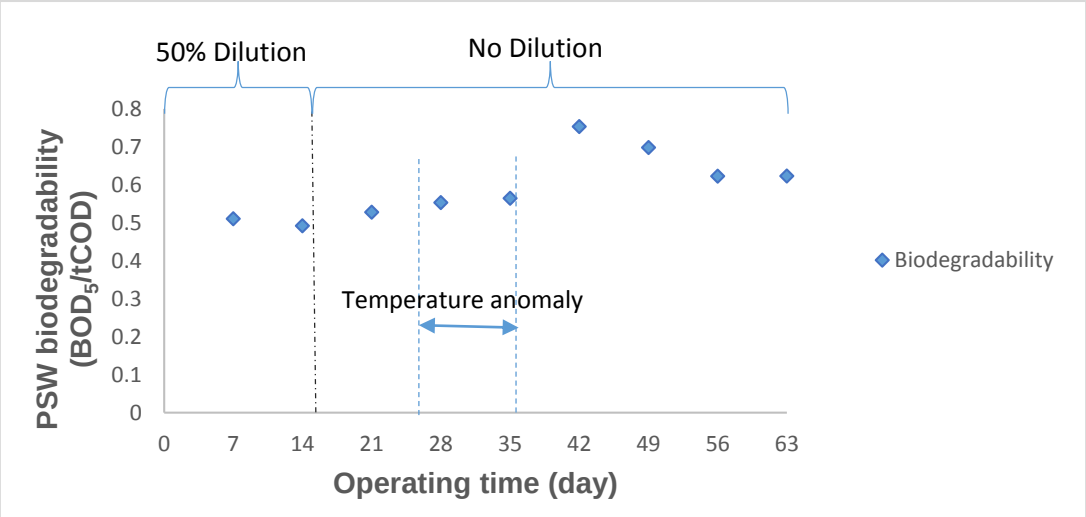


Figure 6.10: Variation of the PSW biodegradability during the DEGBR operation

6.4 Evaluation of the effects of the variation of the HRTs, OLRs and recycle stream up-flow velocity on PSW treatment using the DEGBR

One factor that motivates for the selection of HRABs is their robust minimisation and response to variations, including high OLRs, which can be modified for a specific type of wastewater by changing the HRT. Numerous HRTs (n=4) were utilised throughout this study, i.e. 35, 40, 30 and 24 hours. It was observed that this change in HRT didn't produce a similar trend to that observed for OLRs, although the reduction of the HRT, to some extent, contributed to an increase in the response of the OLRs into the system. This differentiation in trends for the two parameters, (OLR and HRT) can be explained by the tCOD concentration of the influent that was an independent input parameter, i.e. varying in concentration due to a variation in the PSW quality during sample collection from the slaughterhouse. This is due to COD concentration variation due to fluctuations of the number of birds processed and therefore the amount of organic matter in the wastewater. It is also logical to assume that the wastewater sampling during the bleeding of the birds resulted in a PSW with a higher organic matter concentration than in PSW sampled during operations such as packaging, cutting and deboning.

Another parameter that strongly influences the OLRs is the dilution of the feed during acclimatisation, which lasted 15 days. Dilution of the PSW contributes to reduced OLRs by reducing the tCOD concentration of the influent, with the tap water being used having minimal concentrations of tCOD. The ascending profile of the OLR in Fig. 6.11 on day 15 was not related to the progressive reduction of the dilution ratio, but rather to the progressive increase of the concentration in tCOD in the samples collected from the slaughterhouse; an indication of the direct influence of operational activities within the slaughterhouse on the quality of the PSW collected.

As the initial HRT of the PSW in the system was very low, implementation of a PSW dilution operational strategy was advisable. By inoculating the diluted PSW at 15 days, consistent system performance quantified using effluent turbidity and other parameters, was indicative of conserved high removal efficiency, irrespective of heating, which was reflected by temperature maintenance anomalies experienced, as depicted in Fig. 6.14. After an increase in the HRT due to an operational problem related to the operational temperature offset, the system adaption was swift, with minimal consequent effects due to the offset, even after 7 days. The continuous stability of the system even under decreased HRT, i.e. with increased OLR, is depicted in Fig. 6.11.

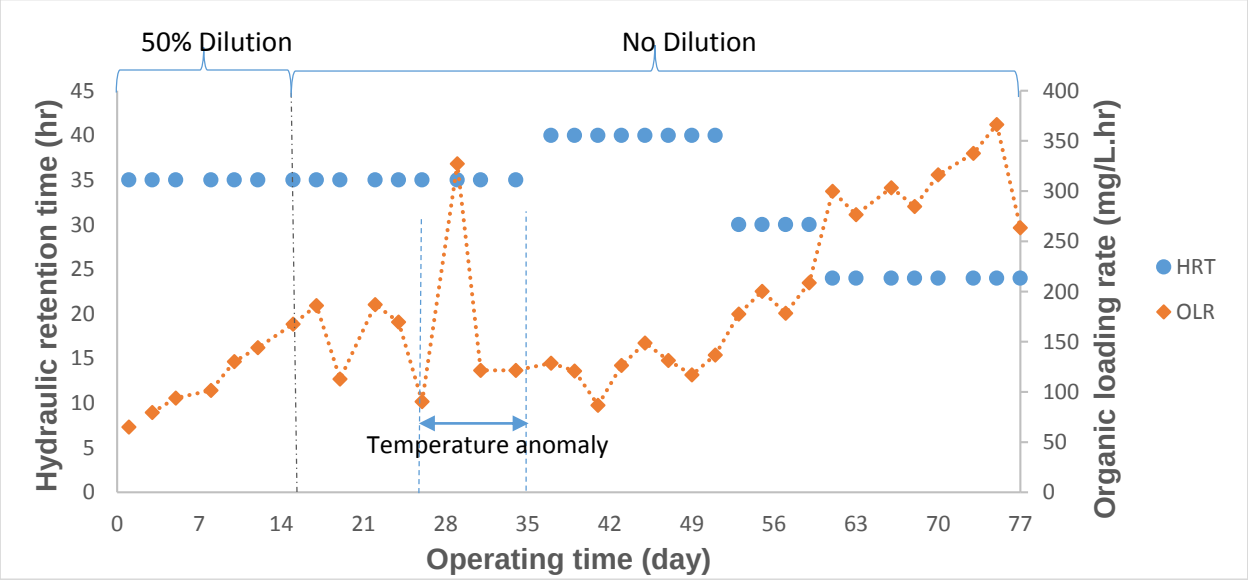


Figure 6.11: Variation of the HRT and OLR during the DEGBR operation

As with the HRT, the recycle stream up-flow velocity was progressively changed throughout the operation of the DEGBR. The recycle stream was utilised to improve the distribution of the substrate across the anaerobic granular bed, culminating in partial fluidisation; with minimal consequential influences on the tCOD degradation from day 56 to 77. Since the up-flow velocity did not influence the OLR of the system, it was expected to contribute to the

improvement of substrate distribution and thus availability for biodegradation. Figure 6.12 illustrates the comparative recycle stream up-flow velocity with OLRs used in this study.

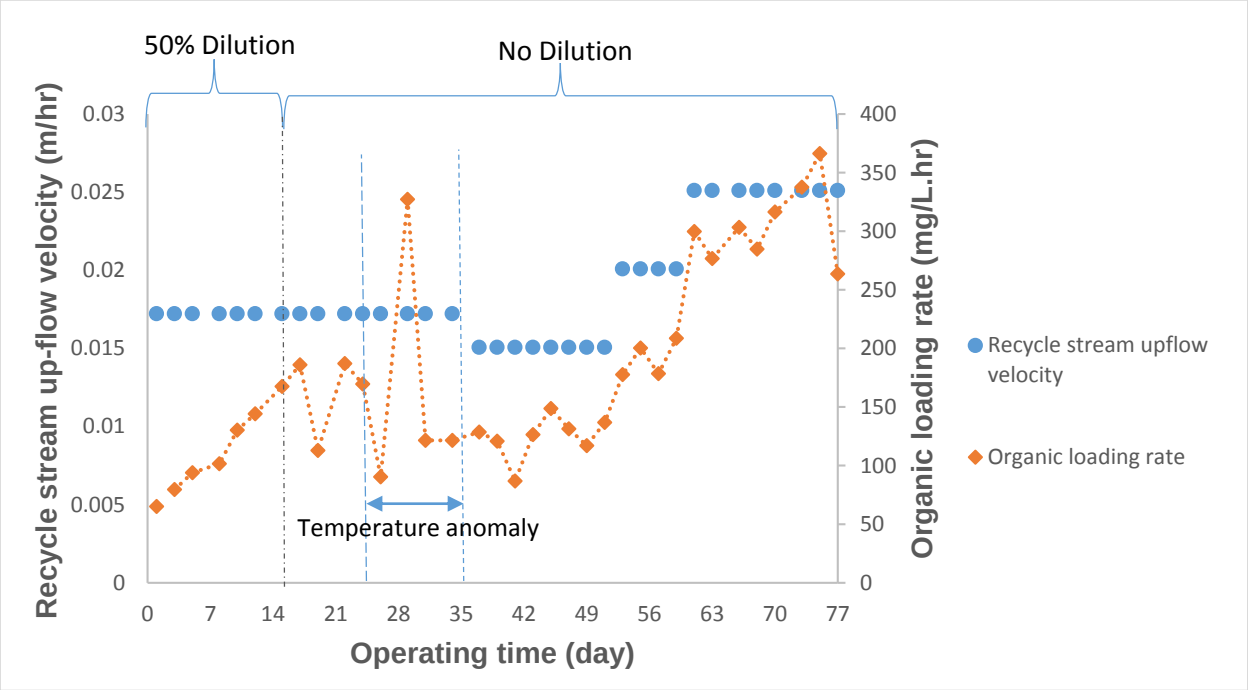


Figure 6.12: Variation of the recycle stream up-flow velocity and the OLR during the DEGBR operation

The initial recycle stream up-low velocity was 0.017 m/hr, which was reduced to 0.015 m/hr during the periodic temperature anomaly, which further increased to 0.02 m/hr and finally 0.025 m/hr after day 60 of operation. The effect of the recycle stream up-flow velocity on the overall performance of the DEGBR is illustrated in Figure 6.13, with the implementation of incremental changes in the up-flow velocity, resulting in a minute reduction of the performance of the DEGBR, particularly when the sCOD was used as the monitored parameter, i.e. whose percentage removal was reduced, indicative of reduced biomass (anaerobic sludge) organic matter content, thus reduced bio-catalytic activity. This deviation was not observed after the decrease of the up-flow velocity, suggesting that the quality of the distribution of the organic matter and thus interaction with the biomass has direct effects on the performance of the bioreactor. Overall, the variation in the recycle stream up-flow velocity didn't adversely affect the performance of the DEGBR, with increment increases being within a range suitable for a slight granular bed expansion and a corresponding increase in activity of anaerobic granules, which hypothetically improved the contact time between the organic matter and the biomass, which in turn could result in an improved degradation of the organic matter and therefore improved removal of tCOD from the PSW.

The configuration of the DEGBR combines the organic matter distribution features of down-flow and up-flow HRABs for improved operability and the resolution of some challenges associated with head losses across anaerobic granules, as demonstrated in Chapter 5.

Furthermore, the effect of the OLR on the overall performance of the DEGBR was minimal (Figure 6.14), with lower performance efficiency when the PSW was diluted with higher percentage removal (75 to 99.8%).

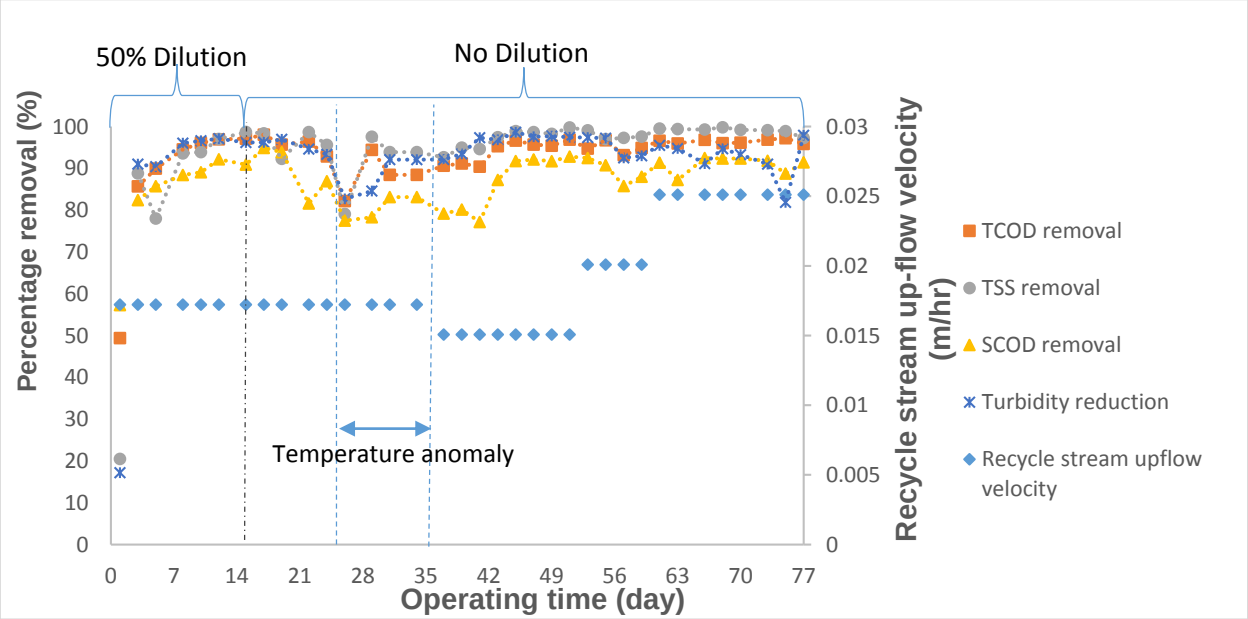


Figure 6.13: Variation of organic matter removal with changes in the recycle stream up-flow velocity during the DEGBR operation

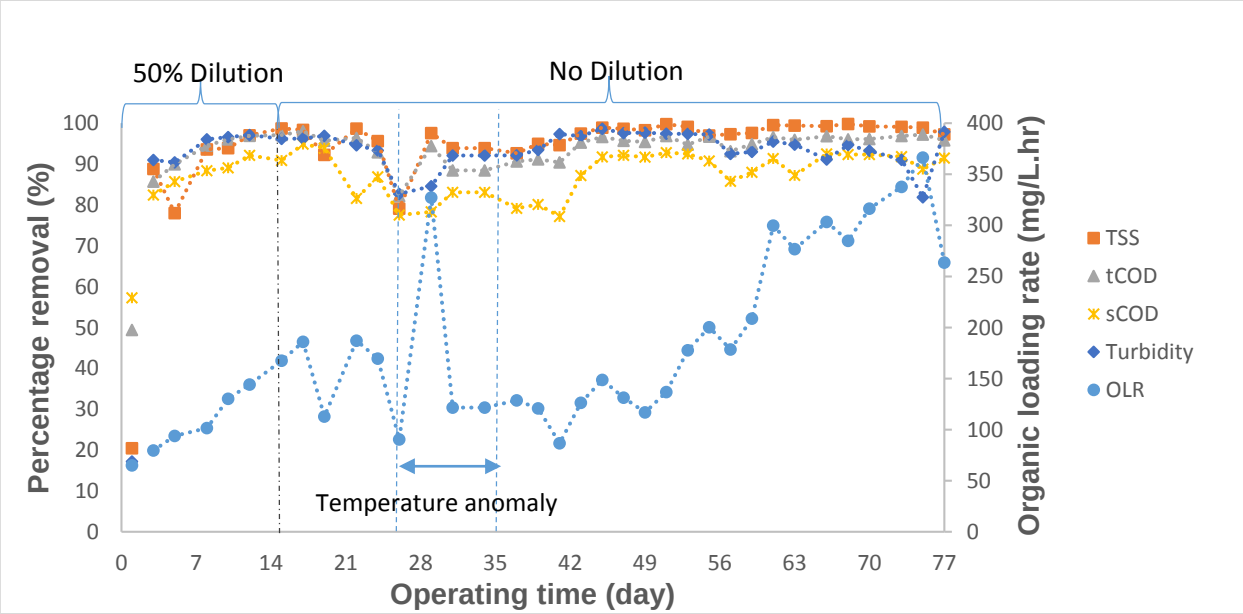


Figure 6.14: Quantification of organic matter removal with the change of OLR during the DEGBR operation

6.5 Variation of the conductivity and the salinity of the DEGBR influent and effluent during the PSW treatment

The ability of a substance to conduct electricity is measured in terms of conductivity. The CCT imposes an effluent conductivity discharge limit of 500 mS/m, which corresponds to a value of 5000 µS/cm. The highest effluent conductivity value reached during the DEGBR operation was 3770 uS/cm (see Fig.15). During the DEGBR's operation, conductivity was not directly related to reduction of TSS, FOG or COD, as these were significantly reduced during the DEGBR operation, with minimal influential and/or associated variation in the conductivity observed in the influent. Since conductivity has a linear relation with the concentration of dissolved ions (Metcalf & Eddy *et al*., 2003), as demonstrated in Figure 6.16, a correlative TDS concentration reduction in the effluent from the DEGBR operation.

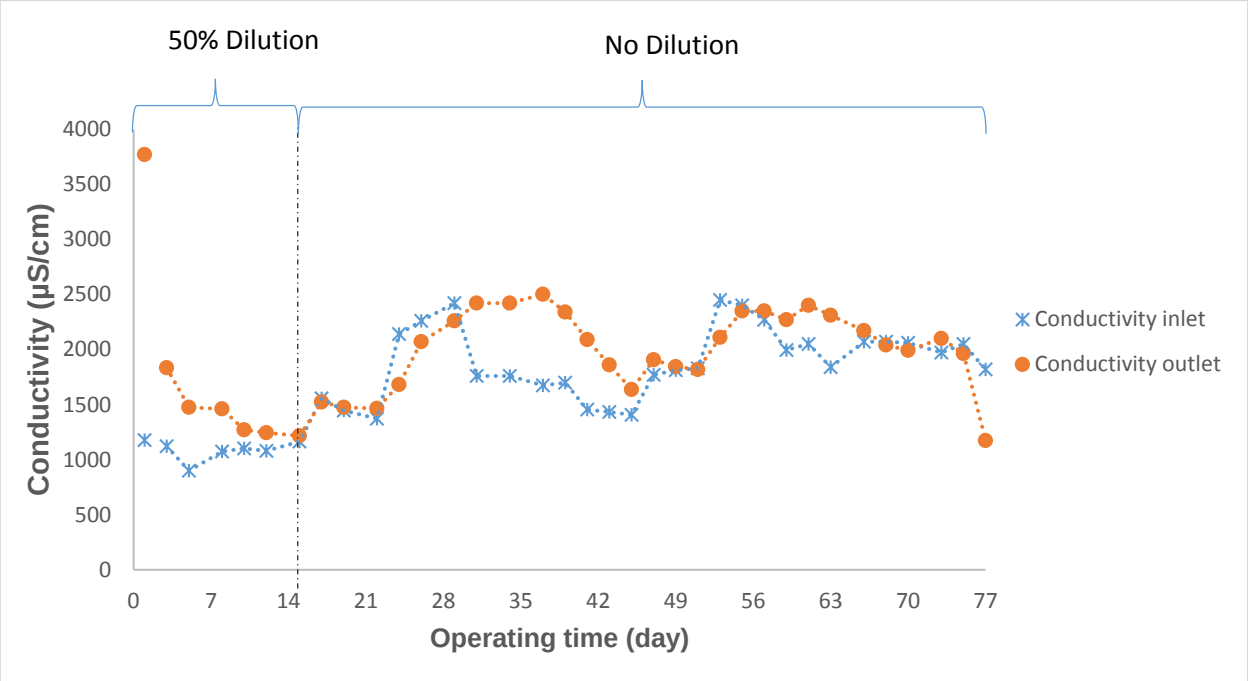


Figure 6.15: Variation of the conductivity of the DEGBR influent and effluent during the operation of PSW treatment

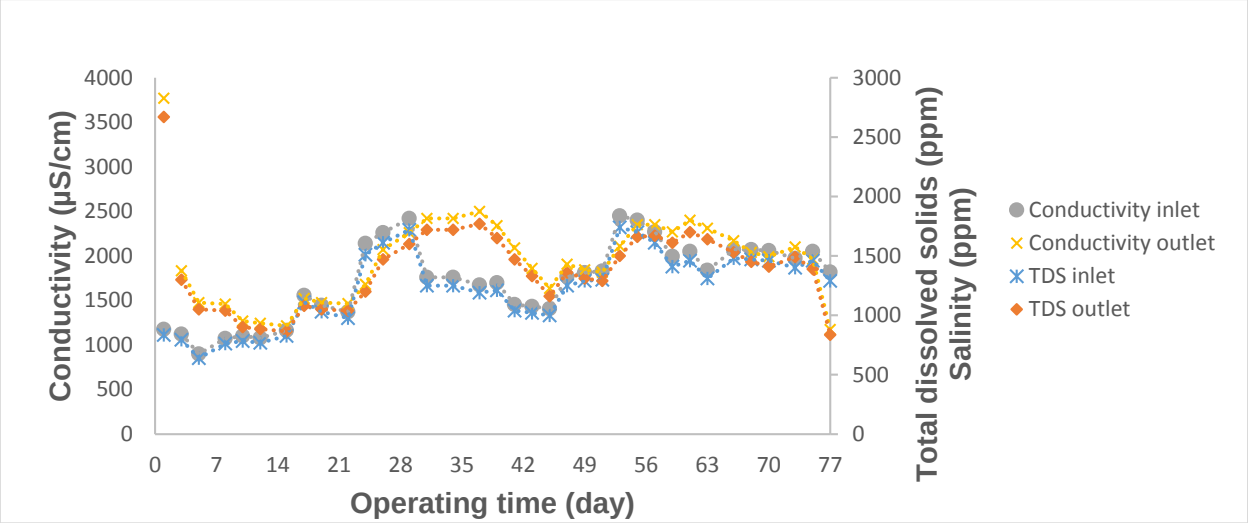


Figure 6.16: Comparison of the variation of the influent and effluent conductivity values and TDS concentration

Furthermore, it was observed (Fig. 6.17) that the variation of salinity in the DEGBR effluent depicts a similar response profile to that of conductivity, suggesting causality effects between the salinity and thus conductivity, although there was a reduction of the concentration of the BOD_5, COD, FOG and TSS, and inorganic salt accumulation could result in the regenerative ability of the granular sludge used. Although the evaluation of the salinity remains an important parameter to monitor, its influence on the PSW tCOD reduction was not directly observed (Metcalf & Eddy *et al*., 2003). Thus, the accumulation or transformation of some dissolved salts during the PSW digestion, which can be attributed to plumping of poultry products, must be evaluated in further studies, as high salinity may reduce the treatment efficiency of HRABs operated for elongated periods, with negative effects on the ecosystem.

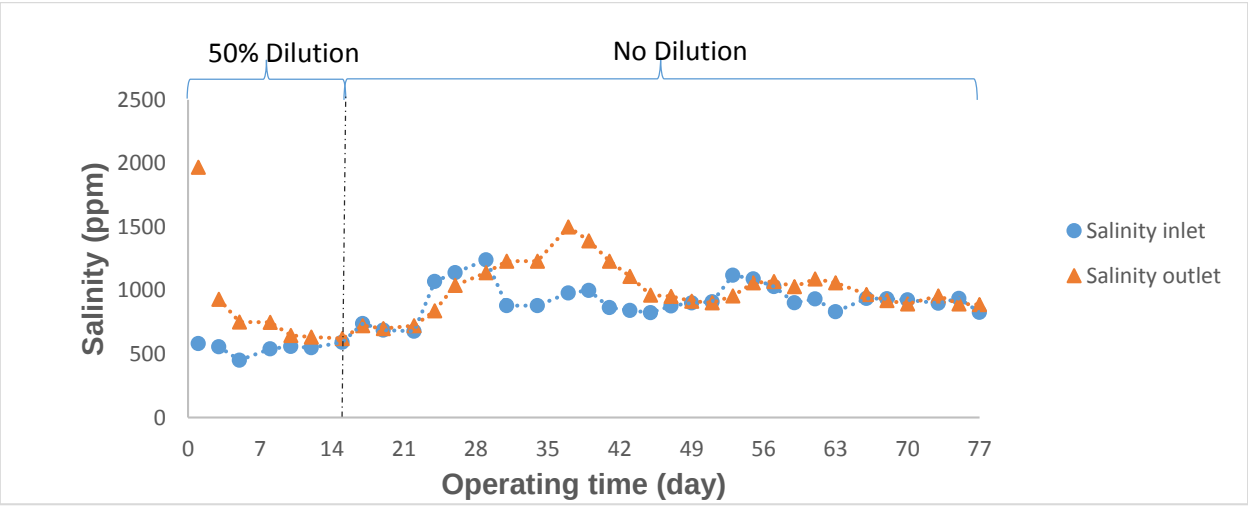


Figure 6.17: Variation of the DEGBR influent and effluent salinity during the operation of PSW treatment

6.6 Auxiliary parameter outcomes used for DEGBR performance evaluation in PSW treatment

6.6.1 Turbidity reduction

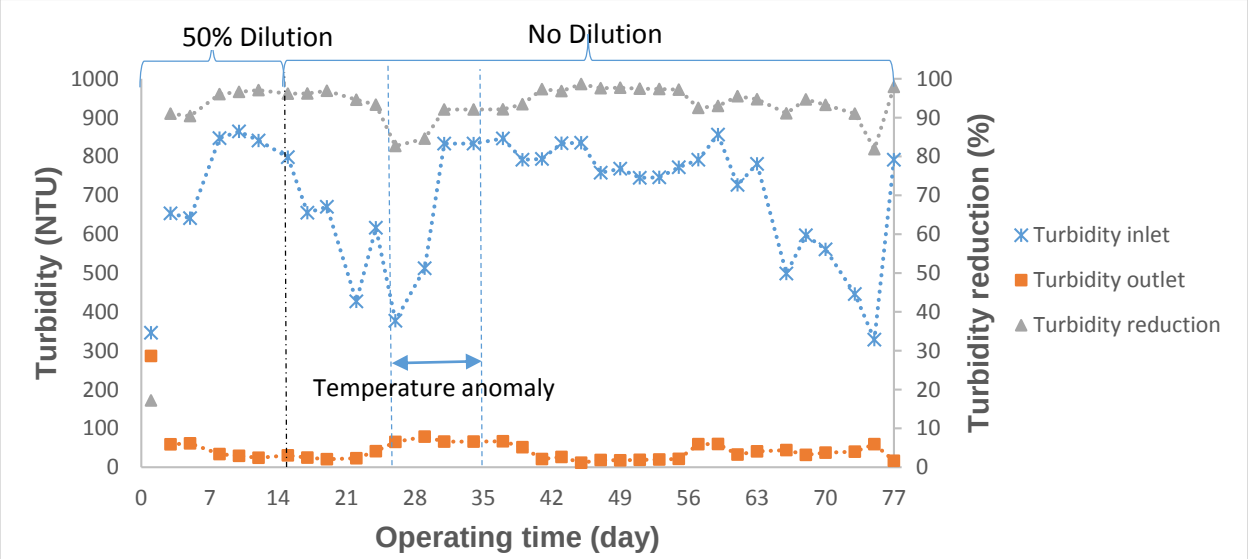


Figure 6.18: Variation of the turbidity during the DEGBR operation

Turbidity removal using the DEGBR showed appreciable results throughout its operation, with removal efficiency being 82 to 98.6%, (see Figure 6.18). Although turbidity values of the influent fluctuated, the outcomes were consistent with effluent NTU values, an indication of adequate stability of the system. Furthermore, the pre-treatment operation applied, i.e. filtration prior to the feed being stored in a holding tank, contributes to system stability. The filtration unit, consisting of 9.51 mm mesh, enabled the retention of coarse solids, feathers and a portion of the FOG. From day 3, turbidity was under 100 NTU, which was below the recommended level required for industrial effluent. Furthermore, as depicted in Figure 6.20, samples of the influent and effluent taken randomly during the operation of the DEGBR on PSW treatment, illustrated the turbidity difference, and thus calorimetric appeal, between the bioreactor feed and product, with the DEGBR product demonstrating greater clarity than the feed.

Furthermore, the percentage turbidity reduction was compared to the percentage removal of the TSS, as these parameters are interrelated, as both do have an impact on the appearance and therefore the clarity of the PSW. Figure 6.19 displays similar phase variations and thus response throughout the DEGBR operation, suggesting that the TSS and turbidity are semi-dependent parameters, with the TSS concentration having a direct influence on the turbidity values. The deviation observed at day 29 was directly influenced by temperature offset (anomaly initiated at day 26), with the deviation observed at day 5 being directly related to the

acclimatisation phase, whereby the granular sludge was inoculated, thus being exposed to atmospheric oxygen, and culminating in the outer biomass being sloughed off due to deactivation.

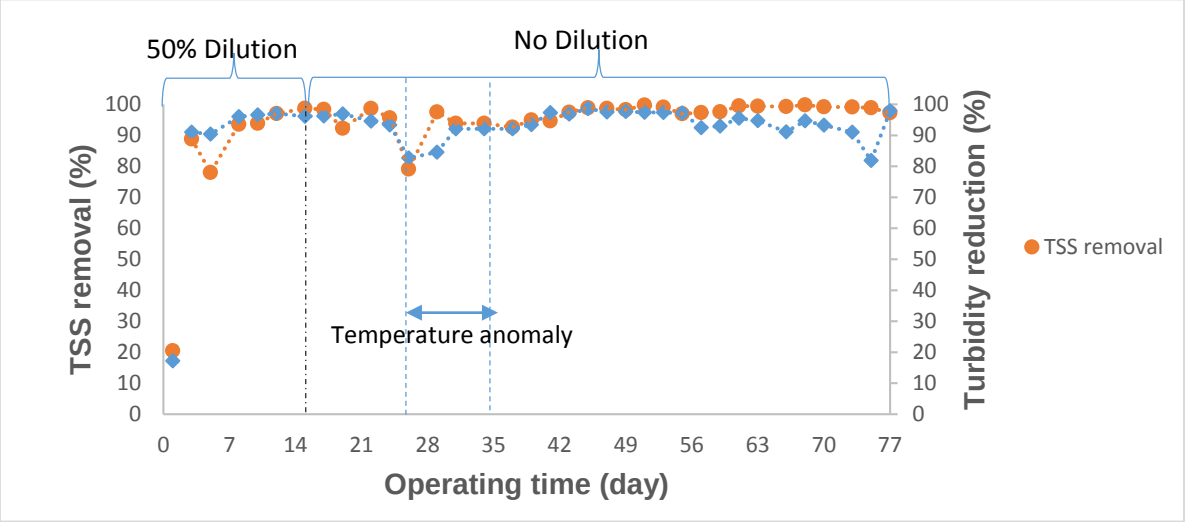
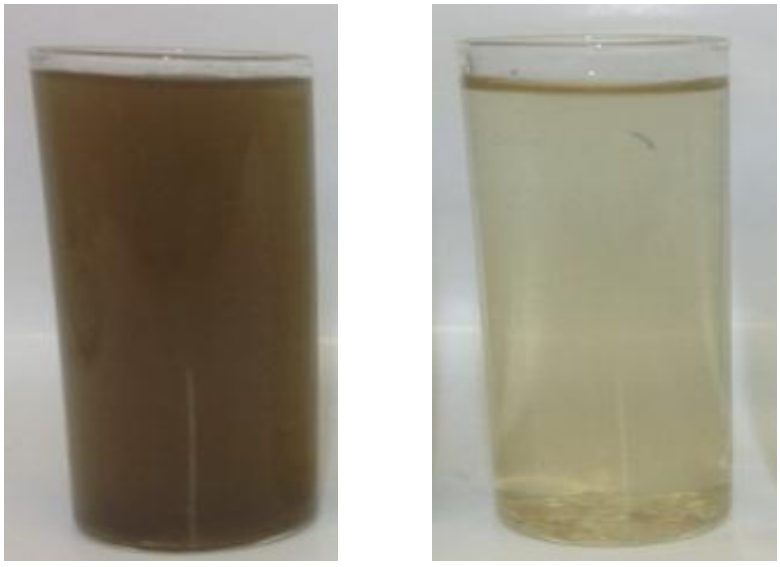


Figure 6.19: Comparison between the variation of the TSS removal and the turbidity reduction during the DEGBR operation



(a) DEGBR Feed (b) DEGBR product

Figure 6.20: Illustration of the change of the quality of the PSW after treatment

6.6.2 Total suspended solids and total dissolved solids removal

The TSS concentration plays a significant role in the appearance of the PSW and consists of particles > 2 µm. Particles < 2 µm are considered as dissolved solids. Algae, bacteria and other inorganic matter can also be assumed to constitute and/or contribute to the composition of TSS (Metcalf & Eddy *et al*., 2003). However, for the pre-treated PSW, most of this particulate matter would have been organic in composition with their size comprising material larger than the aperture size of the screen used. For PSW, the suspended solids include meat debris, solidified FOG, feathers, and faeces. However, minute quantities of inorganic particles such as dunes can be found in the influent PSW, as these accumulate in wastewater while sanitising the poultry slaughterhouse facilities and/or equipment. Generally, in wastewater treatment, the concentration of TSS gives an estimate of the quantity of undissolved and polymeric materials. For Fig. 6.21, the lowest values of TSS removal were observed during the acclimatisation phase, until day 15, when undiluted PSW was used in the system, obtaining a satisfactory result of 98.7% TSS removal by day 15, an outcome maintained at above 90% for the remainder of the experiment. Small deviations were observed at day 26 due to temperature related to temporary disruptions, with a TSS reduction to 79%, with minimal severity. This shows that the anaerobic granular biomass remained sufficiently active under various temperature conditions, defined as psychrophilic, mesophilic or thermophilic conditions, though the mesophilic conditions were previously cited as the most suitable for methanogenic activity (see section 6.7).

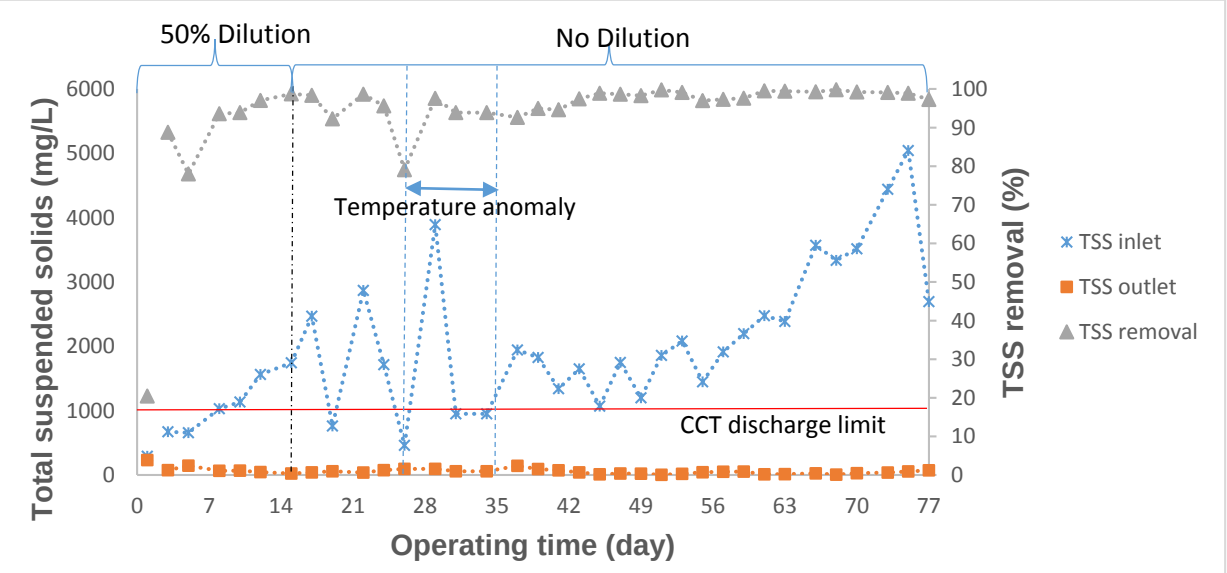


Figure 6.21: Variation of the TSS concentration during DEGBR operation

Furthermore, due to the stability of the process designed, minimal impact was observed even when TSS fluctuations were observed in the influent. It is recommended that future studies assess even higher influent TSS concentrations.

CCT discharge standards were met, with TSS concentration always being within the recommended discharge limits of 1000 mg/L, as depicted in Figure 6.21 and 6.22, although the PSW quality characteristics were observed to exceed this value during the processing of a large quantity of birds, i.e. slaughtering.

For TDS concentration (Figure 6.22), the reduction was minute, an indication of soluble inorganic salt having a higher traversing potential and thus reduced reductability using the DEGBR. Furthermore, anomalies were observed, as the TDS concentrations of the effluent were cyclically higher than those of the influent during some phases, depicting ab-/de-sorption phases previously unreported in HRABs. Variability can hypothetically be directly influenced by sloughing-off of biomass and EPS due to biotic salinity intolerance, thus biomass analysis, as observed by smaller particle dejection that contributed to an increase in the concentration of TSS, when inorganic salt accumulation exceeds the osmotic pressure tolerance of the external biomass of the granules.

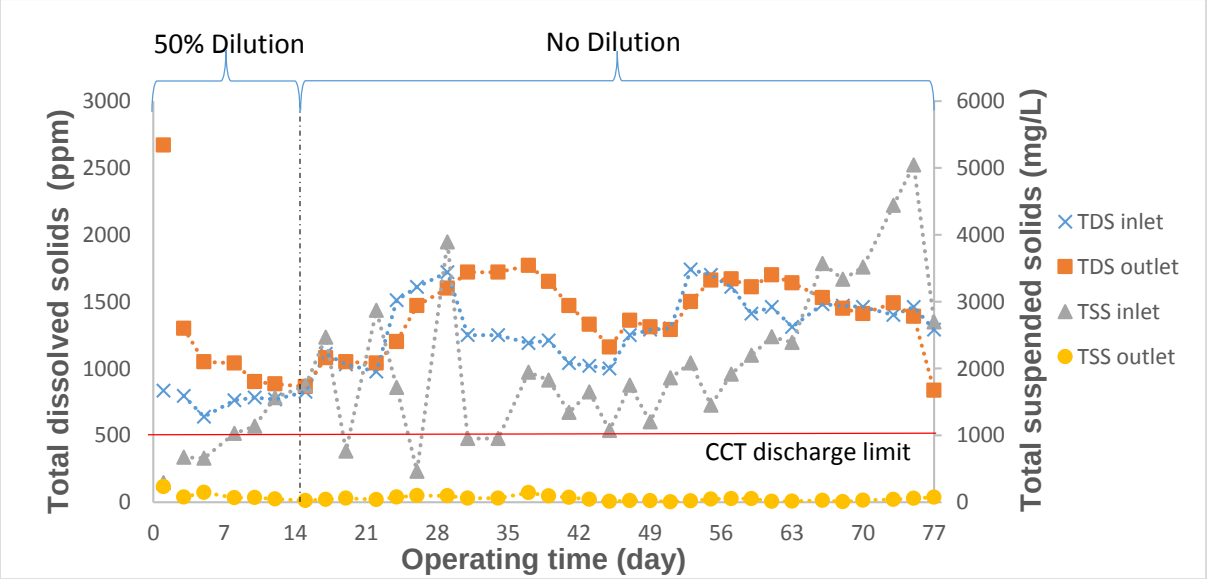


Figure 6.22: Comparison between the variation of the TDS and TSS concentrations during the DEGBR operation

6.7 Evaluation of the DEGBR biogas production during PSW treatment

A water displacement system, described in section 4.4.4, enabled the collection of biogas from the DEGBR during its operation. Figure 6.23 depicts the variation in the production of biogas, along with the variation of the removal percentage of tCOD and sCOD. A significant variation

or decrease in biogas production was noticed from day 26, which corresponded to the day at which a significant temperature offset affected the stability of the DEGBR. Increasing the influent HRT to allow the system to adjust from the interruptive change, did not consequentially result in increased biogas, but rather a weakened production of biogas, confirming that it was an important design parameter to maintain mesophilic conditions adequate for methanogenic activity and therefore biogas production.

Furthermore, competitive analysis between biogas production and OLR (Figure 6.24) post the acclimatisation phase, was indicative of the production of biogas to the OLR. Similarly, temperature anomalies (day 28), which had a direct influence on the methanogenic activity, influenced OLR conversion rates and therefore biogas production. For other HRABs, biogas entrapment within the granular bed, as detailed in Chapter 5, remains a challenge. This might be ameliorated with the DEGBR, since it incorporates features such as the reduction of biogas entrapment. As discussed in section 2.8, the biogas production can be related to the tCOD degradation from the influent, hence the correlation between the OLR and the biogas production. Moreover, biogas production is a critical factor demonstrating good anaerobic and methanogenic activity, as a lack of production of biogas highlights an operational problem that can be related to the accumulation of toxicants, a change in operational temperature or a weak distribution of the substrate. The common challenge of weakened distribution of organic matter to the biomass was previously discussed in Chapter 5 and resolved through the recycling of the PSW to other sections of the granular bed, whereby minimisation of the pressure drop across the granular bed could easily minimise biogas entrapment. Subsequently, the effect of the anaerobic granules packing to the percolation of biogas bubbles was explained in Chapter 5 as one of the factors preventing the evolution of biogas bubbles in static granular beds, requiring intermittent fluidisation of the granular bed to enable the dispersion of toxic substances and the collection of biogas bubbles. Thus, these considerations allowed a continuous and consistent collection of biogas during the DEGBR operation.

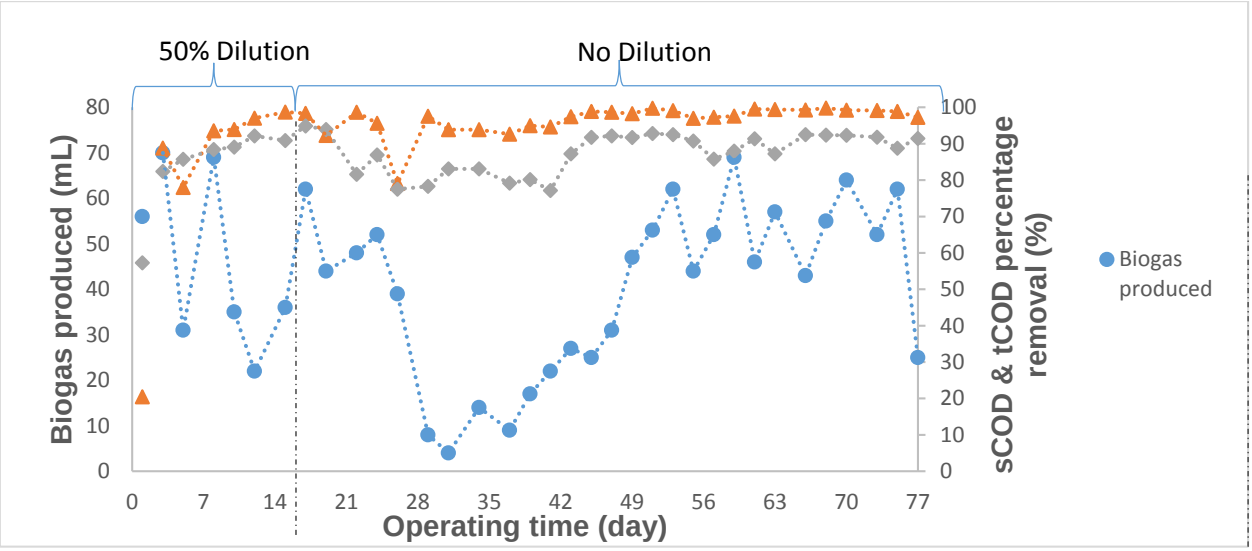


Figure 6.23: Evaluation of the biogas produced in relation to the sCOD and tCOD percentage removal during the DEGBR operation

The biogas produced was collected in a Tedlar bag and analysed for its composition. The outcome from these analyses indicated that the biogas was composed of:

- 40.8% of CH_4,
- 3.6% of CO_2,
- 12.1% of O_2,
- 0.5 % of H_2,
- 0% of H_2S, and
- 43% of other gases.

It was noticed from this composition that the yield of methane was not as high as expected (60 to 75%), and could be justified by the presence of oxygen, which was as high as 12.1% of the volume of biogas analysed. This high concentration of oxygen was probably related to either unpurged content with nitrogen prior to its operation, as nitrogen purging enables the removal of undesirable gases such as oxygen prior to the operation of the bioreactor, or the inadequacy of the method used for biogas collection causing oxygen leakage into the system. However, the high production of methane suggested a good methanogenic activity that was promoted by the lack of dissolved oxygen in the granular bed. Thus, the high concentration of oxygen in the biogas might also be justified by the penetration of air through the upper part of the bioreactor, or through the water displacement system, highlighting the importance of ensuring that the bioreactor remain completely sealed during the anaerobic digestion. Following the assumption of air entering through a part of the system, nitrogen might be the gas dominating the composition of the 42.8% of other gases composing the analysed biogas.

Scrubbing reduced H_2S to a minimum desired from these analyses, suggesting that the biogas scrubber efficiently removed the H_2S, although traces H_2S are always listed in the composition of the biogas (Gerardi, 2003; Metcalf & Eddy *et al.,* 2003). Furthermore, 3.6% of CO_2 was indicative of effective conversion by the barrier solution (5% solution of KOH).

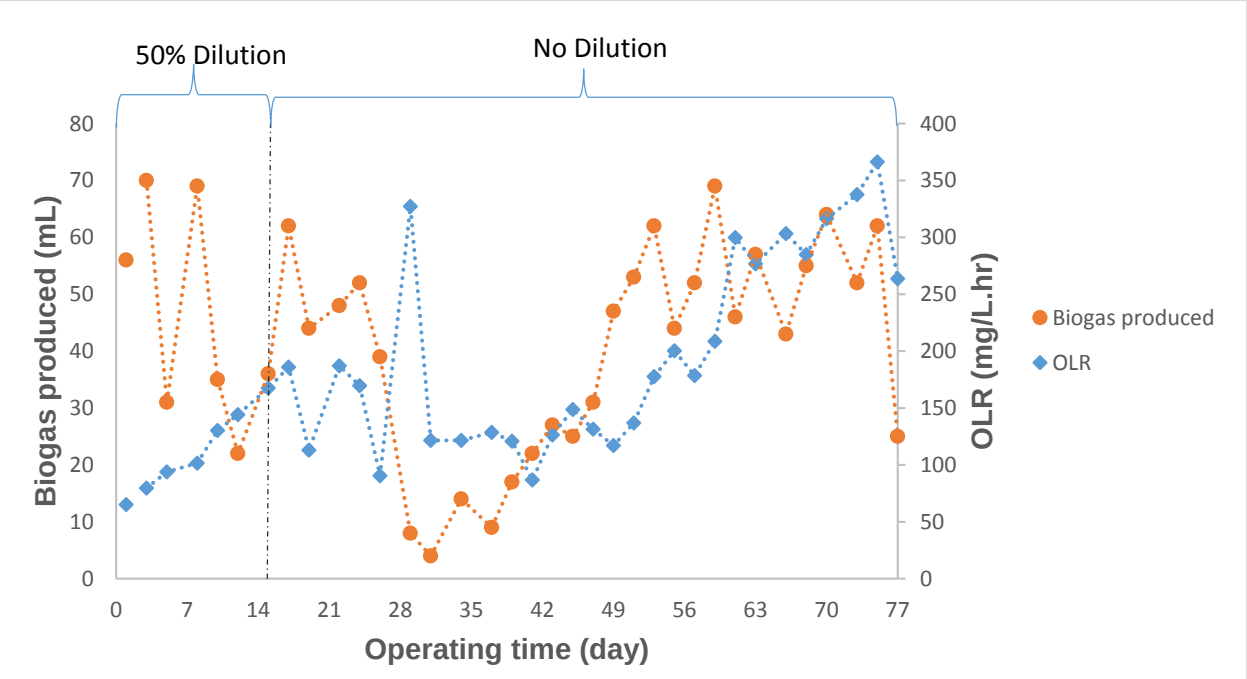


Figure 6.24: Evaluation of the biogas produced with the variation of the OLR during the DEGBR operation

6.8 Summary

Overall, the DEGBR showed results deemed appropriate for an effectively designed HRAB for the treatment of PSW. A comparison between Table 2.7 and 6.1, which respectively provide the performance of technologies used for the treatment of PSW and the summary of the performance of the DEGBR on the treatment of PSW, proved that the DEGBR is an option which can further improve the treatment of PSW or similar types of wastewater, as its performance remained steadily robust for the different parameters analysed. Furthermore, there was a consistent production of biogas, with mesophilic temperature range being proven suitable for biogas production. Moreover, these results served to confirm the assumptions made in Chapter 1 and thus satisfied the objectives of this research.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

Chapter 7 : CONCLUSIONS AND RECOMMENDATIONS

The development of HRABs enables the improvement in performance of such systems for the treatment of various types of wastewater. The treatment of PSW was studied for this research study, and shortcomings, such as the washout of anaerobic granules in up-flow HRABs, the weak distribution of the organic matter to the biomass, biogas entrapment within the anaerobic biomass, the poor dispersion of toxicants, as well as the clogging of the underdrain system in down-flow HRABs, were considered for the design of the DEGBR, which is a novel HRAB.

The evaluation of the suitability of packing materials (ceramic marbles, white pebbles, small and medium-sized pumice stones and pea gravel) for the underdrain system was implemented through a series of methods, such as the determination of the porosity, the permeability, the pressure loss induced, as well as the sludge retention capacity, which were used to complement factors such affordability, availability and the inertness of the materials used. Results from this evaluation indicated that the medium-sized pumice stones were the most suitable packing materials for use in the underdrain system for the DEGBR.

This packing material evaluation was followed by the study of the effects of the head loss across a static anaerobic granular bed, with demonstrations indicating that the substrate was not evenly distributed as the pressure drop induced by the packed anaerobic granules reduced the kinetic energy of the PSW flowing through the bioreactor. This effect was demonstrated by Eq. 5.8, which showed that the velocity of the PSW was being reduced by the height of the granular bed, thus highlighting the importance of a recycle stream to improve the distribution of the substrate, as implemented with the DEGBR configuration.

Furthermore, it was also demonstrated that the packed structure of the granular bed affected the evolution of the biogas bubbles as the height of the granular bed increased, a phenomenon which must be overcome by biogas bubbles, as elucidated by the Laplace-equation and Eq. 5.8. Therefore, intermittent expansion of the granular bed using a PSW redistribution system, designed to expand it, was suggested to overcome this challenge of biogas entrapment, which could also result in the poor dispersion of toxicants, as gases such as H_2S and NH_3, which are considered toxic to anaerobic biomass, could accumulate in the methanogenic bed, and thus sour it. Therefore, the release of biogas bubbles also enabled the dispersion of other gases that may inhibit methanogens through adequate shifting of the operational pH.

Moreover, the evaluation of the performance of the DEGBR for the treatment of PSW was executed, with the result that the bioreactor showed excellent performance in the removal of

organic matter from the PSW, with average percentage removal of 95.68 ± 3.63%, 88.75 ± 5.12, 98.59 ± 4.54%, 93.77 ± 3.57% and 97.44 ± 5% for the tCOD, sCOD, BOD_5, FOG, and TSS, respectively. This performance translated to average biogas production of 44 ± 18.55 mL/day, for a biogas composition that included 40.8% of CH_4, 3.6% of CO_2, 12.1% of O_2, 0.5% of H_2 and 0% of H_2S, suggesting a good efficiency from the biogas scrubber. The high concentration of oxygen suggested that part of the DEGBR had atmospheric leakage, or that nitrogen purging was required prior to the operation of the DEGBR, as the continuous presence of dissolved oxygen in the anaerobic granular bed would have inhibited the methanogens, and thus limited the production of biogas. Therefore, it is recommended to ensure that the DEGBR is completely anaerobic to minimise oxygen penetration into the system during the operation, or that nitrogen purging could be implemented.

Overall, the DEGBR showed good results when compared to other HRAB technologies used for the treatment of PSW in previous studies, suggesting that its use for the treatment of such types of wastewater could obviate the recourse to potable water.

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APPENDICES

Appendix A: SELECTION OF THE SUITABLE PACKING MATERIAL FOR THE UNDERDRAIN SYSTEM

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

Packing material	Pea gravels	White pebbles	Glass 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 Gravels	Glass 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^{o}C (Pa.s)	0.000726
Water density at 35^{o}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	Glass 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)	Glass 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)	Glass 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

Table A.6: Determination of the minimum fluidising velocities

Type of anaerobic granules	**Type 1**	**Type 2**	**Type 3**
Water viscosity @ 35^{o}C (Pa.s)	0.0007255	0.0007255	0.0007255
Water density @ 35^{o}C (kg.m^{3})	993.95	993.95	993.95
specific gravity of granules	1.028	1.05	1.075
Density of granules (kg/m^{3})	1021.7806	1043.6475	1068.49625
diameter of granules 1 (m)	0.00115	0.0026	0.00425
Porosity 1	0.9	0.71	0.64
Bed Height (m)	0.025	0.025	0.025
inner diameter reactor (m)	0.086	0.086	0.086
Area (m^{2})	0.0058088	0.0058088	0.0058088
working volume (m^{3})	0.003	0.003	0.003
minimum fluidising velocity (m/s)	**0.009069084**	**0.013033533**	**0.019154481**
g (m/s^{2})	9.81	9.81	9.81
g(ρ_P-ρ_F)	273.018186	487.532475	731.2987125
Left term	273.0182046	487.5325155	731.2985896

Table A.7: Determination of the head losses across the anaerobic granules varying with superficial velocities and HRTs

Superficial velocity (m/s)	Type 1 Pressure drop (Pa)	Type 2 Pressure drop (Pa)	Type 3 Pressure drop (Pa)	Reynolds number	Q (m^3/s)	HRT (hrs)
0.000003855	0.000108862	0.00036476	0.000287274	0.060736442	2.23929E-08	37.21414
0.000004	0.00011296	0.000378488	0.000298087	0.063020951	2.32352E-08	35.86512
0.000005	0.000141226	0.000473177	0.000372679	0.078776189	2.9044E-08	28.6921
0.000006	0.000169502	0.000567894	0.0004473	0.094531427	3.48528E-08	23.91008
0.000007	0.000197789	0.000662638	0.000521948	0.110286664	4.06616E-08	20.49436
0.000008	0.000226086	0.000757409	0.000596624	0.126041902	4.64704E-08	17.93256
0.000009	0.000254393	0.000852207	0.000671329	0.14179714	5.22792E-08	15.94006
0.00001	0.000282711	0.000947032	0.000746061	0.157552378	5.8088E-08	14.34605
0.000011	0.000311039	0.001041885	0.000820822	0.173307615	6.38968E-08	13.04186
0.000012	0.000339378	0.001136764	0.000895611	0.189062853	6.97056E-08	11.95504
0.000013	0.000367727	0.001231671	0.000970428	0.204818091	7.55144E-08	11.03542
0.000014	0.000396086	0.001326604	0.001045273	0.220573329	8.13232E-08	10.24718
0.000015	0.000424456	0.001421565	0.001120146	0.236328567	8.7132E-08	9.564033
0.000016	0.000452836	0.001516553	0.001195047	0.252083804	9.29408E-08	8.966281
0.000017	0.000481226	0.001611568	0.001269977	0.267839042	9.87496E-08	8.438853
0.000018	0.000509627	0.00170661	0.001344934	0.28359428	1.04558E-07	7.970028
0.000019	0.000538038	0.001801679	0.00141992	0.299349518	1.10367E-07	7.550553
0.00002	0.00056646	0.001896775	0.001494933	0.315104755	1.16176E-07	7.173025
0.000021	0.000594892	0.001991898	0.001569975	0.330859993	1.21985E-07	6.831452
0.000022	0.000623334	0.002087049	0.001645045	0.346615231	1.27794E-07	6.520932
0.000023	0.000651787	0.002182226	0.001720143	0.362370469	1.33602E-07	6.237413
0.000024	0.00068025	0.002277431	0.001795269	0.378125706	1.39411E-07	5.977521
0.000025	0.000708723	0.002372663	0.001870423	0.393880944	1.4522E-07	5.73842
0.000026	0.000737207	0.002467922	0.001945605	0.409636182	1.51029E-07	5.517711
0.000027	0.000765701	0.002563208	0.002020816	0.42539142	1.56838E-07	5.313352
0.000028	0.000794206	0.002658521	0.002096054	0.441146657	1.62646E-07	5.123589

Superficial velocity (m/s)	Type 1 Pressure drop 1 (Pa)	Type 2 Pressure drop 2 (Pa)	Type 3 Pressure drop 3 (Pa)	Reynolds number	Q (m^3/s)	HRT (hrs)
0.000029	0.000822721	0.002753861	0.002171321	0.456901895	1.68455E-07	4.946914
0.00003	0.000851246	0.002849228	0.002246615	0.472657133	1.74264E-07	4.782017
0.000031	0.000879781	0.002944623	0.002321938	0.488412371	1.80073E-07	4.627758
0.000032	0.000908328	0.003040044	0.002397289	0.504167609	1.85882E-07	4.483141
0.000033	0.000936884	0.003135493	0.002472668	0.519922846	1.9169E-07	4.347288
0.000034	0.000965451	0.003230968	0.002548075	0.535678084	1.97499E-07	4.219426
0.000035	0.000994028	0.003326471	0.00262351	0.551433322	2.03308E-07	4.098871
0.000036	0.001022615	0.003422001	0.002698973	0.56718856	2.09117E-07	3.985014
0.000037	0.001051213	0.003517558	0.002774465	0.582943797	2.14926E-07	3.877311
0.000038	0.001079822	0.003613142	0.002849984	0.598699035	2.20734E-07	3.775276
0.000039	0.00110844	0.003708753	0.002925532	0.614454273	2.26543E-07	3.678474
0.00004	0.001137069	0.003804392	0.003001108	0.630209511	2.32352E-07	3.586512
0.000041	0.001165709	0.003900057	0.003076711	0.645964748	2.38161E-07	3.499037
0.000042	0.001194358	0.00399575	0.003152343	0.661719986	2.4397E-07	3.415726
0.000043	0.001223019	0.004091469	0.003228003	0.677475224	2.49778E-07	3.336291
0.000044	0.001251689	0.004187216	0.003303691	0.693230462	2.55587E-07	3.260466
0.000045	0.00128037	0.00428299	0.003379407	0.7089857	2.61396E-07	3.188011
0.000046	0.001309061	0.004378791	0.003455152	0.724740937	2.67205E-07	3.118706
0.000047	0.001337763	0.004474619	0.003530924	0.740496175	2.73014E-07	3.052351
0.000048	0.001366475	0.004570474	0.003606725	0.756251413	2.78822E-07	2.98876
0.000049	0.001395197	0.004666356	0.003682553	0.772006651	2.84631E-07	2.927765
0.00005	0.00142393	0.004762265	0.00375841	0.787761888	2.9044E-07	2.86921

Appendix B: AUXILIARY PARAMETERS USED FOR THE DEGBR OPERATION

B.1 Preparation of the barrier solution

The barrier solution used in the water displacement set was prepared every week. It consisted of a 5% (W/V) solution of potassium hydroxide (KOH). The percentage weight per volume is given by 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 weekly, therefore the mass of the solute required was determined by Equation B.2 that is 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 introduced into 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 is the period of time a given volume of substrate is retained in the bioreactor. It 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 is given 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 research project, the performance of the bioreactor is evaluated through the deduction of the percentage removal of some parameters such as the TSS, the COD and the BOD_5. The correlation that serves for this evaluation is given 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 up-flow velocity is determined by Equation B.6 and B.7:

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

Or,

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