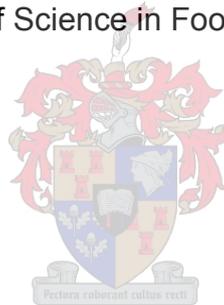


**INVESTIGATING THE EFFECT OF MAGNETISABLE GLASS FOAM
PARTICLES (MGFP) IN AN UASB REACTOR TREATING SYNTHETIC
WINERY WASTEWATER BY MONITORING BIOFILM DEVELOPMENT
AND ACTIVITY OF COLONISED MGFP**

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Thesis presented in partial fulfilment of the requirements for the degree of
Master of Science in Food Science



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Declaration

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Abstract

The growing of grapes for wine production is a generational, agricultural practice that has been associated with generating large revenue in South Africa. However, due to one of the outcomes of wine production, i.e. the generation of heavily contaminated wastewater, wine industries often face the obligation to treat wastewater prior to discharge to the municipality or irrigation. In addition, with the pressing matter of water scarcity at hand in South Africa, stricter regulations have been imposed on the treatment of winery wastewater (WWW), considering that wine production generates bulk volumes of wastewater. Since this winery wastewater is partially acidic and is characterised by high amounts of organic matter, inorganic ions, total suspended solids and polyphenols, these substances contribute to the pollution of water. Therefore, it is crucial for WWW to be depolluted to the standard specifications lest it negatively disrupts the ecosystem upon its discharge. Among the winery wastewater treatment methods developed and used in the wine industries, biological treatment methods (e.g. anaerobic digestion) are regarded as the most effective in treating winery wastewater (80 - 98% efficiency). The Upflow Anaerobic Sludge Blanket (UASB) bioreactor, which was used in this study, is one of the successful biological methods widely used at a lab-scale and commercial scale. The UASB reactor is primarily centred on the breakdown of organic matter to produce methane, a source of energy. However, the operation of UASB reactors often has the problem of sludge washout, which, consequently, results in reactor performance deterioration. Hence, in an attempt to prevent this problem, biofilm carrier particles known as magnetisable glass foam particles (MGFP) were used in this research. The study was focused on comprehensively investigating the effects of MGFP in an UASB reactor treating synthetic winery wastewater (SWWW) and monitoring biofilm development and activity of the colonised MGFP. The SWWW, which mimics the industrial winery wastewater, was used to make the substrate. The study was divided into two phases, whereby the first phase was aimed at treating SWWW at a gradually increasing organic loading rate (OLR) ranging from 0.5 to 5.0 kgCOD.m⁻³.d⁻¹. Phase 2 was also focused on treating SWWW at a constant OLR (5.0 kgCOD.m⁻³.d⁻¹), which resembles the common industrial OLR used in wine industries. Phase 1 was characterised by a high treatment efficiency with an increase in OLR. During Phase 1, from day 0 – 107, the COD reduction ranged from 71.4 to 97.7% in both R_{control} and R_{mgfp}. The alkalinity, which indicates the strength of the buffer system, ranged from 450 to 3 075 mgCaCO₃.L⁻¹ in both R_{control} and R_{mgfp}. The pH and concentration of volatile fatty acids (VFA), which also determine reactor

stability and performance, were also within the standard specifications. The average VFA concentration in R_{mgfp} and R_{control} ranged from 25 – 425 mg.L⁻¹, which was within the optimal standard of < 500 mg.L⁻¹ in both reactors, while the average pH ranged from 7.48 – 8.40 in R_{control} and R_{mgfp} . There was a stable production of a high methane biogas by both reactors (> 55% methane), which ranged from 63 – 74% in R_{control} and R_{mgfp} . The concentration of total suspended solids (TSS) measured in the effluent gradually increased with increase in OLR. The TSS increased from 100 - 380 mgTSS.L⁻¹ in R_{control} and from 80 – 400 mgTSS.L⁻¹ in R_{mgfp} . Nevertheless, towards the end of the Phase 1, there was a reactor performance disturbance due to sludge washout caused by a high biogas production and this reduced the treatment efficiency of the reactors. Optimal reactor performance was restored in Phase 2 (day 108 - 180) due to improved settling of the sludge bed, moreover, due to stable operation of the reactors. During Phase 2, the water quality parameters were within the optimal standards for operating the UASB. The average COD reduction, alkalinity and pH ranged from 71.4 - 97.7%, 1 500 – 2 750 mgCaCO₃.L⁻¹ and 7.53 - 8.25 in R_{control} and R_{mgfp} . The average TSS concentration in both reactors reduced from 380 to 360 mgTSS.L⁻¹ in R_{control} and 720 to 160 mgTSS.L⁻¹ in R_{mgfp} . When R_{mgfp} , R_{control} , control granules and colonised MGFP were analysed with a scanning electron microscope (SEM), a dense biofilm coverage was observed from the second month until the sixth month of the UASB operation. Both cocci and rod-shaped bacteria were observed in all of these samples, except for the control MGFP. In addition, the presence of bacteria and methanogens was corroborated under the fluorescence microscope where normal bacteria were distinguished from methanogens. The normal bacteria illuminated green while methanogens were blue as they auto-fluoresce. After performing granule activity tests, the results noted indicated that, overall, the colonised MGFP had the highest biological activity and acidogenic activity. This was presumably as a result of the presence of iron in the particles that aid in biogas production. However, R_{control} granules had the highest biological activity when an acetic acid media was used, thus presumably suggesting that the sample had the highest population of active acetoclastic methanogens. Although, the magnetisable particles had negligible effects on the treatment efficiencies of the reactors, overall, the incorporation of the MGFP in an R_{mgfp} reactor had a positive impact, as an active anaerobic biofilm attached to the particles and produced a higher methane biogas. More so, due to the magnetic properties of the MGFP it was also feasible to extract them with a magnetic rod so that they could potentially be used as a source of multiplying active biomass to either seed another treatment process or be stored for cases of emergency (i.e. reactor failure or loss of biomass).

Uittreksel

Die verbouing van druiwe vir wynproduksie is 'n landboupraktik wat oor generasies verband hou met die generering van groot inkomste in Suid-Afrika. Nietemin, as gevolg van die generering van swaar gekontaminateerde afvalwater tydens wynproduksie, is die wynbedryf dikwels genoodsaak om die afvalwater te behandel voor dit aan die munisipaliteit vrygestel of vir besproeiing gebruik word. Met die drukkende kwessie van waterskaarsheid op hande in Suid-Afrika, is strengere regulasies op die behandeling van wynkelderafvalwater (WWW) opgelê, aangesien wynproduksie groot volumes afvalwater genereer. Aangesien hierdie wynkelderafvalwater gedeeltelik suur is en gekenmerk word deur hoë hoeveelhede organiese stowwe, anorganiese ione, totale gesuspendeerde vastestowwe en polifenole, dra hierdie stowwe by tot die besoedeling van die water. Daarom is dit van kardinale belang vir WWW om gereinig te word tot die standaard spesifikasies sodat dit nie die ekosisteem negatief ontwig tydens afvoer nie. Tussen die kelderafvalwater-behandelingsmetodes wat ontwikkel is en gebruik word in die wynbedryf, word biologiese behandelingsmetodes (bv anaërobiese vertering) beskou as die mees doeltreffende metode in reiniging van kelderafvalwater (80 - 98% doeltreffendheid). Die Upflow Anaerobic Sludge Blanket (UASB) bioreaktor, wat tydens hierdie studie gebruik is, is een van die suksesvolle biologiese metodes wat grootliks op laboratorium en kommersiële skaal gebruik word. Die UASB reaktor is hoofsaaklik gesentreer om die afbreek van organiese materiaal om metaan, 'n bron van energie, te produseer. Die werking van UASB reaktors het egter 'n groot en algemene probleem van slykuitspoeling, wat gevolglik lei tot die agteruitgang van reaktor prestasie. Dus, in 'n poging om hierdie probleem te voorkom, is biofilm draerpartikels, bekend as magnetiseerbare glas skuimparkel (MGFP) in hierdie navorsing gebruik. Hierdie studie het gefokus op die omvattende ondersoek van die uitwerking van MGFP binne 'n UASB reaktor wat sintetiese kelderafvalwater (SWWW) behandel en die monitering van biofilmontwikkeling en aktiwiteit van die gekoloniseerde MGFP. SWWW, wat die industriële wynkelder afloopwater naboots, is gebruik om die substraat te maak. Die studie is hoofsaaklik verdeel in twee fases, waar die eerste fase gefokus het op die behandeling van SWWW teen 'n geleidelik-toenemende organiese laaikoers (OLR) wat gewissel het tussen 0.5 en 5.0 kgCOD.m⁻³.d⁻¹. Die tweede fase het ook gefokus op die behandeling van WWW teen 'n konstante OLR (5.0 kgCOD.m⁻³.d⁻¹), wat soortgelyk is aan die algemene industriële OLR in die wynbedryf gebruik word. Fase een is gekenmerk deur 'n hoë behandelingsdoeltreffendheid met 'n toename in OLR. Tydens fase 1, van dag 0 - 107, het

die COD afname gewissel van 77.1% tot 97.7% en 71.4 tot 97.7% in R_{control} en R_{mgfp} , onderskeidelik. Die alkaliniteit, wat die sterkte van die buffersisteem aandui, het gewissel van 450 tot 3 075 $\text{mgCaCO}_3\cdot\text{L}^{-1}$ in beide R_{control} en R_{mgfp} . Die pH en konsentrasie van vlugtige vetsure (VFA), wat ook reaktorstabiliteit en werkverrigting bepaal, was ook binne die standaard spesifikasies. Die gemiddelde VFA konsentrasie in R_{mgfp} en R_{control} het gewissel van 25 - 425 $\text{mg}\cdot\text{L}^{-1}$, wat binne die optimale standaard van $<500 \text{ mg}\cdot\text{L}^{-1}$ in beide reaktors was, terwyl die gemiddelde pH gewissel het van 7.48 – 8.40 in R_{control} en R_{mgfp} . Goeie reaktor werking is ook bewys deur die stabiele produksie van hoë metaan biogas deur beide reaktors ($> 55\%$ metaan). Die gemiddelde metaan persentasie het gewissel van 66 - 74% in R_{control} en 63 - 73% in R_{mgfp} . Die konsentrasie van die totale gesuspendeerde vastestowwe (TSS) gemeet in die uitvloeisel, het geleidelik toegeneem met toename in organiese laaikoers. Die TSS het toegeneem van 100 – 380 $\text{mgTSS}\cdot\text{L}^{-1}$ in R_{control} en van 80 – 400 $\text{mgTSS}\cdot\text{L}^{-1}$ in R_{mgfp} . Nietemin, aan die einde van die eerste fase was daar 'n reaktor werkingsversteuring weens slykuitspoeling wat veroorsaak is deur 'n hoë produksie van biogas en dit het die behandelingsdoeltreffendheid van die UASB reaktore verminder. Optimale reaktor werking is herstel in die tweede fase (dag 108-180) as gevolg van die verbeterde vestiging van die slykbodem, en ook as gevolg van die stabiele werking van die reaktors. Tydens die tweede fase was die kwaliteit van die water parameters binne die optimale standarde vir die gebruik van die UASB. Die gemiddelde COD afname, alkaliniteit en pH het gewissel van 71.9 – 97.7%, 1 500 – 2 750 $\text{mgCaCO}_3\cdot\text{L}^{-1}$ en 7.53 - 8.25 in R_{control} en R_{mgfp} . Die gemiddelde TSS konsentrasie in beide reaktors het verminder van 380 tot 360 $\text{mgTSS}\cdot\text{L}^{-1}$ in R_{control} en 720 tot 160 $\text{mgTSS}\cdot\text{L}^{-1}$ in R_{mgfp} . Toe die R_{mgfp} , R_{control} , kontrole korrels en gekoloniseerde MGFP met 'n skandeerelektronmikroskoop (SEM) ontleed is, is 'n digte biofilmbedekking waargeneem vanaf die tweede maand tot en met die sesde maand van UASB werking. Beide kokki en staaf-vormige bakterieë is in al hierdie monsters waargeneem, behalwe in die kontrole MGFP. Die teenwoordigheid van bakterieë en metanogene was ook gestaaf onder die fluoressensiemikroskoop waar daar tussen normale bakterieë en metanogene onderskei is. Die normale bakterieë het groen verlig terwyl metanogene blou was omdat dit auto-fluoresseer. Na granule aktiwiteitstoetse, het die resultate getoon dat gekoloniseerde MGFP oor die algemeen die hoogste biologiese aktiwiteit en asidogeniese aktiwiteit gehad het. Dit was waarskynlik as gevolg van die teenwoordigheid van yster in die partikels wat help met die produksie van biogas. Nietemin, R_{control} granules het die hoogste biologiese aktiwiteit getoon wanneer 'n asynsuur medium gebruik is, wat waarskynlik voorstel dat die monster die hoogste populasie van aktiewe asetoklastiese metanogene besit het. Nietemin die magnetiseerbare partikels het

weglaatbare effekte op die behandelingsdoeltreffendheid van die reaktors gehad. Oor die algemeen het die inkorporasie van die MGFP in 'n R_{mgfp} reaktor 'n positiewe impak gehad, omdat 'n aktiewe anaerobiese biofilm vasgeheg het aan die partikels en 'n hoër metaan biogas geproduseer het. Meer so as gevolg van die magnetise eienskappe van die MGFP was dit ook haalbaar om dit uit te trek met 'n magnetise staaf sodat hulle moontlik gebruik kan word as 'n bron vir die vermeerterings van aktiewe biomassa om òf ander behandelingsprosesse te saai òf gestoor te word vir noodgevalle (m.a.w reaktor mislukking of verlies van biomassa).

Dedications

To my dearest loving family:

Robson & Grace Dzviti in Zimbabwe, Ruramayi (sister) in Australia, Dzidzai (brother) in Canada, Imelda (sister) in Spain.

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This thesis is presented in the format prescribed by the Department of Food Science at Stellenbosch University. The structure is in the form of one or more research chapters (papers prepared for publication) and is prefaced by an introduction chapter with the study objectives, followed by a literature review chapter and culminating with a chapter for elaborating a general discussion and conclusion. Language, style and referencing format used are in accordance with the requirements of the *International Journal of Food Science and Technology*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

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Abbreviations of key terms

ABR	Anaerobic baffled reactor
AD	Anaerobic Digestion
AMBBR	Anaerobic moving bed biofilm reactor
BOD	Biological oxygen demand
BSF	Biological sand filter
COD	Chemical oxygen demand
DWA	Department of Water Affairs
EC	Electrical conductivity
HRT	Hydraulic retention time
JLR	Jet-loop activated sludge reactor
MGFP	Magnetisable foam glass particles or magnetisable particles
MBR	Membrane bioreactors
OLR	Organic loading rate
R_{control}	Control reactor
R_{mgfp}	Test reactor, reactor with MGFP
SAR	Sodium adsorption rate
SBR	Sequencing batch reactor
SRT	Solids retention time
TSS	Total suspended solids
UASB	Upflow anaerobic sludge blanket
VFA	Volatile fatty acids
VSS	Volatile suspended solids
WWW	Winery wastewater
SWWW	Synthetic winery wastewater

Chapter 1

Introduction

The impact of water scarcity and water quality deterioration worldwide has been of great concern considering the negative effects it has been posing on human health, environment and socio-economic status (Rocha & Soares, 2015). Water scarcity is currently increasing as a result of rapid population growth, urbanisation and industrialisation (Shevah, 2014). Furthermore, misuse and contamination of present water resources have been worsening the problem (Hoekstra *et al.*, 2012; Clarke, 2013). It has been documented that agricultural practices, inconsistent hydrologic patterns due to global warming and uneven distribution of rainfall per region are also contributing factors to water scarcity (McDonald *et al.*, 2011; Clarke, 2013). These problems all contribute to a precipitous decrease in the quality and quantity of the available freshwater (Shevah, 2014). The repercussion of this will affect 3.2 billion people who will suffer from chronic water scarcity by 2025 (especially those in Middle East and Africa) as explained by Hoekstra *et al.* (2012). Currently, about 505 million people are being affected by this problem (McDonald *et al.*, 2011). People residing in the arid or semi-arid regions are highly affected by water scarcity and therefore are most likely to be affected by drought and water related illnesses (Hoekstra *et al.*, 2012; Shevah, 2014).

In South Africa, agricultural practices (including wine production) consume approximately 57% of the freshwater resources while the municipality and other industries use 35% and 8%, respectively (Anon., 2014). Wineries generate varied volumes of wastewater (0.7 - 14 L for every litre of wine), all depending on the type of wine being produced (Da Ros *et al.*, 2016). The type of wine being produced consequently influences the processing techniques involved and is also dependant on the size of the winery (Andreottola *et al.*, 2009; Devesa-Rey *et al.*, 2011). Winery wastewater (WWW) usually has high levels of COD due to the presence of ethanol and organic macromolecules (Myburgh *et al.*, 2015). It also comprises high levels of inorganic ions (potassium, phosphorus, nitrogen and sodium) and recalcitrant contaminants such as polyphenols and tannins (Valderrama *et al.*, 2012; Myburgh *et al.*, 2015). Because of the high contamination associated with WWW it is essential and mandatory to depollute WWW prior to discharge as suggested by various legislative boards (DWA, 2013).

As highlighted by Basset *et al.* (2016) and Tee *et al.* (2016), there are various methods that are feasibly and effectual in the treatment of WWW. These methods include

physiochemical, advanced oxidation processes and biological methods (aerobic and anaerobic). Among these, anaerobic digestion (AD) is a successful biological treatment method that has been used for the past century to treat winery wastewater (Basset *et al.*, 2016). Anaerobic digestion involves the conversion of organic matter to biogas by bacteria in the absence of oxygen (Khalid *et al.*, 2011). This method is beneficial as it generates methane rich biogas, which can be used as a source of fuel (Da Ros *et al.*, 2016). Anaerobic digestion achieves energy conservation due to the fact that there is no energy required to initiate this process and the methane produced can be used in the process (Basset *et al.*, 2016; Da Ros *et al.*, 2016). More so, there is minimal sludge production (7-10% sludge produced) because of ATP energy consumed during the numerous stages of AD (Basset *et al.*, 2016). Therefore, there is only a small amount of energy available for the production of sludge (Da Ros *et al.*, 2016).

The anaerobic bioreactor, known as the upflow anaerobic sludge blanket (UASB). This bioreactor works on the principle of developing flocs or granules of biomass with high biological activity (Huang *et al.*, 2005). The upward flow of the influent and biogas production ensures maximal contact between the biomass and the substrate (Jih *et al.*, 2003). This circulation promotes breakdown of macromolecules into biogas by bacteria (Huang *et al.*, 2005). Moreover, the bioreactor works on the principle of separating the biogas, produced from the breakdown of organic matter, from the substrate (Huang *et al.*, 2005; Chernicharo, 2006). One of the main reasons this method is superior to the other anaerobic methods is that the granular sludge used in the bioreactor has excellent flocculating and settling properties, which can retain various microbial species (Huang *et al.*, 2005).

Nevertheless, the use of UASBs has its own disadvantages, as biomass washout and foaming are likely to occur (Van Der Westhuizen, 2014). Biomass washout is the result of a high upflow rate, high organic loading rate and high biogas production, which cause the granular sludge bed to be excessively, suspended causing the methane-producing bacteria to be washed out (Van Der Westhuizen, 2014). The loss of methanogens consequently leads to incomplete digestion of organic matter, which often results in build-up of intermediary products such as volatile fatty acids (Massalha *et al.*, 2015). The rate of volatile fatty acids production exceeds the rate of volatile fatty acids consumption (Van Der Westhuizen, 2014). This has detrimental effects on the performance of the UASB reactors due to the acidic environment created that inhibits methanogenesis, the crucial stage of AD (Massalha *et al.*, 2015). Since there will be insufficient bacteria in the reactor, the process of anaerobic digestion will be at a slower rate (Chernicharo, 2006).

To counter these problems various immobilising biofilm carriers such as alumina-based ceramics, clay montmorillonite, polymeric materials and magnetisable glass foam particles (MGFP) are used (Hellman *et al.*, 2010; Wang *et al.*, 2016). MGFP are extremely versatile as they can be made with different weights, densities and particles sizes (Ramm *et al.*, 2014). The MGFP contain iron, making them magnetisable. Their porous nature and iron content make them an ideal attachment media for bacteria, specifically methanogens (Ramm *et al.*, 2014)

The main aim of the study was thus to investigate the effect of seeding MGFP into a UASB reactor treating synthetic winery wastewater (SWWW) and monitoring biofilm development and activity of the colonised MGFP. The first objective was to compare treatment efficiency of two parallel lab-scale UASBs (one with MGFP and one without acting as the control), treating SWWW at a temperature of 35°C. The treatment efficiency of the UASB reactors was evaluated by measuring various water quality parameters such as chemical oxygen demand (COD) reduction, pH, alkalinity and volatile fatty acids (VFA) and biogas composition. The second objective was focused on monitoring biofilm development on the MGFP, by using scanning electron microscopy (SEM) and fluorescence microscopy. Analysis using scanning electron microscopy was conducted to study the surface morphology of the control and biofilm colonised MGFP (Yang *et al.*, 2015). The presence of microbes was verified by staining nucleic acids with fluorescent dye SYTO 9 at an excitation wavelength of 488 nm to obtain emission at 576 nm. The presence and growth of methane producing bacteria was achieved by triggering auto-fluorescence at an excitation wavelength of 405 nm and emission was detected at 476 – 585 nm. Methanogens auto-fluoresce due to the presence of coenzymes that partake in the redox reactions of methane production (Valle *et al.*, 2015). The third and final objective was to compare the activity of the MGFP biofilm and control granules with the initial granule activity. The activity was determined using standardised activity tests to measure biogas production and composition (CO₂ and methane content).

1.1 REFERENCES

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Chapter 2

Literature Review

2.1 WATER

2.1.1 SIGNIFICANCE OF WATER

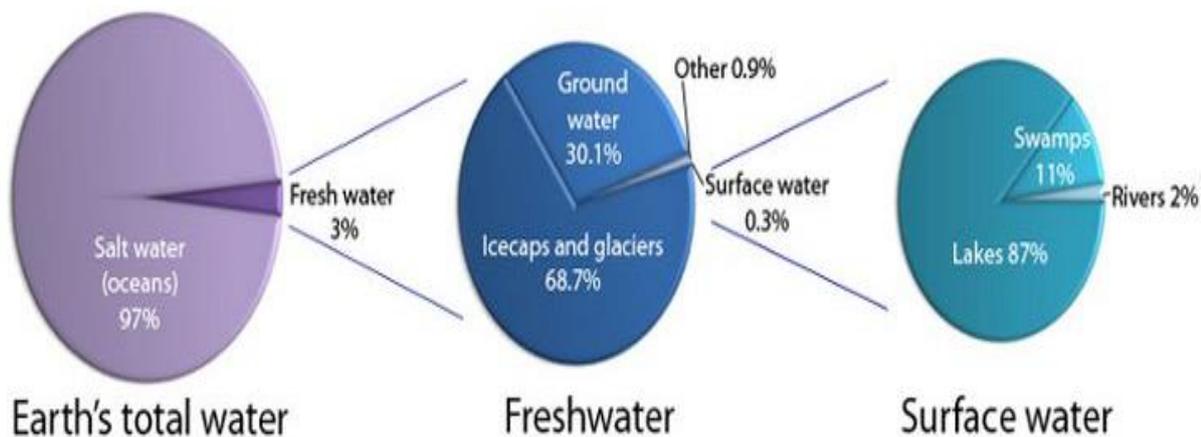
Approximately 70% of the earth is covered by water; this unique and versatile liquid is a basic necessity required daily to sustain the life of humans, animals and vegetation (Postel & Richter, 2012). Water has many applications in the household, agriculture and food and beverage industries. It is useful for human consumption, irrigation, food processing as well as non-alcoholic and alcoholic beverage production (Teklehaimanot *et al.*, 2014). To maintain 70% of water (by mass) in a human being, one to two litres of water needs to be consumed daily by an individual (Jequier & Constant, 2010). Water also directly affects the wellbeing of an environment as it supports various biochemical reactions that take place within the environment (Vasudevan & Oturan, 2014). Furthermore, the survival of animals and plants in an ecosystem is also sustained by water (Postel & Richter, 2012). However, due to the growing agriculture as a result of rapidly increasing population, inconsistent rainfall patterns, urbanisation and other factors, the available water has been inadequate to support the population and the ecosystems (Buhaug & Urdala, 2013). These problems have led to South Africa being classified as a water scarce country (Otieno & Ochieng, 2007; Hoekstra *et al.*, 2012).

2.1.2 WATER SCARCITY

Freshwater only covers 1% of the globe, as seen in Figure 2.1; this is a minute volume relative to the rest of the water in oceans and seas (Vasudevan & Oturan, 2014). The available limited freshwater is unevenly distributed among the continents and within provinces of a country and this is one of the reasons why this resource is scarce in South Africa (Otieno & Ochieng, 2007). Water scarcity refers to a situation when a large group of people lack adequate, safe freshwater to support their daily livelihood (Rijsberman, 2011). This is a typical concern for South Africa (SA) as 65% of the country is semi-arid (Otieno & Ochieng, 2007; Snyman, 2008). The available water resources are sparsely distributed as they do not match with the location of the demand (Vasudevan & Oturan, 2014). It is therefore challenging for the government to create sustainable water development and

management systems that can improve the accessibility of water to all the locations within the country (Snyman, 2008).

South Africa has a yearly average rainfall of 450 mm, which is far below the global average yearly rainfall of 860 mm (Anon, 2014). The rainfall received in SA is therefore inadequate to support the growing population (Snyman, 2008; Hoekstra *et al.*, 2012). Thus, factors influencing the problem of water scarcity should be assessed.



Earth's water distribution

Figure 0.1 Diagrammatic representation of the distribution of earth's water (USGS, 2016).

2.1.3 FACTORS AFFECTING WATER SCARCITY

As discussed by Hoekstra & Wiedmann (2014), freshwater is a finite renewable resource and its accessibility is limited across South Africa. Water scarcity is an acute growing problem that has been aggravated mainly by changes in climatic conditions (Baguma *et al.*, 2013). Water scarcity is identified by the reduced water tables, shrinkage of lakes, pollution of rivers and wetlands that continue to disappear (Baguma *et al.*, 2013). Several factors such as deforestation, excessive evaporation, rapid urbanisation and inconsistent water supply contribute to the problem of water scarcity (Postel & Richter, 2012).

Water scarcity is arising due to human induced living patterns, for instance the cutting down of forests and clearing off of vegetation (Bogino *et al.*, 2015). This is leading to an increase in carbon dioxide, methane and other pollutant gases, which are increasing global warming, and thus, resulting in inconsistent rainfall patterns (Gebrehiwot *et al.*, 2014). Furthermore, the land is being cleared off to expand mining, agricultural activities and urban residential areas as well as to remove invasive alien plant species which draw so much

water relative to indigenous species (Binns *et al.*, 2001; Van Luijk *et al.*, 2013). The Working for Water Programme (WWP) had a goal of removing invasive alien plant species to increase water security, however, it was observed that desertification started increasing in vast parts of the land (Binns *et al.*, 2001, Turpie *et al.*, 2008). Without afforestation or reforestation the problem of water scarcity continues to increase due to inadequate and unreliable rainfall (which is influenced by vegetative cover) thus, leading to drought which results in poor food security (Binns *et al.*, 2001; Bogino *et al.*, 2015; Araujo *et al.*, 2016).

Moreover, the population of the world is rapidly growing to an extent that the demand for freshwater is higher than the supply (Hoekstra *et al.*, 2011; Baguma *et al.*, 2013). Water scarcity has been worsened by suburbanisation, which, through current modernisation in Africa, has rapidly increased (Jacobsen *et al.*, 2013). People are migrating from underdeveloped rural areas to urban areas to search for employment (Van Leeuwen, 2015). The author further reported that 50% of people in developing countries live in cities and this will increase to 67% by 2050 (Van Leeuwen, 2015). It is therefore, not surprising that the pressure on the available water resources is escalating beyond the predicted expectations (Gleick, 2014). Furthermore, due to urbanisation there have been conflicts over water allocation to the household, industrial and agricultural sectors as all entities have been expanding (Baguma *et al.*, 2013; Van Leeuwen, 2015). As a consequence, the consistency of water supply services has decreased across the country, pushing the low-income people to secure water from boreholes and wells (Baguma *et al.*, 2013; Jacobsen *et al.*, 2013; Bagley *et al.*, 2014). Despite the fact that boreholes and wells are now the common water source in SA, these water resources are relatively unsafe since the maintenance of these infrastructures is insufficient (Gleick, 2014). It can therefore be assumed that in most cases the water is no longer safe for consumption.

Another factor affecting water scarcity is the unreliable, inadequate rainfall and geomorphology of a specific location (Gilliland *et al.*, 2016; Oueslati *et al.*, 2016). SA is a semi-arid country where 20% of the area receives below 200 mm of rainfall yearly (Van Luijk *et al.*, 2013). Due to minimal and unpredictable rainfall patterns experienced across the provinces, some communities are receiving excess water while the majority of the communities where industrial activities are, is receiving the least rainfall (Gilliland *et al.*, 2016). There is a high demand for freshwater in wine estates located in the Western Cape region which receives low rainfall (<400 mm of rainfall yearly) in the winter season (Van Luijk *et al.*, 2013; Chase *et al.*, 2015). This is regarded as too low to sustain the daily activities in the winery, hence, the reason why it generates highly polluted water, which cannot be reused for household chores (Araujo *et al.*, 2016). Contrary to this, the Gauteng Province

is located on the interior plateau of SA where summer rainfall (over 700 mm) is experienced (Chase *et al.*, 2015; Dyson, 2015). There are higher rates of evapotranspiration during summer season which results in a decrease in run-off levels and obtainability of surface water for human consumption and irrigation agriculture (Binns *et al.*, 2001). Moreover, during winter, in the mountainous areas, there are immense volumes of snowfall (Chase *et al.*, 2015). However, as spring season starts, this snow melts and flows into the nearest rivers (Dixon & Wilby, 2016). The recollection and redistribution of this water on the land increases the traction of mineral ions, nutrients and pollutants through evaporative water losses and contact with soils (Oueslati *et al.*, 2016). Thus, water scarcity is exacerbated by the high evaporation rates, reduced and unevenly distributed rainfall, high demand from a growing population and limited freshwater due to highly polluted water sources (Van Leeuwen, 2015).

Water scarcity is also worsened by the excessive contamination emerging and already existing in water resources in SA (Vasudevan & Oturan, 2014). Water pollution is mainly caused by the impact of overexploitation of surface and groundwater resources, industrial wastewater (WW) discharge, domestic sewage discharge and organic and inorganic ions from agricultural runoffs into freshwater sources (Jiang, 2009). The problem with water pollution is that the water has no economic value since it is not tradeable and cannot support agriculture (Jiang, 2009). More so, it has been reported that poor water quality is associated with high mortality rates as a result of cancers of the stomach, bladder, and liver as reported by Jiang, (2009). There is, then, a restricted amount of water available for use when water is contaminated and this worsens the problem of water scarcity (Dyson, 2015).

Several strategies have been drawn up by the SA government to minimise the problem of water scarcity. Programs focussing on the construction of more and larger man-made dams, river diversions and groundwater wells have been implemented (Liu *et al.*, 2013; Macian-Sorribes *et al.*, 2014). Large canals have also been built as they distribute and divert water between and within river basins (Macian-Sorribes *et al.*, 2014). However, in addition to constructing water sites it would be ideal to focus on involving and informing community members about the management of integrated water resources through forming some associations that can address the problems (Liu *et al.*, 2013; Macian-Sorribes *et al.*, 2014). More dams can be built but this has a downside because safe and affordable water is not accessible to a large part of the population, as discussed by Macian-Sorribes *et al.* (2014). Sustainable solutions to this problem would be to recycle, reuse, and conserve present water systems. The use of effective and cost efficient methods can be used to treat the water so that it can be reusable and consumable (Liu *et al.*, 2013). Some countries have

established water-pricing policies; this has helped making water users aware of their water usage (Jiang, *et al.*, 2009; Macian-Sorribes *et al.*, 2014). These policies could help in water saving and improve efficiency of water use (Jiang, 2009; Bagley *et al.*, 2014).

2.1.4 SOURCES OF POLLUTION

Water is polluted due to various domestic, industrial, agricultural and food and beverage industries (especially wine production), as pointed out by Peng *et al* (2013). Water authority boards have set strict regulations which industries are obliged to comply before they discharge wastewater, but often these standards are not met (DWA, 2013). Water is polluted as a result of a high load of organics, microbes and chemicals among other sources, as highlighted by Valipour *et al* (2012). Water pollutants can be classified as active water pollutants as seen in the Table 2.1 below (Gupta *et al.*, 2012).

Table 0.1 Active pollutants found in water (DeBlonde *et al.*, 2011; Das & Adholeya, 2015; Gavrilesco *et al.*, 2015).

Pollutant	Examples
Pathogens	Bacteria, viruses and protozoa
Inorganic pollutants	Acids, salts and toxic metals
Anions and cations	Nitrates, phosphate, sulphates, Ca^{2+} , Mg^{2+} and F^{-}
Toxic metals	Arsenic, iron, cadmium, nickel
Organic compounds	Oil and pesticides
Emerging pollutants	phthalates, pharmaceuticals compounds, polycyclic aromatic hydrocarbons, polychlorinated biphenyls , Bisphenol A

Liquid water disposal is often as a result of wastewater effluent being discharged by the municipality (Peng *et al.*, 2013). Pathogens, toxic metals and organic compounds are usually released in excessive amounts, rendering the water polluted (Harrison *et al.*, 2012). Liquid waste disposal is primarily from sewage sludge and wastewater sludge from home septic tanks as well as illegal dumping in water sources (Gupta *et al.*, 2012). Owing to the fewer and less frequently maintained septic tanks in rural areas, septic tanks are often overloaded with wastewater, which will result in surface water run-off and direct penetration of

wastewater into the ground (Ongley & Wang, 2004). In this manner phosphorus, nitrogen and pathogens from faecal matter are usually the components in the wastewater (Valipour *et al.*, 2012).

The literature on the effects of emerging pollutants that are produced from numerous anthropogenic sources is limited (Gavrilescu *et al.*, 2015). These limitations have made it challenging to understand the depth of how adverse these compounds can impact human health, the environment or the water itself in the short-term or long-term (DeBlonde *et al.*, 2011; Das & Adholeya, 2015). In addition to the emerging pollutants listed in Table 2.1, other contaminants found in wastewater or the environment are part of the drugs prescribed to people (Rosal *et al.*, 2010). These drugs include the antibiotics, analgesics, anti-convulsants, anti-cancer agents, beta-blockers, contrast agents, disinfectants, hormones and lipid-regulators (DeBlonde *et al.*, 2011; Gavrilescu *et al.*, 2015). The toxic dosage of these substances is still debatable since there is minimal information about their toxicity (Rosal *et al.*, 2010). Therefore, wastewater from pharmaceutical industries ought to be thoroughly depolluted to eradicate prospective toxicants (Gavrilescu *et al.*, 2015). Nevertheless, the efficacy of the current treatments is not well understood, as the treatment plants were initially developed with less intention of eradicating emerging pollutants like xenobiotics (DeBlonde *et al.*, 2011).

With the dramatically growing world population, agriculture has grown to assure food security (Zhang *et al.*, 2012). However, in the process of clearing land by deforestation and using natural and synthetic fertilisers, water pollution has risen to alarming levels (Jordan *et al.*, 2014). Deforestation (without afforestation or reforestation programs) due to agricultural activities is expanding the amount of land exposed (Zhang *et al.*, 2012; Jordan *et al.*, 2014). It is thus increasing the surface water run-off and soil erosion, consequently, increasing the level of turbidity and siltation at the bottom of rivers (Imeson, 2012; Jordan *et al.*, 2014). The cutting down of trees and agricultural tillage has encouraged high speed water run-off which carries sediments to rivers (Jordan *et al.*, 2014). Soil erosion is also being worsened by the removal of vegetation cover as roots aid in binding soil particles together (Imeson, 2012; Zhang *et al.*, 2012)

The frequent and excessive use of fertilisers in the form of pesticides or manure negatively impacts the environment and water (Schwarzenbach *et al.*, 2010). Highly mineralised pesticide can be discharged into water bodies, which communities use to access water for their daily needs (Zhang *et al.*, 2012). More so, with run-off water, especially containing nutrients such as nitrogen and phosphorus, can give rise to eutrophication which gives rise to rapid algae growth (Gilbert, 2013). This consequentially causes deoxygenation

and death of aquatic life, as highlighted by Jordan *et al.* (2014). Furthermore, the presence of excess inorganic ions stated in Table 2.1, often results in an off-taste and odour in public water sources (Gilbert, 2013). Additionally, the pesticides blown in the form of a dust contaminates surrounding water sources, ultimately increasing the water salinity of the water body (Schwarzenbach *et al.*, 2010). There can be run-off of manure over frozen ground during the winter season in the arctic regions, which eventually causes water pollution upon thawing (Azizullah *et al.*, 2011).

Other industries such as mining and food and beverage industries generate voluminous amounts of wastewater containing high amounts of mineral ions and organic ions, respectively (Hoekstra, 2015). Water run-off from mines, mine wastes, quarries and well sites containing physical sediments, acids, toxic metals, oils and organic substances are some of the substances that can cause water pollution, if they are not removed (Harrison *et al.*, 2012; Malm *et al.*, 2013). Failure to reduce these contaminants to the recommended levels will result in degradation of water quality rendering the water useless for reuse (Vasudevan & Oturan, 2014; Hoekstra, 2015) without further treatment.

Agriculture utilises approximately 60% of the freshwater available and food industries also use a significant amount of water during production and cleaning operations (Azizullah *et al.*, 2011; Ozturk, 2015). Due to these various processes in the industries large volumes of wastewater, with different chemical properties are generated. Wastewater from food industries is mainly generated from the cleaning, sanitising and cooling operations as well as steam generation (Hoekstra, 2015). Wastewater comprises of suspended solids, organic sugars and infrequently residual pesticides (Harrison *et al.*, 2012). The solid wastes are usually organic matter from mechanical preparation processes, such as rinds, seeds, and skins from raw materials (Conradie, 2014). Ineffectual physical or chemical treatment of the wastewater reduces the quality of water (Harrison *et al.*, 2012). Water pollution also arises as a result of accumulation of disinfectants, residual pesticides, organic and inorganic ions (Schwarzenbach *et al.*, 2010). Furthermore, the food and beverage industry incorporates the use of boiled water in processing (Herath *et al.*, 2013). Therefore, the slightest malfunctioning of heating equipment can cause water not to reach the temperature proposed to kill bacteria (Malm *et al.*, 2013). Consequently, the growth of pathogens is stimulated to an extent that water is microbially unsafe (Valipour *et al.*, 2012).

For the dairy industry, it is estimated that 0.2 to 10 L of wastewater is generated for every litre of milk processed (Qasim & Mane, 2013). The wastewater is high in organic matter and is slightly alkaline although it rapidly turns acidic due to fermentation of lactose to lactic acid. More so, dairy wastewater has a strong pungent odour because of the high

concentration of butyric acid (Qasim & Mane, 2013). On the other hand, the sugar confectionery industry produces heavily polluted acidic wastewater mainly comprising of high levels of carbohydrates, fats and oils (von Sperling, 2007). The sugar confectionery industry generates about 5 to 25 litres of wastewater for every kilogram of sweets produced (von Sperling, 2007). In the beverage industry, solid wastes such as spent grains and materials used in the fermentation process usually contaminate water (Harrison *et al.*, 2012). Although, the beverage industry generates relatively lower wastewater volumes, the fermentation processes cause the wastewater to be high in COD and biological oxygen demand (BOD) compared to food industries (Aulakh *et al.*, 2009). The COD represents the amount of organic matter in wastewater, but is determined by measuring the amount of oxygen required to chemically breakdown organic matter in wastewater (Gatti *et al.*, 2015). BOD is a measure of the amount of dissolved oxygen demanded by aerobes to degrade organic matter (von Sperling, 2007).

Wastewater generated from soft drink industries is characterised by high amounts of soluble sugar, high pH, high suspended solids as well as polyethylene glycol, a detergent used during washing and rinsing of bottles and equipment (Marsland & Whiteley, 2015). Approximately 2 to 5 L of wastewater is produced for every litre of soft drink produced (von Sperling, 2007). Alcoholic drinks, which include wine, are said to be one of the main contributors of water pollution (Herath *et al.*, 2013). These industries generate acidic wastewater with high organic matter and microorganisms (used during fermentation), as highlighted by Gatti *et al.* (2015). South Africa (SA) is in the southern hemisphere therefore, the harvest of grapes is usually done from late January to April (Gatti *et al.*, 2015). Due to the variability of chemical constituents of wine during the harvesting season and also the volume and type of wine being produced, more pollution is recorded during the harvesting season (Latif *et al.*, 2011). Furthermore, the degree of pollution and water-usage is dependent on the unique processes conducted at the specific winery estate (Arienzo *et al.*, 2009). Buelow *et al.* (2015) highlighted that the extent of pollution of the water also differs with the type of wine being produced (red or white wine). This is due to the different times in which a particular wine is produced, but more so because of exclusive wine production methods among the wineries (Gatti *et al.*, 2015).

2.2 WINERIES

2.2.1 BACKGROUND

The art of wine production initially started over six millenniums ago in the European and Mediterranean regions (Goosen, 2014; Duarte, 2015). It was developed to serve as a beverage drink during socio-religious commemorations and also because of the health benefits linked to it (Aleixandre *et al.*, 2016; Lamuela-Raventós & Estruch, 2016). South Africa is among the different regions where wine production is being practised, with the Western Cape Province being the top producer of wine in the country because of the semi-Mediterranean climate. Due to the expanding wine industry in the Western Cape and high wine exports, wine production is providing 8% employment and contributing considerably to the gross domestic product, respectively (Araujo *et al.*, 2016). Furthermore, with the growth of modernisation across the world, wine consumption has also expanded among different age groups (Duarte, 2015).

The art of wine making is known as viticulture and it involves making wine through fermentation of grapes (Goosen, 2014 & Parenti *et al.*, 2015). South Africa produces both red and white wine, however, white wine, particularly the Chenin Blanc is the widely produced wine relative to the red wine (SAWIS, 2014). The production flow of red and white wine is relatively similar, but particularly differs during the fermentation stage (Goosen, 2014). During production of red wine, the grape juice, grape skin and pieces of grape are used for fermentation, while during the production of white wine the grape skin is removed; only the grape juice is fermented to produce white wine (Swami *et al.*, 2014). So, due to this difference, the level of tannins (polyphenols) and sugar content in red and white wine differs, consequently causing differences in volumes and quality of wastewater generated (Mills *et al.*, 2008).

Wine production is generally classified into two stages i.e. the harvest and post-harvest period (Oliveira & Duarte, 2016). Solid and liquid wastes, which are winery wastewater constituents are generated during these seasons (Da Ros *et al.*, 2016). There is a larger and more concentrated volume of solid waste (SW) and wastewater during the harvesting stage comparative to the post-harvest stage (Oliveira & Duarte, 2016). The solid waste is produced during the destemming, pressing and settling stages of wine making (Mosse *et al.*, 2012). This waste comprises of the stems or stalks, grape marcs, which consists of pressed skins and seeds, lees containing dead yeast cells and sediments as well as filtration earths (Mosse *et al.*, 2012; Oliveira & Duarte, 2016). On the contrary, liquid wastes (LW) are predominantly generated from the initial stage, were grapes are received

and washed then during crushing of grapes, sedimentation and decanting of juice as well as during the filtration stage (Fig. 2.2) (Mosse *et al.*, 2012). Liquid wastes include fining agents, filtration earths, cleaning and disinfection products such as NaOH and KOH contribute to the pollution of winery wastewater (Mahajan *et al.*, 2010). Inattention to abide to the set standards for winery wastewater discharge (Table 2.2) could result in adverse responses in the surrounding environment, human health and aquaculture (DWA, 2013).

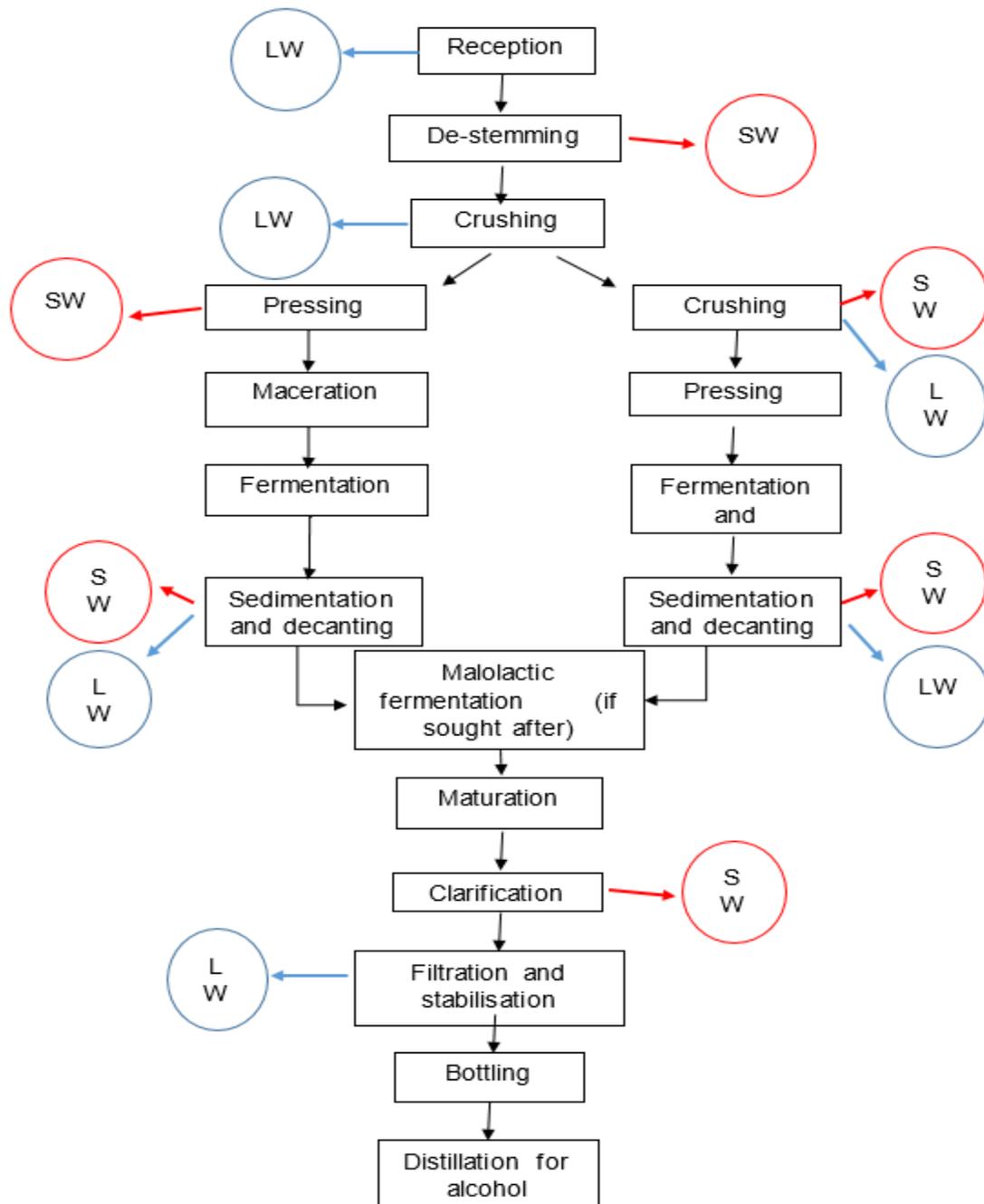


Figure 0.2 Diagrammatic representation of the different stages of wine making (Mills *et al.*, 2008; Swami *et al.*, 2014, De Kock, 2015).

Table 0.2 The legal stipulated parameters for reuse of treated wastewater in crop irrigation in different countries (Oliveira & Duarte, 2016).

parameter	European Union regulations / recommendations									
	California	EPA Guidelines	WHO Guidelines	Portugal	Greece	France	Spain	Germany	Turkey Cyprus	
pH	-	6.0–9.0	6.5–8.0	4.5-9.0	-	-	-	6.0-9.0	6.5- 8.5	-
BOD (mg.L ⁻¹)	-	30	<500	-	-	100-400	-	20	25- 50	10
TSS (mg.L ⁻¹)	-	30	<50	60	20-35	150-500	20-35	30	30	10
faecal coliforms (CFU.100 mL ⁻¹)	2.2-23	200	<1000	<100	100- 1000	<1000	200- 1000	100	2-20	<15
Helmith egg	<2	-	<1	<1	-	<1	<1	-	-	-

2.2.2 VOLUMES OF WINE PRODUCED

Wine production is one of the major agricultural activities done worldwide with France, Italy and Spain being the top producers (Goosen, 2014; Botha, 2015). South Africa is amongst the top ten wine producing countries (SAWIS, 2011). Locally, the production of wine has increased over the years, due to the large demand for wine by consumers. Wine consumption has increased from 346 million litres to 375 million litres between 2010 and 2014 (Esterhuizen, 2014). So, due to the increasing demand for wine, wine industries have also increased wine production from 831.2 to 915.5 million litres from 2011 to 2013 (SAWIS, 2014).

Presently, the area allocated to viticulture covers an area larger than 800 km² producing over 6 800 different wines (Goosen, 2014; SAWIS, 2014). Of the numerous wine varieties currently available, 54.6% and 45.4% are cultivated for the white and red wines, respectively (SAWIS, 2014). Chenin Blanc is the white variety extensively planted whilst Cabernet Sauvignon is the red wine variety mostly produced (Goosen, 2014; Weightman, 2014).

Due to the production of different wines, bulk volumes of wastewater with varying properties are generated during the different steps of wine making (Goosen, 2014). Valderrama *et al.* (2012), stated that 1 to 4 m³ of WWW is produced for every cubic metre of wine made, of which 60 to 70% of this volume is generated during the vintage period (Gupta *et al.*, 2012).

2.2.3 COMPOSITION OF WINERY WASTEWATER

WWW generated by each wine estate varies from the other due to the uniqueness in the processing techniques, region of farming and cultivar of grapes used. Table 2.3, displays the average values of water quality parameters measured from several wine making farms at different wine making stages (Melamane *et al.*, 2007; Bustamante *et al.*, 2011; Mosse *et al.*, 2011; Ioannou *et al.*, 2015).

Table 0.3 The average water quality parameter values measured during different phases of wine making process (Melamane *et al.*, 2007; Bustamante *et al.*, 2011; Mosse *et al.*, 2011, Ioannou *et al.*, 2015)

	Unit of measurement	Mean	Range
pH	-	5.5	3.6 – 11.8
COD	mgO ₂ .L ⁻¹	49 105	738 – 296 119
BOD	mgO ₂ .L ⁻¹	22 418	125 – 130 000
Polyphenols	mg.L ⁻¹	140	29 – 474
SS	mg.L ⁻¹	5 137	226 – 30,300
VS	mg.L ⁻¹	12 385	661 – 54 952
TS	mg.L ⁻¹	18 336	1,602 – 79,635
Density	g.cm ⁻³	1.010	1.002 – 1.054
Total nitrogen	mg.L ⁻¹	35.4	0.0 – 142.8
Oxidisable organic carbon	mg.L ⁻¹	2.16	0.11 – 9.18
Na	mg.L ⁻¹	158	7 – 470
K	mg.L ⁻¹	270	29 – 353
P	mg.L ⁻¹	35.4	3.3 – 188.3
Ca	mg.L ⁻¹	545	187 – 2,203
Mg	mg.L ⁻¹	36	16 – 87
Fe	mg.L ⁻¹	12	1 – 77
Mn	µg.L ⁻¹	310	< 200 – 1 740
Cu	µg.L ⁻¹	790	< 200 – 3 260
Zn	µg.L ⁻¹	580	90 – 1 400
Co	µg.L ⁻¹	170	110 – 300
Cr	µg.L ⁻¹	150	< 200 – 720
Pb	µg.L ⁻¹	1 090	550 – 1 340
Ni	µg.L ⁻¹	120	<200 – 650

Although the majority of the wine regions in South Africa continually attempt to improve and maintain effective irrigation agriculture by use of drip line irrigation the solution to improving water security is still unfulfilled (Garcia *et al.*, 2012; Romero *et al.*, 2014). Winery wastewater is acidic, contains a high organic load, sodium and sulphide and is of variable salinity (Table 2.3) (Mosse *et al.*, 2011). As seen in Table 2.4, the negative effects of these properties highlight the need for wine estates to comply with the stipulated treatment practices. Most (80 to 85%) of the wastes produced in a cellar are organic wastes produced from the grapes and wine (Ruggieri *et al.*, 2009; Valderrama *et al.*, 2012). The high COD is attributable to the grape skins getting in contact with wastewater systems, the residues on the floors of the cellar and during the pressing stage, which causes pH variations (Latif *et al.*, 2011; Fourie *et al.*, 2015).

Furthermore, the post-fermentation of grape juice and lees sediments at the bottom of the wine tank will influence the organic content of wastewater (Mosse *et al.*, 2011). Unavoidable chemical reactions result in the varied composition of the organic material in wastewater (Andreottola *et al.*, 2009; Mosse *et al.*, 2011). Alcohols, organic acids, polyphenols and esters are also constituents in winery wastewater (Mosse *et al.*, 2012). The organic composition of WWW is as follows: ethanol (80.3%), organic acids (9.4%), glucose and fructose (7.3%) and glycerol (3.1%), as said by Filladeau *et al.* (2008) and Conradie (2014).

In addition, inorganic ions are also constituents of winery wastewater, where over 50% of the ions are mainly from cleaning agents used in wineries with the exception of potassium, which is found in grape juice (Mosse *et al.*, 2011; Fourie *et al.*, 2015). As stated earlier, the differences in winemaking processes will result in different constituents and concentrations of pollutants found in wastewater (Botha, 2015; De Kock, 2015). Potassium (80 - 180 mg.L⁻¹), sodium (4 - 8 mg.L⁻¹), calcium (13 - 40 mg.L⁻¹) and magnesium (6 - 50 mg.L⁻¹) are some of the inorganic ions found in winery wastewater (EPA, 2004; Mosse *et al.*, 2012). The inappropriate discharge of WWW containing these ions gradually increases soil salinity and soil sodicity, which can result in dispersion (Bustamante *et al.*, 2011)

Table 0.4: Components found in wine effluent and their influence on the environment (EPA 2004; Winewatch, 2009)

Constituent	Indicators	Effects
Organic matter	Biochemical oxygen demand, total organic carbon and chemical oxygen demand.	Depletes oxygen when discharged into water leading to the death of fish and other aquatic organisms. Odours are generated by anaerobic decomposition of waste. Phenolic compounds may reduce light transmission in water.
Acidity	pH	Kills aquatic organisms at extreme values. Affects crop growth, microbial activity in biological wastewater treatment processes and heavy metal solubility.
Salinity	Electrical conductivity and total dissolved salts.	Imparts undesirable taste to water, is toxic to aquatic organisms and affects water uptake by crops.
Nutrients	Nitrogen, phosphorus, and potassium.	Can lead to eutrophication or algal blooms when discharged into water or stored in lagoons; algal blooms can cause undesirable odours. Toxic to crops in large doses. Nitrates and nitrites in drinking water can be toxic to infants.
Heavy metals	Cd, Cr, Co, Cu, Ni, Pb, Zn, Hg.	Toxic to plants and animals.

2.2.4 REASONS FOR MANAGING WINERY WASTEWATER

The quantity of contaminants found in winery wastewater (WWW) renders it unsuitable for direct discharge as it has detrimental effects on the soil and immediate environment (Da Ros *et al.*, 2015). Reasonable amounts of winery waste and wastewater can be beneficial to the soils as they enrich and fertilise the soils' organic material due to the soluble organic carbon in WWW (Bustamante *et al.*, 2011; Mulidzi *et al.*, 2015). However, constant contact with organic material can lead to organic overload that can reduce soil porosity because of the organic matter that blocks soil pores, thus lowering the quality of the soils immensely (Mosse *et al.*, 2012; Da Ros *et al.*, 2015). Another disadvantage of the organic matter in wine waste is that it increases soil salinity and soil sodicity, which can result in dispersion (Bustamante *et al.*, 2011).

Therefore, after scrutinising the volumes and composition of winery wastewater generated by winery estates, it is clear that winery wastewater management practices should be implemented and thoroughly monitored to achieve water sustainability (Mulidzi *et al.*, 2015). One of the practices that can aid in the management of WWW is including a separate tank to temporarily store the thick fermented lees which is characterised by a COD of 76 000 mg.L⁻¹ (Conradie, 2014). A separate tank is essential as it prevents contamination in the WWW treatment plant by any possible spillage of lees into the treatment system (Fillaudeau *et al.*, 2008; Conradie *et al.*, 2014).

In addition, there has been a continuous rise in wine production due to the increased demand and this has put a significant amount of pressure on industries and agriculture sectors to adopt the Reduce, Recycle and Reuse (RRR) management practice (EPA, 2004). This was notably proposed as one of the effective WWW management practises by Klemeš (2012) and Piadeh *et al.* (2014). As part of RRR, effective cleaning methods can be applied as a way to reduce the amount of water used since this stage generates the highest volume of WWW. It was reported that changing cleaning strategies requires minimal capital input to achieve 30% water volume reduction (Klemeš, 2012). Effective cleaning strategies would require auditing the amount of water used at each stage by installing water meters (Van Schoor, 2005). Furthermore, water can be saved by planning and monitoring water usage through implementing the use of nozzles on water pipes to prevent water wastage (Wineland, 2016). Sweeping floors with brooms and squeegees prior to washing floors will also promote water-saving, as some of the solid and fine organic matter are removed (Wineland, 2016). More so, the amount of organic matter in wastewater can be lowered, to reduce pollution (Dillion, 2011; Conradie *et al.*, 2014). This can be achieved through putting

mesh sieves on the floors in the crushing and pressing section to capture the skins, stalks and pips to prevent them from passing into wastewater systems (Dillion, 2011; Conradie *et al.*, 2014). In addition, implementing the use of in-line detectors for finer solids will reduce contamination, especially during the first stages of WWT treatment (Dillion, 2011). Furthermore, the winery staff should be made aware of water usage, water wastage and the importance of water-saving through regular training workshops (Van Schoor, 2005; Wineland, 2016). With these strategies it is possible for wineries to consider the reuse of water as well as recycling water post-treatment (The Water Wheel, 2008; Klemeš, 2012).

Recycling water refers to reusing depolluted wastewater for valuable activities such as agricultural irrigation and increasing water basin levels (Christen *et al.*, 2013). This practice improves water sustainability in addition to aiding in cost and water-saving (Piadeh, *et al.*, 2014). Water recycling can be designed to achieve water qualities desired for a particular use, such as irrigation (Christen *et al.*, 2013). However, the legal required water quality parameters for the reuse of treated wastewater in crop irrigation should comply with the set standards in Tables 2.2 and Table 2.5, depending on the location. Viticulture is continually expanding worldwide and with the demand for more water in this sector water recycling is the ideal solution to this problem as WWT requires considerably lower treatment if its end use is irrigation than consumption (Botha, 2015). However, due to the poor water quality of recycled water, it is not suited for consumption as it is not microbially safe (Mulidzi *et al.*, 2015; Wineland, 2016).

Developing and enforcing winery wastewater management practices will help reduce the water scarcity problem (Igbinosa & Okoh, 2009). Due to the fast growing population, the agriculture sector, which includes wine production, is expanding, as previously explained. More water is being used during the different stages of wine production, subsequently increasing the volumes of wastewater generated and thus, exacerbating the pressure needed to depollute water (Ioannou *et al.*, 2015). As a consequence of using large volumes of water to produce wine, the WWT generated is not being effectively depolluted, resulting in poor water quality (Mulidzi *et al.*, 2015). Therefore, it is important for effective and eco-friendly WWT management practices to be implemented to reduce water usage during wine production, which will also enable the easy recycling and reuse of water (Klemeš, 2012).

Table 0.5 South African Legislation standards set for irrigation-recycled wastewater. (DWA, 2013)

1

Parameter	< 50 m ³ .day ⁻¹	< 500 m ³ .day ⁻¹	< 2000 m ³ . ⁻¹
COD	5 000 mg.L ⁻¹	400 mg. L ⁻¹	75 mg. L ⁻¹
Faecal coliforms	1 000 000 per 100 ml	100 000 per 100 ml	1 000 per 100 ml
pH	6 - 9	6 – 9	5.5 – 9.5
EC ¹	200 mS.m ⁻²	200 mS.m ⁻²	70 – 150 mS.m ⁻²
SAR ²	< 5	< 5	Other criteria apply
Suspended solids	< 25 mg.L ⁻¹		
Nitrate/Nitrite as nitrogen	< 15 mg.L ⁻¹		
Ammonia as Nitrogen	< 3 mg.L ⁻¹		
Soap, oil or grease	< 2.5 mg.L ⁻¹		
Chlorine	< 0.25 mg.L ⁻¹		

¹Electrical conductivity (EC) is a measure of the amount of total dissolved salts in wastewater

² Sodium adsorption ratio (SAR) expresses the sodium hazard of irrigation water as a proportion of sodium to calcium and magnesium

Water legislative boards have stipulated that WWW ought to have pH and COD within 5.5 - 7.5 range and below 75 mg.L⁻¹, respectively (DWA, 2013) prior to discharge. Nevertheless, WWW is characterised by a pH of 3 - 4, 738 - 296 119 mg.L⁻¹ COD, contingent of the harvesting season and processing techniques involved (Malandra *et al.*, 2003). Failure to comply with these set standards results in wineries being given penalties and moreover, this has notable negative impacts on the environment (Malandra *et al.*, 2003; DWA, 2013). Due to discharge of high amounts of nitrogen in WWW, the process of eutrophication is stimulated in water resources e.g. rivers, wetlands and dams and this reduces the water quality (Ioannou *et al.*, 2015). As a result of the high BOD in WWW, upon discharge of untreated of WWW, as aquatic plants respire, the dissolved oxygen is rapidly depleted, thus, causing aquatic life to die due to insufficient oxygen levels (Mohana *et al.*, 2009). Furthermore, inappropriate discharge of WWW, can change the water quality parameters (pH, colour and EC) of groundwater (Kumar *et al.*, 2009). Mulidzi *et al.* (2015) explained that, the low pH of WWW could alter the microbial consortia in the soils and thus, reduce

plant growth. The high EC can disturb the water uptake in seeds and consequently, reduce the germination process (Mohana *et al.*, 2009). More so, the high organic content and presence of several mineral ions in WWW, further inhibits plant growth (Mulidzi *et al.*, 2015).

Regardless of the fact that the WWW has various ecological risks should it be discharged in the inappropriate way, several waste products can be recovered from WWW and used for other industrial purposes (Ioannou *et al.*, 2015). For instance, ethanol, malate, tartaric acid, flavanol, citrates and tannins can be recovered from grape marcs and lees (Oliveira & Duarte, 2016). Furthermore, polyphenols extracted from grape skin can be useful as food additives as they are dense in anti-inflammatory and antioxidant properties (Lamuela-Raventós & Estruch, 2016). In addition, upon distillation, ethanol and tartrates can be obtained from the grape marc (Yalcin *et al.*, 2008). Nutritional supplements such as flavanols can also be extracted (Oliveira & Duarte, 2016).

It is now clear that the treatment of WWW at wine estates is mandatory should it be reused or used for irrigation, in view of the negative effects associated with WWW on the environment (Fourie *et al.*, 2015). Use of efficient, eco-friendly and flexible (withstand WWW with different characteristics) treatment methods need to be investigated.

2.3 TREATMENT METHODS OF WINERY WASTEWATER

The volumes of winery wastewaters generated worldwide have been escalating and various treatment systems have been incorporated to depollute winery wastewater (Conradie *et al.*, 2014; Matheyarasu *et al.*, 2014). Winery wastewater is high in colour, has a low pH, high in COD and potentially has phenolic compounds that can hinder the performance of biological treatment systems (Kumar *et al.*, 2009; Arienzo *et al.*, 2012; Ioannou *et al.*, 2015). As such, depending on the end use of the treated water, the choice of treatment is highly dependent on a number of factors. These factors include the type of contaminants (suspended, colloidal or dissolved), biodegradability, toxicity of contaminants, desired effluent quality, costs of treatment as well as size and land availability (Kumar *et al.*, 2009; Matheyarasu *et al.*, 2014).

Treatment methods have been grouped into three major categories, namely: primary, secondary and tertiary treatment methods, however, these methods are further classified into physicochemical, biological and chemical treatment methods (Figure 2.3). The primary treatment of WW involves its collection and preparation by removing solids before biological treatment methods are utilised (Kumar *et al.*, 2009; Gupta *et al.*, 2012). During this treatment, there is the screening of large solid particles (>0.5 mm). Moreover, grape skins, pomace, seeds, wine lees, fats and oils are eliminated through flotation, sedimentation,

coagulation and filtration methods (Matheyarasu *et al.*, 2014). Approximately 25% of organics and nearly all non-organic solids are removed during this phase (Gupta *et al.*, 2012). The WWW pH is also adjusted by neutralisation methods, i.e. by adding sodium bicarbonate, sodium hydroxide, lime or potassium bicarbonate (Matheyarasu *et al.*, 2014).

According to Cai *et al.* (2013), secondary treatment methods are the subsequent depollution methods. Kumar *et al.* (2009), described the secondary treatment method as a phase that involves the catabolism of macromolecules (non-settleable solids) to simpler molecules (settleable-solids) through aerobic or anaerobic treatment methods to reduce BOD. Microbes and suspended solids are left to settle for a particular period prior to treatment (Kumar *et al.*, 2009). This treatment involves the partial treatment of the bacterial sludge to remove toxic mineral ions (Matheyarasu *et al.*, 2015). Some of the examples of secondary treatment methods include aerobic/anaerobic lagoons and bioreactors such as sequencing batch reactors and UASBs (Kumar *et al.*, 2009).

The final stage of treatment, i.e. tertiary treatment, involves eliminating the extra fine and colloidal solids through filtration and adsorption, respectively (Matheyarasu *et al.*, 2015). The tertiary treatment method mainly involves the disinfection of the WW after primary and secondary treatment to produce potable water (Gupta *et al.*, 2012). This is achieved using chlorination and ozonation methods (Kumar *et al.*, 2009; Gupta *et al.*, 2012). Moreover, macromolecules, nitrogen as well as phosphorus can be removed during this stage in order to control odours (Gupta *et al.*, 2012). However, the tertiary treatment methods for WWW include constructed wetlands and filtration. Constructed wetlands are dependent on sub-surface flows where WWW flows through gravel beds or the wastewater is exposed to air. In this manner, the COD, suspended solids and ions (e.g. nitrogen and phosphorus) are considerably reduced, thus making water easy to recycle for irrigation.

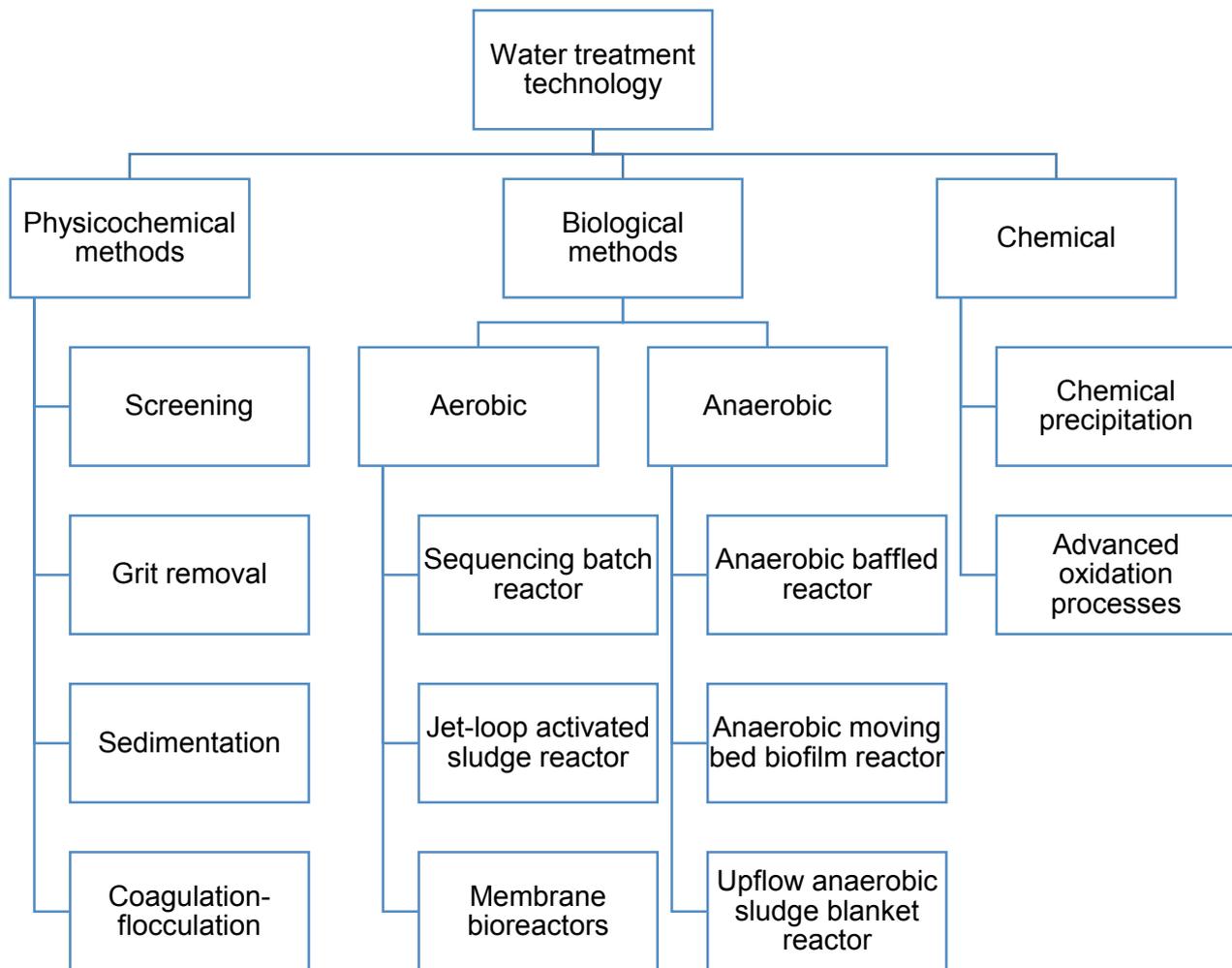


Figure 0.3 The different primary, secondary and tertiary treatment methods of wastewater classified under physicochemical, biological and chemical treatment methods (von Sperling, 2007).

2.3.1 PHYSICOCHEMICAL TREATMENT METHODS

These methods focus on the removal of heavy solid materials, suspended solids, fats and oils, heavy metals, inorganic ions (e.g. nitrogen and phosphorus) (von Sperling, 2007). Physicochemical treatment methods are particularly used as a pretreatment technique for wastewater treatment or for final treatment of wastewater prior to recycling or reuse (Ioannou *et al.*, 2015). There are several physicochemical treatment methods that can be applied to WWT, including screening, accelerated gravity separation, grit removal, sedimentation, coagulation-flocculation, dissolved air flotation and chemical precipitation treatment methods (Valderrama *et al.*, 2012).

Screening, accelerated gravity separation, grit removal

According to Gupta *et al.* (2012), screening is often the initial stage of WWW treatment, where a particular size of screen is used to eliminate large pieces of solids, such as corks, wood and fibres. There are two major types of screens, namely the coarse screen and fine screen. The coarse screen, e.g. bar racks, fixed and rotary screens, have holes which have a pore size of 0.6 mm (EPA, 2003, Ioannou *et al.*, 2013). They separate debris and other big solids from WWW. On the contrary, fine screens have pore sizes ranging between 1.5 to 6 mm and they are primarily designed to treat wastewater which has not gone through primary treatment (EPA, 2003). They eliminate the discrete particles that may cause operational problems such as pipe clogging (Ioannou *et al.*, 2013). In addition, there are finer screens, which are characterised by pore sizes ranging between 0.02 to 1.5 mm (EPA, 2003). The fine screens can also be used in place of the sedimentation process as they remove grits and algae which might cause blockages.

The subsequent stage is accelerated gravity separation, whereby organic particles such as stems, seeds and skins are removed by altering the gravitational field and centrifugal acceleration force (Gupta *et al.*, 2012). Centrifugation is also done to eliminate the settle-able solids, especially the stillage suspended solids from wine. Approximately 10 000 mg.L⁻¹ of suspended solids can be reduced to 1 000 mg.L⁻¹ during this process (Gupta *et al.*, 2012). The density of the solids and speed of the centrifuge influences the efficacy of the treatment method, denser suspended solids tend to be removed first (Ioannou *et al.*, 2013). Grit removal involves the removal of heavy solid particles such as sand, grit and gravel from winery waste using a de-gritting devices such as the mechanically cleaned channels (Andoh, 2015). The grit is removed by air being blown into the treatment system that creates a perpendicular motion, which causes the heavy solid materials to drop to the bottom of the treatment system, while the less dense materials remain suspended due to circulating air, until a point where they leave the treatment system (Andoh, 2015; De Kock, 2015). This treatment method is advantageous as the accumulation of grit in pipes, which consequently causes pipe clogging is prevented (Andoh, 2015).

Sedimentation

Sedimentation is another useful physical treatment method that focuses on using force of gravity to separate solids from winery wastewater, due to their different densities (Robertson, 2014). The water velocity is reduced to an extent where particles cannot stay in suspension, thus causing the solids to settle (Arvaniti *et al.*, 2014). This treatment system is used to settle unstable suspended solids such as grits, sand and biological flocs before the

filtration method (Arvaniti *et al.*, 2014). The process of sedimentation is dependent on the concentration (concentrated or dilute suspensions), characteristics (flocculating or discrete particles) of the suspended solids, temperature (low temperature, slow sedimentation) and presence of currents, as discussed by Arvaniti *et al.* (2014). Although, the sedimentation process is simple, affordable and reduces turbidity in WWT, it has a downside, in that, it is a lengthy operation that does not effectively remove all suspended solids (Mosse *et al.*, 2011).

Coagulation – flocculation

Coagulation–flocculation is a method employed by wineries and functions on the principle of destabilising colloidal particles by the addition of a coagulant e.g. chitosan, which in turn results in sedimentation (Oncel *et al.*, 2013). Following coagulation, is the flocculation of the unstable particles into bulky flocs, this is done to enlarge the particle size (Meraz *et al.*, 2016). The operation of this method is dependent on pH adjustment and supplementing with ferric or alum salts as the coagulant, to weaken the repulsive forces linking the particles (Liang *et al.*, 2014). Studies done by Rizzo *et al.* (2010) using chitosan as the coagulant, in the treatment of WWT, with an influent COD of 1 550 mg.L⁻¹ resulted in high reduction efficiencies of COD (73%), TSS (80%) and turbidity (92%). However, 20 mg.L⁻¹ was reported to be the optimal dose for the coagulant as no further changes were observed above this concentration, as there was clumping of particles (Rizzo *et al.*, 2010). In another study reported by Braz *et al.* (2010), the combination of coagulation and flocculation methods yielded results that are more positive. When WWT with an influent COD of 31 369 - 38 391 mg.L⁻¹ was treated, the removal of TSS and turbidity reached 95.4% and 92.6%, respectively, at a pH of 5, using 5% (w.v⁻¹) of Ca(OH)₂ and Al₂(SO₄)₃ coagulants (Braz *et al.*, 2010). The COD reduction was higher, at about 68%, and this was comparatively higher than the COD reduction recorded when the coagulant was just chitosan (Braz *et al.*, 2010).

Dissolved air flotation (DAF)

The dissolved air flotation is a process aimed at eliminating oils, fats and grease by blowing compressed air through the wastewater (Edzwald, 2010). A bubble attachment is used to separate solutes or dispersed liquids from a liquid phase (Kim *et al.*, 2015). Due to buoyancy, the bubble will move to the top, thus allowing the attached particles to move away from each other (Kim *et al.*, 2015). The foaming phase allows for the separation of floating substances from bulk water (Oncel *et al.*, 2013). The advantages of using this technique are that smaller particles that cannot be removed by other methods can easily be removed by flotation; it is

affordable and the treatment has a shorter hydraulic retention time (Edzwald, 2010). Although the application of the DAF is still in preliminary stages in winery wastewater treatment, when DAF was used to treat winery wastewater, 90 - 95% of TSS and 90 - 95% of BOD was removed (Chrobak & Ryder, 2005; Donaldson *et al.*, 2012). However, the application of DAF is more effective when used in combination with other treatment methods such as filtration or powder activated carbon (Edzwald, 2010; Kim *et al.*, 2015).

Chemical precipitation (CP)

Chemical precipitation is a treatment method advancing in the wine industry. It is a treatment method that involves using chelating agents such as trimercaptotriazine or lime to reduce the turbidity as well as inorganic concentration in WWW (Oncel *et al.*, 2013; Ioannou *et al.*, 2015). According to Ioannou *et al.* (2015), the WWW is adjusted to an alkali pH of 11, which allows the metal ions dissolved to be changed to the insoluble solid phase (hydroxide salt), which can easily be removed by filtration methods. Shih *et al.* (2013), reported that, the use of lime as a precipitant can reduce the levels of zinc, cadmium and manganese ions in WW to acceptable levels as recommended by Thai Pollution Control Department but not recommended by US EPA (Oncel *et al.*, 2013). It is therefore recommended to apply other treatment methods in combination with chemical precipitation for effectual removal of other inorganic ions. Moreover, when Andreottola *et al.* (2009), used chitosan as the coagulant, about 90% TSS, 76% Zn and 96% Cu were effectively reduced from WWW, although the COD reduction (9%) was relatively low. This was as a result of the high solubility of the WWW, for which CP is ineffective.

Table 2.6 displays the general advantages and disadvantages commonly found in the physicochemical treatment methods listed above. It should be noted that the lone use of physicochemical methods was deemed inadequate for the treatment of the winery wastewater as there are mainly focused on the removal of heavy metals and not organic matter which is the main component to be removed in WWW Kurniawan *et al.*, 2006; (Arvaniti *et al.*, 2014).

Table 0.6 Advantages and disadvantages of various physicochemical methods (Kurniawan *et al.*, 2006; Arvaniti *et al.*, 2014)

Type of treatment	Target of removal	Advantages	Disadvantages
Screening, accelerated gravity separation, grit removal	Suspended solids	All the systems are easy to operate and monitor, they can be used in small treatment systems with low-turbidity substrate.	The systems are labour intensive as most of the systems are manually operated.
Coagulation–flocculation	Heavy metals and suspended solids	Shorter time to settle out suspended solids Improved sludge settling	Sludge production Extra operational cost for sludge disposal
Dissolved air flotation	Heavy metals and suspended solids	Low cost Shorter hydraulic retention time	Subsequent treatments are required to improve the removal efficiency of heavy metal.
Sedimentation	Suspended solids	Simple, low cost, reduces the turbidity of water and can remove some pathogens.	Some smaller pathogens are not completely removed, post treatment is required since the process does not remove all the solid matter
Chemical precipitation	Heavy metals Divalent metals	Low capital cost Simple operation	Sludge generation Extra operational cost for sludge disposal

2.3.2 CHEMICAL TREATMENT METHODS

The application of chemical treatment methods on wastewater is mainly done to improve the quality of water, by removing heavy metals and hydrolysing bio-recalcitrant compounds (Gupta *et al.*, 2012). However, since WWW contains low levels of heavy metals, the widely used chemical treatment method for WWW is through the advanced oxidation process (Kurniawan *et al.*, 2006). This process involves the use of strong oxidising ions to break down complex macromolecules in wastewater and kill any pathogens present (Oller *et al.*, 2011).

Advanced oxidation processes (AOP)

Winery wastewater contains high amounts of organic matter as well as bio-recalcitrant compounds such as polyphenols. As a result, AOPs have been developed to degrade these high molecular weight recalcitrant compounds (polyphenols and tannins), alcohols and acids which cannot be eliminated by the lone application of biological treatment methods in wastewater, to simple biodegradable compounds (Oller *et al.*, 2011; Ioannou *et al.*, 2015). AOPs are based on the use of strong oxidising agents (Table 2.7) which enable the effective conversion of bio-recalcitrant and toxic compounds to biodegradable compounds.

Table 0.7 The relative oxidation power of various oxidants (Oller *et al.*, 2011)

Oxidising species	Relative oxidation power
Chlorine	1.00
Hypochlorous acid	1.10
Permanganate	1.24
Hydrogen peroxide	1.31
Ozone	1.52
Atomic oxygen	1.78
Hydroxyl radical	2.05
Positively charged hole on titanium dioxide, TiO^{2+}	2.35

The advanced oxidation processes involve the oxidising agents reacting with organic macromolecules to produce biodegradable intermediates (Lucas *et al.*, 2010).

Subsequently, through a process known as mineralisation, oxidising agents react with intermediary products to produce carbon dioxide, water and inorganic salts (Oller *et al.*, 2011). Some of the AOP methods include ozonation and ozonation in combination with UV-C radiation and/or peroxidation (Oller *et al.*, 2011; Ioannou *et al.*, 2015). These methods have been successful in the treatment and biodegradability enhancement of wastewater with high polyphenol content such as winery wastewater (Lucas *et al.*, 2010).

The polyphenolic content of WWW can be reduced by ozonation in the presence of titanium dioxide (Mosse *et al.*, 2011) and this process further reduces the COD of WWW when UV-A irradiation is combined with it due to the synergistic effects (Gimeno *et al.* 2007). The biodegradability of the organic molecules is associated with the reduction in phenols and the process is enhanced with a UV-ozone combination (Gimeno *et al.* 2007). In addition, when heterogeneous photo-Fenton oxidation was used to treat diluted red winery wastewater (influent COD of 3 300 to 5 530 mg.L⁻¹), 55% of the total organic carbon (TOC) was removed (Gimeno *et al.*, 2007). High ozone doses (above 0.7 g.L⁻¹) were required to treat pure winery wastewater (influent COD = 21 715 ± 1 236 mg.L⁻¹) to obtain a 37% COD reduction using ozonation (Gimeno *et al.*, 2007). Furthermore, increasing the temperature from 10 °C to 30 °C improved COD reduction from 12% to 20% (Souza *et al.*, 2013).

2.3.3 BIOLOGICAL TREATMENT METHODS

Several biological treatment technologies have been assayed to remove the macromolecules in WWW (Andreottola *et al.*, 2009; Oliveira & Duarte, 2011). These systems work on the principle of using aerobic or anaerobic bacteria to convert organic matter to simpler, biodegradable molecules (Mittal, 2011). Thus, biological treatment methods have been categorised into two main groups, that is, the aerobic and anaerobic treatment methods (Eckenfelder *et al.*, 2016) and the differences have been summarised in Table 2.8.

Table 0.8 The differences between aerobic and anaerobic biological treatment methods (adapted from Cakir & Stenstrom, 2005; Chernicharo, 2007; Mittal, 2011; Eckenfelder *et al.*, 2016)

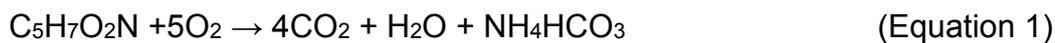
Parameter	Aerobic	Anaerobic
Process principle	Organic matter broken down by bacteria in the presence of oxygen.	Organic matter broken down in a chain reaction by four different physiological groups of bacteria.
By-products	Carbon dioxide (40 - 50%) Effluent water (5 - 10%) Biomass	Biogas i.e. methane and carbon dioxide (70 - 90%) Effluent water (10 - 30%) Biomass
Application	For low to medium strength wastewater and for wastewater that are difficult to biodegrade due to presence of bio-recalcitrants e.g. municipal sewage	For medium to high strength wastewater that easily biodegradable e.g. winery wastewater
Reaction kinetics	Fast	Slow
Nutrient requirements	High	Low
Start-up period	Short 2 – 4 weeks	Long 3 – 8 months
Process stability	Moderate – high	Low - medium
Odour	Low	High
Alkalinity requirements	Low	High

Net sludge yield	High 50 - 60% sludge production	Low (7 - 10%)
Post treatment	Possible for direct discharge or minimal treatment by filtration or disinfection.	Anaerobic digestion followed by aerobic treatment.
Foot print	Large	Small and compact
Degree of BOD removal	High	Low
Degree of Nitrogen removal	+	-
Degree of Phosphorus removal	+	-
Power input	-	+
Heat input	+	-
Examples	Sequencing batch reactor, Jet-loop activated sludge reactor, Membrane bioreactors, Biological sand filters.	Anaerobic baffled reactor, Anaerobic moving bed biofilm reactor, Upflow anaerobic sludge blanket.

+ Presence - Absence

2.3.3.1 AEROBIC MICROBIOLOGICAL TREATMENT PROCESSES

Aerobic treatment processes depend on the presence of oxygen to allow for the breakdown of organic matter (commonly measured as biochemical oxygen demand) by heterotrophic aerobes (Mosse *et al.*, 2011). As seen in Equation 1, organic matter is catabolised in the presence of oxygen to produce CO₂, water and nitrogenous compounds. Furthermore, during this process sludge and non-degradable materials such as polysaccharides, hemicellulose, and cellulose are produced (Water Environment Federation, 2007).

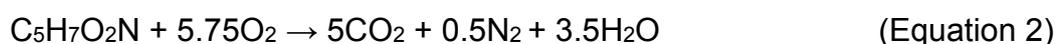


Breakdown of organic matter during aerobic digestion

Most aerobic treatment systems are based on oxygen being introduced into the system either through surface aerators or into diffused aeration systems (Eckenfelder *et al.*, 2016). For surface aerators, numerous evenly spaced units placed at the surface of the system are used, while for diffused aerators, oxygen is provided through multiple blowers or compressors and air is introduced into the treatment system via subsurface pipes, which ensure the uniform distribution of air in the treatment system (Eckenfelder *et al.*, 2016). A typical aerobic treatment system mainly consists of four units namely: the pretreatment tank, aeration chamber, settling chamber and dispersal component (Lesikar, 2008). The pretreatment tank eliminates the solid materials which microorganisms cannot break down (Lesikar, 2008). Then, the aeration chamber consists of air pumps that compress air, which then flows into diffusers that blow air through pipes into the water, thus enabling aerobes to degrade organic matter (Buchanan & Seabloom, 2004). The air forms bubbles which rise to the surface of the chamber allowing the mixing of wastewater. Then, it is the settling chamber, where the aerobic bacteria and biological solids settle at the bottom of the chamber due to gravitational forces (Lesikar, 2008). The settling chamber is also known as the clarifier due to the separation of the bacteria from the wastewater (Buchanan & Seabloom, 2004). Next is the dispersal component, where the treated wastewater is discharged either to the surrounding environment for reuse or discharged through a spray field that comprises of a disinfection system and distribution heads, for irrigation (Buchanan & Seabloom, 2004; Lesikar, 2008). The end-use of the water determines which application of discharge is used (Lesikar, 2008).

The microorganisms (aerobes) use carbon as an energy source and convert it to biomass and CO₂ using oxygen as an electron acceptor (Marin *et al.*, 2016). This process

is favourably effective but it leads to enormous amounts of excess sludge production, which will contain microbial biomass (Mosse *et al.*, 2011). This, therefore, requires regular maintenance of the system as it becomes time consuming and increases operational costs to dewater and discharge the sludge (Melamane *et al.*, 2007). Aerobic digestion generates high energy which allows for a wide range of metabolic processes (denitrification and nitrification) to occur (Daverey *et al.*, 2013). Therefore, aerobic microbial consortiums thrive under low energy levels, different environmental conditions and variable influent composition, which makes aerobic digestion an indispensable process for wastewater treatment (Sivanandan, 2011). However, under depleted substrate levels, the aerobes obtain energy from degrading their protoplasm, which subsequently lowers the concentration of the sludge's volatile solids by 40 – 50% (Sivanandan, 2011). Aerobic treatment methods also support nitrification processes, which convert ammonium ions to nitrite ions and consequently to nitrate ions (Daverey *et al.*, 2013; Marin *et al.*, 2016). Furthermore, as seen in Equation 2, in the absence of molecular oxygen, there is denitrification process where the nitrate is converted to nitrogen gas (Buchanan & Seabloom, 2004).



Nitrification and denitrification

The operation of aerobic treatment systems is simple as they attain an average of 90% COD reduction in pilot studies (Montalvo *et al.*, 2010). These methods have been implemented in wineries for the treatment of the wastewater due to the ease of use, ease of adaptability and high efficiency of the method (Alla, 2013). Some of the advantages of aerobic digestion are: 1) higher COD reduction than anaerobic treatment systems and thus they are more suitable for the final wastewater treatment, as a polishing step, 2) the sludge produced is an odourless, good fertiliser, 3) low costs of equipment, and 4) it is relatively easy to operate compared to anaerobic methods (Sably *et al.*, 2009). The disadvantages of aerobic treatment methods are that high power costs are required for the supply of oxygen, the rapid production of sludge which has poor dewatering properties and there is consistent decrease of alkalinity in the system, (Shammas & Wang, 2007). In addition, the aerobic treatment systems are prone to upsets under abrupt changes in organic loading rates and when under-maintained (Carvalho *et al.*, 2013). The aerobic treatment systems also produces wastewater with a high load of nitrates, which can pose a negative impact on

the environment (Carvalho *et al.*, 2013). Some of these aerobic methods include sequencing batch reactor, jet-loop activated sludge reactors, membrane bioreactors and biological sand filters (Ioannou *et al.*, 2015).

Sequencing batch reactor (SBR)

The sequencing batch reactor is a time-oriented, compact and effective variant of an activated sludge treatment system that was initially developed to depollute municipal and industrial wastewater with varying or low flow rates (Mahvi, 2008; Fernandes *et al.*, 2013). Wastewater is treated in one tank through four phases namely: fill, react, settle and decant. The series of the four phases make a complete cycle, which is repeated. The first phase is an aerated fill where wastewater is pumped in batches into a partly filled reactor system, where acclimatised microorganisms in the sludge are present. Subsequently, in the second phase, when the reactor is full, the aerobes react with wastewater to remove the carbonaceous BOD, COD, nitrogen and phosphorus (Flores-Tlacuahuac & Pedraza-Segura, 2016). Air is introduced into the system using mechanical devices or through diffused air. As air is added to the system, there is an acclimation of the microbes present, which utilise the organic matter as a source of energy (Fernandes *et al.*, 2013; Marin *et al.*, 2016). Simultaneously, ammonia is converted to oxidised nitrite and nitrate forms, in a process commonly known as nitrification (Marin *et al.*, 2016). Alum (aluminium sulphate) can be added to the wastewater to produce insoluble solids, this allows the removal of phosphorus based molecules (Alla, 2013).

The third phase is settling, where aeration and mixing of wastewater and biomass stops to allow sedimentation of the bio-solids and sludge (Marin *et al.*, 2016). Clear water moves to the top of the tank as the sludge settles (Flores-Tlacuahuac & Pedraza-Segura, 2016). However, the growth of aerobes continues until a point when all the oxygen is completely consumed (Alla, 2013). Upon depletion of oxygen, there is denitrification, where nitrogen is used as the terminal oxygen acceptor (Fernandes *et al.*, 2013). The nitrogen is converted to a gaseous nitrogen oxide or molecular nitrogen (Marin *et al.*, 2016). There is gradual accumulation of the waste sludge as some microorganisms simultaneously multiply and die (Marin *et al.*, 2016). The fourth phase is decanting, where the clarified effluent is discharged to a lagoon where it is temporarily stored before being discharged to water bodies or wetlands (Alla, 2013).

The SBR has mostly been used to treat other wastewaters, e.g. textile, dairy and slaughterhouse wastewater where it achieves a COD reduction and NH₃ removal of 90 -

95% and 80 – 90%, respectively (Rada *et al.*, 2013; Guieysse & Norvill, 2014). However, the use of the SBR is expanding in the wine industry due to its efficiency. SBR can achieve COD reduction of 95% given an influent with COD of 5 200 - 17 900 mg.L⁻¹ in winery wastewater (Oliveira & Duarte, 2011; Ioannou *et al.*, 2015). The treatment system can also achieve 97.5% reduction of BOD (Oliveira & Duarte, 2011). In addition, 86 – 99% COD reduction was achieved when winery wastewater with an organic loading rate of 6.3 kgCODm⁻³.d⁻¹ and high total suspended solids concentration (4-5 kgTSS.m⁻³) was treated (Torrijos & Moletta, 1997).

Jet-loop activated sludge reactor (JLR)

The jet-loop activated sludge reactor works on the principle of forcing wastewater with a high flow rate through a nozzle, thus, creating a high mixing and turbulence to achieve the necessary mass transfer between air and wastewater, which allows the breakdown of organic matter (Ioannou *et al.*, 2015). The JLR is capable of treating wastewater with high organic loading rates as a result of the high efficiency of oxygen transfer, turbulence and thus, high mixing achieved (Patil & Usmani, 2014). JLRs are normally associated with treating low winery wastewater volumes, which means, minimal area for workspace is needed (Kurniawan *et al.*, 2006). There are low initial costs of installation and maintenance and minimal energy is consumed (Ioannou *et al.*, 2015).

The JLR is used in several applications to treat various wastewaters. When the JLR was used to treat cheese whey wastewater with an organic loading rate of 22.2 kgCOD.m⁻³.d⁻¹, a COD reduction of 82% was achieved (Farizoglu *et al.*, 2004). Furthermore, when this treatment system was used to depollute urban wastewater with an OLR of 6 - 13 kgCOD.m⁻³.d⁻¹, a COD reduction of 95% was achieved (Holler & Trösch, 2001). As reported by Petruccioli *et al.* (2000) and Eusébio *et al.* (2000), the jet-loop activated sludge reactors can achieve the reduction of 80 - 90% organic matter given that the influent COD is 800 – 27 200 mg.L⁻¹ in winery wastewater. More so, 85% total phosphorus and 75% total phenolic compounds (TPh) can be successfully removed from winery wastewater (Ioannou *et al.*, 2015). It is possible that, high TPh removal efficiency is as a result of a syntrophic relationship between microbes (e.g. *Pseudomonas* species) that degrade phenolic-based molecules (Ioannou *et al.*, 2015). In addition, the presence of optimal oxidising conditions generated by the vigorous turbulence and aeration promotes the treatment process (Robinson *et al.*, 2001). The major drawback found when using JLR is that there is a need to improve sludge settling properties (Kurniawan *et al.*, 2006). The JLR can also achieve a

high COD reduction of 90% when treating winery wastewater, as highlighted by Oliviera & Duarte (2011).

Membrane bioreactors (MBR)

The membrane bioreactor is a pressure-driven treatment method, based on the use of ultrafiltration and microfiltration principles (Rodriguez-Caballero *et al.*, 2012). MBR consists of a suspended growth activated sludge system that makes use of microporous membranes for solid and liquid separation, by creating a negative pressure on the permeate (Valderrama *et al.*, 2012). Thus, wastewater flows through a membrane, under pressure, to separate dissolved solids and biomass from the liquid waste (Rodriguez-Caballero *et al.*, 2012). The combination of ultrafiltration and microfiltration membranes (microporous membranes) develops a barrier to particular chlorine resistant microbes such as *Cryptosporidium* and *Giardia* (Rodriguez-Caballero *et al.*, 2012). Ultrafiltration membranes can remove 0.01 to 0.1 μm molecules such as large organics, while microfiltration membranes remove $> 0.1 \mu\text{m}$ macromolecules such as suspended solids and microbes from wastewater (Kader, 2007). Furthermore, MBR consists of a unit where the organic matter is broken down by bacteria (Valderrama *et al.*, 2012). The advantages associated with the membrane bioreactors are: high process stability, shorter HRT, lower sludge production and minimal risks of process upsets relative to conventional suspended systems like SBR (Rodriguez-Caballero *et al.*, 2012). In spite of this, MBRs are highly costly because of the membrane modules. There is limited information on the membrane shelf life, therefore, membranes are constantly replaced which makes the treatment relatively costly (Ramond *et al.*, 2013). The membrane scouring is energy consuming relative to conventional processes (Ioannou *et al.*, 2015). Lastly, dewatering of waste sludge from the membrane process could be challenging to overcome (Ioannou *et al.*, 2015).

When MBRs were used to treat WWW, a 97% COD reduction was achieved when the influent COD ranged from 1 000 to 13 448 $\text{mg}\cdot\text{L}^{-1}$ (Valderrama *et al.*, 2012; Ioannou *et al.*, 2015). However, the build-up of solutes in terms of volatile suspended solids (VSS) in the reactor, can promote a decrease in the oxygen potential of the system during the process (Ioannou *et al.*, 2015). In addition, the MBRs are capable of treating winery wastewater of varying organic loading rates, 0.2 – 19.7 $\text{kgCOD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$, achieving a COD reduction of over 90% (Melamane, 2007). When the MBR was used to depollute WWW in the studies done by Oliviera and Duarte (2011), a 97% COD reduction was achieved. Similarly to the previous study, when winery wastewater was treated by this system a COD reduction of 97% was

achieved at an organic loading rate of $3.4 \text{ kgCOD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ (Basset *et al.*, 2016). At this organic loading rate, 85 – 88% of methane was produced by the reactors (Basset *et al.*, 2016).

Biological sand filters (BSF)

The biological sand filter treatment system works on the principle of allowing wastewater to flow through a biosand filter periodically or in batches (Ioannou *et al.*, 2015). The biosand filter is created from the biological layer of microbes, sediment and slime that forms on the surface of the sand. Subsequently, the organic matter attaches to the sand substratum due to static charges, which provides a large surface area for biofilm attachment and may contain co-factors required for microbial metabolism (Oller *et al.*, 2011). Therefore, as wastewater flows through a BSF, bio-solids and organic matter are removed through biological (aerobic digestion) and physical processes (filtration) that happen on the biological layer. This process does not need any power as the flow of WWT is facilitated by gravity (Robinson *et al.*, 2001). The aerobic bacteria consume the organic matter on the surface of the sand, thus, reducing the organic load in WWT (Robinson *et al.*, 2001). Air is supplied to the biological layer by the dissolved oxygen from the wastewater as it passes through the filter or through diffusion from the air when wastewater is not flowing through the biological layer (Ramond *et al.*, 2013). The treated water then exits the system via an upright pipe into a storage container (Ramond *et al.*, 2013). BSF was initially developed for household use, however, due to its effectiveness in removing suspended solids, nitrogen and phosphorus and reducing COD, its use has been extended to the treatment of WWT (Rodriguez-Caballero *et al.*, 2012). When the BSF was operated for 112 days, to treat WWT with an influent COD of $2\,304 \text{ mg}\cdot\text{L}^{-1}$, the COD reduction and total phenols removal was over 98% (Ioannou *et al.*, 2015).

2.3.3.2 ANAEROBIC TREATMENT METHODS

Anaerobic digestion (AD) is a biological treatment method where organic molecules are catabolised in multi-stages to biogas and biomass, in the absence of oxygen, in a closed system to capture methane produced (Van Lier *et al.*, 2015). Anaerobic treatment systems have been widely implemented to treat low to high strength wastewater (Couras *et al.*, 2015). The main microorganisms in the biomass are the methanogens and acid forming bacteria (Ariunbaatar *et al.*, 2014). The acid forming bacteria are bacteria that produce organic acids from the simple macromolecules while methanogens produce methane from organic acids

(Sankarana *et al.*, 2014). The main advantages associated with these systems in comparison to aerobic treatment systems is their simplicity and capability to depollute heavily contaminated WWT at high organic loading rates (Ariunbaatar *et al.*, 2014; Da Ros *et al.*, 2014). Since the system does not require oxygen, the energy demand is lower relative to aerobic treatment methods, which require power input to pump air into aerobic treatment systems (Sankarana *et al.*, 2014). In addition, over 50% of the COD can be converted to biogas, which is used as a source of fuel (Ariunbaatar *et al.*, 2014). The biogas contains 55 - 75% of methane and 25 - 45% CO₂ (Vindis *et al.*, 2009). Methane is an ignitable gas, which upon combustion releases energy (Ariunbaatar *et al.*, 2014). Furthermore, depending on the anaerobic treatment method, for every kilogram of COD removed, nearly 1 kWh is saved due to the production of methane (Van Lier *et al.*, 2015). The breakdown of 1 kg of COD using anaerobic treatment methods yields 13.5 MJ of methane, which is equal to 1.5 kWh electricity (Van Lier *et al.*, 2015). Another advantage of the system is the minimal production of excess sludge, which reduces the costs involved in handling, stabilisation and discarding of the waste sludge (Mosse *et al.*, 2011). More so, there is minimal production of malodorous gases, minimal land requirements and slow build-up of biomass within the digester (Sankarana *et al.*, 2014).

However, the use of anaerobic treatment systems is associated with several disadvantages. The treatment systems seldom act in accordance to the discharge standards set by environmental associations (DWA, 2013). Anaerobic treatment systems produce water with high total suspended solids and mineralised ions such as nitrates and phosphates (Mosse *et al.*, 2011). Therefore, further post-treatment of the effluent using aerobic treatment systems is recommended to remove residual contaminants (Sankarana *et al.*, 2014). Furthermore, high strength wastewaters e.g. WWT contain bio-recalcitrants such as polyphenols, which can be lethal to microbial growth thus, preventing the process of anaerobic digestion (Kleerebezem *et al.*, 2015). Another disadvantage is that, due to the seasonal variability of wine production, the diversity of the microbial groups changes, which consequently has a negative impact on AD as, long incubation periods for acclimatisation will be required during the start-up phase by microbes (Pohl *et al.*, 2013; Zhang *et al.*, 2014). In addition, anaerobic treatment methods are associated with the slow growth rate of methane producing bacteria and the loss of biomass in high hydraulic rate systems that reduces reactor performance (Zhang *et al.*, 2014).

The two major anaerobic treatment systems are the batch and continuous reactors, where in a batch system the granular sludge is inoculated into a reactor, which is completely

sealed throughout the treatment process (Li *et al.*, 2011). Wastewater is fed into the reactor intermittently (Khalid *et al.*, 2011). The operation of the batch system is relatively simple and less costly relative to the continuous system (Khalid *et al.*, 2011; Nasir *et al.*, 2012). However, the main disadvantage of this process is the production of malodorous gases (Nasir *et al.*, 2012). The continuous systems have granular sludge already inoculated into the anaerobic reactor and wastewater, which is the substrate, is constantly fed to the treatment system (Khalid *et al.*, 2011). Therefore, due to the continuous feeding of the reactor, the by-products, i.e. biogas are removed constantly to be used as a source of energy (Li *et al.*, 2011; Nasir *et al.*, 2012). Some of the common anaerobic treatment systems implemented by wine industries include the anaerobic baffled reactor, anaerobic moving bed biofilm reactor and upflow anaerobic sludge blanket reactor (Latif *et al.*, 2011; Chai *et al.*, 2014; Hahn *et al.*, 2015).

Anaerobic baffled reactor (ABR)

According to Hahn *et al.* (2015), ABR is one of the high rate anaerobic reactors used to treat wastewater. The treatment system consists of a series of compartments or tanks with standing and hanging baffles, under which, winery wastewater is forced to flow (Jianzheng *et al.*, 2013). Organic matter is broken down by microorganisms through anaerobic digestion. Immobile solids which are part of the influent amass in the first tank and subsequently sink to the bottom, while, solutes with a lower density than water rise to the top due to buoyancy and form the scum (Hahn *et al.*, 2015). The treatment is more successful when the solutes have the same density as water, as they remain suspended in the reactor and flow out as part of the effluent (Jianzheng *et al.*, 2013). More so, the treatment is catalysed by increasing the flow rate of the wastewater with the sludge (Zwain *et al.*, 2013). The advantages of ABR are: lower risk to organic and hydraulic shock loadings and improved biomass retention times (Jianzheng *et al.*, 2013). In addition, it is possible to separate the different stages of AD because of the presence of different tanks (Hahn *et al.*, 2015). Some of the disadvantages of using the ABR include: the system is prone to sludge washout and the need for longer time to get to the maximal treatment capacity because of the slow growth rate of anaerobes in the sludge that first have to develop in the reactor (Thanwised *et al.*, 2012; Hahn *et al.*, 2015). The ABR is currently being used to treat WWW. When winery wastewater with an influent COD of 20 000 mg.L⁻¹ was treated by this anaerobic digester, the COD reduction was 85% (Ioannou *et al.*, 2015).

Anaerobic moving bed biofilm reactor (AMBBR)

AMBBR is one of the effectual and successful anaerobic treatment methods that has been used to depollute low and high flows of high strength wastewater such as winery wastewater (Ioannou *et al.*, 2015). The AMBBR is a continuous process that works on the principles of two technologies i.e. of the conventional activated sludge and bio-trickling filters (Chai *et al.*, 2014). Wastewater flows into a reactor inoculated with biomass of suspended flocs and biofilm colonised carriers allowing the breakdown of organic matter through anaerobic digestion (Chai *et al.*, 2014). The biomass is kept moving using biogas recirculation or a mechanic stirrer (Chai *et al.*, 2014). Low density and high surface area properties typify the ideal biofilm carrier particles to be used in the reactor (Ioannou *et al.*, 2015). More so, these carriers are kept from being washed out of the reactor by means of a rectangular or cylindrical sieve (Sheli & Moletta, 2007). The advantages of the AMBBR are, its resistance to organic overloads, better sludge settling characteristics, low risk of high biomass production and bulking (Sheli & Moletta, 2007). In addition, it is a simple and small sized treatment plant, with a high treatment efficiency and it also can be inoculated with high volumes of biomass (Chai *et al.*, 2014). When the AMBBR was used to treat WWW with an influent COD of 15 140 - 44 180 mg.L⁻¹, the COD reduction was 89.2% (Sheli & Moletta, 2007). Moreover, under aforementioned conditions, the system produced methane rich biogas (CH₄% 45.5 - 82.2%).

Upflow anaerobic sludge blanket (UASB)

The technology of UASB was introduced during the 1970's by Lettinga and Van Velsen at the Wageningen University in the Netherlands and has since been the widely used biological treatment method for low to high strength wastewaters (Abbasi *et al.*, 2012). UASB can depollute wastewater with a high organic loading rate, 10 to 15 kg.m³.d⁻¹ (Lim, 2011). The system is based on the formation of granular sludge with good settling properties that can effectively retain intricate microbial groups, without attachment media to facilitate immobilisation (Latif *et al.*, 2011; Lim, 2011). At start-up, the UASB is inoculated with 10 to 30% by volume of anaerobic granules (Abbasi *et al.*, 2012). Higher volumes of anaerobic sludge will result in higher volumes of wastewater being treated (Lamprecht, 2009). The anaerobic granules are in the form of spherical aggregates of diverse microbial groups and their diameter ranges between 0.14 to 5 mm (Lamprecht, 2009; Abbasi *et al.*, 2012). Wastewater enters the reactor from the bottom and flows upwards through a dense bed of anaerobic sludge where the microorganisms in the sludge come into contact with the

wastewater substrate (Robertson, 2014). The soluble organic material in the substrate is broken down by microorganisms to produce biogas, which subsequently exits the reactor and is often used to provide energy (Rosa *et al.*, 2015). The natural turbulence caused by the WWF flow rate and biogas production provides good wastewater to biomass contact in a UASB reactor (Latif *et al.*, 2011).

Advantages

- The UASB is a simple treatment system which does not need any high-tech machinery (Kongjan *et al.*, 2011);
- The UASB is a high rate treatment system characterised by a high COD reduction (90%) (Ioannou *et al.*, 2015);
- The system is independent of mechanical agitation to mix the biomass and WWF, as production of biogas promotes mixing of reactor contents (Robertson, 2014; Ioannou *et al.*, 2015; Rosa *et al.*, 2015);
- The UASB can retain high biomass concentrations due to the excellent settling properties of the sludge (Chen *et al.*, 2014);
- The UASB's have a high loading capacity and can withstand high organic shock loads (Latif *et al.*, 2011; Chen *et al.*, 2016);
- The UASB aids in decolourisation and reducing polyphenol content (67% TPh reduction), as observed by Ioannou *et al.* (2015);
- There is low production of bio-solids waste (7-10%) relative to aerobic treatment methods because of maximal conversion of organic matter to biogas, which results in low energy for new cell growth (Latif *et al.*, 2011; Chen *et al.*, 2016);
- The UASB is a sustainable traditional treatment method, it has low operational costs as it produces the highest volume of methane relative to the other anaerobic treatment methods, methane can be easily recovered and used as a source of thermal and electrical energy (Robertson, 2014);
- Minimal working space area is required (Latif *et al.*, 2011);
- Minimal energy required for the operation due to the production of biogas, which can be used as an energy source (Chen *et al.*, 2016);
- UASB's remain stable under varying environmental conditions such as pH and hydraulic retention time (Kongjan *et al.*, 2011).

Disadvantages of UASBs

- The start-up period is long taking about 3 – 8 months to acclimatise the granules (Rosa *et al.*, 2015);
- There is a high risk of flotation when granules start disintegrating causing build-up of sludge. This, further leads to biomass washout. (Kongjan *et al.*, 2011);
- There is poor reactor performance under low temperatures due to the process of hydrolysis being inhibited due to slow reaction kinetics. The optimal temperature for the UASBs is the mesophilic temperatures of 30 – 40 °C (Kongjan *et al.*, 2011; Rizvi *et al.*, 2015);
- The operation needs personnel with expertise of the system (Hamza *et al.*, 2016);
- High levels of TSS gradually accumulate forcing the less dense active sludge to be regularly removed (Rosa *et al.*, 2015). Consequently, the solid retention time starts dropping, thus, reducing the activity of methanogens, further leading to low COD reduction and reactor performance (Mosse *et al.*, 2012);
- The effluent produced does not comply with the stipulated discharge standards, as the BOD, NH_4^+ and S^- are higher than the dosages recommended for discharge, post-treatments are therefore recommended (Rizvi *et al.*, 2015);
- UASB's are unstable at high upflow velocities (above $1.5 \text{ m}\cdot\text{h}^{-1}$). High upflow velocities lead to biomass washout (Abbasi & Abbasi, 2012; Guerrero *et al.*, 2013);
- Probable emissions of odorous gases e.g. hydrogen sulphide as well as ammonia under wrong design and operation of the UASB (Mosse *et al.*, 2012; Makadia *et al.*, 2016).

When the UASB was used to depollute winery distillery wastewater with an influent COD of 20 000 – 30 000 $\text{mg}\cdot\text{L}^{-1}$, 90% COD reduction was attained (Wolmarans & De Villiers, 2002). Furthermore when Kalyuzhnyi *et al.* (2001), treated WWW with an influent COD of 90 000 – 196 000 $\text{mg}\cdot\text{L}^{-1}$, a 70% COD reduction and decolourisation of the WWW was achieved. More so, approximately 70% of the total phenols were removed. In addition, a COD reduction over 90% was achieved when WWW was treated by the UASB reactor (Pant & Adholeya, 2007; Latif *et al.*, 2011; Mosse *et al.*, 2012), corroborating how effective this treatment system in depolluting WWW. The COD reduction ranged between 88 – 92% when winery wastewater with an OLR of $3.7 \text{ kgCOD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ was treated by the UASB reactors (Sigge, 2005). Furthermore, Van Der Weisthuizen (2014) reported similar results of high COD reduction (85%) when the UASB reactor was used to depollute grain distillery

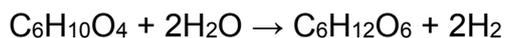
wastewater with an organic loading rate of $5.5 \text{ kgCOD.m}^{-3}.\text{d}^{-1}$. According to studies done by Kalyuzhnyi *et al.* 2001, the COD reduction was up to 70% when this digester was used to treat winery wastewater with an influent COD of $90\,000 - 196\,000 \text{ mg.L}^{-1}$. Moreover, 67% of the total phenolics were successfully removed from this wastewater (Kalyuzhnyi *et al.*, 2001). The UASB reactor was also effective in treating winery wastewater with an influent COD of $20\,000 - 30\,000 \text{ mg.L}^{-1}$, achieving a 90% COD reduction (Wolmarans & De Villiers, 2002). In addition, when the UASB was used to treat WWW in Sekerdağ, with an OLR of $2.5 - 8.5 \text{ kgCOD.m}^{-3}.\text{d}^{-1}$, the COD reduction ranged between 60 – 80% (Pant & Aholeya, 2007). More so, Akarsubasi *et al.* 2006, reported a COD reduction of over 90% when WWW with an OLR of $6 - 11 \text{ kgCOD.m}^{-3}.\text{d}^{-1}$ was treated by a UASB.

2.4 MICROBIOLOGY OF ANAEROBIC DIGESTION

Niu *et al.* (2015) stated that anaerobic digestion is a multi-stage metabolic process in which large organic molecules sequentially go through hydrolysis, acidogenesis, acetogenesis, and methanogenesis in the absence of oxygen (Fig. 2.4). These biological processes are facilitated by various microbial species, which are generally categorised into acidogenic bacteria and methanogenic archaea (Makadia *et al.*, 2016). These bacteria exist in a mixed anaerobic consortium but they vary in their pH and nutritional requirements, tolerance to varying environmental fluctuations and growth kinetics (Niu *et al.*, 2015). Anaerobes break down the waste organic material in water, into biogas and a liquefied effluent during biogas production (Keating, 2015)

Hydrolysis

During the first stage, which is, the liquefaction stage (hydrolysis stage), bacteria convert insoluble, complex materials e.g. carbohydrates, fats and proteins into soluble molecules permeable to the cell membranes of acidogens (Equation 3) (Li *et al.*, 2011). Complex molecules are broken down through a series of steps by hydrolytic enzymes (Da Ros *et al.*, 2016; Makadia *et al.*, 2016). These enzymes (glucosidases, lipases, and proteases) can convert macromolecules and recalcitrant molecules to simple soluble molecules (Yang *et al.*, 2016). With reference to Li *et al.* (2011), although, a mixed-enzyme group achieves maximal hydrolysis, the presence of amylase was said to have a higher hydrolytic efficiency comparative to the protease and lipase enzyme. As a result of the complexity of other macromolecules, hydrolysis is incapable of catabolising all the polymers, so, the molecules produced either accumulate in the UASB reactor or flow out of the digester (Lettinga, 1992).



(Equation 3)

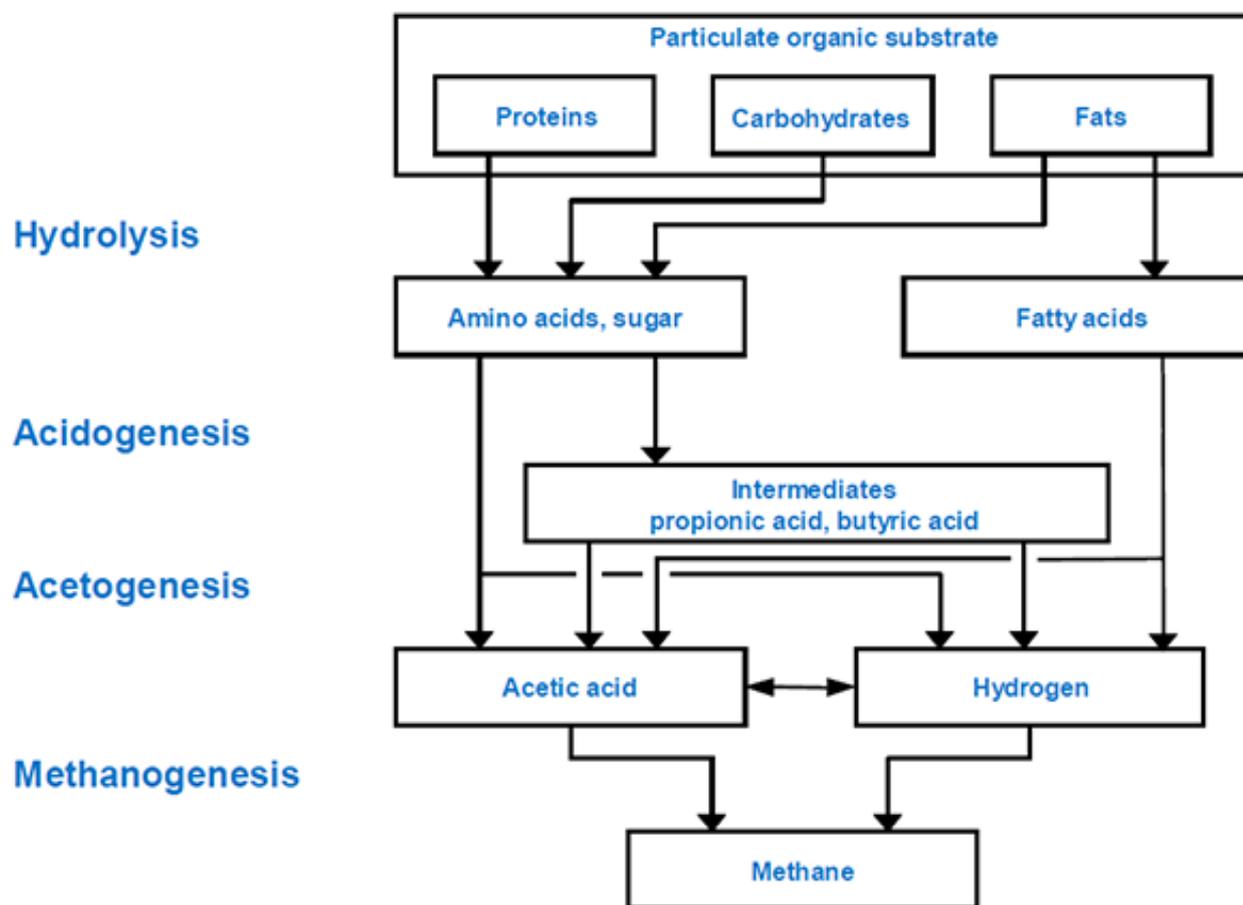


Figure 0.4 The diagrammatic illustration of the flow chart of the different stages of anaerobic digestion (Ostrem, 2004).

Acidogenesis

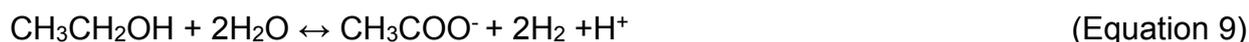
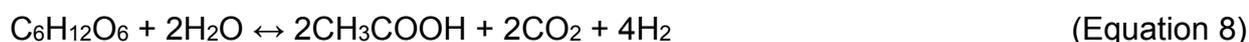
This is a stage characterised by fermentation of simple molecules (sugars, amino acids and fatty acids) produced from hydrolysis into ethanol and short chain fatty acids such as acetate, propionate, formate, butyrate as well as valeric acid (Keating, 2015; Nguyen *et al.*, 2015). The typical acidogenesis reactions involve the production of ethanol, propionate and acetic acid, respectively (Equation 4, 5 and 6). This process is carried out by acidogens which originate from diverse group of anaerobic fermentative bacteria (Zhao *et al.*, 2015). The bacteria are also spore formers, implying that, they are resistant to harsh environmental conditions (Keating, 2015). The acidogens are the largest trophic group in granules and can survive temperature fluctuations, a wide range of pH conditions, thrive without or with oxygen and can live on various organic matter as a food source (Chen *et al.*, 2014; Keating,

2015). Some of these acidogens are possibly from *Bacteroidaceaea* family, which inhabits the gut system, hydrolysing amino acids and simple sugars (Zhao *et al.*, 2015). Exoenzymes secreted from the fermentative microbes are involved in the breakdown of these polymers (De Lemos Chernicharo, 2007). Once the products of acidogens are formed they are subsequently secreted by the cells of the acidogens (Makadia *et al.*, 2016).



Acetogenesis

The third reaction, involves the acetogens breaking down the short chain organic acids produced during acidogenesis i.e. acid fermentation (Latif *et al.*, 2011). It is an intermediary stage of anaerobic digestion, whereby syntrophic acetogens convert the products from acidogenesis, by oxidation, to create substrate for the subsequent stage of methanogenesis (Nguyen *et al.*, 2015). Acetic acid, carbon dioxide and hydrogen are produced, causing a pH drop as a result of the high concentration of hydrogen ions in the system generated from the formation of propionic, butyric, valeric and acetic acid (Zhao *et al.*, 2015). Accumulation of these acids has detrimental effects on the activity of methanogens (Zhao *et al.*, 2015). The Equations 7, 8 and 9 show the anaerobic oxidation of propionate, glucose and ethanol during acetogenesis.



Methanogenesis

This stage is characterised by the anaerobic catabolism of organic acids to biogas containing 48 - 65% methane, 36 - 41% CO₂, 17% nitrogen, <1% of oxygen, 32 - 169 ppm of H₂S and traces of other gases (Khalid *et al.*, 2011; Keating, 2015). Some of the substrates

used during methanogenesis include acetic acid, H_2/CO_2 , CO, formic acid, methyl amines and methanol (Da Ros *et al.*, 2016; Makadia *et al.*, 2016). In general, large numbers of methanogens are said to be stable to hydraulic overloads, sudden increases in temperature, fluctuations in fatty acids and NH_3 concentrations; they can withstand the stress they are subjected to better than small numbers of methanogens (De Lemos Chernicharo, 2007; Lappa *et al.*, 2015).

It can be seen in Figure 2.5, there are various groups of methanogens, each group is substrate specific (Nguyen *et al.*, 2015). For that reason, the methanogens consortia ought to comprise of a number of distinctive bacterial groups for complete conversion of acetogenesis products to biogas (De Lemos Chernicharo, 2007).

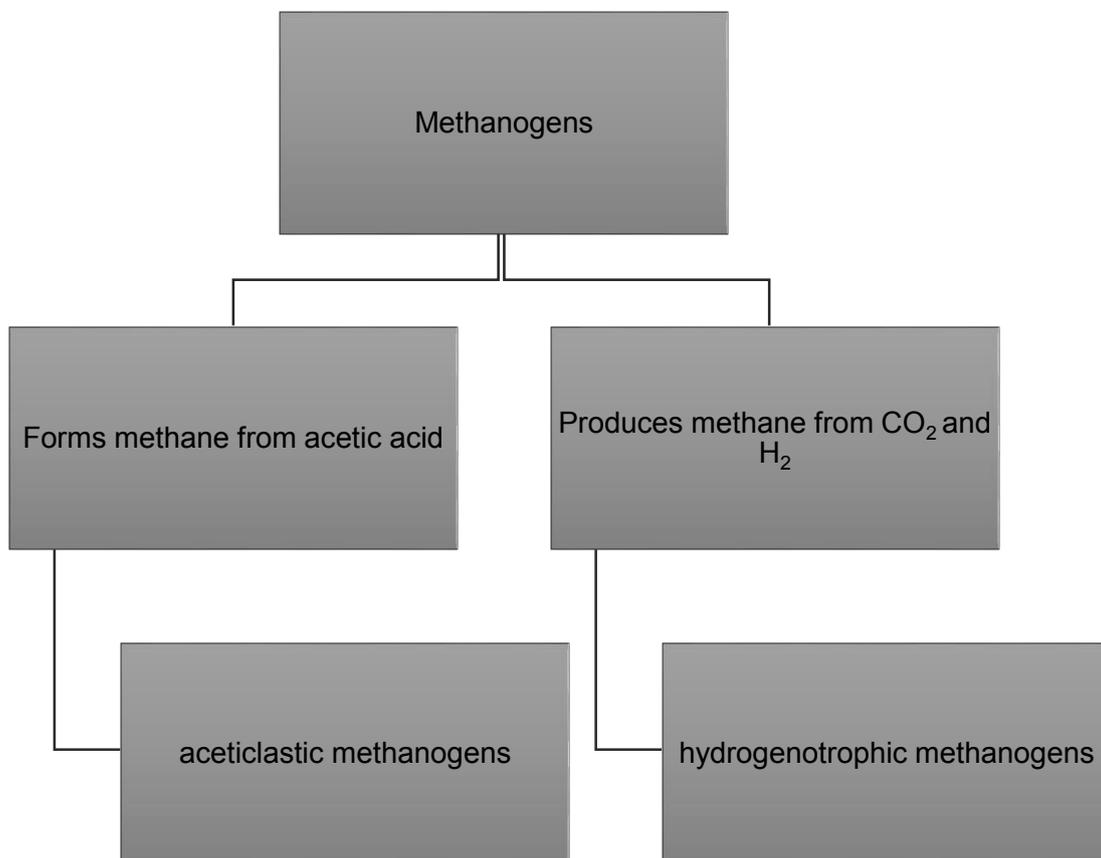
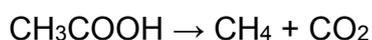


Figure 0.5 Diagrammatic representation of the two main different classes of methanogens (Lappa *et al.*, 2015).

- Acetoclastic methanogens



(Equation 10)

Only a limited number of methanogens have the capability of converting acetate to methane and carbon dioxide (Equation 10) (Calderón *et al.*, 2013). Aceticlastic methanogens carry out approximately 70% of methanogenesis (Erdogan, 2014; Robertson, 2014). Two different genera of methanogens convert acetate to methane, that is, the *Methanosarcina* which use 10^{-3} M of acetate and *Methanosaeta* which use a slightly lower concentration of the acetate (De Lemos Chernicharo, 2007). It is presumed that, the *Methanosaeta* are more susceptible to pH fluctuations and hence, produce less methane comparative to *Methanosarcina* (Schmidt & Ahring, 1996). Generally, methanogens have a slow growth-rate but, the *Methanosaeta* have a distinctively slower growth rate, entailing that the UASB reactor needs longer solid retention time for methanogens to grow (Gerardi, 2003). Unlike the *Methanosaeta*, the *Methanosarcina* have an enhanced growth rate, lower affinity for acetate and consumes the substrate better than *Methanosaeta* (Calderón *et al.*, 2013). In addition, *Methanosarcinas* are the most versatile group of methanogens, characterised by forming packages of cocci shaped microorganism (Calderón *et al.*, 2013). *Methanosaetas* are characterised by having long filaments which aid in the development of the texture of the bacteria in the granules (De Lemos Chernicharo, 2007).

- Hydrogenotrophic methanogens



Hydrogenotrophic methanogens, include all the methanogenic species that produce methane from CO_2 and H_2 (Equation 11) (Lamprecht, 2009). Some of the genera extracted from the reactors include *Methanobacterium*, *Methanospirillum* and *Methanobrevibacter* (Gerardi, 2003). As soon as methane is produced, it is immediately separated from the effluent because of its low solubility properties (Lamprecht, 2009). Since CO_2 is partially soluble in water, it escapes from the reactor in its gaseous state or dissolved in the liquid effluent (Keating, 2015).

Sulphate reduction

The presence of sulphate or sulphite containing molecules promotes the growth of sulphate reducing bacteria (Moestedt *et al.*, 2013). Sulphate reducing bacteria (SRB) use sulphate and sulphite ions as electron acceptors when organic materials are oxidised (Lettinga *et al.*, 1992). The SRB are versatile since they consume volatile fatty acids, aromatic acids, amino

acids, hydrogen, ethanol and methanol to produce hydrogen sulphide (Gerardi, 2003). The main disadvantage of the presence of sulphate is that, it accelerates the growth of SRB which out-compete methanogens for substrates (H_2 and acetate), as highlighted by Zhang *et al.* (2013). Furthermore, H_2S can instantly enter the cellular membrane and denature proteins within the cytoplasm, which create disulphide and sulphide cross-linkages between polypeptide chains (Moestedt, *et al.*, 2013; Zhang *et al.*, 2013). More so, sulphides are also involved in the precipitation of non-alkali metals in bioreactors thus restricting their availability for absorption by methanogens (Zhang *et al.*, 2013). Furthermore, the presence of sulphate ions in WWT leads to the consumption of some intermediary molecules such as VFA by SRB thus, causing negative alterations in the metabolic pathway (De Lemos Chernicharo, 2007). Subsequently, there is lower production of biogas as the SRB will be competing with acetogens, methanogens and fermentative bacteria for the substrates present (Zhang *et al.*, 2013).

Factors affecting anaerobic digestion

Temperature is an important parameter that affects the microbial activity, methane production, process kinetics as well as stability of anaerobic digestion (Keating, 2015). With reference to the Arrhenius Equation, apart from affecting the rate of AD processes, temperature also influences the degree of breakdown of macromolecules (Moestedt, *et al.*, 2013). Psychrophilic temperatures (below 15 °C) are known to reduce growth of bacteria, rate of utilisation of substrates and production of methane (Khalid *et al.*, 2011). On the other hand, extreme elevated temperatures, above optimum temperature, reduce the yield of biogas production and methanogen activity due to bacterial decay (Keating, 2015). The best operational temperature for AD was said to be in the mesophilic range, 30 - 45 °C (Khalid *et al.*, 2011). Thermophilic temperatures however, have a more rapid organic matter breakdown, more biomass and biogas production, enhanced dewatering characteristics and improved pathogen removal (Khalid *et al.*, 2011). Therefore, due to improved dewatering characteristics, there are lower costs incurred in sludge management (Nguyen *et al.*, 2015). Use of thermophilic temperatures also results in the production of pathogen free sludge, which is environmentally friendly (Khalid *et al.*, 2011). Also, a high methane yield is expected due to the increased degree of hydrolysis per unit of macromolecules added (Khalid *et al.*, 2011). Temperature also has external effects on the microbes of the system, as it affects the rate of enzyme reaction and the rate of diffusion of substrates (Gerardi, 2003). The rate of reaction doubles with every 10 °C increase (Keating, 2015). Nevertheless, very high

temperatures (above 65 °C), can denature the three-dimensional structure of enzymes and render them inactive for use in anaerobiosis (De Lemos Chernicharo, 2007).

The different physiological bacterial groups present during anaerobic digestion work within a specific pH range, which is determined by the phase of AD (Khalid *et al.*, 2011; Nguyen *et al.*, 2015). The overall optimal pH range for AD is between 6.0 and 8.0 (Khalid *et al.*, 2011). However, different stages of AD require a specific pH range, as displayed in Table 2.9. The methanogens are quite sensitive to low pH (acidic pH) compared to the acidogens (Moestedt, *et al.*, 2013). The pH in UASBs is primarily regulated by ratios of $\text{CO}_2 \leftrightarrow \text{HCO}_3^- \leftrightarrow \text{CO}_3^{2-}$, $\text{NH}_4^+ \leftrightarrow \text{NH}_3$ as well as $\text{CH}_3\text{COOH} \leftrightarrow \text{CH}_3\text{COO}^-$ species (Daverey *et al.*, 2015). In addition, pH increases as a result of the production ($(\text{NH}_4)_2\text{CO}_3$) and the elimination of carbon dioxide, due the conversion of CO_3^{2-} and 2H^+ to carbon dioxide and hydrogen sulphide (Goosen, 2014). However, acidogenesis is the main process occurring at low pH, promoting rapid fermentation of simple molecules to fatty acids (De Lemos Chernicharo, 2007). Moreover, imbalanced organic digestion results in rapid build-up of volatile fatty acids, which consequently results in a drop in pH, reducing the buffer or alkalinity of the UASBs (Keating, 2015). To correct this; lime, KOH, NaOH, sodium and potassium bicarbonate can be used to restore optimal pH (Khalid *et al.*, 2011). Lime is the cheaper alternative and rapidly restores stability from organic overload (Keating, 2015). However, in excess, lime is associated with inhibiting metabolic processes (Moestedt, *et al.*, 2013). Unwanted precipitates of calcium carbonate are formed, which leads to the removal of carbon dioxide from biogas thus, leading to a decrease in biogas yield (Keating, 2015).

Table 0.9 The optimal pH range for the different phases of anaerobic digestion (Kumar *et al.*, 2009; Khalid *et al.*, 2011)

Phase of anaerobic digestion	pH range
Hydrolysis	5.2 – 6.3
Acidogenesis	5.2 – 6.3
Methanogenesis	6.5 – 7.5

The nature, complexity and availability of the substrate affects the rate of anaerobic digestion (Khalid *et al.*, 2011). Microorganisms are substrate specific, so, the type of

macromolecule affects the growth of a specific consortium of microbes and thus, AD (Latif *et al.*, 2011). Also, the substrate must be well analysed to evaluate the amount of carbohydrates, proteins and lipids present prior to AD processes (Khalid *et al.*, 2011). This would be beneficial as it will predict the volume of methane to be yielded from the substrate (Abbasi & Abbasi, 2012). Usually, carbohydrates are the most preferred and consumed substrate during anaerobic digestion (Khalid *et al.*, 2011).

Upflow velocity is another factor influencing the rate of anaerobic digestion and efficiency of the UASB reactor (Abbasi & Abbasi, 2012). Moderate upflow velocity is required to achieve excellent settling properties of the granular sludge, as, high velocity promotes the shearing and disintegration of granules, which consequently results in poor reactor performance (Gustina & Marinšek-Logar, 2011). Moderate upflow velocity is required to enhance maximal contact between the WWW and the granules (Keating, 2015). Moreover, moderate upflow velocity will help reduce short circuiting in the reactor which is caused by the gas bubbles formed which separate the granular bed (Abbasi & Abbasi, 2012).

The hydraulic retention time (HRT) further influences the rate of anaerobic digestion, as discussed by Gustina & Marinšek-Logar (2011). HRT refers to the total length of time the substrate i.e. WWW resides in the reactor for depollution (Gerardi, 2003). According to (Goosen, 2014), high COD reduction is achieved at very short hydraulic retention time. However, the main carbon source in the WW influences the HRT, as carbohydrates take shorter HRT relative to fats (Keating, 2015). A COD reduction of over 90% was attained at an estimated HRT of 10 h (Keating, 2015).

Nitrogen is an important parameter influencing the process of AD, as it facilitates the production of proteins and provides the nutritional requirements for bacteria during AD (Khalid *et al.*, 2011). Proteins are nitrogenous molecules in organic matter that are converted to ammonium during AD (Latif *et al.*, 2011). With that said, nitrogen in ammonium helps stabilise the alkalinity in the bioreactor (Khalid *et al.*, 2011). More so, the presence of ammonium aids in the production of new cell mass (Gustina & Marinšek-Logar, 2011). For maximal production of methane in high strength wastewater e.g. WWW, a 1 000: 7: 1 ratio should be present for C: N: P, respectively (Khalid *et al.*, 2011). However, a high concentration of ammonia is reported to inhibit the bacterial metabolism and thus, the growth of methanogens, when the concentration is above 100 mM (Khalid *et al.*, 2011).

2.4.1 GRANULATION

Granulation is a process whereby biomass agglomerates and develops into compact aggregates or pellet like structures known as granules (Liu *et al.*, 2009; Latif *et al.*, 2011). The fundamental principle of this process is the clumping of bacteria into a symbiotic multilayer structure (Fig. 2.6) and retention of substantially active biomass with good settling properties (Lamprecht, 2009; Gupta *et al.*, 2012).

Granulation involves four main steps as discussed by Lamprecht (2009). These stages, in order of occurrence involve:

1. Transfer of cells to the exterior inert surface of uncolonised matter
2. Reversible adsorption to the granular sludge by physicochemical forces
3. Irreversible adhesion of the microorganisms to the granular sludge by means of polymers or filamentous structures attaching the bacteria to the granules. This process needs strong specific bonds (Van Der Waals forces) that allows the granules and bacteria to attach to each other
4. Proliferation of the bacteria and growth of the sludge

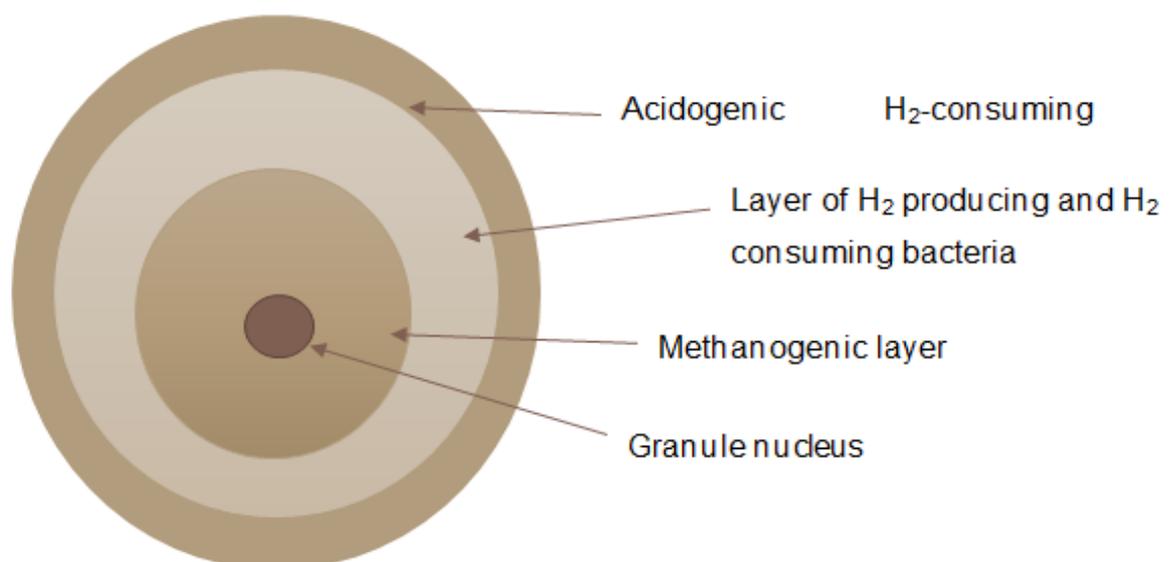


Figure 0.6 The pictorial diagram of a typical anaerobic granule, showing the different bacterial groups present (Gupta *et al.*, 2012).

2.4.1.1 FACTORS AFFECTING GRANULATION PROCESS

Similarly, to anaerobic digestion, numerous factors affect the rate and degree in which granulation takes place in the UASB.

Firstly, the temperature used in UASB reactors has a greater influence on the metabolic activities of methanogens relative to acidogens (Van Lier *et al.*, 2015). The optimal temperature for maximal granulation is a mesophilic temperature, ranging between 30 to 40°C (Keating, 2015). Granules subjected to mesophilic temperatures are very sensitive to changes in temperature conditions, such that, an increase in operational temperature can result in granule fragmentation (Tiwari *et al.*, 2006). This has a consequence of disturbing the reactor performance (Keating, 2015). However, granules subjected to mesophilic temperature show quick and high stability properties during start-up compared to granules subjected to thermophilic temperatures (Tiwari *et al.*, 2006). On the other hand, thermophilic temperatures result in an increased reaction rate and better biodegradability of organic matter (Latif *et al.*, 2011; Alla, 2013).

Microbial consortiums especially of, the methanogens are very sensitive to pH (Van Der Weisthuizen, 2014). Therefore, to develop a desirable granular sludge there ought to be a stable pH (within 6.5 to 8.5 range) and high partial pressure of hydrogen (Lamprecht, 2009). While Show *et al.* (2014), suggests that the optimal pH for granulation ranges from 6.6 to 7.5. Acidogens are less susceptible to abrupt changes in pH relative to methanogens, which function within a strict optimal pH range (Abbasi & Abbasi, 2012). This, therefore, entails that acidic pH simultaneously favours the growth of acidogens and inhibits methanogenic growth and consequently inhibits granule formation (Tiwari *et al.*, 2006). So, to achieve maximum granulation, pH should be within a range of 6.5 to 8.5, higher pH will result in smaller sized granules being formed (Show *et al.*, 2014).

Organic Loading rate (OLR) also affects the microbial consortium and functioning of a UASB (Latif *et al.*, 2011). Differences in OLR's are as a result of different influent COD's (Tiwari *et al.*, 2006). Low OLR has negative effects on large granules, as granules are fragmented due to mass transfer limitation (Alla, 2013). The granules disintegrate and lose their stability, as a result of the decay that develops from the nucleus of the granule due to limitation of diffusion of the substrate (Lamprecht, 2009; Dobre *et al.*, 2014). In addition, the production of biogas increases with an increase in OLR, until a phase when methanogens cannot effectively convert all the available acetic acid to methane (Latif *et al.*, 2011).

The upflow rate of the influent and the gas production rate influences the granulation process (Keating, 2015). An upflow rate above 1.5 m.h⁻¹, may cause the granules to fragment due to friction between the granules, resulting in smaller sized granules being formed (Khan *et al.*, 2015). The optimal range of upflow rate for UASBs', as discussed by Latif *et al.* (2011), is 0.5 to 1.5 m.h⁻¹. Small granules are prone to be washed out of the

reactor resulting in a decrease in the SRT (Abbasi & Abbasi, 2012). At high OLR and high biogas production, bacterial cells shear-off from the surface of the granules (Latif *et al.*, 2011).

The nature of the consortia of microorganisms also affects granulation (Abbasi & Abbasi, 2012). Acetogens and some methanogens such as *Methanosaeta sp.* form part of the microbial population during granule formation (Gustina & Marinšek-Logar, 2011). The rate of granulation is high when the microbial population comprises of *Methanosaeta sp.* and syntrophic organisms (Tiwari *et al.*, 2006). On the contrary, *Methanosarcina* and acidogens decrease the rate of granule formation (Tiwari *et al.*, 2006). It has been reported that *Methanosarcina* is not involved in the first stages of biofilm formation as it does not have the ability to attach to hydrophilic or hydrophobic structures (Show *et al.*, 2014; Lu *et al.*, 2015).

Another factor influencing the granulation process is the presence of exo-cellular polymers (ECP) (Van Lier *et al.*, 2015). Exo-cellular polymers are macromolecules produced by bacteria to retain the structure of granules in the reactor (Van Lier *et al.*, 2015). ECP's are macromolecules that build up and surround the surface of microorganisms (Tiwari *et al.*, 2006). In addition, they are functional groups that can facilitate the cell to cell interactions of bacteria (Schmidt & Ahring, 1996). Furthermore, ECP are normally bound to calcium and magnesium, which act as bridging ions consequently creating network in which cells are embedded (Schmidt & Ahring, 1996). ECP also aids in protecting microbes from toxic molecules (Van Lier *et al.*, 2015). Moreover, it was emphasised that excessive amounts of ECP have a negative impact on the rate of floc formation in the active granules (d'Abzac *et al.*, 2013).

2.4.2 PROBLEMS ASSOCIATED WITH ANAEROBIC DIGESTION

There are several challenges faced when operating anaerobic bioreactors, which result in poor COD conversion, reactor instability and eventually reactor failure (Zhang *et al.*, 2013).

The presence of long chain fatty acids present in lipids can have inhibitory effects on the hydrolysis stage of anaerobic digestion (De Lemos Chernicharo, 2007). The hydrolysis stage therefore becomes the rate limiting step due to the difficulty of degrading LCFA and slow acclimatisation of granules to LCFA wastewater (Zhang *et al.*, 2013). Moreover, this results in sludge flotation, which disturbs the sludge settling properties, consequently leading to sludge washout (Moestedt, *et al.*, 2013).

The excessive production of ammonia (>100 mM) and hydrogen sulphide (50 – 400 mg.L⁻¹), above the satisfactory amounts for optimal reactor performance is another common

problem in anaerobic reactors (Chen *et al.*, 2014). Ammonia is produced from the oxidation of ammonium compounds, which increases the reactor pH to an alkali pH (Khalid *et al.*, 2011). The optimal ammonia and hydrogen sulphide concentrations for effective anaerobic digestion are 200 mg.L^{-1} and 25 mgS.L^{-1} , respectively (Chen *et al.*, 2008). Hydrogen sulphide is produced by sulphate reducing bacteria, in reactors depolluting sulphur containing wastewater such as winery wastewater (Chen *et al.*, 2008). Not only does the excessive production of these gases disturb microbial metabolic reactions, but results in odour emissions (Moestedt, *et al.*, 2013; Zhang *et al.*, 2013).

Furthermore, another problem of AD is as a result of incomplete conversion of intermediary compounds to biogas, which subsequently leads to accumulation of volatile fatty acids (Khalid *et al.*, 2011). Incomplete digestion of intermediary compounds is often caused by abrupt changes in the organic loading rate (Abbasi & Abbasi, 2012). So, a sudden increase in the OLR will result in poor breakdown of organic matter, resulting in low COD reduction (Keating, 2015). The volatile fatty acids produced during acidogenesis and acetogenesis continually accumulate, consequently causing the pH to drop below 7 (Keating, 2015). An acidic pH has adverse effects to the microbial metabolic reactions, as these reactions are pH specific (Abbasi & Abbasi, 2012). More so, in attention to monitor the reactor pH will inhibit and suppress the growth of the methane producing bacteria (Chen *et al.*, 2014).

Granular sludge washout is another common problem of anaerobic digestion processes (Moestedt, *et al.*, 2013). This problem is attributed to a high upflow rate (above 1.5 m.h^{-1}) which disturbs the settling property of the granules (Abbasi & Abbasi, 2012). In addition, sludge washout can be as a result of rapid production of biogas (Chen *et al.*, 2008). The presence of biologically active methanogens, as a result of good granulation promotes the conversion of acetate and hydrogen to methane gas (Moestedt, *et al.*, 2013). However, if the production is too rapid, it excessively lifts the granular sludge bed causing some of the biomass to be washed out (Abbasi & Abbasi, 2012). According to Abbasi & Abbasi (2012), this has a negative impact on the reactor as the SRT is reduced, entailing that, the biomass left will be inadequate to achieve expected COD reduction. Thus, low SRT leads to accumulation of organic acids, which reduces the reactor pH (Khalid *et al.*, 2011). Sludge washout is also caused by the inevitable abrasion of granules against each other, thus, causing the granules to fragment or become smaller, further, increasing the risk of washout due to low density and poor settling properties (Lamprecht, 2009). Fragmentation of granules reduces the water quality as a result of an increase in the concentration of

suspended solids i.e. an increase in turbidity (Khemkha *et al.*, 2015). Furthermore, the problem of high TSS further aggravates the operation of bioreactors, as there is a high likelihood of pipe clogging (Hannouche *et al.*, 2011; Daverey *et al.*, 2015).

Therefore, to counteract the problem of biomass washout, the use of using biofilm carrier particles has been successful in retaining biomass in UASB reactors (Khalid *et al.*, 2011; Daverey *et al.*, 2015).

2.5 ATTACHMENT MEDIA

Various attachment media have been developed and these have been categorised as porous or non-porous media (Mohana *et al.*, 2009). Some of the non-porous media include glass beads, sand and numerous plastics (Mohana *et al.*, 2009). Plastics have been the most common non-porous media used at pilot plants and large-scale operations (Zhang *et al.*, 2014). On the contrary, porous media include needle-punched polyester, polyurethane foam, ceramic, sintered glass as well as magnetic foam particles (Ramm *et al.*, 2014). Other biofilm carriers that have been used in wastewater technology include dried-up pieces of stem of *Opuntia imbricata cacti*, strips of polyvinyl chloride sponge, natural rice husk, fireclay, fibre threads, bamboo charcoal and peach pit (Hu *et al.*, 2011). It is therefore important to review the characteristics of biofilm carrier particles as they influence the choice of carrier, due to their unique adhesion patterns (Mohana *et al.*, 2009; Hellman *et al.*, 2011). Studies done by Chauhan *et al.* (2005), pointed out that increased attachment of various species of methanogens is observed when porous and hydrophobic carriers are used. To maximise attachment of methanogens to the carriers, it is preferential to choose Fe-containing carriers as they have a high affinity to attach to these carriers (Glass & Orphan, 2012). The factors affecting the choice of attachment media include the porosity, type of media, size, as well as the surface area of the attachment material (Jo *et al.*, 2015).

Therefore, attachment media ought to have a certain surface roughness to lower the thermodynamically considered free energy of preliminary microbial adhesion (Jo *et al.*, 2015). The speed at which biomass attaches and accumulates on the attachment media is affected by the surface roughness of the media, with enhanced attachment on particles with a high surface roughness (Jo *et al.*, 2015).

The attachment media chosen should also be porous, as high porosity provides a large surface area for microbial attachment (Ramm *et al.*, 2014). The presence of pores in porous media reduces the actual amount of media needed in the reactor, as bacteria first occupy the cavities (Ramm *et al.*, 2014). Unlike non-porous media which have operational

problems like clogging and short circuiting, these problems are less likely to happen because of the high porosity presented by the media (Anderson *et al.*, 1994). These studies further showed that porous media have a bulk of biomass growing within and over them at higher organic loading rates compared to non-porous media (Bassin *et al.*, 2016). Non-porous media are unstable at high OLR's (Ramm *et al.*, 2014). From the studies done by Anderson *et al.* (1994), the dense biomass grew within the pores, while the less dense biomass grew over the media. Furthermore, Anderson *et al.* (1994) highlighted that at high OLR there was low biomass detachment from the porous sintered glass media relative to non-porous media. This is so because non-porous media does not have the capability to allow biomass to grow within its structure, so biofilm attaches to its surface due to the presence of crevices and this enables ease detachment of biomass from the attachment media (Jo *et al.*, 2015). There is effective attachment of biomass if the porous media are 1 to 5 times bigger in diameter than the size of the media (Zhang *et al.*, 2014).

Silva *et al.* (2006) showed that that alumina-based ceramics presented the best adhesion of methanogenic consortia, while Ramm *et al.* (2014) identified the best adhesion to be on clay montmorillonite. The surface of iron (ferrite) particles is characterised by having hydrophilic surfaces and porous particles, which provide surface pronounced roughness (Hellman *et al.*, 2011). Magnetite is another possible attachment media consisting of magnetic iron oxide, which showed the highest adhesion values for several microbial strains when it was compared to other metal oxides (Cheng *et al.*, 2014). These favourable adhesion characteristic were attributed to the relatively hydrophobic nature and high surface roughness of iron oxide (Hu *et al.*, 2012). Moreover, the surface magnetite comprises of thick layers of different iron oxides that provide a large number of positive charges and thereby enable better electrostatic attraction of negatively charged microorganisms (Cheng *et al.*, 2014). More so, since the surface charge on the microorganisms is predominantly negative, the attachment media with a positive surface charge (e.g. magnetite) are ideal, as they enable minimal repulsion between the two surfaces (Cheng *et al.*, 2014)

Based on the beneficial properties of biofilm carriers in stabilising bioreactor performance, through retaining biomass within the reactor (Bassin *et al.*, 2016), it was crucial to determine the effect of seeding magnetisable glass foam particles, biofilm carrier particles, on the performance of UASB reactors.

2.5.1 MAGNETISABLE GLASS FORM PARTICLES, MGFP

MGFP were used as an attachment media to allow biomass colonisation. MGFP are mainly made from foamed soda-lime silicate glass which has a porous surface (Ramm *et al.*, 2014). According to Ramm *et al.* (2014), to obtain the magnetisable particles, Bayoxide E AB 21, a magnetic iron powder is blended into the foam glass during the MGFP production process. Bayoxide E AB 21 is known to be an active pigment and colorant comprising of mostly Fe_2O_3 and heavy metals such as copper, lead, mercury and cadmium (Fabis & Jennrich, 2005). Binding, expansion agents and recycled glass are also added to Bayoxide E AB 21 during its production (Ramm *et al.*, 2014). Pores are thus developed through the drying process when the particles will be expanding (Jiang *et al.*, 2013).

It should be noted that, MGFP are not necessarily magnetic, as the UASB reactors cannot work effectively with magnetic particles that can permanently clump together (Ramm *et al.*, 2014). However, the particles are rather magnetisable, suggesting that they become magnetic only when there are subjected to a strong magnetic field (Fabis & Jennrich, 2005). They lose their magnetic properties the very moment there are removed from a strong magnetic field (Ramm *et al.*, 2014). This technique is advantageous as it can accommodate various wastewater to be treated. Winery wastewater has a thin consistency, does not contain long fibres, which makes it suitable to use MGFP as the biofilm carrier in the reactors (Huysman *et al.*, 1981). In addition, magnetisable particles potentially promote the stability of the reactors (Hellman *et al.*, 2011). Recent studies show that, when a concentration of 1% w/w of the magnetic particles was added to a continuous stirred tank reactor, the methanogenic rate increased from 1.34 to 1.42 $\text{L} \cdot \text{L}^{-1} \cdot \text{d}^{-1}$ (Ramm *et al.*, 2014), thus indicating how the particles positive influence reactor performance. Magnetisable particles will enhance the activity of methanogens due to the presence of Fe in MGFP (Jiang *et al.*, 2013; Zhang *et al.*, 2013).

2.6 CONCLUSION

For the past 6 000 years people have been struggling to maintain water resources, to the extent that water scarcity problems have escalated to unprecedented levels (Clarke, 2013). Notable conventional treatment methods and structures have been constructed to improve water supply (Litaor *et al.*, 2015), but, all these measures have not been successful in curbing the current problem of water scarcity. Water scarcity became the most pressing global risk in the year 2015, according to the World Economic Forum (2015) statistics. This

has pushed water regulatory bodies to implement more strict rules on water usage and wastewater discharge standards (World Economic Forum, 2015).

Agriculture consumes approximately 60% of surface water resources and wine production is a chief agricultural practice utilising vast amounts of land and water in the Western Cape region of South Africa (Goosen, 2014). With that said, it is of no doubt that wineries are contributing to the growing problem of water pollution (Conradie *et al.*, 2014). Water pollution in wineries is mainly due to cleaning operations, spillages and bottling operations (Mosse *et al.*, 2012). An estimation of 0.8 to 14 litres of wastewater is produced for each litre of wine produced (Conradie *et al.*, 2014; Litaor *et al.*, 2015). This estimation varies from winery to winery due to different processing techniques and type of wine being produced among other factors (Mosse *et al.*, 2012). Winery wastewater is characterised by a bad odour, brown colour, relatively low pH (3.3 - 4.5) and a high COD (Litaor *et al.*, 2015). All these characteristics are far above legal standards and thus make winery wastewater unsuitable for discharge prior to treatment (Conradie *et al.*, 2014). Therefore, various combinations of treatment systems have been implemented to reduce contamination to legally acceptable values for discharge (Ioannou *et al.*, 2015). The Upflow Anaerobic Sludge Blanket (UASB) reactor is a predominant method used by several wineries to reduce the COD to less than 5 000 mg.L⁻¹ as required by legislation (Republic of South Africa, 2004). The UASB reactor involves biological treatment of wastewater through anaerobic digestion (Khalid *et al.*, 2011).

The underlying principle of the UASB is to have an anaerobic sludge, which exhibits good flocculating and settling properties that efficiently retains biomass (Latif *et al.*, 2011). Anaerobes are located in the granular sludge and they appear in concentric layers with the strict anaerobes, that is, methanogens at the nucleus of the granule (Van Lier *et al.*, 2015). During the operation of the UASB reactor, organic molecules are broken down by anaerobic bacteria through various stages (hydrolysis, acidogenesis, acetogenesis and methanogenesis) to produce mainly biogas, which is a renewable resource (Khalid *et al.*, 2011). Nevertheless, the UASB commonly has a major problem of biomass washout, which consequently has detrimental effects to the performance of the reactor (Robertson, 2014). Biomass washout is often caused by rapid production of biogas, which causes the sludge bed to be excessively lifted causing the loss of different species of bacteria responsible for the conversion of organic matter to biogas. Consequently, this reduces the treatment efficiency of the UASB reactors due to unstable reactor conditions, i.e. build-up of volatile fatty acids, pH drop, decrease in buffer strength and an increase in total suspended solids

in the effluent. Therefore, magnetisable glass foam particles (MGFP) can be used as a biofilm carrier material to increase biomass retention and improve stability of the reactor.

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Chapter 3

Investigating the effect of magnetisable glass foam particles (MGFP) in an UASB reactor treating synthetic winery wastewater by monitoring biofilm development and activity of colonised MGFP

3.1 SUMMARY

Two 2.3 L UASB reactors were each seeded with 350g of granular sludge (with a volatile suspended solids content of 0.1 gVSS.g^{-1} granules) to treat synthetic winery wastewater (SWWW). One of the reactors was seeded with biofilm carrier particles known as magnetisable glass foam particles (MGFP), while the other reactor was not seeded with these magnetisable particles. The reactors were fed on a daily basis, semi-continuously with SWWW substrate, at a pH of 7.5, daily, using an automated peristaltic pump. The operation of the UASB reactors was done in 180 cycles and was mainly divided into two phases. During the first phase, the organic loading rate (OLR) was gradually increased from 0.5 to $4.5 \text{ kgCOD.m}^{-3}.\text{d}^{-1}$. This phase lasted from day 0 to 92. During the second phase, the OLR was steadily increased and maintained at $5 \text{ kgCOD.m}^{-3}.\text{d}^{-1}$. This phase lasted from day 93 to 180. The treatment efficiencies of the reactors was then determined by measuring several water quality parameters (chemical oxygen demand reduction, volatile fatty acids, pH, alkalinity and total suspended solids). In addition, the growth of biofilm around the biofilm carrier particles, MGFP was analysed periodically, using the scanning electron microscope and fluorescence microscope. Overall, in spite of the slight reactor disturbances experienced during the UASBs' operation, the treatment efficiency of both reactors was relatively high and different groups of bacteria were observed under microscopy.

3.2 INTRODUCTION

A vast amount of land in South Africa (i.e. 99 463 hectares) has been dedicated to grape production which is intended to produce wine (WOSA, 2014). Most of the wine farms are situated in the Western Cape, which is characterised as a semi-arid region, with a mean yearly rainfall of less than 400 mm (SAWIS, 2015). Bulk volumes of water are used and

generated during the production of wine and it has been estimated that 0.3 to 14 L of wastewater are generated for every litre of wine produced (Oliveira & Duarte, 2016). This wastewater is characterised by a low pH, high COD and high total suspended solids (Mosse *et al.*, 2011). This has driven most wineries to implement effective wastewater treatment plants onsite prior to discharge. With the strict laws often imposed by regulatory bodies, wastewater ought to have a neutral pH, COD below 300 mg.L⁻¹ and low TSS upon discharge (Republic of South Africa, 2013). Failure to comply with these regulations subsequently results in wineries being issued with fines (Bustamante *et al.*, 2011).

Several treatment technologies, i.e. aerobic and anaerobic treatment methods can be used to reduce the microbial, organic and inorganic load in wastewater. Aerobic treatment methods are dependent on the presence of oxygen to allow for the catabolism of organic matter by heterotrophic aerobes to carbon dioxide, water and nitrogenous compounds (Sigge, 2005). These treatment methods include, amongst others, the membrane reactors, jet-loop activated sludge reactor, biological sand filter and the moving bed biofilm reactor. On the contrary, anaerobic treatment methods involve the breakdown of macromolecules through four different sequential stages to produce biogas and biomass, in the absence of oxygen (Van Lier *et al.*, 2015). Some of the anaerobic treatment methods used in wineries include the anaerobic sequencing batch reactor, anaerobic baffled reactor and the upflow anaerobic sludge blanket reactor (UASB). The UASB reactor is a biological treatment method based on anaerobic digestion (Bhatti *et al.*, 2014). Bacteria present in the form of granules or pellet structures break down the organic molecules in the effluent to produce a combustible biogas mainly consisting of methane (Ioannou *et al.*, 2015). The bacteria appear in concentric layers and breakdown macromolecules through four sequential phases i.e. hydrolysis, acidogenesis, acetogenesis and methanogenesis (Abbasi & Abbasi, 2012). The UASB reactor has several advantages, which include that it is a simple, high-rate treatment system, which produces biogas, a source of energy, it also has minimal working space and also, the operational costs are relatively low. Nonetheless, the UASB reactor often has one major drawback during operation, biomass can wash out in these bioreactors and consequently results in poor treatment performance (Lamprecht, 2009). The loss of biomass results in rapid and constant accumulation of volatile fatty acids which will manifest in the system getting sour (Keating, 2015). Methanogens that are responsible for converting organic acids to biogas have a markedly slow growth, so incomplete digestion results in organic acids' build-up (Nguyen *et al.*, 2015). To prevent this problem, biofilm carrier particles e.g. magnetisable glass foam particles can be used. These carriers are very

light in weight and density. Moreover, the carriers are magnetisable, which enables ease recovery upon biomass washout, using a magnetic rod (Ramm *et al.*, 2014).

The following study was aimed at investigating the effect of incorporation of magnetisable foam glass particles (MGFP) in an UASB reactor treating synthetic winery wastewater. The first objective was to compare the effect of MGFP incorporation in the control UASB and a UASB with MGFP added. The treatment efficiency of the UASB reactors was evaluated by monitoring various water quality parameters such as chemical oxygen demand (COD) reduction, pH, alkalinity and volatile fatty acids (VFA) content and biogas (production and composition). The second objective was to monitor biofilm development on the MGFP, to determine whether and where methanogens and other bacteria colonised the MGFP. This was done using scanning electron microscopy (SEM) and fluorescence microscopy. The presence of microbes was verified by staining nucleic acids with fluorescent dye SYTO 9 at an excitation wavelength of 488 nm to obtain emission at 576 nm. The presence and growth of methane producing bacteria was achieved by triggering auto-fluorescence at an excitation wavelength of 405 nm and emission was detected at 476 – 585 nm. The third and final objective was to compare the activity of the MGFP biofilm, granules from the control and MGFP reactors to the activity of the control granules (i.e. the activity was determined using activity of the granules inoculated into both UASB's initially. Standardised activity tests to measure biogas production and composition (CO₂ and methane content).

3.3 MATERIALS AND METHODS

3.3.1 EXPERIMENTAL DESIGN

Two parallel lab-scale UASB bioreactors were seeded with granular sludge from James Sedgwick Distillery in Wellington, South Africa. Furthermore, one of the reactors, R_{mgfp} , was seeded with the carrier particles made of magnetisable glass foam particles (MGFP), while the control reactor, $R_{control}$, was not seeded with the magnetisable particles. Synthetic winery wastewater substrate (2 L) was fed semi-continuously to each of the reactors on a daily basis using a peristaltic pump. The operation of the reactors was divided into two main phases. The first phase was the start-up and incremental phase, where the organic loading rate (OLR) was increased steadily from 0.5 to 5.0 kgCOD.m⁻³.d⁻¹, to stabilise the reactors through maintaining optimal reactor conditions (pH of 7.5, volatile fatty acids <500 mg.L⁻¹ and alkalinity 1 000 – 3 000 mg.L⁻¹). The first phase lasted from day 0 to 107. The second

phase was the stable load phase, where the OLR was maintained at $5.0 \text{ kgCOD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$. This phase lasted from day 108 to 180. The substrate was pumped into the reactor, from the bottom, and flowed upwards through a dense granular sludge bed. During the non-feeding periods, the organic matter in the substrate was broken down to biogas, which escaped the reactor from an exit at the top of the reactor. In addition, the volume of biogas was measured daily using an electronically controlled manometric counter. The effluent produced flowed out of the reactor via a U-tube manometer into a Schott bottle (Fig. 3.1b). Synthetic winery wastewater was used as the substrate to reduce any variability that could possibly arise when water quality parameters were measured. Actual winery wastewater gives inconsistent results due to the large variability of chemical constituent concentrations during the vintage period. In addition, the reactors were only fed to an influent COD of $5\,000 \text{ mg}\cdot\text{L}^{-1}$ to obtain a consistent wastewater composition and eliminate any possible variables.

3.3.2 UASB REACTOR SET UP

Two 2.3 L UASB reactors (R_{control} and R_{mgfp}) were erected in parallel as illustrated in Fig. 3.1a and depicted in Fig. 3.1b. A temperature of 35°C and hydraulic retention time of 24 h was maintained throughout the operation of the UASB's. The bioreactors were heated by a heating tape that was connected to an electronic control unit to maintain an optimal temperature. SWWW was semi-continuously pumped into the bottom of the reactors and this was achieved by the use of a peristaltic pump (Watson-Marlow 323) that was controlled by an electronic timer. Both reactors were fed by means of the same pump, fitted with a dual pump head, to ensure equal feeding rates. An upflow velocity of $0.75 \text{ m}\cdot\text{h}^{-1}$ was maintained in both reactors by means of a circulating peristaltic pump (one pump with a dual pump head again responsible for equal pumping rates). Effluent was collected at the top exit of the reactor via a U-tube to preclude any possible inflowing atmospheric oxygen from the bioreactor, thus maintaining the anaerobic state. The effluent was collected in 2 L Schott bottles. Furthermore, the volume of the biogas produced was measured by an electronically controlled manometric counter.

3.3.3 UASB START UP

Anaerobic granular sludge (350 g with a volatile suspended solids (VSS) content = $0.1 \text{ g}\cdot\text{g}^{-1}$ granules) was seeded in each bioreactor ($13.04 \text{ kgVSS}\cdot\text{m}^{-3}$) at start-up. The granules were sourced from a UASB reactor treating distillery wastewater at James Sedgwick Distillery in

Wellington, South Africa. During the first two days, the UASB reactors were fed with tap water containing 500 mg.L⁻¹ urea ((NH₂)₂CO) and 500 mg.L⁻¹ di-potassium hydrogen orthophosphate (K₂HPO₄), for stabilisation. Following this, a substrate composed of 90% sterilised synthetic glucose substrate (1 000 mg.L⁻¹) (Table 3.1) and 10% of SWWW (1 000 mg.L⁻¹) was fed to the reactors. Initially, the SGS had a concentration of 10 727 mg.L⁻¹ while the SWWW had a concentration of 71 000 mg.L⁻¹, the two compounds were diluted separately to 1 000 mg.L⁻¹ using tap water, prior to mixing. The 9:1 ratio (SGS: SWWW) was gradually decreased during the first two weeks, by increasing the amount of SWWW and decreasing the amount of SGS. The ratio was only decreased further if the COD reduction achieved was ≥ 80%. Throughout the two weeks, the influent COD concentration was maintained at 1 000 mg.L⁻¹ and only the ratio of the SGS to SWWW changed. The substrate or influent was maintained at a pH 7.5, by use of 10% (w/w) acetic acid and 2 M potassium hydroxide (KOH) throughout the start-up.

Table 0.1: Composition of sterilised synthetic glucose substrate (Buys, 2015)

Chemical	Concentration (g.L ⁻¹)
Glucose	1
Yeast extract	20
Urea	0.5
di-Potassium hydrogen orthophosphate	0.5
Trace element solution	1 mL

3.3.4 SYNTHETIC WINERY WASTEWATER (SWWW) AND UASB SUBSTRATE

A SWWW stock solution (Table 3.2) was prepared according to a standardised formula modified from Malandra *et al.* (2003). The compounds listed in Table 3.2 were prepared by dissolving them in distilled water. This substrate stock solution was developed to mimic industrial winery wastewater. Although the theoretical COD of the stock solution prepared in the 5 L Schott bottle was 100 000 mg.L⁻¹ (Malandra *et al.*, 2003), the actual COD for the stock solution was approximately 71 000 mg.L⁻¹. The pH of the stock solution was partially acidic, ranging between a pH of 4.00 and 4.86 with a total suspended solids content of 1.4 g.L⁻¹. Therefore, to make up the daily UASB substrate of 2 L per reactor, a calculated volume of the stock solution was diluted with tap water and reactor effluent to obtain the desired influent COD concentration. The reactor effluent was also used to dilute the SWWW stock

solution, during start-up (from day 28) to increase alkalinity and to prevent the accumulation of VFA being produced (Buys, 2015). The concentration of the UASB substrate was adjusted from 500 to 5000 mg.L⁻¹ during the first phase, while in the second phase it was maintained at 5 000 mg.L⁻¹. Furthermore, 2 M potassium hydroxide was used to increase the pH to 7.5 while urea ((NH₂)₂CO), di-potassium orthophosphate (K₂HPO₄) and potassium hydrogen carbonate (KHCO₃) was added to the substrate to develop and maintain a strong buffer system. The ratio of the compounds added to the substrate was dependent on the measured reactor effluent, but ranged from 0.2 – 0.5 g.L⁻¹, each, for urea and di-potassium orthophosphate. The concentration of potassium hydrogen carbonate added to the daily substrate ranged from 0.1 – 0.4 g.L⁻¹. A trace element solution containing micronutrients (Table 3.3) was added once a week to the substrate to enhance granulation and microbial growth. 1 mL of trace element solution was added for every litre of substrate used.

3.3.5 MAGNETISABLE GLASS FOAM PARTICLES

The MGFP were developed and sourced from Poraver GmbH (Postbauer-Heng, Germany). The MGFP were produced from soda lime silicate glass and magnetic iron powder (Bayoxide E AB 21) to attain porosity and magnetisability, respectively (Ramm *et al.*, 2014). Bayoxide E AB 21 is a colouring agent dye that consists of 97.5% Fe₂O₃ and low amounts of heavy metals like Cd, Cu, Hg and Pb (Ramm *et al.*, 2014). To produce the uneven circular particle, magnetic iron powder was melted into the foam glass to generate the magnetisable particles (Ramm *et al.*, 2014). Binding agents, expansion agents, water and recycled glass are also added during production to get porous surface particles (Ramm *et al.*, 2014). The MGFP can be referred to as magnetic particles but it should be noted that they are not magnetic *per se*, but in the presence of strong magnetic field show magnetisable properties (Ramm *et al.*, 2014). When the magnetic field is withdrawn MGFP lose their magnetism. Two different sizes of MGFP were added to the UASB reactors in the ratio 7:3 (Fig. 3.2a and Fig. 3.2b). Approximately 70% of the biofilm carrier particles had a diameter above 1.6 mm (Fig. 3.2a), whilst 30% of the MGFP had a diameter of < 850 µm < MGFP < 650 µm (Fig. 3.2b).

3.3.6 MGFP AND GRANULE ANALYSIS

3.3.6.1 MGFP RECOVERY FROM UASB BIOREACTOR

After every two months interval of the UASB operation (6 months), MGFP were extracted from the granular sludge by immersing a 9 000 gauss magnetic rod (Sesotec GmbH, Schönberg, Germany) into R_{mgfp} and subsequently removing the magnetisable particles attached to it. The magnetisable particles extracted were divided into two portions of approximately 1 g each and temporarily stored in sealed petri dish. The MGFP were then prepared for microscopic analysis. Scanning electron microscopy was employed to examine the attachment and to visualise different consortia surrounding the colonised magnetisable particles. On the other hand, fluorescence microscopy was employed to elucidate the spatial distribution of bacteria and specifically, methanogens, within the biofilm by excitation of the fluorochrome, SYTO 9 at different wavelengths.

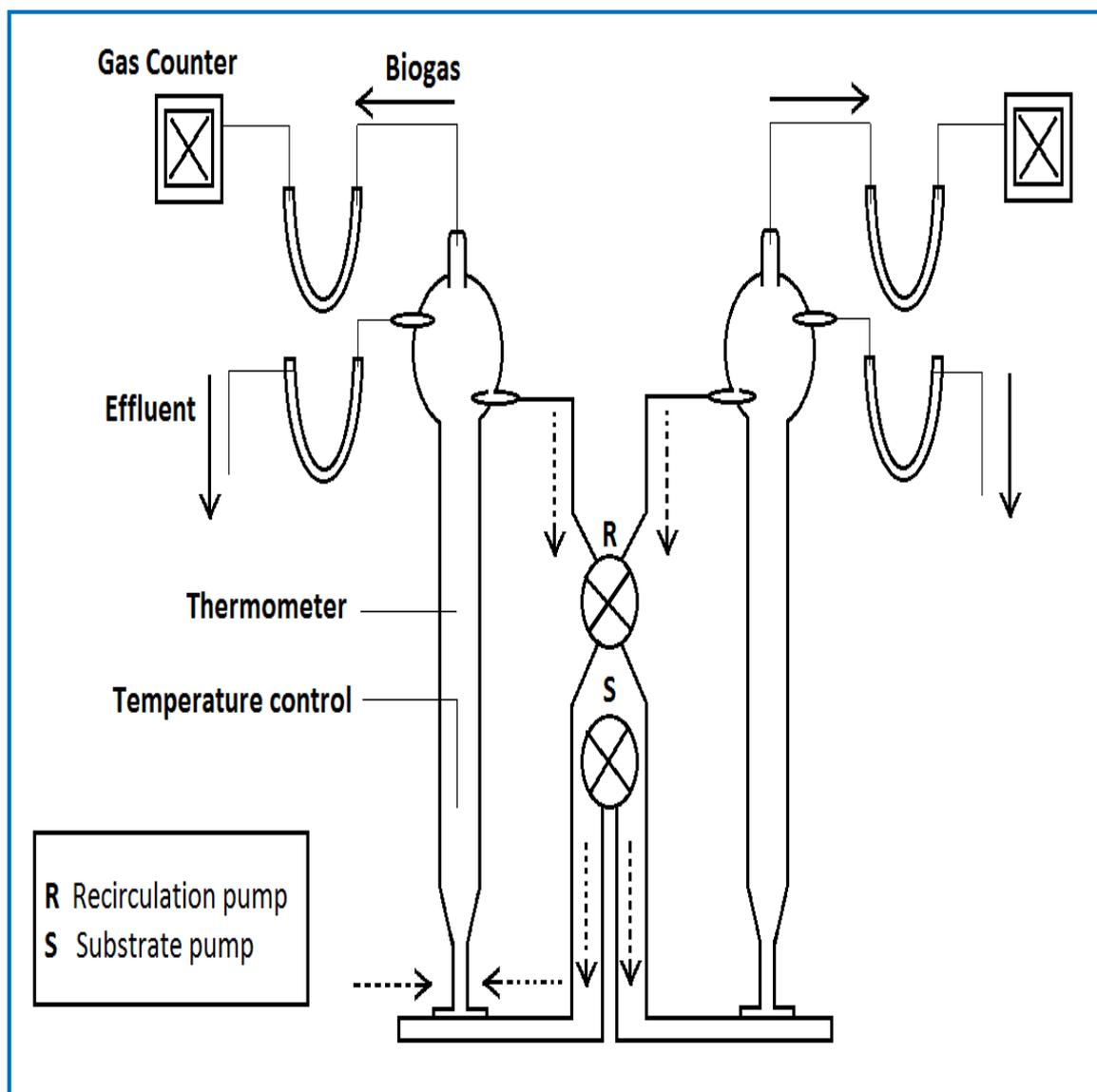
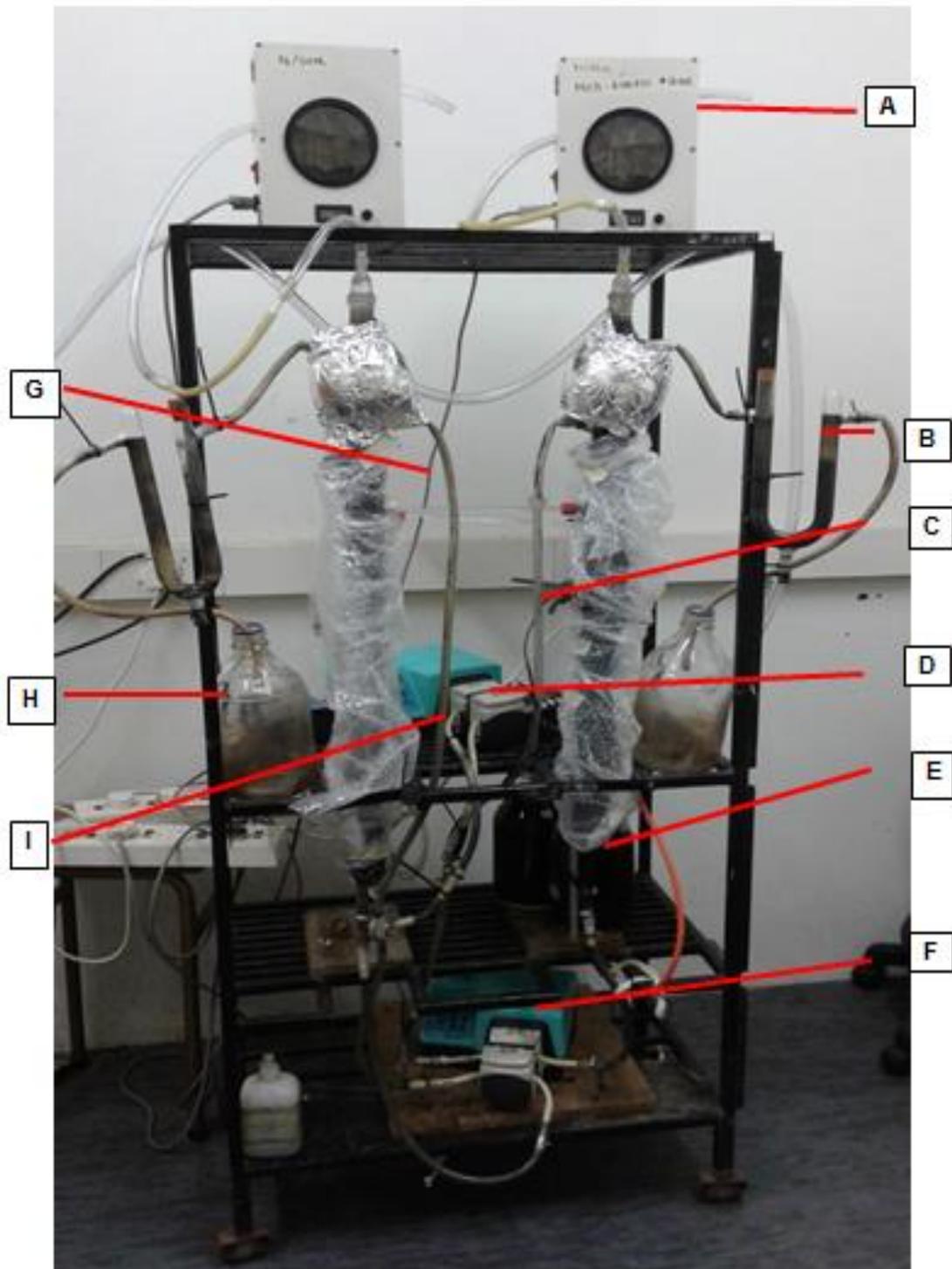


Figure 3.1a Illustrating the design of the UASB bioreactors (adapted from Robertson, 2014).



A = Gas meter, B = U-tube manometer, C = Heating tape, D = Peristaltic feeding pump, E = Substrate/ SWWW, F = Recirculation pump, G = Recirculation pipe, H = Effluent bottle, I = Feeding pipe

Figure 3.1b The experimental set-up of the two parallel Upflow Anaerobic Sludge Blanket (UASB) reactors.

Table 0.2 Chemical constituents used to prepare standardised stock synthetic wastewater solution (Malandra *et al.*, 2003)

Chemical	Concentration in synthetic winery wastewater stock solution	Theoretical concentration COD contributed by the pure solution (mg.L⁻¹)
Synthetic stock solution		100 000
Glucose	20.45 g.L ⁻¹	23 863.34
Fructose	20.45 g.L ⁻¹	22 727.27
Yeast extract	19.32 g.L ⁻¹	
Propanol	17.93 µL.L ⁻¹	45.45
Butanol	14.03 µL.L ⁻¹	45.45
i-amyl alcohol	53.31 µL.L ⁻¹	125
Ethanol	144.75 µL.L ⁻¹	3 409.09
Lactic acid	18.8 µL.L ⁻¹	34.09
Acetic acid	2.71 mL.L ⁻¹	2 954.55
Ethyl acetate	50.51 µL.L ⁻¹	
Citric acid	11.36 mg.L ⁻¹	11.36
Tartaric acid	22.73 mg.L ⁻¹	45.45
Malic acid	22.73 mg.L ⁻¹	22.73
Propionic acid	91.82 µL.L ⁻¹	
Valeric acid	12.22 µL.L ⁻¹	
Hexanoic acid	6.11 µL.L ⁻¹	
Octanoic acid	8.74 µL.L ⁻¹	
Grape skin extract	5.68 g.L ⁻¹	

Table 0.3 The composition of the trace element solution added per litre of the synthetic winery wastewater (Nel *et al.*, 1985)

Trace element	Concentration (mg.L ⁻¹)
CaCl ₂	36
MgCl ₂ .6H ₂ O	24
MnSO ₄ .5H ₂ O	0.241
ZnCl ₂	0.202
H ₂ SeO	0.092
CoCl ₂	0.091
AlCl ₃	0.081
MoO ₃	0.066
H ₃ BO ₃	0.0124
NiCl ₂	0.006
SiO ₂	0.004
Na ₂ WO ₄ .2H ₂ O	0.002



Figure 3.2 The two sizes of MGFP used as biofilm carrier particles in R_{mgfp} **a).** diameter above 1.6 mm **b).** diameter of $< 850 \mu\text{m} < \text{MGFP} < 650 \mu\text{m}$.

3.3.6.2 SCANNING ELECTRON MICROSCOPY (SEM)

The MGFP preparation for SEM analysis consisting of firstly freezing the particles at -80°C . Thereafter, the frozen MGFP were moved to the cold stage of the freeze dryer operating at -71°C for approximately 18 h until dried. The MGFP were then taken to the Central Analytical Facility at Stellenbosch University for analysis by SEM in the back scatter mode. Prior to imaging, the samples were fixed on a carbon glue coated 10 mm aluminium pin stub and then coated with a thin layer of gold in order to establish conductivity. In-lens surface imaging (using a secondary electron type detector) was accomplished using a Zeiss MERLIN FEG[®] Scanning Electron Microscope with a GEMINI II[®] column. Beam conditions during the image analysis were 5 kV, with a working distance of 4 mm and approximately inlens-probe current of 250 pA. The samples were examined at 200 μm , 100 μm , 10 μm and 2 μm magnifications.

3.3.6.3 FLUORESCENCE MICROSCOPY

Fluorescence microscopy was used for a qualitative test to monitor and examine growth and development of biomass and all bacteria and specifically the methanogenic bacteria on MGFP. To achieve this, samples of MGFP had to be removed for analysis at the specific intervals. Therefore, approximately 1 g of the magnetisable particles were extracted from the granular bed using a 9 000 gauss magnetic separator (Sesotec GmbH, Schönberg, Germany). This was done at two months' intervals throughout the trial (2, 4 and 6 months). The MGFP were temporarily stored in a basic test medium (BTM) solution at a pH of 7.0 and at a temperature of 4°C until required for analysis. A fresh, new BTM solution was prepared daily, while the old media was decanted. The 1 g of MGFP was then transferred to a 2 mL Eppendorf and stained to enable viewing of the bacteria around the magnetisable particles. A stock solution of the dye SYTO 9 in dimethyl sulphoxide (5 mM) was diluted in phosphate buffer saline in the ratio 1:1000 (34854, Molecular probes, Oregon, USA). The phosphate buffer solution was prepared by dissolving the salts (Table 3.4) in 800 mL of distilled water and the pH was adjusted to 7.4 using hydrochloric acid (Promega, 2012). About 2-3 drops of the dye was added to submerge the particles in the Eppendorf and incubated for 10 min at room temperature. Subsequently, a fraction of the MGFP (enough to cover the base of the well) was transferred to the 8-well chambered cover glass system (155411, Lab- Tek, Nunc, NY, USA), to preserve the three dimensional shape of the MGFP. Using a LSM780 ELYRA PS1 confocal microscope (Zeiss, Oberkochen, Germany), equipped with a GaAsP detector, image stacks were acquired with a plan apochromat 100x

/ 1.46 oil objective, To observe the methanogens which are auto-fluorescent the sample was excited with a 405 nm laser and emission was detected in the range 476 - 535 nm. Conversely, to observe all bacteria, SYTO 9 was excited with a 488 nm Argon laser and emission was detected between 509 – 646 nm (Zeiss, Oberkochen, Germany). The image stacks with no more than 12 images and with a step width of 1 μm , were captured with ZEN 2012 software at a resolution of 1024 x 1024 pixels resulting in an image size of 84.94 μm x 84.94 μm (Zeiss, Oberkochen, Germany).

Table 0.4 The composition of a phosphate buffer saline solution

Chemical	Amount (g.L ⁻¹)	Concentration (mM)
NaCl	8	137
KCl	0.2	2.7
Na ₂ HPO ₄	1.44	4.3
KH ₂ PO ₄	0.24	1.4

3.3.6.4 GRANULE ACTIVITY

Granule activity was measured according to the method described by O’Kennedy (2000). Initial activity of the granules (Fig. 3.3) received from the anaerobic digesters from James Sedgwick Distillery was determined before seeding the two reactors with these granules. A comparison of the granular activity was performed between the control granules and granules from R_{mgfp} and R_{control} . This was done by sampling granules from both reactors (R_{mgfp} and R_{control}) at the end of the 180 d trial and determining the activity in terms of biogas production and biogas composition. Biogas production was expressed as cumulative biogas while composition was expressed as the percentage methane of the biogas (CH₄%). The methane percentage of the four samples (i.e. control granules, colonised MGFP and granules from R_{mgfp} and R_{control}) was measured after 25 h incubation time. Basic test medium (BTM), glucose test medium (GTM) and acetic acid medium (ATM) were used to measure activity of all bacteria, acidogens and acetoclastic methanogens, respectively. The composition of the media is given in Table 3.5 and Table 3.6.

Prior to the activity test, at a temperature of 35°C, the control, R_{mgfp} and R_{control} granules were incubated for 48 hours in 1 L of activation media (Table 3.7). The activation

media was decanted daily and was replaced with fresh activation media. Following the 48 h incubation, 3 g of each granule sample was transferred to a 20 mL glass vial. The specific test medium (13 mL) was added to the vials leaving 6 mL for headspace. Butyl septa fitted into aluminium caps were used to seal the vials. After filling the glass vials with granules and test media, these vials were incubated for 25 h at 35°C. The biogas volume was measured after 5, 10 and 25 h in a free moving 10 mL syringe fitted with a 12-gauge needle. Biogas volume was measured by inserting the needle through the butyl septa and the volume was measured when the piston had stopped moving. Analysis of the granule activity was performed in triplicate, while the activity of the MGFP samples were analysed in duplicate.



Figure 3.3 The size distribution of the initial granules used in the operation of the UASB reactors.

Table 0.5 Basic test medium (BTM) composition

Compound	Concentration (g.L⁻¹)
Glucose	2.0
Di-potassium hydrogen orthophosphate	1.0
Potassium di-hydrogen orthophosphate	2.6
Urea	1.1
Ammonium chloride	1.0
Sodium sulphide	0.1
Magnesium chloride	0.1
Yeast extract	0.2
pH	7

Table 0.6 Three different test media used to measure the activity of different bacterial consortiums

Compound	Microbial group
Basic test medium (BTM)	Control
Glucose test medium (BTM + 2.0 g.L ⁻¹ glucose) (GTM)	Acidogens
Acetic acid test medium (BTM + 2.0 g.L ⁻¹ acetic acid) (ATM)	Acetoclastic methanogens

Table 0.7 Concentration of the components in the activation media used

Compound	Concentration (g.L⁻¹)
Glucose	1.0
Di-potassium hydrogen orthophosphate	0.5
Urea	0.5

3.3.7 ANALYTICAL METHODS

Total suspended solids (TSS), volatile suspended solids (VSS), volatile fatty acids and alkalinity (as mg CaCO₃.L⁻¹) of the granules, substrate and effluent were measured

according to the Standard Methods (APHA, 2005). The pH of the influent and effluent was measured using a senTix®41 electrode (Altmann Analytik GmbH, Germany). The influent chemical oxygen demand (COD) which ranged from 500 to 10000 mg.L⁻¹ was measured colorimetrically, using a DR2000 spectrophotometer (Hach Co. Loveland, CO). The effluent chemical oxygen demand that ranged from 25 – 450 mg.L⁻¹ was measured by first, filtering it using a Whatman filter paper no. 1, then colorimetrically, using a DR2000 spectrophotometer (Hach Co. Loveland, CO). The chemical oxygen demand of the synthetic winery wastewater solution was measured in Spectroquant® Cell Test kits (Merck, South Africa) using a Nova 60 spectrophotometer. To measure the biogas composition (i.e. methane and carbon dioxide), first, the biogas was withdrawn from the latex pipe connected to the UASB system and then 0.2 mL of biogas was analysed using a Varian 3300 gas chromatograph at a temperature of 55°C (Varian Inc., Palo Alto, CA). Analysis of the biogas composition was carried out in duplicate. The gas chromatography system was fitted with a 2.0 × 3.0 mm i.d. Hayecep Q (Supelco, Bellefonte, PA) 80/100 mesh packed column and a thermal conductivity detector. The carrier gas, helium was at a flow rate of 30 mL.min⁻¹.

3.4 RESULTS AND DISCUSSION

3.4.1 THE EFFECT OF MGFP ADDITION ON UASB REACTOR EFFICIENCY

3.4.1.1 PHASE 1

It can be seen in Figure 3.4 that as the influent COD was gradually increased, from 500 to ca. 2 000 mg.L⁻¹, the COD reduction initially varied between day 0 and 40. During this period, COD reduction ranged from 61.8 – 93.6% in R_{control} and from 68.9 – 97.7% in R_{mgfp}. Although the COD reduction was relatively high sometimes, there was an unsteady COD reduction in both reactors, especially in the control reactor (Fig. 3.4). This unsteadiness is common during start-up, as bacteria will be acclimatising to the new environmental conditions (Rizvi *et al.*, 2015). However, the R_{mgfp} had a more stable COD reduction, possibly indicating a positive influence of the biofilm carrier particles on reactor performance. This could possibly be ascribed to the presence of the iron in the magnetisable particles, which promotes continuous growth of bacteria, specifically the methane-forming bacteria (Ramm *et al.*, 2014). The combination of the iron content and the MGFP surface, which is conducive to biofilm attachment, could possibly have contributed to immobilising some free-floating bacteria, which might otherwise have been washed out, thus slightly improving the reactor stability. In addition, during this start-up, from day 0 – 28, the alkalinity continuously dropped

from 1 300 to 400 mgCaCO₃.L⁻¹ in R_{control} and from 1 375 to 400 mgCaCO₃.L⁻¹ in R_{mgfp} (Fig 3.5). This was presumably attributable to the gradual depletion of the alkalinity. Alkalinity represents the strength of the buffer system, it also indicates the ability of the buffer system to neutralise organic acids that have an inhibitory effect on methane production (De Kock, 2015; Keating, 2015). Alkalinity also possibly gradually decreased because of the rapid and constant production of volatile fatty acids (VFA) (Fig. 3.5). The rapid production of VFA is as a result of a high rate of hydrolysis of organic molecules during anaerobic digestion (Umaiayakanjaram & Shanmugam, 2016). Although the VFA concentration was well within the optimal standard (< 500 mg.L⁻¹) (Ward *et al.*, 2008), the VFA's which were not quickly converted to methane were using up the alkalinity, but not to the point that the pH was affected. The gradual production of the VFA therefore did not cause a pH drop. Figure 3.5 shows that the pH ranged from pH 7.38 - 8.37 in R_{control} and from 7.08 – 8.55 in R_{mgfp}. According to Wijetunga *et al.* (2010), optimal alkalinity ought to be within 1 000 to 3 000 mgCaCO₃.L⁻¹, therefore, during this stage the alkalinity in both reactors was insufficient to optimise the buffer system. In spite of the alkalinity decreasing between day 0 and day 28, the pH remained in the alkaline range in both reactors. The pH ranged from pH 7.38 - 8.37 in R_{control} and from 7.08 – 8.55 in R_{mgfp} (Fig. 3.5), implying that the buffer system in the reactors was high enough to prevent accumulation of VFA which would cause the pH to decrease. However, the alkalinity started increasing from day 29 – 40. The alkalinity gradually increased from 625 to 1 375 mgCaCO₃.L⁻¹ in R_{control} and from 625 – 1 500 mgCaCO₃.L⁻¹ in R_{mgfp}. This increase in alkalinity was also because of the supplementation of the potassium hydrogen carbonate and using the reactor effluent to dilute SWWW stock solution.

Moreover, as the microorganisms adapted to the reactor's environmental conditions, organic acids were efficiently converted to methane and hence there was an increase in alkalinity again. During this period, pH ranged from 7.60 – 7.88 in R_{control} and from 7.51 – 7.83 in R_{mgfp}. The slight increase in pH correlates with the increase in alkalinity concentration since pH and alkalinity are dependent on each other. In addition, due to the reactor instability during the first days of the start-up, biogas composition was only determined from day 28.

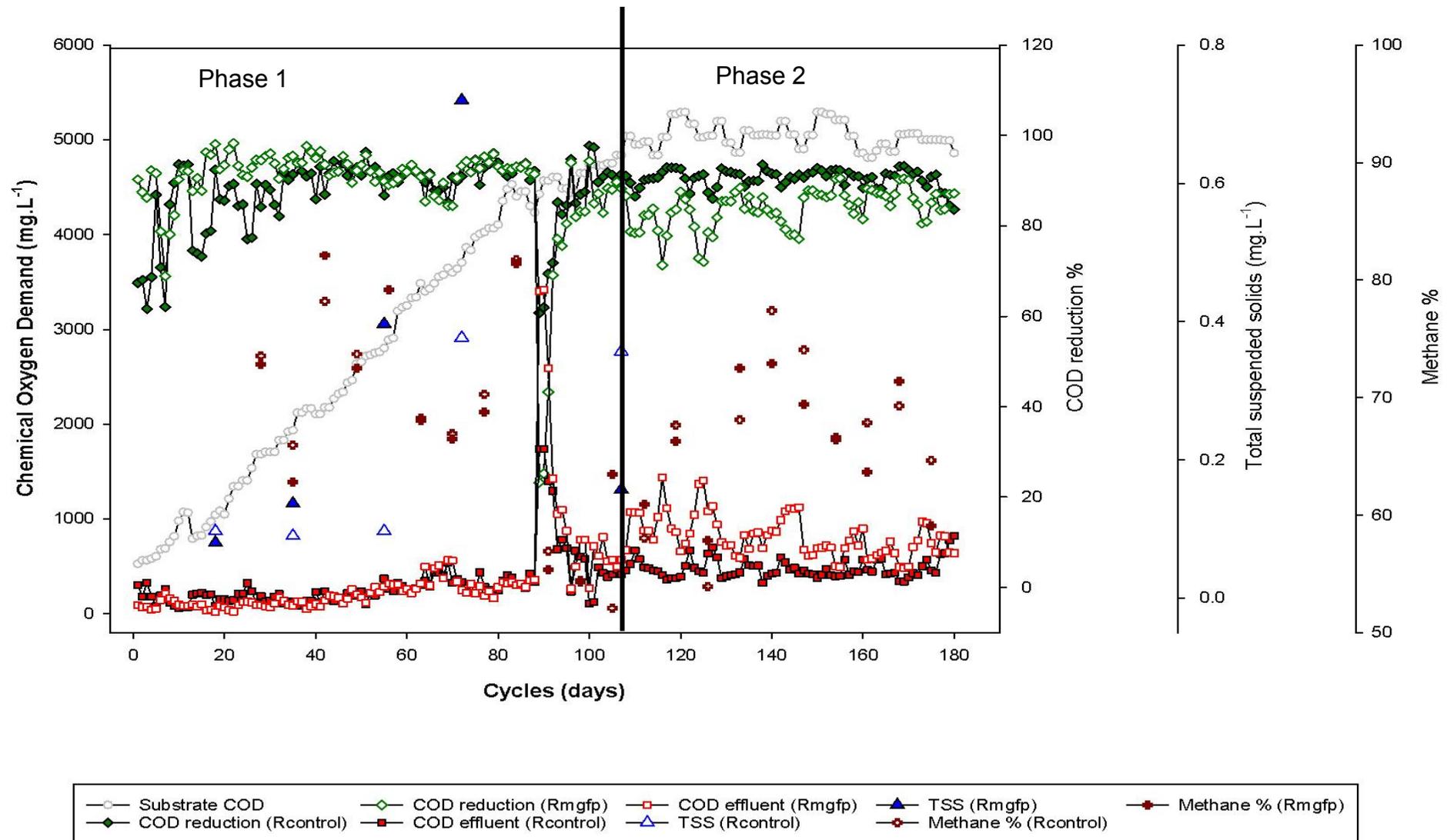


Figure 3.4 Diagrammatic representation of COD substrate, COD effluent and COD reduction in R_{mgfp} and $R_{control}$, methane percentage and total suspended solids' concentration.

The methane percentage from day 28 – 40 ranged between 66 – 74% in R_{control} and 63 – 73% in R_{mgfp} . In spite of the unsteadiness in reactor performance, the methane percentage was well within the optimal standard of 50 – 75% (Khalid *et al.*, 2011; Makadia *et al.*, 2016). The volume of biogas produced could not be quantified reliably as a result of malfunctioning gas meters. The biogas composition was, however, measured regularly. In addition, total suspended solids (TSS) of the effluent was determined whenever the influent COD concentration had increased by a further 1 000 mg.L⁻¹, to determine the amount of solid, fine particulate organic matter. At low organic loading rates (i.e. 0.5 to 2 kgCOD.m⁻³.d⁻¹), there was a relatively low TSS in the effluent (Fig. 3.4). The TSS was 100 mgTSS.L⁻¹ in R_{control} and 80 mgTSS.L⁻¹ in R_{mgfp} on day 25 at an influent COD of 1 000 mg.L⁻¹, but steadily increased to 140 mgTSS.L⁻¹ in R_{mgfp} and remained at 90 mgTSS.L⁻¹ in R_{control} , when the influent COD was 2 000 mg.L⁻¹ on day 55 (Fig. 3.4). The low TSS indicated stable settling properties of the sludge bed and effective breakdown of organic matter. The TSS measured was potentially as a result of the washout of fluffy, less dense biomass. From day 36 - 82 (OLR 2.0 to 4.5 kgCOD.m⁻³.d⁻¹), the influent COD was further increased, step-wise, from \pm 2 000 to 4 500 mg.L⁻¹. Both reactors gradually developed a stable COD reduction, which ranged from 77.1 – 96.0% in R_{control} and 84.4 – 97.7% in R_{mgfp} (Fig. 3.4). There were negligible differences in the COD reduction between the two reactors; however, the R_{mgfp} had a slightly higher COD reduction (Fig. 3.4). This COD reduction indicated the stability of the reactors, further implying that macromolecules were effectively catabolised to produce biogas (Keating, 2015). The stability of the reactors during this stage (day 36 – 82), correlated with the increase in alkalinity in the UASB reactors from day 41 to 74 (Fig. 3.5). Alkalinity gradually increased to 2 750 mgCaCO₃.L⁻¹ in R_{control} and to 2 625 mgCaCO₃.L⁻¹ in R_{mgfp} . The alkalinity of the system was boosted by incorporating potassium hydrogen carbonate, urea and di-potassium hydrogen orthophosphate in the substrate.

Moreover, effluent was used to dilute the SWWW stock solution to also increase the alkalinity. Although optimal reactor pH is 6.5 to 8.0 (Lu *et al.*, 2015), the pH from day 36 - 82 ranged from 7.5 – 8.1 in R_{control} and 7.5 – 8.2 in R_{mgfp} (Fig. 3.5). There was a high pH in both reactors because of the high influent high pH of 7.5, moreover, due to the high strength of the buffer system in the reactors. The high pH had negligible effects on the COD reduction, as the COD reduction remained high (77.1 – 96.0% in R_{control} and 84.4 – 97.7% in R_{mgfp}) (Fig. 3.4). Furthermore, from day 28 - 84 the methane percentage ranged from 67 – 82% in R_{control} , while in R_{mgfp} it ranged from 67 – 81% (Fig. 3.4). However, towards the end of this period, from day 88 – 92, there was a process instability (Fig. 3.4). The COD reduction

drastically dropped from 92.1% to 60.7% in R_{control} and from 91.5% to 23.2 % in R_{mgfp} (Fig. 3.4) and this was due to biomass washout and pipe clogging. Furthermore, this had a negative impact on the production of methane, as it reduced to 56.9% and 55.4% in R_{control} and R_{mgfp} , respectively (Fig.3.5). The TSS concentration had gradually increased in both reactors during the UASB operation; but the TSS was the highest just before the process instability experienced on day 88 – 92. The TSS increased from 100 mgTSS.L⁻¹ on day 55 (influent COD = 3 000mg.L⁻¹) to 380 mgTSS.L⁻¹ on day 72 (influent COD 4 000 mg.L⁻¹) in R_{control} and from 400 mgTSS.L⁻¹ on day 72 (influent COD = 3 000 mg.L⁻¹) to 720 mgTSS.L⁻¹ (influent COD = 4 000 mg.L⁻¹) in R_{mgfp} (Fig. 3.4). This steep increase in TSS from an influent COD of 3 000 mg.L⁻¹ to 4 000 mg.L⁻¹ in both reactors was presumably as a result of the biomass washout caused by the high biogas production. The high biogas production, which was visually observed, caused a turbulence within the reactors. The biomass washout and pipe clogging during this phase was ascribed to the rapid and high production of biogas, which caused the sludge bed to be excessively lifted, further causing the less dense biologically active granules to flow into the recirculation pipe, thus causing clogging and, subsequently biomass washout. Moreover, the biomass washout was as a result of the build-up of pressure in the recirculation pipes due to the accumulation of granules in the pipes, which then fragmented and were washed out. In addition, the notable increase in TSS in the R_{mgfp} was also potentially due to the continuous breakdown of granules as a result of constant abrasion between MGFP and the granules and this was further enhanced by the extra mixing caused by higher biogas production (Tang *et al.*, 2011; Intanoo *et al.*, 2016). This caused more biomass to be washed out increasing the COD of the effluent and thus reduced the COD reduction as biomass was being lost. Although the clogged pipes and COD reduction was quickly resolved, the gas production and shearing of granules caused larger losses of biomass as seen by the high TSS in the R_{mgfp} at an influent COD of 4 000 mg.L⁻¹ on day 72. More so, this reactor disturbance further caused the alkalinity to drop, the concentration started decreasing from day 74 to day 80, dropping to 1 375 mgCaCO₃.L⁻¹ in both reactors as a result of the build-up of intermediary products, i.e. organic acids which were ineffectively broken down (Lu *et al.*, 2015). Due to biomass washout, there was lesser microorganisms to breakdown organic acids; hence, the rate of the production of the VFA was higher than their consumption (Latif *et al.*, 2011; Wang *et al.*, 2015). This consequentially caused the alkalinity to decrease and thus weakened the buffer system (Fig. 3.5). However, from day 92 the reactor performance again improved. The alkalinity increased from 1 375 to 2 550 mgCaCO₃.L⁻¹ in R_{control} and from 1 375 to 2 500 mgCaCO₃.L⁻¹

¹ in R_{mgfp} . The increase in alkalinity was as a result of increasing the concentration of the urea, di-potassium orthophosphate and potassium hydrogen carbonate addition in the influent. In addition, the pH was still in the alkaline range, i.e. 7.82 – 8.36 in $R_{control}$ and 7.85 – 8.29 in R_{mgfp} (Fig. 3.5). Throughout the operation of the UASB reactors, from day 0 to 180, the VFA ranged from 50 – 200 mg.L⁻¹ in both reactors (Fig. 3.4) and was therefore within the optimal standard of < 500 mg.L⁻¹ (Nguyen *et al.*, 2015). The alkalinity dropped again from 2 375 to 1 500 mgCaCO₃.L⁻¹ in both $R_{control}$ and R_{mgfp} , from day 91 – 100 (Fig. 3.5). This decrease in alkalinity was potentially as a result of the high biomass washout in both reactors from day 88 - 92. Biomass washout causes the loss of biologically active bacteria which are responsible for the conversion of intermediary products to biogas. Therefore, upon biomass washout organic acids are rapidly produced and gradually accumulate which reduces the strength of the buffer system, i.e. lowers the alkalinity. On the other hand, the COD reduction started increasing from day 92 – 107, towards the end of the phase. Although the COD reduction was erratic during this period, the COD reduction increased from 71.9 – 97.7% in $R_{control}$ and from 69.1 – 94.3% in R_{mgfp} (Fig. 3.4). This increase in COD reduction was partly attributable to the addition of 50 g of granules to each of the reactors to make up for the losses of biomass during the washout as these granules had good settling ability, there was less biomass washout and a more stable reactor efficiency could be observed.

3.4.1.2 PHASE 2

It can also be observed in Figure 3.4 that as the influent COD reached 5 000 mg.L⁻¹ from day 108 to 180, there was a gradual process stability (Fig. 3.4). The COD reduction continually increased from 82.6 - 93.6% in $R_{control}$ and from 71.4 – 88.5% in R_{mgfp} . Although the COD reduction increased in both reactors, the COD reduction in the R_{mgfp} was now observably lower, relative to the COD reduction of $R_{control}$ in the first phase (Fig. 3.4). Furthermore, the COD reduction was unsteady in R_{mgfp} comparative to the $R_{control}$. This unsteadiness was potentially as a result of the abrasion of the magnetisable particles with granules, which resulted in an increase in TSS, consequently resulting in more biomass being turned to a non-granular form, which is less efficient in reducing COD (Lamprecht, 2009; Wang *et al.*, 2016). Furthermore, alkalinity started increasing and ranged from 1 500 – 2 250 mgCaCO₃.L⁻¹ in $R_{control}$ and 1 625 – 2 375 mgCaCO₃.L⁻¹ in R_{mgfp} . Thus, well within the optimum suggested level of 1 000 to 3 000 mgCaCO₃.L⁻¹. As illustrated in Fig. 3.5, the pH ranged from 7.54 – 8.20 in $R_{control}$ and 7.53 – 8.25 in R_{mgfp} . The high pH (Fig. 3.5) had negligible effects on the COD reduction, as the COD reduction was still relatively high (Fig.

3.4). The high and stable pH (pH = 7.53 – 8.14 in R_{control} and pH = 7.46 – 8.16 in R_{mgfp}) recorded from day 108 – 180 (Fig. 3.5) was due to a strong buffer system, as alkalinity is directly correlated to pH stability (Umaiyanjaram & Shanmugam, 2016). From day 108 – 180, the methane percentage ranged from 52.1 – 77.4% in R_{control} and 54.3 – 72.9% in R_{mgfp} as a result of the high reactor performance (Fig. 3.4). During this phase, on day 107, when the influent COD was $\pm 5\,000\text{ mg.L}^{-1}$, the TSS dropped from 380 to 360 mgTSS.L^{-1} in R_{control} and from 720 to 160 mgTSS.L^{-1} in R_{mgfp} (Fig. 3.4). The decrease in TSS was possibly due to improved and stable operation of the reactors, moreover, due to improved settling properties of the granules left in the reactor.

Therefore, the operation of the UASB reactors for 180 days at an organic loading rate ranging between 0.5 to 5.0 $\text{kgCOD.m}^{-3}.\text{d}^{-1}$ was successful as seen by the high COD reduction (61.8 – 97.7% for R_{control} and 68.9 – 97.7% in R_{mgfp}) throughout most of the study. Furthermore, the volatile fatty acids were kept within the operational standard of less than 500 mg.L^{-1} , while the alkalinity gradually increased from 500 to 3 075 $\text{mgCaCO}_3.\text{L}^{-1}$ in R_{control} and from 450 to 2 625 $\text{mgCaCO}_3.\text{L}^{-1}$ in R_{mgfp} . The pH ranged between 7.60 – 8.36 in R_{control} and from 7.49 – 8.29 in R_{mgfp} . Throughout most of the 180 d of the study, both reactors had an alkalinity and pH within the optimal standard of 1 000 to 3 000 $\text{mgCaCO}_3.\text{L}^{-1}$ and 6.5 – 8.5, respectively (Nguyen *et al.*, 2015; Lu *et al.*, 2015). The methane percentage (52 – 74% in R_{control} and 54 – 73% in R_{mgfp}) recorded in both reactors from 28 – 180, also confirmed that the UASB reactors were operating well within the optimal standard of 50 – 75% methane. The incorporation of the magnetisable glass foam particles was also successful as a biofilm attachment media due to its unique surface characteristics. The MGFP have a high a surface roughness and high porosity (Ramm *et al.*, 2014) which allowed for the adhesion of biofilm. However, towards the end of the UASB reactor operation, day 170 – 180, the R_{mgfp} (80.6 – 90.5%) had a lower treatment efficiency comparative to the R_{control} (83.6 – 93.3%) (Fig. 3.4). This was possibly as a result of a high biogas production which caused constant abrasion of granules against the MGFP, which consequently resulted in biomass washout and thus an increase TSS in the effluent. Therefore, the microorganisms responsible for the conversion of organic molecules to biogas were washed out such that there was a low population of active bacteria that could treat the synthetic winery wastewater. In spite of this, the UASB reactors were successfully operated for 6 months.

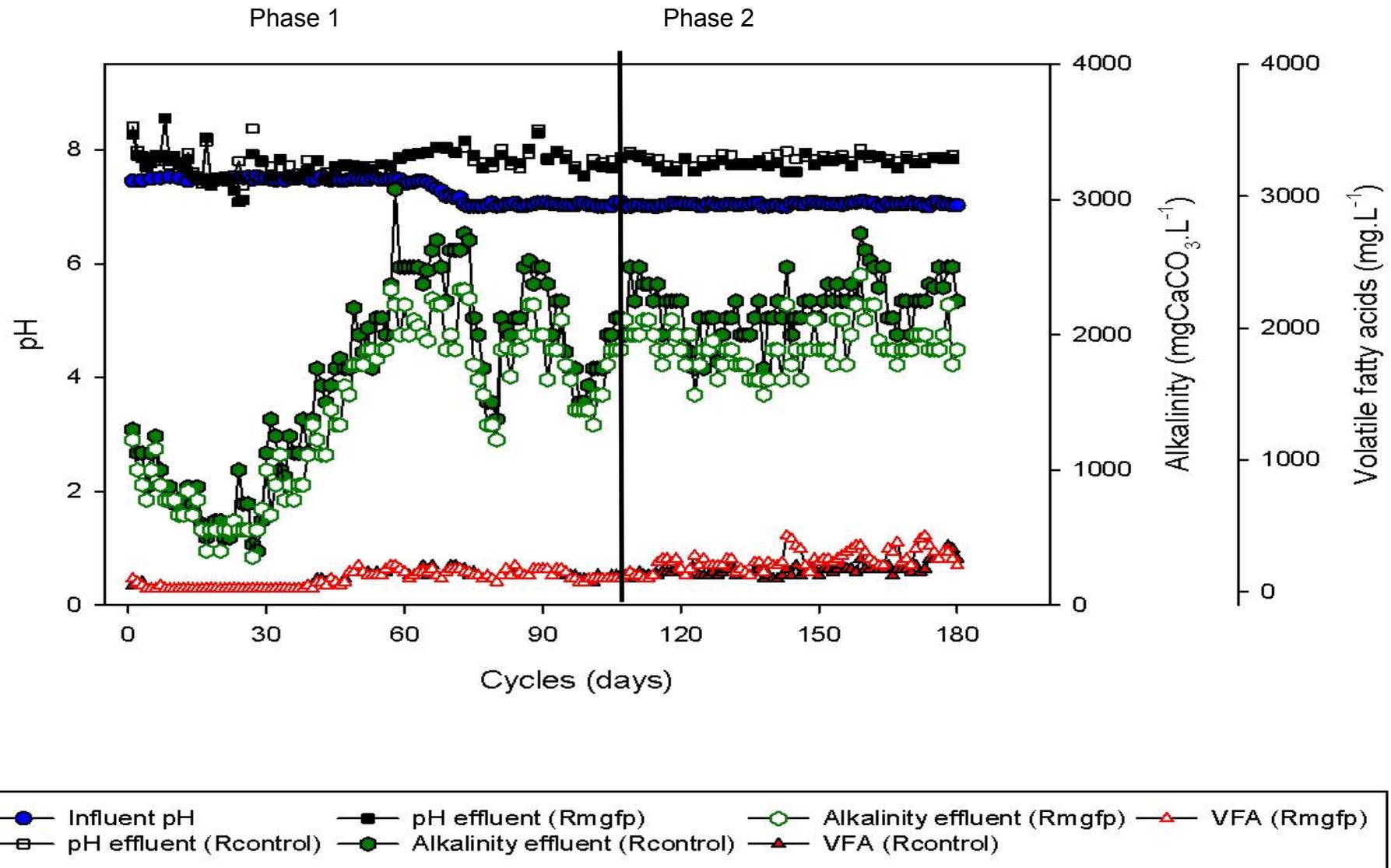


Figure 3.5 Diagrammatic representation of substrate pH, effluent pH and volatile fatty acids and alkalinity of R_{mgfp} and $R_{control}$.

3.4.2 SCANNING ELECTRON MICROSCOPY (SEM)

Scanning electron microscopy in back scatter mode was used to analyse the surface structure of both the granules and the magnetisable glass foam particles. From the images captured by SEM, it was observed that MGFP are biofilm attachment media with an interporous, rough and uneven surface area (Fig. 3.6 & Fig. 3.7). The presence of a rough surface area promotes microbial attachment by lowering the thermodynamic energy needed for initial adhesion (Hellman *et al.*, 2011). Furthermore, MGFP are small which increases the surface area for biofilm attachment. The presence of many pores aids in rapid microbial attachment in the cavities. Therefore, it is presumed that initial microbial adhesion takes place in cavities and crevices of carrier particles due to an increased surface area (Hellman *et al.*, 2011).

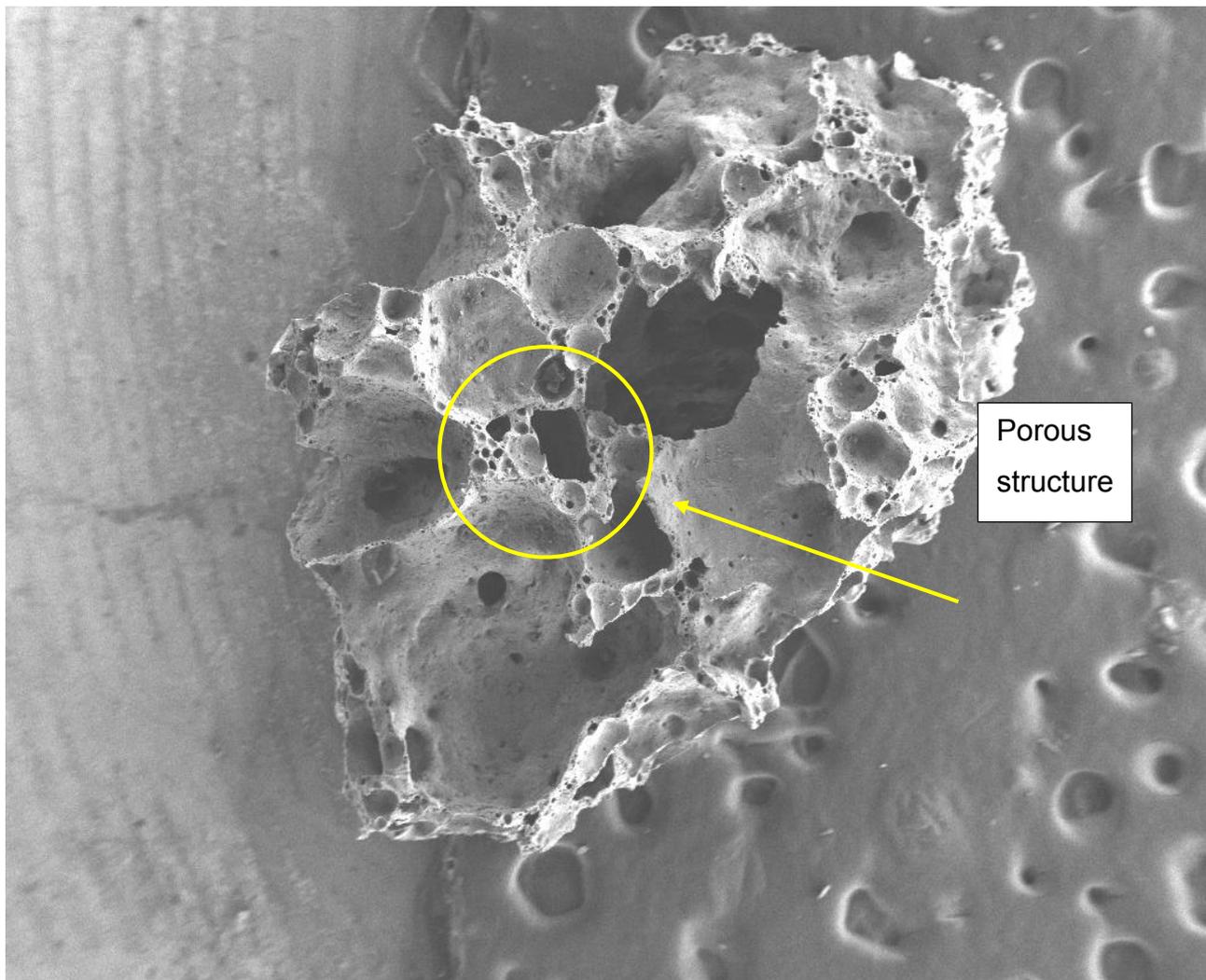


Figure 3.6 The exterior surface of the smaller sized uncolonised magnetisable particle.

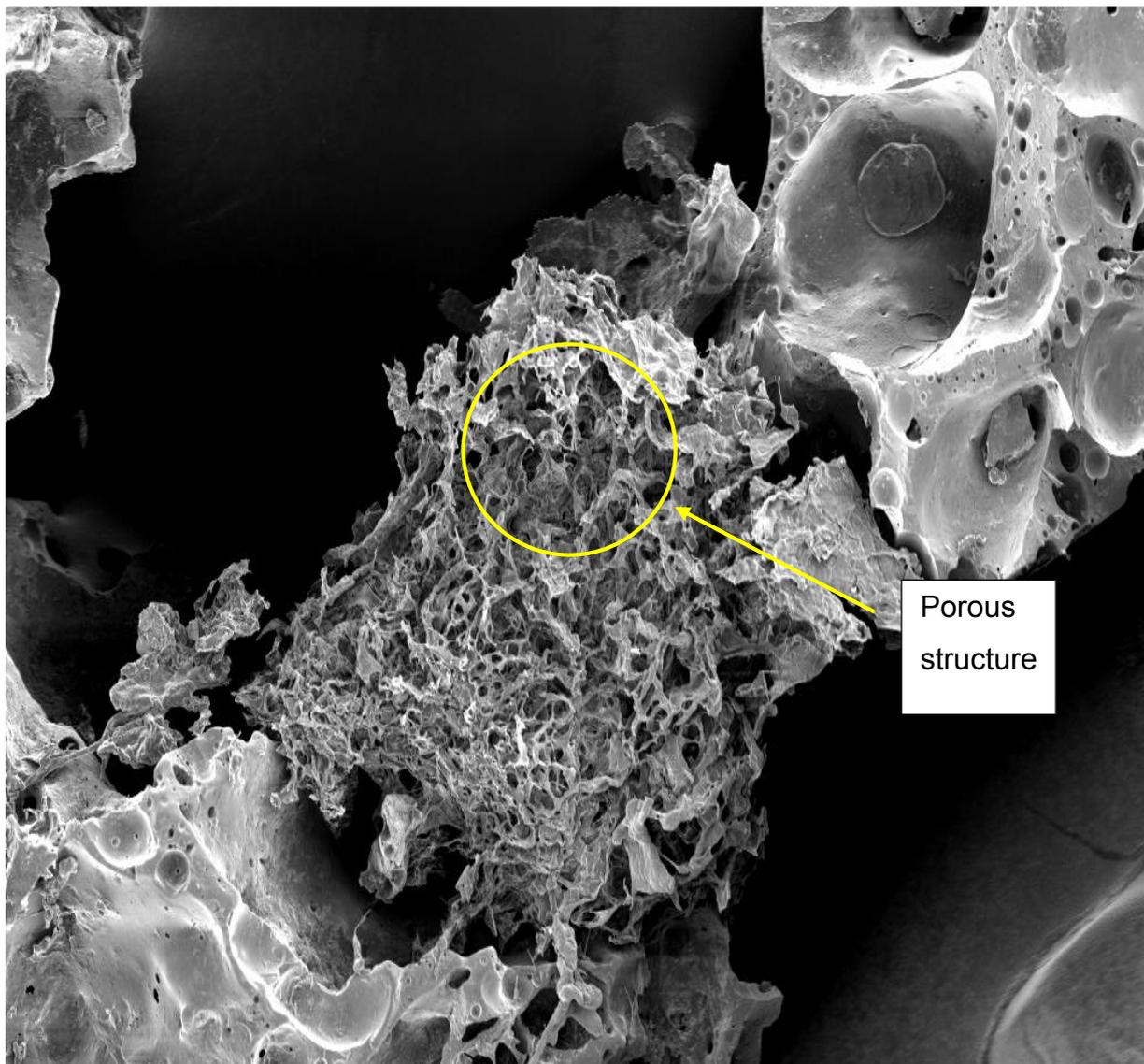


Figure 3.7 Image depicting the exterior surface of the bigger sized uncolonised magnetisable particle showing numerous cavities that could facilitate microbial attachment.

Scanning electron microscopy in the back-scatter mode was also used to analyse the MGFP extracted from the UASB reactor at 2, 4 and 6 months, after seeding. There was a dense biofilm coverage on the 2 months colonised MGFP (Fig. 3.8). Initial microbial adhesion presumably took place in the cavities and crevices of carrier particles due to an increased surface area (Hellman *et al.*, 2011). Consequently, fewer pores can be observed on the magnetisable particle (Fig. 3.8). The biofilm attachment was presumably as a result of the production of high molecular weight macromolecules known as extracellular polymeric substances (EPS) by different microbial species (Wang *et al.*, 2015).

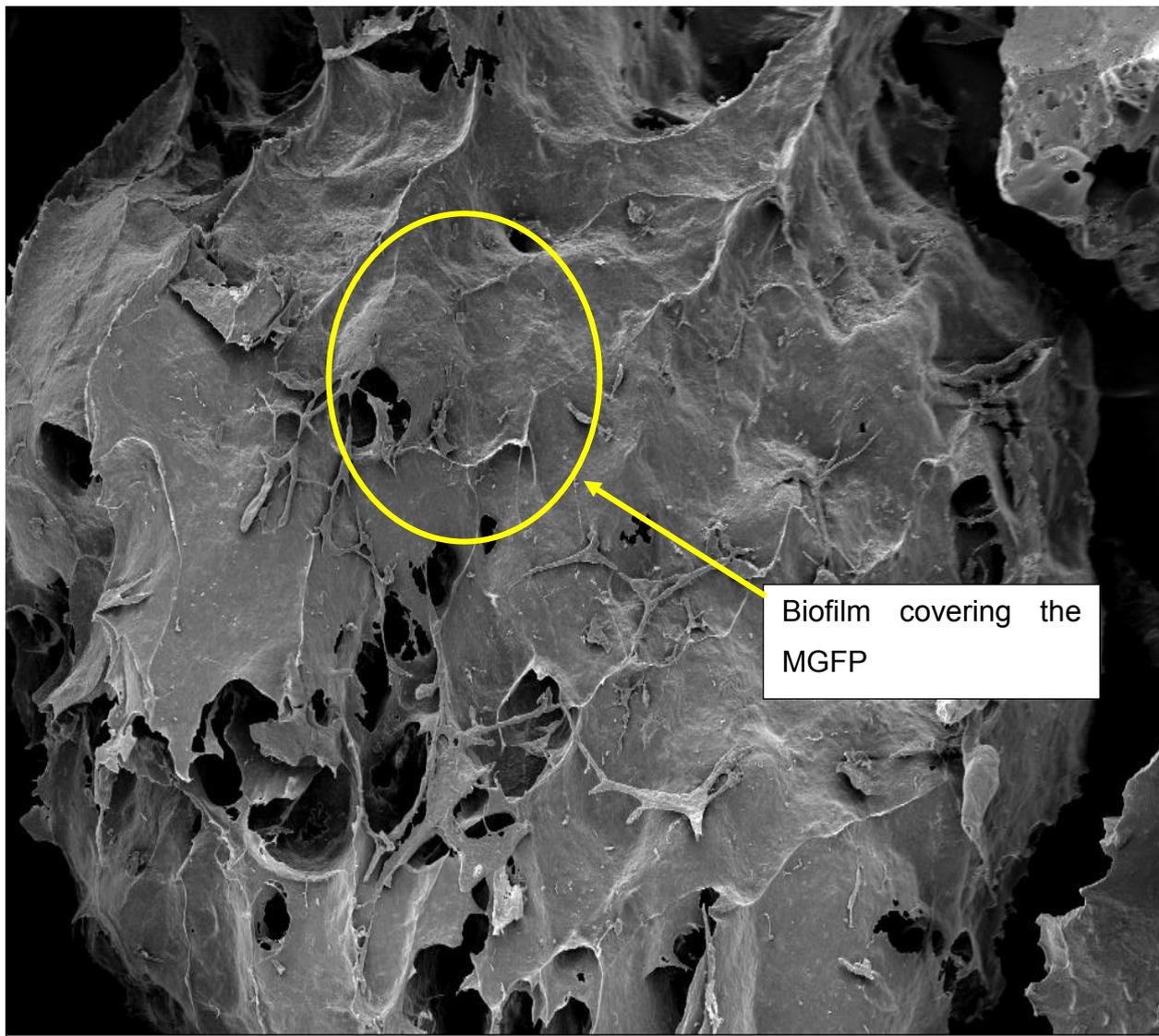


Figure 3.8 The 2 months colonised MGFP extracted from R_{mgfp} .

As the reactor cycles progressed, more EPS was presumably produced and this possibly the reason for the dense biofilm attachment on the magnetisable particles at 4 months (Fig. 3.9). Most of the MGFP were surrounded by a biofilm. There were fewer pores on the surface of the 4 months colonised magnetisable particles compared to at 2 months, which were probably created through the escape of biogas during methanogenesis (Fig. 3.9). Furthermore, due to the continuous growth of biofilm around the magnetisable particles, the other fraction of MGFP that was floating in the reactor at the start of the UASB operation became heavier and sank to the bottom of the reactor. It could be observed that the 4 months colonised MGFP had a denser biofilm compared to the 2 months colonised MGFP (Fig. 3.8 & Fig. 3.9). This was possibly attributable to the increase in growth of the microorganisms, over the 4 months, in the biofilm, which possibly produced more EPS and thus resulted in a denser biofilm around the magnetisable particles.

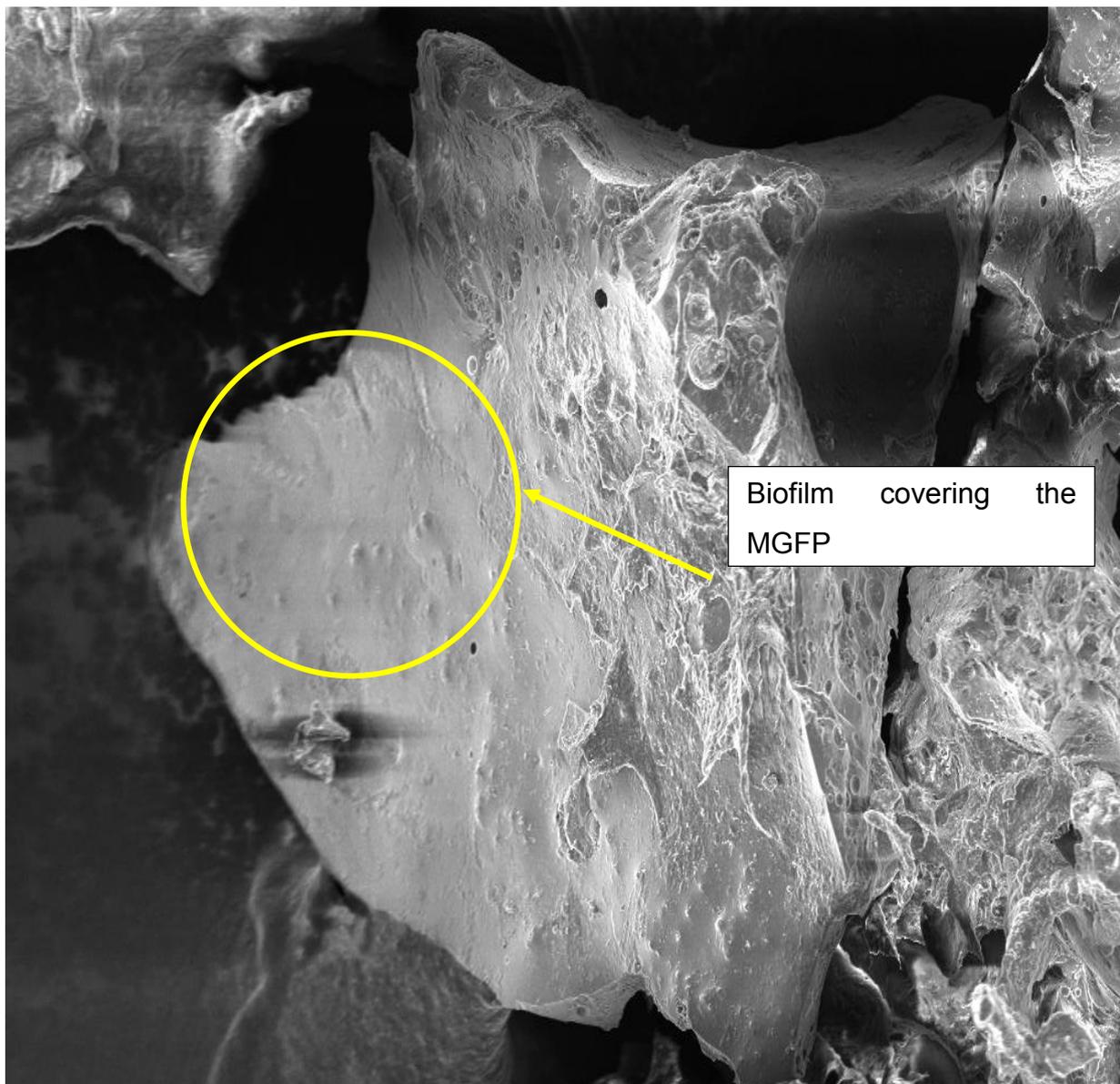


Figure 3.9 The 4 months colonised MGFP extracted from the R_{mgfp} .

However, at 6 months, the biofilm attached to the colonised magnetisable particles was loose and porous (Fig. 3.10). This was highly unexpected as the biofilm around the magnetisable particles was presumed to increase further from 4 to 6 months. The specific biofilm on the 6 months magnetisable particles displayed in Figure 3.10 was less than that of the magnetisable particles shown in Figure 3.8 (2 months MGFP) and Figure 3.9 (4 months MGFP). This porous structure in the 6 months magnetisable particle was presumably as a result of a higher methanogenesis and biogas production rate at 6 months, which resulted in a higher biogas production, which consequently created pores on the biofilm as the gas was escaping from the biofilm (Lu *et al.*, 2015). Furthermore, due to the

high biogas production, the magnetisable particles rapidly bumped into the granules and thus resulted in friction between the two, which further led to abrasion between the carrier particles and the granules. Consequently, this contributed to the detachment of the biofilm surrounding the magnetisable particle.

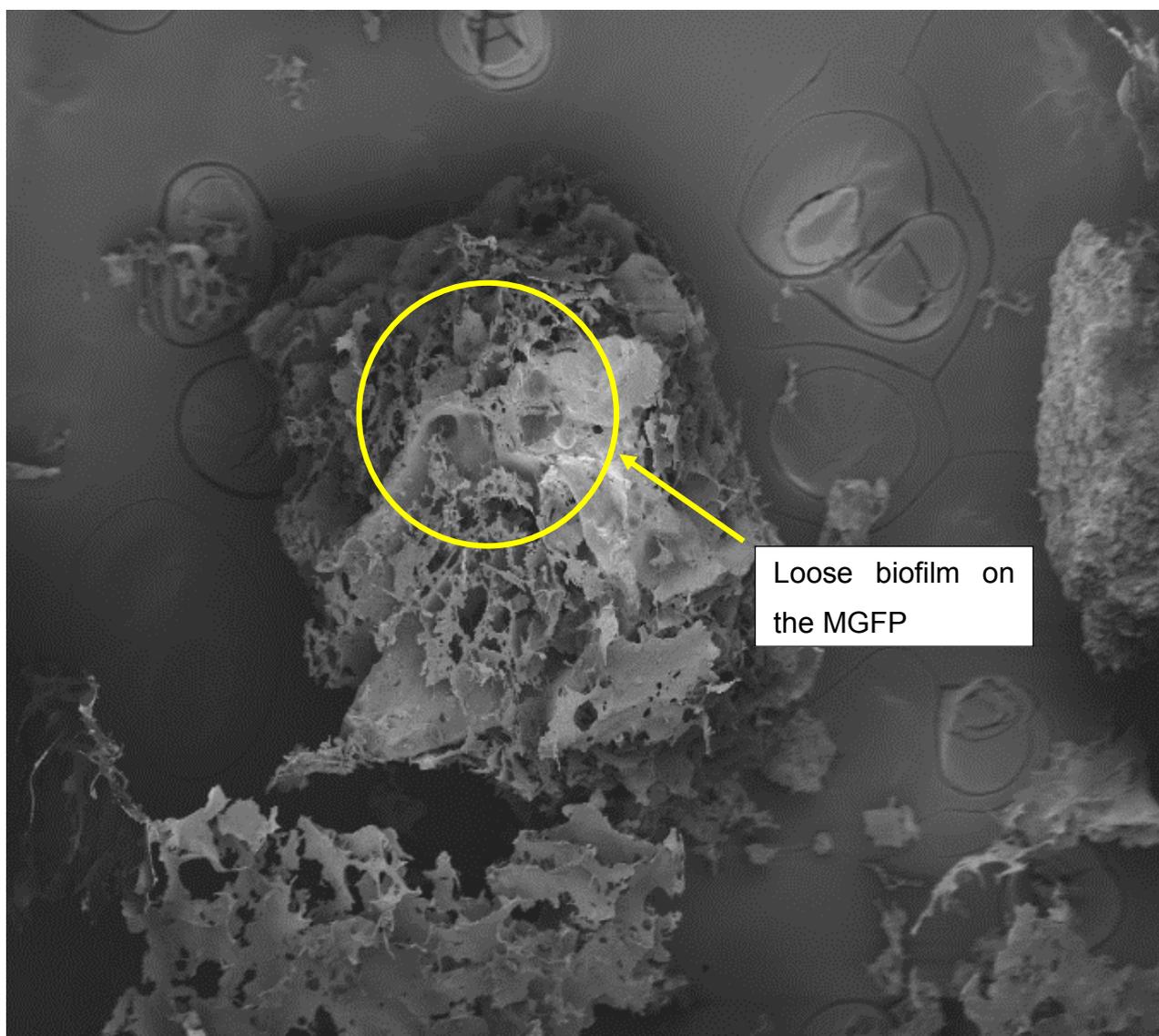


Figure 3.10 The 6 months colonised MGFP extracted from the R_{mgfp} .

Rod-shaped bacteria were observed on 4 and 6 months colonised magnetisable particles (Fig. 3.11 & Fig. 3.12), but more notably on the 6 months colonised magnetisable particles (Fig. 3.12). Rod-shaped bacteria could not be detected at 2 months, possibly because during this period there was a slow microbial growth rate, as microbes were still acclimatising to the reactor conditions. There were more rod-shaped bacteria on the 6 months colonised MGFP (Fig. 3.12) than 4 months MGFP (Fig. 3.11), possibly due to the fact that at 4 months

magnetisable particles were not yet fully colonised. At 6 months, more bacteria had acclimatised to the reactor conditions and thus there was a bigger population of these rod-shaped bacteria (Fig. 3.12). The rod-shaped bacteria were presumably *Methanotrix*, which are commonly found at the bottom of UASB reactors (Mach & Ellis, 2000). where most of the dense granules were situated. The presumable presence of these *Methanotrix* in the reactor possibly suggests that these bacteria were responsible for the granulation or biofilm formation process and facilitating close interaction of microorganisms, thus, consequently resulting in a higher conversion rate of organic matter (Mach & Ellis, 2000).

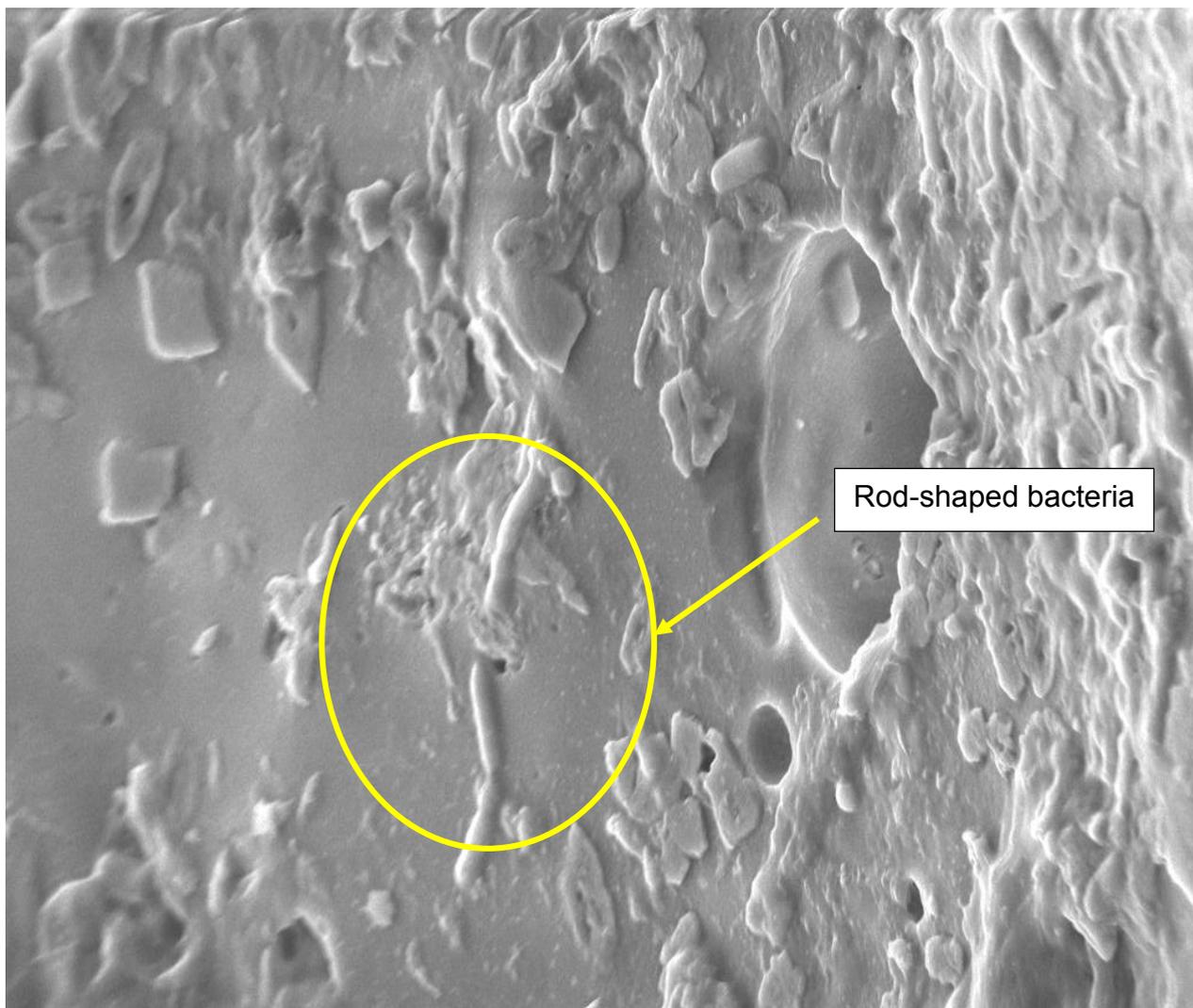


Figure 3.11 The 4 months colonised MGFP showing the rod-shaped bacteria.

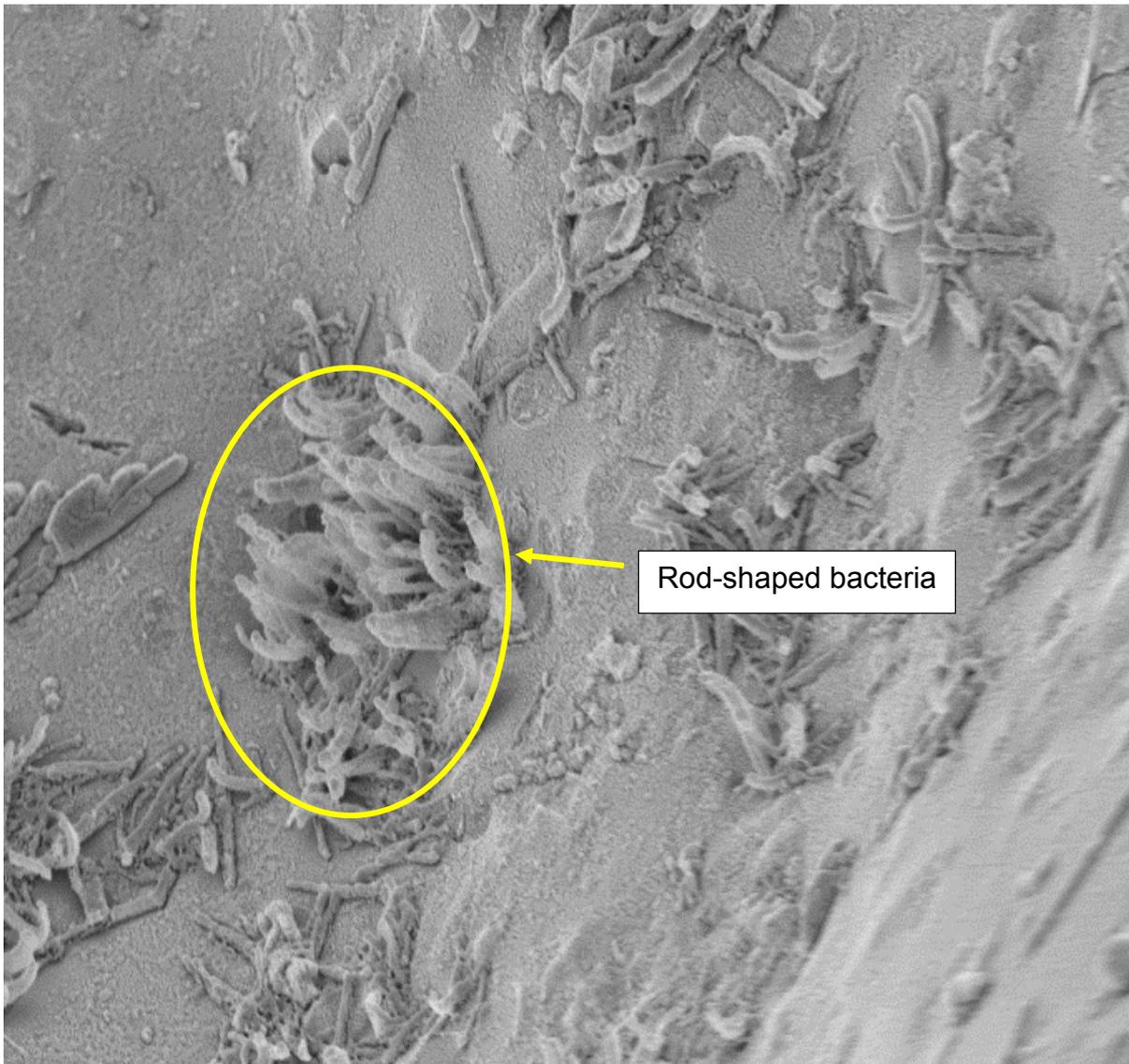


Figure 3.12 The 6 months colonised MGFP showing the rod-shaped bacteria.

The images displayed in Fig. 3.13 shows the initial full control granule. This control granule (Fig. 3.13) has a porous structure and a more distinct circular surface area (Jiang *et al.*, 2015). The presence of pores allows for the transport of the substrate within the granule and escape of biogas from the granule (Jiang *et al.*, 2015).

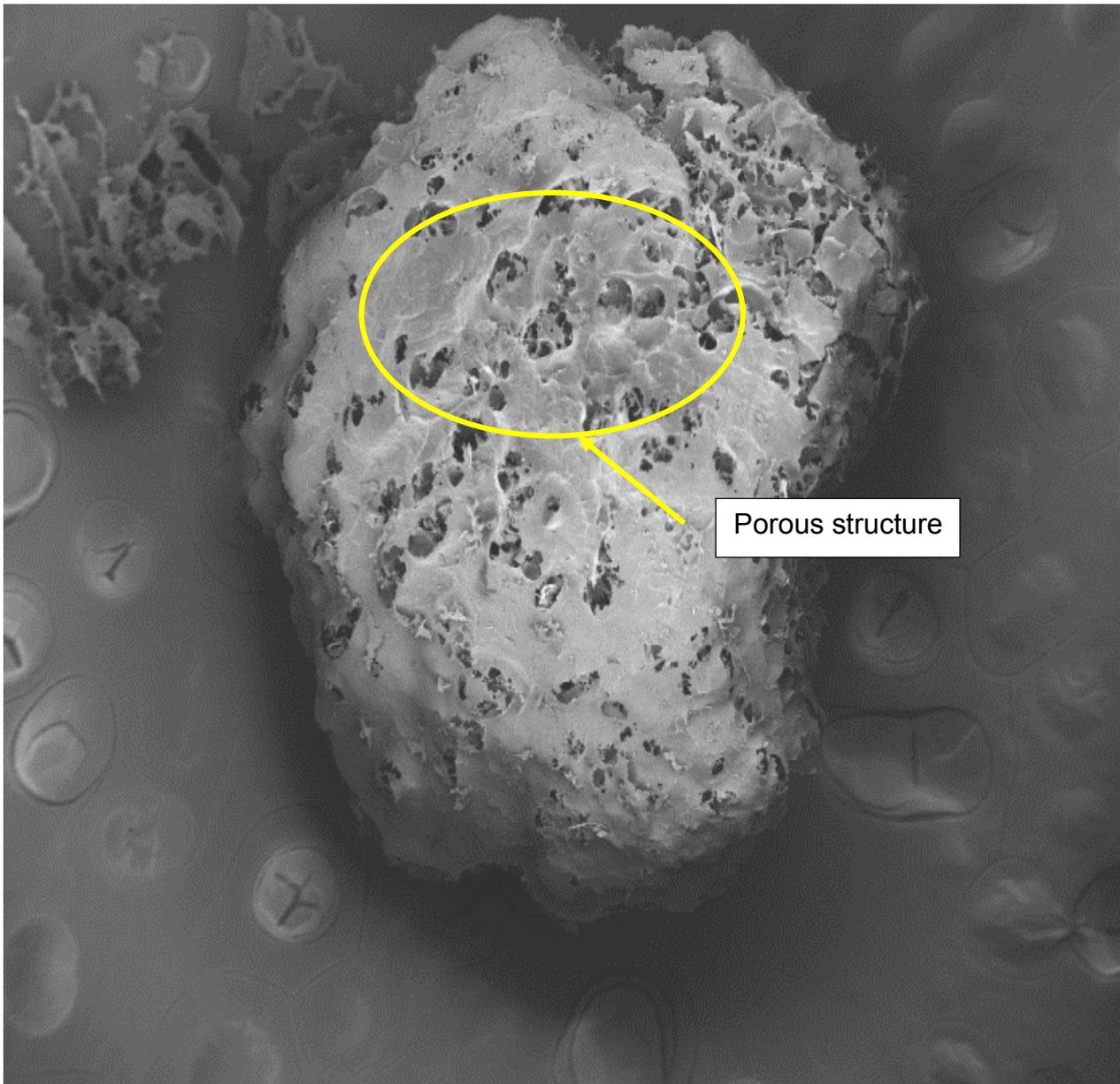


Figure 3.13 The full control granule prior to seeding into the reactor.

Figure 3.14 shows the internal structure of the control granule, upon splitting it in half. The demarcation between the different layers of bacterial groups is not very clear; however, the exterior of the granule where most of the hydrolytic and acidogenic bacteria are located is denser relative to the nucleus (Fig. 3.14).

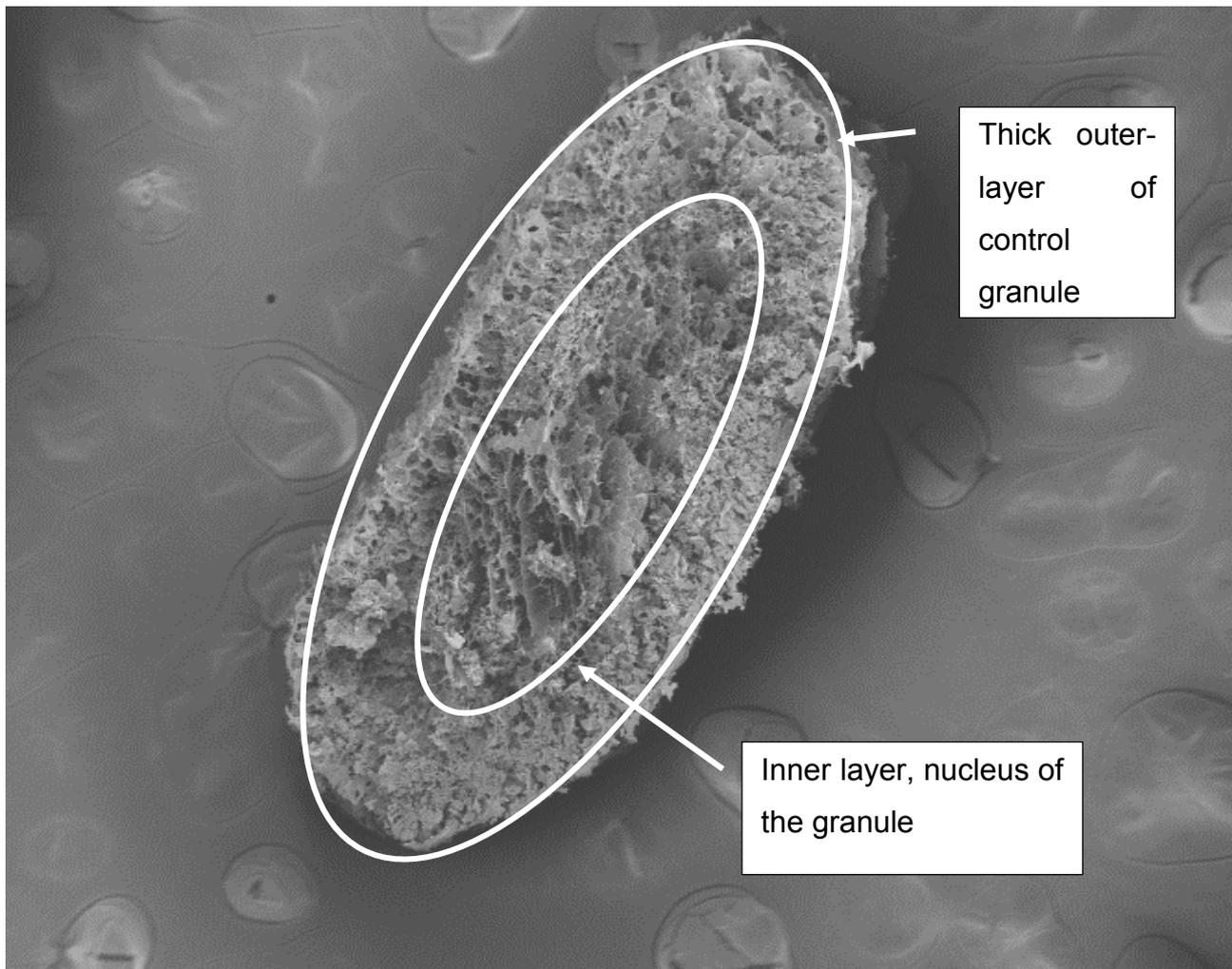


Figure 3.14 The initial control granule showing its internal structure.

Filamentous structures were observed on control granules (Fig. 3.15). The presence of filaments could indicate the presence of extracellular polymer substances produced by a variety of microbes (Wang *et al.*, 2015). *Methanosaeta concilii* and *Methanothrix soehngenii* were possibly the microbes responsible for developing these structures (Melidis *et al.*, 2003). These bacteria are acetoclastic methanogens, which are commonly known to form the nuclei of granules during the first stages of granulation, which further allows for the attachment of other bacteria thus creating a network of filaments that gives stability to the granules (Lamprecht, 2009). *Methanosaeta concilii* is known to be the key bacteria in the granulation process (Hulshoff Pol *et al.*, 2004). These bacteria are also commonly known to produce filaments. The presence of the filaments on the control granules was important as it would potentially give a long-term stability to granules, aid in granulation, give mechanical support to bacteria and facilitate in cell-cell adhesions (Lu *et al.*, 2015; Wang *et al.*, 2015). Moreover, the presence of filaments presumably acted as the matrix upon which the biofilm structure would develop first (Weber *et al.*, 2007). More so, this would subsequently increase

the conversion rate of the organic matter to biogas, as more bacteria would be in close association to each other and work synergistically to breakdown organic matter (Mach & Ellis, 2000). Cocci-shaped bacteria were also seen in the control granules (Fig. 3.15). The coccoid bacteria were presumably the acetoclastic methanogens, i.e. the *Methanosarcina* e.g. *Methanococcus*, *Methanogenium* and *Methanlobus*. In addition, these bacteria were possibly the *Methanosarcina* as they formed clumps through a network of filaments (Lamprecht, 2009). These bacteria are also known to be present from the initial stages of granulation. These bacteria were presumably responsible for the production of 70% of the methane (Jiang *et al.*, 2015).

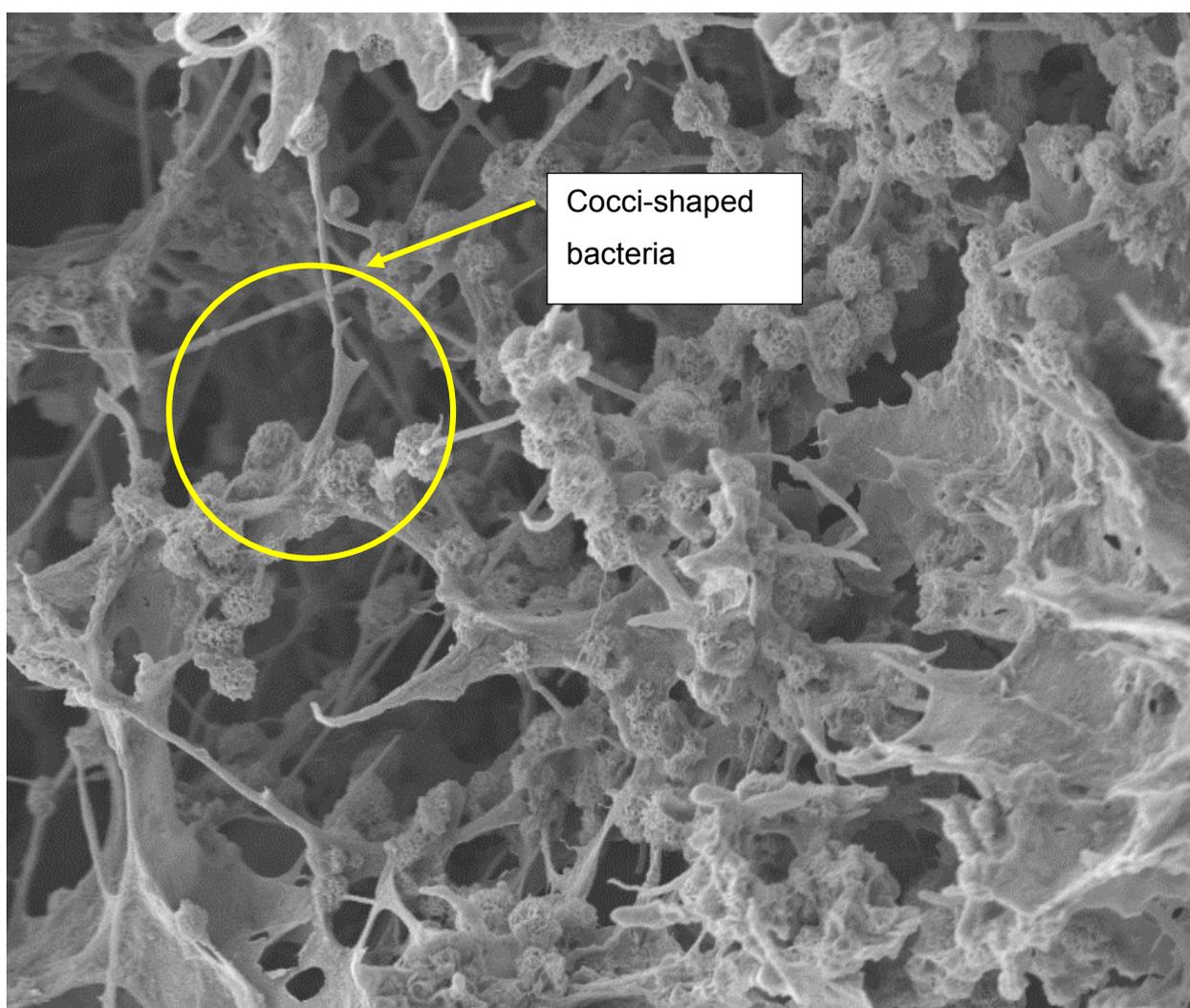


Figure 3.15 The area circled on the initial control granule showing cocci-shaped bacteria interlinked with filaments.

Upon analysing the R_{control} granules with the scanning electron microscope, similar results were observed between the control granules and R_{control} granules (Fig. 3.13 & Fig. 3.16). The control granules had a porous structure with a distinct circular surface area similarly to the

R_{control} granules as seen in Figure 3.8a, the R_{control} granules show a circular, smooth and inter-porous structure. The porous structure allows for the escape of biogas during methanogenesis and for the transport of the substrate within the granule (Herrling *et al.*, 2015).

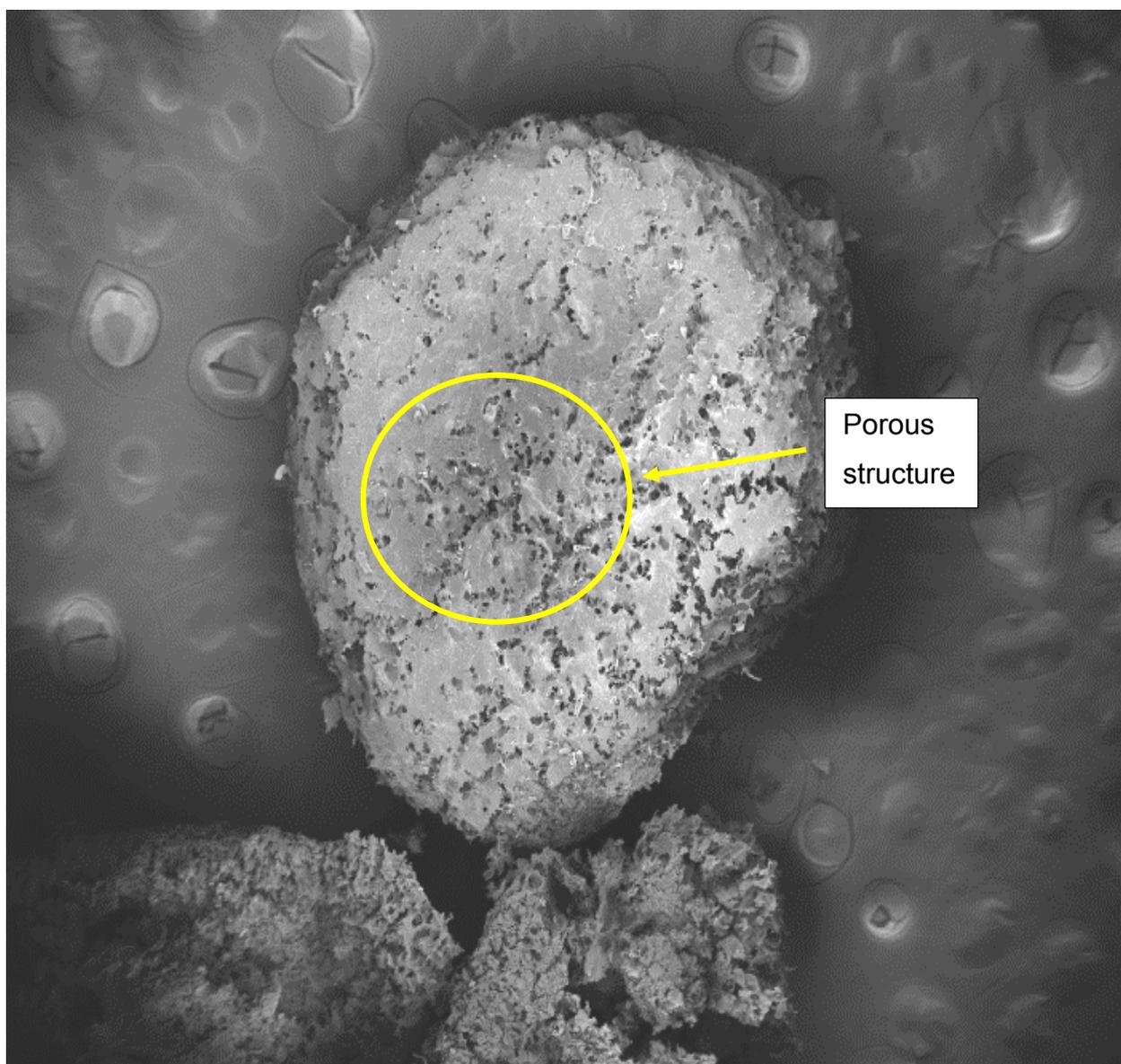


Figure 3.16 The full granule extracted from R_{control} showing a smooth, circular and porous surface area.

There was no visible demarcation between the different physiological groups of bacteria on the granules (Fig. 3.17). Nevertheless, after 180 days, the centre of the granule displayed in Figure 3.17, commonly known as the core zone, was less dense than the exterior of the granules (fringe zone). The fringe zone of granules is commonly associated with the hydrolytic and acidogenic bacteria which are responsible for the breakdown of complex

organic matter and simple molecules, respectively (Jiang *et al.*, 2016). The core zone is commonly associated with the methane-forming bacteria. Therefore, from Fig. 3.17 it can be presumed that the R_{control} granule had different physiological groups of bacteria.

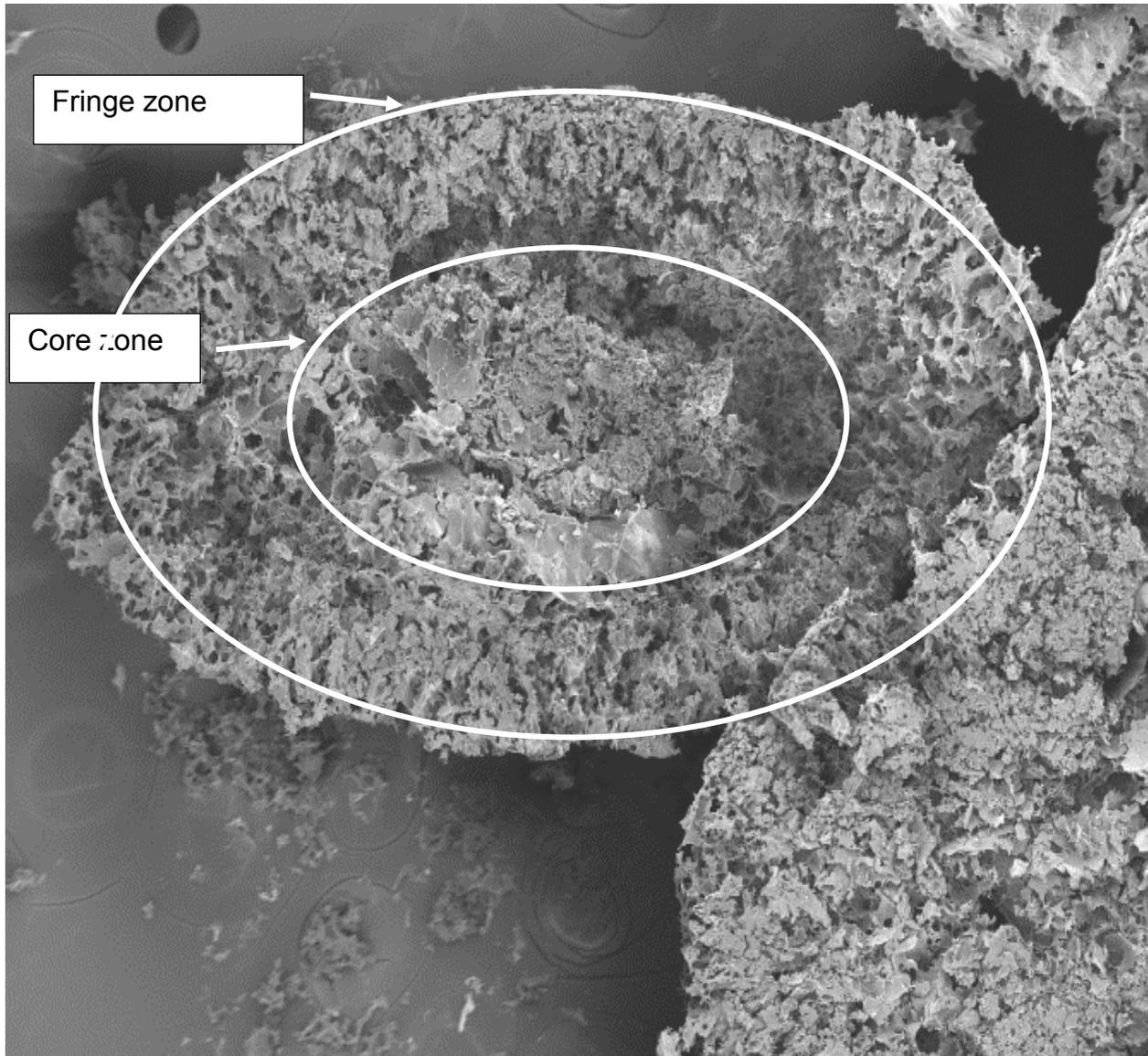


Figure 3.17 The internal structure of the R_{control} granule after 180 days of the UASB reactor operation.

The presence of filaments was also seen in R_{control} granules (Fig. 3.18). The growth of these filamentous structures indicated the presence of extracellular polymer substances produced by bacteria (Wang *et al.*, 2015). The filaments were responsible for giving long-term stability and also facilitated in cell-cell adhesions (Lu *et al.*, 2015; Wang *et al.*, 2014). *Methanosaeta concilii* and *Methanotherix soehngeni* were possibly the microbes responsible for developing these structures (Melidis *et al.*, 2003). These bacteria are acetoclastic methanogens from

the nuclei of granules during the first stages of granulation, which further allows for the attachment of other bacteria through the network of filaments created, which gives stability to the granules (Lamprecht, 2009). The presence of the network-like structure of filaments also allowed for the transport of substrates from one microorganism to the other (Lu *et al.*, 2015). *Methanosaeta concilii* is known to be the key bacteria in the granulation process (Hulshoff Pol *et al.*, 2004). Hence, the R_{control} granules still had a round and firm structure after the 180 days of UASB reactor operation.

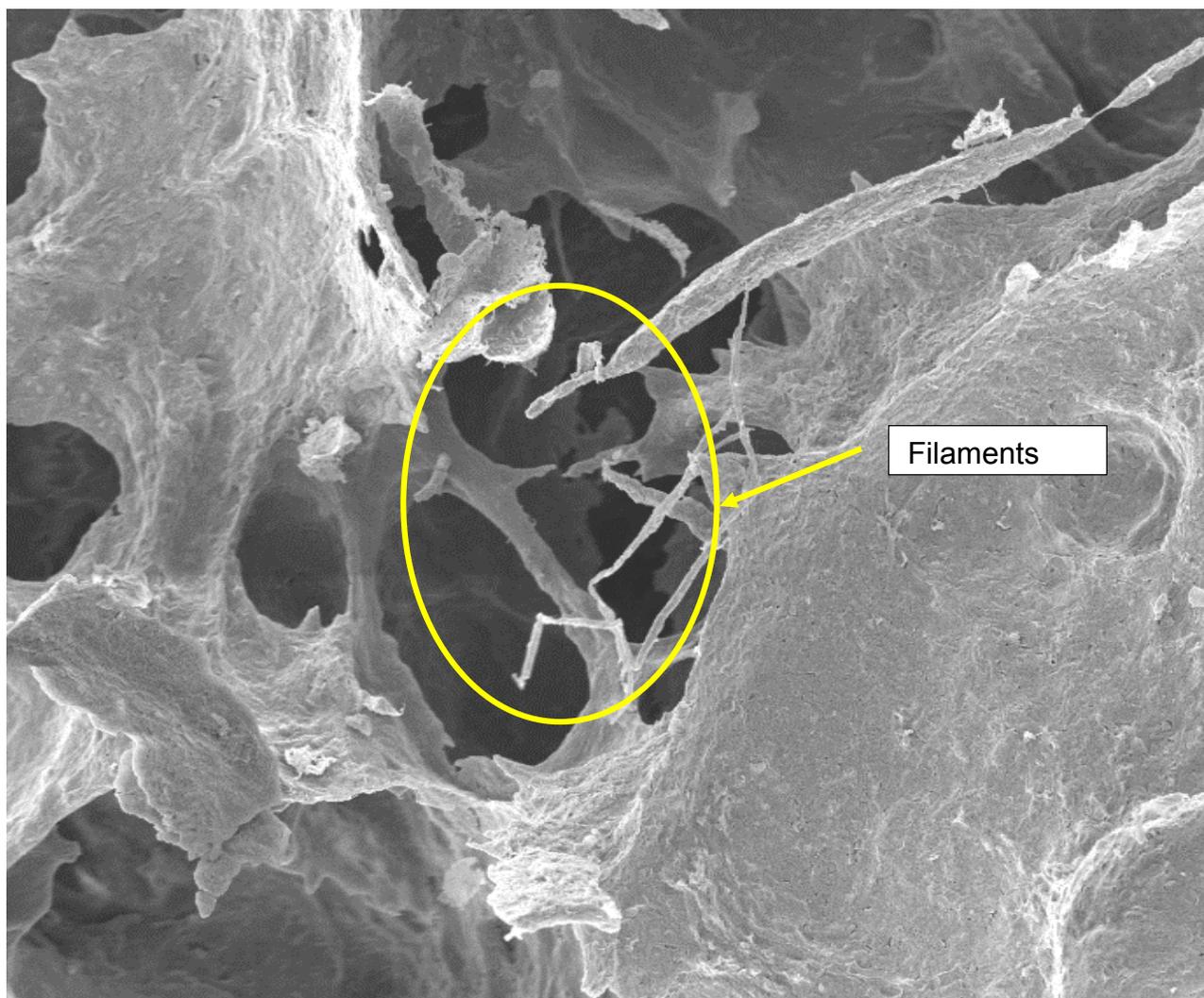


Figure 3.18 The circled area showing filamentous structures in the R_{control} granule.

Due to the fact that the granules recovered from the R_{mgfp} were disintegrated to flocs due to the abrasion between them and the magnetisable particles, it was difficult to isolate individual granule-like structures, therefore, only flocs were isolated (Fig. 3.19). Flocs have a disadvantage in the operation of UASB reactors as they have a lower methanogenic rate and also they have poor settling abilities which then results in biomass washout (Lamprecht,

2009). Figure 3.19 is dissimilar to images of other complete granules (Fig. 3.13 & Fig. 3.16). Fig. 3.19 shows the disintegrated MGFP extracted from the R_{mgfp} , showing a striated surface structure. Figure 3.13 and Figure 3.16 shows the initial control granule and $R_{control}$ granule, respectively, both of the granules show a smooth, round and porous surface area unlike the R_{mgfp} granule.

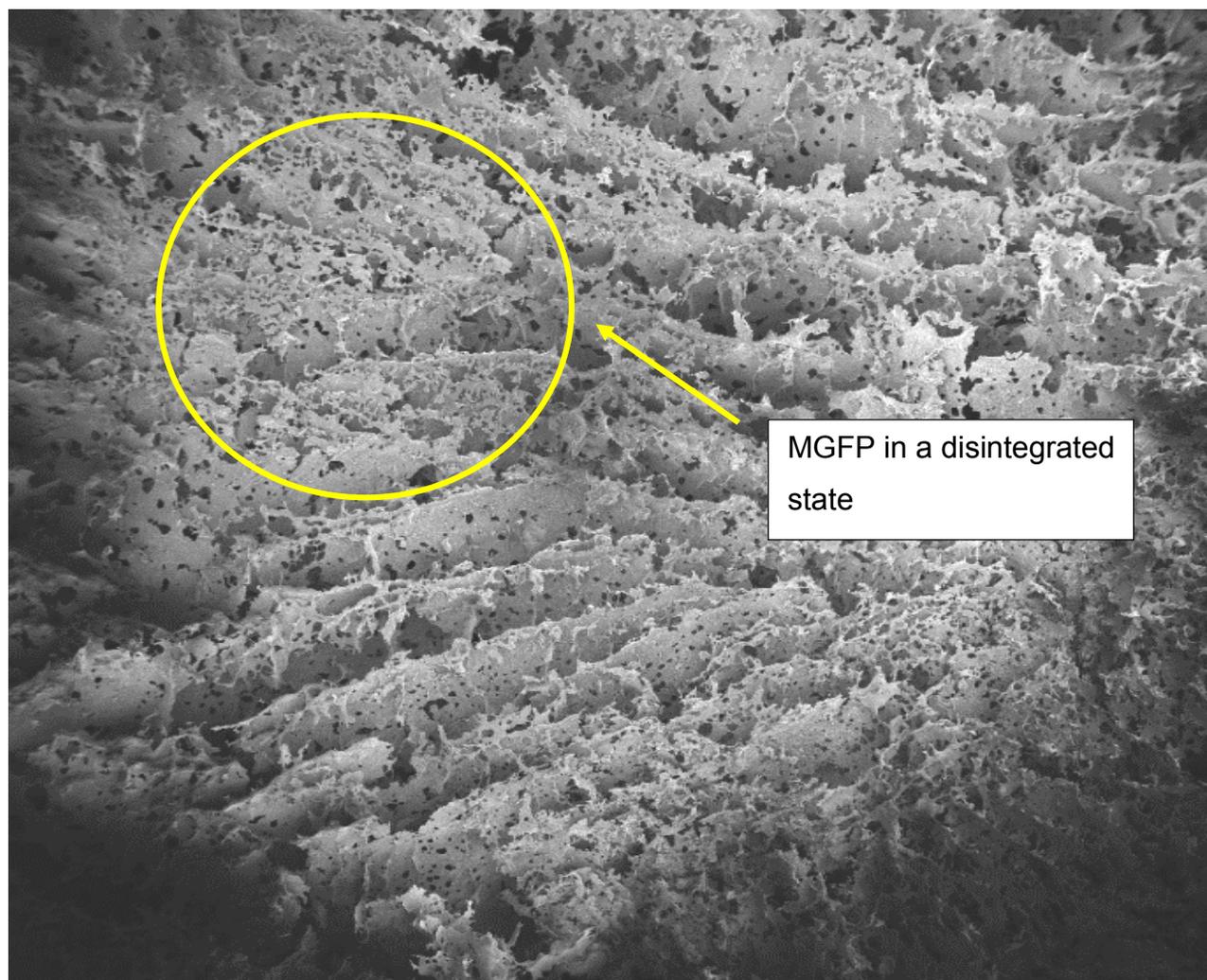


Figure 3.19 The surface structure of the R_{mgfp} granules showing its fragmented state after the UASB operation.

In spite of the condition of the granules, coccoid and rod-shaped bacteria were also present on R_{mgfp} granules after the 180 d of UASB reactor operation (Fig. 3.20). The rod-shaped bacteria were presumably *Methanotrix*, which are commonly found at the bottom of the reactor (Lu *et al.*, 2015), were the majority of the dense granules and carrier particles were situated. While the coccoid bacteria were presumably the *Methanosarcina* e.g. *Methanococcus*, *Methanogenium* and *Methanolobus*. Nevertheless, it was observed that the

majority of the bacteria were rod-shaped (Fig. 3.20). These bacteria are part of the acetoclastic methanogens that might have been responsible for the production of methane during methanogenesis (Latif *et al.*, 2011). Furthermore, these bacteria are known to be present during the initial stages of granule development and form the basis upon which other bacteria can attach (Lamprecht, 2009). In addition, it was possibly these aforementioned groups of bacteria, due to the higher concentration of volatile fatty acids in R_{mgfp} (Fig. 3.5), which presumably was acetic acid that promotes the growth of these bacteria (Lamprecht, 2009), thus more rod-shaped bacteria could be observed.

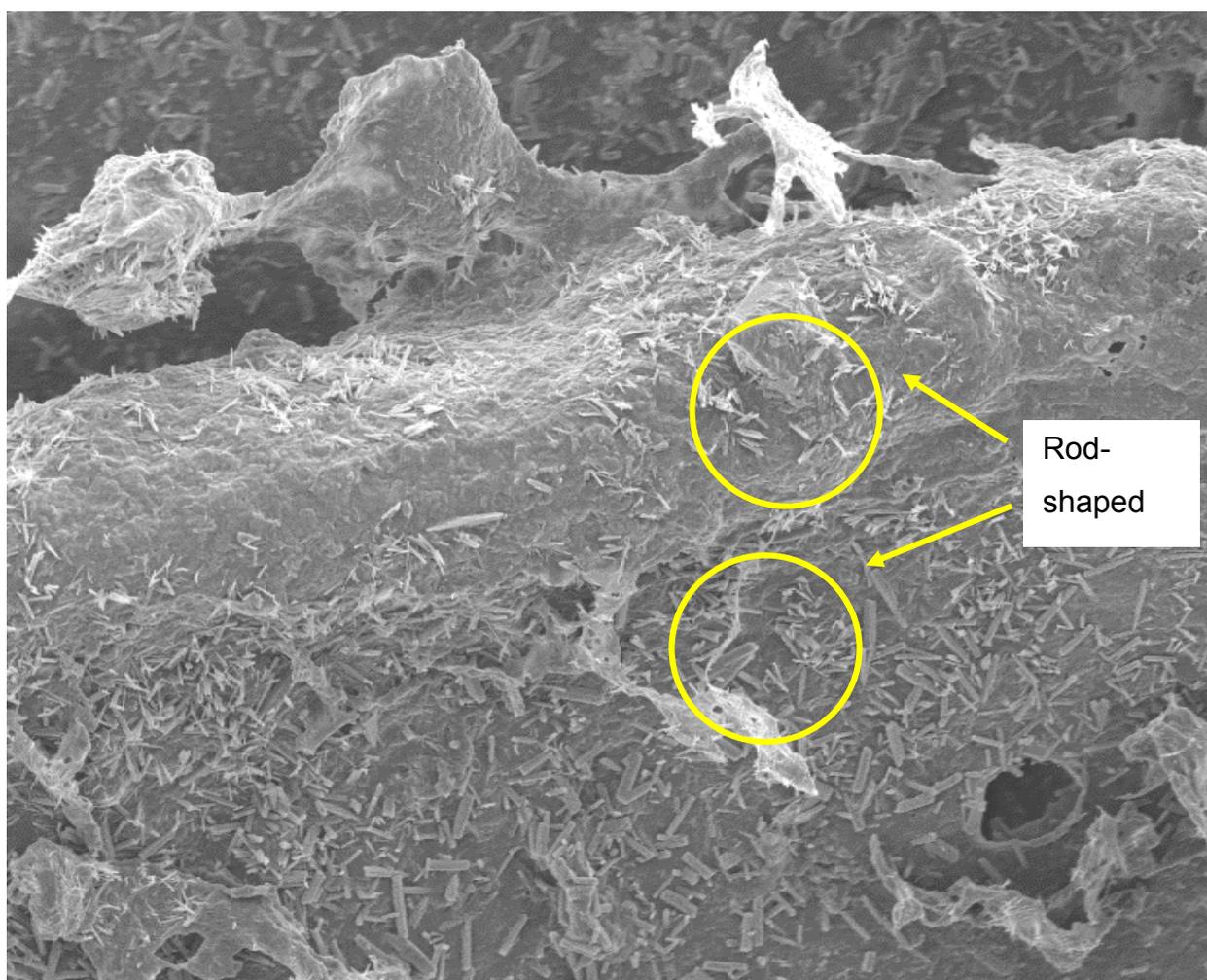


Figure 3.20 The circled areas showing the rod-shaped bacteria surrounding R_{mgfp} granule.

3.4.3 FLUORESCENCE MICROSCOPY

Colonised magnetisable glass foam particles were analysed after 2, 4 and 6 months. SYTO 9 dye was used to stain the MGFP to observe growth of microorganisms while methane-forming bacteria were observed by triggering auto-fluorescence. Within these periods, i.e.

2, 4 and 6 months, rod and cocci-shaped methane-producing bacteria and normal bacteria were observed (Fig. 3.21; Fig. 3.22; Fig. 3.23). Figure 3.21 shows colocalisation at 2 months, indicating the spatial distribution of bacteria (green) and methanogens (blue) on the colonised MGFP. It could be seen that at 2 months the population of methane-forming bacteria was lower than that of the other bacteria, as seen by green fluorescence, which was more dominant than the blue fluorescence (Fig. 3.21). This was presumably because of the slower growth rate of methanogens hence there was more green fluorescence (Abbasi & Abbasi, 2012). Moreover, the green fluorescence was as a result of the other bacteria, which include the hydrolytic, and acidogenic bacteria, which have a rapid growth rate (Lu *et al.*, 2015). The growth of bacteria was attributed to a rough and large surface area on the magnetisable particles, which allowed for the adhesion of the bacteria onto the MGFP (Ramm *et al.*, 2014). Furthermore, the upflow velocity of the substrate was at low rate, which facilitated for the gradual attachment of bacteria on the magnetisable particles (Abbasi & Abbasi, 2012). In addition, the substrate, synthetic winery wastewater that composed of high amounts of simple sugars that acted as the food source of the bacteria and thus allowed for their growth. The blue auto-fluorescence indicated the presence of methane-forming bacteria (Fig. 3.10a-c). Auto-fluorescence is triggered by the presence of coenzyme M, coenzyme F₄₂₀ and methanopterin, which participate in the reduction-oxidation metabolic processes in methanogenic cells (Kim *et al.*, 2014; Pronk *et al.*, 2015).

Figure 3.22 shows the colonised magnetisable particles at 4 months, with blue fluorescence more dominant than the green fluorescence. As the reactor cycles progressed, after 4 months the growth of methanogens had increased as they had acclimatised to the reactor environmental conditions. The rod-shaped bacteria were possibly from the *Methanosarcina* group, which includes the *Methanobacterium* and *Methanobrevibacter* (Kedar, 2014; Stantscheff *et al.*, 2014). The *Methanosaeta* group, on the other hand, possibly represented coccoid bacteria (Melidis *et al.*, 2003; Kedar, 2014). Conversely, green fluorescence (Fig. 3.10a-b) was observed after incubating MGFP stained with SYTO 9 dye, for 10 min. The green fluorescence corroborated the presence of any other bacteria other than methanogens (Valle *et al.*, 2015). The bacteria in Fig. 3.22 could have possibly have been rod and cocci-shaped as highlighted by Kedar, 2014. It is evident that the population of methanogens considerably amassed from 2 months until 6 months' operation, as shown by the dense methanogenic growth after a period of six months (Fig. 3.23b & Fig. 3.23c). More methanogens could be observed at 4 and 6 months (Fig. 3.22 & Fig. 3.23b) possibly due to the presence of iron in the magnetisable glass foam particles that promoted the

growth of these bacteria. Moreover, due to the stable operating conditions of the UASB reactor, the continuous growth of methanogens was achieved.

At 6 months, in spite of the disintegrated state of the colonised MGFP, there was a higher growth bacteria and methanogens comparative to the magnetisable particles at 4 and 6 months. Figure 3.23a-c shows green fluorescence, blue auto-fluorescence and colocalisation, respectively. At 6 months, the population of methane-forming bacteria around the MGFP was more dominant than the other bacteria and this was observed upon colocalisation (Fig. 3.23d). This was highly expected because of the presence of iron in the magnetisable particles, which enhances the growth of methane-forming bacteria (Melidis *et al.*, 2003; Pronk *et al.*, 2015). The higher growth of bacteria was attributable to the continuous growth of the bacteria throughout the UASB operation. Again, rod-shaped and cocci bacteria were observed on the magnetisable particles (Fig. 3.23a-c).

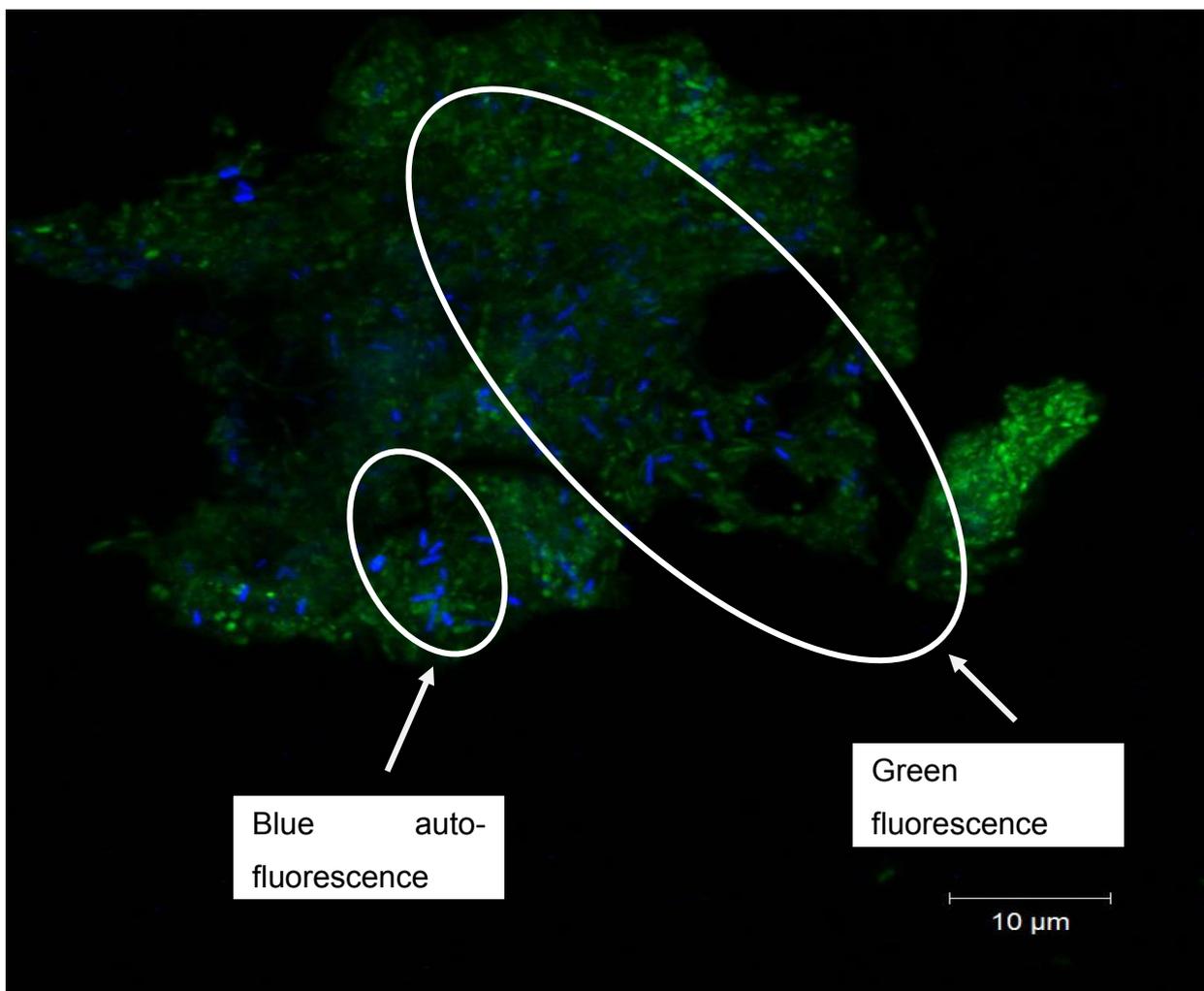


Figure 3.21 Fluorescence microscopy image of the colonised magnetisable particles at 2 months.

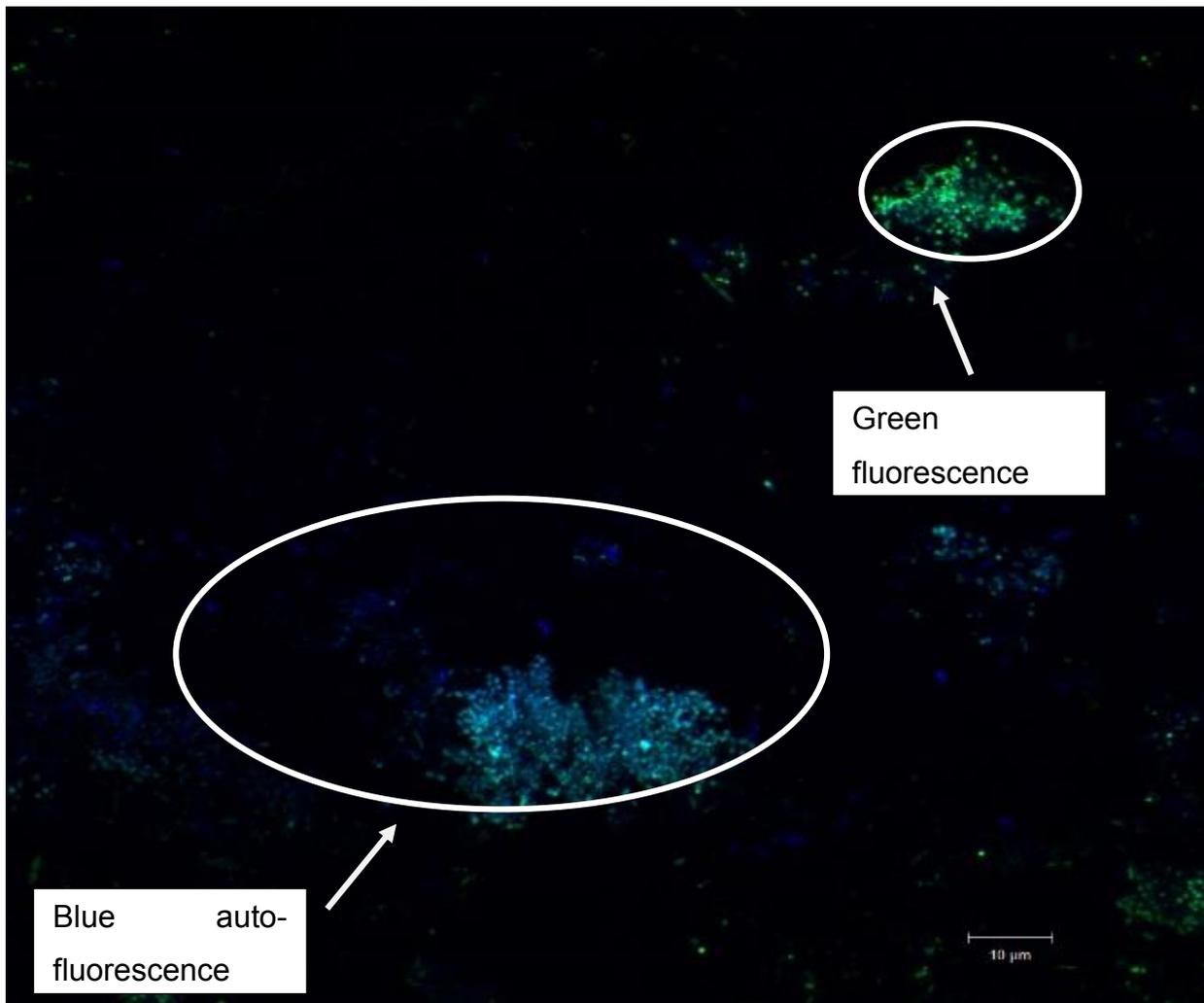


Figure 3.22 Fluorescence microscopy image of the colonised magnetisable particles at 4 months.

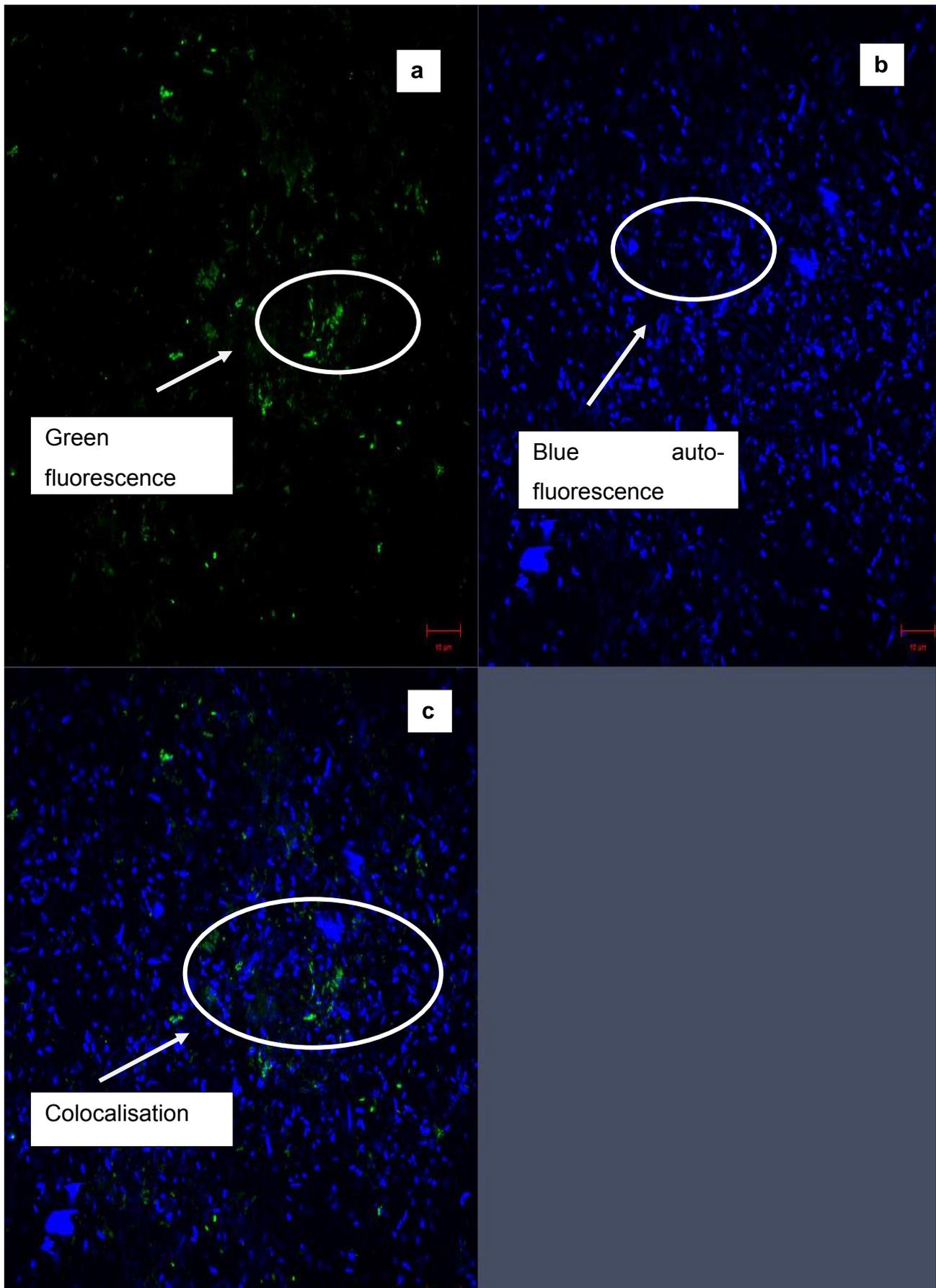


Figure 3.23 The 6 months colonised MGFP showing **a).** Green fluorescence and **b).** Blue auto-fluorescence **c).** Colocalisation

3.4.4 GRANULE ACTIVITY TESTS

Granule activity tests were performed after 180 d of operating the UASBs to determine the biological activity of the biofilm surrounding the magnetisable particles, granules from the control and MGFP reactors and compare them to the initial activity of the control granules (at day 0) using standardised activity tests. These tests were done by measuring the biogas production and composition (carbon dioxide and methane content) using three different test media, i.e. basic test media, glucose test media and acetic acid media.

3.4.4.1 BASIC TEST MEDIA (BTM)

Basic test media solution was used to measure the general activity of the microbial consortiums in the samples (Van Der Weisthuizen, 2014). Figure 3.24 depicts the average cumulative biogas volume produced over 25 h using basic test media results of the activity tests carried out in BTM. At 5 h the colonised MGFP (9.9 mL.gVSS^{-1}), R_{mgfp} granules (8.1 mL.gVSS^{-1}), control granules (9.3 mL.gVSS^{-1}) and R_{control} granules (6.9 mL.gVSS^{-1}) had a similar biological activity as evidenced by the overlapping error bars. However, as the incubation time progressed to 10 h, the colonised MGFP ($12.7 \text{ mL.gVSS}^{-1}$) and R_{mgfp} granules ($11.5 \text{ mL.gVSS}^{-1}$) had a comparatively similar biological activity while the control granules ($10.1 \text{ mL.gVSS}^{-1}$) and R_{control} granules (9.8 mL.gVSS^{-1}) had a similar biological activity. After 25 h incubation time, the biological activity in descending order was colonised MGFP ($14.5 \text{ mL.gVSS}^{-1}$), R_{mgfp} granules ($12.5 \text{ mL.gVSS}^{-1}$) then lastly the R_{control} granules ($10.6 \text{ mL.gVSS}^{-1}$) and control granules ($10.5 \text{ mL.gVSS}^{-1}$) which had a similar biological activity (Fig. 3.24). Therefore, colonised MGFP had the highest biological activity relative to the other samples thus suggesting that it possibly had the most active bacteria after the UASB reactor operation. Overall, the colonised magnetisable particles after 25 h had the highest biological activity, potentially as a result of the presence of a high iron content in the carrier particles which enhances the methanogenesis rate (Ramm *et al.*, 2014). In addition, the R_{mgfp} granules had a relatively higher biological activity than the control and R_{control} granules because there were closely associated with the magnetisable particles, which contain iron, which promotes the growth of the methane-forming bacteria. Therefore, the R_{control} granules and control granules had the lowest biological activity possibly as a result of the absence of the iron which promotes growth of methanogens.

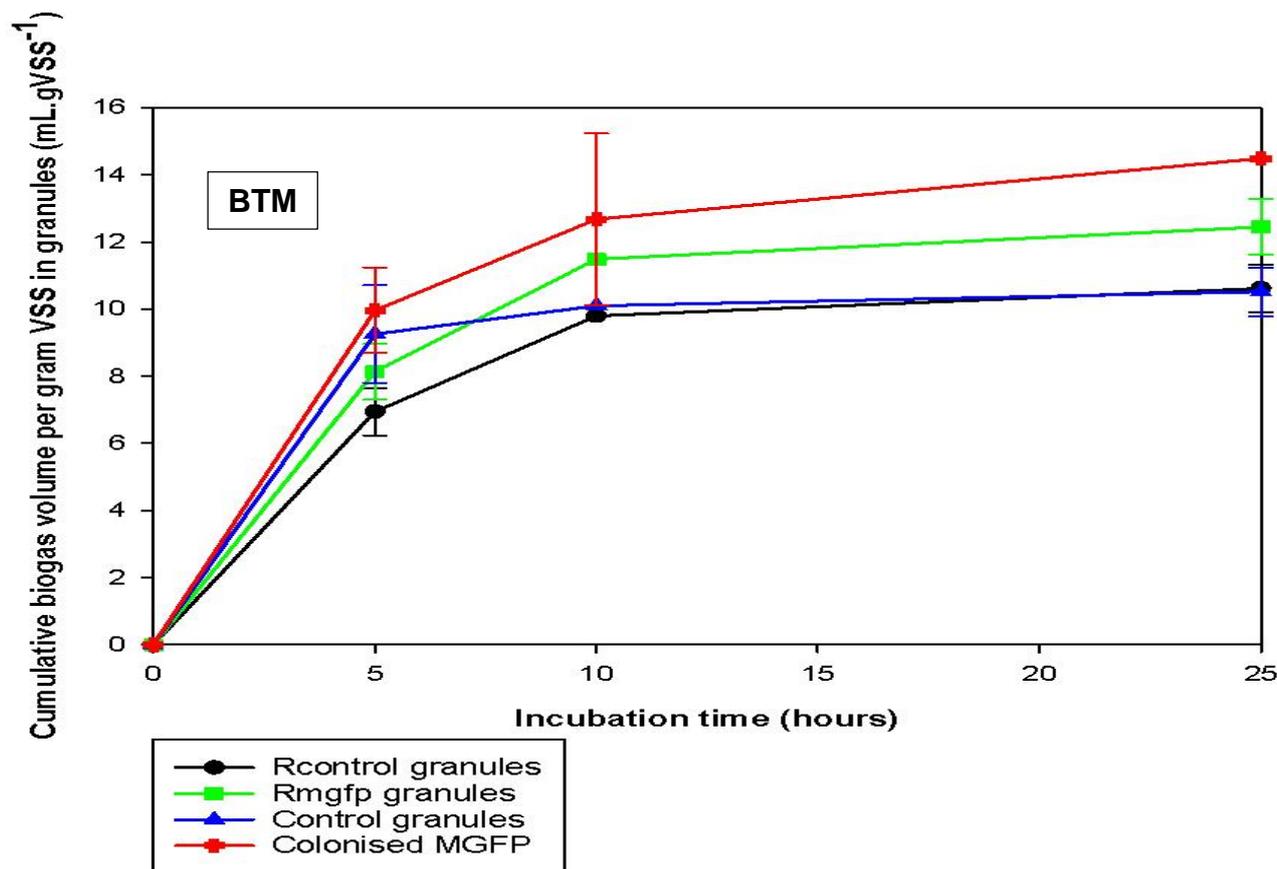


Figure 3.24 The average cumulative biogas volume produced over 25 h using basic test medium.

3.4.4.2 GLUCOSE TEST MEDIA (GTM)

Glucose test media solution was used to measure the activity of the acidogenic bacteria, which convert simple organic molecules to volatile fatty acids and alcohols during acidogenesis (Cameroon, 2000). Figure 3.25 depicts the average cumulative biogas volume produced over 25 h using glucose test media. From Fig. 3.25, after 5 h, the colonised MGFP ($14.5 \text{ mL.gVSS}^{-1}$), R_{control} granules ($13.9 \text{ mL.gVSS}^{-1}$), R_{mgfp} granules ($12.5 \text{ mL.gVSS}^{-1}$) and control granules ($10.9 \text{ mL.gVSS}^{-1}$) all had a similar biological activity, i.e. acidogenic activity. However, the control granules ($14.3 \text{ mL.gVSS}^{-1}$) had the lowest acidogenic activity after 10 h, while there were minimal differences among the R_{mgfp} ($21.6 \text{ mL.gVSS}^{-1}$), R_{control} granules ($21.7 \text{ mL.gVSS}^{-1}$) and colonised MGFP ($21.7 \text{ mL.gVSS}^{-1}$). This indicated that after 10 h the biological activity in the R_{mgfp} , R_{control} granules and colonised MGFP was similar (Fig. 3.25). After 25 h the colonised MGFP ($29.0 \text{ mL.gVSS}^{-1}$) also had the highest biological activity thus indicating that it possibly had the highest population of active acidogens while the control granules ($16.8 \text{ mL.gVSS}^{-1}$) had the lowest population of these bacteria. Moreover, although

the colonised MGFP had the highest acidogenic activity, the biological activity between the R_{mgfp} granules ($24.9 \text{ mL.gVSS}^{-1}$) and $R_{control}$ granules ($23.9 \text{ mL.gVSS}^{-1}$) was relatively similar (Fig. 3.25). In addition, since GTM contained an additional 2.0 g.L^{-1} of glucose, a higher biological activity was reported compared to the BTM results (Fig. 3.24; Fig. 3.25). Therefore, the high biological activity recorded for the colonised MGFP is possibly attributable to presence of iron, which also promotes the growth and activity of acidogenic bacteria (Herrling *et al.*, 2015). Although the R_{mgfp} granules ($24.9 \text{ mL.gVSS}^{-1}$) and $R_{control}$ granules ($23.9 \text{ mL.gVSS}^{-1}$) had a similar biological activity, the R_{mgfp} granules had a relatively higher biological activity. This was attributable to the possible close association of the R_{mgfp} granules with the carrier particles, which contain iron that promotes the activity of acidogenic bacteria (Herrling *et al.*, 2015).

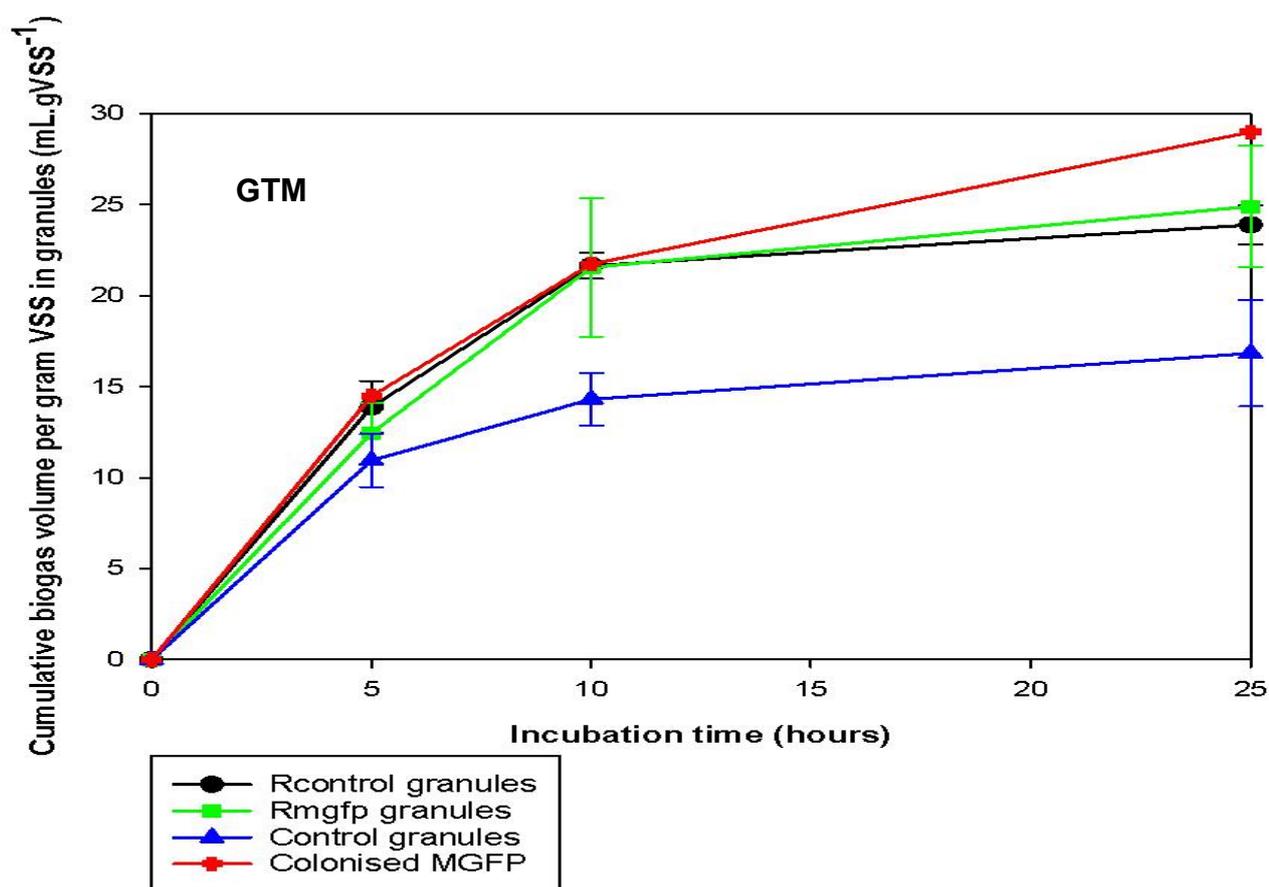


Figure 3.25 The cumulative biogas volume produced over 25 h using glucose test medium.

3.4.4.3 ACETIC ACID TEST MEDIA (ATM)

The end product of anaerobic digestion is methane, which is predominantly produced by the acetoclastic methanogens (Van der Weisthuizen, 2014). As such, determining the

methanogenic activity is one of the evaluations that should be conducted after a UASB operation (Van der Weisthuizen, 2014). Figure 3.26 shows the average cumulative biogas volume produced over 25 h using acetic acid test media. ATM was therefore used to measure the activity of the acetoclastic methanogens that convert acetate to methane and carbon dioxide. Figure 3.26 depicts results of the activity tests carried out in acetic acid test media. After 5 h there were minimal differences in biological activity between the R_{control} granules (6.9 mL.gVSS^{-1}) and colonised MGFP (7.3 mL.gVSS^{-1}). These samples had the highest biological activity at 5 h. The R_{mgfp} granules (5.8 mL.gVSS^{-1}) had the second highest biological activity while the control granules (5.1 mL.gVSS^{-1}) had the least biological activity (Fig. 3.26). After 10h the R_{control} granules (9.8 mL.gVSS^{-1}) had the highest biological activity while, the biological activity rate in colonised MGFP (9.1 mL.gVSS^{-1}) decreased such that it had a similar biological activity as the R_{mgfp} granules (8.8 mL.gVSS^{-1}) (Fig. 3.26). The control granules (5.1 mL.gVSS^{-1}) had the lowest biological activity among the samples (Fig. 3.26). Furthermore, the biological activity after 25 h, in descending order was, the R_{control} granules ($20.4 \text{ mL.gVSS}^{-1}$) and R_{mgfp} granules ($20.1 \text{ mL.gVSS}^{-1}$) then the colonised MGFP ($12.7 \text{ mL.gVSS}^{-1}$) and lastly, the control granules (7.2 mL.gVSS^{-1}) (Fig. 3.26). The high biological activity in R_{control} and R_{mgfp} granules possibly suggests that they had the highest activity of the acetoclastic methanogens. It could be observed that the R_{control} ($20.4 \text{ mL.gVSS}^{-1}$) had sound and dense granules, which possibly allowed for the maximum growth of methanogens and thus resulted in a high biological activity. In addition, the R_{mgfp} granules ($20.1 \text{ mL.gVSS}^{-1}$) also had a relatively higher biological activity presumably as a result of its association to the magnetisable particles, which contain a high amount of iron, which promotes the activity of methanogens (Ramm *et al.*, 2014). However, the colonised magnetisable particles ($12.7 \text{ mL.gVSS}^{-1}$) had a lower biological activity than R_{control} and R_{mgfp} granules presumably because of the small biofilm fraction around the carrier particles that was still acclimatising to the reactor conditions. Moreover, the low biological activity of the colonised MGFP was possibly due to the slow grow rate of methanogens (Ward *et al.*, 2008) and as such, it had a lower biological activity. The ATM in general resulted in lower biogas volumes (Fig. 3.26) and consequently lower methane percentages comparative to the results noted for the BTM and GTM tests (Fig. 3.24 & Fig. 3.25). The control granules had the lowest biological activity, possibly due to the fact that the granules had not been acclimatised to reactor conditions. Therefore, there was possibly a slower metabolic activity of methanogenic bacteria, which resulted in the low biological activity of the control granules. The cocci-shaped bacteria, which were presumably the *Methanosarcina* e.g. *Methanococcus*, *Methanogenium* and

Methanoblobus were possibly responsible for the production of methane in the samples (Jiang *et al.*, 2015)

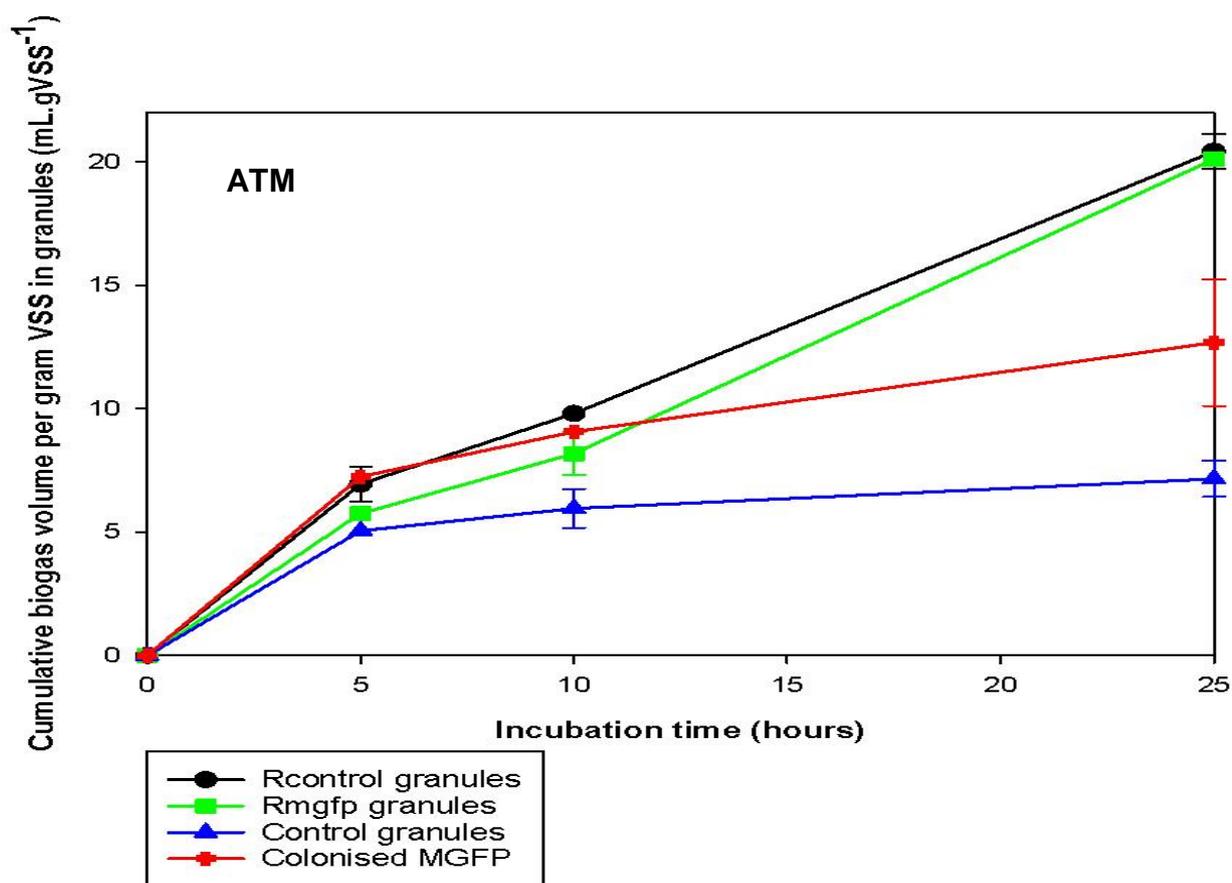


Figure 3.26 The average cumulative biogas volume produced over 25 h using acetic acid test medium.

3.4.4.4 METHANE PERCENTAGES AFTER 25 H INCUBATION TIME

After 25 h of conducting granule activity tests on 6 months colonised MGFP, R_{mgfp^-} , $R_{control}$ and control granules, methane percentage was measured to determine the methane percentage of the biogas.

Basic test media

Figure 3.27 also shows the average methane percentage recorded after the incubation time 25 h using basic test media. The colonised MGFP produced biogas with the highest methane percentage (82.4%), comparative to the R_{mgfp} granules (77.2%), control granules (76.4%) and $R_{control}$ granules (73.9%) (Fig. 3.27). This high methane percentage recorded for colonised MGFP was an expected outcome as Feng *et al.* (2014) and Zhang *et al.* (2014)

highlighted that the presence of iron in MGFP enhances the conversion of acetate to methane. Furthermore, due to the surface properties of the MGFP, i.e. small, high surface roughness and porous, there was a high surface area for adhesion which promoted the attachment of more bacteria including the methanogens. There were minimal differences with regards to methane percentage in $R_{\text{control-}}$ (73.9%), $R_{\text{mgfp-}}$ (77.2%) and control granules (76.4%), as indicated by the overlapping error bars, thus suggesting that after 25 h incubation time these granules had similar methane percentages.

Glucose test media

Figure 3.27 shows the average methane percentage recorded after 25 h incubation time using glucose test media. As seen in Fig. 3.27, although there were no significant differences, in methane percentage after 25 h among the $R_{\text{mgfp-}}$, control granules and colonised MGFP. The R_{mgfp} granules had the highest methane percentage (84.7%) relative to colonised MGFP (78.2%) and R_{control} granules (78.4%). The high methane percentage in R_{mgfp} was possibly as a result of the presence of iron in the MGFP that enhanced methanogenesis and thus caused a high methane biogas to be produced. There was a slightly lower methane percentage in colonised MGFP possibly due to the fact that the biofilm around the particles was still acclimatising to the reactor's environmental conditions such that it could not allow for the production of a high methane percentage biogas (Feng *et al.*, 2014; Zhang *et al.*, 2014). More so, since the methane percentage of the colonised MGFP (78.2%) was lower than $R_{\text{mgfp-}}$ (84.7%) and R_{control} granules (78.4%) (Fig. 3.27), it can be presumed that the ratio of the ECP was higher than that of the bacteria in the biofilm, possibly indicating that the methanogenic bacteria present were insufficient to produce a high methane percentage biogas as in the other samples. The R_{control} granules had the lowest methane percentage after the incubation time (65.6%) (Fig. 3.27). The low methane percentage was possibly attributable to the fact that the control granules were not acclimatised to the UASB reactor conditions such that there were less acetoclastic methanogens to produce biogas with a high methane percentage as in the R_{control} , R_{mgfp} granules and colonised MGFP.

Acetic acid test media

Figure 3.27 shows the average methane percentage recorded after 25 h incubation time using acetic acid test media. Unlike the results reported from BTM and GTM tests, ATM test results indicated that R_{control} granules (66.7%) had the highest methane percentage

compared to the control granules, which produced the lowest methane percentage (38.8%) (Fig. 3.27). Overall, the ATM tests reported the lowest methane percentage compared to the BTM and GTM test results (Fig. 3.11d). This was due to the extra 4.0 g.L⁻¹ of acetic acid (carbon source) that was added to BTM, for which the reason was limited bacterial groups that could metabolise the acetate to methane (Cameroon, 2000). However, there were minimal differences in methane percentage after the incubation time, 25 h, among the R_{mgfp}- (60.7%), R_{control} granules (66.7%) and colonised MGFP (61.3%). The control granules had the lowest methane percentage after 25 h (Fig. 3.27). The lower methane percentage reported from the ATM results possibly suggest that the methanogens were incapable of fully converting the acetic acid to biogas and, thus there was a lower methanogenic activity. The lower methanogenic activity was potentially ascribed to the biomass washout during the late stages of phase one. As such, at the end of the UASB operation a fraction of the active methanogens had been lost which resulted in the lower methane activity.

There was a direct correlation between the biogas volume produced and the methane percentage in the samples (Fig. 3.26). For instance, the high biogas volume (8.5 mL) recorded in R_{control} granules was related to the high methane percentage produced (66.7%) (Fig. 3.26; Fig. 3.27). The high biogas activity in R_{control} granules suggest that the sample had high acetoclastic methanogens' activity compared to the other samples measured. The high biological activity observed in R_{control} granules thus suggested that this sample had the highest population of acetoclastic methanogens as it produced the highest methane (Fig. 3.26).

Overall, after using all the three test media, the biological activity of the R_{mgfp} granules, R_{control} granules and colonised MGFP was slightly higher than that of the control granules. This highlighted that the bacteria in the biomass were active in spite of the fact that some granules had disintegrated (Fig. 3.28b; Fig. 3.28c) unlike the control granules (Fig. 3.28a). This was possibly because the control granules had not been acclimatised to the UASB reactor conditions, such that there was less growth of bacteria, which subsequently resulted in less biological activity. The disintegration was presumably ascribed to the high turbulence in the reactors caused by biogas production (Keating, 2015). More so, the inevitable abrasion between particles and the granules during the operation possibly worsened the problem (Tang *et al.*, 2011; Intanoo *et al.*, 2016).

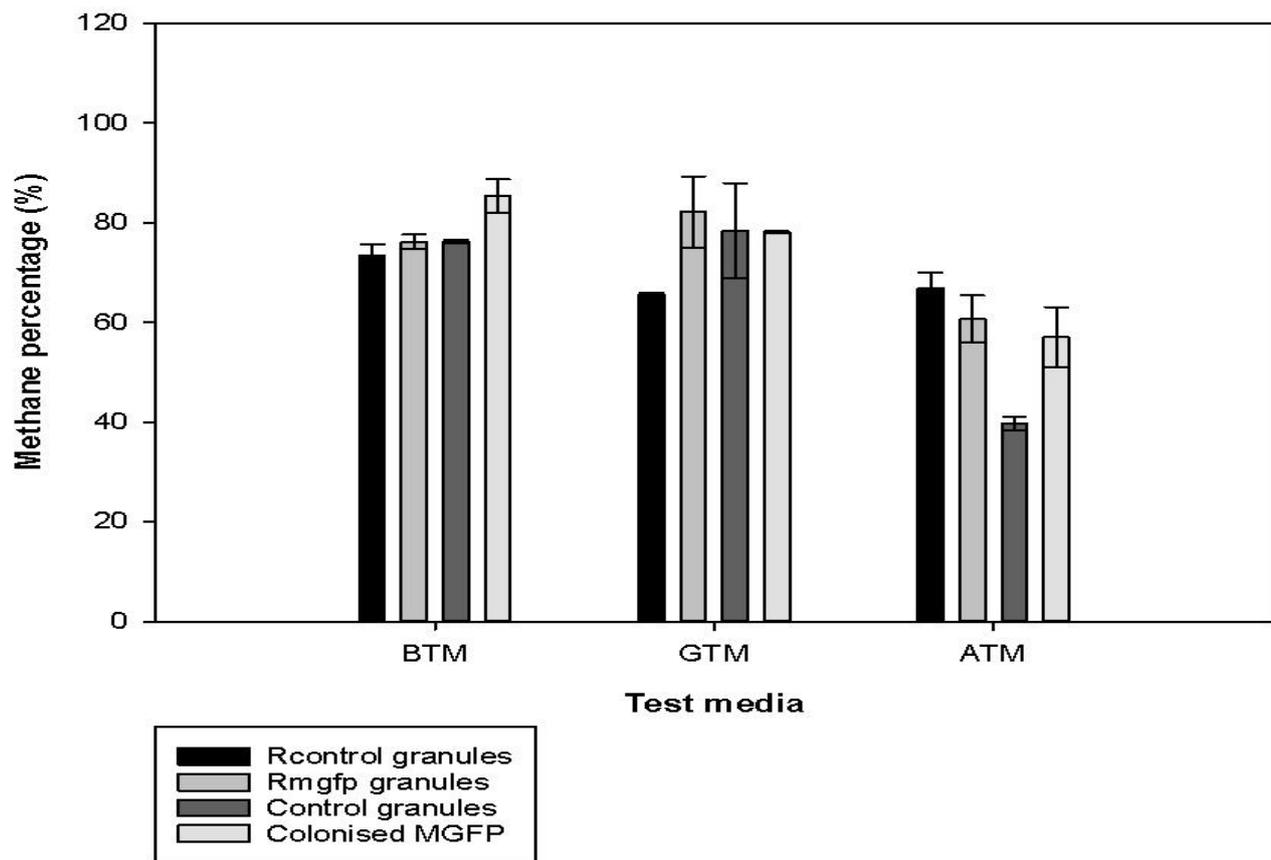


Figure 3.27 The methane percentage recorded after 25 h incubation time.



Figure 3.28 The pictorial view of a). The control granules b). R_{mgfp} granules c). R_{control} granules after 180 days of UASB operation.

The control-, R_{mgfp} - and $R_{control}$ granules had a similar VSS concentration (0.1 gVSS.g^{-1} granule) (Table 3.8), unlike the colonised MGFP which had a lower VSS content (0.09 gVSS.g^{-1} granule) (Table 3.8). VSS is a measure of the amount of active bacteria in granules (Contreras *et al.*, 2002). Thus, meaning that the control-, $R_{control}$, and R_{mgfp} granules had a slightly higher amount of active bacteria in comparison to colonised MGFP. However, the high VSS did not necessarily correlate with the bacterial activity. The colonised MGFP had a slightly lower VSS because of the lower ratio of biofilm matrix to MGFP, suggesting that there were less bacteria to contribute to the VSS comparative to the full granules of the initial control-, $R_{control}$ - and R_{mgfp} granule. This was also attributed to the fact that some microorganisms specifically the methanogens have a slower growth rate, so there was lesser bacteria (Khalid *et al.*, 2011).

Table 3.8 The concentration of the volatile suspended solids in four test samples

Sample	VSS (gVSS.g^{-1} granule)
Control granules	0.1
R_{mgfp}	0.1
$R_{control}$	0.1
Colonised MGFP	0.09

3.5 CONCLUSION

The main objective of this study, which was to investigate the effect of magnetisable particles on the efficacy of an UASB reactor treating synthetic winery wastewater was successfully achieved by monitoring water quality parameters in R_{mgfp} and $R_{control}$. The further objectives of monitoring biofilm development on the MGFP and the activity of colonised MFPG was also achieved.

Both reactors, R_{mgfp} and $R_{control}$ displayed high treatment efficiencies and stable reactor conditions. The $R_{control}$ and R_{mgfp} both had relatively good COD reduction efficiencies, in spite of the untimely reactor disturbances at day 88 to 92. Overall, the COD reduction ranged from 77.1 to 97.7% and 71.4 to 97.7% in $R_{control}$ and R_{mgfp} , respectively. Stable reactor conditions were evidenced by the alkalinity and pH of both bioreactors that were well within the optimal specifications. The alkalinity indicates the strength of the buffer system and ranged from 500 to 3 075 $\text{mgCaCO}_3.\text{L}^{-1}$ in $R_{control}$ and 450 to 2 625 $\text{mgCaCO}_3.\text{L}^{-1}$ in R_{mgfp} . Although the pH was within the optimal standard of 6.50 to 8.50 (7.60 – 8.36 in $R_{control}$

and 7.49 – 8.29 in R_{mgfp}) according to Umaiayakanjaram and Shanmugam (2016), the average pH for UASB reactors ranges from pH 6.8 – 7.2 as described by Lu *et al.* (2015). Nonetheless, the pH had negligible effects on the treatment efficiencies. Furthermore, stable reactor conditions were also observed in terms of the volatile fatty acids' concentration that ranged between 25 to 450 mg.L⁻¹ in both R_{mgfp} and $R_{control}$ throughout the study, which fits into the operational standard recommended for the operation of a UASB (< 500 mg.L⁻¹) (Nguyen *et al.*, 2015). Good reactor performance was also evidenced by the stable production of biogas (by both reactors) which contained over 55% methane, the optimal standard for methane content in the operation of a stable UASB bioreactor (Nguyen *et al.*, 2015).

In addition, the further aim of the study was to monitor biofilm development, periodically throughout the six months UASB reactor operation. This was achieved by using scanning electron microscopy and fluorescence microscopy. After analysing R_{mgfp} , $R_{control}$, control granules and colonised MGFP under a scanning electron microscope, a dense biofilm coverage was observed from the second month until the sixth month of the operation. The biofilm consisted of both cocci and rod-shaped bacteria. In addition, fluorescence microscopy was also conducted to monitor the growth of methanogens throughout the study. The presence of other bacteria and methanogens was distinguished using SYTO 9 dye to identify general bacteria and auto-fluorescence to identify methanogens. There was a distinctive gradual positive change in the growth of these methanogens, which were identified by the blue auto-fluorescence.

The final aim of the study was to determine the metabolic activity of R_{mgfp} , $R_{control}$, control granules and colonised MGFP using basic, glucose and acetic acid test media. Overall, the colonised MGFP showed the highest biological activity when BTM and GTM were used, thus signifying that this sample presumably had the highest population of active general bacteria and acidogens. There were minimal differences in terms of biological activity noted in $R_{control}$, R_{mgfp} and control granules. Thus suggesting that these three samples had a comparable bacterial activity. The $R_{control}$ granules had the highest activity of acetoclastic methanogens when ATM solution was used. Methane percentage was also evaluated after the 25 h incubation time. The volume of biogas produced correlated with the methane percentage measured. Thus meaning that the high biogas volume produced by a sample was directly proportional to the high methane percentage measured in that sample.

The volatile suspended solids were also measured in R_{mgfp} , $R_{control}$, control granules and colonised MGFP to estimate the amount of bacteria present. The control granules had

the highest VSS among all the samples, while the colonised MGFP had the lowest VSS content. After carrying out all the objectives it was also crucial to determine the amount of colonised MGFP that could be seeded to a UASB to treat SWWW in the absence of granules. The dose of colonised MGFP was obtained by comparing the biological activity of colonised MGFP to that of control granules. Presumably, an initial load of 427.8 g colonised MGFP ought to be added to a UASB reactor in the absence of granules.

In conclusion, it can be presumed that the use of MGFP as a biofilm carrier has a potential in the operation of the UASB reactors. There were several advantages associated with the incorporation of the MGFP. The magnetisable particles had negligible effects on the treatment efficiencies, as they were relatively similar in R_{control} and R_{mgfp} . The treatment efficiencies were relatively high and stable throughout most of the 180 d of the UASB operation. In addition, the presence of the magnetisable particles was beneficial to the R_{mgfp} as it produced a higher methane biogas than R_{control} , this was attributable to the presence of iron which promotes methanogenesis. Furthermore, due to the unique properties of the MGFP, i.e. high surface roughness and porosity, an active dense biofilm composing of various microbial species attached to the magnetisable particles. Due to the stable reactor conditions there was a gradual growth of the methane-forming bacteria as evidenced by the fluorescence microscopy. In addition, as a result of effective conversion of organic matter to biogas, the R_{control} and R_{mgfp} had stable reactor conditions (i.e. optimal pH, VFA, alkalinity and methane production).

However, in spite of positive outcome of this study, there were some several setbacks to the study. It was observed that the close interaction of the MGFP and granules caused continuous abrasion between them, which consequently led to the fragmentation of the granules. Furthermore, the abrasion also caused the crumbling of the magnetisable particles, which caused some of the MGFP to be washed out because of their small size. This increased the effluent total suspended solids, which slightly reduced the treatment efficiency of the reactors. Therefore, for future research, it is recommended to use lower quantities of MGFP since, in high quantities, they might negatively impact the structure of the granules, causing them to disintegrate which reduces water quality. Furthermore, future research can possibly use a separate UASB reactor to colonise MGFP, which could then be used as inocula in a treatment process. This would be achieved by feeding a lower concentration substrate to biomass in the presence of MGFP, ultimately focusing on the immobilisation of biomass on the particles rather than wastewater treatment. Thus, it could be feasible to colonise MGFP with an active anaerobic biofilm, which is also easily

collectable due to the magnetisable nature. This could ultimately serve as a source of multiplying active biomass to either seed another treatment process or be stored for cases of emergency (i.e. reactor failure or loss of biomass).

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Chapter 4

General Discussion and Conclusion

The quantity and quality of water in South Africa (SA) has gradually decreased in the past decades, to the extent that the country has been listed in the world's top 30 driest countries (Anonymous, 2016). This has been worsened by the fact that approximately 98% of the freshwater resources are exploited yearly and the subsequent result has been drought (Anonymous, 2011). According to McDonald *et al.* (2011), the major factors that have caused water scarcity are a combination of inconsistent and low rainfall as well as extreme high temperatures, El-Nino. In addition, to complicate this water scarcity issue, the Western Cape where extensive wine production is practised, has recorded the least rainfall (200 mm) in comparison to other provinces (Hawker, 2015). During the vintage season, the production of wine generates large volumes of heavily polluted acidic water containing organic matter, inorganic ions, suspended solids as well as recalcitrant polyphenols (Gupta *et al.*, 2012). For every litre of wine produced, 0.3 to 14 L of wastewater is generated (Oliveira & Duarte 2016). Therefore, it is obligatory for winery wastewater to be treated in order to improve the water quality and quantity.

Different treatment methods are used to depollute WWW, but this study focused on the use of two parallel lab-scale UASB reactors, which are based on anaerobic digestion (Kongjan *et al.*, 2011). The UASB reactor is a simple, biological and high rate treatment system that can achieve a 90% efficiency (Ioannou *et al.*, 2015). It is also a sustainable traditional treatment method with low operational costs as it produces the highest volume of methane relative to the other anaerobic treatment methods (Chung *et al.*, 2016). Methane can be easily recovered and used as a source of thermal and electrical energy (Alavi *et al.*, 2014). However, apart from the system having a lengthy start-up period, the major downside of the UASB is sludge washout (Alavi *et al.*, 2014). Sludge washout is usually caused by the crumbling of granules and high biogas production, which cause the sludge bed to be excessively lifted and thus, sludge washout (Lu *et al.*, 2015). The prevention of sludge washout can be achieved by seeding biofilm carrier particles e.g. MGFP in reactors (Ramm *et al.*, 2014).

This research investigated the effect of MGFP in an UASB reactor treating synthetic winery wastewater by monitoring biofilm development and the activity of colonised MGFP. The first aim of the study was to determine the effect of seeding MGFP on the treatment

efficiency, in a UASB reactor, treating synthetic winery wastewater by measuring and monitoring the water quality parameters of the effluent such as the pH, alkalinity, volatile fatty acids and chemical oxygen demand. Monitoring the growth of methanogens was a secondary aim, which was achieved by analysing the biofilm on MGFP using scanning electron microscopy and fluorescence microscopy. The final aim of the study was to compare the biological activity of the colonised MGFP with the control, R_{control} , and R_{mgfp} granules. This would give insights into the possibility of using colonised MGFP in anaerobic treatment processes

The operation of the UASBs lasted 180 days. The OLR was steadily increased from $0.5 \text{ kgCOD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ to $5.2 \text{ kgCOD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ (Fig. 3.4). All the water quality parameters were well within the standard operation. Overall, the COD reduction ranged from 77.1% to 97.7% and 71.4 to 97.7% in R_{control} and R_{mgfp} , respectively (Fig. 3.4). According to studies done by Ioannou *et al.* (2015) on UASB reactors, the reactor COD reduction efficiencies were relatively high. The alkalinity ranged from 500 to 3 075 $\text{mgCaCO}_3\cdot\text{L}^{-1}$ in R_{control} and 450 to 2 625 $\text{mgCaCO}_3\cdot\text{L}^{-1}$ in R_{mgfp} , while the pH ranged from 7.60 to 8.36 in R_{control} and 7.49 to 8.29 R_{mgfp} , respectively (Fig. 3.5). The volatile fatty acids concentration ranged from 25 – 350 $\text{mg}\cdot\text{L}^{-1}$ in R_{control} and 25 – 425 $\text{mg}\cdot\text{L}^{-1}$ in R_{mgfp} (Fig. 3.5). The average methane percentage ranged from 52 – 82% in R_{control} and 54 - 82% in R_{mgfp} (Fig. 3.4). The TSS in the effluent ranged from 100 – 380 $\text{mgTSS}\cdot\text{L}^{-1}$ in R_{control} and 80 – 720 $\text{mgTSS}\cdot\text{L}^{-1}$ in R_{mgfp} .

Upon analysis of the colonised MGFP using a scanning electron microscope (SEM), a dense biofilm coverage was observed every second month until the sixth month of the operation. Extracellular polymeric substances formed by the microorganisms embedded in the biofilm, were responsible for developing the biofilm around the MGFP. Furthermore, when R_{control} , R_{mgfp} granules and colonised MGFP were viewed under SEM, both cocci and rod-shaped bacteria were observed on all of these samples, except the uncolonised MGFP as they were not put in the reactor. In addition, when colonised MGFP were extracted every second month of the UASB operation and viewed under the fluorescence microscope, the growth of methanogens was detected upon auto-fluorescence, while the other bacteria were observed when the magnetisable particles were stained with SYTO 9 dye.

When granule activity tests were conducted on colonised MGFP, R_{control} , R_{mgfp} and control granules, the colonised MGFP had the highest activity among all the samples. This indicated that the colonised carrier particles had the highest concentration of active bacteria, acidogens and acetoclastic methanogens. The high activity was possibly as a result of the presence of iron in the MGFP which aids in acidogenesis and acetogenesis (Lu *et al.*, 2015).

It could therefore be concluded that the use of colonised MGFP in combination with granules could potentially be more advantageous compared to the use of granules alone.

Overall, investigating the effect of MGFP on the treatment efficiency of UASB reactors had a positive outcome. The use of MGFP as a biofilm carrier is possibly feasible in the operation of the UASB reactors, as an active biofilm manifested itself on the surface of the magnetisable particles. The biofilm attachment was enhanced by the presence of crevices and pores, more so, by the rough surface area on the MGFP. The presence of an active biofilm allowed for the production of a higher methane biogas in R_{mgfp} comparative to $R_{control}$, which can be beneficial for industries which need methane as a source of electrical energy. The production of a higher methane biogas was possibly as a result of the presence of iron in MGFP which promotes the conversion of acetate to biogas. In addition, although there were negligible differences with regards to treatment efficiencies between both reactors, the active biofilm on the magnetisable particles slightly improved the treatment efficiency in R_{mgfp} , during Phase 1 compared to $R_{control}$. The treatment efficiencies were relatively high and stable throughout most of the 180 d of the UASB operation. Overall, the water quality parameters (pH, VFA, alkalinity) and methane production were in their optimal range throughout the study, which created stable reactor conditions in $R_{control}$ and R_{mgfp} . Furthermore, as a result of the stable reactor conditions there was a gradual growth of the methanogens as evidenced by the fluorescence microscopy.

Although the operation of the study was successful as indicated by the high COD reduction (Fig. 3.4), there was a downside to the operation of the reactors. As a result of high biogas production, the main challenge of the study was the constant abrasion between the magnetisable particles and granules, which subsequently caused their disintegration and thus increased the effluent total suspended solids concentration due to the subsequent washout. Furthermore, some of the MGFP which disintegrated could not be detected in the effluent because of their small size. Therefore, the following recommendations could potentially improve further studies:

1. Using more improved MGFP with better resistance to crumbling at high shear forces. This would help minimise sludge washout, as the colonised MGFP will remain intact at high frictional forces.
2. Using a higher amount of bigger MGFP, as some of the smaller particles were washed out and undetectable in the effluent. This would allow for a higher biofilm

attachment on the particles, which will presumably enhance the treatment efficiency of wastewater.

3. Using a separate reactor to colonise MGFP, in a batch system, to prevent rapid shearing off the particles. This would be achieved by feeding a low concentration substrate to biomass in the presence of MGFP, ultimately focusing on the immobilisation of biomass on the particles rather than wastewater treatment.
4. Using a lower influent pH would help reduce industrial operational costs (i.e. reduce costs of neutralising agents), as the effluent alkalinity recorded remained relatively high to buffer the reactor and prevent the negative effects of the continuous production of volatile fatty acids (e.g. souring of the reactor).

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