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TREATING WASTEWATER USING AN INTERNAL CIRCULATION ANAEROBIC DIGESTER (IC-AD)



By

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A Dissertation submitted in the fulfilment of the requirements for degree of Master of Science to the School of Chemistry and Environmental Analytical Chemistry Research Group, University of the Witwatersrand, Johannesburg.

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DECLARATION

I, Natasja Raijmakers, declares that this Dissertation is my own work. It is being submitted for the Degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



N Raijmakers

20th day of March 2019 in Vanderbijlpark

ABSTRACT

Anaerobic treatment technologies are becoming increasingly more popular for the treatment of high organic strength wastewater. Investigating the Internal Circulating Anaerobic Digestion (IC-AD) technology is of importance as it is more cost effective, the reactor aids in the production of high quality effluent which reduces the cost of a polishing step and produces an effluent that complies to discharge limits. The following parameters were used to evaluate the daily operation of the Internal Circulating Anaerobic Digestion reactors; temperature, pH, alkalinity, COD (Chemical Oxygen Demand) and Volatile fatty Acids (VFA). This study presents the anaerobic treatment of synthetically prepared Fischer-Tropsch (FT) process wastewater using Laboratory Scale (LS) and Pilot Scale Internal Circulating Anaerobic Digestion (PS IC-AD) reactors. The reactors were inoculated with granular sludge obtained from reactors that were treating brewery wastewater and pulp and paper wastewater, respectively. The results indicated that the IC-AD technology was most likely a viable option for treating high strength COD wastewater at higher Organic Loading Rate (OLR) compared to other conventional anaerobic technologies. During this study the function and structural integrity of the granules were investigated. From the granules used to inoculate the LS reactors, 90% (d_{90}) of the granules sampled consisted of granules smaller than between 1248 µm and 1348 µm. Granules sampled from the LS IC-AD after treating synthetically prepared FT process wastewater for 100 days, indicated that in Run 1, 90% (d_{90}) of the granular sludge sample consisted out of granules smaller than 874 µm, whereas 90% (d_{90}) of the granular sludge sample during Run 2 consisted out of granules smaller than 660 µm. Visual observations (SEM images) demonstrated a probable shift in the microbial population relative to the granules used during the inoculation process; this shift was probably ascribed to the substrate being fed to the reactors. The reactors were probably not able to sustain the formation of granules, increase the granular size and form

new granules. Furthermore, the reactors had to be re-seeded each time operational upsets occurred, this probably resulted in the microbial population within the granules not being adapted to the synthetically prepared FT process wastewater and possibly compromised the structural integrity of the granules.

The LS IC-AD reactor studies were evaluated for a period of 100 days and divided into two sections, Run 1 and Run 2. In Run 1 the influent COD concentration was maintained constant at approximately 22000 mg/L, whereas in Run 2 the influent COD concentration was started at about 1000 mg/L and increased with time to about 6000 mg/L. Although the reactor in Run 1 was not able to achieved stable operation, due to OLR being increased continuously (state of ramp up), the assumption was made that stable operation was achieved from day 80 onwards. This assumption was based on the fact that both IC-AD reactors had obtained a similar OLR of approximately 10 kg COD/m^3.d. The IC-AD reactor operated during Run 2 achieved stable operation on day 51. During Run 1 the reactor obtained an average OLR of 10 kg COD/m^3.d with a removal efficiency of 98%. In Run 2 the reactor obtained an average OLR of 11 kg COD/m^3.d with a removal efficiency of approximately 94%. During the Pilot Plant Internal Circulating anaerobic Digester (PS IC-AD) evaluation period of 544 days the reactor could obtain an OLR of between 30 to 40 kg COD/m^3.d. The PS IC-AD yielded an effluent COD of 732 mg/L with a removal efficiency of >90%.

Keywords: Anaerobic treatment, synthetically prepared FT process wastewater, Internal Circulation Anaerobic Digestion reactor, granular function, granular integrity

Preliminary results presented at a conference proceeding (WISA 2008).
Albertus, RMC; Riedel, KH; Van Greunen, L; **Raijmakers-Rossouw, N**; Ting, MB; Nel, D; Fasemore, O; Driesen, W and Vogelaar, J; Anaerobic treatment of low temperature Fischer-Tropsch (F-T) coal to liquids(CTL) reaction water using the Paques Biopaq®IC internal circulating anaerobic technology; Proceedings of the WISA 2008 Biennial Conference (conference paper), Sun City, South Africa, 2008.

Biological anaerobic treatment of industrial effluents is becoming increasingly popular due to the low sludge yields, the ability to treat high-strength organic effluents and the production of methane which can be captured and used. Various high rate anaerobic treatment technologies have recently been developed. The Paques BIOPAQ®IC technology was of particular interest to Sasol for the treatment of low temperature Fischer-Tropsch (FT) reaction water from coal-to-liquids (CTL) production facilities.

A 60 L Paques BIOPAQ®IC anaerobic bioreactor, two-stage Up-flow Anaerobic Sludge Blanket (UASB) configured reactor was used for this pilot-scale study to evaluate the suitability of using this particular technology. The bioreactor was seeded with granular sludge obtained from a bioreactor treating pulp and paper wastewater and acclimatised at a low organic load on the intended reaction water, which consists predominantly of volatile fatty acids. Once the system reached steady state, the organic load was increased step wise to 30 kg COD/m^3.d by reducing the Hydraulic Retention Time (HRT) from 0.5 days to 0.2 days, while maintaining a constant Chemical Oxygen Demand (COD) feed concentration. Nutrient and alkalinity requirements were subsequently optimised. At steady state operation, the reactor efficiency in terms of organic removal efficiency, alkalinity requirement and granule integrity

were evaluated. Performance studies were carried out for approximately six months.

At an OLR of 30 kg COD/m^3.d, a COD removal efficiency of greater than 90% was achieved. The effluent alkalinity and Total Suspended Solids (TSS) were approximately 2000 mg/L and less than 150 mg/L, respectively. The specific alkalinity requirement at this OLR was approximately 0.11 kg NaOH / kg COD removed. Although a shift in the structural diversity of the microbial community of the granules from a diverse Methanococcus- and Methanosaeta-like structure to a predominantly Methanosaeta-like structure was observed, no indication of granule disintegration was evident. Preliminary results indicate that the BIOPAQ®IC anaerobic technology could be successfully applied for the treatment of the low temperature FT reaction water at significantly greater loading rates than conventional UASB and packed-bed technologies.

Dedicated to my friend Dr Lynn Raijmakers

~ I know that you always supported me, thank you ~

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My family; for your continued support and love.

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CHAPTER ONE - INTRODUCTION

Interest in the application of anaerobic digestion of wastewater in the paper and pulp, dairy, food and chemical industries has increased over the past few years (Macarie, 2000; Mahadevaswamy et al., 2004). The nature of the effluents produced in these industries favours the use of anaerobic digestion as a result of the high organic content present.

Chemical and petrochemical industries produce effluents (wastewaters) when producing fuels and chemicals. The wastewater can be treated using conventional activated sludge systems in order for the water to be re-used as process cooling water make-up. Alternatively, treatment technologies such as anaerobic treatment technologies have also been identified for treating wastewater. These technologies have various advantages over aerobic biological treatment processes and are less energy intensive, have low power consumption, produces less sludge, have a small/compact designs and produces methane gas (Wilson, 2014).

The wastewater produced during the liquefaction of the hydrocarbons, for example can be characterised by the high Chemical Oxygen Demand (COD) concentration. The resulting wastewater consists of 85% alcohols, 10% short chain fatty acids, 4.5% hydrocarbons *(*Majone, et al., 2010) and a negligible amount of nutrients, inorganic salts, etc. In order for biological anaerobic conversion to take place, nutrients need to be dosed to the synthetic wastewater. Anaerobic digestion is a process by which micro-organisms break down biodegradable material in the absence of oxygen. In general the anaerobic digestion process is facilitated by a methanogenic environment and the micro-organisms

depend on fermentation reactions to biodegrade the organic substrates (Metcalf and Eddy, 2014).

Extracellular Polymers (ECP) are metabolic products that are produced by bacteria and accumulate in the bacteria cell surface. Extracellular polymers are one of the most important and abundant species present within granules. The ECP is of high importance to maintain the structural integrity of the anaerobic granules (Schmidt et al., 1996). The granules can be defined as a symbiotic community of anaerobic micro-organisms that forms during the treatment of wastewater.

The anaerobic treatment technologies are becoming increasingly popular due to the ability to treat high-strength organic effluents (elevated organic content), produce low sludge yields and methane gas. According to literature various technologies including Up-Flow Anaerobic Sludge Blanket (UASB), fixed and/or packed bed configurations have been developed over the last few years (van Lier et al., 2015). Although excellent performance has been observed using fixed and packed bed reactor configurations, the time required to start-up these reactors are lengthy since biofilm establishment is required. Up-flow anaerobic sludge blanket technology can be considered to be the most popular system for treating wastewater due to the advantages when compared to other conventional anaerobic digesters. Some advantages of the UASB reactor are that anaerobic granulation seems to develop faster (Hulshoff et al., 2004), obtain organic removal efficiencies >89% and Organic Loading Rate (OLR) of between 10 to 15 kg COD/m^3.d (Driessen et al., 1999).

The UASB reactor is a modified fluidised bed reactor (Fogler, 1999*)*. In this modified reactor the solid phase catalyst has been replaced with granulated anaerobic sludge and the liquid phase with wastewater. A gas cap has been placed on top of the UASB reactor in order to serve as a biogas collector. The biogas produced during the anaerobic process consists mainly of carbon dioxide (CO_2) and methane (CH_4) (Grant et al., 2002*)*. The biogas is produced during the stepwise decomposition of carbohydrates and proteins that are present within the granules. The UASB reactors can achieve organic removal efficiencies of between 89% and 93%, OLR of 10 to 15 kg COD/m^3.d at Hydraulic Retention Time (HRT) of four to five hours (Driessen et al., 1999).

The OLR and Hydraulic Loading Rate (HLR) are the most critical design criteria for up-flow sludge bed reactors. Thus, to overcome the OLR and HLR limitations of these conventional treatment technologies, high rate anaerobic treatment systems were designed (Driessen et al., 1999). These high rate anaerobic reactors have increased height as well as diameter ratios. The technology is based on the UASB technology that consists of three phase separators for the biogas, granular sludge and liquid which represents two UASB reactors stacked on-top of one another. Therefore, these reactors can operate at higher up-flow velocities and OLRs, resulting in an increase in biomass activity due to more contact between the wastewater and biomass. The Internal Circulating Anaerobic Digester (IC-AD) is an example of such a technology and can achieve COD removal efficiencies of greater than 80% with an OLR of between 15 to 35 kg COD/m^3.d (Mutombo, 2004). The Internal Circulating (IC) technology can obtain higher OLR, are more cost effective and produces high quality effluents.

When comparing the IC-AD to the fixed bed and packed bed reactor configurations, one of the unique technical advantages of the IC-AD is the faster start-up time. The UASB technologies are more commonly applied to low-medium strength wastewaters with COD concentrations of between 3000 to 7000 mg/L, OLR of greater than 15 kg COD/m^3.d with an up-flow velocity of 1.5 m/h (Driessen et al., 1999). The IC-AD reactor can obtain an OLR of up to 35 kg COD/m^3.d and up-flow velocities that range between 3 to 9 m/h (Driessen et al., 1999). Literature indicated that when using an IC-AD, the circulation could assist with producing a higher quality effluent. This technology could reduce the production costs of a potential polishing step (Driessen et al., 1999). The IC-AD process could be used for the treatment of wastewater.

This study made use of a LS IC-AD reactor (4 L) and a pilot scale IC-AD reactor (60 L) to evaluate the granule function and granule structural integrity within the bioreactor.

CHAPTER TWO – LITERATURE REVIEW

2.1 Introduction

Interest in the application of anaerobic digestion of wastewater in the chemical industry, paper and pulp, dairy and food has increased over the past ten to fifteen years (Macarie, 2000; Mahadevaswamy et al*.,* 2004). The nature of the effluents produced in these industries favours the use of anaerobic digestion as a result of the high organic content present within these effluents.

Anaerobic digestion (AD) is becoming increasingly popular as it has the ability to treat high-strength organic effluents (elevated organic content), produces low sludge yields and methane gas (van Lier et al., 2015*)*. The successful application of anaerobic treatment technologies could be ascribed to the development of new and innovative reactor designs (Surampalli and Tyagi, 2004). According to literature various technologies including Up-Flow Anaerobic Sludge Blanket (UASB), fixed and/or packed bed configurations have been evaluated over the last few years. Although excellent performance has been observed using fixed and packed bed reactor configurations, the time required to start-up these reactors are lengthy since biofilm establishment is required. Up-flow anaerobic sludge blanket technology can be considered to be the most popular system for treating wastewater due to the advantages when compared to other conventional anaerobic digesters *(*van Lier et al*.,* 2015*)*.

The aim of this chapter will be to review the topics that form the basis of anaerobic digestion:

(i) definition of anaerobic digestion,

(ii) history of anaerobic digestion,
(iii) fundamental principles of anaerobic digestion,
(iv) anaerobic reactor types,
(v) wastewater, and
(vi) process conditions.

2.2 Definition of anaerobic digestion

Anaerobic digestion is a process where micro-organisms break down biodegradable material in the absence of oxygen. The anaerobic process entails the degradation of complex high molecular weight organic compounds into methane (CH_4) and carbon dioxide (CO_2) *(*Bitton, 2005*)*. The anaerobic process is widely used for treating wastewater with high organic concentrations as it significantly reduces the mass of the input materials (Haandel et al., 1994*)*.

2.3 History of Anaerobic digestion

According to historical evidence, the anaerobic digestion (AD) process has been one of the oldest technologies used in the world. The industrialisation of the AD process started in Bombay in 1859 with the first AD plant. The first biogas produced as a result of anaerobic digestion was recovered from a sewage treatment plant in England in 1895 (Meynell, 1976 and Monnet, 2003). Anaerobic bacteria and the conditions to promote methane gas production were discovered in 1930 (Monnet, 2003). The methane in the biogas can be used as an energy source for electricity generation and heating.

Anaerobic digestion has been a known wastewater treatment process from the late 19th and early 20th century. Anaerobic digestion was introduced at a later stage as a wastewater treatment process for sludge digestion. The types of wastes treated using anaerobic digestion have expanded and these anaerobic digestion systems have become more complex as an industrial application for secondary treatment. The anaerobic digestion process has found widespread application in municipal as well as industrial wastewater treatment with over 1500 full scale commercial installations currently in operation (Grant et al., 2002*).*

The use of anaerobic digestion throughout Europe has proven to be successful as a wide range of wastes, inclusive of farm, industrial and municipal wastes could be treated. Some facilities have been operational for more than 20 years and include more than 600 farm-based digesters. These digesters have been successful as a result of their simplicity and in the last few years more than 250 systems were installed in Germany *(*Monnet, 2003).

One of the countries using anaerobic digestion with the most experience in using large-scale digestion facilities is Denmark. In Denmark alone, there are 18 large centralised plants in operation which co-digest manure, organic industrial wastes and municipal solid waste (Danish Ministry of Energy and Environment (1996)).

2.4 Fundamental Principles of Anaerobic Digestion

In general the anaerobic digestion process is facilitated by a methanogenic environment where inorganic electron acceptors are absent; hence the micro-organisms depend on fermentation reactions to

biodegrade the organic substrates. These fermentation reactions primarily yield methane and carbon dioxide. The conversion of organic polymers to methane and carbon dioxide in the absence of oxygen occurs very slowly. The growth rates of the methanogenic species are very slow and the organic polymers cannot be completely converted into water (H_2O) and oxygen (O_2) (Metcalf and Eddy, 2014). This results in slower sludge accumulation within the reactor. Full conversion to methane and carbon dioxide results from a biological chain reaction where one type of organism generates the substrate which is consumed by the subsequent organism in the chain. This reaction chain includes five basic steps namely hydrolysis, fermentation (also known as acidogenesis), acetoclastic methanogenesis and hydrogenotrophic methanogenesis. However, the mechanism to achieve anaerobic oxidation depends on the wastewater to be treated (illustrated in Figure 2.1).

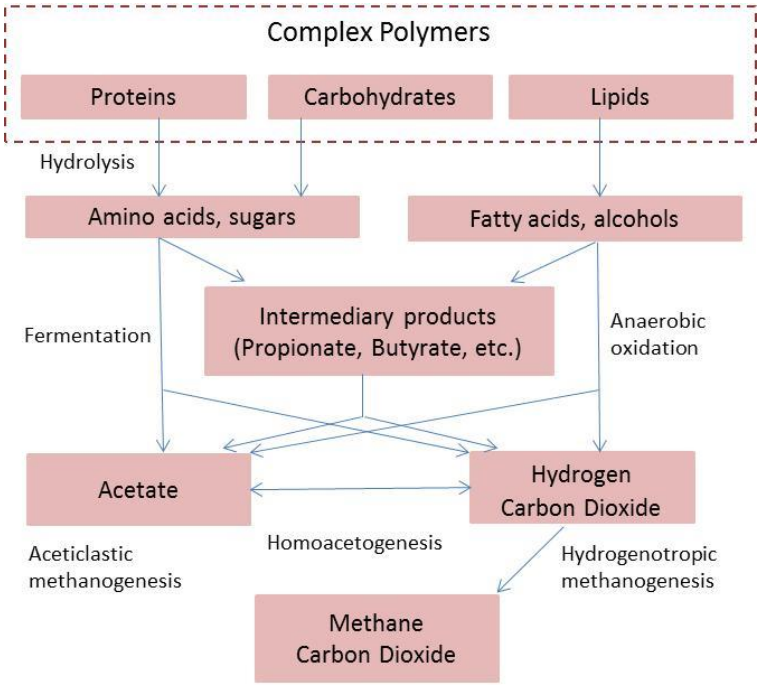


Figure 2.1: Simplified schematic representation of wastewater degrading an anaerobic consortium (van Lier et al., 2015)

Hydrolysis – this is a process where particulate material is converted to soluble compounds that can be hydrolysed further to simple monomers (Metcalf and Eddy, 2014). The extra cellular enzymes that are excreted by acidogenic biomass give rise to dead cell complex organisms to be degraded into organic product groups such as soluble biodegradable fermentable organics, unbiodegradable solubles and unbiodegradable particulates. During this conversion the Chemical Oxygen Demand (COD) cannot be reduced as the organics are not mineralised (Lorestani, 2006).

Fermentation/Acidogenesis – in this process amino acids, sugars and some fatty acids are degraded further and the organic enriched substrate then acts as both the electron donor and acceptor. This process is an anaerobic acid producing microbial process where dissolved hydrogen gas $H_{2\ (aq)}$ acts as an electron acceptor. Due to the higher free energy yields, the reactions can occur at higher hydrogen partial pressures and biomass yields. The biodegradable products from the hydrolysis process are ingested by acidogenic organisms and fermented intracellularly to the short chain fatty acids such as propionic acid, acetic acid, carbon dioxide and hydrogen gas.

Throughout this step, the hydrolysis products are absorbed by the cells of the fermentative bacteria to be fermented or anaerobically converted into compounds such as alcohols, short-chain fatty acids, formic acid, carbon dioxide and hydrogen. These organic substrates can serve as electron donors and acceptors. The final products of the metabolic activities of these bacteria depend upon the initial substrate (Figure 2.1) as well as the environmental conditions. As an example, consider the following reactions of glucose metabolism (Mosey, 1983).

$C_6 H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$ (2.1)

$C_6 H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O$ (2.2)

$C_6 H_{12}O_6 \rightarrow 2CH_3CH_2CH_2COOH + 2CO_2 + 2H_2$ (2.3)

Reaction (2.1) is the preferred reaction and produces acetic acid which is the major precursor of methane. As soon as an accumulation of hydrogen within the reactor is observed, reaction (2.2) and (2.3) will take place. It can be seen from the reactions above that reaction (2.2) utilises more hydrogen and therefore the acid load to the reactor will be lower (Lorestani, 2006).

Acetogenesis – methanogenic organisms cannot utilize short chain fatty acids with a carbon chain longer than two for the production of methane. Therefore, the acetogenesis plays an important role in the conversion of the short chain fatty acids (propionic acid, butyric acid and valeric acid) (Refer to Figure 2.1).

The sensitivity of the hydrogen partial pressure is determined by the amount of hydrogen produced during the acetogenic step. If the forward reaction for the breakdown of propionic and longer chain fatty acids are thermodynamically unfavourable, the propionic and butyric acids remain unchanged within the reactor. Thus, resulting in an increase in the short chain fatty acid concentration, this inhibits other processes such as acetoclastic and hydrogenotrophic methanogenises and increases alkalinity consumption.

Acetoclastic Methanogenesis – the acetoclastic methanogens will be the predominant biomass due to the concentration of the acetic acid. The acetoclastic methanogens are more robust and will not be affected by

the hydrogen partial pressure as easily as the acetogenic biomass, but could be affected by pH. The acetic acid is converted to methane in the following reaction (2.4):

$$CH_3COOH \rightarrow CH_4 + CO_2 + \text{biomass} \qquad (2.4)$$

There are two kinds of acetoclastic methanogens that can convert acetic acid into methane; one being the methanosarcina (>60 mgAc/L) and the other the methanosaeta (<60 mgAc/L). Methanosarcina can operate at low concentrations of acetic acid although they are sensitive to pH fluctuations and have slow growth rates (Batstone et al., 2002).

Hydrogenotrophic Methanogenesis – During an oxidation step the acetate is degraded by higher organic acids that have no electron acceptors. This results in the utilisation of an additional electron acceptor such as hydrogen ions to produce the hydrogen gas required by the oxidising organisms. In order for the $H_{2(aq)}$–oxidation reaction (2.5) to be thermodynamically possible the metabolic product ($H_{2(aq)}$) has to be present in low concentrations.

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O + \text{Biomass} \qquad (2.5)$$

In addition, bacteria within anaerobic processes, termed Acetogens, are also capable of using carbon dioxide to oxidise hydrogen and form acetic acid, which is further converted into methane; however this reaction is considered to be of minor importance (Metcalf & Eddy, 2014). If the wastewater consists mostly of short chain volatile fatty acids, it is expected that the majority of anaerobic processes would occur in the acetogenesis and methanogenesis stages. Limited hydrolysis of biomass could occur under certain operating conditions.

The group of non-methanogenic micro-organisms responsible for hydrolysis and fermentation consists of facultative (function under anoxic conditions) and obligate (function under anaerobic conditions) anaerobic bacteria. The micro-organisms responsible for the production of methane are classified as archaea and are strict obligate anaerobes. In general, many of the methanogenic organisms identified in an anaerobic environment are similar to those found in the stomachs of ruminant animals and in organic sediments taken from lakes and rivers (Metcalf & Eddy, 2014). The principal genera of micro-organisms that have been identified at mesophillic conditions include *Methanobacterium, Methanobacillus, Methanococcus, Methanothrix* and *Methanosarcina*. *Methanosarcina* and *Methanothrix* are the only organisms able to use acetate for the production of methane and carbon dioxide (Metcalf and Eddy, 2014).

2.5 Stoichiometry of Anaerobic Fermentation and Oxidation

A limited number of substrates are used by methanogenic organisms.

$$C_4H_5O_2 + H_2O \rightarrow 2.5CH_4 + 1.5CO_2 \quad (2.6)$$

$$C_3H_6O_2 + 0.5H_2O \rightarrow 1.75CH_4 + 1.25CO_2 \quad (2.7)$$

$$C_2H_4O_2 \rightarrow CH_4 + CO_2 \quad (2.8)$$

$$4H_2^* + CO_2 \rightarrow CH_4 + 2H_2O \quad (2.9)$$

* metabolic intermediate

The COD loss in the anaerobic digestion process is accounted for by the methane production i.e. the COD of methane is the amount of oxygen required to oxidize methane to carbon dioxide and water in the following way:

$$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O \quad (2.10)$$

Hence, from this reaction (2.10) the COD per mole of methane is 64 g O_2/mole. In addition, the volume of methane produced at standard conditions (STP) is 22.414 L; hence the theoretical methane equivalent of COD is 0.35 L CH_4/g COD (0.35 m^3/kg COD). Thus, the theoretical COD can be calculated using reaction (2.10) (Metcalf and Eddy, 2014; Henze et al., 2008).

2.6 Major Advantages and Disadvantages of Anaerobic Digestion

2.6.1 Advantages of Anaerobic Digestion

- The AD process can be regarded as an energy converting process due to the production of a methane rich gas. The trapped energy within the organics of the wastewater is converted to methane and carbon dioxide. This presents an opportunity for the reuse of the methane gas.
- When comparing the activated sludge process to the AD process, the AD process produces only 10% of the biomass per unit of substrate fed.
- The addition of nutrients can be directly linked to the amount of biomass formed, therefore less biomass is produced in the AD process which significantly lowers the amount of nutrients required when compared to aerobic processes.
- Newly developed AD processes can operate at higher biomass concentrations and treat higher volumetric capacities, thus reducing the process footprint.

2.6.2 Disadvantages of Anaerobic Digestion

- The AD process, requires a significantly longer start-up period when compared to aerobic processes. The time required to recover from process upsets are longer compared to that of aerobic processes. In addition, if unacclimatised seed sludge is used during start-up conditions, the start-up time will increase even further since most specific anaerobic micro-organisms are capable of degrading a limited range of substrates. This implies that seeding an anaerobic reactor for the treatment of a wastewater with biomass obtained from another process significantly decreases the acclimatisation time.
- The AD process typically produces an effluent with higher concentrations of organic matter when compared to aerobic processes. This implies that a polishing step such as aerobic biological treatment is usually required to reduce the effluent concentrations to acceptable levels.
- The AD process usually also requires the addition of alkalinity to maintain a pH of approximately seven due to the high gas phase carbon dioxide concentration and resultant carbonic acid (H_2CO_3). Depending on the oxidation state of the organic pollutants and the wastewater strength, the biogas will typically contain approximately 35% to 40% of carbon dioxide. Hence to maintain a pH of approximately seven at this concentration, 40 equivalents of bicarbonate per cubic meter of wastewater must be present.

2.7 Anaerobic Reactor Types

In this section the various types of suspended growth anaerobic reactors and attached growth anaerobic reactors will be listed for reference purposes.

2.7.1 Suspended Growth Anaerobic Reactors

In principle the suspended growth anaerobic reactor, is designed as an aerobic activated sludge process and operates as a fluidised reactor in the absence of oxygen using the generated biogas as a fluidisation medium. The most commonly used suspended growth anaerobic reactors are the Continuously Stirred Tank Reactor (CSTR) and the Up-flow Anaerobic Sludge Bed reactor (UASB).

a) Continuously Stirred Tank Reactor (CSTR)

The CSTR was the first type of anaerobic bioreactors to be built and are still widely used for the treatment of municipal wastewaters. Anaerobic digestion using CSTR reactors (as illustrated in Figure 2.2) are generally used to treat wastes with high particulate matter such as sewage sludge and fractions of municipal solid waste (Kleerebezem, 2003). The growth rate of the slowest growing micro-organism determines the Hydraulic Retention Time (HRT) of the system. Therefore, a HRT of up to 25 days are generally required resulting in this technology to be less feasible for the treatment of wastewater streams primarily containing dissolved organic compounds (4 to 30 kg COD/m^3 of wastewater). The separation of biomass from the biomass-wastewater mixture can be achieved by using a settler. The settled biomass is fed back to the CSTR, thus increasing the volumetric degradation rate in the system. This approach is called the Contact Process and is comparable to the aerobic activated sludge process. The maximum biomass concentration achievable is usually between 4 to 6 kg COD/m^3 of reactor attributed to the poor settling characteristic of methanogenic sludge as well as the formation of biogas in the settler (Kleerebezem, 2003). Because of the large footprint and poor settling ability of the dispersed anaerobic biomass, the anaerobic contact process has found only limited applications for the full scale.

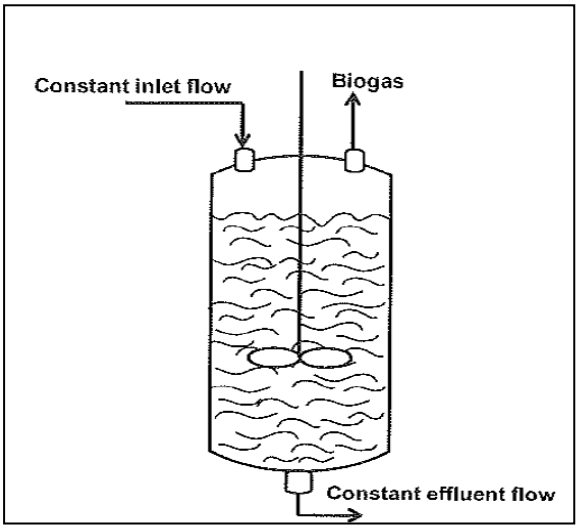


Figure 2.2: Schematic representation of a typical anaerobic CSTR process configuration (Metcalf & Eddy, 2014)

b) Anaerobic Sequencing Batch Reactor (ASBR)

The operation of the ASBR consists of four steps: influent, reaction, settling and effluent decanting (Korzak, 2008). In the first phase some mixing occurs to ensure uniform distribution of the substrate and solids as illustrated in Figure 2.3. A critical feature of the ASBR is the settling velocity of the sludge during the settling period before the effluent is withdrawn. The settling time is approximately 30 minutes (Metcalf and Eddy, 2014). At the completion of the reaction period, the removal efficiencies and gas production rates are lower in order to improve the settling of the solids.

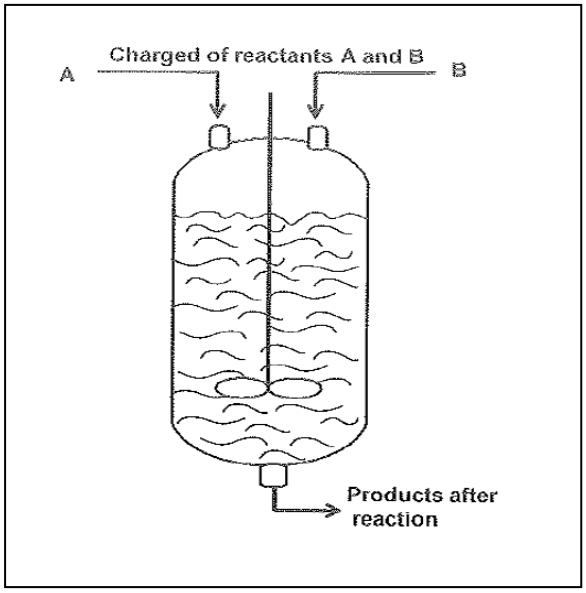


Figure 2.3: Schematic representation of a typical anaerobic ASBR process configuration (Metcalf & Eddy, 2014)

c) Up-flow Anaerobic Sludge Blanket reactor (UASB)

Anaerobic processes can be used to form a blanket of granular sludge. The wastewater that flows through this granular blanket is processed by the micro-organisms. The blanketing of the sludge results in a duel solid liquid retention time within the reactor. The upward flow and settling action of gravity, suspends the blanket which reaches maturity within three months. The formation of granules takes place as the surface areas are covered in aggregates of bacteria. The flow conditions create an ideal environment for the micro-organisms that attach to each other, survive and proliferate. The aggregates form dense biofilms and are known as granules. Under reduced hydrodynamic shear, coagulation within granular sludge cannot occur and the granules settle much faster than activated sludge flocs.

Anaerobic processes using UASB reactors (as illustrated in Figure 2.4) combine a reaction compartment with an internal settler and a biogas separator (Kleerebezem, 2003). The influent wastewater is distributed

evenly into the bottom of the reaction compartment and travels in an up-flow mode through the sludge bed where the organics are converted into biogas. The up-flow of the wastewater through the granular sludge bed together with the mixing resulting from the biogas bubbles provides sufficient up-flow to keep the granular sludge bed in a suspended state. Biogas is collected in the three phase separator that is operated at a low overpressure to increase the gas-to-liquid exchange surface area. The sludge/water mixture flows to the settler section where biomass is allowed to settle and return to the reaction compartment. High biomass concentrations (20 to 30 kg/m^3 of reactor) can be achieved with moderate strength wastewaters (5 kg COD/m^3 of wastewater) at a low HRT (10 h) and high Sludge Retention Time (SRT) e.g. 50 days. These high biomass concentrations can be attributed to the spontaneous formation of large, dense biomass conglomerates or methanogenic granular sludge. The density of the sludge is slightly higher than water which enables good mixing in the reactor compartment and good settling in the settler compartment. In general, the combination of a simple construction and high volumetric treatment capacity has enabled granular sludge bed reactors like the UASB to become a dominant reactor type for the treatment of wastewaters (Kleerebezem, 2003). High rate configurations of the UASB type reactors have been developed in recent years of which the Expanded Bed Biothane® and Paques Internal Circulating BIOPAQ® (van Lier et al., 2008) technologies are well known in the treatment of paper mill effluent and brewery effluents.

Limitations to UASB

The UASB process is mostly applied on medium strength industrial effluents having COD concentrations in the range of 3000 to 7000 mg/L. The organic loading capacity and the hydraulic capacity are the most critical design criteria for up-flow sludge bed reactors. The reactor

design for treating low strength effluents is mostly hydraulically limited, whereas for high strength effluents the system is limited by its organic loading capacity (Driessen et al., 1999). In order to overcome hydraulic and organic loading limitations of UASB reactors, new high rate reactor concepts with in an increased height/diameter ratio have been developed. The Expanded Granular Sludge Bed (EGSB) and the Internal Circulation reactor (IC) are examples thereof. These systems operate with higher up-flow velocities and organic loading rates. Therefore, increased biomass activity has been achieved due to the maximum contact between wastewater and biomass.

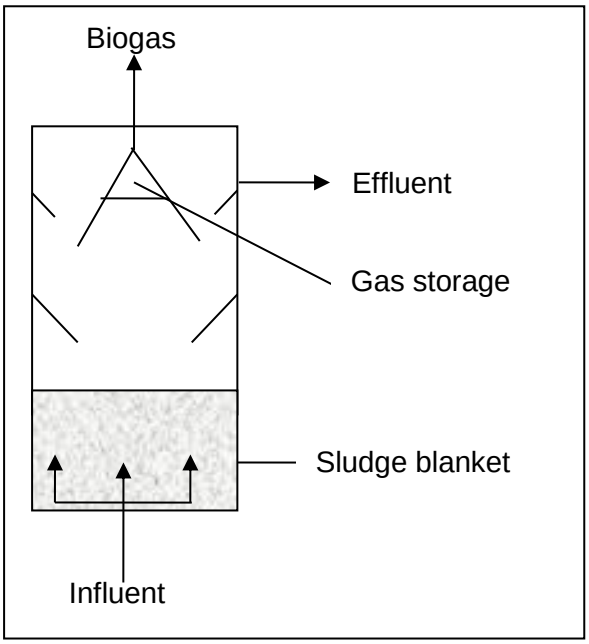


Figure 2.4: Schematic representation of a typical UASB reactor configuration (Metcalf and Eddy, 2014)

2.7.2 Granular reactors

The granular reactors are anaerobic reactors used for "high rate" anaerobic treatment of wastewater. This concept originated from the UASB reactor. The wastewater passes upwards through an anaerobic sludge bed allowing the micro-organisms within the sludge to come in

contact with the wastewater substrates. At the top of the reactor, the water phase is separated from sludge solids and gas in a three-phase separator (also known the gas-liquid-solid separator).

a) Expanded Granular Sludge Bed (EGSB)

The expanded granular sludge bed reactor (EGSB) (Figure 2.5) is a modification of the UASB reactor; it was primarily developed to improve the substrate-biomass contact within the treatment system by expanding the sludge bed and intensifying the hydraulic mixing (Kato et al., 1994). Similar to the UASB technology, the EGSB reactor is reliant on the self-immobilisation properties of micro-organisms and the development of a well-settling granular biomass within the reactor. The wastewater enters at the bottom of the reactor via an influent distribution system and then moves upward through the anaerobic sludge bed where conversion of organic matter to biogas takes place. However, unlike the UASB, the EGSB reactor operates at high superficial velocities (>7 m/h), which are obtained either by effluent recirculation and/or a tall narrow reactor design (McHugh et al, 2003). A granular sludge can develop and be maintained under high liquid up-flow velocities (<12.5 m/h) (McHugh et al., 2003) and gas (>7 m/h) in the EGSB reactor. The intensified hydraulic mixing within the reactor results in an increased substrate-biomass contact, which consequently enhances the reactor performance and stability.

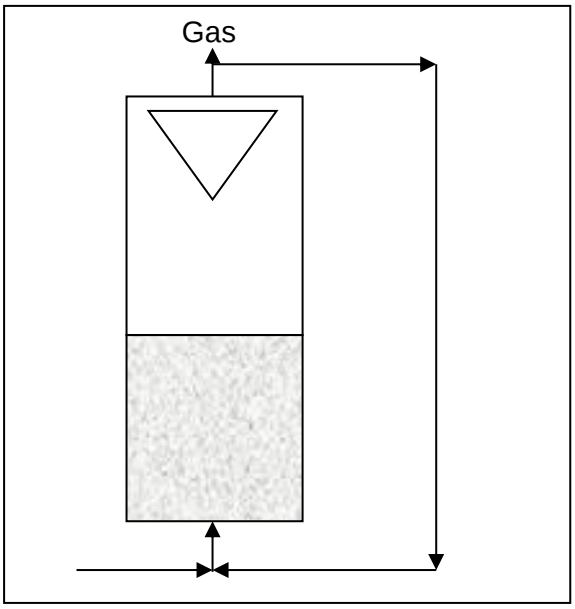


Figure 2.5: Schematic representation of an EGSB reactor *(*Tielbaard et al., 2002)

b) Internal Circulation Anaerobic Digester (IC-AD)

When compared to single-stage reactors, such as the UASB and EGSB, the IC reactor offers considerable performance advantages by allowing high levels of biomass retention, coupled with elevated mixing intensities within the system. The flow-rate of the internal circulation subsequently depends on the influent COD and is therefore self-regulating: at high influent COD concentrations, more is circulated while at lower influent concentrations, less is circulated. In particular, the Internal Circulating (IC) reactor displays superior performance levels to the UASB, achieving equal removal efficiencies at considerably higher reactor loading rates (Pereboom et al., 1994). Biomass retention in the second stage is not hindered by excessive gas production and hydraulic retention times of <2.5 h can therefore be applied to the system (Driessen et al., 1999). Since the internal circulation flow does not pass through the second reactor compartment, it does not lead to an increased superficial liquid velocity in the separator at the top of the reactor (Driessen et al., 1999), which permits extreme high effluent recycle rates. The effective mixing

of the bulk liquid phase for substrate-biomass contact results in a high sludge activity. Comparison tests showed that the anaerobic granular sludge from the IC reactors often have higher acetoclastic methanogenic activities than anaerobic sludge from UASB reactors. In comparison to single stage separator concepts like the UASB and other EGSB systems the IC reactor can be operated at higher organic and hydraulic loading rates (Driessen et al., 1999).

The IC reactor is a granular sludge bed reactor that makes provision for higher up-flow velocities and is characterised by the two-stage separation system to achieve the best sludge retention (Figure 2.6) (Tielbaard et al., 2002). Due to the construction of the reactor the up-flow velocity within the top of the reactor is not affected. As a result of this the reactor has excellent sludge retention at high up-flow velocities and organic loading rates. The name for the IC reactor originated from the gas lift (biogas) driven internal circulation reactor.

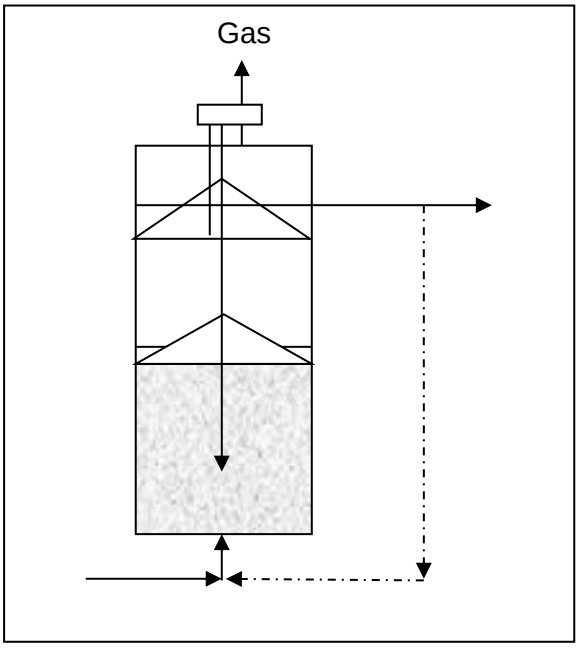


Figure 2.6: Schematic representation of an IC reactor (Tielbaard et al., 2002)

2.7.3 Attached Growth Anaerobic Reactors

The attached growth anaerobic reactor, unlike the suspended growth anaerobic reactor, includes a packing medium to which the micro-organisms attach. The attached growth anaerobic reactor typically consists of either an up-flow packed anaerobic reactor or a down-flow packed anaerobic reactor configuration.

a) Up-flow packed bed attached growth reactor (UPBR)

The UPBR has various reactor configurations; however, the critical difference between these reactor types is the type of packing used and the degree of bed expansion. A typical reactor schematic is illustrated in Figure 2.7. Whilst the first up-flow anaerobic reactors contained rock as a packing medium, current designs employ a variety of plastic type packing media. The most common packing is corrugated plastic, cross-flow or tubular modules and plastic pall rings (Metcalf & Eddy, 2014). The packing media is inserted into the reactor i.e. the packing material could be through the entire depth of the reactor or only the upper 50% to 70% of the reactor (hybrid designs) (Metcalf and Eddy, 2014). When these hybrid configurations are applied, the wastewater flows up through the interstitial spaces. The reactor configuration is typically cylindrical or rectangular with widths or diameters of 2 to 8 m and heights ranging from 3 to 13 m (Metcalf and Eddy, 2014). An example of using an UPBR for treatment of chemical wastes at a temperature of 37°C has proven that a COD load of between 12 to 15 kg COD/m^3.d, a HRT of 1.2 days and a COD removal efficiency of 80% to 90% were achieved (Metcalf & Eddy, 2014). The UPBR can treat waters with high COD organic loading rates, with a small reactor volume and by simple operation. The UPBR process is suitable for the treatment of wastewaters with low concentrations of Total Suspended Solids (TSS).

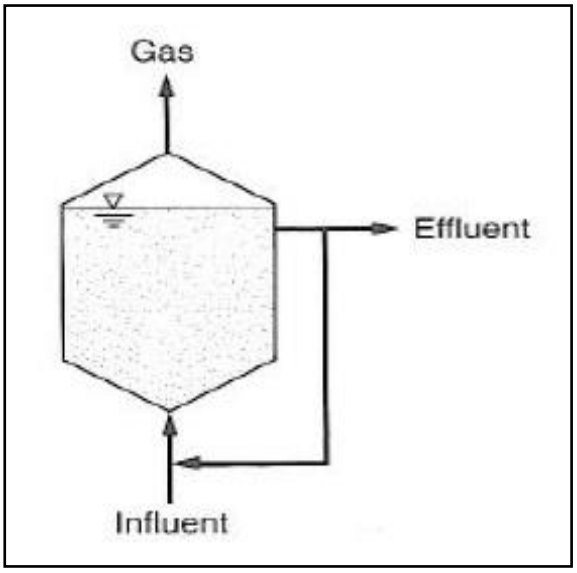


Figure 2.7: Schematic representation of a typical UPBR reactor configuration (Metcalf and Eddy, 2014)

b. Anaerobic Fluidised Bed Reactor (AFBR)

The AFBR configuration, although similar to the UPBR, typically utilises silica sand as a packing medium. The smaller packing medium provides a greater surface area per unit volume, which in theory supports a greater amount of biomass but results in a packing void fraction of 50% when expanded (Metcalf & Eddy, 2014). As a result, expanded operation is necessary to prevent plugging. Plugging occurs when the solids and biomass accumulate within the packing material. However, due to partial fluidisation some solids are trapped and degradation occurs. Bench scale experiments treating domestic wastewater have shown that a maximum COD removal efficiency of 89% and a loading rate of approximately 4.4 kg COD/m^3.d were achieved at 20°C (Metcalf & Eddy, 2014). Because of the success of granular anaerobic systems, this process has fallen to the wayside over the past three decades.

c) Down-flow attached growth process reactor (DFAR)

The DFAR configuration (as illustrated in Figure 2.8) is commonly used to treat high-strength wastewaters using various types of packing material. The packing heights are in the range of 2 to 4 m (Metcalf & Eddy, 2014). The reactor design allows for the recirculation of the effluent. The DFAR can be operated at an OLR of 5 to 10 kg COD/m^3.d (Metcalf & Eddy, 2014). Packing with high void volume is recommended due to the fact that the packing could be plugged. The main advantages of DFARs when high void packing material is used include the following; no plugging occurs, operation is simple and the inlet flow distribution design is simpler (Metcalf & Eddy, 2014). High COD loads can be treated with relatively small reactor volume sizes. The high costs of packing material and treating wastewaters with lower OLRs are some of the disadvantages of the down-flow attached growth reactors (Metcalf & Eddy, 2014).

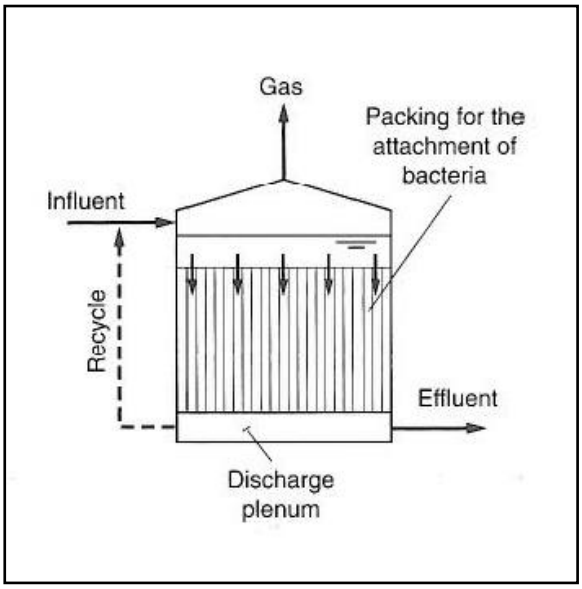


Figure 2.8: Schematic representation of a typical DFAR configuration (Metcalf & Eddy, 2014)

d) Fluidised-Bed Reactor (FBR)

The design is similar than that of the physical design of the UPBR. The FBR (as illustrated in Figure 2.9) is operated at higher up-flow liquid velocities (20 m/h) (Metcalf and Eddy, 2014; Korzak, 2008). In order to achieve sufficient up-flow velocities the effluent is recycled. The reactor depth can range from four to six meters. Various packing materials could be used in the FBR, such as sand, diatomaceous earth, anion and cation exchange resins and activated carbon. Many FBRs that were used to treat industrial and hazardous waste streams were operated using activated carbon. The disadvantage of using activated carbon is the high cost, although some wastewater streams require the use of activated carbon. Due to the high turbulence and the thin biofilm that develops in the FBR, the solids that are captured are kept to a minimum and therefore this process could be used to treat wastewaters with mainly soluble COD concentrations. The FBR can obtain an organic loading rate of 10 kg COD/m^3.d at 35°C within three months with a COD concentration of 5000 mg/L (Metcalf and Eddy, 2014). The COD removal efficiency for the FBR is approximately 96%. The advantages of the FBR are that it can produce high biomass concentrations, high organic loading rates, high mass transfer, the ability to handle shock treatments and minimal space is required for the reactor. This process is suitable for soluble wastewaters as a result of the ability to capture solids. Some of the disadvantages are the pumping power to operate the fluidised bed, the cost of packing and the long start-up time required.

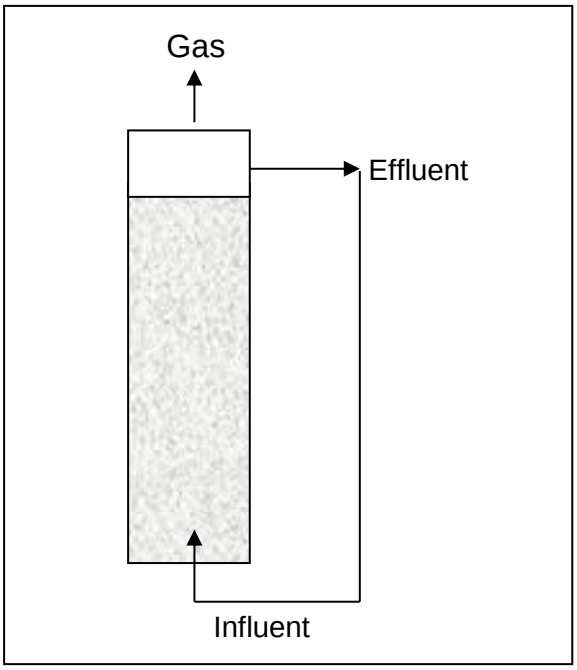


Figure 2.9: Schematic representation of a typical FBR configuration (Metcalf and Eddy, 2014)

2.8 Process Considerations for LS IC-AD and PS IC-AD

The main parameters used to measure the reactor operating conditions include flow and loading variations, temperature, pH, alkalinity, nutrients, gas production and Ripley Ratio (RR). These parameters are discussed in the paragraphs that follow.

2.8.1 Flow and loading variations

Variations in the influent flow, hydraulic loads and organic loads can upset the balance between acid fermentation and methanogens (Metcalf and Eddy, 2014). This implies that during unstable conditions the rate of volatile fatty acid production (propionic and acetic acid) can be faster than their removal, resulting in increasing concentrations and low pH levels (Habeeb, 2011). The combined effect will inhibit the acetogenic and methanogenic phases (pH 7.0) i.e. the rate limiting steps. Thus,

volatile acids (e.g. propionic acid) can be used as an indicator of reactor performance.

Digester sizing is determined by sufficient residence time in a well-mixed reactor which could allow for destruction of Volatile Suspended Solids (VSS). The following sizing criteria could be used (i) hydraulic retention time (HRT) and (ii) solid retention time (SRT);

(i) The Hydraulic Residence Time

The average length of time that a soluble compound will remain in a reactor is measured by the hydraulic residence time and is normally expressed in hours (h) or days (d) (Abdelgadir et al., 2014). Hydraulic retention time can be determined by dividing the reactor volume (m^3) with the influent flow-rate (m^3/d). Hydraulic retention time is easy to control and considered as a good operational parameter.

(ii) Sludge Retention time

The amount of time sludge remains within the reactor is known as sludge retention time. The SRT for a soluble substrate can be calculated by dividing the mass of solids in the reactor by the mass of the solids that are removed on a daily basis. The hydrolysis, fermentation and methanogenesis are directly related to the SRT. An increase or decrease in the SRT results in an increase or decrease in each reaction (Metcalf and Eddy, 2014). Should the SRT be less than the minimum SRT, the bacteria cannot grow rapidly enough and will result in the digestion process to fail in time. In order to achieve a stable, reliable performance within a reactor at 35°C, an SRT of 15 to 20 days are required, although many high-rate reactors could exceed 30 to 50 days.

The two parameters discussed above relate to time within a system. The length of time biomass remains in a reactor (SRT) and the time soluble compounds remain in the reactor (HRT). When solid liquid separation occurs in a reactor, the liquid and solid retention time are separated and the SRT > HRT. It is expected that longer sludge ages result in larger sludge masses within a reactor, which then results in larger reactor volumes. Thus, a longer SRT causes a longer HRT. Therefore, the link between SRT and HRT are not proportional or linear, but depends on the organic concentration of the wastewater and the suspended solid concentration (Henze et al., 2008).

2.8.2 Temperature

Although organics in wastewaters can be anaerobically degraded at temperatures between 0°C to 69°C, the digesting bacteria will decrease at temperatures below 16°C. Anaerobic bacteria can be classified according to the basis of the maximum temperature. For reactor temperatures of 25°C to 40°C, mesophillic bacteria are generally preferred to support more ideal biological reaction rates and to provide more stable operation (Abdelgadir et al., 2014; Jiang et al., 2015). The thermophiles exist at thermophillic temperatures around 50°C to 60°C (Abdelgadir et al., 2014; Jiang et al., 2015). Lower temperatures in the range of 10°C to 20°C are generally undesirable due to slower reaction rates and longer HRTs which require larger reactor volumes and lower organic COD loadings. At higher temperatures the reaction rates are much faster resulting in a more efficient operation of the reactor (Abdelgadir et al., 2014).

2.8.3 pH and Alkalinity

The pH measurement is used to describe the intensity of the basic or acid condition of a solution. The methanogens present in wastewater treatment systems are more active in the neutral pH range (7.0). Most organisms prefer a suitable pH range of between 6.0 and 9.0, although digestion can still proceed beyond this range but with less efficiency. Thus, if the wastewater to be treated is of high acidity it will result in a decrease in the reactor pH. This could result in a slower digestion until the bacteria has absorbed the acids within the influent. Ideally anaerobic digestion occurs at a pH of between 6.8 and 8.0 in order to maintain the high levels of activity in methane-forming micro-organisms. Methane forming bacteria are sensitive to pH and are inhibited at pH values less than 6.2. It is thus important to maintain the operating pH near neutral (7.0).

Therefore, it is of great importance to know what the concentration of the reserve alkalinity is within the reactor before the pH decreased to below the safe threshold value of approximately 6.2 to 6.5. A decrease in the pH could lead to low methanogenic activity which results in the accumulation of Volatile Fatty Acids (VFA) and hydrogen. This could lead to the inhibition of propionic acid degrading bacteria which in turn results in an increase in the VFA concentration and a decrease in the pH to below the safe threshold value. This could result in reactor failure and is known as “SOUR” or “STUCK”.

A well-established reactor could have an alkalinity of between 1000 to 5000 mg/L. Sufficient alkalinity (derived from the breakdown of organics and which is primarily present in the form of bicarbonates) is essential to maintain the correct pH in the system. Alkalinity is important as it

represents the ability of the digester to neutralise the volatile fatty acids formed during digestion (Jiang et al., 2015; Rajeshwari et al., 1999).

2.8.4 Nutrients

In order to maintain the ideal methanogenic activity, liquid phase macro-nutrients are required (nitrogen and phosphorus). In addition, trace elements (iron, cobalt, and zinc) are generally required to stimulate methanogenic activity. The exact requirement of trace nutrients varies for different wastewaters. A recommended dose per litre of reaction volume is 0.1 mg of each of $FeCl_2$, $CoCl_2$, $NiCl_2$, and $ZnCl_2$ (Speece, 1996).

2.8.5 Gas production

Anaerobic reactors stabilise the biodegradable organic matter within a reactor by converting it into methane gas. At standard temperatures and pressures, 0.35 m^3 of methane can be produced per kg of COD removed. At removal efficiencies of 80% to 90%, the biogas production is approximately 0.5 m^3/kg COD removed, which is equivalent to the methane production of 0.35 m^3/kg COD removed (Hall, 1992).

The micro-organisms responsible for methane production are classified as archaea and are strict obligate anaerobes. These micro-organisms produces biogas as a waste product which consist mostly of CO_2 (25% to 40%) and CH_4 (60% to 75%). Small amounts of hydrogen (H_2) and hydrogen sulphide (H_2S) can also be present in the biogas. In a bacteria population growth phase the biogas production is at its highest and tapers down once all the material has been exhausted. The production

of gas is more rapid between 29°C to 41°C (mesophillic) and 49°C to 60°C (termophillic). The increasing or decreasing OLR has an immediate increase or decrease in the production of the biogas. Although the reactor response to biological conversion of the OLR can be determined by the volumetric gas measurement an indication of the healthiness of the system is given by the CO_2 to CH_4 ratio of the biogas. If the CO_2 fraction is higher than the methane, this is a sure sign of the reactor upset.

2.8.6 Liquid-solid separation

Dispersed AD sludge usually has poor settling properties, leading to biomass washout. This combined with low biomass yields results in poor solid retention in disposed systems and low stable OLR.

2.8.7 Volatile acids and total alkalinity ratio (Ripley Ratio)

The first indication of an upset in the digestion process is the measurable changes that take place between the volatile acids and the total alkalinity. However, both these parameters change on a daily basis. As the volatile acids rise and the alkalinity decreases, the ratio will change faster than the individual values thus indicating an impending problem.

The Ripley Ratio (RR) is an empirical relationship that indicates the state of the anaerobic digestion process and this can warn of any impending upsets. The RR changes much faster than indicated by the pH in the reactor. Anaerobic digesters perform at its best when the RR is maintained at approximately 0.3 (Ross, 1992). A low RR is an indication

that the operating conditions of the reactor are good, whereas an increase in the RR indicates that an upset has occurred.

2.9 Wastewater

Literature describes that the type of wastewater used in wastewater treatment has a greater influence on granulation and granular quality compared to the design of the reactor or the operational conditions (Jhung et al., 1995; Batstone et al., 2001; Zhou et al., 2006). Anaerobic granulation seems to develop faster within USAB reactors that use carbohydrate-rich influents (Hulshoff Pol, 1989). These carbohydrate-rich influents typically include wastewaters from industries such as pulp and paper, slaughterhouse, cheese whey, textile, coffee, brewery, olive oil, sugar mill, distillery, yeast factories and sugar beet- and potato-processing factories (Lin et al., 1991).

Wastewater that originates from a petrochemical industry can typically be characterised as a medium to high COD concentration content. During the Fischer-Tropsch (FT) process a mixture of carbon dioxide and hydrogen are converted into a range of hydrocarbons. The liquefaction of these hydrocarbons results in an aqueous wastewater stream. The typical composition of the synthetically prepared FT process wastewater stream is summarised in Table 2.1 (Majone et al., 2010).

Table 2.1: Composition of synthetically prepared Fischer-Tropsch process wastewater stream (Majone et al., 2010)

Compounds	% COD
Short chain alcohols (SCA)	76.6
Long chain alcohols (LCA)	8.2
Volatile fatty acids (VFA)	10.7
Hydrocarbons (HCH)	4.5
Total COD	100

The composition of the biogas can be calculated for each of the compounds in the synthetically prepared FT process wastewater. The definition below is a good approximation of the biogas composition within a wastewater with a known chemical composition. The stoichiometry for anaerobic digestion has been defined by Tarvin and Buswell, (1934) (Nielfa et al., 2014).

$$C_nH_aO_b + (n-a/4-b/2)\ H_2O \rightarrow (n/2+a/8-b/4)\ CH_4 + (n/2-a/8+b/4)\ CO_2 \quad (2.11)$$

The amount of methane expected after complete conversion of the organic compounds within the substrate, is referred to as theoretical methane and the expected biogas. As illustrated in equation 2.11, the higher the oxidation state of the carbon in the substrate, the lower the proportion of methane in the biogas.

The biogas composition of the synthetically prepared FT process wastewater was calculated using the Buswell formula and is summarised in Table 2.2

Table 2.2: Calculated biogas composition of synthetically prepared FT process wastewater (refer to equation 2.11)

Compound	n	a	b	Mol CH_4	Mol CO_2	CH_4 (%)	CO_2 (%)
Acetic acid	2	4	2	1	1	50.0	50.0
Propionic acid	3	6	2	1.75	1.25	58.3	41.7
Butyric acid	4	8	2	2.5	1.5	62.5	37.5
Valeric acid	5	10	2	3.25	1.75	65.0	35.0
Methanol	1	4	1	0.75	0.25	75	25
Ethanol	2	6	1	1.5	0.5	75	25
Propanol	3	8	1	2.25	0.7	75	25
n-Butanol	4	10	1	2.5	1.5	75	25
n-Prentanol	5	12	1	3.25	1.75	75	25
Influent biogas composition						72.1	27.9

Wastewaters consisting of high COD concentrations and biodegradable characteristics can be treated using anaerobic treatment technologies. Due to the high strength character of these wastewaters, biological treatment systems are the most appropriate treatment technologies (Rajeshwari et al., 2000). There could be differences in the dominant methanogen populations depending on the type of wastewater treated. The methanogenic component of granules used to treat carbohydrate- and protein-based wastewaters are mainly dominated by members of *Methanosaetaceae* and *Methanobacteriales* whereas ethanol-cultivated granules are dominated by *Methanosaetaceae* (Molina et al., 2008).

Liquid phase macro-nutrients that sustain growth as well as granulation are nitrogen and phosphorous whereas cobalt, iron and nickel are obligatory micro-nutrients necessary for the ideal functioning of methanogenic enzyme systems *(*van Lier et al., 2015*).* Other micro-nutrients that can contribute to methanogenesis and granulation include

calcium (Ca), magnesium (Mg), molybdenum (Mo), tungsten (Tu), selenium (Se), potassium (K), barium (Ba), manganese (Mn), zinc (Zn), sulphur (S), copper (Cu) and aluminium (Al) (Nel et al., 1985; Goodwin et al., 1990; Singh et al., 1999; Gerardi, 2003). The macro-nutrient requirements are determined by the C:N:P ratio of the reactor influent and as the COD increases so does the need for nitrogen and phosphorous requirement. The exact requirement of trace nutrients varies for different wastewaters (Metcalf and Eddy, 2014). The macro-nutrients that are critical to sustained growth and granulation are nitrogen (N) and phosphorous (P). The micro-nutrients necessary for the functioning of the methanogenic enzyme system are cobalt (Co), iron (Fe) and nickel (Ni) (Metcalf and Eddy, 2014).

The methanogens present in wastewater treatment systems are more active in the neutral pH range (7.0). Most organisms prefer a suitable pH range of between 6.0 and 9.0, although digestion can still proceed beyond this range, but with less efficiency. Thus, if the wastewater to be treated is of high acidity it will result in a decrease in the reactor pH. This could result in a slower digestion until the bacteria has absorbed the acids within the influent.

2.10 Granular formation and composition

In this section granular structure, formation according to the different granular development theories and the metabolic pathways of granules will be discussed.

2.10.1 Defining granular sludge

Granular sludge can be defined as a symbiotic community of anaerobic micro-organisms that is formed during the treatment of wastewater in an environment with a constant up-flow velocity.

2.10.2 Granular structure

Granular sludge has a typical spherical form with diameters from 0.14 to 5 mm which allows for maximum micro-organism to space ratio. Granular sludge comprises of a granule population with a size fraction of 80% less than 0.5 mm and a larger size granule population that represent 20% of the population. These granules are dense pellets with black or brown exteriors in sizes that vary from 0.25 to 5 mm in diameter. The granules generally have a sponge like structure of 0.1 mm in the outer layer. The inner core of the granules consists of crystalline precipitates and is normally black in colour (Uemura and Harada, 1995).

The bacterial profiles of granules are dependent on the substrate concentration, mass transfer and concentration of the metabolites within the granule. In the outer prominent layer of the acidogenic species the simple sugars are degraded, producing a waste (VFA) that diffuse inward as a result of the concentration gradients. The VFAs are then broken down by the acetogenic species, forming the substrate which the methanogenic bacteria use within the core of the granule. Normally the granule surface consists of gram negative bacteria in a matrix of gram positive microbes. The core is more homogeneous and contains gram negative microbes of the *Methanothrix* family which has an ideal growth pH in the range between 7.1 and 7.8. (Britz et al., 1999) (Flanders Health Blog).

The inorganic content of the granules varies between 10% and 90% of dry weight of granules. The most prominent inorganic species that occur in granules are calcium and phosphate (Britz et al., 1999). Calcium-bound phosphorous is found more prominently in the core of the granule. The interior core of the granules consists of crystalline precipitates such as calcium carbonate. Although there are other inorganic species (Mg, Fe, S, Al, Si, K, Na and Cu) present within the core of the granule they are of less importance.

The granules also consist of organic compounds. The Extracellular Polymers (ECP) is one of the most important and abundant species present in the granules. Extracellular polymers consists mainly of proteins (asparagines, glutamine and aniline) and carbohydrates (mannose and ramose). Compounds such as Deoxyribonucleic Acid (DNA), Ribonucleic Acid (RNA) and lipopolysaccharides can also be found in the granules. The largest fraction of the inorganic elements in ECP is calcium and phosphates.



Figure 2.10: Typical example of granules from a UASB anaerobic reactor (Hulshoff et al., 2004)

The composition of the granules various according to the type of wastewater treated as well as the operational conditions. Typically the

granules are made up of various inorganic components, extracellular polymers and micro-organism groups. These micro-organisms groups form different layers within the granule that comprise of acidogens and hydrogen-consuming micro-organisms in the outer layer. From Figure 2.11 it can be seen that in the intermediate layer, hydrogens producing acetogens are present and the core consists of Methanotrix-like cells (methanogens). In the interior of the granules, holes or cavities can be found which indicates vigorous gas production (MacLeod et al., 1990).

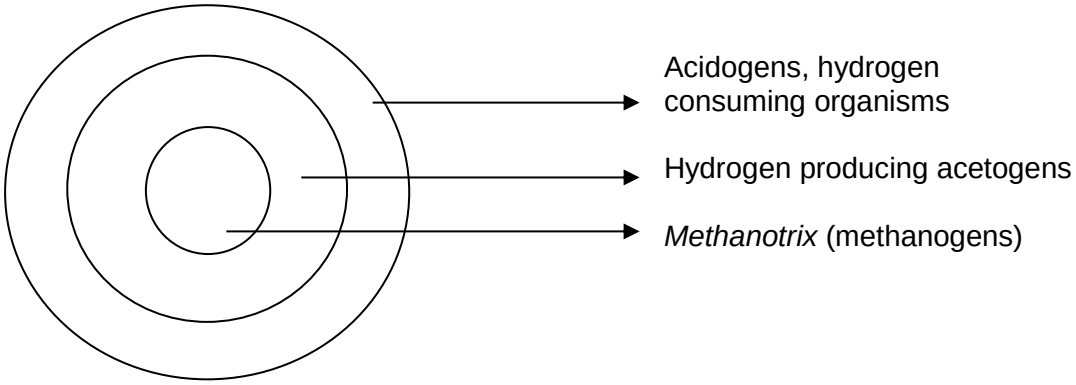


Figure 2.11: Schematic illustration of the different layers of micro-organisms within a granule (MacLeod et al., 1990)

Granular sludge has good separation properties, settling properties, high activity, high biomass retention and the ability to withstand high OLRs. The extracellular polymers within the granular sludge are of high importance as these aids with the structure and maintenance of the granules. The content of the ECP fluctuates from 0.6% to 20% of the Volatile Suspended Solids (VSS) and consists primarily of proteins and polysaccharides (Schmidt et al., 1996).

2.10.3 Granulation process

The anaerobic granulation process is influenced by the bacterial growth as organic and inorganic compounds excreted during bacterial metabolism promote nuclei formation. According to literature, the structural maintenance of mature granules is influenced by the interactions amongst the individual microbial species that are involved in anaerobic digestion (Korsak, 2008). Granulation could be enhanced significantly by manipulating the environmental conditions, so as to create the ideal growth conditions for the slow-growing acetoclastic methanogens bacteria (Hulshoff Pol et al., 2004).

During granular formation there are three distinctive granules that could be formed:

(i) Compact spherical granules – rod shaped bacteria resembling *Methanothrix soehngenii* which are short chain or single cells (rod granules),

(ii) Spherical granules – consisting of intertwined filamentous bacteria attached to inert particles resembling *Methanothrix soehngenii* (filamentous granules), and

(iii) Compact spherical granules composed predominantly of *Methanosarcina* bacteria type.

According to Hulshoff et al., (2004) several theories exist on anaerobic sludge granular formation and these can be divided into three groups: physical, microbial and thermodynamic.

Physical theories

The main physical theories that occur in the reactor during granulation are the “growth of colonised suspended solids” and the “selection pressure” theories. Granules are formed by small particles (micro-organisms) that can be released from larger granules or particles. These suspended micro-organisms can attach to themselves and in so doing form new granules. A fraction of the micro-organisms may be lost due to washout. The micro-organisms can also attach themselves onto suspended solids that are present in the influent and thus form new granules.

Microbial theories

Micro-organisms can adhere to themselves and trap dispersed micro-organisms within their sticky structure resulting in the formation of granules. The main microbial theories are divided into two, the extracellular polymer bonding model and the spaghetti theory.

i) Extracellular Polymer bonding model

According to the ECP model, two cells can connect to either each other or to inert particulate matter by way of changing the negative surface charge of the bacteria. An increase in the OLR can lead to an increase in *Methanosarcina* bacteria growth resulting in the bacteria secreting ECP and creating larger groups/clumps. Figure 2.12 illustrates a schematic representation of the ECP bonding model (Liu et al., 2003).

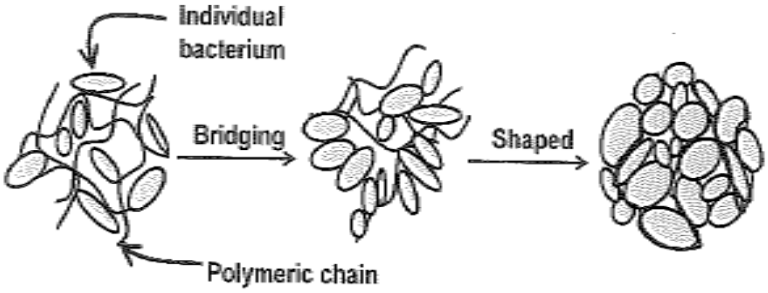


Figure 2.12: Extracellular Polymer bonding model (Adapted from Liu et al., 2003)

Extracellular polymers are metabolic products produced by bacteria and accumulate in the bacteria cell surface. Carbohydrate and protein analysis play an important role in bacterial ECP. Extracellular polymer is composed of various substances such as extracellular polysaccharides (EPS), carbohydrates, polypeptides (protein), humic substance, uronic acid and DNA (Fang et al., 2002; Veiga et al., 1997). Extracellular polymers form a protective layer around the cells in order to protect the cells against the harsh external environment. Extracellular polymer forms a crucial microstructure of methanogenic activated sludge as it aids with the adhesion of the micro-organisms. The ECP has shown to be composed of ESP and polypeptides.

Dispersed bacteria are negatively charged and there is electrostatic repulsion between the cells. The production of ECP can change the surface charge of the bacteria. The chemical composition of the ECP will affect the surface properties of the bacterial flocs and according to literature ECP mediates adhesion of bacteria in natural ecosystems. (Schmidt and Ahring, 1994).

The reported ECP content of granules are between 0.6% and 20% of the VSS content and consists mainly of protein and polysacharrides, typically in a ratio of 2:1 to 6:1 (Schmidt and Ahring, 1996). It is uncertain whether specific species produce ECP, or several or all species in the granular sludge are able to do so. However, Schmidt and Ahring, (1996) have reported that the protein and polysaccharide content decreases when the wastewater influent changes from a sugar containing wastewater to a synthetic wastewater containing acetate, propionate and butyrate. These results disclosed that the production of ECP, in particular polysaccharide production, was restricted in methanogenic and acetogenic systems. Acidogenic populations have a greater influence on the production.

ii) Spaghetti theory

The formation of granules within a UASB reactor occurs by way of the 'Spaghetti theory'. Figure 2.13 illustrates the formation of the granules. The 'spaghetti theory' leads to sludge granulation within a reactor as the acidified water and/or mixtures of VFA are predominantly treated by *Methanothrix* bacteria which has an ideal growth pH range between 7.1 and 7.8 (Flanders Health Blog). Figure 2.13 illustrates how the *Methanosaeta* can attach to bacteria, dispersed matter or other structures that are produced as a result of turbulence due to biogas production. These filaments form a network which traps the bacteria. The multiplication of the entrapped bacteria allows the structure to grow which forms a "spaghetti ball". The density of the 'spaghetti ball' increases as the rod-shaped micro-organisms is formed, resulting in the formation of anaerobic granules (Liu et al., 2003).

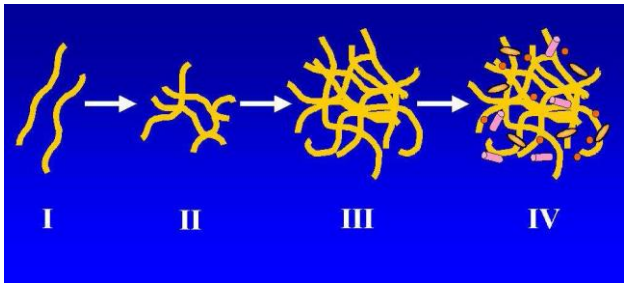
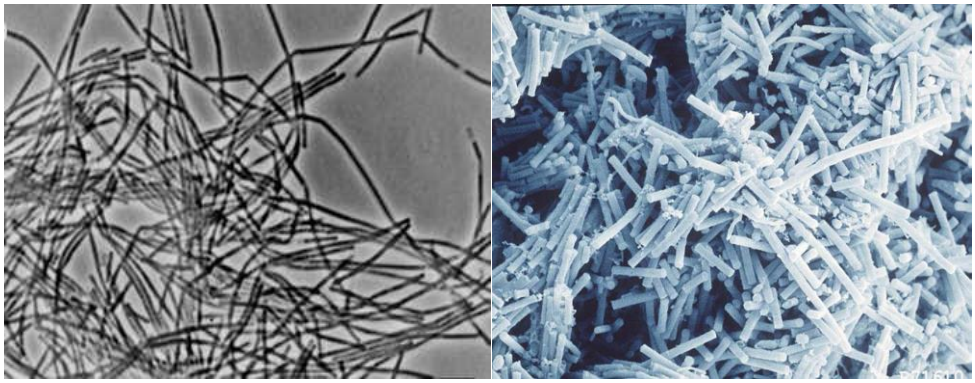


Figure 2.13: Granular formation using the spaghetti theory (Ahmed, 2014)
I) Filamentous *methanosaeta*, II) Network formation, III) Multiplication results in spaghetti balls, and IV) Mature granules, with attachment of other anaerobic Microorganisms

Figures 2.14 (a) and (b) illustrates the rod like shaped *Methanosaeta sp.* and the *Methanosaeta* that forms when the bacteria treats an acetate substrate, respectively.



(a) (b)

Figure 2.14: (a) *Methanosaeta sp.* and (b) *Methanosaeta* treating an acetate substrate (Ahmed, 2014)

Thermodynamic theories

During the granulation process one must consider the granule charge. There are three theories that can be used to describe the granule charge: the four-step model, the proton translocation-dehydration model and the multi-valance ion-bonding model.

(i) Four-step model

This model describes granulation by the transportation of cells to the surface of an inert material (substratum). As the cells collide with each other, reversible adsorption occurs and plays the role of the nucleus to the substratum. The irreversible adhesion of the cell to the substratum occurs when a strong bond forms between the microbe and polymer. Thereafter, the cells multiply resulting in the development of the granules (Schmidt et al., 1996).

(ii) Proton translocation-dehydration model

The formations of granules start with the dehydration of the bacterial surface due to proton translocation activity. During embryonic granule formation the complex organic compound degrades to form substrates for acetogens and methanogens which results in bacteria multiplication. Once the embryonic granules are formed, particles could attach to them resulting in granular maturation. The mature granules are maintained by the proton translocation activity.

(iii) Multi-valence ion-bonding model

The theory of this model is based on the electrostatic interaction between the negatively and positively charged bacteria. Under normal pH conditions the bacteria are negatively charged and multi-valent positive ions favour the attachment between the bacteria. These ions include calcium, aluminium, magnesium and ferric ion (Liu et al., 2003; McHugh et al., 2003).

2.10.4 The influence of metabolic pathways in the determination of the ultra-structure of a granule

The degradation of a substrate has a major influence in terms of the catabolic pathway and ultimately the spatial orientation (ultra-structure) of micro-organisms within in a granule. The mechanisms for the formation of granules in up-flow reactors are not known in full detail. The methanogenic granules are produced during self-immobilisation of the different metabolic groups into bacterial aggregates. Based on a microscopic examination of the UASB granules, Fang, (2000) proposed that the microbial distribution of the UASB granules strongly depends on the degradation thermodynamics and kinetics of an in individual substrate. Therefore, it appears that different catabolic pathways may give rise to different structural granules. The bacteria in a UASB granule are not randomly distributed but rather organized to best meet the needs of each for a defined organic substrate, i.e. the spatial organisation of the UASB granules is developed to cope with the constraints imposed by the substrate fed and corresponding metabolic processes (Liu et al., 2003). It can be expected that the concentration of acetate inside the granules will vary, giving a higher concentration of acetate near the surface than near the centre of the granules. Therefore, different metabolic pathways co-exist in different areas of the granules. This phenomenon of mixed metabolism was observed when sewage sludge digester was either enriched by acetate oxidizing co-culture or *acetotrophic Methanosarcina* depending on the acetate concentration (Schmidt and Ahring, 1993). This study indicated that an increase in hydrogenotrophic methanogens after removing the sludge was due to the increasing size of the granules, resulting in a lower concentration of acetate in the centre of the granules, favouring syntrophic acetate oxidation.

A study from Ahring et al., (1993) investigated the effect of removing granular sludge from a stable operated reactor. After 649 days of stable operation, 60% biomass was removed. This 60% showed that *Methanosarcina* appeared as a cellular blanket rolled upon itself and the remaining biomass after three months formed large aggregates near the granular surface. The inner structure of the granules changed after sludge removal. The granules had actually increased in size, proven by finding an increase in hydrogenotrophic methanogens. This increase was due to a lower concentration of acetate in the centre of the granules, favouring syntrophic acetate oxidation. These changes were accompanied by modifications of the micro-anatomy of the granules. The results indicated that different metabolic pathways most likely co-exist in different areas of the granules. Because the concentration of acetate inside the granules will vary, there is a mixed metabolism within a granule (mixed morphologies of granules). Under conditions where there is high acetate, aceticlastic reaction is favoured and therefore acetoclastic methanogens such as M*ethanosaeta* or *Methanosarcina* predominate. Under acetate conditions, limited acetate oxidation is favoured and hydrogenotrophic methanogens predominate. Different catabolic pathways dominate in different regions of the granules which ultimately determine the spatial orientation of the underlying structure of the granule. This means that acetate oxidation takes place in the middle of the granules where there is a low acetate concentration. The aceticlastic reaction occurs in the outer surfaces, with a high acetate concentration.

2.10.5 Granulation accelerators and inhibitor

According to literature the following constituents were identified as granulation enhancers or inhibitors.

(i) High energy sugars can be considered to be one of the primary factors for influencing the growth of granular sludge. The effluent from a peach cannery effluent was found to be a granulation enhancing carbon source.

(ii) The hydrogen concentration and pH was proven to be the primary limiting factors in the anaerobic digestion and granulation process. The acetogenic ethanol conversion can be limited by the high hydrogen concentrations whereas the pH inhibits the methanogenises. It was also found that the methanogenic step can be inhibited by ethanol, butyrate and propionate (Kalyuzhnyi, 1996).

(iii) Although the granulation process can be inhibited by heavy metals, it was found that inhibition decreases in the following order: Ni>Cu>Cd>Cr>Pb. The following constituents have an inhibitory tendency that affects the granulation rate: ammonia concentration, dispersed solids, organic solvents and long chain volatile fatty acies (Britz et al., 1999). Therefore, foreign substances and heavy metals also have the tendency to inhibit granulation.

(iv) Calcium and magnesium supports the granulation process by neutralising the negative charge on the cell surface or functions as a cationic bridge between bacteria. Thus, calcium and magnesium encourages in granule formation.

CHAPTER THREE – RESEARCH OBJECTIVES

3.1 General objective

This study was aimed at evaluating the function and structural integrity of the granules within the Internal Circulating Anaerobic Digester (IC-AD) treating synthetic wastewater. The structural integrity refers to the ability to hold together a 'structure' (granule) resisting breakage or excessive deformities.

3.2 Specific objectives

i) To optimize the IC-AD so as to remove organic carbon concentration in the synthetically prepared Fischer-Tropsch (FT) process wastewater from between 10000 to 20000 mg/L to 1000 mg/L.

ii) To calculate the removal efficiency of the organic carbon in the synthetic wastewater in excess of 85% at ideal conditions,

iii) To determine the alkalinity requirements in the IC-AD for the removal of organic carbon from synthetically prepared FT process wastewater,

iv) To determine the structure and/or composition of the granules within the reactor,

v) To determine the size of the granules,

vi) To determine the shape of the granules, and

vii) To determine the volume of the granules.

3.3 Hypothesis

An Internal Circulating Anaerobic Digester (IC-AD) reactor that was fed with synthetically prepared FT process wastewater will produce granules, alter or change the size of granules and change the granular function.

3.4 Justification and novelty

A high rate Internal Circulating (IC) reactor was used for evaluating anaerobic treatment of synthetically prepared FT process wastewater during the IC anaerobic treatment. Investigating this technology is of high importance as:

(i) This technology is more cost effective than aerobic treatment because of the lower energy input and the reduced biomass produced which could affect the further costs for disposal or treatment.
(ii) The biomass can be retained within the reactor independently from Hydraulic Retention Time (HRT), a higher microbial activity and Organic Loading Rate can be achieved.
(iii) The reactor is equipped with a three phase separator module that aids in the production of higher quality effluent and reduces the costs of a possible polishing step.
(iv) This technology produces an effluent that complies with the discharge limits.

The treatment of wastewater from a chemical industry using an IC-AD technology is a novel process due to the fact that:

(i) Higher organic loading rates can be obtained without the use of a support medium (packing) resulting in reduced operating costs,
(ii) High organic strength wastewater can be treated,
(iii) Higher organic loading rate can be maintained, and
(iv) This technology has a smaller footprint when compared to that of aerobic technologies and conventional anaerobic treatment technologies (e.g. packed bed or continuously stirred tank reactor)

CHAPTER FOUR – MATERIALS AND METHODS

4.1 Introduction

Chapter four focused on the composition of the synthetically prepared Fischer-Tropsch (FT) process wastewater to be treated, reactor design, the methods used for data capturing and the operating philosophy for the anaerobic reactors.

A description on the different parameters and analytical methods used to capture the data will also be discussed in the chapter. The parameters analysed during this study include pH, alkalinity, Chemical Oxygen Demand (COD), Volatile Fatty Acids (VFA), surface morphology, proteins and carbohydrates. pH, alkalinity, Organic Loading Rate (OLR), COD and VFA were evaluated daily for the Laboratory Scale (LS) and Pilot Scale (PS) reactors while the protein and carbohydrate were only measured at the completion of the different phases for the Pilot Scale Internal Circulating Anaerobic Digester (PS IC-AD) reactor.

4.2 Wastewater

4.2.1 Laboratory scale (LS) reactor

The synthetically prepared FT process wastewater influent to the Laboratory Scale Internal Circulating Anaerobic Digester (LS IC-AD) reactor was prepared in 25 L drums. The nutrients such as nitrogen, phosphate and micro-nutrients were dosed into the 25 L drum as a once off dosage per batch of influent prepared. The pH of the influent was adjusted using a 45% sodium hydroxide (NaOH) solution and/or 32%

hydrochloric acid (HCl). The initial pH set point of the influent was approximately 4.6.

4.2.2 Pilot scale (PS) reactor

The synthetically prepared FT process wastewater was stored in a 1000 L storage tank. This wastewater was pumped into the Pre-Acidification Tank (PAT), where other nutrients such as nitrogen, phosphate and micro-nutrients were dosed. Calcium hydroxide ($Ca(OH_2)$) was dissolved in the wastewater to a concentration of 150 mg/L calcium. This was done for pH correction, as well as for providing calcium for good granule formation. The PAT pH was adjusted using a 10% sodium hydroxide (NaOH) solution. The initial pH set points of the influent during the start-up phase was approximately 3.6 and that of the PAT 5.0.

4.3 Granular sludge

4.3.1 Granular sludge for Laboratory Scale reactor

The granules used to inoculate both LS IC-AD reactors (Run 1 and Run 2) degraded complex polymeric influents consisting of sugars, carbohydrates and polysaccharides and therefore consisted of a distinct Extracellular Polymer (ECP) matrix. The granular sludge used to inoculate the reactors was obtained from a brewery in KwaZulu Natal (KZN).

4.3.2 Granular sludge for pilot scale reactor

The granules used to inoculate the PS IC-AD reactor mainly degraded complex polymeric influents consisting of sugars, carbohydrates and polysaccharides and therefore consisted of a distinct ECP matrix. The granular sludge was obtained from a paper mill effluent in the Netherlands.

4.4 Internal Circulating Anaerobic Digester Reactor

For both the LS and PS IC-AD reactors, the mixing was as a result of up-flow velocity supplied by the recirculation flow-rate.

4.4.1 Laboratory Scale reactor

The LS reactor was a vertical cylindrical tank constructed from glass. The LS reactor had a volume of four litres with a diameter of 15 cm and a height of 33 cm (as illustrated in Figure 4.1).

The reactor was sealed from the environment with a lid on which the inlet and outlets were mounted. The reactor was maintained at 37°C using heating tape that not only holds the thermocouple in place, but also enabled heating of the reactor.

The granular sludge was introduced into the reactor using a Watson-Marlow pump (620S). A peristaltic pump (Watson–Marlow 101) was used to introduce the pre-conditioned synthetically prepared FT process wastewater influent from an influent drum into the reactor. The granular

sludge inside the reactor converted the synthetically prepared FT process wastewater into methane (CH_4), carbon dioxide (CO_2), bicarbonate (HCO_3^-) and new biomass. The biogas produced was collected in the top phase of the reactor. The amount of biogas produced was measured with a Ritter gas flow meter which was open to the atmosphere (outside of the building). A gas sampling point was installed before the gas flow meter in order to allow for sampling of the biogas. The effluent drained from the reactor was collected in a 10 L effluent drum on a daily basis.

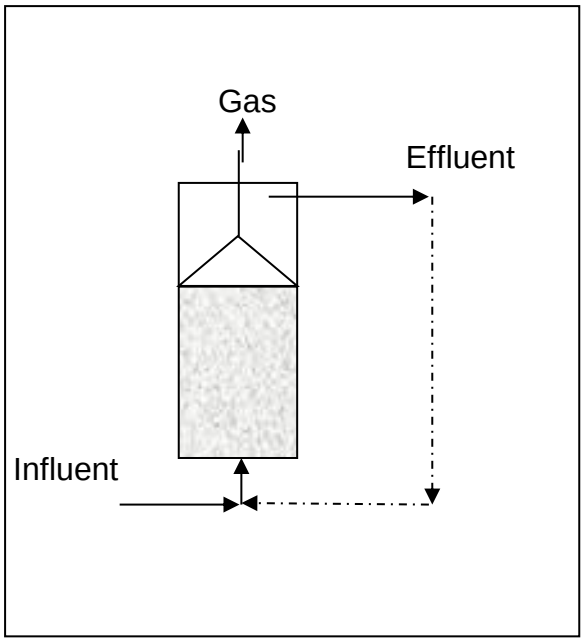


Figure 4.1: Illustration of a Laboratory Scale Internal Circulating Anaerobic Digester

4.4.2 Pilot scale reactor

The PS reactor consists of a 60 L IC-AD reactor with a PAT, influent pumps, recirculation pump, nutrient pumps, a temperature control unit, a pH control unit, biogas meter and a Programmable Logic Controller (PLC) unit for fully automated operation. The unit is constructed on a compact stainless steel frame.

The IC-AD reactor is a vertical cylindrical tank made of perspex with a double wall to ensure heating with water (Tielbaard et al., 2002). The reactor temperature was maintained at 37°C. On top of the lowest part of the reactor a polypropylene 3-phase separator is mounted. This separator enabled maximum separation of the biogas and sludge. From the settler, recirculation of the effluent takes place by means of an external recirculation pump. On top of the highest part of the reactor, where polishing of the effluent takes place, a second polypropylene 3-phase separator was mounted, which enables maximum separation of biogas, sludge and final effluent (Grant et al., 2002). The lowest part of the skid was used for storage of the chemicals (NaOH and HCl) and nutrients.

The granular sludge was introduced into the reactor using a Watson-Marlow pump (620S). The pre-conditioned synthetically prepared FT process wastewater was pumped from the PAT into the reactor using a Watson-Marlow pump 101. The granular sludge inside the reactor converted the synthetic wastewater into methane (CH_4), carbon dioxide (CO_2), bicarbonate (HCO_3^-) and new biomass. The biogas produced was collected at the middle and top 3-phase separators and expelled through a water lock (refer to Figure 4.2). The amount of produced biogas was measured with a Ritter gas flow meter and vented to the open atmosphere (outside of the building). A gas sampling point was installed before the gas flow meter for sampling of the biogas. Although the effluent left the reactor at the top, a part of the effluent was recycled back to the PAT for conditioning of the raw influent. The treated effluent flowed over to the effluent drum and into the sewer system.

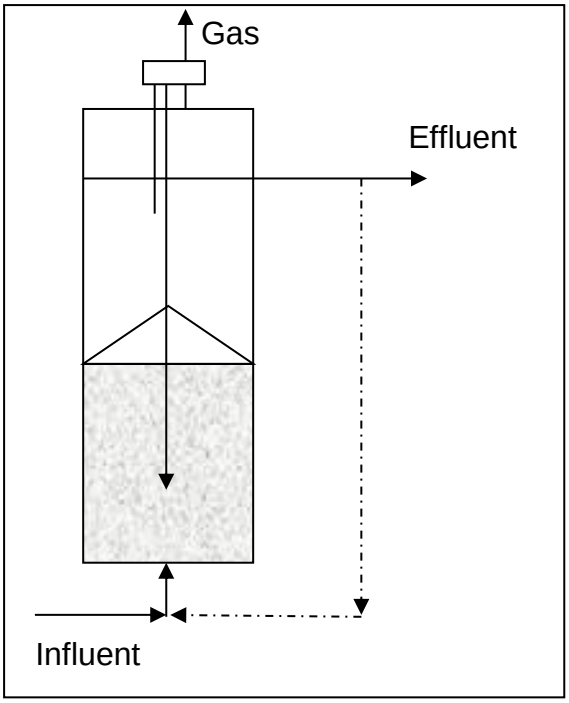


Figure 4.2: Illustration of an IC reactor indicating the important parts namely; lower reactor component which is the first biogas zone (influent enters here and is mixed with the granular sludge). The second zone is after the first separator which includes the polishing compartment because the biogas leaves through a second separator

4.5 Analytical Methodology

This section entails a discussion on the chemicals, instruments and relevant analytical methods used during this study. The samples that were collected during this study include the influent synthetic wastewater, effluent and granules. These samples were analysed for COD, pH, VFA and alkalinity according to the Standard Methods.

4.5.1 Laboratory Scale reactor

A grab sample (1 L) of the influent was taken daily from the influent drum. Another grab sample (1 L) of the effluent was taken daily from the composite drum. The initial sample of granules obtained from a brewery

was collected with start-up. Thereafter, the granules were collected at different intervals for analyses from the reactor.

4.5.1.1 Reagents/Chemicals/Solutions

The Buffer solution 4 and 7 was used in order to calibrate the pH meter (Mecosa, Randburg, South Africa).
The determination of the VFA was done by titration using Hydrochloric acid (Sigma Aldrich, Johannesburg, South Africa).
The COD measurements were performed using Nanocolor Test kit 0-28 (1000 to 15000 mg/L) and Nanocolor Test kit 0-29 (100 to 1500 mg/L) (Separations, Randburg, South Africa).
The alkalinity was determined by titration using Sulphuric acid (Sigma Aldrich, Johannesburg, South Africa).

4.5.1.2 Equipment/Instruments

A WTW Multiliner P4 portable pH meter and Sentix 41 WTW pH330 sensor probe were used for the measurement of pH (South Africa).
The alkalinity and VFA concentrations were determined by titration using a Methrohm 785 DMP tirtino and 760 sample changer (Methrohm, Johannesburg, South Africa).
The COD concentrations were determined using a Nanocolor® 500D spectrophotometer (Separations, Randburg, South Africa).
The biogas composition was determined on an Agilent Technologies 7890A Gas Chromatography (GC) System (Chemetrix, South Africa).
The biogas production was measured with a Ritter drum type gas meter (Poly Test Instruments C, Farrarmere, South Africa)
The granular surface morphology was determined using a FEI QUANTA FEG 250 Environmental scanning electron microscope.

The individual VFA concentration was determined using a High Performance Liquid Chromatography (HPLC)) (Chemetrix, South Africa)

4.5.1.3 Analytical analyses

The following parameters were analysed in order to assess the reactor performance.

4.5.1.3.1 pH

The pH was measured in accordance with Standard methods for examination of water and wastewater, method nr. 4500-H^+ B, Electrometric Method (STANDARD METHOD, 1995).

The pH meter was calibrated with buffer solutions (4 and 7), the probe rinsed with distilled water and dried with a soft tissue. Thereafter, the pH of the influent and effluent were determined by adding 80 mL of sample into a 100 mL glass beaker. The electrode was immersed in the sample and the pH measured, the probe rinsed with distilled water and dried with a soft tissue.

The precision of the pH meter is ± 0.02 pH unit with an accuracy of ± 0.05 pH unit. However, a 0.1 pH unit represents the limit of accuracy under normal conditions for water or poorly buffered solutions. A buffer solution with a pH 7.3 was analysed electrometrically by 30 laboratories with a standard deviation of 0.13 pH unit.

4.5.1.3.2 Alkalinity

The alkalinity was measured in accordance with Standard methods for examination of water and wastewater, method nr. 2320 (STANDARD METHOD, 1995).

The alkalinity was determined by potentiometric titration. A chosen sample volume (50 mL or 100 mL) was titrated using a 0.02N sulphuric acid solution. The sample was titrated to an end point of pH 4.3 and 4.5 which resulted in the m-alkalinity. The results were computed by a pre-programmed instrument at completion of the analyses.

In a laboratory a sample was analysed and using the same calibration had a standard deviation of 0.21 mg/L and a coefficient of variation of 1.05% with respect to repeatability. This sample had a standard deviation of 1.67 mg/L and a coefficient of variation of 8.04% with respect to reproducibility.

4.5.1.3.3 Volatile fatty acids

Determination of total VFA concentration (titration method)
The samples were titrated from the initial pH to four further points. Unfiltered samples of the liquid influent and effluent were analysed for VFA concentrations. The fatty acids were measured using a five point titration method. This titration method was performed at pH values 6.7, 5.9, 5.2 and 4.3. A chosen sample volume was added into a 100 mL glass beaker. The sample was titrated using a 0.01N HCl solution. Once the titration was completed, the VFA results were calculated using

a computer calculation program (TITRA4 and TITRA5). The precision for this test has not been determined.

Determination of individual VFA compounds (High Performance Liquid Chromatography (HPLC))

The HPLC was calibrated with an external standards prepared in the mobile phase containing different concentrations of the organic acids.

The C_1 to C_5 organic acids were measured using a reversed phase HPLC, equipped with a Variable Wavelength Detector (Ultra Violet (UV) detector) at 210 nm using a C18 (100 mm x 4.6 mm, 2.7 µm)

The repeatability precision of the five acids was determined from ten replicates of a reaction water sample. The sample was injected by the same analyst using the same instrument on the same day.

4.5.1.3.4 Total Suspended Solids (TSS)

The suspended solids were measured in accordance with Standard methods for examination of water and wastewater, method nr. 2540D (STANDARD METHOD, 1995).

The glass fibre filter disk was washed, dried for an hour at 103°C to 105°C and weighed. This process was repeated until the filter disk obtained a constant weight. The sample volume was chosen to yield a dried sample of between 2.5 and 200 mg dried residue.

A 100 mL sample was filtered through the prepared filter disk. The measuring cylinder that contained the sample was washed with three successive 20 mL of distilled water to ensure that all the solids were transferred to the filter disk. The suction was continued for a three minute period once filtration was completed to remove all the traces of water. The filter disk was removed from the filtration unit and placed on a weighing dish, dried for at least two hours at 103°C to 105°C, placed in a desiccator and weighed until a constant weight was obtained.

In the laboratory two analysts obtained a standard deviation of 5.2 mg/L at 15 mg/L (coefficient of variation 33%), 24 mg/L (10%) at 242 mg/L and 13 mg/L (0.76%) at 1707 mg/L for four sets of 10 determinations each.

4.5.1.3.5 Chemical oxygen demand

The chemical oxygen demand was measured in accordance with the German Institute for standardisation International Organisation for Standardization 15705 method (DIN ISO 15705).

Determination of COD from 100 to 1500 mg/L (Test 0-29)
The test tube was opened and 2 mL of unfiltered sample was slowly added to the test tube without mixing. The cap was screwed back onto the test tube. The test tube was held by the cap while shaking the test tube. The test tube was placed in a heating block (148°C) for two hours to allow for digestion. After two hours the test tube was removed from the heating block. The test tube was allowed to cool to room temperature and the COD concentration was read at an absorbance of 620 nm using a Nanocolor® spectrophotometer.

Determination of COD from of 1000 to 15000 mg/L (Test 0-28)
The test tube was opened and 0.2 mL of unfiltered sample was slowly added to the test tube without mixing. The cap was screwed back onto the test tube. The test tube was held by the cap while shaking the test tube. The test tube was placed in a heating block (148°C) for two hours to allow for digestion. After two hours the test tube was removed from the heating block. The test tube was allowed to cool to room temperature and the COD concentration was read at an absorbance of 605/620 nm using a Nanocolor® spectrophotometer.

A sample was analysed daily by different analysts and under different conditions. A verification standard was used and the analyst obtained a standard deviation of 3.93 mg/L O_2 (RSD 3.88%) with a precision limit of 10.89 was obtained.

4.5.1.3.6 Biogas composition

The methane and carbon dioxide content was measured with an Agilent Technologies 7890A Gas Chromatography (GC) system equipped with a Thermal Conductivity Detector (TCD) detector. The column was a 6 Ft x ⅛ in Porapak Q column. The temperature for the injection point, detector and column were 150°C, 200°C and 50°C, respectively. The carrier gas used was helium at a flow-rate of 2 mL/min (The precision for this test has not been determined). The biogas production was measured with a Ritter drum type gas meter with a deviation of +0.14%.

4.5.1.3.7 Granules surface morphology determination

The objective of the Scanning Electron Microscope (SEM) analyses was to investigate the various microbial population dynamics within the granules. The SEM analyses were conducted with the assistance of the North West University (NWU).

The granular samples were analysed under the SEM. The material was fixed in 70% ethanol for two hours and dehydrated in an ethanol series of 70%, 80%, 90% and twice in 100% ethanol. The dehydration process was performed over a period of 15 minutes for each of the percentage values in the ethanol series. The samples were then placed in a critical point drier. The dried samples were mounted on SEM stubs and viewed under a scanning electron microscope. Precision is not applicable.

4.5.1.3.8 Particle Size Distribution (PSD)

The objective of the Particle Size Distribution (PSD) analyses was to determine the size distribution of the granules. Laser diffraction technique was used to determine the PSD. The PSD analyses were conducted with the assistance of Group Technology, Sasol.

This method was adapted from the International Organization of Standardization (ISO) method 13320:2009

The instrument was pre-set using the parameters listed below:

- Flow-rate: 3.0 L/min
- Obscuration 35% - set on auto dilute
- Refractive Index: 1434
- Absorption coefficient: 0.1

The granular samples were circulated for 90 seconds. A 100 mL of the sample was pipetted from the beaker and placed in the sampling unit of the Micrometrics Saturn Digiziser 5205. The Refractive Index and the absorption coefficient were set to the values mentioned above in order to ensure a goodness to fit of <1% (only 1% of the collected data do not fit optical model based on refractive index and absorption coefficient). As soon as the obscuration of 35% was obtained, the instrument collected the data and an average of five tests was reported by the software. The precision for this test has not been determined.

4.5.2 Pilot scale reactor

A grab sample (1 L) of the influent was taken daily from the influent drum. Another grab sample (1 L) of the effluent was taken daily from the composite drum. The initial sample of granules obtained from a paper mill was collected with start-up. Thereafter, the granules were collected at different intervals for analyses from the reactor.

4.5.2.1 Chemicals/Reagents/Solutions

The proteins were determined by using the following solutions:

- Solution A (sodium tartrate and sodium carbonate dissolved in sodium hydroxide) (Sigma Aldrich, Johannesburg, South Africa)
- Solution B (sodium tartrate and copper sulphate dissolved in distilled water) (Sigma Aldrich, Johannesburg, South Africa)
- Solution C (Folin-Ciocalteu dissolved in distilled water) (Sigma Aldrich, Johannesburg, South Africa)

The carbohydrates were determined using the following solutions:

- Athrone reagent in ethanol diluted with sulphuric acid (Sigma Aldrich, Johannesburg, South Africa)
- Glucose solution, glucose dissolved in distilled water and benzoic acid (Sigma Aldrich, Johannesburg, South Africa)
- Sulphuric acid (75% v/v) (Sigma Aldrich, Johannesburg, South Africa)
- Sodium chloride (0.85% v/v) (Sigma Aldrich, Johannesburg, South Africa)

4.5.2.2 Instruments

A Jenway 6300 spectrophotometer and cuvettes were used for determining the proteins and carbohydrates (Aqualytics, Pretoria, South Africa).

4.5.2.4 Analytical analyses

The following parameters were analysed in order to assess the reactor performance.

4.5.2.4.7 Protein test

The protein was measured in accordance to a modified Lowry method obtained from the Jenkins manual, pp. 19, Table 2.13 (Jenkins et al., 2003).

The granules from the PS IC-AD reactor were washed and crushed. Thereafter, one mL of sample containing 15 to 100 µg protein was added

to the test tube, followed by the addition of 0.9 mL of solution A. The test tube was placed in a water bath at 50°C for 10 minutes, removed and allowed to cool to room temperature before adding 0.1 mL of solution B. The test tube was allowed to stand at room temperature for 10 minutes. Solution C was added and mixed thoroughly. The test tube was placed in a 50°C water bath for 10 minutes, removed and allowed to cool at room temperature. The absorbance was read at 650 nm using a one centimeter cuvette. The granules were sonificated in order to ease the extraction of the protein compounds. A standard curve of the absorbance versus the micrograms protein was plotted. The precision for this test has not been determined.

4.5.2.4.8 Anthrone test for Carbohydrates

The carbohydrates were measured in accordance to the Jenkins manual, pp. 18, Table 2.12 (Jenkins et al., 2003).

The granules were washed, crushed, pipette into boiling tubes and the final volume was adjusted to 1 mL using distilled water. Three to four glucose standards were prepared with a final volume of 1 mL and one 1 mL distilled water blank. The test tubes were cooled in an ice bath. The absorbance was read at 625 nm and distilled water was used as a blank. A standard curve of absorbance versus glucose concentration was plotted. The precision for this test has not been determined.

4.6 Operating philosophy

4.6.1 Laboratory Scale Internal Circulating Anaerobic Digester

The reactor was started-up with a diluted synthetically prepared FT process wastewater influent. During commissioning the reactor was inoculated with 2.0 L of granular sludge obtained from a brewery in KwaZulu Natal (KZN). The operating parameters are summarised in Table 4.1.

Table 4.1: Operating conditions during the Laboratory Scale Internal Circulation Anaerobic Digester operation (Run 1 and Run 2)

Parameter	Value
OLR (kg COD/m^3.d)	20
Temperature	37°C
pH	6.8 – 7.2

During Run 1 the COD concentration was maintained at approximately 22000 mg/L. The influent to the reactor was fed with a peristaltic pump (Watson-Marlow 101) with a variable flow-rate. The OLR during Run 1 was increased by increasing the influent pump flow-rate. The influent flow-rate was increased incrementally once the effluent COD concentration was below 100 mg/L.

During Run 2 the influent COD concentration was increased incrementally. The influent to the reactor was fed with a peristaltic pump (Watson-Marlow 101) that was kept at a constant flow-rate of 5.4 mL per minute. The influent COD concentration was increased during this operation. The OLR was increased by increasing the COD concentration

of the influent. The influent COD concentration was increased in increments once the effluent COD concentration was below 100 mg/L. The COD concentration range was between 987 and 6300 mg/L.

In order to maintain maximum methanogenic activity, liquid phase nutrients were required (nitrogen and phosphorus). In addition, trace elements (iron, cobalt, and zinc) were generally required to stimulate methanogenic activity. Urea and phosphoric acid were dosed to maintain sufficient nitrogen and phosphate levels in the effluent and a micro-nutrient solution was also added to maintain a C:N:P ratio of 100:1:0.25. It was thus important to maintain the operating pH near neutral (7.0). Sufficient alkalinity was essential to maintain the correct pH in the system and represent the ability of the digester to neutralise the volatile acids formed during digestion. Therefore the influent pH was adjusted using a 45% sodium hydroxide (NaOH) solution.

4.6.2 Pilot Scale Internal Circulating Anaerobic Digester

Phased approaches were applied during the study using the PS IC-AD. First, a commissioning phase was used for the start-up period and to stabilise the IC-AD reactor.

In the first phase the reactor was commissioned by inoculating the reactor with 25 L of acclimatised granular sludge obtained from a paper mill in the Netherlands. The operating parameters are summarised in Table 4.2.

Table 4.2: Operating conditions during the commissioning phase (PS IC-AD)

Parameter	Value
Influent flow-rate (L/h)	150 – 160
OLR (kg COD/m^3.d)	20 – 25
Recycle flow-rate (L/h)	150
pH	6.8 – 7.2

During the stabilisation phase the reactor was fed with a diluted synthetic wastewater mixture with a COD concentration of approximately 6500 to 7500 mg/L. The operating conditions for the stabilisation phase are summarised in Table 4.3.

Table 4.3: Operating conditions during the stabilisation phase (PS IC-AD)

Parameter	Value
Influent flow-rate (L/h)	180 – 195
OLR (kg COD/m^3.d)	25 – 30
Recycle flow-rate (L/h)	150
pH	6.6 – 7.5

The commissioning phase was followed by the stabilisation phase to generate the relevant data and evaluate the process efficiency. The operating parameters for the synthetic wastewater using the PS IC-AD reactor are presented in Table 4.4.

Table 4.4: Operating parameters for the Pilot Scale Internal Circulating-Anaerobic Digester (PS IC-AD)

Parameter	PS IC-AD Reactor
Design COD loading rate (OLR)	30 kg COD/m^3.d
Maximum COD loading rate (OLR)	30 - 40 kg COD/m^3.d
pH	6.6 – 7.5
Influent : recycle ratio	No recycle (only required when COD >10000 mg/L)
Temperature	36°C – 42°C
C:N:P ratio	500 : 5 :1

The PS IC-AD reactor was operated at 30 kg COD/m^3.d after commissioning and maintained at this loading rate for a period of six to eight weeks to evaluate the treatment of synthetic wastewater under stable operation.

During the third phase the reactor had to be re-commissioned. The operating conditions for the re-commissioning phase are summarised in Table 4.5.

Table 4.5: Operating conditions during re-commissioning of the reactor (PS IC-AD)

Parameter	Value
Influent flow-rate (L/h)	130
OLR (kg COD/m^3.d)	15 – 20
Recycle flow-rate (L/h)	150
pH	6.6 – 7.5

In order to maintain a maximum methanogenic activity, liquid phase nutrients were required (nitrogen and phosphorus). In addition, trace elements (iron, cobalt, and zinc) were generally required to stimulate methanogenic activity. Urea and phosphoric acid were dosed to maintain sufficient nitrogen and phosphate levels in the effluent and a micro-nutrient solution was also added to maintain a C:N:P ratio of 500:5:1. Methane producing bacteria are sensitive to pH and are inhibited at pH values less than 6.2. It was thus important to maintain the operating pH near neutral (7.0). Sufficient alkalinity (derived from the breakdown of organics and which is primarily present in the form of bicarbonates) was essential to maintain the correct pH in the system. Alkalinity is important as it represents the ability of the digester to neutralise the volatile acids formed during digestion. Therefore, the influent pH was adjusted using a 10% sodium hydroxide (NaOH) solution. The average Ripley Ratio (RR) during the stabilisation period was 0.02. According to literature the ideal RR should be 0.3 (Ross, 1992).

CHAPTER FIVE – RESULTS AND DISCUSSIONS

The results obtained during this study and the performance of the Internal Circulation Anaerobic Digester (IC-AD) is presented in this chapter. These results are separated into two sections; Laboratory Scale (LS) and Pilot Scale (PS) internal circulating anaerobic digester. For each section; (i) analytical parameters and (ii) Scanning Electron Microscopy (SEM) study results are presented. The pilot scale internal circulating anaerobic study includes the protein and carbohydrate measurements.

Part A: Laboratory Scale Internal Circulating Anaerobic Digester (LS IC-AD Run 1)

This is divided into two sections, Run 1 and Run 2. In Run 1, the influent COD concentration was maintained constant at approximately 22000 mg/L, while in Run 2 the influent COD concentration was stared at about 1000 mg/L and increased with time to about 6000 mg/L.

5.1 Influent and effluent characteristics of the Laboratory Scale Internal Circulating Anaerobic Digester (LS IC-AD Run 1)

The average range of the minimum and maximum composition of the synthetically prepared Fisher-Tropsch (FT) process wastewater influent is provided in Table 5.1. The synthetically prepared FT process wastewater influent pH was controlled by adjusting the pH in the influent tank with hydrochloric acid (HCl) or sodium hydroxide (NaOH). The synthetic wastewater influent was supplemented with nutrients such as micro-and macro-nutrients, urea and phosphoric acid as mentioned in the experimental section (refer to section 4.6.1). The nutrient levels were

controlled within the influent tank. The average range of composition of the parameters after LS IC-AD treatment is given in Table 5.2.

Table 5.1: Average range composition of the synthetically prepared Fisher-Tropsch process wastewater influent before treatment (LS IC-AD Run 1)

Parameters	Range of values	Average value ± SD	n
pH	3.6 – 4.7	4.5 ± 0.2	100
COD (mg/L)	20800 – 23600	22517 ± 568	100
Alkalinity (mg/L)	187.0 – 923.0	620.0 ± 204.0	53
Formic acid (mg/L)	0 – 44.0	1.5 ± 7.98	33
Acetic acid (mg/L)	124.0 – 1226.0	928.2 ± 201.0	33
Propionic acid (mg/L)	355.0 – 782.0	616.1 ± 99.7	33
Butyric acid (mg/L)	206.0 – 449.0	331.9 ± 56.7	33
Valeric acid (mg/L)	71.9 – 217.0	116.7 ± 38.5	33

SD - Standard Deviation

Table 5.2: Average range composition of the effluent after treatment (LS IC-AD Run 1)

Parameters	Range of values	Average value ± SD	n
pH	6.9 – 8.0	7.5 ± 0.2	100
Effluent COD (mg/L)	165 – 4500	1263 ± 1183	98
Alkalinity (mg/L)	187.0 – 923.0	641.0 ± 176.0	53
Total Suspended Solids (TSS) (mg/L)	2 – 2210	404 ± 557	46
Total VFA (mg/L)	30 – 186	68 ± 32.0	33
Formic acid (mg/L)	0 – 60.0	2.05 ± 11.12	33
Acetic acid (mg/L)	2.0 – 34.0	10.97 ± 8.6	33
Propionic acid (mg/L)	4.2 – 31.9	14.6 ± 5.2	33
Butyric acid (mg/L)	2.5 – 65.8	13.3 ± 11.6	33
Valeric acid (mg/L)	0 – 40.2	5.5 ± 7.5	33
Biogas (L/d)	0 – 54.0	11 ± 11	100
Methane (%)	21.0 – 78.0	66.0 ± 9.0	70
Carbon dioxide (%)	4.0 – 25.0	17.0 ± 6.0	70
Organic Loading Rate (kg COD/m^3.d)	1 – 11	5 ± 3.0	100
Removal efficiency (%)	81 – 100	94 ± 5.0	100

5.2 Performance of the Laboratory Scale internal Circulating Anaerobic Digester (LS IC-AD Run 1)

The performance and results of the LS IC-AD (Run 1) was studied over a period of 100 days. These results were separated into different analytical parameters for discussion. The influent to the reactor was prepared in batches to maintain an influent Chemical Oxygen Demand (COD) concentration of approximately 22000 mg/L. The influent adjustments to the reactor were done by increasing or decreasing the influent pump flow-rate. Throughout section 5.2 of the study, the reference to COD effluent will be referring to unfiltered COD concentration measured in the effluent.

5.2.1 Influent and effluent COD concentration

The influent and change in effluent COD concentration during the operation of the LS IC-AD reactor is depicted in Figure 5.1. The influent COD concentration varied between 22200 mg/L to 23800 mg/L as a function of batch influent preparation. The reactor was started up at an influent flow-rate of 0.8 L/d. The effluent COD concentration increased gradually to about 3200 mg/L on day five and peaked at 4500 mg/L on days seven. The increase in the effluent COD concentration was probably attributed to the increase in the Total Suspended Solids (TSS) (>500 mg/L) due to wash-out of fine particles (Figure 5.2). The wash-out of solids detected in the effluent was probably attributed to the poor adaptation of the granules. According to Mahmoud (2008), the TSS was removed from the UASB reactor by being washed out in the effluent during experiments conducted with a similar reactor. Thereafter, the influent flow-rate to the reactor was halved to 0.4 L/d on day six. Over the next ten days the effluent COD concentration decreased steadily. The decrease in the effluent COD concentration during the next few days

most likely resulted in an increase in the COD removal efficiencies to 90%. Findings from studies conducted by Goodwin and Stuart (1994), indicted that COD removal efficiencies of 90% were achieved using an UASB reactor to treat distillery effluent. The lower effluent COD concentration measured on day 17 was an indication that the reactor performance was probably improving (Figure 5.1). The COD removal efficiency increased to above of 95% during this time period.

The effluent COD concentration remained below 500 mg/L for the period between days 20 to 38. As the COD effluent concentration decreased to below 500 mg/L, the influent flow-rate was increased to 0.48 L/d. As a result of the influent flow-rate being steadily increased to 0.79 L/d, an increase in the effluent COD concentration was observed. The effluent COD concentration was highly variable and remained high (between 1200 and 3500 mg/L) from days 39 to 64. This increase was most likely ascribed to the temporary wash-out of fine particles which most likely resulted in an increased TSS concentration (Figure 5.2). The washout of the solids detected in the effluent was probably attributed to the poor adaptation of the granules. Similar observations were made by Mahmoud (2008), as TSS was removed from the UASB reactor by being washed out in the effluent. In conjunction with the effluent COD concentration decreasing to below 300 mg/L on day 65 subsequently did the wash-out of the sludge particles decreased (TSS below 100 mg/L). Furthermore, the higher COD effluent concentration observed when increasing the Organic Loading rate (OLR) may possibly be explained by an increase in the Volatile Fatty Acid concentration (VFA).

The decrease observed in the effluent COD concentration on day 65 indicated that the reactor performance was improving and that the microbial population within the granules had probably adapted to the

influent substrate. The effluent COD concentration remained below 500 mg/L for the next few days (Figure 5.1).

The unexpected increase in the effluent COD concentrations on days 82 and 88 was probably ascribed to the increase in the influent flow-rates from approximately 2.0 L/d to 2.2 L/d on day 82 and from 2.4 L/d to 2.8 L/d on day 88 (Figure 5.1). The slight decrease in the influent COD concentration from approximately 22500 mg/L to 21000 mg/L noted on day 94 most likely stemmed from the influent being prepared in batches.

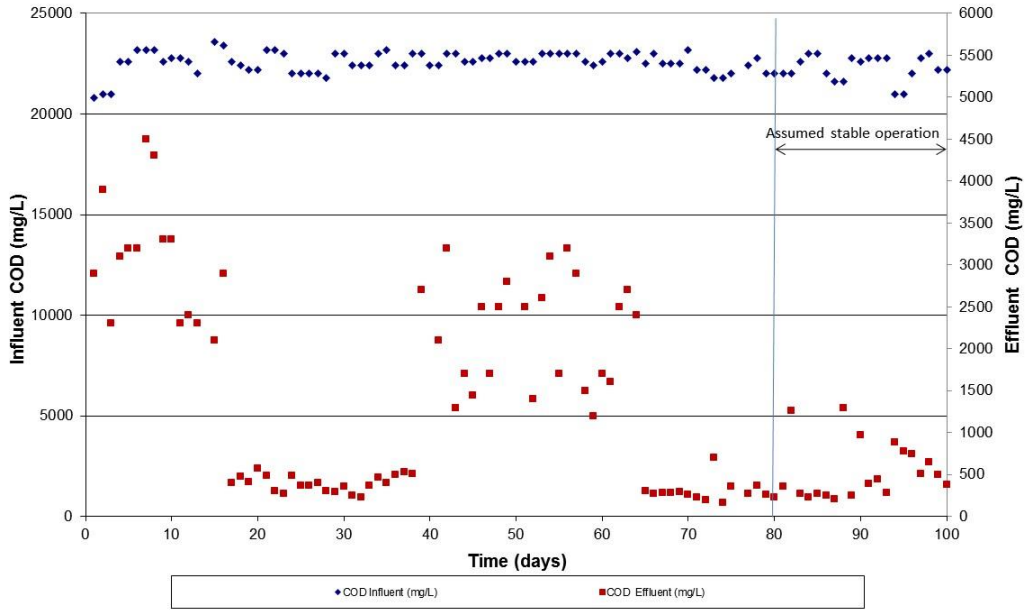


Figure 5.1: Comparison of influent and effluent COD concentration during operation of the reactor (LS IC-AD Run 1)

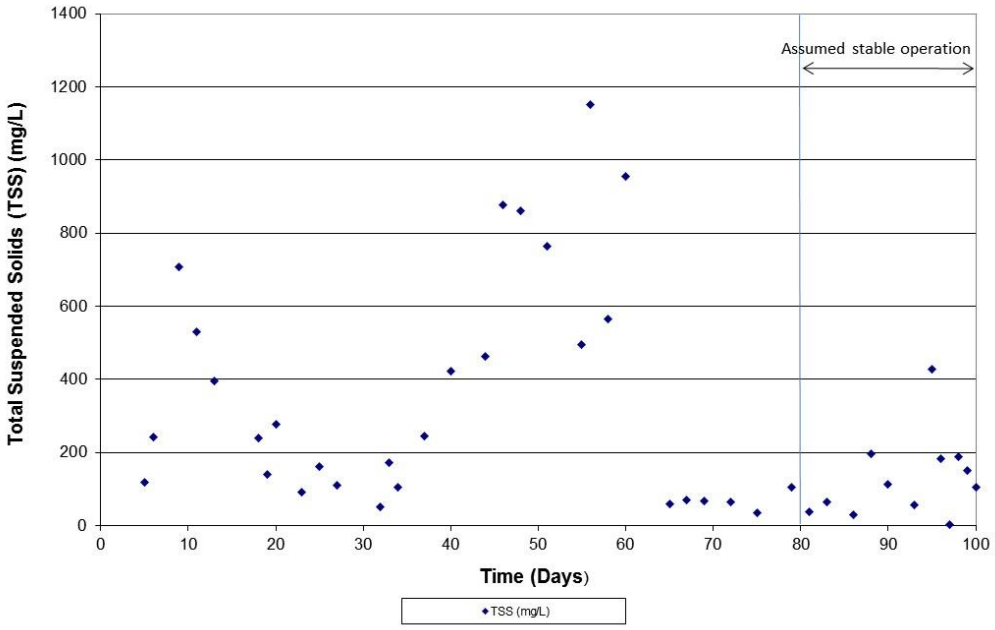


Figure 5.2: Total Suspended Solids (TSS) concentration during operation of the reactor (LS IC-AD Run 1)

The percentage COD removed in an ideal system is independent of the flow-rate within the system, subsequently after an increase in the COD concentration the effluent COD always spikes (lower removal efficiencies) and then returns back to a removal efficiency percentage of >90%. The expected flow-rate within an ideal system ranges between 5.5 and 9.6 L/d for synthetically prepared FT process wastewater with a typical COD of 22000 mg/L. The flow-rates obtained during this study mostly ranged between 0.4 and 2.8 L/d for an average COD concentration of 22500 mg/L.

An increase in the influent flow-rate most likely increased the OLR which resulted in an increased effluent COD concentration or poorer removal efficiency. It can be seen in the calculated data summarised in Table 5.3 what the expected flow range and OLR could be for the IC-AD technology. During this study (Run 1) the assumed stable operation

period (day 80 – 100) achieved an OLR of between 8 -11 kg COD/m^3.d, however based on literature the expected OLR could still have increased should the experimental trial been completed. The study in Run 1 never achieved stable operation, but was continuously in a state of ramp up, although the removal efficiencies continued to improve over time as the OLR was increased.

Table 5.3: Expected flow range and organic loading rates for the Internal Circulating Anaerobic Digester technology (LS IC-AD Run 1)

Constant influent COD (mg/L)	Flow-rate (L/d)	OLR (kg COD/m^3.d) (calculated)	Influent COD obtained (mg/L)	Flow-rate (L/d)	OLR (kg COD/m^3.d) (calculated)
22000	0.4	1.47	22500	0.4	1.50
22000	0.8	2.93	22500	0.8	3.00
22000	1.0	3.67	22500	1.0	3.75
22000	1.2	4.40	22500	1.2	4.50
22000	1.4	5.13	22500	1.4	5.25
22000	1.6	5.87	22500	1.6	6.00
22000	1.8	6.60	22500	1.8	6.75
22000	2.0	7.33	22500	2.0	7.50
22000	2.2	8.07	22500	2.2	8.25
22000	2.6	9.53	22500	2.6	9.75
22000	2.8	10.27	22500	2.8	10.50
22000	5.5	20.17	22500	5.5	20.63
22000	6.9	25.30	22500	6.9	25.88
22000	8.2	30.07	22500	8.2	30.75
22000	9.6	35.20	22500	9.6	36.00

5.2.2 Organic Loading Rate and Removal efficiency

The Organic Loading Rate (OLR) is dependent on the influent COD concentration. The OLR and removal efficiency obtained for the LS IC-AD are shown in Figure 5.3.

The reactor was stared-up at an OLR of between 2.7 to 3.8 kg COD/m^3.d. During this period the removal efficiency remained above 80%. Although the OLR was decreased to 1.5 kg COD/m^3.d on day five the removal efficiency remained above to 80%. A similar trend was observed by Wolmarans et al., (2002) during the start-up phase of an USAB treatment plant. At an OLR of approximately 1.7 kg COD/m^3.d (day 13) an increase in the removal efficiency from 81% to 90% was observed. The OLR varied consistently with the removal efficiency for the first 30 days of operation. These observations bear a resemblance to the results attained by other research workers (Wolmarans et al., 2002).

Figure 5.3 shows an increase was observed in the removal efficiency to 98% on day 20 and remained more stable during the next 20 days of operation. During LS studies conducted by Standbauer et al., (1994), removal efficiency in the range of 90% was observed which was in concurrence with the removal efficiency attained during this study.

The variation in the removal efficiency that was observed from days 39 to 64 was most likely due to an increase in the effluent COD concentration during this period. As the effluent COD concentration decreased, the removal efficiency increased and stabilised at 99% on day 65.

The OLR was increased steadily over a period of 13 days (day 20 to 33) from 1.85 and 3.36 kg COD/m^3.d as a result of the influent flow-rate being increased from 0.4 L/d to 0.6 L/d. The influent flow-rate was further increased from 0.8 L/d to 2.3 L/d which resulted in an increase in the OLR from approximately 2.4 to 8.4 kg COD/m^3.d. However, when the OLR was increased to 8.4 kg COD/m^3.d it appeared that the COD

removal efficiency deteriorated. Similar findings were reported in a study treating distillery wastewater (Wolmarans et al., 2002). According to findings made by Moosbrugger et al., (1993), plant performance correlates directly with COD removal efficiency.

The decrease in COD removal efficiency may possibly be accompanied by an increase in the effluent VFA concentrations. Similar observations were made by de Graaff et al., (2010) in a study using an USAB which was treating concentrated black water. During environmental stress, the methanogenic system was most likely stressed and the reactor was probably overloaded with easily degradable organic matter resulting in the accumulation of propionic acid.

The expected OLR range within an ideal system is between 20 to 30 kg COD/m^3.d with a percentage COD removal efficiency of between 90% and 95%. Although this study had not reached stable operation an average OLR of 5 kg COD/m^3.d with an average removal efficiency of >90% was achieved (100 days).

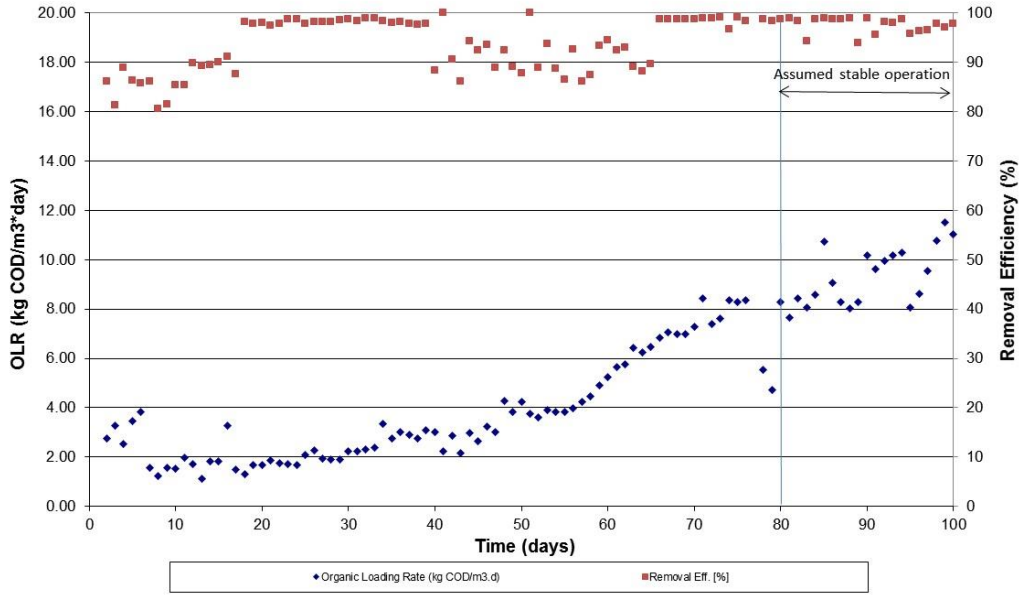


Figure 5.3: Organic Loading Rate (kg COD/m^3.d) and Removal Efficiency (%) during operation of the reactor (LS IC-AD Run 1)

5.2.3 Biogas Production

The biogas produced was depended on the COD conversion rate and the main constituents were methane (60% to 86%) and carbon dioxide (14% to 28%). An increase or decrease in the OLR probably had an immediate increase or decrease in the production of the biogas. However, during digester overload the carbon dioxide content in the biogas most likely increased as the methane content decreased. Although the reactor response to biological conversion of the OLR was determined by the volumetric gas measurement, an indication of the healthiness of the system was given by the methane to carbon dioxide ratio of the biogas. If the carbon dioxide fraction was higher than the methane, this was most likely a sure sign of the reactor upset.

The biogas production rate is summarized in Figure 5.4. The biogas produced in the first 19 days was between 5 to 6 L/d. A decrease in the biogas production rate was observed on day seven and probably resulted in the influent flow-rate being reduced. As a result of the influent flow-rate being reduced, the Hydraulic Retention Time (HRT) reduced and therefore the methanogens had a shorter time to convert the organics into biogas. The biogas production decreased to approximately 3 L/d.

On day 38 a faulty Ritter gas meter resulted in problems with measuring the biogas concentration. Thereafter, the biogas production rate remained unstable for a period of time. This could be attributed to the micro-organisms utilising all the influent in a short period of time. As the influent flow-rate was increased at shorter time intervals, the biogas production increased to above 10 L/d on day 70.

Although the biogas production was erratic as indicated in Figure 5.4, an increase was observed from day 79 onwards. The methane content of the biogas declined from 67% to 60% during this time frame with a corresponding increase in the carbon dioxide. This slight increase in the carbon dioxide content was probably as a result of the increasing OLR. The possibility exists that the carbon dioxide solubility increased the amount of carbon dioxide going into the biogas phase, thus decreasing the methane concentration in the biogas. The decrease observed in the methane concentration may possibly be as a result of the methanogenic activity decreasing.

In an ideal system the expected methane produced is in the range of 60% to 75% and the carbon dioxide is in the range of 25% to 40%. The

expected biogas produced in an ideal system is in the range of 0.2 to 0.6 L/g COD. The maximum biogas produced in this system was probably 0.6 ± 0.2 (n = 96) L/g COD (100 days) whereas the maximum methane produced was probably 0.4 ± 0.1 (n = 98) L/g COD (100 days). During this study (100 days) the methane and carbon dioxide produced was between 66% to 75% and 17% to 23%, respectively.

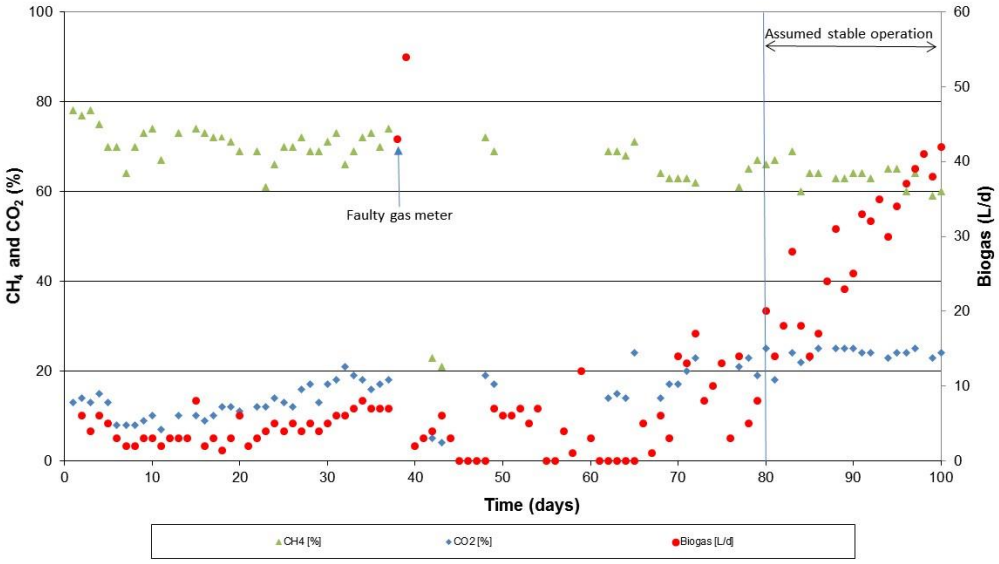


Figure 5.4: Total biogas production (L/d), methane and carbon dioxide concentration (%) observed during operation of the reactor (LS IC-AD Run 1)

5.2.4 Influent and effluent pH

The influent and effluent pH are summarised in Figure 5.5. The influent pH was adjusted from 3.2 to 3.5 using a 45% sodium hydroxide solution. The influent pH was maintained at this pH for the first five days. Thereafter, the influent pH was increased to 4.0 as a result of the effluent pH decreasing to below 6.9. The decrease observed in the reactor pH was most likely due to the high acidity of the synthetically prepared influent or as a result of VFA being produced. Therefore, the influent pH was further increased to 4.6 on day eight. The effluent pH increased

from 7.4 to 7.7 from days 13 to 18, a slight decrease in the effluent pH was observed on day 19.

During this study the effluent pH was maintained at a pH between 7.4 and 7.7, which is the ideal growth pH range for methane-forming bacteria such as Mathanothrix, *Methanospirillium* and *Methanococcoides* (Flanders Health Blog) (illustrated in Figure 5.5), hence ensuring that the required pH for effective anaerobic digestion was maintained. According to a study by Beccari et al*.,* (1996) the methanogenic activity will be affected by low pH values. The best pH for microbial growth is between 6.8 and 7.2 which is the ideal growth pH for methane-forming bacteria such *Methanosphaera, Methanothermus, Methanolacinia, Methanospirillium, Methanococcoides, Methanogenium and Methanothri* (Flanders Health Blog)*. T*herefore, the variation in pH probably resulted in the methanogenic activity decreasing as a result of the methanogens ability to metabolise the organic acids fast enough. Similar observations in pH measurements were made by Keyser et al., (2003) during the operation of a bioreactor.

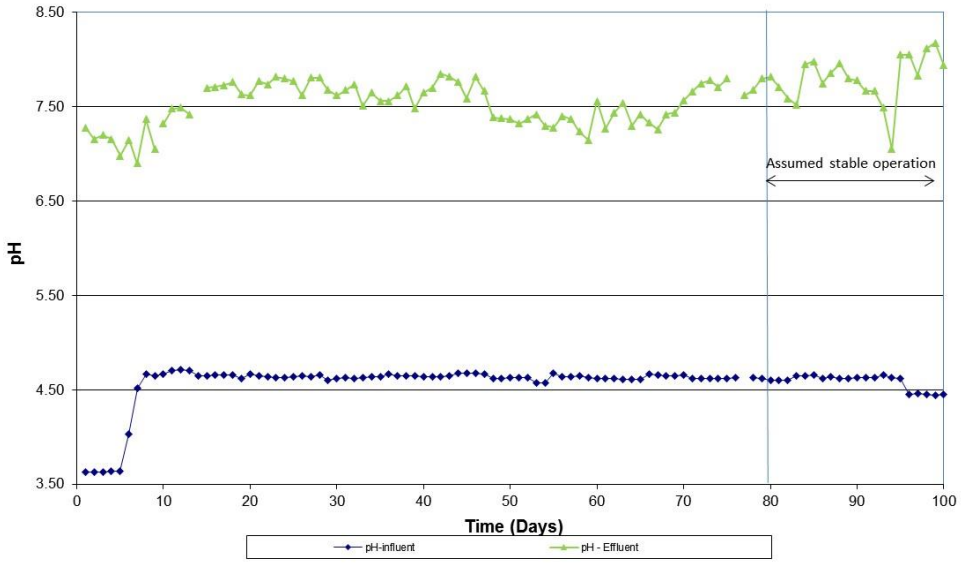


Figure 5.5: Influent and effluent pH observed during operation of the Reactor (LS IC-AD Run 1)

5.2.5 Alkalinity

The synthetically prepared FT process wastewater had a pH range of between 3.6 and 4.7 which was in an acidic pH range. Therefore, its addition into the reactor lead to the consumption of the alkalinity, bicarbonate (HCO_3^-) as shown in equation 5.1.

$$ROO^-H^+ + HCO_3^- \longrightarrow CO_2 + H_2O \quad (5.1)$$

(organic acid) (alkalinity in reactor)

As a result of the above mentioned reaction (5.1), the pH within the anaerobic digester decreased. The addition of alkalinity in the form of calcium hydroxide was therefore required to buffer the anaerobic digester to optimal operating pH conditions.

In addition to the above mentioned reaction, the micro-organisms within the anaerobic digester produced biogas which consisted of methane and carbon dioxide as the synthetically prepared FT process wastewater was degraded. The carbon dioxide within the reactor shifted the pH of the reactor into a lower pH range.

$$\text{Biomass + Organics} \longrightarrow \underset{\text{(in reactor)}}{CO_{2(g)}} + H_2O \longrightarrow \underset{\text{(carbonic acid)}}{H_2CO_3} \longleftrightarrow H^+ + HCO_3^- \qquad (5.2)$$

Since the anaerobic digester was at a pH range of between 6.9 and 8.0 the alkalinity in the system was maintained in the form of bicarbonate (HCO_3^-) (illustrated in Figure 5.6) (D. LAB 4. Acid-base titrations).

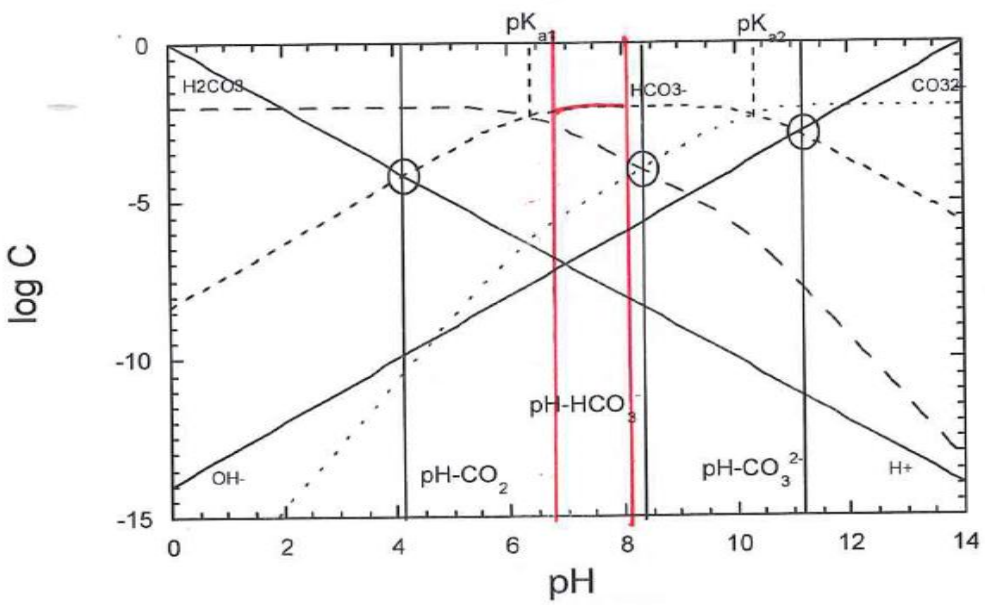


Figure 5.6: Distribution of the carbonate species with the pH in a closed system, the red lines indicate the pH range where the LS IC-AD reactors were operated (D. LAB 4. Acid-base titrations)

Alkalinity serves as a buffer to prevent rapid pH fluctuations (Figure 5.7). Therefore, sufficient alkalinity within the digester is essential in order to maintain proper pH control. The reserve bicarbonate alkalinity was defined as the concentration of bicarbonate alkalinity that is available for the anaerobic process to neutralise the additional free VFA. Therefore, it

was of great importance to know what the concentration of the reserve alkalinity was before the pH decreased to below the safe threshold value of approximately 6.2 to 6.5.

The average alkalinity measured during the 100 day run was 641 mg/L. As the alkalinity steadily increased during the first 10 days of operation so the effluent pH increased to above 7.3. As a result of the alkalinity increasing on day 20, an increase in the effluent pH was observed.

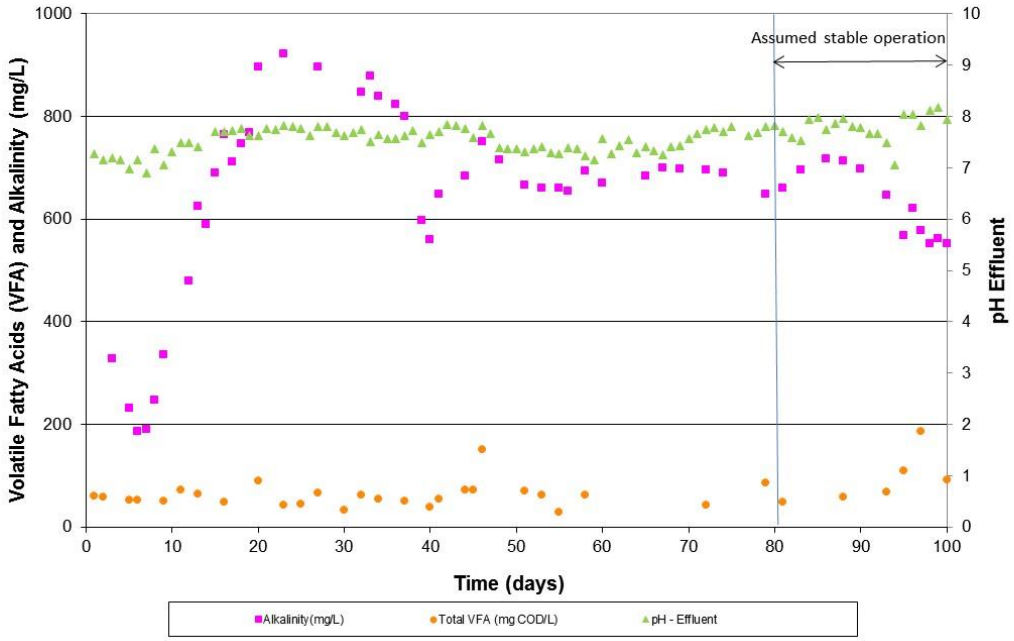


Figure 5.7: Alkalinity and effluent pH observed during operation of the reactor (LS IC-AD Run 1)

The RR was calculated by dividing the VFA concentration in the effluent with the effluent alkalinity. Obtaining a RR of 0.1 indicates normal conditions within the reactor. The RR obtained during Run 1 was between 0.04 and 0.32 (illustrated in Figure 5.8). According to Ross (1992), anaerobic digesters perform best at RR of 0.3 and at low RR the reactor operating conditions are good. On days 20 and 46 the RR

increased and this was most likely ascribed to the increase in the VFA concentration. The increase observed on day 97 to 0.32, most likely indicated that the reactor was not performing as expected. This increase in the RR was likely ascribed to increase in the VFA (110 to 180 mg/L) which indicated that the microbial population was likely under 'stress' and the reactor performance was not at its best. The alkalinity concentration remained unchanged and indicated that the system possibly had a greater capacity for resisting pH changes.

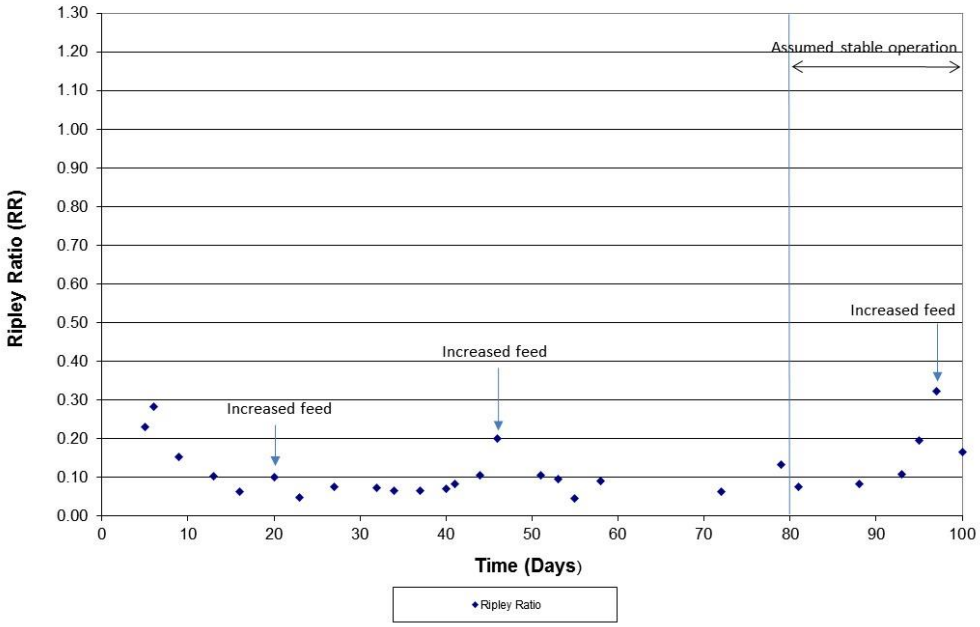


Figure 5.8: Ripley Ration (RR) observed during operation of the reactor (LS IC-AD Run 1)

5.2.6 Scanning Electron Microscopy (SEM) analyses for Laboratory Scale IC-AD (Run 1)

For both runs using the LS IC-AD, the reactors were commissioned using granules obtained from a brewery in KZN.

The granules used to inoculate the reactor as well as the granules that have been treating synthetically prepared FT process wastewater were analysed using SEM.

The granules used to inoculate the LS IC-AD had a mean and median (d_{50}) size of 1248 and 1258 µm, respectively. Although 90% (d_{90}) of the granular sludge sample consisted of granules smaller than 1870 µm, 28% of the granules were smaller than 1000 µm. The particle size distribution of the granules used to inoculate the reactor as well as the granules sampled after a 100 days are illustrated in Figure 5.9.

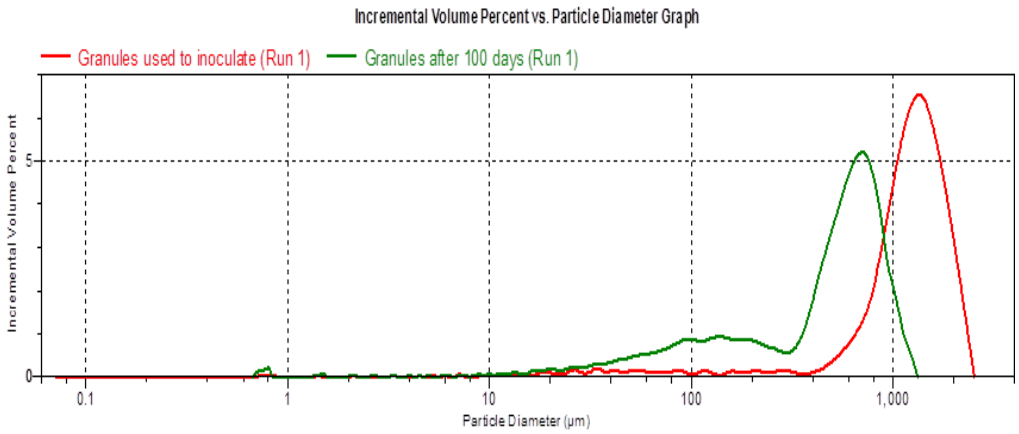
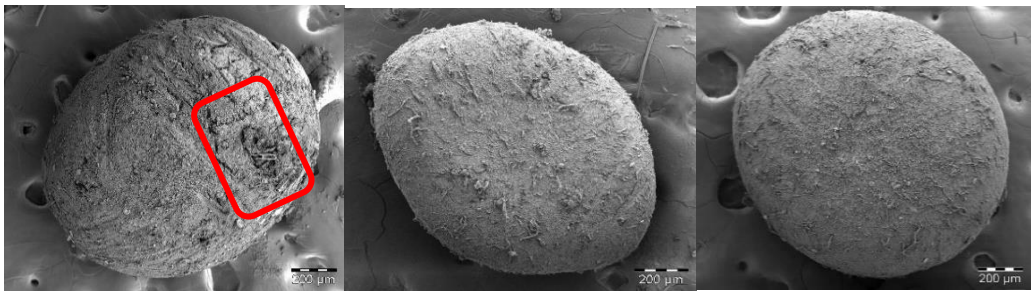


Figure 5.9: Particle size distribution for the initial granules and granules after 100 days (LS IC-AD Run 1)

The SEM images in Figure 5.10 (a-c); depict images of various different granules obtained from the initial batch of granules. The granular sludge had a typical spherical shape and seemed to have a sponge like structure with a surface made up of aggregates of micro-organisms.

The inset from Figure 5.10 (a) shows the presence of a diatom skeleton on the surface of the initial granule obtained from the brewery. With the completion of the life cycle of the diatom, the diatom organic matter

decomposes and washes away. The diatom skeleton (a silica skeleton) remains as an inorganic mineral and results in the formation of sedimentary deposits. Diatoms are single cell algae that are enclosed with a cell wall made of silica (frustule) and could be present in all aquatic environments. Diatoms can be subdivided into two groups, centric and pennate diatoms, depending on the form of the frustule.



(a) (b) (c)

Figure 5.10 a-c: SEM images of granules used to inoculate the reactor. The red inset illustrates the presence of a diatom skeleton (LS IC-AD Run 1)

The inset from Figure 5.11 shows a higher magnification of the diatom skeleton present on the surface of the KZN Brewery granules.

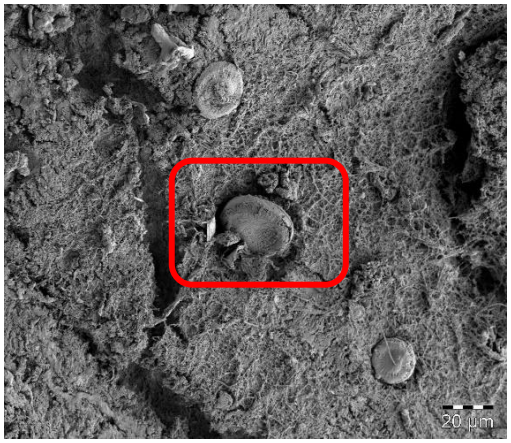
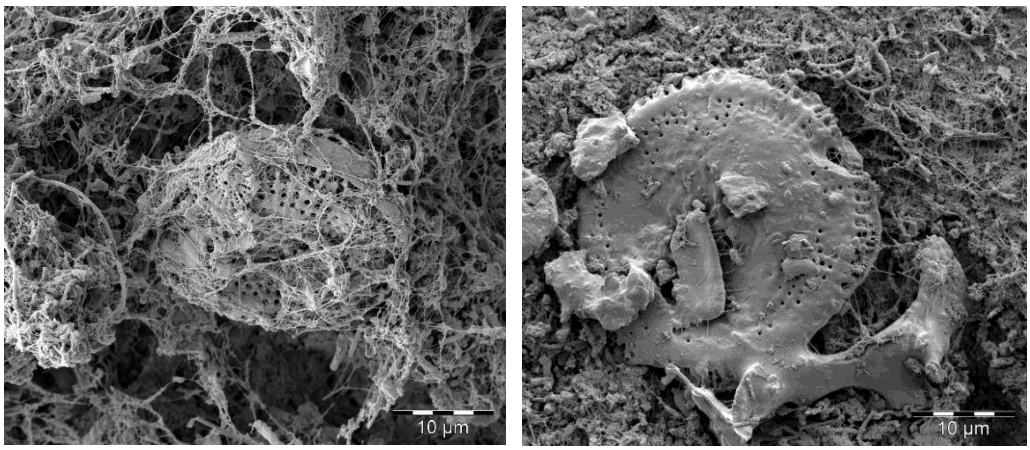


Figure 5.11: Magnification of a diatom skeleton present on the surface of a KZN Brewery granule (LS IC-AD Run 1)

Diatomaceous earth is a chalky sedimentary rock comprised of skeletal remains of diatoms. These inorganic minerals are used as a filtration medium due to it being inert, having a low density and high porosity. These skeletons are processed by drying, milling and calcination. The products formed are classified and used for water filtration.

The diatom skeleton (centric) within the initial granules was present as the KZN Brewery uses a diatomaceous earth filtration process for the purification of fluid (Figure 5.12). Similar observations were made by Mach et al., (2000) in a study treating low to medium strength brewery wastewater in a Static Granular Bed Reactor (SGBR).

The ECP matrix that surrounded the diatom skeleton was visually visible as shown in the SEM image in Figure 5.12 (a). It was anticipated that the fermentative acedogenic bacteria was probably responsible for the production of the ECP. The ECP matrix aids in the bacterial cells agglomerating and aids with structural integrity (MacLeod, 1995).



(a) (b)

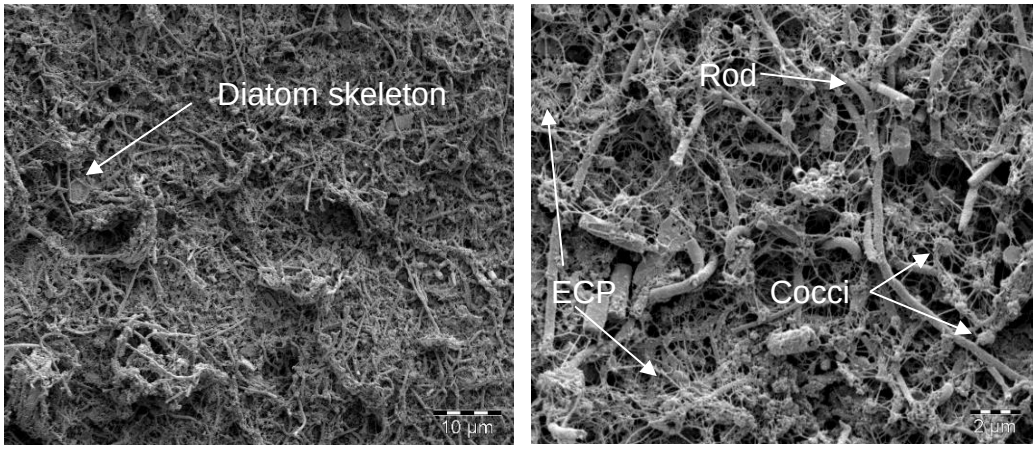
Figure 5.12: Closer view of centric diatom skeletons present on the surface of the KZN Brewery granule (LS IC-AD Run 1)

The microstructure of the granule was examined using SEM and the images are shown in Figure 5.13 (a) and (b). These images indicate that the initial granules had a distinct micro-organism morphology. The ECP within the granule was clearly visible and indicated that bacterial adhesion within the granule was present, aiding with the maintenance of the granule structure. According to Morgan (1990) the accumulation of ECP on the surface was most likely due to metabolic products produced by bacteria.

The distinct ECP matrix clearly visible in the granules could be ascribed to the original granules received from the brewery. These granules were mainly degrading complex polymeric influent consisting of sugars, carbohydrates and polysaccharides. The formation of the ECP matrix was due to the presence of fermentative and hydrolytic bacteria degrading the substrate into simple compounds (VFA, etc.) which are excreted (van Lier et al., 2008).

The distinct ECP matrix tightly linked and embedded various Cocci and rod shaped micro-organisms (Figure 5.13 (a) and (b)). The two morphological shapes of Cocci and rod shaped micro-organisms were in all probability known as M*ethanococci* and *Methanosaete,* respectively*.* As illustrated in the SEM images, the micro-organisms were present in a mixed metabolism configuration in close proximity to one another. The internal structure of the granule is dependent on the substrate being degraded. Due to the complex nature of the wastewater, the bacterial groups involved in the anaerobic digestion process are most likely hydrolytic and acidogenic fermentative bacteria, which had been reported to produce ECP*.* The hydrolytic bacteria most likely consist of members of the genera *Streptococcus* and *Entero* bacterium. The lengthy rod shaped bacteria and the presence of the ECP aided with the

initial granulation process, granule development and maintaining the granule structural integrity.



(a) (b)

Figure 5.13: SEM image illustrating the morphology of the inner core of the initial KZN Brewery granules (LS IC-AD Run 1)

The visual observation of the morphology of a granule that has been treating synthetically prepared FT process wastewater for 16 days using a LS IC-AD reactor (Run 1) can be seen in Figure 5.14. The granule had a spherical shape.

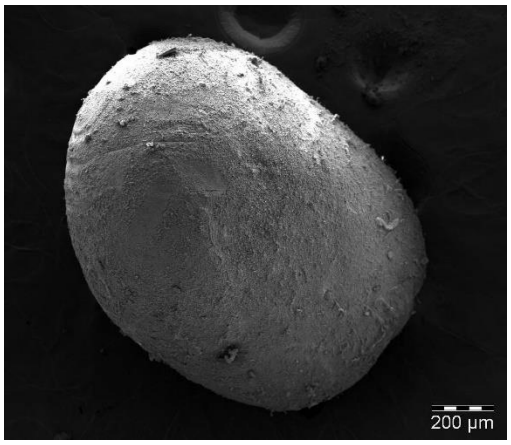


Figure 5.14: SEM image of a granule treating synthetically prepared FT process wastewater for 16 days (LS IC-AD Run 1)

The SEM observations indicate that the morphology of the granule mainly consisted of rod shaped bacteria and some ECP matrix. The morphology of the visually observed bacteria in the granule (Figure 5.15) demonstrated a shift in the microbial population. The rod shaped bacteria were visibly shorter than those present in the initial granules (Figure 5.13 (b)) and a decrease in the ECP matrix was apparent. Relative to the granules used during the inoculation process, visually the morphology of the granule had changed due to the presence of Cocci being reduced (see Figure 5.13 (b)). This could be ascribed to the change in the composition of the wastewater being fed to the granules. This changed from a brewery complex wastewater containing sugars and carbohydrates to a synthetic wastewater made up of VFAs (acetate, propionate and butyrate). Given the absence of the complex substrate in the wastewater influent, the hydrolytic and acidogenic bacteria no longer occupy the functional niche in the granules and the granules consisted primary of acetogenic and methanogenic bacteria (refer to Figure 2.1). The acetogenic bacteria may possibly consist of members of the genera *Acetomaculum, Clostridium, Syntrophococcus, Syntrophobacter* and *Syntrophus* (Lamb, 1995).

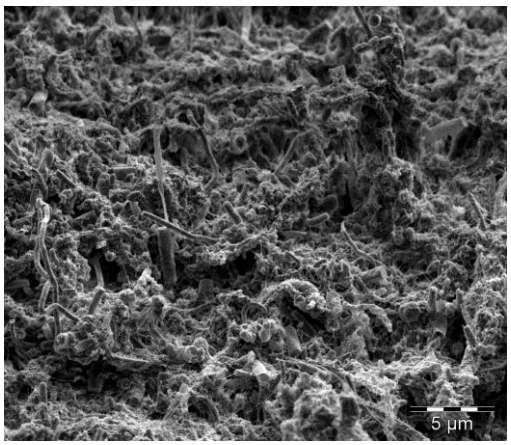


Figure 5.15: SEM image showing the morphology of a granule that has been treating wastewater for 16 days (LS IC-AD Run 1)

Figure 5.16 shows a granule that has been treating synthetically prepared FT process wastewater for 38 days using a LS IC-AD reactor (Run 1). From the SEM image it appeared that the shape of the granule was unchanged.

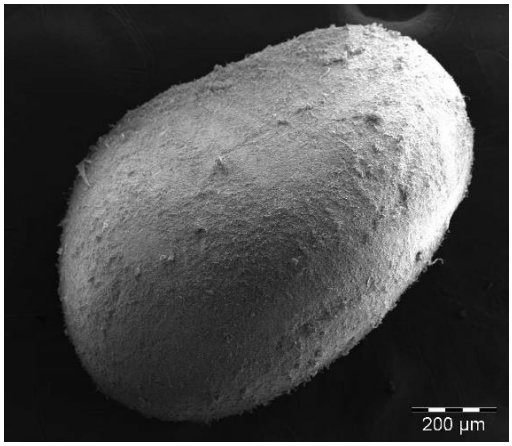


Figure 5.16: The SEM image of a granule that has been treating wastewater for 38 days (LS IC-AD Run 1)

Although the morphology of the visually observed bacteria mainly consisted of rod shaped bacteria and some ECP matrix, no additional shift in microbial population had occurred (Figure 5.17). As reported before (Figure 5.15), the rod shaped bacteria were visibly shorter than those present in the initial granules (Figure 5.13 (b)) and a decrease in the ECP matrix was apparent. Relative to the granules used during the inoculation process, visually the morphology of the granule had changed due to the presence of Cocci being reduced (illustrated in Figure 5.13 (b)). This was probably ascribed to the change in the composition of the wastewater being fed to the granules. This changed from a brewery complex wastewater containing sugars and carbohydrates to a synthetic wastewater made up of VFAs (acetate, propionate and butyrate).

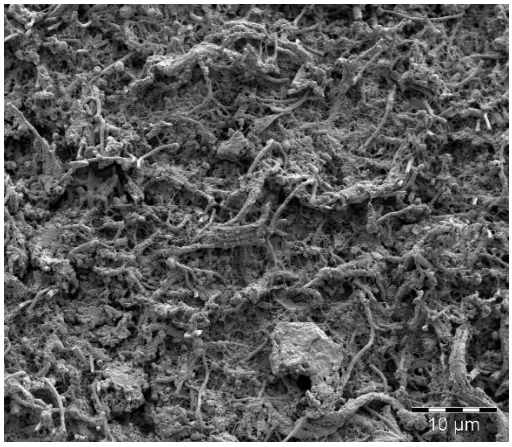


Figure 5.17: SEM image illustrating the morphology of a granule that has been treating synthetically prepared FT process wastewater for 38 days (LS IC-AD Run 1)

Figure 5.18 shows a granule that has been treating synthetically prepared FT process wastewater for 46 days using a LS IC-AD reactor (Run 1). From the SEM image it appeared that the shape of the granule was unchanged.

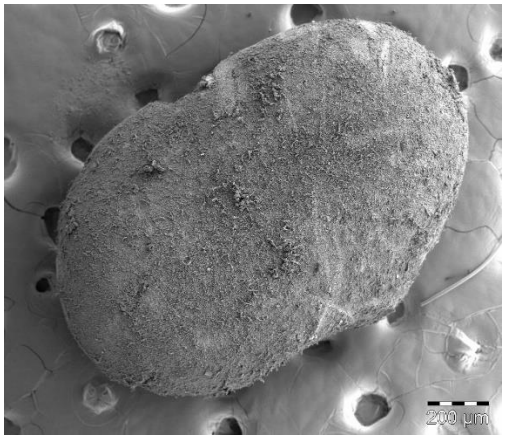
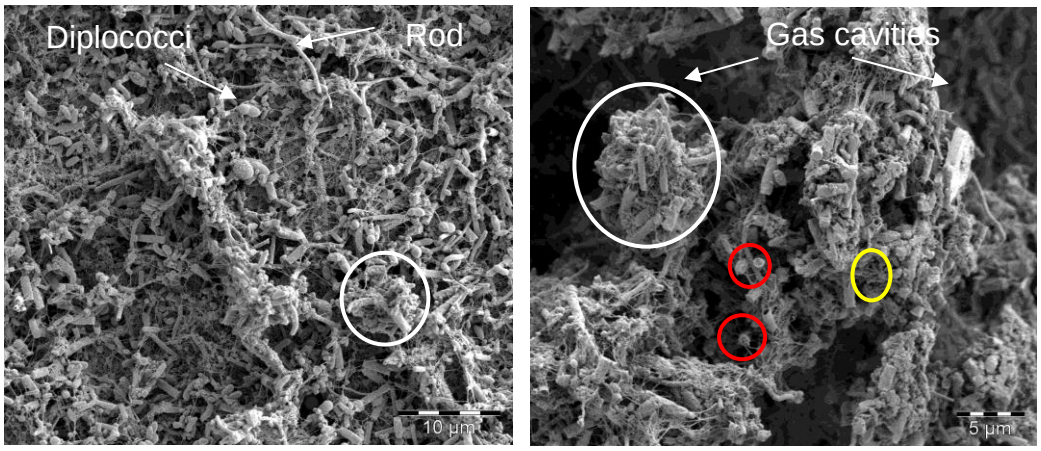


Figure 5.18: SEM image of a granule treating synthetically prepared FT process wastewater for 46 days (LS IC-AD Run 1)

It was evident from the morphology of the inner core of the granule, that Diplococci (Cocci in pairs) and Coccobacilli (round rods) like bacteria were present (Figure 5.19). The area shown in the white circle shows a small group of bacteria that were clumped together inside the granule.

The presence of gas cavities between the clumps of bacteria can be seen in Figure 5.19 (b). In addition, the SEM image shows an area, indicated with a yellow circle, where the ECP structure surrounds the terminal cell and the red circle shows the ECP cap covering the terminal cell.

According to the morphology of these granules it was clear that a change in the bacterial population had occurred. The ECP matrix has decreased and the rod shaped bacteria were shorter. The change observed in the ECP matrix was probably attributed to the synthetic wastewater being degraded by acetogenic and methanogenic bacteria. As the methanogenic system is stressed, the reactor was most likely overloaded with easily degradable organic matter which probably resulted in the accumulation of propionic acid.



(a) (b)

Figure 5.19: SEM image showing the inner core of a granule treating synthetically prepared FT process wastewater (46 days). White circles indicate clumps of bacteria, the yellow circle an ECP structure surrounding the terminal cell and red the ECP caps covering the terminal cells (LS IC-AD Run 1)

The granules sampled from the LS IC-AD after 100 days had a mean and median (d_{50}) size of 508 µm and 553 µm, respectively. Although

90% (d_{90}) of the granular sludge sample consisted out of granules smaller than 874 µm, 95% of the granules were smaller than 1000 µm (Figure 5.09).

A Scanning Electron Microscopy image of the outer layer of a granule that has been treating synthetically prepared FT process wastewater for 100 days is shown in Figure 5.20. The presence of a 'net' like ECP matrix is clearly visible on the surface of the granule. The outer layer of the granule has a sponge like appearance.

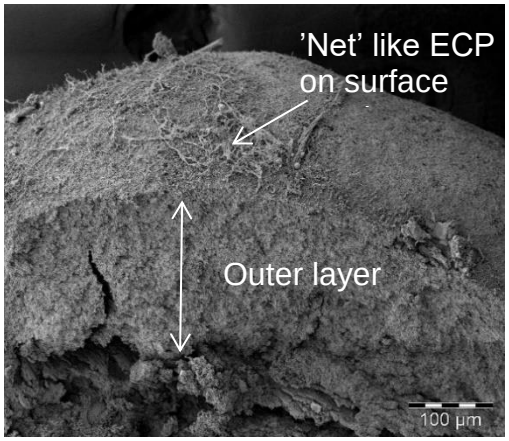


Figure 5.20: A SEM image of the outer layer of a granule treating synthetically prepared FT process wastewater (100 days) (LS IC-AD Run 1)

According to the morphology of these granules it was clear that a change in the bacterial population had occurred as shown in Figure 5.21. The ECP matrix has decreased and the rod shaped bacteria visually appeared longer. The change observed in the ECP matrix could probably be attributed to the synthetic wastewater being degraded by acetogenic and methanogenic bacteria. It was evident from the morphology of the granule, that the microbial population of Diplococci (Cocci in pairs) and Coccobacilli (round rods) were absent when compared to that treating synthetically prepared FT process wastewater

for 46 days (illustrated in Figure 5.19). The ECP within the granule formed part of the granule and assisted with the strength and stability of the granule as illustrated in Figure 5.21.

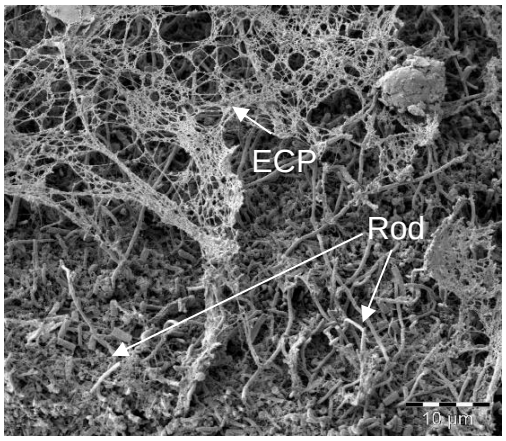


Figure 5.21: The outer layer of a granule that has been treating synthetically prepared FT process wastewater for 100 days (LS IC-AD Run 1)

5.3 Influent and effluent characteristics of the Laboratory Scale Internal Circulating Anaerobic Digester (LS IC-AD Run 2)

The synthetically prepared FT process wastewater influent pH was controlled by adjusting the pH in the influent tank using HCl or NaOH. The synthetically prepared FT process wastewater influent was supplemented with nutrients such as micro-and macro-nutrients, urea and phosphoric acid as mentioned in the experimental section (refer to section 4.6.1). The nutrient levels were controlled within the influent tank.

The average composition of the effluent after treatment is provided in Table 5.4.

Table 5.4: Average range composition of the effluent after treatment (LS IC-AD Run 2)

Parameters	Range of values	Average value ± SD	n
pH	6.5 – 7.5	7.1 ± 0.2	100
Effluent COD (mg/L)	34 – 3200	283 ± 419	99
Alkalinity (mg/L)	90.0 – 518.0	315.0 ± 84.0	99
Total Suspended Solids (TSS) (mg/L)	9 – 1225	109 ± 175	60
Total VFA (mg/L)	4 – 371	82 ± 93.0	35
Formic acid (mg/L)	0 – 3.0	0.4 ± 0.7	40
Acetic acid (mg/L)	0 – 99.0	10.3 ± 21.4	40
Propionic acid (mg/L)	0 – 79.0	10.9 ± 15.6	40
Butyric acid (mg/L)	0 – 96.0	15.1 ± 25.9	40
Valeric acid (mg/L)	0 – 56.0	8.5 ± 15.4	40
Biogas (L/d)	0 – 57.0	12 ± 12.7	96
Methane (%)	31.0 – 76.0	67.9 ± 7.4	86
Carbon dioxide (%)	2.0 - 19.0	13.6 ± 5.4	86
Organic Loading Rate (kg COD/m^3.d)	0 - 13	8 ± 4	99
Removal efficiency (%)	16 - 100	90 ± 9	95

5.4 Performance of the Laboratory Scale internal Circulating Anaerobic Digester (LS IC-AD Run 2)

The performance and results of the LS IC-AD (Run 2) was studied over a period of 100 days. These results are separated into different analytical parameters for discussion. The influent to the reactor was prepared in batches and had a COD concentration was approximately 1050 mg/L. The influent adjustment to the reactor was done by increasing or decreasing the influent COD concentration and maintaining the influent pump flow-rate. The starting influent COD concentration was approximately 1050 mg/L. Throughout section 5.4 of the study, the reference to COD effluent will be referring to unfiltered COD concentration measured in the effluent.

5.4.1 Influent and effluent COD concentration

Figure 5.22 depicts the influent and effluent COD concentration during the operation of the LS IC-AD reactor (Run 2). The influent COD concentration varied between 987 and 6300 mg/L as a function of batch influent preparation. The unexpected increase observed in the effluent COD concentration to greater than 1200 mg/L within the first four days of operation was probably due to a momentary wash-out of the granules (Figure 5.23) from the IC-AD reactor. Similarly, Mahmoud (2008) reported that solids were washed out in the effluent of an USAB reactor used for the treatment of sewage resulting in increased effluent COD concentrations. It was evident that the decrease in effluent COD concentration from 406 mg/L to 176 mg/L (days 6 to 17) may have gone hand in hand with the decrease of the TSS concentration measured in the effluent (<11 mg/L).

The influent concentration was increased steadily by increasing the influent concentration by 10% once the effluent COD concentration decreased to below 200 mg/L. After 20 days of operation, the effluent COD concentration decreased further to below 100 mg/L. The effluent COD concentration remained well below a 100 mg/L for the next 38 days. A similar trend was observed by Moosbrugger et al., (1992) during the start-up phase of an USAB treatment grape wine distillery waste. The lower effluent COD concentration measurement during this time most likely indicated that the reactor performance was improving and that the granules were adapting to the influent.

Thereafter, the effluent COD peaked at 823 mg/L on day 60 and this increase was most likely ascribed to the increased influent concentration. The higher influent concentration possibly gave rise to the temporary

wash-out of fine particles. Subsequently, during the next few days the effluent COD concentration decreased steadily to approximately 160 mg/L. Furthermore, the higher COD effluent concentration observed when increasing the OLR may possibly be explained by an increase in the VFA values.

The average effluent COD concentration during the first 60 days of operation was approximately 180 mg/L which resulted in a COD removal efficiency >90 % during this time period. Similar effects were observed by Yan and Tay (1996), during an examination of an USAB reactor that was treating brewery wastewater.

Thereafter, from day 60 onwards the effluent COD concentration increased with periodic intervals each time the influent concentration was increased. It was evident from the results obtained that the increase in the influent COD may possibly be explained by an increase in the effluent COD, VFA and OLR. The higher COD concentration in the effluent was probably ascribed to the presence of solids being washed (Figure 5.23) out due to the poor adaptation phase of the granules. According to observations made by Mahmoud (2008), the TSS was removed from the UASB reactor by being washed out in the effluent. The effluent COD concentration remained high for two days before decreasing to below 120 mg/L.

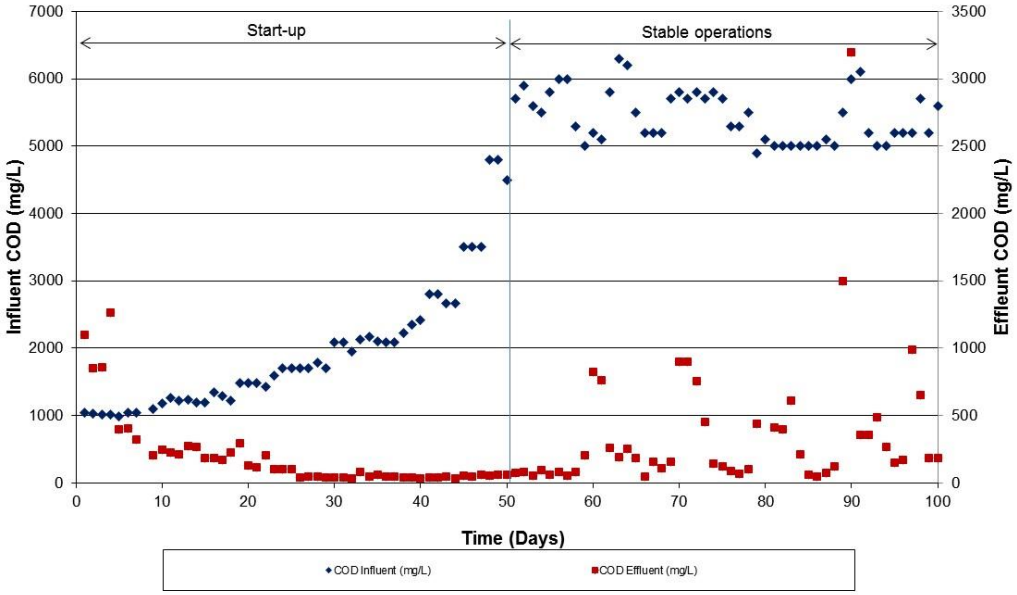


Figure 5.22: Comparison of influent and effluent COD concentration during operation (LS IC-AD Run 2)

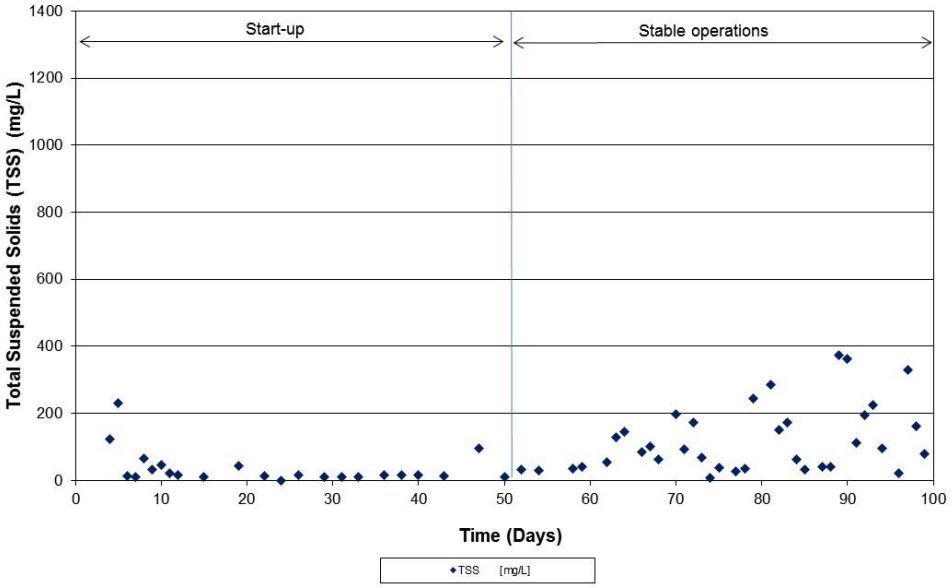


Figure 5.23: Total Suspended solids during the operation of the reactor (LS IC-AD Run 2)

5.4.2 Organic Loading Rate and Removal efficiency

The Organic Loading Rate (OLR) is dependent on the influent COD concentration. The OLR and removal efficiency obtained for the LS IC-AD are shown in Figure 5.24.

The reactor was stared-up at an OLR of approximately 1.5 kg COD/m^3.d. During this period the removal efficiency was below 60%. However, on day nine the OLR was increased to 2.1 kg COD/m^3.d which resulted in an increase in the removal efficiency to 80%. The OLR was increased to 3.0 kg COD/m^3.d (day 20) which resulted in an increase in the removal efficiency to >90%. A similar trend was observed by Wolmarans et al., (2002) during the start-up phase of an USAB treatment plant.

The OLR was increased steadily during the next period of 24 days (day 20 to 44) from 3.0 and 5.4 kg COD/m^3.d as a result of the influent concentration being increased from ~1480 mg/L to ~2600 mg/L. The influent COD concentration was further increased from ~2600 mg/L to ~5900 mg/L which resulted in an increase in the OLR from approximately 5.4 to 11.2 kg COD/m^3.d. During this timeframe the removal efficiency was stable at >95%. During LS studies conducted by Standbauer et al., (1994), removal efficiency in the range of 90% was observed which was in concurrence with the removal efficiency attained during this study. According to findings made by Moosbrugger et al., (1993), plant performance correlates directly with COD removal efficiency.

Thereafter, from days 58 onwards an inconsistent variation in the OLR was observed which also resulted in a variation in the removal efficiency. These observations bear a resemblance to the results attained by other

research workers (Wolmarans et al., 2002). The variation in the removal efficiency that was observed from day 58 onwards was presumably ascribed to the increase in the effluent COD concentration. The decrease in the effluent COD concentration (days 76 and 85) correlated with the increase in the removal efficiency and stabilised at 98% on day 76.

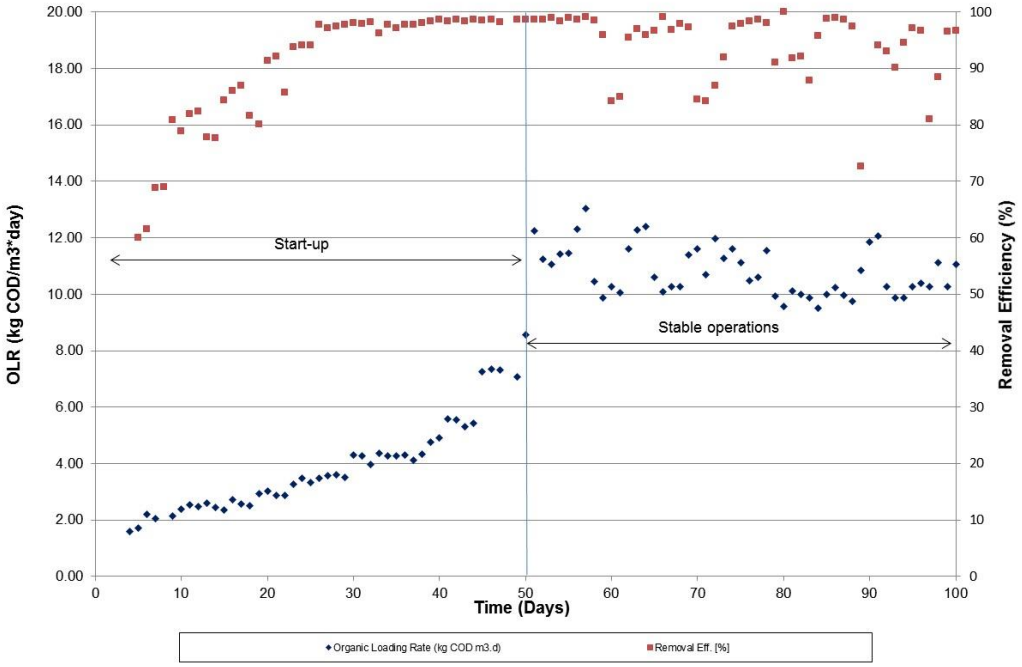


Figure 5.24: Organic Loading Rate (kg COD/m^3.d) and Removal Efficiency (%) during operation of the reactor (LS IC-AD Run 2)

5.4.3 Biogas Production

The biogas, methane and carbon dioxide concentration rates are summarised in Figure 5.25. The maximum biogas produced within the first 30 days of operation was 3 L/d. It was observed from the results, that an increase in the influent flow-rate resulted in an increase in the biogas production. The increase in the influent flow-rate could be directly proportional to the increase of the OLR. Hence, an increase or decrease in OLR had an immediate increase or decrease effect on the production

of the biogas. The CH_4 content of the biogas with an OLR of between 10 to 11.5 kg COD/m^3.d was 68% to 71% and that of the carbon dioxide was 11% to 19%. The decrease in the methane concentration may possibly increase the rate of acidogenesis and non-proportional growth of methanogens.

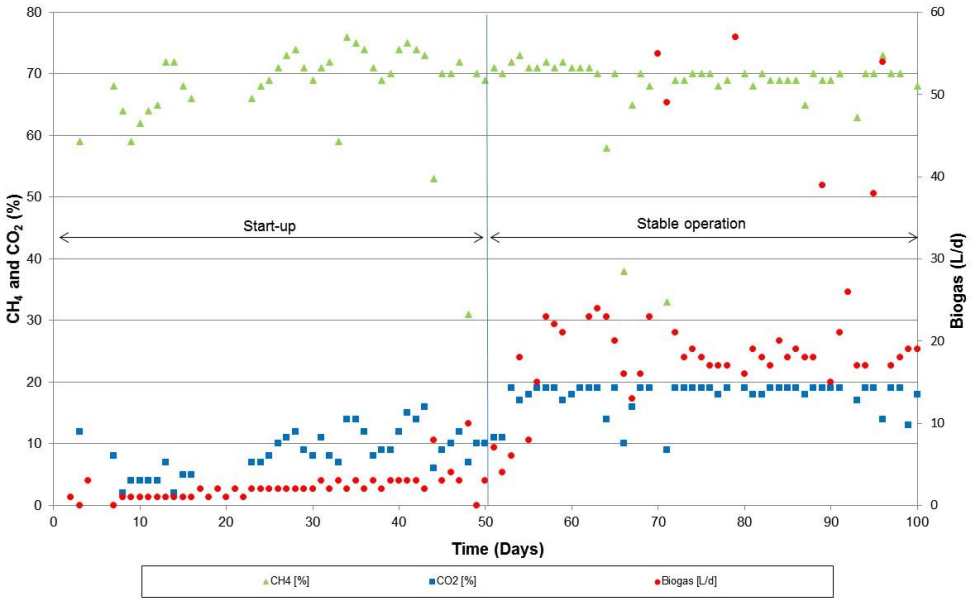


Figure 5.25: Total biogas production (L/d), methane and carbon dioxide concentration (%) observed during operation of the reactor (LS IC-AD Run 2)

5.4.4 Influent and effluent pH

The influent and effluent pH are summarised in Figure 5.26. The influent pH was adjusted from 4.2 to 4.9 using a 45% sodium hydroxide solution. During the first 25 days of operation the influent pH was steadily increased to approximately six in order to maintain the effluent pH at 7.0. A decrease in the reactor pH noticed on day 47 may possibly be due to some build-up of VFA. According to Dohanyos et al., (1985) the accumulation of VFA could lead to a decrease in pH, inhibiting the methanogens which could in turn result in reactor failure. The decrease in the effluent pH to below 7.0 suggested that the conversion of VFA into

methane was slowed down. The influent pH decreased to approximately 5.2 on day 60 as the effluent pH increasing to between 7.5 and 7.6 which falls within the ideal growth pH range for methane-forming bacteria such as *Methanothrix, Methanospirilla or Methanococcoides*) (Flanders Health Blog).

Ideally anaerobic digestion occurs at a pH of between 6.8 and 8.0 which falls within the ideal growth pH range for methane-forming bacteria such as *Methanosphaera, Methanothermus, Methanolacinia, Methanospirillium, Methanococcoides, Methanogenium and Methanothrix* (Flanders Health Blog). Methane producing bacteria are sensitive to pH and are inhibited at pH values less than 6.2 (*Methanomicrobium*) (Flanders Health Blog). It is thus important to maintain the operating pH at 6.8 to 7.5. The average effluent pH value (pH 7.1) obtained compared well with the intended operational philosophy (pH 6.8 – 7.5), hence ensuring that the required pH for effective anaerobic digestion was maintained. According to a study by Beccari et al., (1996) the methanogenic activity will be affected by pH. The ideal pH for microbial growth is between 6.8 and 7.2 falling within the ideal growth pH for methane-forming bacteria such *Methanosphaera, Methanothermus, Methanolacinia, Methanospirillium, Methanococcoides, Methanogenium and Methanothrix* (Flanders Health Blog), therefore the variation in pH could result in the methanogenic activity decreasing. Similar observations in pH measurements were made by Keyser et al., (2003) during the operation of a bioreactor.

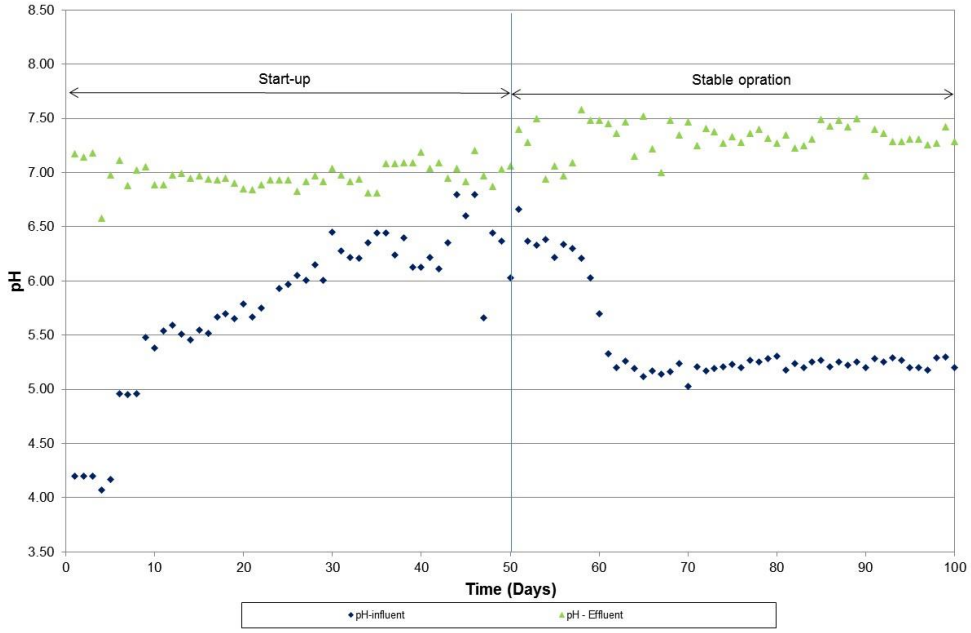


Figure 5.26: Influent and effluent pH observed during operation of the reactor (LS IC-AD Run 2)

5.4.5 Alkalinity

It is of great importance to know what the concentration of the reserve alkalinity would be before the pH decreases to below the safe threshold value of approximately 6.2 to 6.5 (Figure 27).

The synthetically prepared FT process wastewater had a pH range of between 4.0 and 6.9 in Run 2, which was in an acidic pH range. Therefore, its addition into the reactor leads to the consumption of the alkalinity, bicarbonate (HCO_3^-) (refer to Section 5.2.5). At this pH there is no alkalinity present and only free mineral acids and dissociated carbon dioxide.

In addition the micro-organisms in the anaerobic digester produced biogas which comprised of methane and carbon dioxide as the

synthetically prepared FT process wastewater was degraded. The carbon dioxide within the reactor shifted the pH of the reactor into the lower pH range.

Since the anaerobic digester was at a pH range of between 6.5 and 7.5 the alkalinity in the system was maintained in the form of bicarbonate (HCO_3^-) (refer to section 5.2.5).

The alkalinity increased overtime and an average alkalinity of 315 mg/L was measured during the operation of the LS IC-AD (Run 2) (Figure 27). An increase in VFA concentration most likely indicated an imbalance in the acid producers and consumers. This possibly resulted in a breakdown of the buffering capacity as the alkalinity decreased and possibly inhibited the growth of the methanogens.

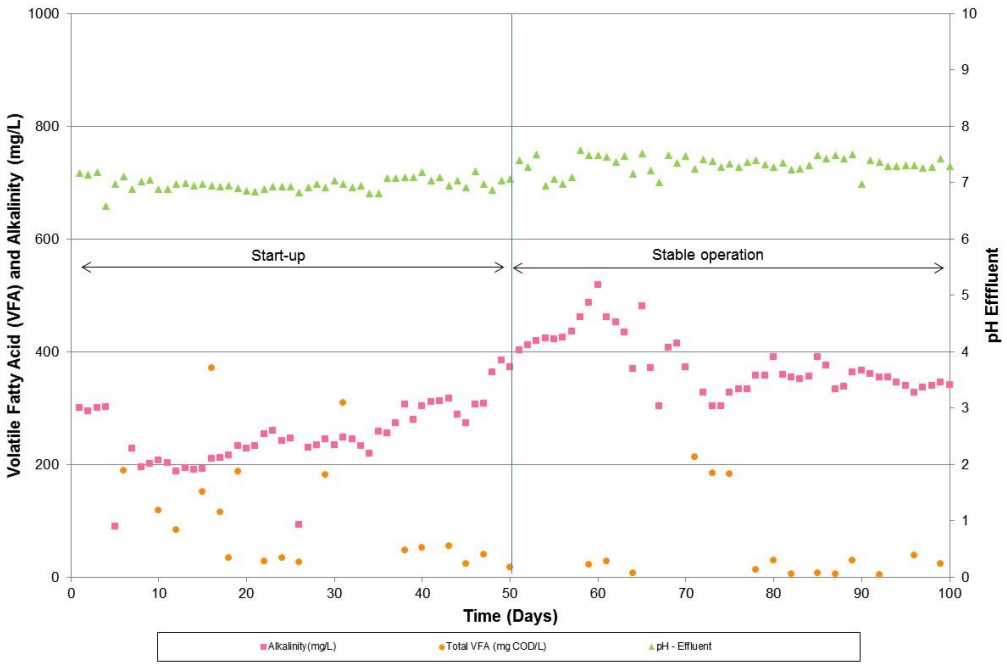


Figure 5.27: Alkalinity and effluent pH observed during operation of the reactor (LS IC-AD Run 2)

The RR obtained during the operation of LS IC-AD Run 2 was between 0.01 and 0.8 (Figure 5.28). The RR increased to above 0.3, which was possibly ascribed to the increase in the VFA concentration and the subsequent decrease in the alkalinity concentration (400 mg/L to 300 mg/l) on the respective days. The decrease in the bicarbonate alkalinity concentration was possibly due to the consumption of bicarbonate ions by the VFA. This most likely indicated that the reactor was operated under 'stress' as the micro-organisms had not acclimatised.

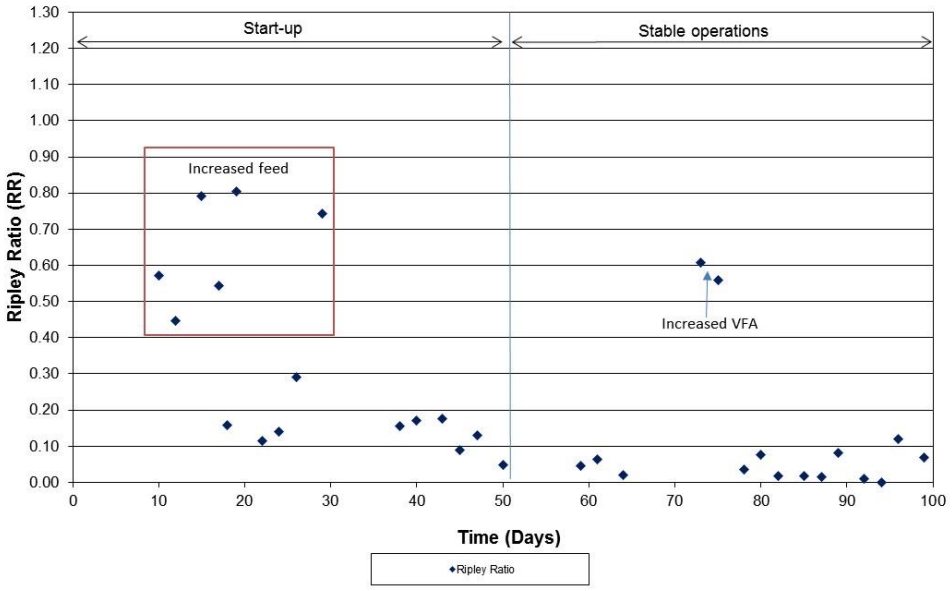


Figure 5.28: Ripley Ration (RR) observed during operation of the reactor (LS IC-AD Run 2)

5.4.6 A summary of the results obtained during Run 1 and Run 2

Although the reactor in Run 1 was not able to achieved stable operation, due to the OLR being increased continuously (state of ramp up), the assumption was made that stable operation was achieved from day 80 onwards. This assumption was based on the fact that both reactors had a similar OLR of approximately 10 kg COD/m^3.d. The reactor operated in Run 2 achieved stable operation on day 51. In order to compare the

results obtained for the assumed stable operation conditions during Run 1 (80 – 100 days) and Run 2 (51 – 100 days), the average results for these periods are summarised in Table 5.5.

The influent COD concentration for Run 1 was approximately four times higher than that of Run 2. The influent COD concentration for Run 2 was increased incrementally thus increasing the OLR. From these results it was evident that the effluent COD concentration in Run 2 decreased and stabilised to below 400 mg/L. A higher, more erratic effluent COD concentration was observed during Run 1 compared to that of Run 2. This behaviour was probably ascribed to the accumulation of VFA in the effluent as a result of the higher COD influent concentration which possibly attributed to an increase in the TSS as a result of the poor adaptation of the granules. Although the average influent COD concentration in Run 1 (22517 mg/L) was significantly higher compared to that of Run 2 (5429 mg/L), the organic loading rate for both reactors were comparable at 10 kg COD/m^3.d, therefor similar COD removal efficiencies were achieved for Run 1 and 2, 98% and 94%, respectively.

The average operational results obtained for the assumed stable operation period for Run 1 and 2 are summarised in Table 5.5. Stable operation for Run 1 and 2 were most likely at 80 and 51 days, respectively. Operational stability was achieved at an OLR of approximately 10 kg COD/m^3.d. During Run 1 the average COD removal efficiency was found to be 98% at a HRT of two days and an OLR of 10 kg COD/m^3.d. Whereas in Run 2 the average COD removal efficiency of 94% was obtained at a HRT of 0.5 days at an average OLR of 11 kg COD/m^3.d. The lower COD removal efficiency of 94% in Run 2 compared to 98% in Run 1 was most likely attributed to the slight wash-out of sludge from the reactor due to the significant lower HRT of 0.5

relative to a HRT of 2 (refer to section 2.8.1). The average TSS obtained for Run 2 was 151 mg/L at a HRT of 0.5 days which was higher relative to the 128 mg/L for Run 1 at a HRT of 2 days. Thus, the possibility exists that the HRT obtained in Run 2 was greater than the SRT, which resulted in the wash-out of sludge. Given the application of suspended growth technology in the reactor, the shorter HRT will impact on the linear up-flow velocity and result in wash-out of biomass resulting in an increase in the SS in the effluent for Run 2 relative to Run 1. The COD removal efficiencies obtained during these studies were >90% and was comparable with the expected values of an ideal system. A lower OLR was obtained during these studies relative to the expected values for an ideal system which ranged between 20 and 30 kg COD/m^3.d.

The pH of the reactor and final effluent was controlled during the operational period by adjustment of the influent pH (refer to section 4.2.1). From the results in Table 5.5 it is evident that the pH values obtained for Run 1 and 2 during stable operation were comparable. The average pH values obtained were representative of the expected range of pH values between 7 and 8 in an ideal system.

The expected amount of biogas produced in an ideal system is in a range between 0.2 to 0.6 L/g COD and the fraction of methane in the biogas is in a range of 0.6 and 0.9 (Henze et al., 2008). The theoretical amount of methane produced per unit of COD utilised under anaerobic conditions equals 0.35 L CH_4/g COD at standard conditions, 0ºC and 1 atmosphere. At 35ºC this equates to 0.40 L CH_4/g COD (Metcalf and Eddy, 2014). From the results summarised in Table 5.5, it can be seen that the amount of methane produced at 35°C during Run 1 and Run 2 were 0.31 L CH_4/g COD and 0.37 L CH_4/g COD, respectively. These values were comparable with the theoretical amount of methane (0.40 L

CH_4/g COD) produced during anaerobic digestion. The actual amount of biogas produced during Run 1 and 2 was 29.2 and 22.4 L/d, respectively. Although the theoretical amount of biogas produced at 35ºC for Run 1 and 2 was calculated to be 32.6 L/d and 21.8 L/d, the amount of biogas produced at STP were slightly lower at 25.7 L/d and 24.1 L/d, respectively, for this IC-AD technology used during this study. The amount of biogas produced at 35ºC during Run 1 and 2 was 0.52 L biogas/g COD and 0.57 L biogas/g COD, respectively and was comparable with the theoretical amount of biogas which ranged between 0.2 to 0.6 L/g COD. Therefore, the actual amount of biogas produced in these systems were 86% and 95% of the theoretical biogas calculated for these systems.

The chemical oxygen demand of a sample indicates the amount of organic matter present in the system. According to van Lier et al., (2008) the substrate is degraded, resulting in the production of more simple intermediates and the mineralisation to methane and carbon dioxide. All the COD that enters the system ends up in an end-product of methane minus the COD incorporated in the new biological mass.

$$COD_{in} = COD_{out} \qquad (5.3)$$

Therefore, for the purpose of this study, equation 5.3 was expanded to the different outlets for the LS IC-AD reactors (Run 1 and Run 2) (illustrated in Figure 5.29).

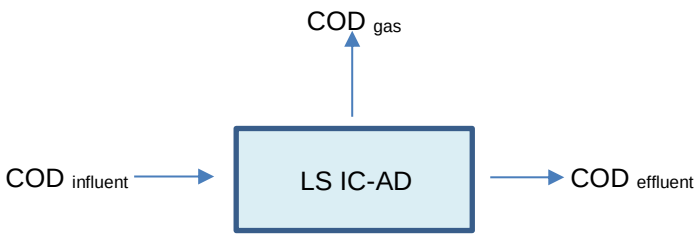


Figure 5.29: A diagram illustrating the different COD outlets for the LS IC-AD reactors (Run 1 and Run 2) (adapted from Van Lier et al., (2015))

The theoretical COD balance for Run 1 was performed for days 80 to 100 based on the measured influent COD concentration and the sum of the effluent COD concentration and calculated COD in the biogas. Based on the calculation a COD balance of 69.1% ± 0.2 (n = 23) was obtained during the assumed steady state conditions. The influent g COD/d was 53.6 ± 4.8 (n = 23), the effluent g COD/d was 1.3 ± 0.7 (n = 23) and the methane g COD/d was 46.6 ± 14.6 (n = 23).

The theoretical COD balance for Run 2 was performed for days 50 to 100 based on the measured influent COD concentration and the sum of the effluent COD concentration and COD in the biogas. Based on the calculation a COD balance of 61.4% ± 0.2 (n = 50) was obtained. The influent g COD/d was 42.2 ± 3.0 (n = 50), the effluent g COD/d was 2.9 ± 4.1 (n = 50) and the methane g COD/d was 35.6 ± 19.2 (n = 50).

Therefore, from the discussion above it can be seen that Run 1 performed better than Run 2. Although the actual measured biogas produced in both runs were comparable to the theoretical biogas, during Run 2 less biogas was produced which was most likely ascribed to the lower removal efficiency (94%) and the higher TSS concentration within the effluent.

Table 5.5: Summarised average assumed stable operation results for (LS IC-AD Run 1 and 2)

Parameter	Run 1	n	Run 2	n
pH	7.7 ± 0.2	23	7.3 ± 0.2	50
OLR (kg COD/m^3.d)	10 ± 1	23	11 ± 1	50
Removal efficiency (%)	98 ± 2	23	94 ± 6	50
COD influent (mg/L)	22517 ± 649	23	5429 ± 382	50
COD effluent (mg/L)	517 ± 363	23	367 ± 513	50
TSS (mg/L)	128 ± 133	13	121 ± 101	36
HRT (d)	2.0 ± 0.2	23	0.5 ± 0	50
Actual biogas @ 35ºC (L/d)	30 ± 8	23	21 ± 12	50
Theoretical biogas @ 35ºC (L biogas/d)	32.6		21.8	
Theoretical biogas @ STP (L biogas/d)	25.7		24.1	
L Biogas/g COD @ 35 ºC	0.52		0.57	
Methane (%)	63 ± 2	19	68 ± 7	50
Carbon dioxide (%)	24 ± 2	19	18 ± 3	50
L Methane/g COD (STP)	0.35		0.35	
L Methane/g COD @ 35 ºC	0.31 ± 0.1		0.37 ± 0.2	

5.4.7 Scanning Electron Microscopy (SEM) analyses for Laboratory Scale Internal Circulating Anaerobic Digester (Run 2)

The LS IC-AD (Run 2) was inoculated with granules that were obtained from a brewery in KZN. The initial granules had a distinct micro-organism morphology as shown in Figures 5.10 to 5.12. The distinct ECP matrix in the granules could be ascribed to the original granules received from the brewery. These granules were mainly degrading complex polymeric influent consisting of sugars, carbohydrates and polysaccharides. The formation of the ECP matrix was due to the presence of fermentative and hydrolytic bacteria degrading the substrate into simple compounds (VFA, etc.) which are excreted (Jules et al., (2008)). Due to the complex nature of the wastewater, the bacterial groups involved in the anaerobic digestion process are most likely hydrolytic and acidogenic fermentative bacteria, which had been reported to produce ECP.

The granules used to inoculate the LS IC-AD had a mean and median (d_{50}) size of 1347 and 1363 µm, respectively. Although 90% (d_{90}) of the granular sludge sample consisted of granules smaller than 1947 µm, 20% of the granules were smaller than 1000 µm (Figure 5.30). The particle size distribution of the granules used to inoculate the reactor in Run 2 as well as the granules sampled after a 100 days are illustrated in Figure 5.30.

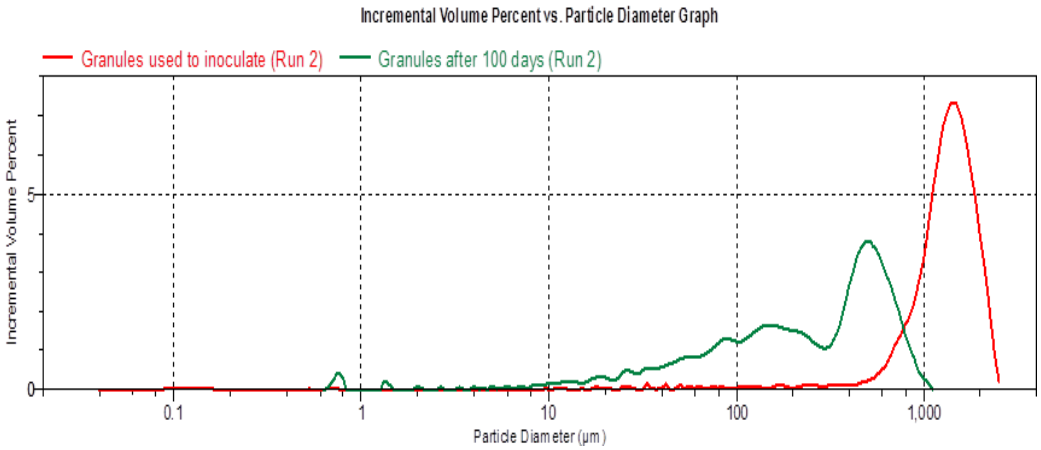


Figure 5.30: Particle size distribution for the initial granules and granules after 100 days (LS IC-AD Run 2)

The SEM image in Figure 5.31 shows the morphology of a granule that has been treating synthetically prepared FT process wastewater for a period of 100 days. The surface of the granule was most likely made up of aggregates of micro-organisms.

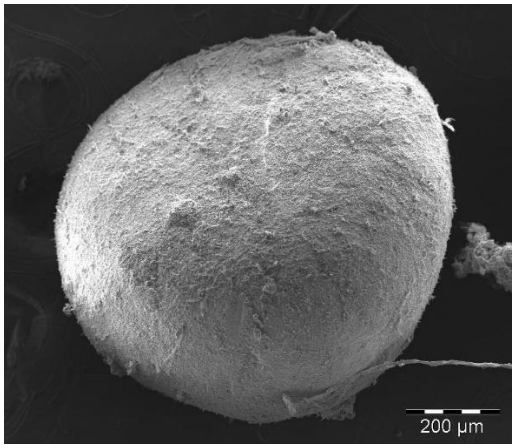


Figure 5.31: SEM image illustrating a granule that has been treating synthetically prepared FT process wastewater for 100 days (LS IC-AD Run 2)

The granules sampled from the LS IC-AD after treating synthetically prepared FT process wastewater for 100 days had a mean and median (d_{50}) size of 319 and 282 µm, respectively. Although 90% (d_{90}) of the granular sludge sample consisted of granules smaller than 660 µm, 99% of the granules were smaller than 1000 µm (refer to Figure 5.30).

Visually the structure of the granule seemed to be sponge like as indicated in Figure 5.32. The surface of the granule seems to be covered by an ECP 'net' like layer. The possibility exists that the organised and layered structure of the granule could be as a result of the methanogenesis stage. The methanogenic bacteria most likely involved was *Methanthrix soehngenii* and *Methanosarcina.* The cross section of the granule illustrated in Figure 5.32 depicts a compact, sponge like outer layer of the granule with a looser inner core.

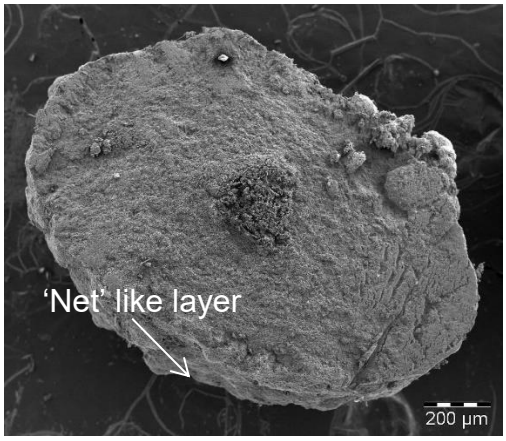
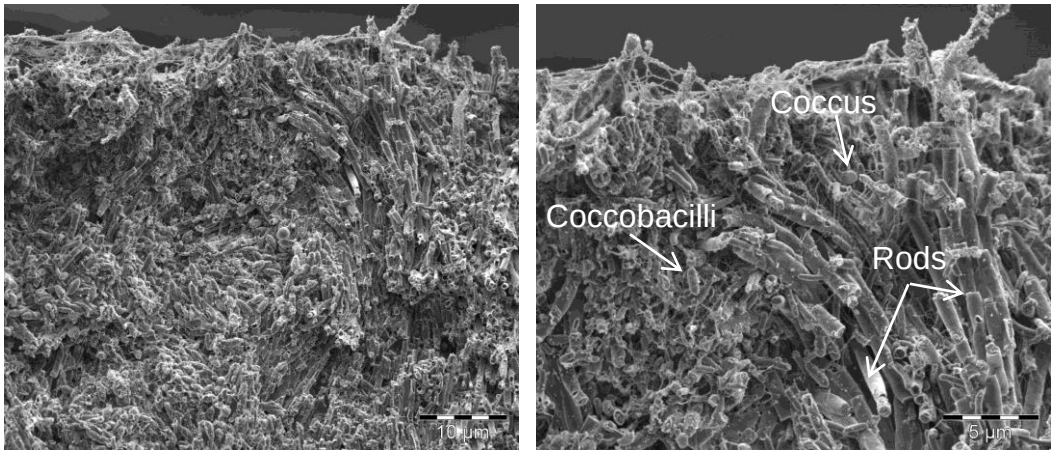


Figure 5.32: Cross section of a granule with a sponge like structure (LS IC-AD Run 2)

The morphology of the outer layer of the granule is presented in Figures 5.33 (a) and (b). According to visual observations the morphology of the bacterial population in the granules changed and consists primarily of rod shaped bacteria with a limited presence of Coccus and Coccobacilli. The morphology of this granule was different to that of the granule used to inoculate the reactor (illustrated in Figure 5.13 (b)).

According to the visual observations, the ECP matrix had decreased and this change was probably attributed to the synthetic wastewater being degraded by acetogenic and methanogenic bacteria. The protective layer of ECP surrounding the granule most likely aided with the adhesion of the bacteria population, granule development and structural integrity (MacLeod, 1995).



(a) (b)

Figure 5.33: SEM image showing the Coccobacilli, Coccus and rods in the outer layer of a granule treating synthetic prepared FT process wastewater for 100 days (LS IC-AD Run 2)

When comparing the visual results obtained for the granules during Run 1 and Run 2 after 100 days of operation, the granules had possibly maintained their spherical shape and sponge like structure. The visual observations obtained from the morphology of the granules, indicated a possible decrease in the ECP matrix for both runs. The change observed in the bacterial population within the granules was most likely the same for Run 1 and Run 2. The results from Run 1 indicated that 90% (d_{90}) of the granular sludge consisted of granules smaller than 874 µm (99% of granules were <1000 µm) (refer Figure 5.9), whereas 90% (d_{90}) of the granular sludge sampled during Run 2 consisted of granules smaller than 660 µm (95% of granules were <1000 µm) (refer Figure 5.30). From these results, it was evident that the granules in Run 2 were most likely smaller than those sampled during Run 1.

Part B: Pilot Scale Internal Circulating Anaerobic Digester (PS IC-AD)

5.5 Influent and effluent characteristics of the Pilot Scale Internal Circulating Anaerobic Digester (PS IC-AD)

The average range of parameter composition of the synthetically prepared FT process wastewater influent is provided in Table 5.6. The synthetically prepared FT process wastewater influent pH was controlled by adjusting the pH in PAT by using HCl or NaOH. The synthetically prepared FT process wastewater influent was supplemented with nutrients such as micro-and macro-nutrients, urea and phosphoric acid.

Table 5.6: Average range composition of the synthetically prepared Fischer-Tropsch process wastewater influent before treatment (PS IC-AD)

Parameters	Range of values	Average value ± SD	n
pH	2.9 – 4.0	3.5 ± 0.1	526
COD (mg/L)	6000 – 10100	7628 ± 617	526
Alkalinity (mg/L)	1.0	1.0 ± 0	487
Formic acid (mg/L)	1.0 – 314.0	23.9 ± 39.5	519
Acetic acid (mg/L)	372.0 – 4931.0	3691.8 ± 522.2	519
Propionic acid (mg/L)	5.0 – 1491.0	905.5 ± 194.8	519
Butyric acid (mg/L)	64.0 – 1529.0	508.7 ± 139.8	519
Valeric acid (mg/L)	56.0 – 831.0	332.1 ± 87.2	519

The average range of composition of the parameters after PS IC-AD treatment is given in Table 5.7.

Table 5.7: Average range composition of the effluent after treatment (PS IC-AD)

Parameters	Range of values	Average value ± SD	n
pH	7.0 – 8.4	7.6 ± 0.2	520
pH_{top}	6.65 – 7.48	7.09 ± 0.18	511
Effluent COD (mg/L)	275 - 6500	732 ± 438	519
Alkalinity (mg/L)	492 - 4200	1919 ± 533	490
Total Suspended Solids (TSS) (mg/L)	25 - 3910	208 ± 299.5	315
Formic acid (mg/L)	1.0 – 126.0	6.6 ± 14.1	315
Acetic acid (mg/L)	4.0 – 1560.0	78.5 ± 116.2	315
Propionic acid (mg/L)	5.0 – 672.0	31.4 ± 54.8	315
Butyric acid (mg/L)	5.0 – 179.0	8.6 ± 19.0	315
Valeric acid (mg/L)	3.0 – 142.0	7.9 ± 15.8	315
Biogas (L/d)	65 – 1 594	179 ± 233	533
Methane (%)	3 - 86	73 ± 12	450
Carbon dioxide (%)	14 - 97	27 ± 12	450
Organic Loading Rate (kg COD/m^3.d)	0 – 37	26 ± 6	530
Removal efficiency (%)	63 – 100	91± 4	524

5.6 Performance of the Pilot Scale Internal Circulating Anaerobic Digester (PS IC-AD)

The performance and results of the PS IC-AD were studied over a period of 544 days. These results are separated into different analytical parameters for discussion.

Each parameter for the operational period of the PS reactor is given under four sections. The start-up phase (0-71 days), up-set conditions and re-commissioning (72-131 days), stable operation (164-256 days) and shock treatment (274-514 days). During shock treatment, the effect of temperature is also included.

5.6.1 Influent and effluent COD concentration

The trend of the influent and effluent COD concentration within the PS IC-AD reactor for the duration of this study is presented in Figure 5.34. This includes all stages of pilot scale study. Throughout section 5.6 of the study, the reference to COD effluent will be referring to unfiltered COD concentration measured in the effluent.

Start-up phase (days 0 – 71)

The fluctuations of the influent COD concentration resulted in a fluctuation in the effluent COD concentration. As a result of this fluctuation the preparation of the influent was standardised in order to maintain an influent COD concentration to approximately 7500 mg/L. The standardisation was achieved by calculating the volume of wastewater required for preparing an influent with a more stable COD concentration.

The sudden increase observed in the effluent COD concentration from 1900 mg/L to 3100 mg/L within the first five days of operation was most likely due to a momentary wash-out of detached sludge from the IC-AD reactor. This washout was most likely ascribed to the decomposed micro-organisms and probably as a result of the unfavourable conditions within the reactor. In order to improve this wash-out, the feed to micro-organism ratio (F/M) needs to be maintained. By maintaining the F/M ratio, the ratio of available food to the mass of micro-organisms, the effluent COD concentration will most probably decrease. Similarly, Mahmoud (2008) reported that solids were washed out in the effluent of an USAB reactor used for the treatment of sewage.

From the results depicted in Figure 5.34, it is clear that the effluent COD concentration measured on day six had decreased to a level of 626 mg/L and decreased even further to 419 mg/L on day 12. These fluctuations observed in the influent and effluent COD concentrations were most probably due to various other factors that occurred during this time frame. These factors included:

(i) the replacement of the 'chip" resulting in the reactor being switched off,
(ii) variation in the influent COD concentration,
(iii) blockages in the nutrient line, and/or
(iv) starting the external recirculation pump.

From Figure 5.34, it is evident that the effluent COD concentration measured was between 980 mg/L and 1359 mg/L for a brief period of time (days 15 to 17). Thereafter, the effluent COD concentration decreased to approximately 392 mg/L on day 18 which most likely was an indication that the reactor was approaching stable operation. Increasing and decreasing COD concentrations in the effluent within the first few days of operation was also reported by Moosbrugger et al., (1993). The effluent COD remained between 300 – 500 mg/L for a few days before the influent pump flow-rate was increased. An unexpected increase in the effluent COD concentration to >1000 mg/L on day 42 was directly attributed to increasing the flow-rate of the influent pump. Although increasing the influent pump flow-rate probably resulted in an unstable reactor for a short period of time, the reactor recovered and stabilised. The decrease observed in the COD concentration indicated that the biomass was able to adjust to the new loading rate within a few days.

Up-set conditions and re-commissioning (days 72-131)

During the up-set conditions there were various contributing factors that could have resulted in the increase and/or decrease of the influent and effluent COD concentrations. The variation in the influent concentration could have been attributed to various parameters that were changed simultaneously, such as:

(i) a variation in the constituents of the wastewater,
(ii) increasing the flow-rate, and/or
(iii) starting the external recirculation pump (day 55).

Although the flow-rates of the influent and external circulation pumps were decreased during the upset conditions, the reactor did however not recover. The reactor was re-seeded by adding 15 L of sludge and re-commissioned on day 72. After re-seeding the reactor, the reactor influent flow-rate was decreased to approximately 130 L/d. The external recirculation flow-rate was set to 130 L/d in order to maintain a ratio of one for the recirculation versus influent.

Once the influent COD concentration was stabilised at approximately 7500 mg/L, the influent COD concentration was more stable and was controlled more accurately. The fluctuation observed in the influent COD concentration was also simulated by the effluent COD concentration. The effluent COD concentration from days 66 to 87 was below 1000 mg/L, at times as low as 312 mg/L which indicated that the reactor was possibly able to adapt to the fluctuating influent COD concentration while still achieving an good removal efficiency.

The increase in the effluent COD concentration to 1225 mg/L on day 122 could have been linked to external factors such as a power trip and damage to the heating pump that occurred on day 117. Furthermore, the COD concentration increased to a maximum of 1434 mg/L which was probably attributed to the increase of the influent flow-rate from 160 L/d to 220 L/d. The effluent COD concentration was unreliable as a result of the influent flow-rate being unstable. After some investigation it was found that the influent flow-rate could not be calibrated as the influent line was blocked. Once the influent line was replaced the reactors performance stabilised.

Stable operation (days 164 – 256)

During the stable operation phase the influent COD concentration remained stable between 7500 – 7600 mg/L throughout the phase. During this phase of the study, the influent flow-rate was maintained at 235 L/d and that of the external circulation at 260 L/d. The results obtained during this phase of the study indicated that the effluent COD concentration was stable between days 164 to 256. A slight increase in the effluent COD concentrations during days 230 to 244 were possibly due to a faulty heating pump (day 234) and a faulty wastewater pump (required to fill the PAT) (day 241).

Shock treatment (days 274 - 514)

Organic Loading Rate (OLR) (days 274 - 432)

As soon as the OLR shock treatment phase was initiated, an increase in the influent COD concentration (from 7600 to 8200 mg/L) was observed (day 274). This increase was most probably attributed to the sequential OLR increase of 1 kg COD/m^3.d which was achieved by increasing the

influent COD concentration. Abdelgadir, (2014) stated that changing the influent COD concentration most likely resulted in changes to the OLR.

The influent COD concentration remained stable at 8200 mg/L for 28 days before being increased to 8400 mg/L on day 302 in order to increase the OLR. From Figure 5.34, it can be seen that the influent COD concentration was increased to 10000 mg/L over a period of 32 days (day 306 to 338) in order to increase the OLR as required. The results indicated that the influent COD concentration decreased to approximately 7200 mg/L at a flow-rate of 235 L/d on day 342 in order to achieve an OLR of 30 kg COD/m^3.d.

It is important to note that the observed increase in the effluent COD concentration was directly proportional to the influent COD concentration increasing. A sudden increase was noted in the effluent COD concentration on day 275 when the OLR was increased. The OLR was increased by increasing the influent COD concentration to 8100 mg/L. A higher COD concentration of between 1080 to 1250 mg/L was observed for three days, thereafter a decline in the effluent COD concentration was likely as a result of the reactor adapting to the higher influent COD concentration. As the OLR was increased from 32 to 39 kg COD/m^3.d over a period of 57 days (day 274 to 341) a steady increase in the effluent COD was observed. At the maximum OLR of 39 kg COD/m^3.d, the effluent COD has increased to between 1000 mg/L to 1200 mg/L. As a result of the propionic acid breakthrough, the OLR in the reactor was decreased.

Temperature effect on system performance (days 432 to 514)

The temperature of the water bath was decreased for 38°C to 32°C on day 431. During this time the effluent COD concentration was approximately 450 mg/L. On day 445 the COD concentration increased to 1600 mg/L was attributed to the propionic acid breakthrough (>90 mg/L) as the influent COD remained at 7500 mg/L. The COD concentration remained high for approximately three days. Thereafter, the reactor temperature was increased to 35°C as from day 448. The increase in temperature resulted in a decrease in the propionic acid concentration.

The reactor was allowed to stabilise for a few days before the high temperature shock treatment commenced. The reactor temperature was increased to 40°C. The effluent COD concentration observed in this time was between 500 to 600 g/L before a breakthrough in propionic acid (>50 mg/L) was observed (days 488 to 514). The breakthrough of propionic acid was most likely as a result of VFA accumulating in the reactor due to the methanogens being inhibited. Higher production of VFA concentration was observed in the higher temperature range (42°C).

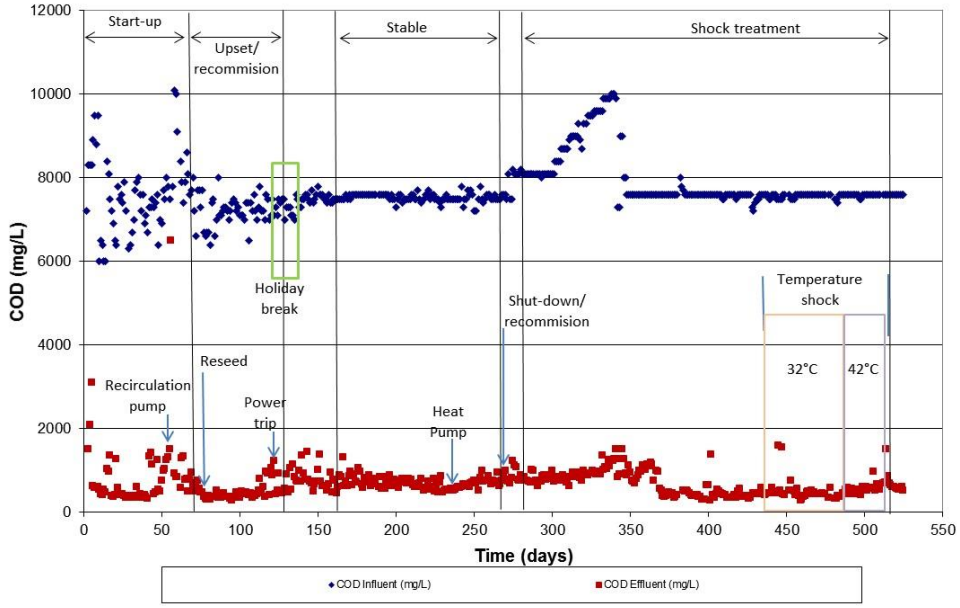


Figure 5.34: Total influent and effluent COD concentration during operation of the PS IC-AD

5.6.2 Organic Loading Rate and Removal efficiency

The trend for the OLR and the removal efficiency within the PS IC-AD reactor for the duration of this study is presented in Figure 5.35 and includes all stages of pilot scale study.

Start-up phase (days 0 – 71)

The removal efficiency showed a gradual decrease from 81% to 64% on day five during the start-up phase (Figure 5.35). Comparable observations were also made by Wolmarans et al., (2002). This decrease was most likely ascribed to the increased loading rate as well as the time required for the reactor to stabilise. The removal efficiency increased to roughly 93% and was maintained for a few days (day 14). On day 16 the fluctuation in the influent concentration resulted in a decrease in the removal efficiency (88%). However, as the reactor

loading rate and the COD influent stabilised, the conversion rate of the reactor increased to 95% and was maintained for a few days (day 20 to 40). On day 42 the removal efficiency decreased to 80%, possibly as a result of the increase in the loading rate. Once the OLR of the reactor was stabilised, the removal efficiency gradually start to increase.

The fluctuation observed in the OLR after the first 20 days of start-up was probably attributed to problems that were experienced with the operating and data collection system (”chip”) for the IC-AD reactor. Furthermore, blockages of the nutrient influent line occurred around day 40. The blockage occurred as a result of a hard white calcium like substance adhering to the walls of the influent line. This resulted in no nutrients being fed to the system during this time frame.

The removal efficiency showed a decrease as the external recirculation pump was started at a flow-rate of 190 L/d on day 55. The influent pump flow-rate was set at 380 L/d. Rizvi et al., (2013) stated that the removal efficiency within an USAB reactor decreased with a higher up-flow velocity, resulting in a lower contact time between the granules and the wastewater.

The OLR decreased to 3.4 kg COD/m^3.d 58 days after start-up, most likely due to re-seeding the reactor. The OLR varied repeatedly with the removal efficiency for the first 60 days of operation. The variation in the OLR was similar to that observed during the operation of the LS IC-AD. According to Wolmarans et al., (2002) these observations also resemble the observations that were made by other research workers.

Up-set conditions and re-commissioning (days 72-131)

During this phase of the study the OLR was increased steadily over a period of time. The OLR was increased to 18 kg COD/m^3.d and maintained for a few days. Thereafter, the OLR was increased to 22 kg COD/m^3.d. During this phase of the study the OLR was increased steadily over a period of time to 32 kg COD/m^3.d.

The results in Figure 5.35 shows a decrease in the OLR to 27 kg COD/m^3.d on day 106 and this was attributed to the decrease in the influent COD concentration to 6500 mg/L. The OLR recovered rapidly to 31 kg COD/m^3.d subsequently of the influent COD concentration that was higher at 7400 mg/L on day 107. The fluctuation in the OLR was probably attributed to the fluctuation of the COD concentration within the influent.

Although a faulty heating pump resulted in the removal efficiency to decrease to approximately 85% on day 117, the COD conversion was stable and ranged between 88% and 96%. The stable removal efficiency was a clear indication that the reactor was degrading the COD adequately and that the reactor was able to deal with the high OLR. During the high OLR the micro-organisms most likely achieved a fast microbial growth and a stronger activity, which most likely increased the COD removal efficiency. Findings from studies conducted by Goodwin and Stuart*, (*1994*)*, indicted that COD removal efficiencies of up to 90% were achieved using an USAB reactor to treat distillery effluent.

The OLR was reduced from 30 kg COD/m^3.d to 20 kg COD/m^3.d on day 118 for a period of 15 days during a holiday break. The results indicated

that the reactor was able to perform well during this time and maintained and OLR of 20 kg COD/m^3.d. The OLR was steadily increased until an OLR of 30 kg COD/m^3.d was achieved once again.

Stable operation (days 164 – 256)

The stabilisation phase was reached on day 164 with an OLR of 30 kg COD/m^3.d and maintained for a period of approximately 84 days (Figure 5.35). During the stabilisation phase the effluent COD concentration decreased on day 206, resulting in the OLR being decreased to 25 kg COD/m^3.d. After the stabilisation phase was completed the reactor was shut-down, cleaned and re-commissioned (day 251). The reactor was operated for approximately 18 days at an OLR of 30 kg COD/m^3.d in order to stabilise the reactor before the shock treatment phase was commenced (days 256 to 274). During studies conducted by Laubscher et al., (2001), it was found that researchers were treating grain distillation water and were able to obtained and maintained removal efficiencies of >90%.

Shock treatment phase (days 274 - 514)

Organic Loading Rate (days 274 - 432)

The shock treatment commenced by increasing the concentration of the influent in order to obtain an OLR of 2 kg COD/m^3.d higher than the OLR obtained in the stable operation phase. Once the OLR was increased, the reactor was left to stabilise for a period of seven days. As soon as the propionic acid concentration remained below 50 mg/L in the effluent, another 2 kg COD/m^3.d increase was made. The result being that the OLR was increased from approximately 30 kg COD/m^3.d to

39 kg COD/m^3.d from days 280 to 325. During this period the reactor was able to operate at the high loading rate. An average OLR of 34 kg COD/m^3.d was achieved, with the highest OLR being 39.1 kg COD/m^3.d during the OLR shock treatment phase. A COD removal efficiency of between 90% and 92% was achieved during this phase. According to Goodwin and Stuart *(*1994*),* COD removal efficiencies of 90% were achieved by using an USAB reactor that was treating distillery effluent.

Temperature effect on system performance (days 432 to 514)

The temperature shock treatment phase was divided into two phases:

(i) low temperature range (33°C), and
(ii) high temperature range (42°C).

The shock treatment phase for the low temperature was conducted in the period of 402 to 446 days. Due to temperature being a critical parameter during anaerobic digestion, the effects of temperature changes were studied, i.e. the lowest would be 33°C and the highest 42°C. The water bath temp will be lowered to 33°C. Once a breakthrough in the VFA concentration (propionic acid) (>50 mg/L) (day 445) was observed, the OLR was lowered to 20 kg COD/m^3.d (day 448). Once the reactor had stabilised at an OLR of 20 kg COD/m^3.d the OLR was further increased to 25 kg COD/m^3.d (days 398 to 402).

Thereafter the OLR was increased to 30 kg COD/m^3.d and the temperature to 40°C until the reactor had once again reached stable operation (days 407 to 428). The water bath temperature was increased from 40°C to 43°C (days 488 to 513). Usually higher temperatures prefer higher OLRs. At the higher OLR a better sludge granulation was probably expected which may increase the characteristics of the

granules. A higher OLR most likely increased the biogas production resulting in an increased agitation and leading to sludge washout. A breakthrough in the VFA concentration (propionic acid) was observed on day 514. The increased VFA concentration possibly caused the methanogens to becoming the rate-limiting step during methane production due to the absence of non-particulate substrates and complex organic material. The accumulation of the VFA is a result of toxic matter entering the reactor or an organic overload Although the temperature within the reactor was decreased to approximately 36°C thereafter, no drastic decrease was observed in the VFA concentration and was ascribed to the methanogens still being active.

The increased VFA as a result of temperature increase was also found by Agler et al., (2010) during a study treating brewery primary sludge. From these results obtained it is evident that an average OLR of 30 kg COD/m^3.d and an average COD removal efficiency of 93% was achieved during the temperature shock treatment phase. No correlation was found between the OLR and the removal efficiency.

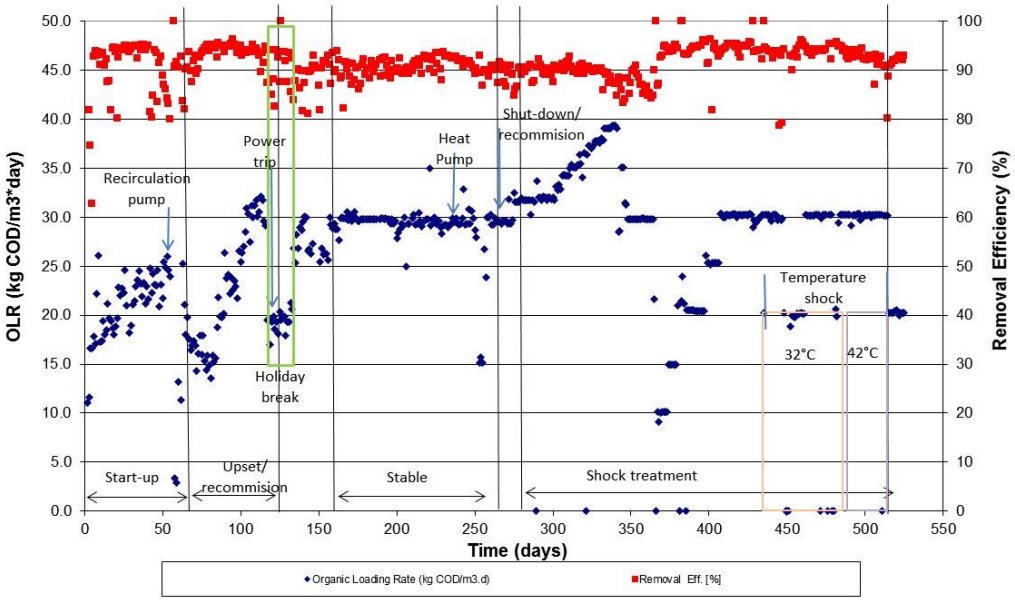


Figure 5.35: Organic Loading Rate (OLR) (kg COD.m^3/d) vs Removal Efficiency (%) (PS IC-AD)

5.6.3 Biogas

The trends for the biogas, methane and carbon dioxide within the PS IC-AD reactor for the duration of this study are presented in Figure 5.36. This includes all stages of pilot scale study.

An increase or decrease in the OLR has an immediate increase or decrease in the production of the biogas. However, during digester overloads the carbon dioxide content in the biogas will increase as the methane content decreases. Although the reactor response to biological conversion of the OLR can be determined by the volumetric gas measurement, an indication of the healthiness of the system is given by the methane to carbon dioxide ratio of the biogas. If the carbon dioxide fraction is higher than the methane, this is a sure sign of the reactor upset.

Figure 5.36 summarises the biogas production rate. The biogas produced in the first 30 days was between 540 to 600 L/d. A decrease in the biogas production rate was observed on day 55 which was probably ascribed to the external recirculation pump being switched on. The biogas production decreased to approximately 220 L/d. On day 56 as the reactor was shut-down and re-started at an influent flow-rate of 180 L/d. This resulted in the OLR decreasing from 24 to 3 kg COD/m^3.d which in turn resulted in a decrease in the biogas production. Although the methane content of the biogas at the lower OLR decreased to approximately 68%, the methane content increased to between 72% and 76% as the OLR was increased. A decrease in the carbon dioxide content was also observed during the same time frame. The increase observed in the methane content was most likely due to less methanogenic bacteria being present and being converted into methane.

From the results depicted in Figure 5.36 it can be seen the biogas production decreased on day 142 as the OLR decreased from 30 to 26.3 kg COD/m^3.d. The methane content of the biogas during this period was approximately 78% and that of the carbon dioxide 21%.

From the results obtained during the OLR test phase, it is evident that the biogas production decreased to below 900 L/d (days 353 to 364) as the OLR decreased from 35 to 29 kg COD/m^3.d. During this test period the methane increased to approximately 80% resulting in a decrease in the carbon dioxide (19%).

As the OLR was increased from 20 to 30 kg COD/m^3.d on days 462, the biogas production increased to greater than 1000 L/d. This resulted in a decrease in the methane content to 77% and the carbon dioxide content increasing to 22%.

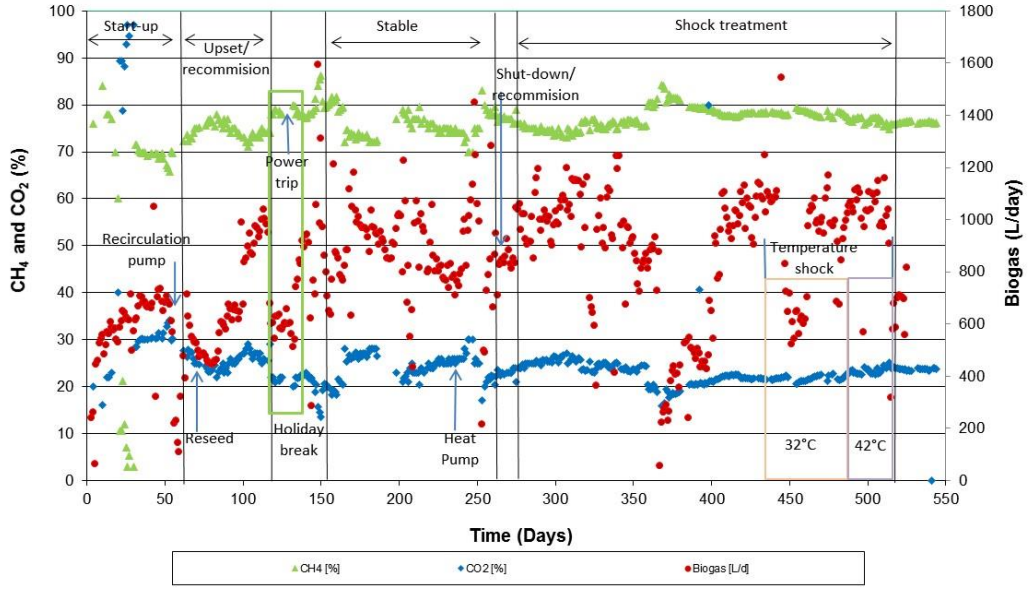


Figure 5.36: Total biogas production (L/d), methane and carbon dioxide concentration (%) observed during operation of the PS IC-AD

5.6.4 Influent and effluent pH

The trend of the influent and effluent pH within the PS IC-AD reactor for the duration of this study is presented in Figure 5.37 and includes all stages of pilot scale study. The measurement of pH is considered to be an important parameter as this aids with assessing the effluent quality.

The results in Figure 5.37 depict the different pH measurements which were taken for the duration of this study. From Figure 5.37 it is evident that four different measurements were taken over this time frame. The first measurement indicated by the line marked pH raw wastewater was to determine the pH of the wastewater being used as influent. The line indicating pH PAT was the pH measurement taken in the pre-acidified tank, where the pH of the influent was corrected to the desired pH. This was done in order to ensure that the reactor was not acidified. From the graph (Figure 5.37) it can be seen that the line indicating pH reactor top was the pH measured at the top of the reactor just before the 'water' leaves the reactor as an effluent and this measurement represents the pH inside the reactor. The line for the pH effluent was the pH of the effluent.

Start-up (days 0 to 71)

Figure 5.37 indicates that the pH of the wastewater remained constant at a pH of between 3.3 to 3.6 during start-up, re-commissioning, stable operation as well as during the shock treatment phases. The pH of the wastewater was an indication of the wastewater high acidity and VFA concentration. The pH in the PAT was adjusted using either hydrochloric acid (HCl) or sodium hydroxide (NaOH). A study by Karim et al., (2017) verified that the pH within the USAB reactor was raised by

adding NaOH to the influent. Although the average pH during this study remained at 6.0, the pH varied between 5.2 and 6.1. The rapid increase observed in the pH PAT measurement on day 55 was most likely due to starting the recirculation pump.

The initial decrease in the effluent pH during the start-up phase (days 1 to 20) was probably as a result of the production of VFAs. Karim et al., (2017) also reported a decrease in the pH resulting from VFAs being formed within the reactor and therefore souring the reactor. The results illustrated in Figure 5.37 highlight that the pH of the effluent and the pH in the top of the reactor followed the same trend throughout this study. Although the average pH measured in the top of the reactor was 7.0 a variation of between 6.9 and 7.3 was observed, which is within the ideal growth pH range for methane-forming bacteria such as *Methanosphaera, Methanothermus, Methanolacinia, Methanospirillium, Methanococcoides, Methanogenium and Methanothrix* (Flanders Health Blog). The variation of the effluent pH (days 22 to 44) between 7.6 and 7.9 was probably attributed to the stripping of carbon dioxide from the effluent.

Up-set conditions and re-commissioning (days 72-131)

During this time frame various adjustments were made to the caustic and acid dosing pumps with the purpose of achieving the correct dosing rate to maintain a stable influent pH. These adjustments result in the pH fluctuations that were observed in the $pH_{(PAT)}$ and the $pH_{(top)}$. The pH of the influent remained at approximately 3.5. As the pH decreased, the methane-forming micro-organisms were less active. The accumulation of VFA in the reactor resulted in the alkalinity and pH decreasing which lead to the inhibition of the methanogenic micro-organisms (Gerardi,

2006). Methanogens are affected by pH and the desired pH for microbial growth is between 6.8 and 7.2. This pH range consist of the ideal growth pH range for methane-forming bacteria such *Methanosphaera, Methanothermus, Methanolacinia, Methanospirillium, Methanococcoides, Methanogenium and Methanothrix* (Flanders Health Blog). As the pH decreased to below 3.5 the pH was not tolerable for microbial growth (Gerardi, 2006), resulting in reactor failure. Values of pH below 6 are restrictive and somewhat toxic to methane-forming bacteria.

Stable operation (days 164 – 256)

The increase of the pH to greater than 8.0 (day 248) resulted from an increase in the Ripley Ratio (RR). The sudden increase in the pH observed on day 281 to approximately 8.42 was as a result of an analytical error or a pH meter not being calibrated as the pH in the PAT was not affected.

Shock treatment (days 274 - 514)

Organic Loading Rate (OLR) (days 274 - 432)

The anaerobic process is most effective within a pH range of 6.8 to 7.2, although the ideal range is within 7.0 to 7.2. The acidogenic populations are significantly less sensitive to pH fluctuations than that of methanogens. The methanogenic activity can be severely limited at pH values below 6.2 and above 8.0. For the duration of the OLR shock treatment phase (days 274 to 370) the $pH_{(top)}$ was well-maintained at approximately 7.0. The average pH value (pH 7.0) attained compared well with the intended operational philosophy (pH

6.8 – 7.2), hence ensuring that the required pH for effective anaerobic digestion was maintained. As observed during this phase of the study, the pH in the top of the reactor ranged from 6.3 to 7.0. Therefore, it is of utter most importance that the pH be maintained in this range as a result of the high loading rates obtained within the reactor.

Temperature effect on system performance (days 432 to 514)

No correlation was found between the pH and temperature.

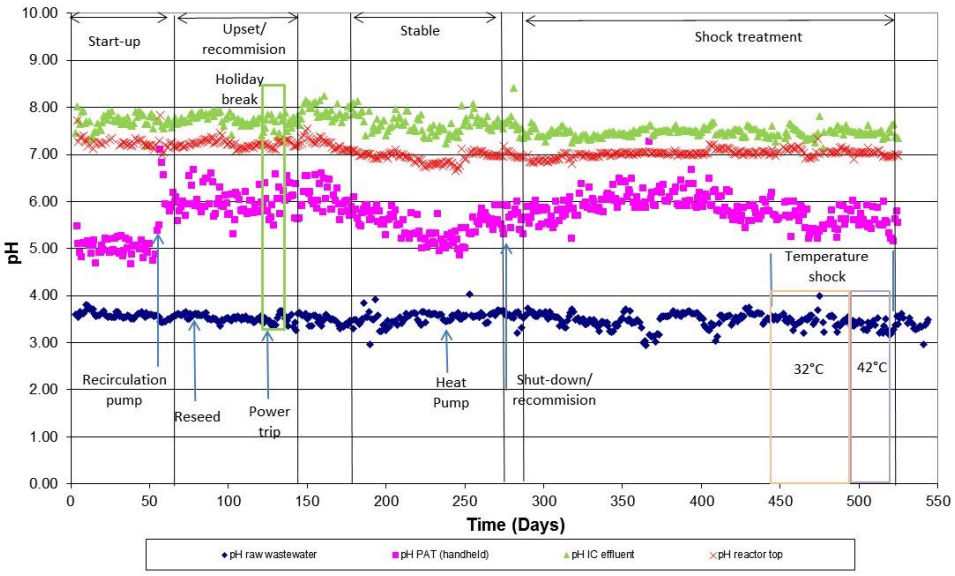


Figure 5.37: Different pH measurements made during the operation of the internal circulating reactor (PS IC-AD)

The pKa values for the different VFA compounds within the synthetically prepared FT process wastewater are summarised in Table 5.8. With a pH of between 7.0 and 8.4, the IC-AD effluent pH (and therefore the reactor internal pH) was much higher when compared to the pKa values of the different VFA compounds.

Table 5.8: The pKa values for the different VFA compounds (www.aqion.de/site/192)

Compound	pKa value
Formic acid	3.75
Acetic acid	4.75
Propionic acid	4.87
Butyric acid	4.76
Valeric acid	5.01

5.6.5 Alkalinity

The synthetically prepared FT process wastewater had a pH range of between 3.6 and 4.7 which is in an acidic pH range. Therefore, its addition into the reactor leads to the consumption of the alkalinity, bicarbonate (HCO_3^-). In addition, the micro-organisms within the anaerobic digester produced biogas which consisted of methane and carbon dioxide as the synthetically prepared FT process wastewater was degraded. The carbon dioxide within the reactor shifted the pH of the reactor into a lower pH range. Since the anaerobic digester was maintained at a pH range of between 6.9 and 8.0 the alkalinity in the system was maintained in the form of bicarbonate (HCO_3^-)

According to Bitton (1999), there are various chemicals such as lime, sodium hydroxide and sodium carbonate that possibly would increase alkalinity and restore the pH balance within the reactor. The effluent alkalinity remained stable throughout the operational period of the reactor (averaging 1919 mg/L with a standard deviation of 533 mg/L). As a result of the stable alkalinity no rapid changes were noticed within the effluent pH (Figure 5.38(a))

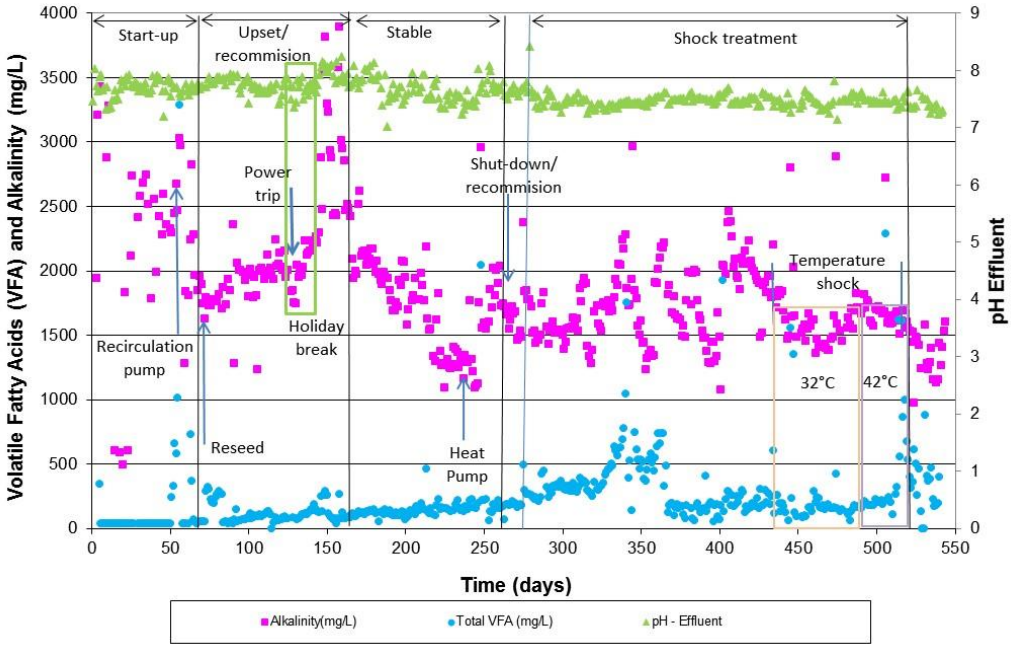


Figure 5.38 (a): Variation in volatile fatty acids, alkalinity and pH of the effluent during anaerobic digestion treatment of wastewater (PS IC-AD)

The RR most likely provided an understanding of the acidity/alkalinity balance within the PS IC-AD reactor. The empirical relationship was probably a measure of the state of the IC-AD reactor and warns of an impending upset. The RR obtained during the operation of PS IC-AD was between 0.01 and 1.08 (refer to Figure 5.38 (b)). The increase in the RR to above 0.3 was most likely ascribed to the increase in the VFA concentration. The increase in the VFA concentration most likely indicated an imbalance in the acid producers and consumers due to the increase in the OLR which indicated that the microbial population was likely under 'stress' and the reactor performance was not at its best.

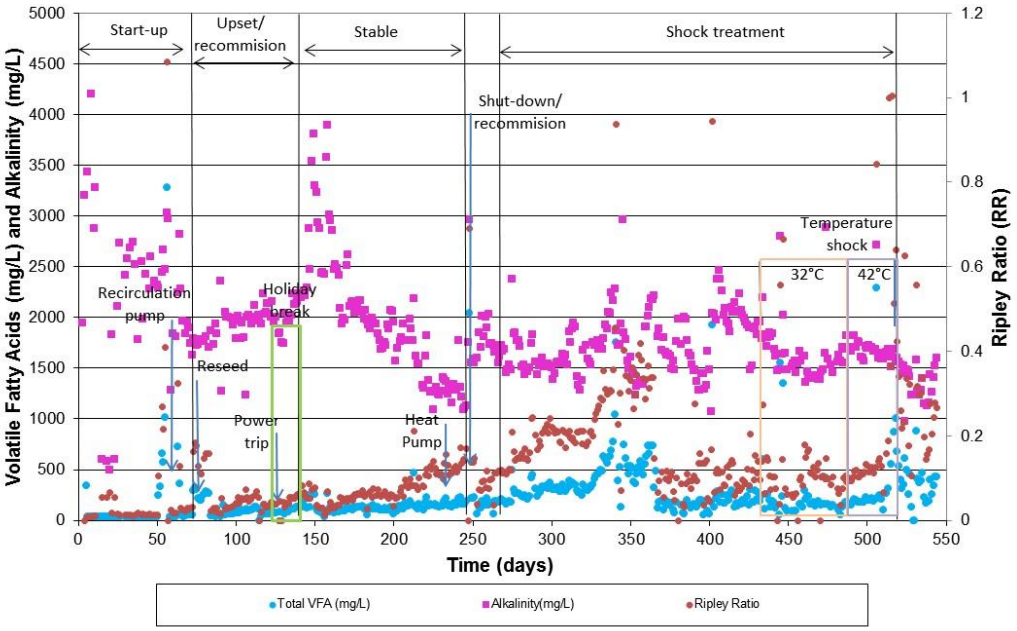


Figure 5.38 (b): Variation in volatile fatty acids, alkalinity and Ripley Ration of the effluent during anaerobic digestion treatment of wastewater (PS IC-AD)

5.6.6 Carbohydrate and Protein Analyses

The carbohydrate and protein analyses are important as it plays a major role in the bacterial Extracellular Polymer (ECP).

Start-up phase (days 0 – 71)

The initial sludge used during the start-up phase had a relatively high carbohydrate content of 0.3 mg/g Volatile Suspended Solids (VSS) presumably due to the high cellulose material content. Approximately three weeks after start-up a sludge sample was taken and analysed for carbohydrates. The carbohydrate concentration of the sludge decreased to 0.06 mg/g VSS (illustrated in Figure 5.39). The protein concentration

of the initial sludge during the start-up phase was 32 mg/g VSS (illustrated in Figure 5.40).

Up-set conditions and re-commissioning (days 72 – 131)

On day 56 acidification of the sludge bed occurred. This upset resulted in increased undisassociated VFA concentrations which in turn negatively impacted on the biomass activity. The protein and carbohydrate concentration decreased significantly to 4 mg/g VSS and 0.007 mg/g VSS, respectively as shown in Figures 5.39 and Figure 5.40. The total VFA concentration in the effluent was 3284 mg/L with a SS of 349 mg/L. From the VSS results it was evident that under stressful conditions the washout of granules effected the protein and carbohydrate concentrations. The reactor was re-seeded with fresh paper mill sludge and stabilised.

During the re-commissioning stage (days 66 to 109) an excellent recovery was displayed as the reactor performance returned back to its ideal performance and the original criteria for stable operation was met. The carbohydrate concentration had significantly increased to 0.13 mg/g VSS on day 72 after re-seeding the reactor. This was most probably attributed to the paper mill sludge providing the essential acidogenic bacteria which influences the ECP production.

However, a month after re-seeding (days 88 to 109) the carbohydrate concentration decreased significantly to 0.07 mg/g VSS. The decrease was likely due to the acidogenic bacteria that had probably died off. From days 88 to 239 no significant changes were observed in the carbohydrate concentration, therefore the carbohydrate concentration

present in the form of ECP was providing structural support for stable granules. From days 261 to 282 a significant negative change in the carbohydrate concentration was observed due to the reactor being subjected to high temperature operational changes. The carbohydrate concentration decreased to 0.02 mg/g VSS.

Stable phase (days 164 – 256)

During the stable operation phase the protein concentration for the initial sample increased to 42 mg/g VSS (as from day 238 to 305) (Figure 5.40). However, the protein concentration decreased significantly to 25 mg/g VSS. This indicates that even a slight upset in the reactor probably affected the protein concentration more than that of the carbohydrate concentration. This was probably be due to ECP formation being a well-integrated matrix of the polymer incorporating various microbes; whereas the protein was more limited to individual cells. Therefore, a slight upset together with cell washout, resulted in a decrease in protein but not necessarily carbohydrates. Thus, a decrease in carbohydrate concentration would be reflected as a gradual decrease in the integrity of the granular make-up and not as a rapid decrease as in the case for proteins.

During stable operation the protein concentration had averaged to 35.02 mg/g VSS in to 33.83 mg/g VSS (days 131 to 153). It was observed from this study that each period of re-seeding was accompanied by a slight increase in carbohydrate concentration; however this was then followed by a significant decrease within in a month after re-seeding.

Shock treatment phase (days 274 – 514)

Organic Loading Rate (OLR)

From days 261 to 370 the variation in organic loading test was started. The reactor was re-seeded and samples were taken (day 522) for analyses. The protein and carbohydrate concentration were 10.92 mg/g VSS and 0.2 mg/g VSS, respectively.

After a series of tests from days 349 to 544, the carbohydrate and protein concentration had been at its lowest at 0.02 mg/g VSS and 1.2 mg/g VSS, respectively.

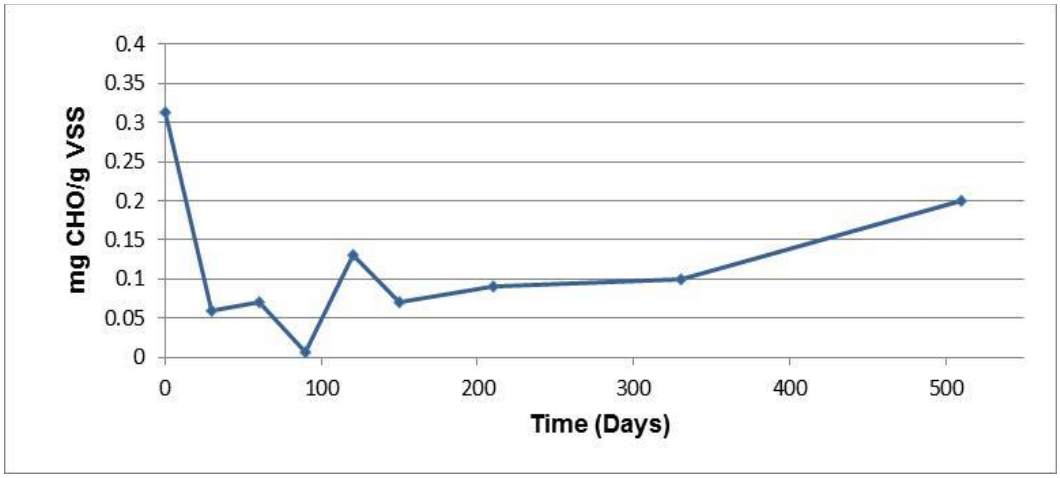


Figure 5.39: Carbohydrate analyses of anaerobic granular sludge relative to the performance/operating period of the PS IC-AD

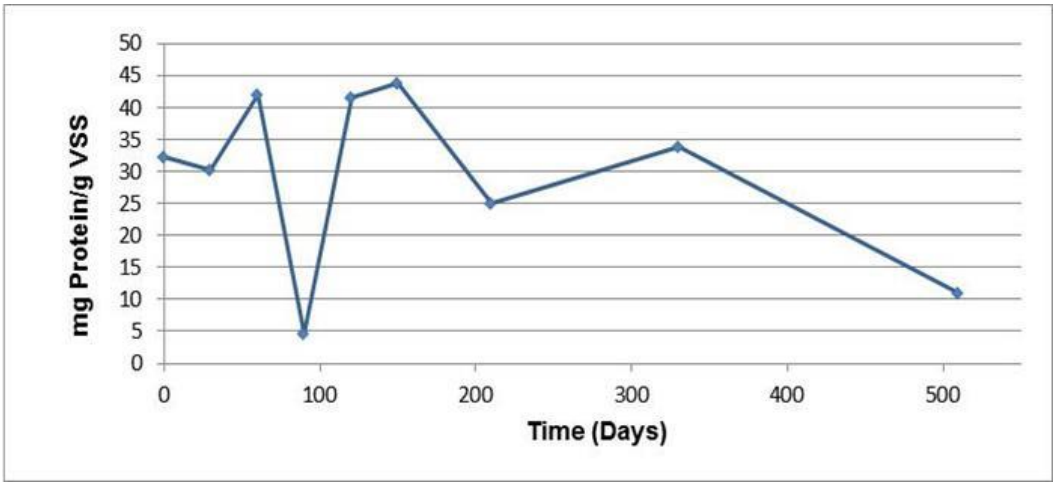


Figure 5.40: Protein analyses of anaerobic granular sludge relative to the performance/operating period of the PS IC-AD

5.6.7 Scanning Electron Microscopy (SEM) analyses for PS IC-AD

Scanning Electron Microscopy images of the initial granules and granules that were treated with wastewater for two months using a PS IC-AD reactor are illustrated in Figures 5.41 and 5.42. The granules used to inoculate the PS IC-AD had an average size of 200 µm. The exterior of the granules had a fluffier loose outer layer.

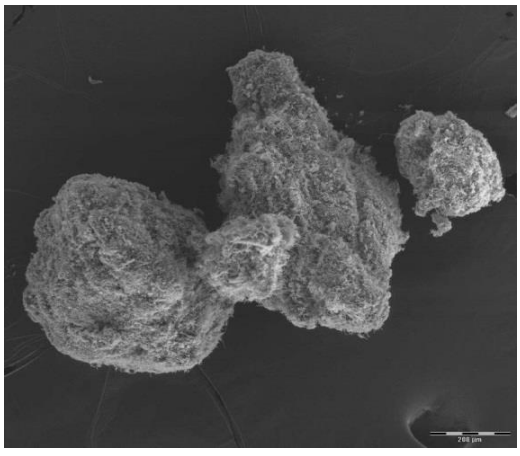


Figure 5.41: SEM image of granules used during inoculation

According to the SEM image shown in Figure 5.42, the granules has grown to approximately 1000 µm two months after the PS IC-AD reactor was started-up. Although the granule has grown considerably, a slight decrease in extra cellular polysaccharide was observed.

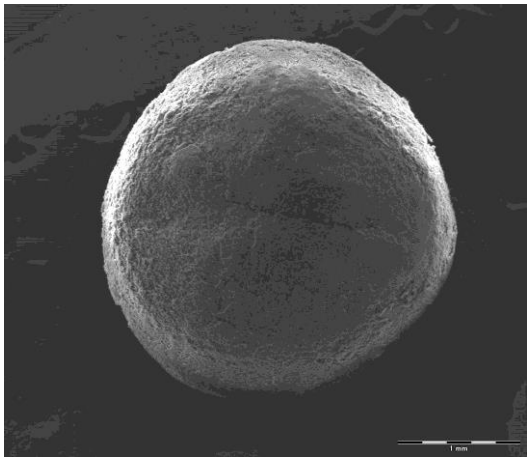


Figure 5.42: SEM image of a granule two months into the experiment (PS IC-AD)

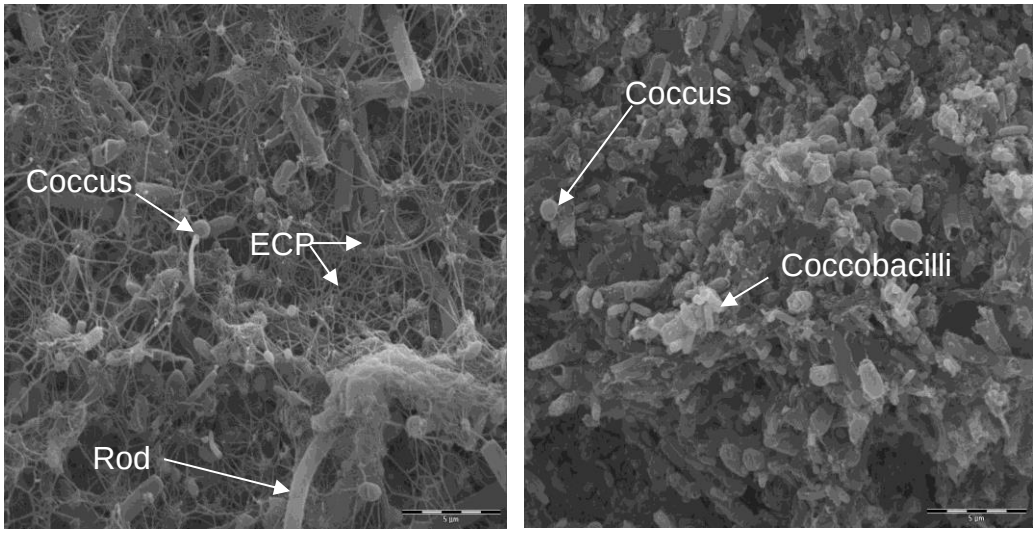
Start-up phase (days 0 – 71)

The initial granules used to inoculate the PS IC-AD had distinct micro-organism morphology (illustrated in Figure 5.43) (days 1 to 45). The SEM images from Figure 5.42 to 5.44 depict the changes observed in the ECP matrix.

According to the SEM image depicted in Figure 5.43 (a), the presence of ECP was clearly visible. The ECP matrix tightly linked and embedded various rod shaped micro-organisms. The internal structure of the granule is dependent on the substrate being degraded. The granules received from the paper mill had been degrading complex effluent wastewaters comprising of sugars, protein and carbohydrates (Mach 2000; Lamb 1995). Due to the complex nature of the wastewater, the

bacterial groups involved in the anaerobic digestion process are most likely hydrolytic and acidogenic fermentative bacteria (Korzak, 2008), which had been reported to produce ECP. The lengthy rod shaped bacteria and the presence of the ECP aided with the initial granulation process, granule development and maintaining the granule structural integrity (MacLeod, 1995).

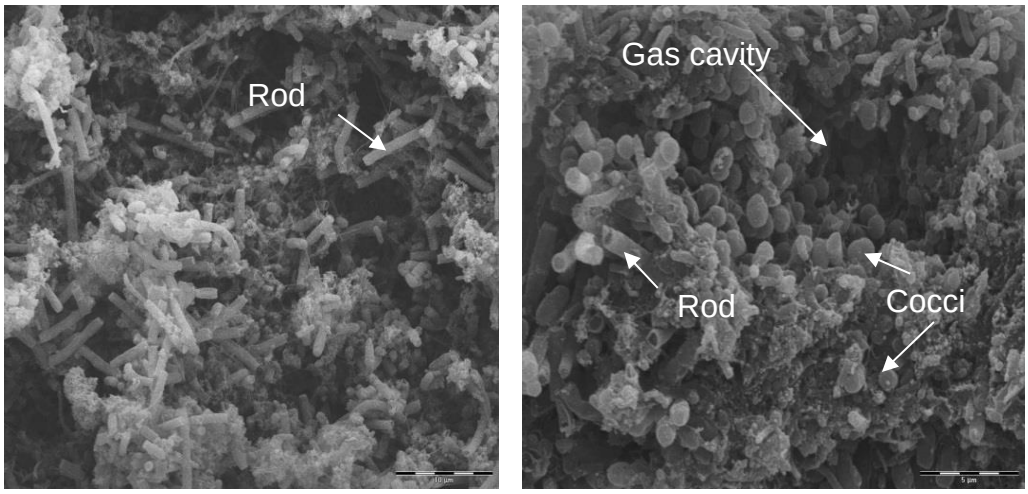
The inner layer or core of the initial granule had a distinct ECP matrix which surrounded various Cocci and rod shaped micro-organisms (Figure 5.43 (a)). These two particular micro-organisms species of Cocci and rod shaped micro-organisms are known as *Methanococci* and *Methanosaete,* respectively. These micro-organisms are normally present in a mixed metabolism configuration in close proximity to one another. The outer layer of the granule was made up of Cocci, Coccobacilli and rod shaped bacteria (Figure 5.43 (b)).



(a) (b)

Figure 5.43: SEM image illustrating the initial granular structure in the inner (a) and outer (b) layers (days 1 to 44) (PS IC-AD)

The outer layer of the granules sampled during days 45 to 65 comprised of layers of Cocci and rod shaped bacteria surrounding a gas cavity (Figure 5.44 (b)). The morphology of the visually observed bacteria within the inner layer of the granule demonstrated a shift in microbial population. Visually the rod shaped bacteria had increased when compared to that of the initial granule (Figure 5.44 (a)) and a decrease in the ECP matrix was apparent. Relative to the granules used during the inoculation process, visually the morphology of the granule had changed due to the presence of Cocci being reduced (illustrated in Figure 5.44 (a)). This was most likely ascribed to the change in the composition of the wastewater being fed to the granules. This changed from a paper mill wastewater containing sugars, proteins and carbohydrates to a synthetic wastewater made up of VFAs (acetate, propionate and butyrate). Given the absence of the complex substrate in the wastewater influent, the hydrolytic and acidogenic bacteria no longer occupy the functional niche in the granules and the granules consisted primary of acetogenic and methanogenic bacteria (illustrated in Figure 2.1). The acetogenic bacteria may possibly consist of members of the genera *Acetomaculum, Clostridium, Syntrophococcus, Syntrophobacter* and *Syntrophus* (Lamb, 1995).



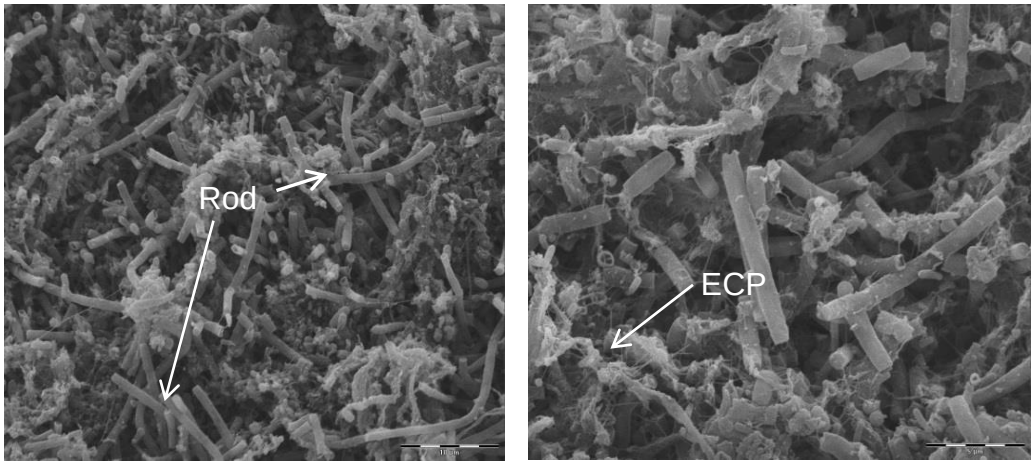
(a) (b)

Figure 5.44: SEM image of the inner (a) and outer core (b) treating synthetically prepared wastewater (days 45 to 65) (PS IC-AD)

Up-set conditions and re-commissioning (days 72 – 131)

It was evident from the SEM image that the ECP matrix of the inner core had considerably changed within the PS IC-AD granules (illustrated in Figure 45 (a)) when compared to that of the original paper mill granules (illustrated in Figure 41 (a)).

The ECP within the granule was visible and indicated that bacterial adhesion within the granule was present, aiding with the maintenance of the granule structure. According to Morgan (1990) the accumulation of ECP on the surface was probably due to metabolic products produced by bacteria. The visual observations of the morphology of the granules sampled during days 88 to109 showed no change when compared to that of the granules sampled during days 45 to 65. The rod shaped bacterial population and the ECP matrix remained unchanged.



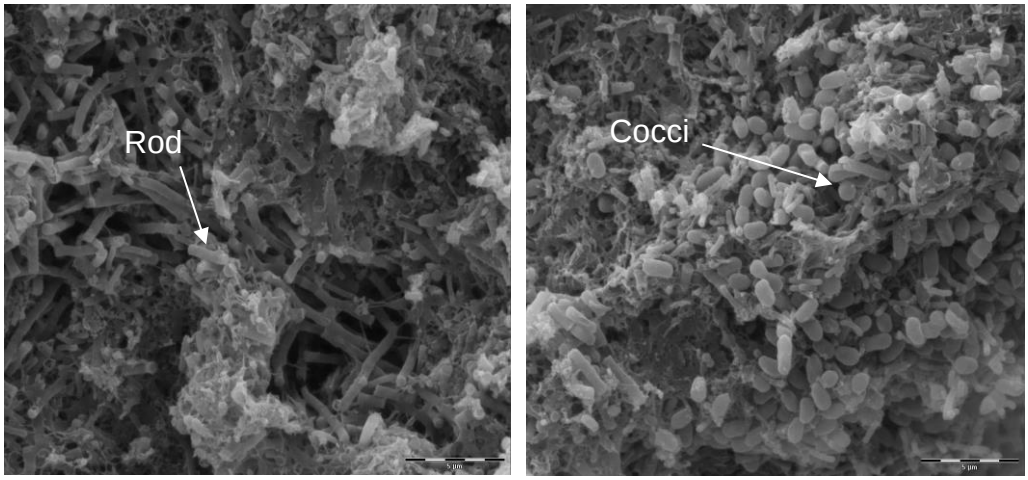
(a) (b)

Figure 5.45: SEM of the inner layer (a) and closer view (b) of the granular sludge (days 88 to 109) (PS IC-AD)

Stable operation (days 164 – 256)

The inner and outer layer of the granule during the re-commissioning phase is illustrated in Figure 5.46.

After the re-commissioning phase the reactor was allowed to stabilise and granules were sampled for analyses. The SEM observation of the morphology of the granule indicated that the inner core of the granule may possibly consist of rod shaped bacteria (Figure 5.46 (a)) whereas the outer layer of the granule probably consisted of Cocci shaped bacteria as displayed in Figure 5.46 (b).



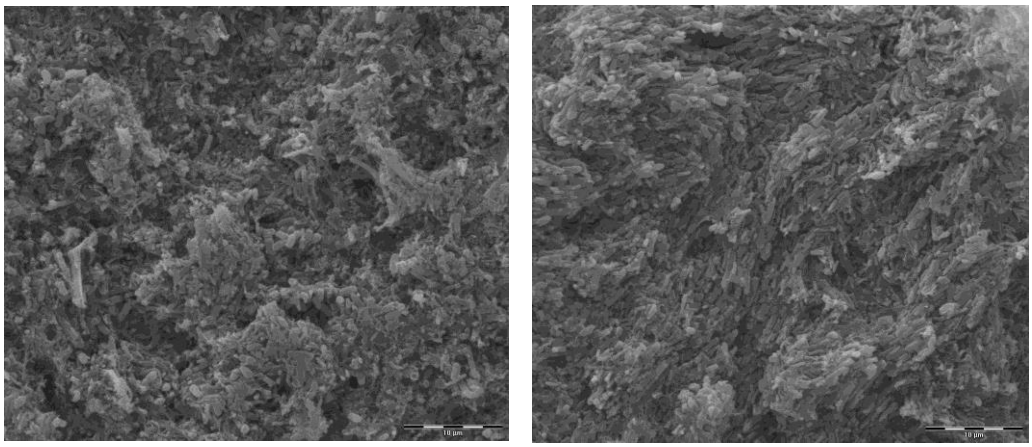
(a) (b)

Figure 5.46: SEM of the inner core (a) and outer layer (b) of the granular sludge from the PS IC-AD for days 131 to 153

During the stable operation phase (days 153 to 239) Figures 5.47 (a) and (b) shows the structure of the inner core and outer layer of the granules.

According to the morphology of these granules it was clear that a change in the bacterial population had occurred. It was evident from the morphology of the inner and outer core of the granule, that rod shape like bacteria was probably present (Figure 5.47 (a) and (b)). From Figure 5.47 (a) and (b) it can be seen that small groups of bacteria were clumped together within the granule. The rod shaped bacteria and the presence of ECP aided with the initial granulation process, granule development and maintaining the granule structural integrity.

Relative to the granules used during the inoculation process, visually the morphology of the granule had changed due to the presence of Cocci being reduced (illustrated in Figure 5.43 (a) and (b)). This was most probably be ascribed to the change in the composition of the wastewater being fed to the granules. This changed from a paper mill complex wastewater containing sugars, proteins and carbohydrates to a synthetic wastewater made up of VFA (acetate, propionate and butyrate).

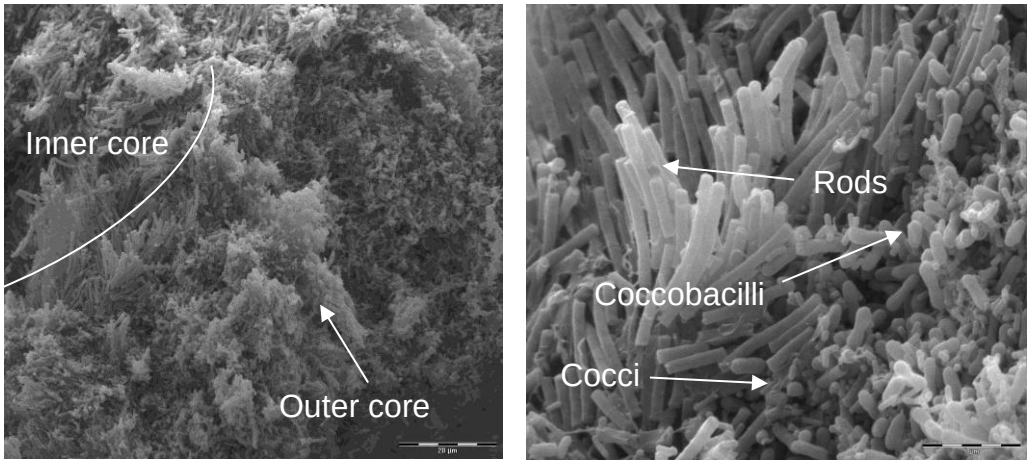


(a) (b)

Figure 5.47: SEM of the inner core (a) and outer layer (b) of the granular sludge from the PS IC-AD reactor

The SEM images obtained from the sample taken at the end of the stable operation phase for days 240 to 260 (Figure 5.48 (a)) showed the

difference in the inner layer and outer layer. A closer view of the granule revealed that the inner core of the granule was most likely made up primarily of rod shaped bacteria whereas the outer layer of the granule in all probability consist of Coccobacilli and Cocci shaped bacteria (Figure 5.48 (b)).



(a) (b)

Figure 5.48: SEM image illustrating the inner and outer core of the granule (a). A closer view of rod shape and outer layer of Coccobacilli and Cocci (b) (PS IC-AD)

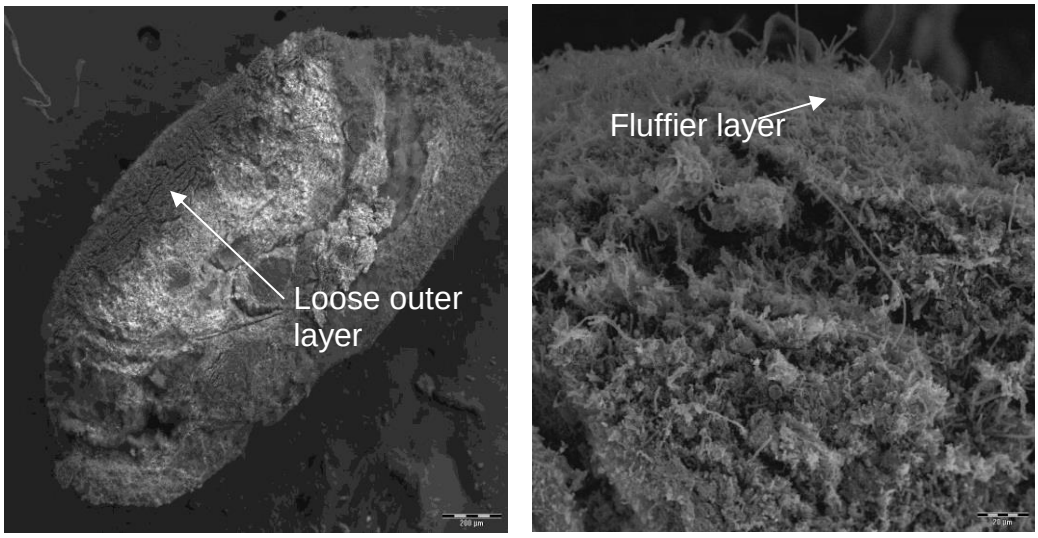
Shock Treatment (days 274 – 514)

Organic Loading Rate

A series of tests which included organic load, high and low temperature followed were performed during the period from days 261 to 544.

During the OLR test period the visual morphology of the granule changed as shown in Figure 5.49. It was evident from the cross section (Figure 5.49 (a)) that the bacteria present on the outer layer appear looser. The outer layer of the granule had changed into a fluffier material which had more spaces in between. The formation of the fluffier

material most likely indicated that a shorter biomass retention time probably existed due to the granules slowly being eroded. This erosion probably resulted in the production of a more filamentous rod shaped bacteria (Figure 5.49 (b)). Visually the cross section (Figure 5.49 (a)) had a loose and disintegrated outer layer. During this part of the study the IC-AD was pushed to the limit regarding the VLR of 40 kg COD/m^3.d.



(a) (b)

Figure 5.49: A cross section of a granule sample taken on days 349 to 370 and the outer core of the granule (b) (PS IC-AD)

Low Temperature

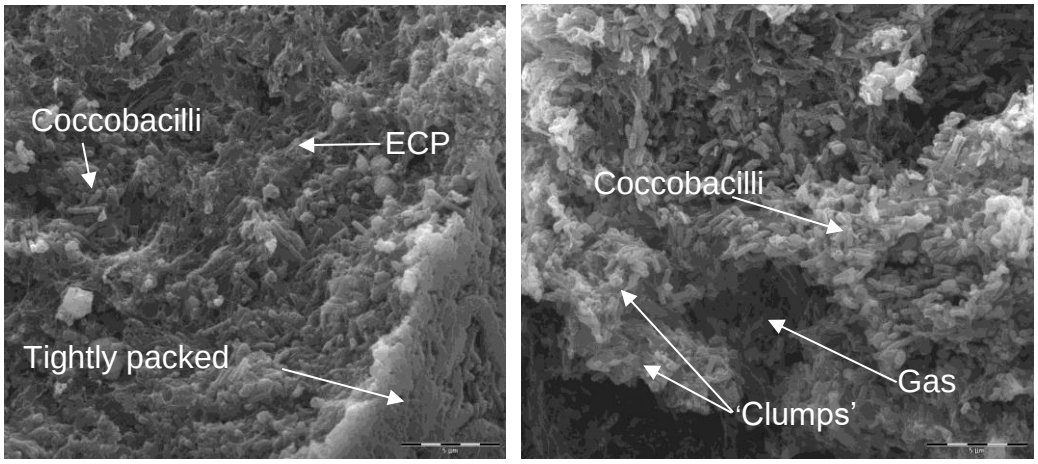
The inner and outer layer of the granule during the low temperature test phase is displayed in Figure 5.50.

It appears from the images that the dead biomass and the sporadic distribution of live biomass which is depicted by the Cocci cells are clearly visible.

During the low temperature test period from days 415 to 435, the outer layer of the granule showed Coccobacilli. The implications of the low

temperature tests on the PS IC-AD most probably had a detrimental effect on the granules. According to the SEM results the methanogenic micro-organisms did not adapt to the continuous VFA influent (low hydrogen partial pressure) during the period and therefore resulted in the micro-organisms dying off. The possible formation of organic salts within the granule, more specifically the inner core, was observed. The organic salts most likely were depicted as a white hard like material (calcium precipitate) in which the cells were embedded.

From a detailed examination of the outer layer a tightly packed biomass is clearly visible. Figure 5.50 (b) shows the core of the granule with a gas cavity which was most likely surrounded by Coccobacilli. These gas cavities probably were shaped when acetogenic bacteria depletes the hydrogen produced by methanogens resulting in the production of methane. These cavities may possibly allow for mass transfer of the substrate or metabolite products in and out of the granule.



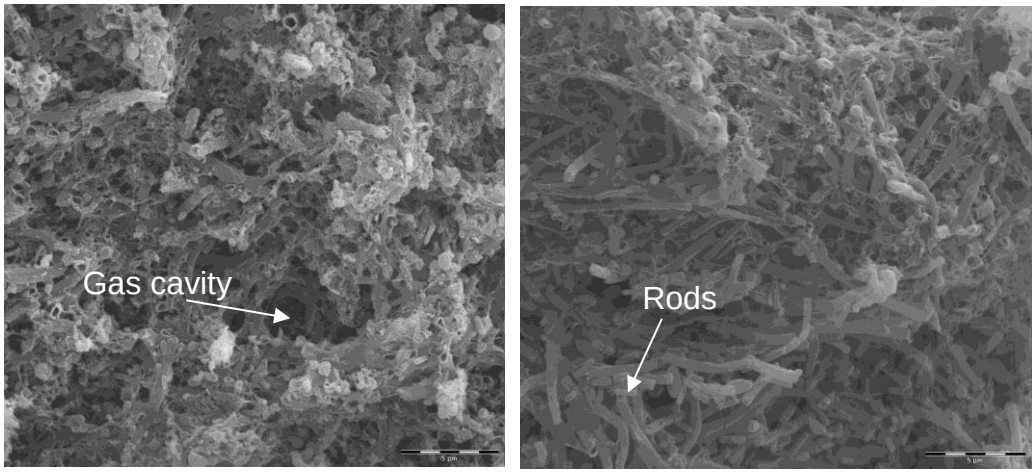
(a) (b)

Figure 5.50: The SEM image of the outer layer (a) and core of the granule (b) (PS IC-AD)

High Temperature

Various pockets of different bacterial organisms that exist between the live and dead biomass can be seen in Figure 5.51.

Over a period of five months during which the effect of lower temperatures were tested, a steady decrease in the protein and carbohydrate concentrations were observed. Although calcium is essential for granular growth, the granules were no longer stable which most likely ascribed to the increased calcium concentration. The granule stability was probably affected as a higher calcium concentration exerts a harmful effect. The calcium probably resulted in a 'cementing 'effect of the granules which led to a loss in mobility or microbial activity.



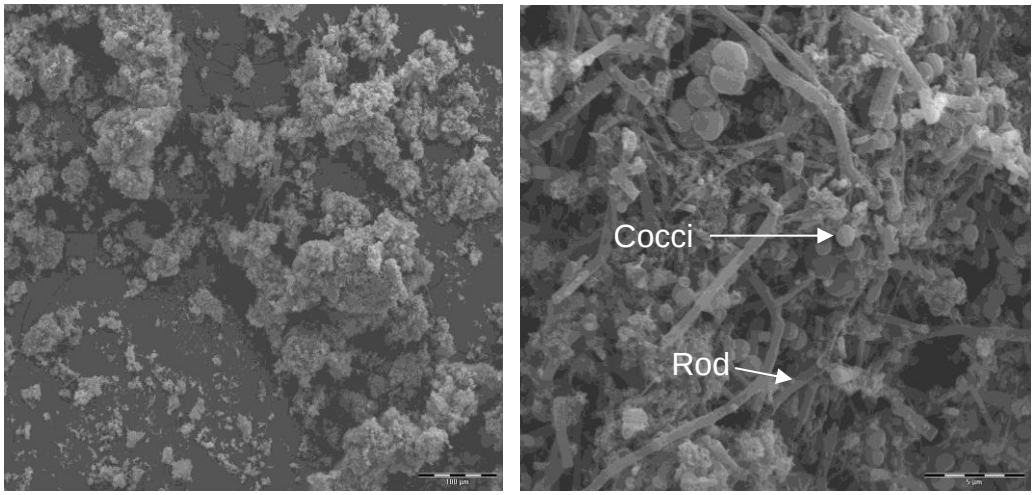
(a) (b)

Figure 5.51: SEM images of the core (a) and edge (b) of the granules during days 513 to 544 (PS IC-AD)

During the high temperature treatment phase, biofilm sludge was taken from the reactor wall and viewed under a SEM (Figure 5.52).

A thin layer of biofilm sludge was found to have attached to the side of the walls of the reactor. This biofilm layer was sampled and sent for SEM analyses (days 531 to 544). The visual observation of the SEM image clearly indicated that the morphology of the sludge sample was different. This sample probably consisted of a mixed population of long rod like and Cocci micro-organisms in a mixed population. The possibility exists that the biofilm layer had adapted to this type of spatial orientation relative to how it gained access to the influent. The 3-phase separator of the IC-AD recycled some of the dispersed biomass back into the reactor which possibly attached to the side of the reactor wall as a biofilm growth (Figure 5.52). Similar findings were reported by Hulshoff et al., 2004 where three distinctive granules were most likely formed in a LS UASB reactor treating digestive sludge with VFA as a substrate.

There was a probability that the biomass retention time for this sludge was most likely too short due to the high up-flow velocity of the influent. This probably resulted in a layer of micro-organisms forming that were most likely composed of mainly bacterial matter that led to no granular formation. These micro-organisms produced conglomerates of bacteria by attaching to inert support particles such as the side walls of the reactor.



(a) (b)

Figure 5.52: Biofilm sludge layer taken from the walls of the reactor (a) and a closer view (b) (PS IC-AD)

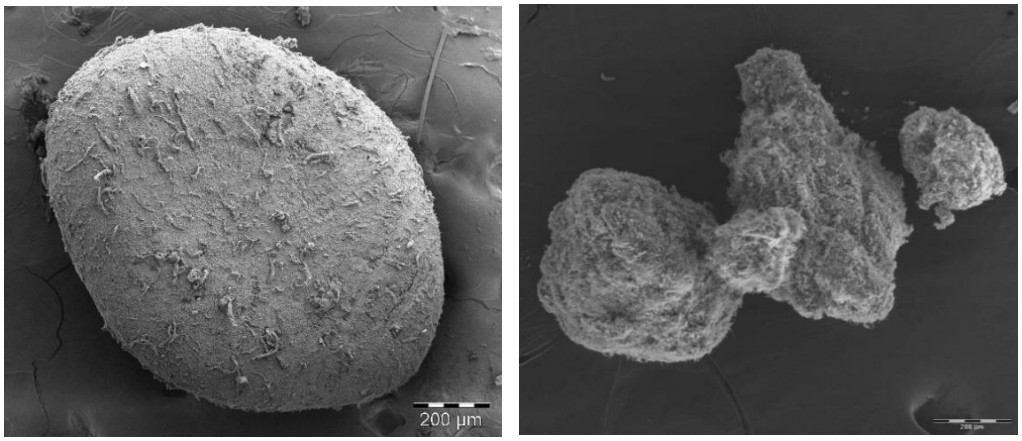
5.7 Comparison between Laboratory Scale and Pilot Scale Internal Circulating Anaerobic Digester reactors

5.7.1 Granule integrity

The granules used to inoculate both the LS IC-AD reactors were received from a brewery in KZN whereas the granules used to inoculate the PS IC-AD reactor were received from a paper mill in the Netherlands.

The SEM images in Figure 5.53 (a) depict images of the granules obtained from the initial batch of granules from the brewery. These granules had a mean size of 1248 and 1347 µm, respectively. The granules seemed to have a typical spherical/rounded shape with a surface made up of aggregates of micro-organisms. The granule seems to be covered with a 'net' like layer.

The SEM images in Figure 5.53 (b) depict the images of the granules obtained from the initial batch of granules from the paper mill. These granules had an average size of 200 µm. The granules from the paper mill were of irregular shape. The exterior of the granules had a fluffier loose outer layer.



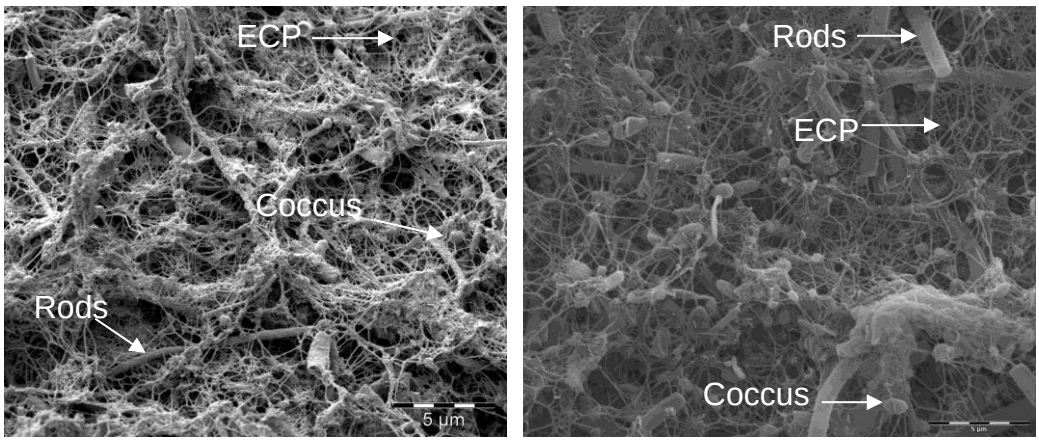
(a) (b)

Figure 5.53: SEM images of a brewery granule (a) and paper mill granule (b) used to inoculate the LS IC-AD and PS IC-AD reactors

The SEM images indicated that the initial granules had a very distinct micro-organism morphology. The ECP within the granule was clearly visible and indicated that bacterial adhesion within the granule was present, aiding with the maintenance of the granule structure. This distinct ECP matrix probably attributed to the original granules received from the brewery and paper mill. These granules were essentially degrading complex polymeric influents consisting of sugars, carbohydrates and polysaccharides. The formation of the ECP matrix was most likely due to the presence of hydrolytic and acidogenic fermentative bacteria degrading the substrate into simple compounds, which are then excreted. Due to the complex nature of the wastewater, the bacterial groups involved in the anaerobic digestion process are most likely hydrolytic and acidogenic fermentative bacteria, which had

been reported to produce ECP. The hydrolytic bacteria most likely consist of members of the genera *Streptococcus* and *Entero* bacterium.

Visual observations revealed that the two morphological shapes of Cocci and rod shaped micro-organisms were most probably known as *Methanococci* and *Methanosaete,* respectively. As illustrated in the SEM images (illustrated in Figure 5.54), the micro-organisms were present in a mixed metabolism configuration in close proximity to one another. The lengthy rod shaped bacteria and the presence of the ECP aided with the initial granulation process, granule development and maintaining the granule structural integrity.

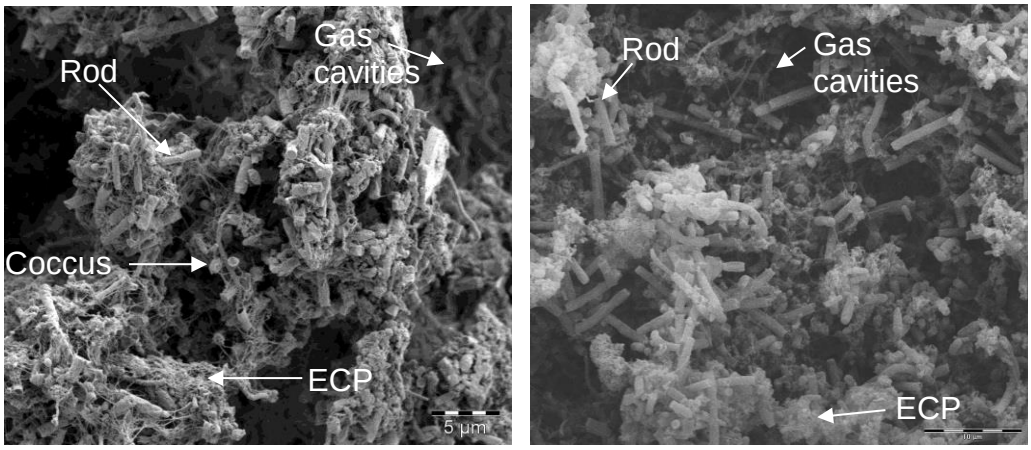


(a) (b)

Figure 5.54: SEM images of an initial granule used to inoculate the LS IC-AD (a) and PS IC-AD (b) reactors

According to the visual observations of the morphology of the granules for both the LS IC-AD and PS IC-AD it was apparent that a change had occurred in the bacterial population. These granules mainly consisted of Cocci and rod shaped like bacteria and some ECP matrix (Figure 5.55 (a) and (b)). Visual interpretations of the granules revealed that the ECP matrix had considerably changed within the LS IC-AD and PS IC-AD compared to that of granules used during the inoculation of the reactors.

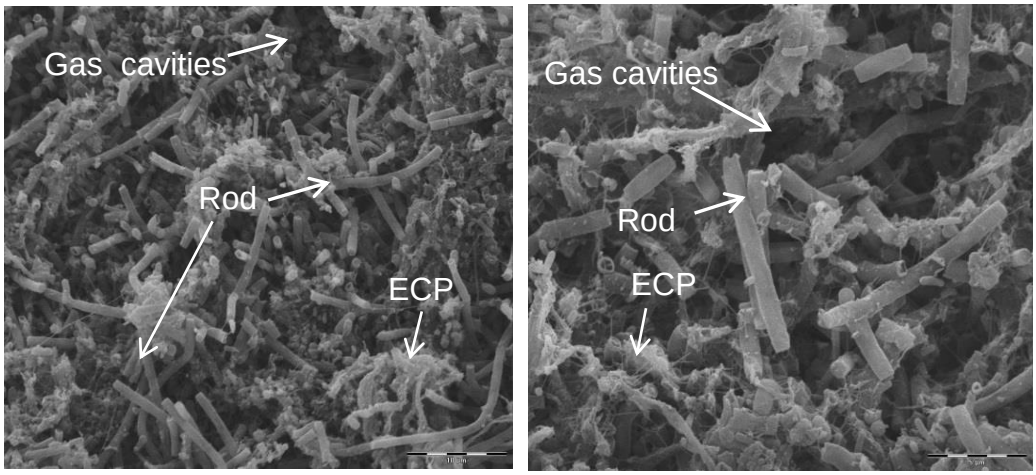
This change most likely ascribed to changing the composition of the brewery and paper mill complex wastewater containing sugars and carbohydrates to a synthetic wastewater consisting primarily of VFAs (acetate, propionate and butyrate). Given the absence of the complex substrate in the wastewater influent, the hydrolytic and acidogenic bacteria no longer occupied the functional niche in the granules and the granules consisted primary of acetogenic and methanogenic bacteria.



(a) (b)

Figure 5.55: SEM images of a granule treating synthetic wastewater in the LS IC-AD (46 days) (a) and PS IC-AD (45 days) (b) reactors

The SEM image in Figure 5.56 (a) and (b) demonstrates the morphology and bacterial population within a granule that had been treating synthetically prepared FT process wastewater for 100 days. According to the visual observations the granules primary comprised of rod shaped bacteria and some ECP for both the LS IC-AD and PS IC-AD reactors. Various gas cavities were present between the clumps of bacteria.



(a) (b)

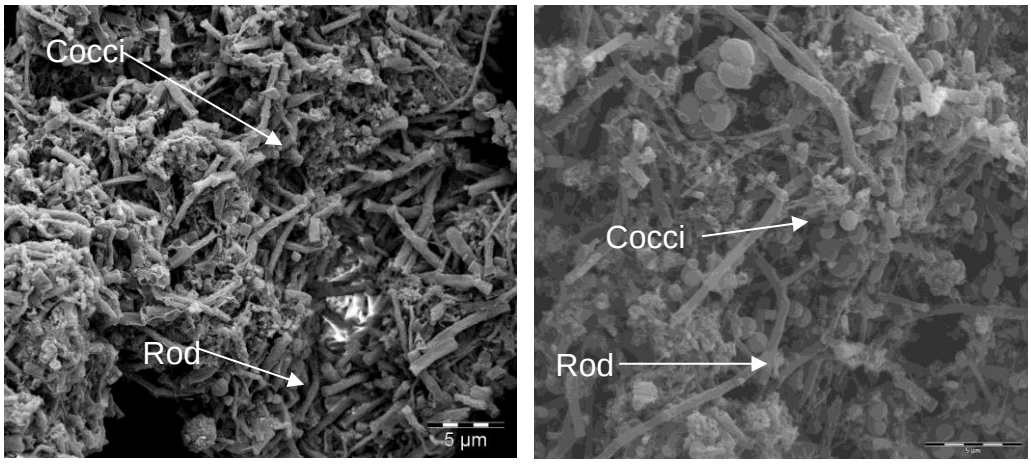
Figure 5.56: SEM images of the LS IC-AD (100 days) (a) and the PS IC-AD (109 days) (b) of the granular sludge treating synthetic wastewater

A thin layer of biofilm sludge was found to have attached to the side of the funnel of the LS IC-AD reactor. This biofilm layer was sampled and sent for SEM analyses. The visual observation of the SEM image clearly indicated that the morphology of the sludge sample did not differ from the granules. The image in Figure 5.57 (a) indicated that a mixed population of rod shaped and Cocci bacteria were most likely present. The possibility exists that the biofilm layer was produced as a result of the granules disintegrating.

A thin layer of biofilm sludge was found to have attached to the side of the walls of the PS IC-AD reactor. This biofilm layer was sampled and sent for SEM analyses. The visual observation of the SEM image clearly indicated that the morphology of the sludge sample was different. The image in Figure 5.57 (b) indicated that a mixed population of rod like and Cocci bacteria were probably present. The possibility exists that the biofilm layer had adapted to this type of influent being fed to the reactor. The 3-phase separator of the IC-AD probably recycled some of the dispersed biomass back into the reactor which possibly attached to the side of the wall as a biofilm growth (Figure 5.57). Similar findings were

reported by Hulshoff et al., 2004. According to Hulshoff (2004), three distinctive granules probably formed in a LS UASB reactor treating digestive sludge with VFA as a substrate.

Presumably the biomass retention time for this sludge was too short due to the high up-flow velocity of the influent. This most probably resulted in a layer of micro-organisms forming which was composed of mainly bacterial matter that most likely had not formed granules. These micro-organisms produced conglomerates of bacteria by attaching to inert support particles such as the side walls of the reactor.



(a) (b)

Figure 5.57: Biofilm sludge layer taken from the walls of the LS IC-AD reactor (a) and PS IC-AD (b)

The Particle Size Distribution (PSD) indicated that the brewery granules used during the inoculation of both the LS IC-AD reactors had a mean size of 1248 and 1348 µm, respectively. At the completion of the LS test run the granules possibly decreased to 874 and 660 µm, respectively. The decrease observed in the granular size was most likely ascribed to the decrease observed in the ECP matrix. This decrease could be ascribed to the shift in the microbial population that occurred as a result

of changing the substrate to a synthetically prepared FT process wastewater.

The size of the paper mill granules used to inoculate the PS IC-AD reactor were determined by measuring the size of the granules while conducting the SEM analyses. The average size of the initial granules was measured as 200 µm. At the completion of the PS test run the granules were not sampled for PSD determinations and therefore the size of the granules were not determined.

CHAPTER 6 – CONCLUSIONS

During this study the function and structural integrity of the granules within the Internal Circulating Anaerobic Digester (IC-AD) treating synthetically prepared Fisher-Tropsch (FT) process wastewater was evaluated. The structural integrity refers to the ability to hold together a 'structure' (granule) resisting breakage or excessive deformities. The following was hypothesized during this study (refer to section 3.3):
"that an Internal Circulating Anaerobic Digester (IC-AD) reactor that was fed with synthetically prepared FT process wastewater that resembles wastewater from a petrochemical process will alter or change the size of granules, change the granular function and produce granules".

During this study Laboratory Scale (LS) and Pilot Scale (PS) anaerobic reactors were used to evaluate synthetic wastewater. The Laboratory IC-AD reactors were operated using granules obtained from a local brewery. Two different runs were conducted using the LS IC-AD (refer to section 4.6.1). Although the reactor in Run 1 was not able to achieved stable operation, due to the Organic Loading Rate (OLR) being increased continuously (state of ramp up), the assumption was made that stable operation was achieved from day 80 onwards. This assumption is based on the fact that both reactors had a similar OLR of approximately 10 kg COD/m^3.d.

(i) Laboratory Scale Internal Circulating Anaerobic Digester (LS IC-AD) (Run1)

The reactor was operated at an average OLR of 10 kg COD/m^3.d and an average removal efficiency of greater than 90% was observed.

Furthermore, an average effluent Chemical Oxygen Demand (COD) concentration of 517 mg/L was obtained. At an average OLR of 10.0 kg/m^3.d the biogas production was found to be 29.2 L/d. The biogas was made up of a methane content between 66% to 75% and a carbon dioxide content of 17% to 23%. As the OLR increased so did the biogas production. The increase in the gas production could possibly have been as a result of the higher OLR resulting in the sludge bed moving due to entrapped and accumulated gas. This might have led to additional turbulence and therefore the disintegration of the granules. The disintegration of the granules could have resulted in the loss of biomass (TSS).

The physic-chemical properties of the granules depend upon the substrate on which the granules are grown. The bacterial population of the original granules were most likely consisted of hydrolytic and acidogenic fermentative bacteria. According to visual observations the morphology of the granules indicated that a shift in the microbial population might have occurred. This shift might have occurred as a result of changing the substrate to a synthetically prepared FT process wastewater. The microbial population within the granules might consist of acetogenic and methanogenic bacteria. Furthermore, the visual observations indicated that the shape of the granules remained rounded and seemed to be covered with a 'net' like layer. In addition, the presence of ECP was clearly visible with rod like bacteria. The presence of the ECP aided with the initial granulation process, granule development and maintaining the granule structural integrity. The results obtained from the Particle Size Distribution (PSD) analyses showed no possible increase in the granular size and no possible formation of new granules.

(ii) Laboratory Scale Internal Circulating Anaerobic Digester (LS IC-AD) (Run 2)

The reactor was operated at an average OLR 11 kg COD/m^3.d and obtained an average removal efficiency of greater than 94%. Furthermore, an average effluent COD concentration of 283 mg/L was obtained. At the average OLR of 11 kg COD/m^3.d the biogas production was found to be 21 L/d. The biogas was made up of a methane content of 68% to 76% and a carbon dioxide content of 14% to 19%. The increase in the OLR resulted in the increase in the biogas production. This increase could be ascribed to the higher OLR which could have resulted in the sludge bed moving due to entrapped and accumulated gas. This might have led to additional turbulence and therefore the disintegration of the granules. The disintegration of the granules could have resulted in the loss of biomass (TSS).

Similar to the first run, the bacterial population of the original granules most likely consisted of hydrolytic and acidogenic fermentative bacteria. The visual observations of the morphology of the granules indicated that a shift in the microbial population might have occurred, this shift might have occurred as a result of the substrate being changed to a synthetically prepared wastewater. The shift in the microbial population could have resulted in the population within the granules to mainly consist of acetogenic and methanogenic bacteria. Furthermore, the visual observations indicated that the shape of the granules remained rounded and seemed to be covered with a ‘net’ like layer. The presence of ECP was clearly visible. The presence of the ECP aided with the initial granulation process, granule development and maintaining the granule structural integrity. The outer layer of the granules was possibly made up of Cocci, Coccobacilli and rods. The results obtained from the

Particle Size Distribution (PSD) analyses showed no possible increase in the granular size and no possible formation of new granules.

Even though the operating conditions (pump flow-rate and influent COD concentration) for the LS reactors differed, it was evident from the results obtained during this study that these conditions did not influence and/or change the granular size, the granular function and granular production.

The PS IC-AD was operated using granules obtained from a paper mill in the Netherlands. The PS IC-AD reactor was operated for a period of 544 days.

(iii) Pilot Scale Internal Circulating Anaerobic Digester (PS IC-AD)

The PS IC-AD reactor commissioning phase was significantly shorter (60 days) when compared to that of the base case fixed media anaerobic reactor at 100 days. The paper mill granules within the IC-AD reactor adapted to the synthetic wastewater that was utilised as influent within a period of two months. The PS IC-AD achieved stable operation in a short period of time (60 days) and a high COD removal efficiency (>90%) was observed. The OLR was increased to determine the upper limit for the OLR of the PS IC-AD reactor. Although an upper limit of 39 kg COD/m^3.d was achieved a significant biomass loss was observed which could have resulted in a reactor failure. Due to the biomass within the IC-AD reactor possibly having poor settleability properties, this biomass could have been easily washed out of the reactor. The Total Suspended Solid (TSS) concentration in the effluent increased with an increase in the VFA concentration of the effluent indicating biomass washout. The removal efficiency for the base case study was 85%,

compared to the higher removal efficiency of >90% that was observed for the IC-AD reactor for the duration of this study. A correlation between the increase and decrease of the COD influent concentration and COD effluent concentration were observed. This was especially evident when the OLR was increased to 39 kg COD/m^3.d by increasing the influent COD concentration.

The anaerobic granules within the IC-AD reactor had successfully remained intact, firm and stable during the commissioning phase. The granules were rounded with some white spots which might be as a result of fresh biofilm growing. The presence of ECP was observed throughout the stable operation phase and contributed to cell structural integrity. The results obtained during this study indicated that no new granules were being formed when using synthetic wastewater. No granular formation or recovery was observed within the IC-AD as a result of the VFA influent characteristics or the reactor design. The reactor performance was dependent on the thin layers of active cells close to the surface of the granules which could primarily be responsible for the degradation of the VFA concentration. Although the methanogens might have been mostly on the outer core influencing on the VFA, the inner core consisted mainly of dead biomass. During upset conditions it seems as if the active micro-organisms were eroded on the exterior part of the granule, which could have resulted in irreversible reactor failure and therefore the reactor had to be re-seeded with paper mill granular sludge.

The results obtained in both the LS runs as well as that of the PS run it was evident that;

- the reactors could not sustain the formation of granules when treating synthetically prepared FT process wastewater,

- re-seeding the reactors each time operational upsets occurred, most likely resulted in the microbial population within the granules not being adapted to the synthetically prepared FT process wastewater and possibly compromised the structural integrity of the granules.
- treating synthetically prepared FT process wastewater in an IC-AD showed no increase in granular size,
- the results obtained during this study indicated that no new granules were formed using synthetically prepared FT process wastewater.

Therefore, the hypothesis of changing the granular size, the granular function and producing granules were not supported during this study.

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