INVESTIGATING THE EFFECT OF WINE AND DISTILLERY WASTEWATER ON THE EFFICACY OF AN UPFLOW ANAEROBIC SLUDGE BLANKET (UASB) AND ENHANCING BIOMASS IMMOBILISATION BY THE ADDITION OF MAGNETISABLE FOAM GLASS PARTICLES (MP)

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DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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ABSTRACT

The wine and distillery industry is one the largest contributors in the production of wastewater worldwide. The effluent produced from this industry is classified as high strength wastewater and does not comply with local regulations. Treatment of these effluents is therefore mandatory if it is to be reused or pumped back into the ecosystem. The upflow anaerobic sludge blanket (UASB) has been found to be one of the most successful technologies in treating high strength wastewater, particularly wine and distillery wastewater. Therefore the first objective of this study was to investigate the effect of combined wine, marula and Brandy wastewater on the operation and efficacy of a UASB reactor.

In order to simulate the production seasons of the three different waste streams, a feeding strategy was developed where the trial was divided up into different phases. The chemical oxygen demand (COD) reduction percentage throughout each phase remained at an average of 85% and above, as the organic loading rate (OLR) increased from 1 kg COD.m^{-3}.d^{-1} (phase one) to 10 kg COD.m^{-3}.d^{-1} (phase four). The biogas production increased from an average of 0.6 L.d^{-1} (phase one) to 10 L.d^{-1} (phase four) as well as the methane percentage that showed a similar trend; as the OLR increased throughout the trial so did the methane percentage. The pH and the alkalinity remained stable throughout the trial; however as the OLR reached 6 kg COD.m^{-3}.d^{-1} some difficulties did occur as the pH dropped to below 5. The reactor was therefore monitored more closely as the OLR increased.

The success of the UASB reactor is found in the retention of anaerobic bacteria that are responsible for the digestion of the substrate. Retention occurs as the anaerobic sludge forms aggregates, also referred to as granules, which can withstand the upflow velocity of the incoming substrate. The loss in biomass does however still occur. Therefore the second objective of this study was to investigate whether added magnetisable foam glass particles (MPs) would be a viable medium for biomass attachment to aid in the immobilisation of granular biomass. The third objective was to investigate whether the added MPs would affect the operation and efficacy of the UASB reactor

Scanning electron microscopy (SEM), fluorescent microscopy analysis and activity tests were done on the MPs after a seven month period within a UASB reactor. SEM results showed microbial attachment and colonisation on the surface of the MPs, a distinct difference was found when comparing an uncolonised MP to a fully colonised MP. A fully colonised particle displayed a large variety of organisms attached to its surface and the

morphology of these organisms gave an indication that *methanobacterium*, *methanoplanus*, *methanosaeta*, *methanobrevibacterium* and *methanosarcina* were present on the surface of the MPs. The attachment and colonisation of bacteria onto the surface of the MPs were confirmed by the results obtained by the fluorescent microscopy analysis. Fluorescence was found after the particles were stained with SYTO 9, a green fluorescent dye that stains the nucleotides of bacterial cells. These results confirmed colonisation of a mixed consortium of bacteria onto the surface of the MPs. Methanogenic attachment was confirmed by autofluorescence; as the MPs were exposed to specific wavelengths of UV a blue colour was observed where methanogenic attachment occurred. Activity tests were performed to investigate whether the MPs produced biogas and methane.   Biogas production was found in all three mediums used, which again confirms both the presence of acidogenic as well as methanogenic activity.

With the addition of the MPs to the one UASB reactor (R_{MP}), there was no initial influence on the operation of the reactor, however as the OLR reached 6 kg $COD.m^{-3}.d^{-1}$ and above the pH and alkalinity of R_{contol} decreased significantly on three different occasions during the trial whereas this was not found in R_{MP}. Another difference between R_{MP} and $R_{control}$ was found at the end of the trial when the granular biomass was removed from the reactor.   The granules in $R_{control}$ were much larger the granules from R_{MP}, furthermore the majority of the biomass was in a floccular form rather than granular.

Based on the data from this study the digestion of combined wine and distillery effluent is possible, however the reactor should be carefully monitored as the OLR increased above 6 kg $COD.m^{-3}.d^{-1}$. This study has also proven that using MPs as a medium to improve biomass retention is a viable option, furthermore the addition of MPs to a UASB reactor might have a positive effect on the digestion of high strength wine and distillery wastewater.

UITTREKSEL

Industrieë wat wyn en gedistilleerde produkte produseer lewer wêreldwyd een van die grootste bydraes tot die produksie van afloopwater. Die afloop wat geproduseer word deur hierdie industrieë word geklassifiseer as hoë sterkte afvalwater en voldoen nie aan die plaaslike regulasies nie. Behandeling van hierdie water is dus verpligtend as die water hergebruik gaan word of as dit in die ekosisteem teruggepomp gaan word. Daar is bevind dat die opvloei-anaërobiese slykkombers (OAS) reaktor een van die mees suksesvolle tegnologieë is in die behandeling van hoë sterkte afvalwater, veral by die afloop van wyn en gedistilleerde produkte. Die eerste doel van hierdie studie was dus om die uitwerking van gemengde afloopwater van wyn, brandewyn en maroela op die werking en doeltreffendheid van 'n OAS reaktor te ondersoek.

Om die seisoenale produksie van die verskillende afloopwaters te simuleer is 'n voerstrategie ontwikkel waar die toets in verskillende fases verdeel is. Die gemiddelde chemiese suurstofbehoefte (CSB) reduksie persentasie van elke fase was 85% en hoër soos wat die organiese ladingstempo (OLT) verhoog is van 1 kg CSB.m^{-3}.d^{-1} (fase een) tot 10 kg CSB.m^{-3}.d^{-1} (fase vier). Die biogas produksie het van gemiddeld 0.6 L.d^{-1} (fase een) tot 10 L. d^{-1} (fase vier) verhoog soos wat die OLT verhoog is deur die loop van die toets, en die metaan persentasie het 'n soortgelyke tendens getoon. Die pH en alkaliniteit het stabiel gebly deur die loop van die toets, maar soos wat die OLT verhoog het tot by 6 kg CSB. m^{-3}.d^{-1} en hoër het die pH en alkaliniteit egter fluktuasies begin toon. Die reaktor is dus meer deeglik gemonitor soos wat die OLT verhoog het.

Die OAS reaktor se sukses word gevind in die vermoë daarvan om die anaërobiese bakterieë wat vir die vertering van die substraat verantwoordelik is te behou. Die behoud van die anaërobiese bakterieë vind plaas soos wat granulasie, of die vorming van sferiese biofilms, plaasvind. Hierdie granules kan die opwaartse vloei van die inkomende substraat weerstaan. Die verlies in biomassa vind egter steeds plaas. Dus was die tweede doel van hierdie studie om vas te stel of magnetiese skuimglaspartikels (MPs) wat by die OAS reaktor gevoeg word 'n geskikte medium sou wees waarop die anaërobiese biomassa kan groei. Die derde doel van hierdie studie was om vas te stel of die bygevoegde MPs 'n invloed op die OAS reaktor se werking sal hê.

Skandeer-elektron mikroskopie (SEM), fluoresserende mikroskopie analise en aktiwiteitstoetse is na 'n periode van sewe maande in die OAS reaktor op die MPs uitgevoer. Die SEM resultate het mikrobiese aanhegting en kolonisasie op die oppervlakte van die MPs getoon, en daar was 'n duidelike verskil tussen partikels met geen aanhegting en partikels wat ten volle bedek was. 'n Partikel wat ten volle bedek was het 'n groot

verskeidenheid van organismes getoon waarvan die morfologie 'n aanduiding gegee het dat die volgende spesies teenwoordig was: *metanobakterium*, *metanoplanus*, *metanosaeta*, *metanobrevibakterium* en *metanosarsiena*.

Die aanhegting en kolonisasie op die oppervlakte van die MPs is bevestig deur die resultate van die fluoresserende mikroskopie analise. Fluoressensie is waargeneem nadat die partikels met SYTO 9 gekleur is, 'n kleurstof wat die nukleïensure van alle bakterieë kleur en groen fluoresseer wanneer dit aan UV radiasie blootgestel word. Die resultate het bevestig dat 'n gemengde konsortium bakterieë aan die oppervlakte van die partikel geheg was. Aanhegting van metanogene is deur outofluoressensie bevestig; soos wat die MPs blootgestel is aan 'n spesifieke golflengte van UV lig is 'n blou kleur waargeneem, en dus bevestig dit die teenwoordigheid van metanogene. Aktiwiteitstoetse is uitgevoer om ondersoek in te stel of die MPs biogas asook metaangas produseer. Die produksie van biogas het plaasgevind in al die mediums, wat die teenwoordigheid van sowel asitogene as metanogene bevestig.

Met die byvoeging van MPs by een van die OAS reaktors (R_{MP}) is geen aanvanklike verskil waargeneem nie. Soos wat die OLT verhoog is tot by 6 kg CSB.m^{-3}.d^{-1} en hoër het die pH en alkaliniteit van $R_{kontrole}$ egter verskille begin toon. Daar was drie gevalle waar die pH en alkaliniteit van $R_{kontrole}$ verlaag het en dit is nie in R_{MP} waargeneem nie. Die ander verskil tussen R_{MP} en $R_{kontrole}$ is aan die einde van die toets waargeneem. Met die verwydering van die biomassa van beide reaktore is 'n beduidende verskil in die grootte van die granules waargeneem, en die meeste van die biomassa in R_{MP} was in 'n geflokkuleerde eerder as 'n gegranuleerde vorm.

Op grond van die data van hierdie studie kan vertering by gemengde alkoholiese afloopwater wel plaasvind, maar die reaktor moet by verhoogde OLT meer deeglik gemonitor word. Hierdie studie het bevestig dat die gebruik van MPs as 'n aanhegtingsmedium effektief is, en verder kan ook bevestig word dat die byvoeging van MPs nie die werking van die OAS reaktor affekteer nie.

To Mom and Dad

(Phyllis & Trevor Buys)

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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 in 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.

CHAPTER 1

INTRODUCTION

The availability and supply of fresh water plays a vital role in sustaining a population (Shannon *et al.*, 2008). Surface water and ground water are the two main sources that supply fresh water to a population; however, the rapid increase of urbanisation and intensive agricultural development are depleting this resource (DEAT, 2006). The amount of fresh water available is one topic of concern but another topic to take into account is the distribution of this resource. A quarter of the world's population live in countries classified as water scarce (Fischer *et al.*, 2011) and many of these countries are found in Africa (Anon, 2014a). South Africa is one of the countries in Africa that is classified as a semi-arid country, where the rainfall per annum is less than half of the worldly rainfall average per annum (Otieno & Ochieng, 2004).

There are six main sectors that are responsible for the water usage in South Africa and irrigation represents 60% of the total water requirements of all six sectors (DWA, 2000). The wine and distillery industry is one of the fastest growing industries in South Africa, according to the South African Wine Industry Statistics (SAWIS) the grapes crushed between 2011 and 2013 has increased by more than 200 000 tons. As a result of the demand increase on wine and distillery products the crop field must therefore also increase (SAWIS, 2014), subsequently increasing the demand on irrigation water. The production of wine and distillery products not only has a high demand on water used for irrigation but also uses large amounts of fresh water during production which results in the production of large amounts of wastewater (Malandra *et al.*, 2003). The wine industry discharges on average 8 – 10L of effluent for every 1L wine produced (Dillon, 2011) and the distillery industry discharges on average 15 – 20L of effluent for every 1L of ethanol (Wilkie *et al.*, 2000).

The effluent produced from wine production has high COD values (300 – 60 000 mg.L^{-1}), a pH of between 3 - 8 and varying concentration of elements (Ca, K, Na and Mg) and solids due to the lees, must and wines that pollute the water during production (Van Schoor, 2005; Brito *et al.*, 2007; Sheridan *et al.*, 2011; Conradie *et al.*, 2013). The effluent produced from distilleries, specifically for Brandy and Amarula production, has high concentrations of solids (TSS: 5 000 – 10 000 mg.L^{-1}), high COD values (30 000 – 70 000 mg.L^{-1}), a low pH (3 - 5) and high phenolic compounds (Chrobak & Ryder, 2005; Musee *et*

al., 2007; Mohana *et al.*, 2009; Strong, 2010). These characteristics of the wastewater do not comply with South African regulation if the reuse of wastewater is considered (Republic of South Africa, 2004), therefore the effluent has to be treated before it can be discharged or used as irrigation water.

Anaerobic digestion (AD) is one of the oldest and most widely applied treatment options used worldwide (Moletta, 2005; Pant & Adholeya, 2007). This technology is mostly implemented in industries that produce high strength wastewater such as the food and beverage industries, the pulp and paper industries, distilleries, chemical industries and for the treatment of domestic waste (Karthikeyan & Kandasamy, 2009; Habeeb *et al.*, 2010).

The success of AD, when operated at its optimal condition, is that it is able to handle high organic loading rates at short hydraulic retention rates, it can also treat a broad range of wastewater, it has low sludge production and low maintenance costs. Furthermore, this process produced biogas of which 60 - 80% is methane that can sequentially be used as energy (Rajeshwari *et al.*, 2000; Els *et al.*, 2005). The wine and distillery industry has widely applied AD as the chosen treatment method for their wastewater (Moletta, 2005), more specifically the use of the upflow anaerobic sludge blanket (UASB) reactor (Habeeb *et al.*, 2010).

The success of the UASB reactor is found in the formation of microbial aggregates called granules (Nuntakumjorn *et al.*, 2008). Granulation of anaerobic sludge results in self-immobilisation of the consortium and can therefore, to some degree, withstand the upflow velocity of the incoming wastewater which in turn prevents them from being washed out (Rajeshwari *et al.*, 2000). The immobilisation of the granules are beneficial to the operation in the UASB reactor, for this increases the contact time between the biomass and the wastewater, consequently reducing the hydraulic retention time (Schmidt & Ahring, 1996). The loss in biomass can cause a drop in pH, which will in turn cause operational problems and eventually death of the biomass. Although the UASB reactor has become one of the most widely applied technologies, it has however, been found to be sensitive to a decrease in pH often caused by washout as well as organic shock loads (Mohana *et al.*, 2009). Organic shock loads often occur due to the variable nature of industrial wastewater. An industry that produces more than one product, which is produced in different seasons throughout the year, will produce wastewater of varying characteristics and this may result in organic overloading as the production of products overlap.

The first objective of this study was to investigate the effect of combined multiple alcoholic beverage waste streams on the efficacy of the UASB reactor. This will be

achieved by monitoring the operational parameters (pH, COD, alkalinity and methane production) of the UASB reactor as the organic loading rate increases with each added waste stream. The second objective of this study was to investigate the effect of added magnetisable foam glass particles, for biomass retention, on the overall performance of the UASB reactor. Two reactors were operated in parallel to investigate the differences. The third objective of this study was to investigate whether added magnetisable particles would be viable as a medium for biomass attachment to enhance biomass retention of anaerobic methanogens in a UASB reactor.

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CHAPTER 2

LITERATURE REVIEW

BACKGROUND

Water is one of the most important resources that sustains life worldwide. It is a renewable source that sustains not only human life but every other ecosystem found on earth. Most of the planet is covered in water; however the majority of the water is unsuitable or unavailable for terrestrial use (Jackson *et al.,* 2001).

Less than three percent of the water on earth is fresh and of that three percent the majority (70%) is locked in glaciers and ice caps (Fig. 1). The rest of the three percent (30%) is available fresh water. Of the total fresh water available one percent is located on the surface of the earth; this includes lakes, rivers, dams, reservoirs etc. The other 99% is ground water which is discussed further in the following section (Flynn, 2009).
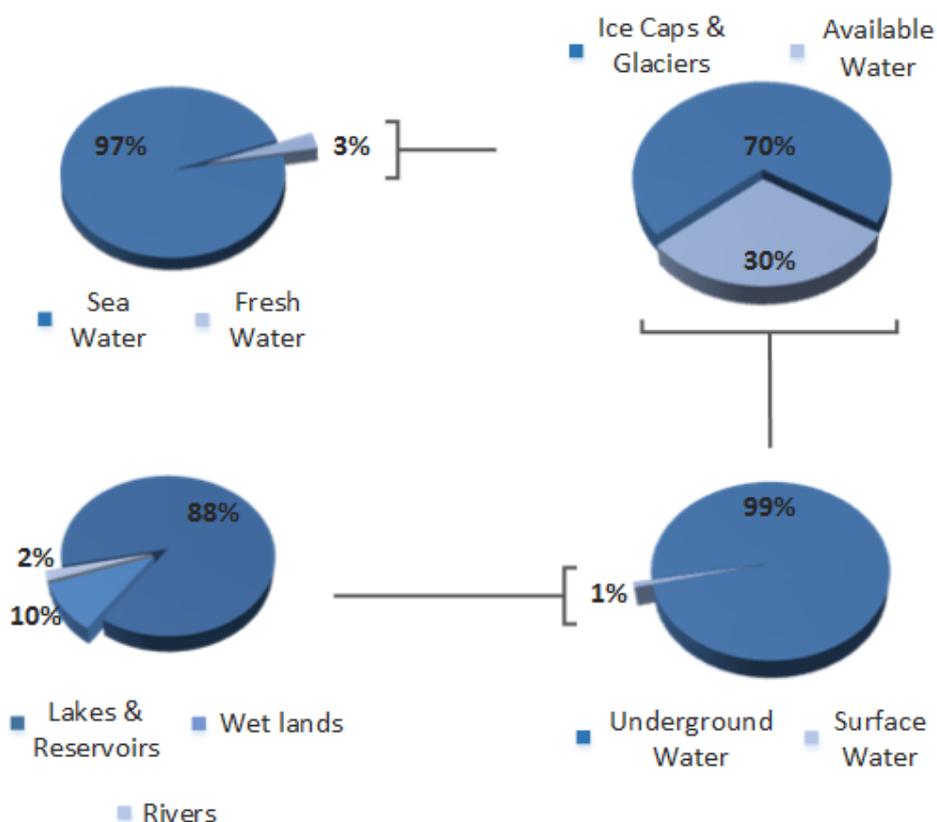

Figure 1 Water availability on earth expressed in percentages (Flynn, 2009).

There are many factors that contribute to the depletion of fresh water sources and one main factor contributing to the replenishing thereof. The main factor in replenishing of fresh water is due to the hydrologic cycle (Jackson *et al.,* 2001).

Replenishing of fresh water

The hydrological cycle is nature's way of recycling water on a global scale (Fig. 2). The water held up in the atmosphere is the source of rain that falls on earth. The majority of the water in the atmosphere is due to evaporation from the sea and only 14% of this water is evaporated from land. Of the water in the atmosphere the majority falls back into the sea and 24% of the water falls onto land. As the precipitation on land is larger than evaporation, a major portion of the precipitated water returns to the sea via rivers and underground aquifers (Pimentel *et al.,* 1997).

The form (snow or rain) in which the precipitate occurs, the timing relative to the season in which it falls and the geomorphology of the region are all factors which influence the availability of the precipitate. For example, in the mountain regions the precipitate usually occurs as snow during the winter seasons. When spring time arrives, the snow melts and this causes a rush of water to run off into the rivers and back into the ocean, not allowing the water to flow back into the underground aquifers. There have been many systems set in place to capture the majority of the spring flood water preventing major runoff back into the ocean (Jackson *et al.,* 2001).
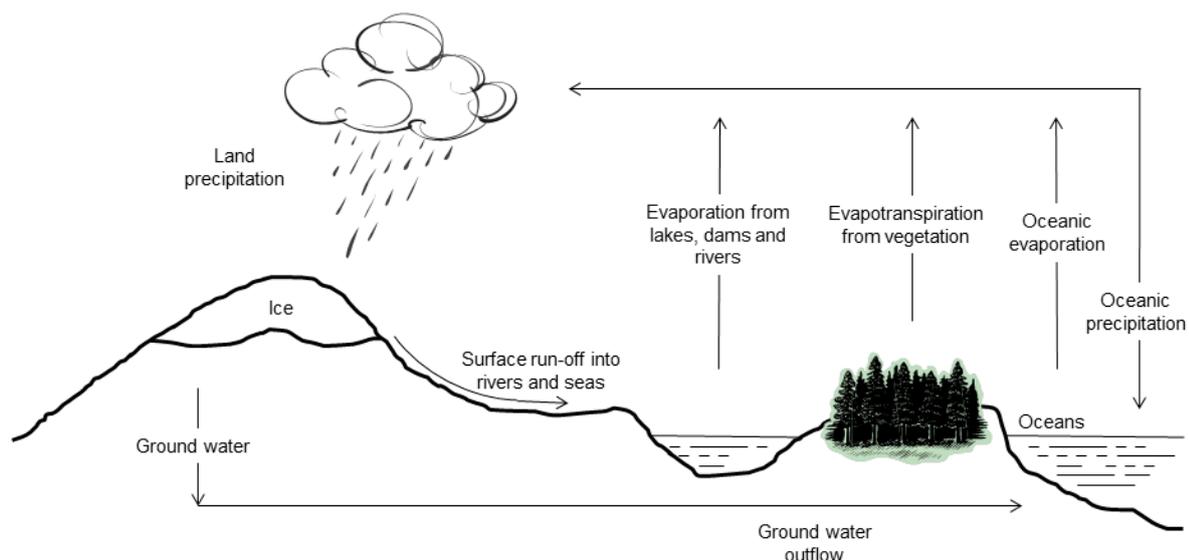

Figure 2 The hydrological cycle, showing how different bodies of water are cycled throughout the seasons (Jackson *et al.,* 2001; Gray, 2010).

Ground water

The term ground water is not as simple as one would think. There are various forms of ground water, but the two main groups can be classified as renewable and non-renewable ground water. The distinction between the two is very important for water management purposes and policies (Jackson *et al.,* 2001).

The hydrologic cycle is the main source for replenishing renewable ground water. As precipitation occurs in the form of rain, the renewable underground aquifers are slowly replenished.   The non-renewable ground water is defined as ground water that is replenished over centuries or more (Jackson *et al.,* 2001). An estimated 20% of water usage globally is from underground sources, renewable as well as non-renewable. Furthermore as the population growth increases rapidly on a global scale, these water sources are being exhausted at a faster rate than which it is replenished (Connor *et al.,* 2009).

Effective water management

As mentioned, water is a primary natural resource and when considering the increasing scarcity of this resource it is of crucial importance that the management and planning around it is optimised. The water shortage crisis can be elevated by proper water management. Water management is not only the regulation and conservation of fresh water, but in addition the management of wastewater. In areas with high wastewater production, it should be considered that the wastewater is a source of water that must be used after proper treatment, instead of considering it as waste that needs to be disposed of (Anon, 2010).

Depletion of fresh water

Factors such as climate changes, run off, erosion, irrigation, population growth, pollution and the wasteful way that water is being used by the communities are all factors contributing to the depletion of fresh water (Pimentel *et al.,* 1997; Connor *et al.,* 2009).

As mentioned, the availability of fresh water for the growing world population is being depleted. Not only is the amount of fresh water that is available a topic of concern, but so is the pollution of this resource becoming an increasing problem. The lack of sanitation in many rural areas, all over the globe, is causing unsuitable and unsafe water for use (Shannon *et al.,* 2008). Open defecation is one of the main problems when it comes to sanitation. It is a high risk for public health; it has largely a negative influence on

the economy as well as a negative social impact on the community (Cross & Coombes, 2013).

Another major problem that is faced with poor sanitation is not only the contamination of the surface water, but also as the communities grow, the volume of contamination increases. This eventually filters through the soil and reaches the underground aquifers. This is a major concern due to, as previously mentioned, the fact that 99% of the fresh water that is available is found in the underground aquifers (Shannon *et al.,* 2008).

Another aspect to consider, rather than the amount of fresh water available, is the distribution of this resource. One quarter of the world's population live in countries classified as physically water scarce (Fischer *et al.,* 2011). There are many countries that face the problem of supply and demand, where the demand for water is higher than what can be supplied. The baseline water stress[1] levels, supplied by AQUEDUCT, (Gassert *et al.,* 2013) from countries in northern Africa and south Asia are 80% and higher, and Mexico, Peru, Chile, Australia and South Africa are between 40-80%.

Many countries in Africa are classified as arid or semi-arid. The term arid and semi-arid areas refers to the rainfall zone in which that area falls; 0-300 mm is termed arid and 300-600 mm is semi-arid (Anon, 2014a). In areas where it is arid or semi-arid, it is difficult to cultivate, due to the fluctuation in climate and unpredictable rainfall patterns. South Africa is one of the countries in Africa that is classified as semi-arid in which the average rainfall per year is currently 450 mm which is well below the worldly rainfall average of 860 mm per year (Otieno & Ochieng, 2004). Due to the limited volumes of rainfall in South Africa as well as area specific precipitation, irrigation is a necessary method that increases crop yield and enables crop growth throughout the country.

South Africa has six main water use sectors namely irrigation; urban use; rural use; mining and bulk industrial use; power generation and afforestation (DWA, 2000). According to the Department of Water Affairs and Forestry (DWA) (2000) irrigation represents 60% of the total water requirements out of all six water use sectors. Furthermore, the main source of irrigation water in South Africa is from the underground aquifers (Oelofse & Strydom, 2010).

There are many different industries within the agricultural industry that use large quantities of water to match the demand of the country. Not only do these industries use fresh water but in addition they produce a lot of wastewater (Gray, 2010).   Each

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[1] A measure of supply and demand for water in a given area, calculated as a ratio of water demand over water supplied (Gassert, 2013).

manufacturer will produce wastewater that differs in composition, quantities and season (Table 1).

One of the vastly growing industries in South Africa, which utilises irrigation as a tool to sustain and develop this industry, is the wine and distillery industry. According to the South African Wine Industry Statistics (SAWIS) the wine and distillery industry of South Africa is growing on a local scale as well as internationally (i.e. exporting), the increase in the amount of grapes crushed (both white and red) between 2011 and 2013 is more than 200 000 tons. As a result of the growing industry, crop fields must therefore also increase to support the demand (SAWIS, 2014), subsequently increasing demand on irrigation water. Furthermore the wine and distillery industry make use of large amounts of fresh water not only for irrigation purposes but also in various production process steps that include crushing and pressing of the fruit, rinsing of fermentation tanks, the general cleaning of the processing area and bottling (Malandra *et al.,* 2003; SAWIS, 2014).

The use of fresh water during the production of wine and distilled products results in the production of large amounts of wastewater per year (Malandra *et al.,* 2003). On average it is found that for every 1L of wine produced between 8 – 10L of effluent is produced (Dillon, 2011) and for every 1L of ethanol produced between 15 – 20L of effluent is produced (Wilkie *et al.,* 2000). In 2013, the total wine produced came to 915 000 kL and the total distilling wine came to 182 000 kL; this equals 9150 000 kL of effluent produced from wine production and 3640 000 kL of effluent from the distilling process. The effluent produced by wine production alone can support a South African family of four for approximately five years (Smith, 2010; SAWIS, 2014). In light of these statistics and considering the water crisis at hand, reusing water produced by these industries is mandatory.

The quantity and quality of effluent produced by wineries and distilleries differ greatly between different producers and different products made and fluctuates throughout the year, as these products are seasonally produced (Melamane *et al.,* 2007). The reuse of wastewater seems mandatory to protect and conserve fresh water in South Africa; however, legal requirements need to be met before effluent can be discharged into the ecosystem (Table 2).

Table 1 Characteristics of industrial wastewater, the scale used is from 1 -10, 1 being low, 5 being intermediate and 10 being extremely high (Adapted from Von Sperling, 2007; Gray, 2010)

Industry type	Specific	Unit	Water consumption per unit (m^3 / unit)	BOD*	COD**	TSS***	pH	Nitrogen	Phosphorus
Food	Fruits and vegetables	1000 kg	4-50	8-10	8-10	1-8	Alkaline	Deficient	Deficient
	Meat and meat products	1 cow, 2,5 pigs	0.5-3.0	8-10	8-10	8	Neutral	Present	Present
	Dairy	1000 L	1-10	5-8	5-8	1-5	Acid-alkaline	Present	Present
	Dairy products	1000 L	2-10	10	10	5-10	Acid-alkaline	Deficient	Present
Beverage	Alcoholic	1000 L	5 – 20	8-10	8-10	1-8	Acid-alkaline	Deficient	Deficient
	Non-alcoholic	1000 L	2-5	5-8	5-8	1-8	-	Deficient	Present
Textiles	Polyester	1000 kg	60-130	8	8	8	Alkaline	Deficient	Present
Paper	Pulp and paper products	1000 kg	15 - 250	5-10	1-8	1-8	Neutral	Deficient	Deficient
Tanning	Leather products	1000 kg hide	20-40	10	10	10	Acid-alkaline	Present	Deficient

*Biological oxygen demand

**Chemical oxygen demand

***Total suspended solids

Legislation

National Water Act (NWA) (Act 36 of 1998)

In Section 21 (e) irrigation is defined as the means to apply the wastewater to any land or property for the production of crops as well as cultivating of pastures or any other suitable purpose. The term wastewater as defined by the NWA in Section 21 (e) is seen as water containing waste, or water that has been in contact with waste.

Table 2 Required properties of wastewater when used for irrigation in South Africa (Republic of South Africa, 2004).

Parameters	Required when irrigating on land		
	2000 $m^3.d^{-1}$	500 $m^3.d^{-1}$	50 $m^3.d^{-1}$
pH between	5.5 – 9.5	6 - 9	6 – 9
COD not exceeding	75 $mg.L^{-1}$	400^2 $mg.L^{-1}$	5000^2 $mg.L^{-1}$
EC^1 not exceeding	70 $mS.m^{-1}$	200 $mS.m^{-1}$	200 $mS.m^{-1}$
Faecal coliforms not exceeding (per 100 ml)	1000	100 000	100 000
Ammonia as Nitrogen, not exceeding	3 $mg.L^{-1}$	-	-
Nitrate/Nitrite as Nitrogen not exceeding	15 $mg.L^{-1}$	-	-
Chlorine not exceeding	0.25 $mg.L^{-1}$	-	-
Suspended solids not exceeding	25 $mg.L^{-1}$	-	-
Phosphorus not exceeding	10 $mg.L^{-1}$	-	-
Fluoride not exceeding	1 $mg.L^{-1}$	-	-
Soap, oil or grease not exceeding	2.5 $mg.L^{-1}$	-	-

[1]Electrical Conductivity

[2]After removal of algae

Sections 21 (e), (f) & (h), (g), and (j) discuss the following respectively, in order as found in the Act: Irrigation on land with waste or water containing waste, discharge of waste or water containing waste into a water source, disposing of waste in a manner which may detrimentally impact on a water source and removing, discharging or disposing of water found underground if it is necessary for the safety of the people.

In view of Sections 21 (e), (f) & (h), (g), and (j) from the National Water Act it is seen that the wastewater generated from the amarula, wine and Brandy production processes are not suitable to be discharged into the environment without being treated.

Wine production and process

Wine is an alcoholic beverage produced from the total or partial fermentation of fresh grapes (Brito *et al.,* 2007). The wine market is very diverse, with different producers in different regions, different grape varieties and each producer having their own style when creating wine, one method cannot be ascribed to the wine making process (Grainger & Tattersall, 2005). However, a basic outline for the production of white wine as well as red wine is given (Fig. 3).

The wine making process starts as soon as the harvested grapes arrive at the cellar where after the grapes, both red and white, are destemmed and crushed. The next step is maceration; this is the process step where the grape skins are in contact with the grape juice so that the nutrients, colourants and flavourings are extracted from the skins, pulp and seeds (Jackson, 2008). This is also the step where the production of white wine and red wine differs. Red wine is fermented during and after maceration whereas white wine is pressed/racked after maceration and then fermented (Brito *et al.*, 2007; Jackson, 2008).

Racking is a process step where the juice of the grapes is transferred from one container to another without pressing; this juice is referred to as *free-run* juice (Grainger & Tattersall, 2005). After the free-run juice has been transferred, skins, seeds and a small fraction of juice are left behind. These constituents will be transferred to the press to further obtain the remainder of the juice (Grainger & Tattersall, 2005).

The last few steps in the wine production process are where the fermented grape juice is clarified and then bottled. Clarification is necessary to remove small suspended particles (colloids) in the wine, which makes the wine opaque (Grainger & Tattersall, 2005; Jackson, 2008). Clarification can be achieved by filtration or by adding a colloidal substance with an opposite charge to bring the unwanted colloids in the wine out of suspension (Grainger & Tattersall, 2005).

Every process step in the production of wine produces waste, in both liquid and solid form. The solids are used for the production of grape distilled products, whereas the liquid fraction has to be treated.

Winery wastewater characteristics

As mentioned, the waste produced from the wine making process is both in liquid and solid form. The majority of the solid waste is used by distilleries; however, a fraction of the

seeds, skins, stems and lees are still found in the liquid fraction of the winery wastewater (Brito *et al*., 2007). Winery wastewater also consists of a fraction of the must and wine that is lost during production, filtration aids and clarification agents used during production and chemicals used to clean the equipment (Brito *et al*., 2007).

Winery wastewater is produced throughout the year; however, the characteristics of the wastewater differ throughout the pre-harvest, early harvest, peak harvest, post-harvest and non-harvest seasons (Conradie *et al*., 2013). The majority of the factors contributing to polluting water used in the wineries occur in the harvest season. The harvest season in the Southern Hemisphere occurs from the end of January until the beginning of May (Conradie *et al*., 2013).

When analysing winery wastewater for the purpose of disposing of it by irrigation, factors such as: pH; chemical oxygen demand (COD); electrical conductivity (EC), sodium adsorption ratio (SAR) and nutrient levels (heavy metals and organic acids) are the main concerns (Sheridan *et al*., 2011). Winery wastewater usually has a low pH, ranging between 3-8 (Van Schoor, 2005; Brito *et al*., 2007). The residue of lees, must and wine that pollutes the water when rinsing the tanks contributes to the level of COD. Furthermore, the COD value will fluctuate during the season depending on the degree of contamination. Values for untreated winery wastewater in South Africa have been found to be between 300 – 60 000 mg.L^{-1} (Van Schoor, 2005; Sheridan *et al*., 2011; Conradie *et al*., 2013).

The concentrations of the elements Ca, Mg, Na and K all play a role in the EC and SAR value (Van Schoor, 2005; Mosse *et al*., 2012). The concentration values of these elements found in winery wastewater vary quite significantly according to different literature sources (Table 3).

Table 3 Cation (Na, K, Ca & Mg) concentrations found in winery wastewater.

| Reference	Element concentration in winery wastewater (mg.L^{-1})			
	Na	K	Ca	Mg
Christen *et al.* (2010)	200 - 900	415	25.3	10.6
Sheridan *et al.* (2011)	54.5	82.9	59.3	12.3
Mosse *et al.* (2012)	99	240	-	-
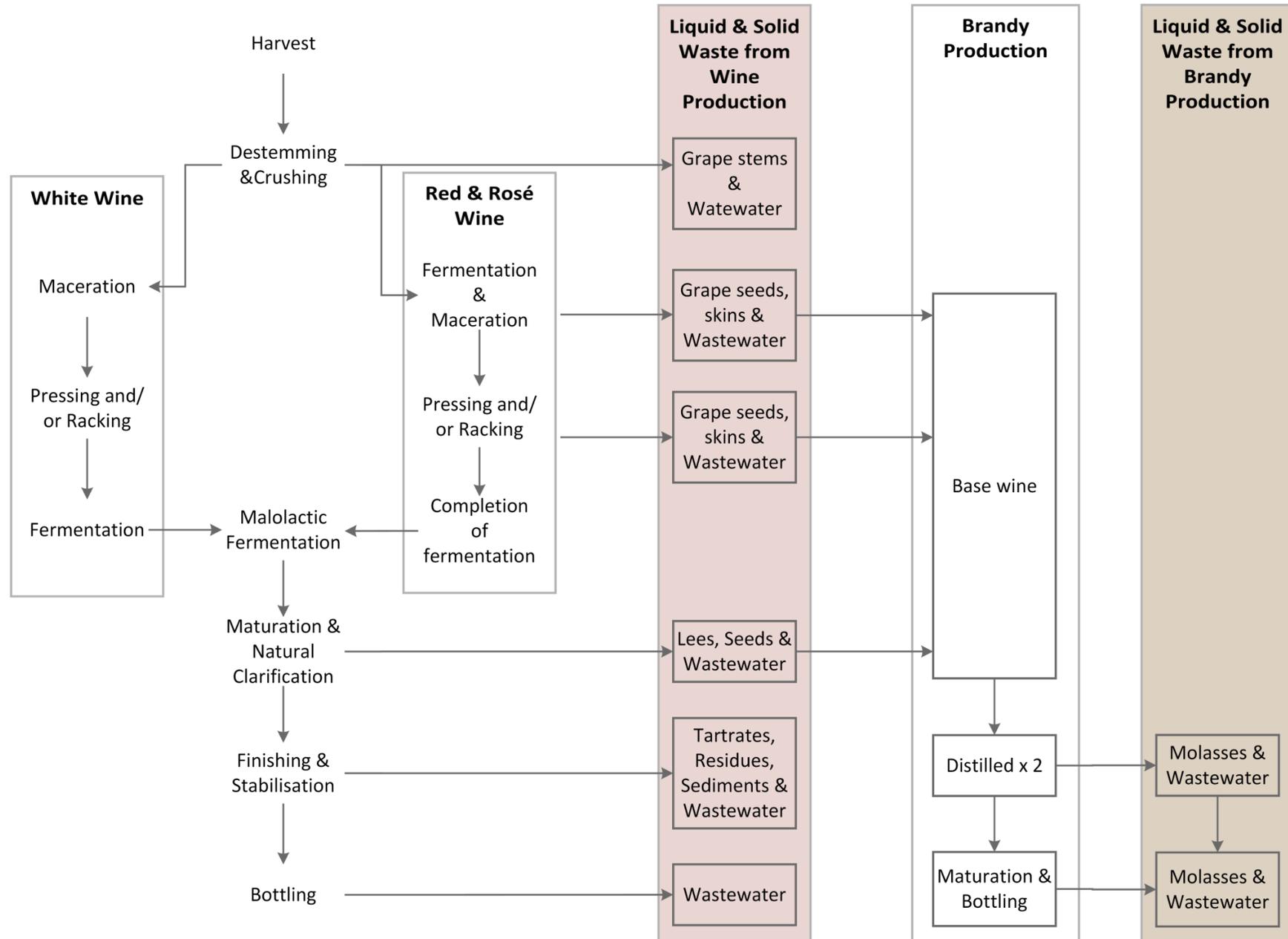
Figure 3 An overview of wine and Brandy production as well as the production of their wastewater respectively (Adjusted from Grainger & Tatter, 2005; Brito *et al.,* 2007; Jackson, 2008; Ramsden, 2012).

Brandy production and process

Brandy is an alcoholic beverage produced from base wine (EPA, 1997). Base wine is the fermented product (juice and skins) obtained from crushed fruit (apples, peaches, apricots or blackberries); however, South African legislation states that all brandies should be produced from grapes (Republic of South Africa, 2014). The base wine is distilled; a process that separates compounds via heat, and the vapour produced is condensed back into a clear white liquid (Ramsden, 2012). Thereafter this liquid is matured for a minimum of three years in oak casks and then bottled (Republic of South Africa, 2014).

The Brandy production process differs between different producers, but the basic outline of the process remains similar. The base wine used to produce the Brandy can be produced at the distillery itself or it can be purchased from a winery (EPA, 1997; Ramsden, 2012) (Fig. 3).

Brandy wastewater characteristics

Waste generated from the Brandy production process is mainly from the product that is left over after distillation. This substance is a thick, dark brown mass that is high in dissolved and suspended solids (TSS: 5 000 – 10 000 mg.L^{-1}), has a low pH (ranging between 3-5), it has high phenolic compounds, it also has a high concentration of nitrogen, phosphorus, ammonia and potassium and a COD ranging between 30 000 – 70 000 mg.L^{-1} (Chrobak & Ryder, 2005; Musee *et al.*, 2007; Mohana *et al.*, 2009).

Fluctuations in the characteristics of the waste that is generated by these industries can be attributed to different methods used by each specific distillery, a specific feedstock that is used to create a specific product and the waste management practices that are implemented by different producers (Musee *et al.*, 2007). The wastewater generated by the Brandy industry has to be treated, for it does not comply with South African legislation as seen in the section discussed previously.

Amarula production and process

Amarula Cream is a liqueur produced in the Republic of South Africa. This liqueur is produced from the pulp of the fruit of a marula tree (*Sclerocarya birrea*) found in various parts of South Africa. It is a small yellow stone fruit, which can be harvested from January until March (Strong, 2010; Anon, 2014b).

After harvesting the fruit it is pitted and pulped, the pulp is then used as the main ingredient for the production of Amarula Cream. The pulp is fermented under similar conditions as the wine fermentation process and thereafter the marula wine is distilled.

The distilled product is aged for two years in oak barrels then further treated to produce a smooth alcoholic beverage. A basic representation of the Amarula Cream process was supplied by Distell (Fig. 4) (Blignaut, J. 2013, Distell, Stellenbosch, South Africa, personal communication).

Marula wastewater characteristics

Amarula Cream, much like the production of wine and Brandy, generates waste at every step of the process (Fig.4). After the fermentation of the marula pulp, the majority of the solid waste is separated and generated into compost; however, the smaller particles and suspended solids are not removed. The fraction of solid waste that remains is distilled with the wine. After distillation a thick, light brown substance with a high amount of suspended and unsuspended solids remains (Blignaut, J. 2013, Distell, Stellenbosch, South Africa, personal communication). There is currently not a lot of scientific literature available on the wastewater characteristic of Amarula Cream. However, a study done by Strong (2010) on the treatment of Amarula Cream wastewater did characterise some of the parameters of the waste. These parameters included a COD of $\pm$ 30 000 mg.L^{-1}, a low pH of between 3 and 4, high suspended solids (10 000 mg.L^{-1}) and high phenolic compounds (866 mg.L^{-1}) (Strong, 2010).

Water purification

There are many different aims to treat wastewater, these include: to reduce the hazardous compounds present in wastewater so that it can safely be disposed of or reused, the protection of the public's health as well as the environment, to recycle and recover a vulnerable resource to comply with the legal standards that are placed on wastewater that is to be discharged (Gray, 2010).

There are many criteria that should be considered before choosing a treatment system for a particular wastewater. Wastewater as a whole can be grouped into domestic waste also known as sewage, industrial waste and municipal waste. Municipal waste is a combination of the former two streams (Ofoefule *et al.*, 2011).

Wastewater treatment can be seen as the removal of solids, bacteria, plants, algae and organic and inorganic compounds by using either physical, chemical or biological methods of purification (Cooke, 2000).
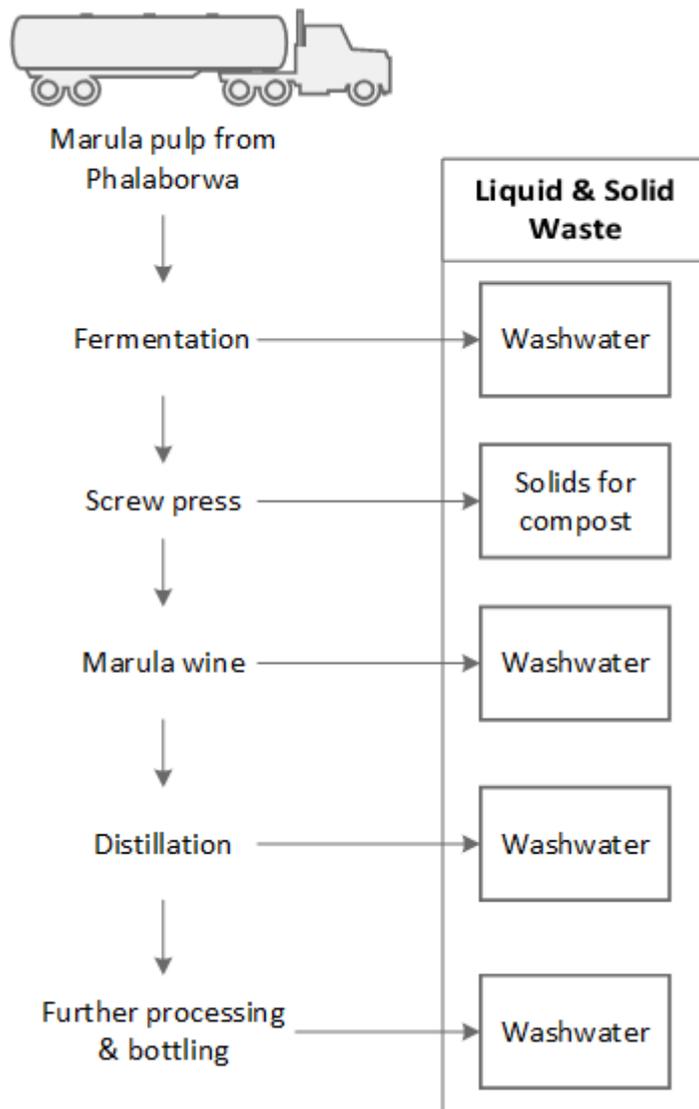
Figure 4 The Amarula Cream production process (Blignaut, J. 2013, Distell, Stellenbosch, South Africa, personal communication).

Treatment options

There are many different treatment technologies developed to treat wastewater from various industries. These include: physical, physicochemical, chemical and biological (Ofoefule *et al.*, 2011). A combination of two or more technologies is often used together as primary, secondary and/or tertiary treatment, to optimise the overall treatment (Fig. 5). Deciding upon a treatment system is dependent on the type of waste that is produced, the conditions of the site, financial resources and local geography (Jhansi & Mishra, 2013).
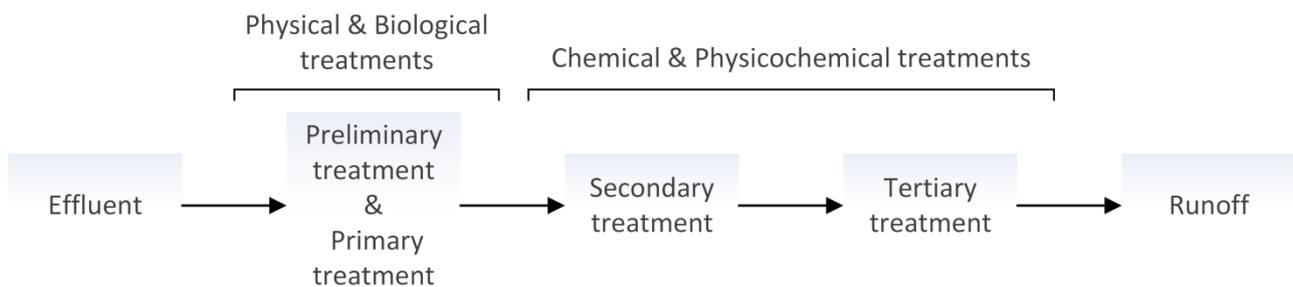
Figure 5 Flow diagram displaying the different treatment options and the different processes used for that treatment (Metcalf & Eddy, 2003).

Physical treatment options

Physical treatment options are often used as the preliminary treatment step in wastewater treatment. This is defined as the removal of large solids and grit or the removal of organic or inorganic suspended solids (Metcalf & Eddy, 2003). This is to either lower the OLR of the wastewater, particularly if a biological treatment option is to be used as the primary treatment, or for mechanical purposes, such as prevention of clogging or damage to pipes and pumps (Brito *et al.*, 2007).

Physical treatment processes include the following: sedimentation, screening, and centrifuging, aeration, filtration, flotation and skimming, degasification and equalisation (Cooke, 2000). These treatment options are strictly physical methods, there are no chemical or biological changes brought to the wastewater (Ofoefule *et al.*, 2011).

Sedimentation

The basic theory of sedimentation can be described as follows: wastewater enters into a tank, this can be in batches or an inline system, suspended particles in the wastewater separate from the water through gravity (Arundel, 1995; Droste, 1997).

Since sedimentation is based on gravitational forces acting on the suspended solids in the wastewater, it is necessary to know the settling velocities of the particles before the basin is designed (Droste, 1997).

There are three types of sedimentation: Type I sedimentation is known as discrete particle settling; this can be described as individual particles settling without interaction with neighbouring particles. Type II sedimentation is known as flocculent sedimentation. It is based on the same principle as Type I sedimentation, except that the particles in the wastewater interact with one another to form larger particles so that their settling velocities will increase. Type III sedimentation is known as zone settling; this is where the interaction between the particles becomes so concentrated that it creates zones of settling. There is a clear zone, a settling zone and a zone of dense concentrated particles (Droste, 1997).

Screening

Screening is a process widely used for reducing solids within the wastewater. There are different technologies available as well as a wide range of screen sizes. The specific device and screen size of choice will depend on the required condition of the wastewater, bearing in mind the treatment process that will follow (Nemerow & Dasgupta, 1991).

There are various types of screening technologies available; one type can be described as a rotary drum that is installed horizontally so that it is in line with the wastewater pipeline or as a rotary drum that is fed from the top (referred to as an overhead-fed unit). Another technology used is called bar rack; this unit is installed vertically at a slight angle, the water runs down the rack and the solids accumulate at the bottom of the rack (Nemerow & Dasgupta, 1991, Anon, 2014c).

Centrifuging

A centrifuge is a mechanical device that uses centrifugal forces to separate materials of different densities; this can be solid-liquid separation or liquid-liquid separation. A centrifuge consists of a chamber where the materials enter, that is spun by an electrical motor. The centrifugal force is proportional to the rotation rate of the rotor (Nemerow & Dasgupta, 1991; Berk, 2009b).

There are different types of technologies available and can be designed according to the required result of the wastewater (Berk, 2009b).

Filtration

As water from the earth`s surface filter through the ground into the aquifers, it undergoes a type of purification. This natural filtration process has been recognised and is now widely

applied in the treatment of wastewater (Vesilind *et al.*, 1994). Filtration can be described as a process which removes solids from either a liquid or a gas phase. This process occurs as the mixture passes through a porous medium (filtration medium) that retains the solid particles (Berk, 2009c).

Filtration can be used as a preliminary treatment to remove suspended solids from the wastewater, or it can be used as a tertiary treatment for further refinement (Droste, 1997). Sand is widely used as a medium for filtration where slow and rapid sand filters are two of the most common methods used (Safari *et al.*, 2013). The two different methods are based on the same basic principle; however, the difference occurs in the design of the filter. The slow sand filter has a longer sand-bed length and concentrates the solids at the top of the filter bed, whereas the rapid sand filter is not as deep and utilises the entire depth of the filter bed for removal of solids (Droste, 1997).

A study done by Prasad *et al.* (2007) used a sand filtration bed as a pre-treatment step to distillery wastewater and found that there was a significant reduction in various physicochemical and biological characteristics. Another study done by Welz *et al.* (2012) used biological sand filters, sand columns and sand microcosms as a process step in the treatment of winery wastewater. It was found that sand proved to be a suitable medium in the removal of phenolic compounds in both biotic and abiotic experiments. Furthermore, the treatment of winery wastewater by biological sand filters has also shown efficient COD, nitrogen and phosphorus removal (Rodriguez-Caballero *et al.*, 2012).

Physicochemical treatment options

Various physicochemical processes are used to treat winery and distillery spent wash. These treatment processes are usually implemented after the primary treatment to further reduce the COD, as well as for colour removal and to reduce the turbidity (Mohana *et al.*, 2009). There are many different physicochemical treatment options available, each one operating in a different manner. Each treatment process has a specific range of particle size it is able to remove. Therefore it is important to understand the mechanism in each process to ensure desired removal of matter. The different physicochemical treatment options that are used in the effluent industry are: coagulation, flocculation, ion exchange, adsorption, reverse osmosis, nanofiltration, microfiltration and ultrafiltration (Shon *et al.*, 2009).

Coagulation and Flocculation

Coagulation and flocculation is a process designed to remove colloids from the wastewater. A colloid can be defined as a particle held in suspension due to its size,

charge and/or hydration. Colloids are particles which are generally smaller than 1 μm, and although they are larger than molecules they still cannot be seen under a microscope. The presence of these small particles within wastewater is responsible for a high BOD, turbidity and colour within wastewater (Nemerow & Dasgupta, 1991; Hughes, 2001).

Coagulation is the first step in this process; it destabilises the colloidal particle so that the surface charge of the particle changes. This causes the particles to attract one another to form larger particles called flocs (Vesilind *et al.*, 1994; Mohana *et al.*, 2009). The chemicals most commonly used for coagulation are: Alum (aluminium sulphate), ferric sulphate, ferric chloride (also known as copperas) and chlorinated copperas (Nemerow & Dasgupta, 1991; Howe *et al.*, 2012).

Flocculation is the second step of the process; this is where bridges are formed between the flocs to form larger agglomerates (Mohana *et al.*, 2009). A flocculator is used to introduce velocity between particles so that there will be more contact between particles to form larger agglomerates (Droste, 1997). These aggregated flocs can then be removed by centrifuging, sedimentation and/or filtration (Howe *et al.*, 2012).

Braz *et al.* (2010) conducted a study on winery wastewater (wastewater from both white and red wine production were used) using coagulation and flocculation as a processing step. It was found that this process successfully decreased the turbidity of the winery wastewater and total suspended solids. It did not, however, have any effect on the reduction of the COD.

The objective of coagulation and flocculation is to enhance other treatment processes like sedimentation and/or filtration (Shammas, 2005).

Ion exchange & Adsorption

Ion exchange and adsorption are two very similar processes. These two processes can be described as the transfer of heavy metals and organic substances from a liquid phase to a solid phase. In ion exchange, zeolites and resins are used as the solid phase (medium), and this process is used for the purpose of demineralisation of wastewater (Droste, 1997; Vassilis, 2010). The medium used can be sensitive to organic matter present in the wastewater, therefore it is recommended that the influent undergoes a pre-treatment process where most of the suspended solids and larger organic particles are removed (Berk, 2009a).

There are two main applications for the use of ion exchange as a treatment option which include water softening (i.e. removing unwanted ions such as magnesium and calcium from the wastewater) and the reduction of acidity (Berk, 2009a).

In adsorption, activated carbon (AC), peat, iron oxide, fly ash and kaolin are used as the medium, and this process is used for the removal of organic substances, such as carbon, and can also effectively remove inorganic substances (Droste, 1997; Vassilis, 2010). Activated carbon is the most common medium used for wastewater treatment due to its broad range of adsorbates, and can be used in two forms; as a granular form (GAC) or in powder form (PAC) (Droste, 1997).

The medium used for both ion exchange and adsorption reaches a point of saturation; this is a term known as "breaking point". The breaking point is an important state to identify before operation begins, for it will give an indication of the volume of wastewater the medium will be able to treat (Vassilis, 2010). The medium for both treatment options can be regenerated, after breaking point has been reached, and reused (Nemerow & Dasgupta, 1991; Vassilis, 2010).

Membrane filtration

Membrane filtration treatment processes are widely used in the water and wastewater industry. This process is used to separate colloidal and dissolved solids from water by means of a membrane and either using pressure as the driving force or an electrical current (Barakat, 2011; Shivajirao, 2012). Many different types of membranes are available and also different techniques. Depending on the composition of the wastewater and size of the particles that need to be removed this will influence the choice of membrane and technique that is used (Droste, 1997; Barakat, 2011; Shivajirao, 2012)

Membrane filtration, as seen in literature, is said to be of increasing popularity especially in the industrial wastewater industry. It has shown great success in the treatment of effluents such as winery, molasses based distillery, dairy and olive mill effluents (Shivajirao, 2012; Ioannou *et al.*, 2013).

Membranes are typically made from polymers like cellulose due to it being so inexpensive, although ceramic or metal oxide membranes are also available as they can withstand high temperatures (Shivajirao, 2012). The process of separation is quite simple; the membrane operates as a filter, letting anything through that is smaller than the pores of the membrane. Furthermore, membrane filtration is classified with regards to the pore size of the medium. The sizes of the different membrane processes are shown in Figure 6. Reverse osmosis (RO), nanofiltration (NF), microfiltration (MF) and electrodialysis are some of the processes used in the treatment of winery and distillery effluents (Droste, 1997; Barakat, 2011; Shivajirao, 2012; Ioannou *et al.*, 2013).
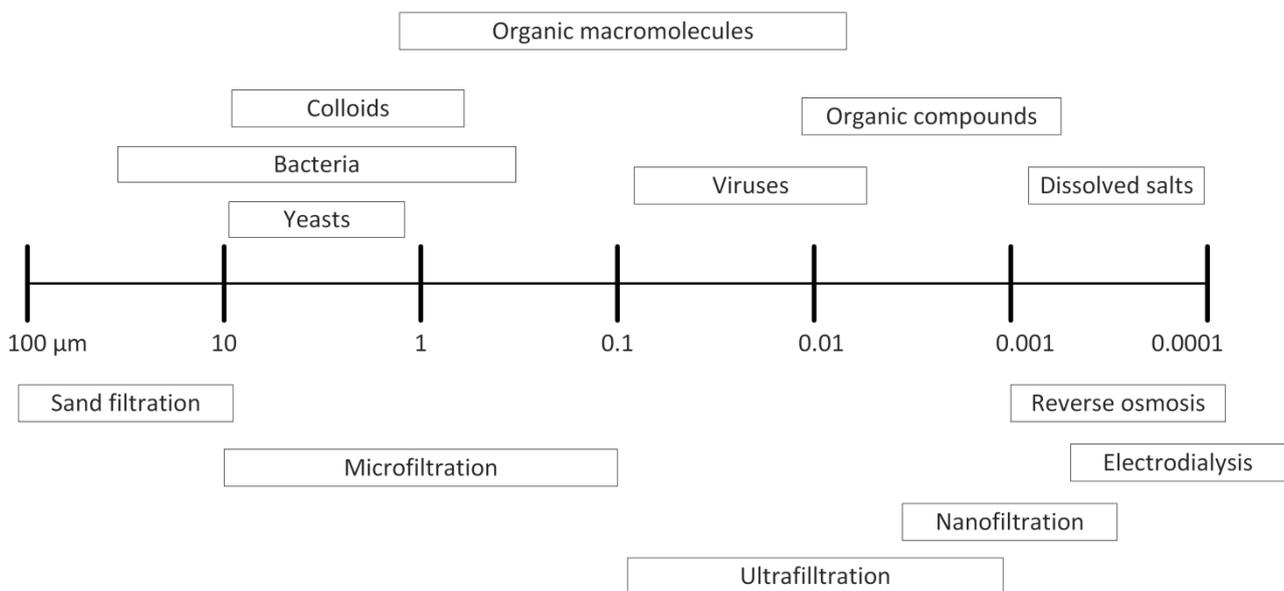
Figure 6 Membrane filtration processes and the corresponding removable particle sizes (adjusted from Droste, 1997; Barakat, 2011; Shivajirao, 2012; Ioannou *et al.,* 2013).

Biological treatment options

All biological treatment systems depend upon a selection of microorganisms to digest complex organic matter. The success in this conversion is influenced by the environment the microbes are kept in (Ofoefule *et al.*, 2011). The environmental factors that influence the operation of the microorganisms are pH; temperature; nutrients of the substrate and the presence of dissolved oxygen.

Biological treatment systems for high strength wastewater can either be aerobic or anaerobic; in some cases these two systems are used in combination (Pant & Adholeya, 2007; Mohana *et al.*, 2009).

Anaerobic vs. aerobic treatment

The basic principles for successful biological digestion are very similar for both the anaerobic process and the aerobic process. Both operations have a microbial consortium that requires a substrate (food) for growth and cell maintenance as well as an environment favourable to allow optimum function of the consortium (Eckenfelder *et al.*, 1988). The main difference between the two processes is that aerobic digestion occurs in the presence of oxygen and anaerobic digestion does not (Els *et al.*, 2005). This difference in operation causes the species within the consortium to change; with an aerated

environment the aerobic and facultative aerobic species become more prominent. Furthermore, as the consortium changes, with an aerated environment, the digestion process changes and as a result the products produced will differ (Parawira, 2004; Els *et al.*, 2005). The aerobic digestion process uses oxygen as an electron acceptor to produce energy during metabolism of the substrate. The majority (60%) of the energy available is used to produce new cells and between 40 – 50% of the carbon source present in the substrate is transferred into carbon dioxide (Fig. 7) (Eckenfelder *et al.*, 1988; Parawira, 2004).

In the anaerobic digestion process an alternative electron acceptor is needed as there is no oxygen available. Often sulphur is used if it is available as well as carbon. The reduction of organic material, as carbon is used as the electron acceptor, results in the formation of methane (50 – 70%) and the oxidation of organic material will result in the production of carbon dioxide (25 – 45%). A very small percentage of the energy produced by the anaerobic digestion process is used for new cells (5 – 10%) (Eckenfelder *et al.*, 1988; Parawira, 2004; Els *et al.*, 2005).
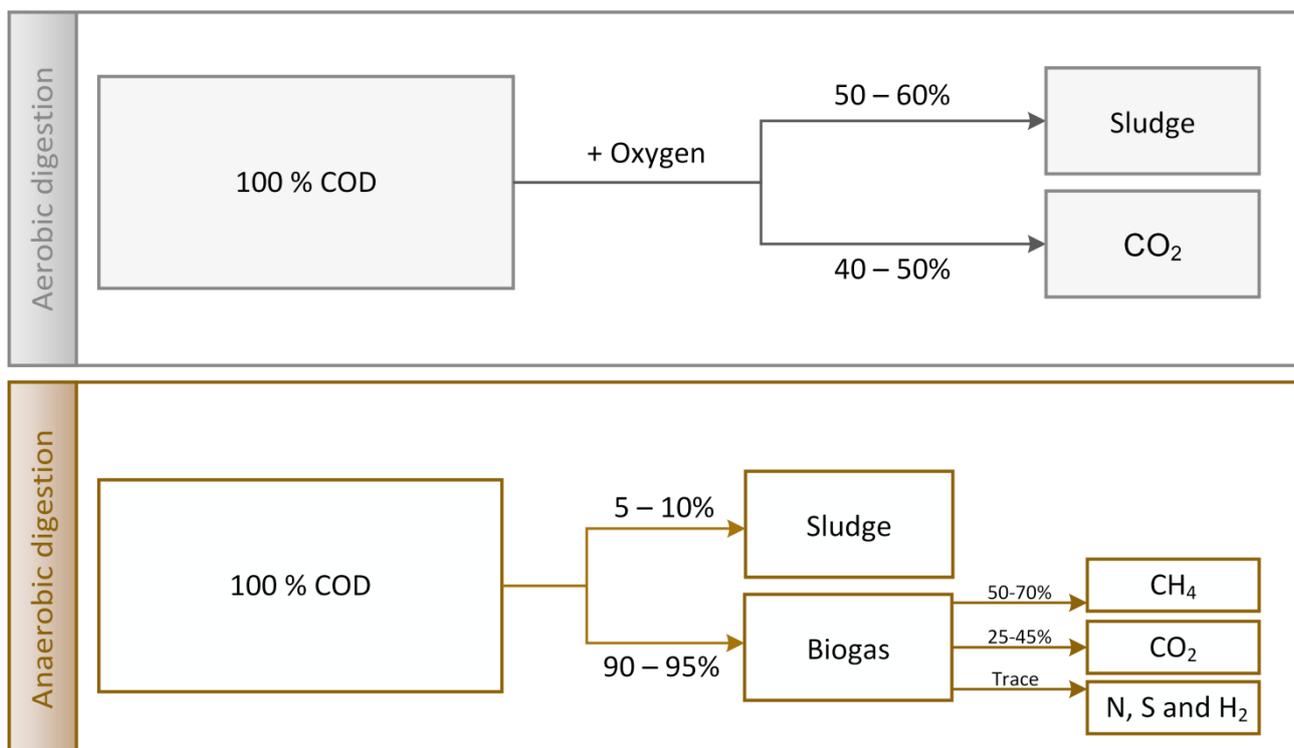

Figure 7 Digestion process of aerobic and anaerobic digestion (adjusted from Eckenfelder *et al.,* 1988; Parawira, 2004; Els *et al.,* 2005).

The production of new cellular mass (sludge) from the aerobic digestion process leads to sludge build up and has to be removed. This is one of the disadvantages of the aerobic process because the disposal of sludge is an added cost. Furthermore, this process also has high operation costs for aeration. An advantage of the aerobic process is that the start-up period is much shorter than in the anaerobic process (Els *et al.*, 2005).

Anaerobic treatment does however show several advantages over aerobic treatment, such as lower energy requirements as no aeration is necessary, it has less sludge production and the anaerobic process produces methane which is a viable energy source (Lettinga *et al.*, 1984; Fang & Liu, 2001; Berni *et al.*, 2014).

Anaerobic digestion

Anaerobic digestion (AD) is one of the oldest and most widely used technologies for the treatment of high strength effluents (Moletta, 2005; Pant & Adholeya, 2007). This treatment method has been traced back to the 1800s where Louis H. Mouras first used it in a septic tank system (Habeeb *et al.*, 2010). In the last few decades there has been an increasing interest in AD and therefore many different technologies have since been developed (Karthikeyan & Kandasamy, 2009; Habeeb *et al.*, 2010).

The use of AD as treatment method has grown widely throughout the industry where waste is concerned. It is mostly implemented in industries with high rate wastewater such as: food and beverage; pulp and paper; chemical; distilleries; treatment of domestic waste; and for the co-digestion of manure (Karthikeyan & Kandasamy, 2009; Habeeb *et al.*, 2010). Lettinga *et al.* (1984) reported that in 1983 just over 60 high rate anaerobic digesters were installed; furthermore, in 1998 a survey showed that 1229 full-scale anaerobic treatment plants have been installed worldwide (as cited by Karthikeyan & Kandasamy, 2009). Today over 8000 anaerobic digesters are installed in the European countries alone (Anon, 2011).

The success of AD is that this system, when operated at optimal conditions, can handle wastes at high organic loading rates; can be implemented for a broad range of wastewaters; has a COD reduction 80-90%; produces only 5-10% sludge; and produces biogas which can be used as an energy source (Rajeshwari *et al.*, 2000; Pant & Adholeya, 2007; Karthikeyan & Kandasamy, 2009).

Anaerobic digestion has been widely applied in the wine and wine distillery waste industry (Moletta, 2005). There has been extensive research done on the AD of the waste produced by these industries, and a few examples are given. Driessen *et al.* (1994) conducted a study on the treatment of effluent from different alcohol producing industries.

They found that effluents with a COD concentration of up to 160 000 mg.L^{-1} showed successful digestion. Furthermore a study by Wolmarans & De Villiers (2002) and Gao *et al.* (2006) on AD of maize and grain distillery, respectively, showed an 80 - 97.3% COD reduction. Successful COD reduction percentages and high methane yield have also been found in the AD of winery wastewater (Moosbrugger *et al.,* 1993a; Malandra *et al.*, 2003; Montalvo *et al.,* 2010).

Microbiology of anaerobic digestion

The digestion of complex organic matter, found in the wastewater, is made possible by an intricate consortium of facultative and obligate anaerobic microorganisms (Ofoefule *et al.*, 2011). These organisms operate in a syntrophic manner; as one group of bacteria degrades a compound the product is utilised as substrate by another group of bacteria (Gerardi, 2003). These organisms all work together in the degradation process to create an anaerobic food chain that can be categorised into four steps: hydrolysis; acidogenesis; acetogenesis; and methanogenesis (Fig. 8) leading to the production of biogas, which consists mainly of methane (CH_4) and carbon dioxide (CO_2), and a small percentage of biomass (Gerardi, 2003; Moletta, 2005; Mohana *et al.*, 2009).

Fermentative bacteria

The first and second step of the digestion process is very closely related to one another, for this reason it is often described as one step. Complex organic matter such as carbohydrates, lipids and proteins that are present in the wastewater are insoluble compounds. Hydrolytic enzymes (lipase, protease, amylase and cellulase) produced by the hydrolytic bacteria are responsible for converting this complex organic matter into smaller monomers (Anderson *et al.*, 2003; Gerardi, 2003). This step is called hydrolysis. Lipase converts lipids into long-chain fatty acids; proteins are hydrolysed by proteases into amino acids and complex carbohydrates are hydrolysed by different enzymes, depending on the degree of polymerisation, into simple sugars such as glucose (Anon, 1997). The hydrolytic bacteria involved in this step include: *Clostridium, Micrococci, Peptococcus, Bacillus* and *Vibrio* (Anon, 1997; Anderson *et al.,* 2003).

After hydrolysis the compounds become soluble, and therefore able to be absorbed by the acidogenic bacterial cell. This step is called Acidogenesis. The soluble compounds produced through hydrolysis are fermented into intermediate products such as: butyrate; propionate; acetate; and a small percentage of hydrogen (Anon, 1997; Gerardi, 2003). Some of the fermentative bacteria are facultative anaerobes and help protect the

methanogens, which are anaerobic bacteria, by consuming traces of oxygen still remaining in the wastewater (Anderson *et al.*, 2003; Gerardi, 2003).

The fermentative microbes involved in this step include *Clostridium, Micrococci, Streptococcus, Pseudomonas, Selenomonas, Bacteroides, Butyribacterium, Bacillus* etc. (Anon, 1997; Anderson *et al.,* 2003; Liu *et al.,* 2009)*.*

Acetogenic bacteria

The third step in the anaerobic digestion process is the acid forming stage; this is where the products of the fermentative bacteria are used as substrates for the acetogenic bacteria. The degradation of alcohols, fatty acids and amino acids result in the formation of hydrogen gas, carbon dioxide and acetate (Gerardi, 2003). These three products are the key compounds used by the methane forming bacteria as substrate to produce methane (Anderson *et al.*, 2003; Gerardi, 2003). There are two main groups of bacteria involved in this step: obligate hydrogen producing acetogens (OHPA) and hydrogen utilising acetogens or homoacetogens. These two groups are in a syntrophic relationship. The OHPA reduces the major fatty acid intermediates into acetate, carbon dioxide and hydrogen. The hydrogen that is produced inhibits the growth of the OHPA and should therefore be removed. The hydrogen is however the substrate for some Methanogenic species and the hydrogen utilising acetogens. This syntrophic relationship is a fragile equilibrium and should be managed carefully to maintain a steady anaerobic digestion process. *Syntrophobacter wolinii* and *Syntrophomonos wolfei* are the species involved in the OHPA group. *Acetobacterium; Acetoanaerobium; Acetogenium; Butribacterium* and the *Clostridiaceae* family utilise the hydrogen as substrate (Anon, 1997; Anderson *et al.,* 2003; Liu *et al.,* 2009).

Methanogenic bacteria

When looking at the phylogenetic tree, methanogenic bacteria are grouped in a kingdom of their own, known as Archaea (ancient), together with halophiles and thermophilic bacteria. These organisms share the trait of growing under harsh conditions. This is due to the specific differences in cell characteristics when compared to eukaryotic bacteria (Hutten, 1982; Anderson *et al.*, 2003; Gerardi, 2003).

Methanogens are the only organisms that produce methane. Methane is the simplest or most reduced compound that is produced by the anaerobic food chain. It is the final step in the degradation process (Gerardi, 2003). This occurs in the last step known as methanogenesis. The three main products used as substrates by the methanogenic bacteria to produce methane are acetate, carbon dioxide and hydrogen. Due to the

methanogens' specificity to their substrate, they are divided into two groups: acetoclastic methanogens and hydrogen-utilising methanogens (Hutten, 1982; Anderson *et al.*, 2003). The acetoclastic methanogens utilise acetate as their main substrate, and 70% of the biogas formed is from this group. The species involved are from these genera: *Methanoseata* (previously known as *Methanothrix*) and *Methanosarcina* (Anderson et al., 2003; Gerardi, 2003). The fermentation of the acetate results in the formation of methane and carbon dioxide, as seen below (Gerardi, 2003):

$$CH_3COOH \rightarrow CH_4 + CO_2$$
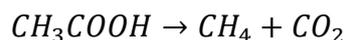
The hydrogen-utilising methanogens use mainly carbon dioxide and hydrogen as their substrate to produce the remaining 30% of the biogas (Anderson *et al.*, 2003). Utilising the hydrogen when oxidising the carbon dioxide also aids in lowering the hydrogen pressure that is required for the activity of the acetoclastic bacteria.

Species involved in the digestion of hydrogen are from these genera: *Methanobacterium, Methanobrevisbacterium* and *Methanoplanus* (Anon, 1997; Borriello *et al.,* 2012)*.*

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$
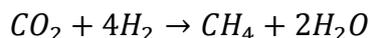
Methane production is seen to be the slowest reaction or rate limiting step when viewing the digestion pathway as a whole. This may result in an acidified environment caused by the acidogenic and acetogenic bacteria. Methanogens are most active in a pH range of between 6.5-8.0, therefore a decrease in the pH of lower than 6.5 will cause the methanogens to become inactive and result in a poor digestion (Kim *et al.*, 2004). This can be prevented by the addition of buffering agents (Anon, 1997; Borriello *et al.,* 2012).

High rate anaerobic reactors

There are many different technologies that can be used for high strength wastewater like wine and distillery effluent (Pant & Adholeya, 2007). These reactors are classified as high-rate reactors. There are three main characteristics that classify their configuration: the bacterial growth system (suspended or fixed film); temperature (psychrophilic; mesophilic or thermophilic); and whether the reactor is single or double phase (Gerardi, 2003; Moletta, 2005).

In single phase reactors the microbial activity occurs in one reactor, and in double phase reactors the microbial activity is divided into two separate systems where the acidogenic phase is separated from the acetogenic and methanogenic phase (Moletta, 2005; Mohana *et al.*, 2009). For industrial purposes the one stage system is preferred due
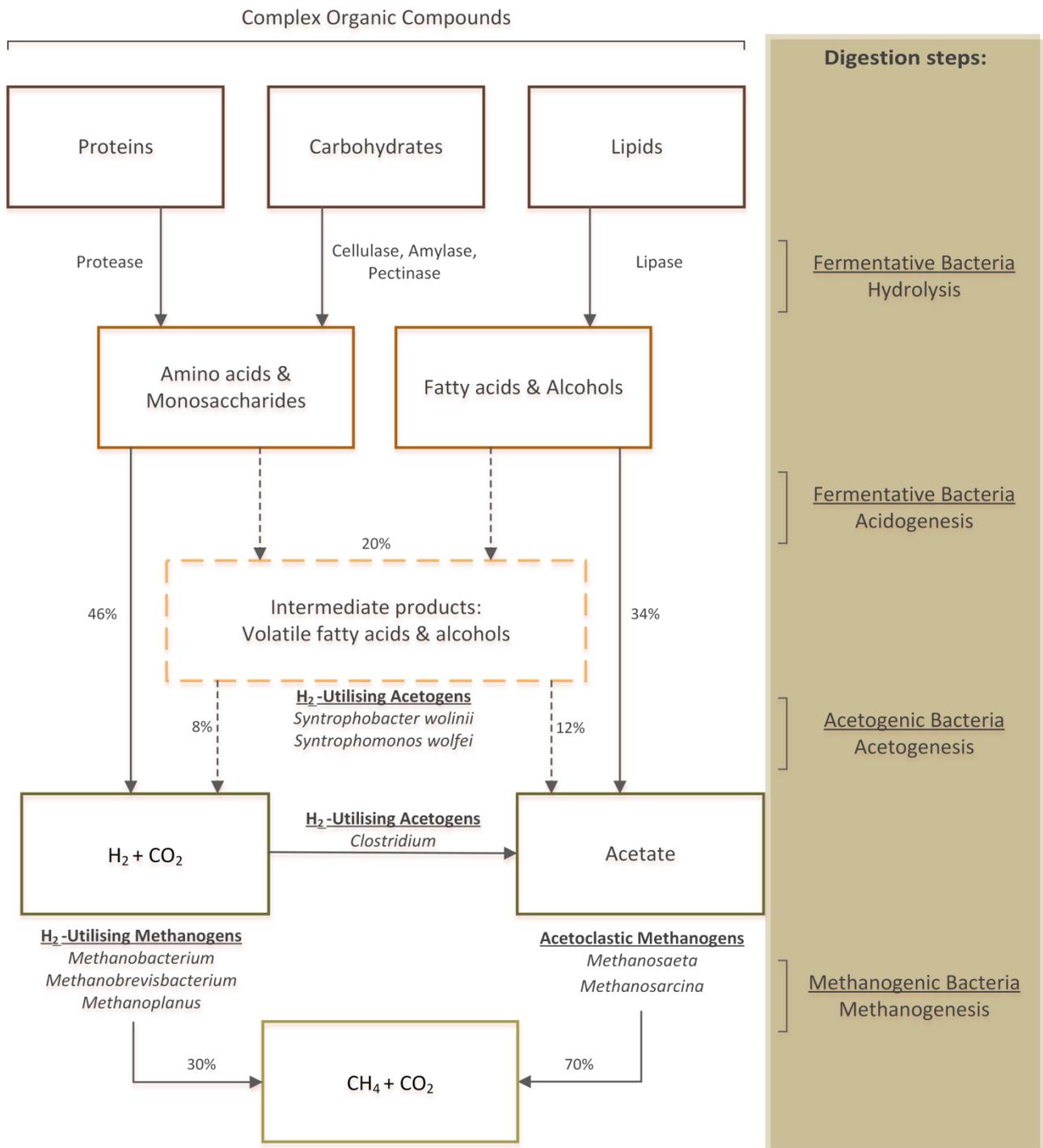
Figure 8 Anaerobic digestion pathway (adjusted from Hutten, 1982; Anon, 1997; Anderson *et al*., 2003; Gerardi, 2003; Liu *et al*., 2009).

to its simple design and lower costs (Bouallagui *et al.*, 2005). The fixed film system has also shown great success in practice. The loss in microbial activity is often found during high peakseasons, due to hydraulic overloading; this results in process failure of the digesters. The fixed film system immobilises the bacteria and is therefore less susceptible for these losses in microbial activity (Kennedy & Van den Berg, 1982; Gerardi, 2003).

The following reactors that are discussed are classified as high rate, single phase, fixed film reactors and are all considered an acceptable system to use for high strength wastewater.

Anaerobic filters

The operation of anaerobic filters (AF) is based on a fixed biological bed. This is achieved by a stationary phase (also referred to as packing material or support media) that is submerged into the reactor on which the biomass attaches (Fig. 9) (Lettinga *et al.*, 1984; Gourari & Achkari-Begdouri, 1997; Moletta, 2005). There are various types of stationary phases that can be used for biofilm attachment in AF and there has been extensive research done on which of these show the most promising results. Studies have also shown that the pore size and geometry of the media are more important than the actual media type (Lettinga *et al.*, 1984; Gourari & Achkari-Begdouri, 1997). However, the type of media used has to be chemically inert and resistant to corrosive material, resistant to friction caused by the flow within the reactor and cost effective.

The support media can be grouped into two categories: conventional or mineral media and fabricated media (Winkler, 1981). Conventional or mineral media is an older technology and includes metallurgical coke, clinker, baked clay and ceramic rings. Fabricated media is usually made from polyvinyl chloride (PVC) plastic and is manufactured in different configurations such as: long or short vertical tubes that are closely packed; corrugated and ribbed vertical sheets; and geometric net media. Other mediums also include etched glass and activated carbon (Winkler, 1981; Gourari & Achkari-Begdouri, 1997; Show & Tay, 1999; Rajeshwari *et al.*, 2000). The AF system can operate in a down flow or an upflow mode; however the majority of the full-scale reactors operate in the upflow mode (Young & Yang, 1989).

Blonskaja *et al.* (2003) used an upflow AF in combination with a UASB reactor to treat wine distillery effluent. The reactor operated at 36°C and the distillery waste used as the effluent had a COD that ranged between 49 000 – 53 000 mg.L^{-1}. The treatment showed a COD reduction of 93% and a significant production of biogas. Another study by Yu *et al.* (2006) also used an upflow AF treating rice wine effluent. The reactor was

operated at 19-27°C and treated effluent with a COD of 8 000 – 25 000 mg.L^{-1}. The reactor reduced the COD by 82% with a short hydraulic retention time of 8 h.

The advantage of the AF system is that it does not require any mixing to keep the biomass in suspension; it is very simple from a design point of view and it can recover rapidly after long periods of starvation (Rajeshwari *et al.*, 2000). There are however a few disadvantages to this design: firstly, due to the stationary phase the reactor requires a large reactor volume to optimise contact between the biomass and the untreated wastewater, and filter clogging can occur if the untreated wastewater contains a lot of solids. There have also been reports that this design requires long start-up periods (Rajeshwari *et al.*, 2000; Els *et al.*, 2005).
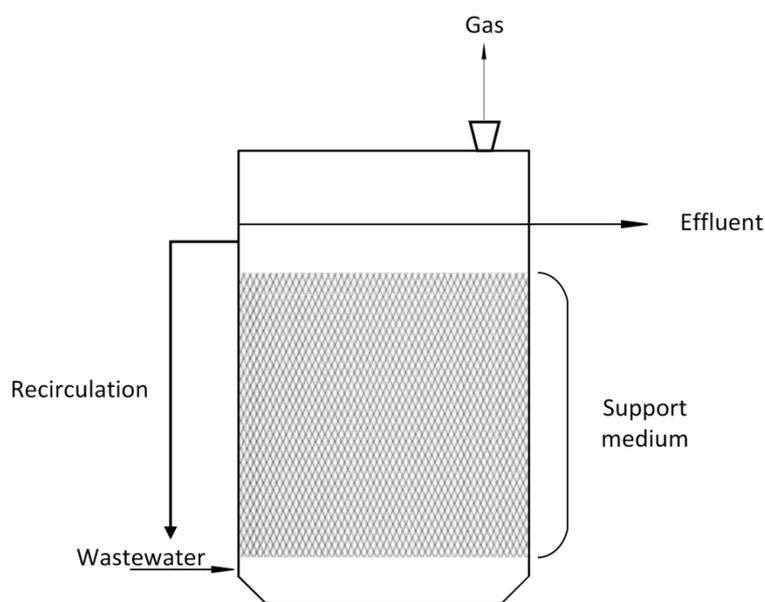

Figure 9 Schematic diagram of an upflow anaerobic filter reactor (adjusted from Moletta, 2005).

Baffled reactor and anaerobic migrating blanket reactor

The anaerobic baffled reactor (ABR) can be described as many UASB reactors placed next to each other. The ABR has a horizontal design with a series of baffles vertically spaced out throughout the reactor. The baffles force the incoming wastewater to move under and over them as it moves through the reactor (Bachmann *et al.*, 1985; Wang *et al.*, 2004). The biomass within the reactor slowly moves horizontally whilst rising due to gas production and then gently settling again. Modifications have been made to the ABR that

include settling tanks to capture solids (hybrid ABR) and packing material placed in the upper part of each chamber has also been used to capture solids. Furthermore mechanical mixers have been added in each compartment to give rise to the anaerobic migrating blanket reactor (AMBR) (Fig. 10) (Angenent & Sung, 2001; Metcalf & Eddy, 2003).

The advantage of this reactor design is its ability to separate the acidogenic stage from the methanogenic stage longitudinally down the reactor, thus creating an environment for each microbial group to develop under the most favourable conditions (Wang *et al.*, 2004). Other advantages claimed for this design is that it requires no special packing material to retain the biomass and no special separator for the biogas. The ABR can also digest a wide range of waste and it is stable to shock loads (Metcalf & Eddy, 2003).
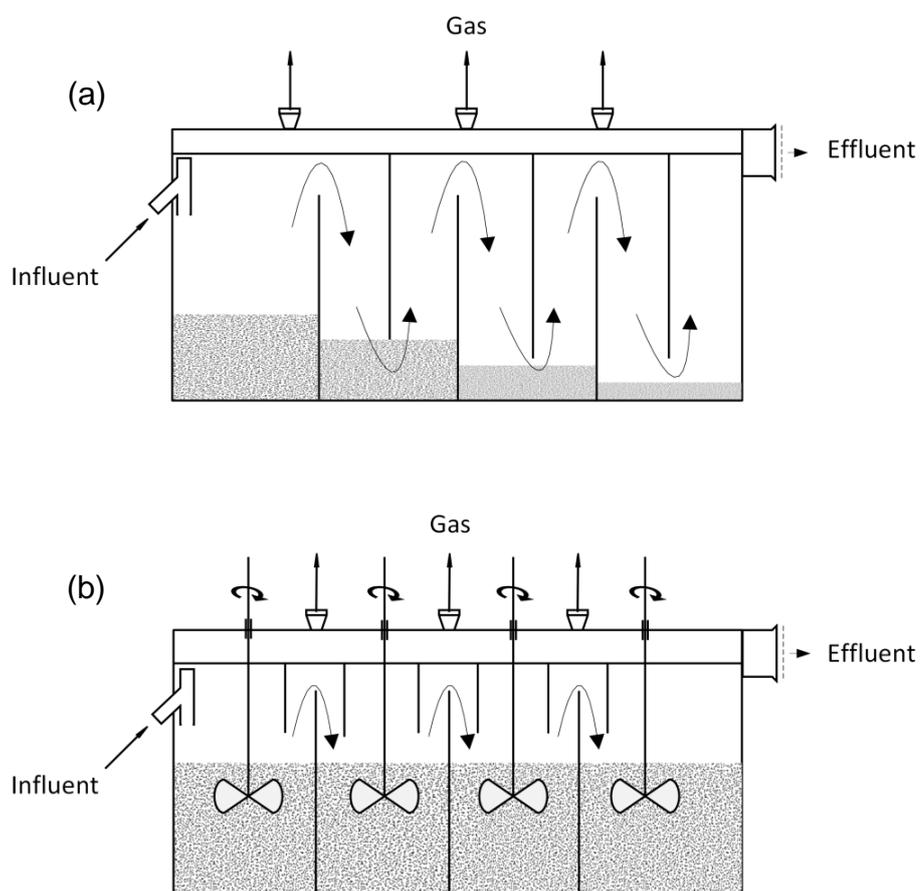

Figure 10 Schematic representations of (a) an anaerobic baffled reactor and (b) an anaerobic migrating blanket reactor (AMBR) (adjusted from Angenent & Sung, 2001; Metcalf & Eddy, 2003).

Anaerobic contact reactor (ACR)

The contact process consists of a main anaerobic reactor with an added sedimentation tank to collect washed out biomass and return it to the main reactor (Fig. 11) (Şentürk *et al.*, 2010).   The ACR operates with mechanical mixing that keeps the biomass in suspension and also aids in increasing the contact time between the wastewater and biomass. Effective mixing increases digestion and therefore increases the gas production; it also ensures a homogenous distribution of the substrate throughout the reactor (Metcalf & Eddy, 2003; Şentürk *et al.*, 2010).

The sedimentation tank attached to the main reactor allows for the main reactor to be seeded with higher volumes of biomass. The washed out biomass settles in the sedimentation tank and is recycled to the main reactor. The advantages of having larger volumes of biomass ensure successful digestion and a high yield in gas production (Şentürk *et al.*, 2010).   Furthermore, it also increases the range of waste that can be treated and ensures short hydraulic retention times. The disadvantage of this process is that digestion still takes place within the settling tank and the production of gas can therefore cause the biomass to rise in the settling tank and be washed out with the effluent (Metcalf & Eddy, 2003; Chernicharo, 2007).
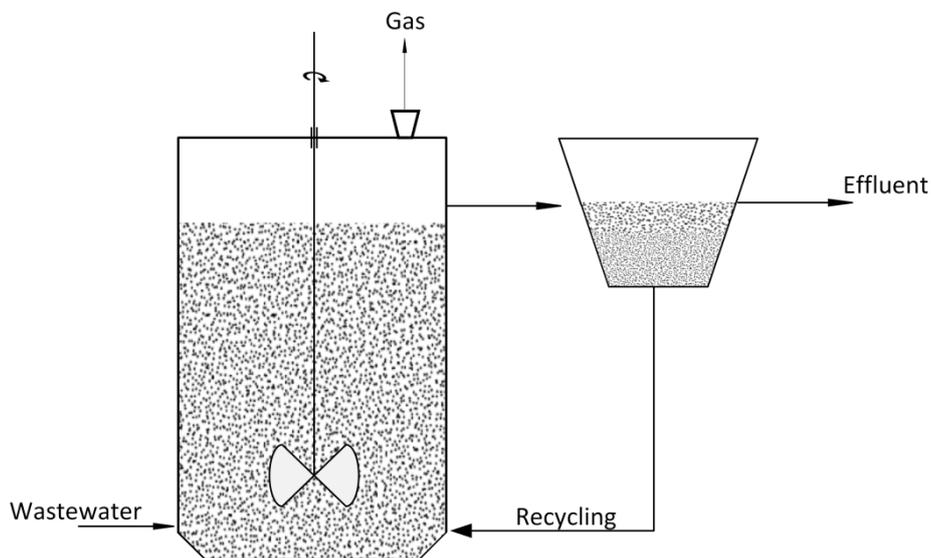

Figure 11 Schematic diagram of an anaerobic contact reactor (adjusted from Metcalf & Eddy, 2003; Moletta, 2005; Cernicharo, 2007).

Anaerobic sequencing batch reactor (ASBR)

The ASBR is a single tank design that operates in a fill-and-draw process. It is a four-step process, which are all carried out in one tank: (1) fill; (2) react; (3) settle and (4) decant (Fig. 12) (Metcalf & Eddy, 2003; Grady *et al.*, 2011). After the tank is filled the wastewater is mixed to allow digestion to take place − this is the reaction step. After sufficient reaction time the sludge is allowed to settle before the treated effluent is decanted (Metcalf & Eddy, 2003; Britz *et al.*, 2004). Due to this process being a batch system, it is important to remember that when the treated effluent is decanted suction will occur; therefore storage of biogas should be provided to equalise the pressure in the tank. While decanting the treated effluent, the gas bags will decrease in volume to compensate for the loss in pressure and as the reactor tank is filled in the feeding step the gas bags will refill (Zaiat *et al.*, 2001; Moletta, 2005).

There are many factors that affect the performance of the ASBR, the most important being the feeding strategy, mixing regime and the ratio of biomass to substrate concentration (Zaiat *et al.*, 2001; Ratusznei *et al.*, 2003). Continuous/semi-continuous flow systems can be implemented with ASBRs (Metcalf & Eddy, 2003); however this will greatly affect the feeding strategy. Furthermore an additional reactor tank will be needed − as the one tank is filled the other can complete the cycle (Metcalf & Eddy, 2003; Ratusznei *et al.*, 2003).

The primary disadvantage of the ASBR process is that the design requires a higher level of sophistication and maintenance. This is associated with the timing and control units and automated switches and valves. There is also potential for floating solids and unsettled biomass to be lost during the decanting step and lastly, due to this system being a batch system, storage units have to be considered to control bulking of untreated effluent (Mahvi, 2008).

Upflow anaerobic sludge blanket (UASB) reactor

The UASB reactor was developed in the late 1900s by Dr. G. Lettinga at the Wageningen University in the Netherlands. Due to its simple design, its cost effectiveness and the fact that it can be applied to a wide range of industrial and municipal wastes (Schmidt & Ahring, 1996; Tiwari *et al.*, 2006), the UASB has become one of the most frequently used reactors worldwide (Lettinga *et al.*, 1980; Habeeb *et al.*, 2010).

The UASB reactor (Fig. 13) is a single phase reactor and operates in a similar manner as the AF, except that it does not have a support medium to retain the biomass (Grady *et al.*, 2011). The biomass is found at the bottom of the reactor and is held in

Wastewater

(1) Fill   (2) React   (3) Settle   (4) Decant
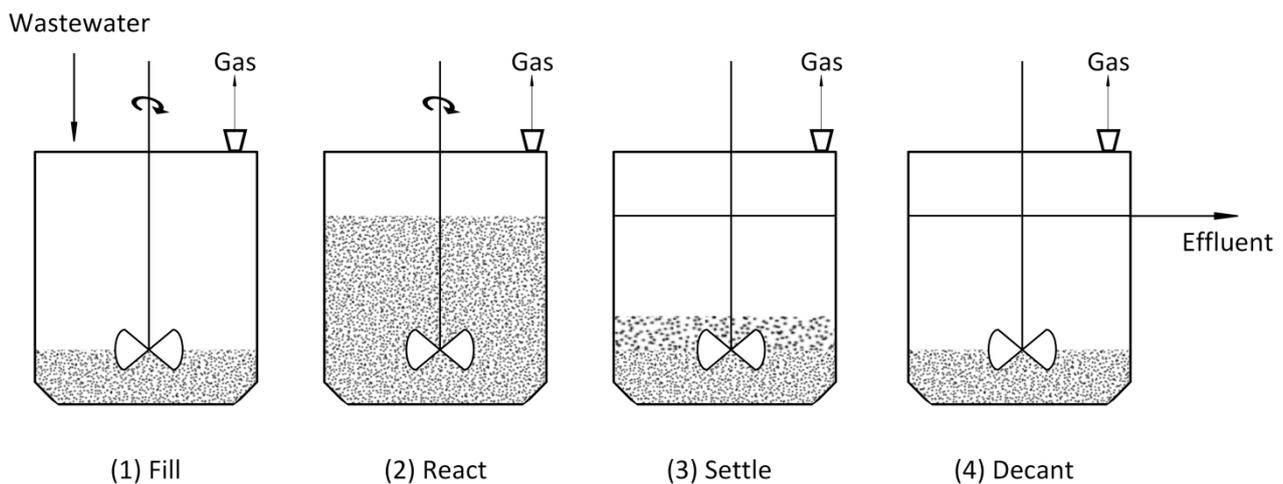
Figure 12 Schematic diagram of an anaerobic sequencing batch reactor and the operational steps (adjusted from Metcalf & Eddy, 2003; Britz *et al.,* 2004; Grady *et al.,* 2011).

suspension by the upflow force of the incoming wastewater as well as the upward motion caused by the formation of methane and carbon dioxide (Tiwari *et al.*, 2006). This upwards motion caused by the biogas can result in biomass washout, therefore a gas-liquid-solids separator (GLS) located at the top of the reactor was invented to reduce this problem (Schmidt & Ahring, 1996; Tiwari *et al.*, 2006; Habeeb *et al.*, 2010). The UASB systems often operate in the mesophilic range (30-35°C) or in the thermophilic range (50-60°C) and therefore needs an external heating unit. This can be achieved by installing a heating unit around the reactor or by heating the incoming wastewater to the appropriate temperature (Habeeb *et al.*, 2010).

As mentioned, the biomass within the reactor is subject to the upflow velocity of the incoming wastewater and causes the sludge level to rise; this can eventually cause the biomass to wash out (Forster, 1991). Washout of biomass can cause potential problems; however, the success of the UASB reactor is found in the formation of dense aggregates referred to as granules (Nuntakumjorn *et al.*, 2008). These granules are able to withstand, to a certain extent, the upflow force of the incoming effluent and can therefore be retained within the reactor. This is beneficial to the anaerobic process because it increases the contact time between the biomass and the incoming wastewater, consequently reducing the hydraulic retention time (Schmidt & Ahring, 1996).

The formation and stability of these aggregates/granules are essential for successful operation of the UASB reactor. The granulation process is therefore a very important part of successful anaerobic digestion.

Granulation

It is well known that microorganisms tend to form flocs and adhere to surfaces to form bacterial biofilms, especially in aquatic environments (Costerton *et al.*, 1987; Hulshoff Pol, 1989). Biofilm formation occurs in a series of steps: association; adhesion; microcolony formation; and finally biofilm formation. The bacteria initially adhere to surfaces in a reversible association and then eventually in an irreversible adhesion (Costerton *et al.*, 1987). Adhesion to surfaces and other bacterial and organic substances is made possible by extracellular polymeric substances (EPS) that form strong bridges between the organisms and their attachment (Schmidt & Ahring, 1996; Anderson *et al.*, 2003).

There are different types of conglomerates/aggregates described by Dolfing (1987) that occur within anaerobic digestion systems: flocs, pellets and granules. Flocs and flocculant sludge are described as conglomerates with a loose structure and after settling they form a homogenous layer. Pellets, however, are conglomerates with a more dense structure than flocs and can be seen as a separate entity when settled. Granules are described as dense pellets that have a near spherical form (Tiwari *et al.*, 2006) and maintain their shape without the presence of water.

According to the anaerobic digestion pathway it is generally accepted that granules which develop in an anaerobic system typically consist of a three-layered structure known as the multi layered-model (Fig. 14) (Liu *et al.*, 2003; Els *et al.*, 2005).

As methanogenesis is the last step of the pathway, it is predicted that the core of the granule consists mainly of species from the methanogenic family, which are strict anaerobes. The middle layer is mainly populated with the syntrophic acetogenic bacteria as this is the third step in the pathway and the outer layer is populated with the acidogenic and fermentative species as this is the second and first step in the pathway respectively. The syntrophic relationship between the organisms within the granular structure creates a settleable dense and stable granule (Fang *et al.*, 1994; Els *et al.*, 2005; Tiwari *et al.*, 2006). However, studies have shown that the microbial structure of the granule depends mainly on the species present within the feed sludge that has been used and the composition of the substrate (Leitão *et al.*, 2006).
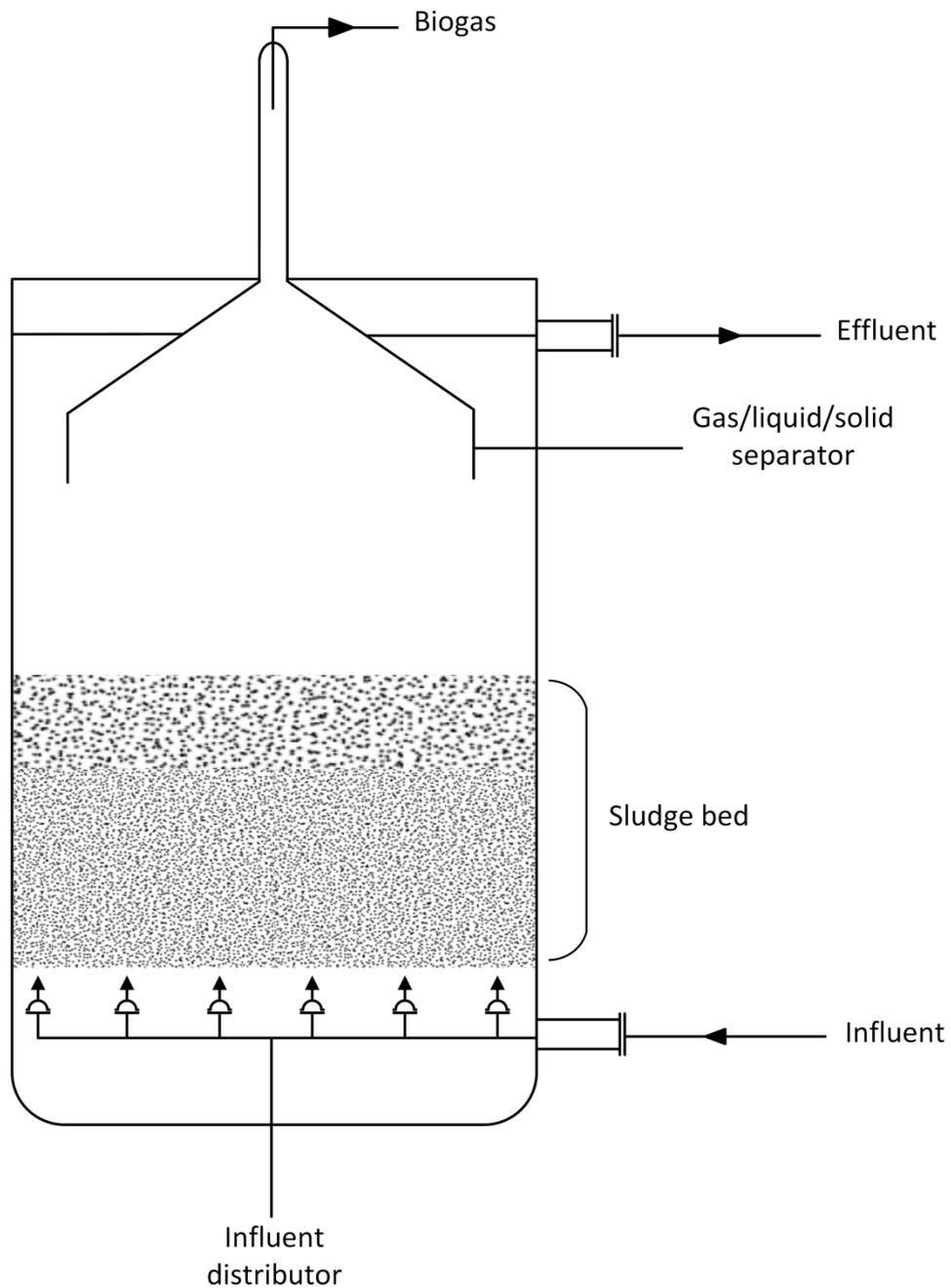
Figure 13 Schematic design of an upflow anaerobic sludge blanket reactor (adjusted from Forster, 1990; Schmidt & Ahring, 1995; Habeeb et al., 2010).

Fang *et al.* (1994) conveyed a study on the microbial structure and activity of three UASB granules treating different substrates. Sucrose, glutamate and brewery wastewater were used as a substrate under mesophilic conditions. Results showed that the granules' microstructure differed between the different substrates. The granules which digested glutamate displayed a uniform structure whereas the granules which degraded carbohydrate rich substrates had a layered structure. Bhatti *et al.* (1995) also confirmed that the mineral concentration within the substrate had an overall effect on the composition of the granular sludge and that the uptake of certain minerals such as iron, magnesium and phosphorus were preferred depending on the operational and environmental conditions.

Studies have also found a large variation in the size of granules used in anaerobic treatment, ranging from 0.1 - 5.0 mm (Kosaric *et al.*, 1990; Schmidt & Ahring, 1996; Yan & Tay, 1997). There have also been many different models designed to explain how granulation occurs and to determine where certain species are located throughout the granule itself (Zhou *et al.*, 2006).

Other than the substrate and the seed sludge, many environmental factors such as pH, alkalinity, temperature, nutrients and the hydraulic force play a role in the development of the granule. The specific consortium of the feed sludge and the development EPS also play a significant role in the granulation process (Bhatti *et al.*, 1995; Schmidt & Ahring, 1996; Tiwari *et al.*, 2006).
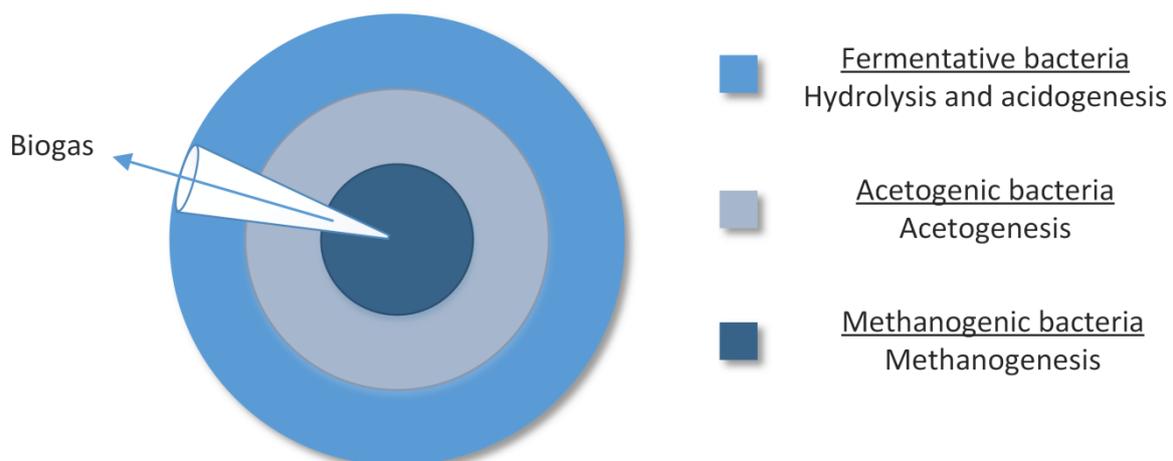

Figure 14 Illustration of a granule and its three-layered structure on microbial level. (adjusted from Fang *et al.,* 1994; Els *et al.,* 2005).

Factors affecting the granulation process

Temperature

Microorganisms are grouped into three temperature range categories: psycrophilic (5-25˚C); mesophilic (30-40˚C), and thermophilic (50-60˚C) (Gerardi, 2003; Chou *et al.*, 2004). Optimal temperature for the digestion of wastewater is a very important factor to control. The temperature affects the activity of the microbial population and slight fluctuations can inhibit the granular activity or cause granule disintegration (Metcalf & Eddy, 2003; Tiwari *et al.*, 2006).

Temperature fluctuations have different effects on the different groups of bacteria within the granule. The hydrolytic bacteria group is not greatly affected by changes in temperature, whereas most methane forming bacteria are active in the mesophilic and thermophilic range, while the acetogenic bacteria can operate at temperatures as low as 21˚C. The temperature should therefore never drop below 30˚C to avoid volatile fatty acid build up that will cause a drop in the pH (Gerardi, 2003; Chou *et al.*, 2004).

An increase in temperature shows an increase in microbial activity (i.e. a higher methane yield and a more effective degradation of wastewater); therefore it is assumed that anaerobic systems should be operated in the thermophilic ranges (Tiwari *et al.*, 2006). However, the majority of the full-scale UASB reactors are operated within the mesophilic range. It is found that the operation of a full scale thermophilic reactor does not maintain a stable temperature which in turn results in bad quality effluent. Furthermore it also requires more energy to operate at such high temperatures (Anderson *et al.*, 2003; Show, 2006a).

Alkalinity and pH

Methanogenic bacteria are more sensitive to pH fluctuations than the hydrolytic and acetogenic bacteria. The optimum pH range for methane forming bacteria range from 6.8-7.2 (Gerardi, 2003; Show, 2006a), the acid forming bacteria can operate under low pH ranges whereas the activity of the methane forming bacteria will be inhibited. This will cause an increase of VFA and furthermore decrease the pH. The methanogens will eventually cease to operate and the granular structure will disintegrate (Anderson *et al.*, 2003; Tiwari *et al.*, 2006).

It is therefore essential to maintain a stable environmental pH to facilitate optimal conditions for the methane producing bacteria. The pH control in a UASB reactor can be achieved naturally by the bacterial consortium or it can be added as chemical buffering agents, the buffering capacity of the reactor is known as alkalinity (Anderson & Yang,

1992a; Gerardi, 2003). Alkalinity is a measure of the total bicarbonate present at that time within the system (Anderson & Yang, 1992a).

Alkalinity can be produced by the bacterial consortium in various ways: form ammonia as protein is degraded, as sulphides form sulphates and as bicarbonates that are in equilibrium with carbon dioxide that form as biogas (Moosbrugger *et al.*, 1993b; Gerardi, 2003). This is however only possible if the substrate contains these specific substances; therefore the degree of natural buffering is subject to the substrate (Moosbrugger *et al.*, 1993b). If the substrate is poor in these substances then alkalinity can be added to the digester in the form of chemicals, and the most commonly used chemicals include: sodium bicarbonate ($NaHCO_3$) (Anderson & Yang, 1992b; Moosbrugger *et al.*, 1993b; Somasiri *et al.*, 2008) and calcium carbonate, also known as lime ($CaCO_3$) (Moosbrugger *et al.*, 1993b; Işık & Sponza, 2005). Other alternatives mentioned by Gerardi (2003) include: potassium bicarbonate ($KHCO_3$); sodium carbonate (Na_2CO_3); potassium carbonate (K_2CO_3); calcium hydroxide ($Ca(OH)_2$); and sodium nitrate ($NaNO_3$).

There are four basic reactions that are of importance in regulating the pH in an anaerobic system: ammonia digestion (1); VFA digestion (2); sulphate digestion (3); and the carbonaceous compounds that form during the production of methane and carbon dioxide (4) (Anderson & Yang, 1992b; Gerardi, 2003). The compounds mentioned below are the buffering ions.

$$NH_3 + H^+ \leftrightarrow NH_4^+ \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots(1)$$

$$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^- \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots(2)$$

$$CH_3COO^- + SO_4^{-2} \rightarrow 2HCO_3^- + HS^- \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots(3)$$

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow H^+ + CO_3^{2-} \dots\dots\dots\dots\dots\dots\dots\dots\dots(4)$$

Nutrients

Macronutrients

The majority of bacterial cell walls contain different ratios of carbon (C); nitrogen (N); phosphorus (P) and sulphur (S). It is necessary for these elements to be available if cell growth is to occur, and a poor availability of one or more could cause rate limitations (Price, 1985; Gerardi, 2003). It is therefore important that the influent contains a sufficient amount of the required nutrients.

The amount of nitrogen and phosphorus needed for a healthy anaerobic reactor is expressed in a ratio depending on the chemical oxygen demand (COD) of the influent: for

high strength wastes the ratio is generally 1000:7:1 (COD:N:P) (Price, 1985; Gerardi, 2003). The necessity and balance of macro- and micronutrients within an anaerobic digester is a very important and delicate system. Although many macro- and micronutrients are essential for microbial growth and operation, these substances in excess can be toxic (Gerardi, 2003; Tiwari *et al.*, 2006).

Nitrogen is mainly utilised by the methane producing bacteria in the form of ammonium (NH_4^+) and serves as an electron donor as well as a buffer. Free ammonia (FA) (NH_3), however, is toxic (Calli *et al.*, 2005; Tiwari *et al.*, 2006). The form in which the nitrogen is present depends on the pH − with an increasing pH the concentration of the FA also increases. At a pH around 7, most of the nitrogen is present as ammonium ions, at a pH above 7.4 the equilibrium shifts and the nitrogen is present as FA; however at concentrations above 3000 $mg.L^{-1}$ ammonia is toxic irrespective of the pH (Anderson *et al.*, 2003; Calli *et al.*, 2005; Tiwari *et al.*, 2006).

Phosphorus is another nutrient essential for bacterial growth (Singh *et al.*, 1999). A study done by Smith & Prairie (2004) verified that bacterial growth and attainable biomass is highly dependent on the availability of soluble phosphorus. Phosphorus accumulating bacteria consume organic matter and store it as poly-β-hydroxybutyrate (PHB) and more specifically, under anaerobic conditions, acetate is stored as PHB (Comeau *et al.*, 1986; Kerrn-Jespersen & Henze, 1993). The energy required to store PHB is gained from polyphosphates. It is also found that the transport of phosphates across the cell membrane helps facilitate the transport of metallic cations via co-transportation (Comeau *et al.*, 1986).

Micronutrients

All microorganisms have mineral requirements needed for growth and survival. Sulphide, cobalt, iron, calcium and nickel are essential metals required especially for anaerobic digestion. The methane forming bacteria have many unique enzymes that require these specific elements for the conversion of acetate to methane (Shen *et al.*, 1993b; Gerardi, 2003). Elements having a cationic charge also aid in the physical binding of the bacterial groups; they bind to the negatively charged cell wall creating a change in charge around the cell, therefore leading to further granulation (Show, 2006a; Tiwari *et al.*, 2006). The total concentration, the form in which these compounds are present and the pH are all factors that could influence whether these metals are beneficial or inhibitory to anaerobic digestion (Chen *et al.*, 2008).

Soluble sulphide is a nutrient required as a growth nutrient in anaerobic digesters (Gerardi, 2003). Sulphate reducing bacteria (SRB) are strict anaerobes and therefore

cannot use oxygen as an electron acceptor. The SRB reduce sulphate to sulphide (S^{2-}) and hydrogen sulphide (H_2S) and, when doing so, use the sulphate radical as an electron acceptor (Price, 1985; Anderson *et al.*, 2003). The reduction of sulphate can however inhibit methane production due to the SRB being in competition with the methanogens for hydrogen as substrate. Furthermore, sulphide can also bind to trace metals and essential micronutrients, to form insoluble metal sulphides (Anderson *et al.*, 2003). Therefore the balance of nutrients between the microbial groups is essential.

Calcium largely plays a role in the granulation process of anaerobic sludge. This was confirmed by Hulshoff Pol (1989) and Yu *et al.* (2001), which found that at certain concentrations of added calcium ions (400 mg.L^{-1} $\geq$ Ca^{2+} $\leq$ 150 mg L^{-1}) the granulation process was enhanced. Calcium enhances initial cell adhesion in binding to the extra cellular polymers (EPS) that act as bridging molecules between the bacteria; the EPS are negatively charged and their attraction towards one another usually requires divalent cations such as calcium (Ca^{2+}) (Yu *et al.*, 2001; Show, 2006b).

There are many processes involved in the digestion of a substrate when viewing the process from a biochemical side (Liu & Whitman, 2008). There are many elements involved in the enzymatic reactions that differ from the first digestion step to the last, especially due to the acidogenic and acetogenic bacteria being much less sensitive to oxygen than the obligate anaerobic methanogens (Liu & Whitman, 2008). The electron donors/acceptors change whether in anaerobic or aerobic conditions, as oxygen cannot be used as an acceptor (Anderson *et al.*, 2003).

Elements from the iron family such as iron, nickel and cobalt are found to be essential for digestion and granulation. These metals are required by specific enzymes involved in energy metabolism (Gerardi, 2003). The addition of metals has a significant effect on the quality of the granular sludge (Osuna *et al.*, 2003).

The addition of iron to anaerobic digester sludge enhanced the granulation process. This is likely due to the divalent charge of the element, enabling it to bind to the negatively charged polymers and form bridges between the bacterial groups (Shen *et al.*, 1993a; Shen *et al.*, 1993b). This was confirmed by Yu *et al.* (2000), which found that the addition of iron at concentrations of 300-450 mg L^{-1} enhanced granulation. Concentrations higher than that had toxic effects. A study done by Hoban and Van den Berg (1979) also found that the addition of iron in a similar concentration range as in Yu *et al.* (2000), markedly increased the conversion of acetate to methane, indicating that iron is used specifically by the methanogenic bacteria.

Physicochemical properties of granulation

Self-immobilisation of microorganisms and the stability of a maturely formed granule cannot be ascribed to a single component or reaction. Many different models have been developed to define granulation from both a biological and physicochemical viewpoint but neither one can solely be accountable for the granulation process. Liu *et al.* (2003) has developed a general model for anaerobic granulation based on a study of previous works. This model can be summarised in four steps: (1) Physical movement of bacteria that initiates bacterium-to-bacterium or bacterium-to-solid surface contact, (2) attractive forces that cause initial bonding between bacteria, these are usually a combination of physical, chemical and biochemical forces, (3) maturity and stability of the granule formed by EPS and microbial growth, and lastly (4) the mechanical effect on the shape and structure of the granule. It is advantageous for a bacterium to be a part of a larger particle, especially in a UASB reactor, since otherwise it will be subject to a high upflow velocity and wash out of the reactor (Dolfing, 1987).

Initial biofilm formation occurs via granulation precursors; this is explained by the model initially designed by Lettinga *et al.* (1980). Microparticles attach to one another to form an initial biofilm called embryonic granules, from these embryonic granules further development occurs from attachment of other organisms. There are many different microbial species involved in biofilms and the formation thereof, the difference in species development occurs due to the substrate and the starting organisms (Melo, 2003). A study done by Zhou *et al.* (2006) found that granules formed on a glucose substrate contained a majority of rod shaped bacteria and granules formed on a skim milk substrate had a majority of cocci microorganisms. Dolfing *et al.* (1985) found that acetotrophic methanogens were predominantly part of the granular consortium of the granules grown on the wastewater from a sugar factory. Seventy percent of the methane produced during anaerobic digestion is from acetate, the main acetogenic methanogens involved under mesophilic condition are: *methanosaeta* (formerly known as *methanothrix*) and *methanosarcina* (Anderson et al., 2003; Van Lier et al., 2008). Acetogenic methanogens especially *Methanosaeta* have been reported to be the key organisms to play a role in the initial granulation process (El-Mamouni *et al.*, 1997). Forster (1991) and Hulshoff Pol (1989) both found that the predominant organism found in stable mature granules was *methanothrix.* After viewing these studies it can be concluded that *methanothrix*/ *methanosaeta* are essential as precursors for the initial granulation process.

EPS also play a significant role in the initial formation and maturation of the granule (Forster, 1991; Schmidt & Ahring, 1996; Tay *et al.*, 2000; Liu *et al.*, 2002). EPS can be described as a fibrous structure at the cell surface. EPS are produced by the bacterial cell and consist of different polymers of protein, lipids, saccharides and nucleic acids (Costerton *et al.*, 1985; Schmidt & Ahring, 1996). The function of EPS is to form a three-dimensional matrix on the surface of the bacteria to capture nutrients, to attract other bacteria or to assist in attaching onto solid particles (Schmidt & Ahring, 1996; Fang, 2000). The surface charge of a bacteria cell is negative, EPS excreted by the bacteria change the negative surface charge and thereby form bridges between other bacteria or solid surfaces (Fig. 15) (Tay *et al.*, 2000). The production of EPS is greatly influenced by the specific bacteria present as well as the substrate (Zhou *et al.*, 2006).
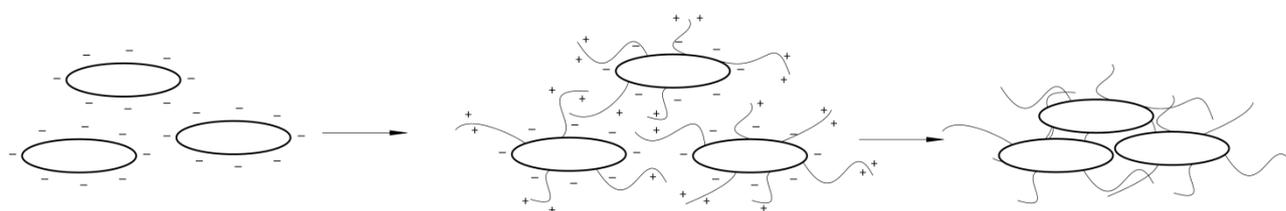

Figure 15 The effect of extracellular polymers (EPS) on the granulation process, adjusted from Schmidt *et al.* (1996) and Lui *et al.* (2003).

General discussion

Treatment of high strength wastewater has become a worldwide priority due to the scarcity of clean and fresh water. The wine and distillery industry makes use of large amounts of water and thereby produces large amounts of wastewater. Untreated effluent generated from this industry generally has a low pH (3-5), (Brito *et al.*, 2007) high concentrations of soluble solids (5 000 – 10 000 mg.L^{-1}) (Strong, 2010; Conradie *et al.*, 2013), high COD values (wine: 300 – 60 000 mg.L^{-1}, distillery: 30 000 – 70 000 mg.L^{-1}) and high concentrations of ions (Mosse *et al.*, 2012). Effluent with these characteristics is classified as high strength waste and has to be treated before it can be discharged.

Anaerobic digestion (AD) is one of the oldest and most widely used methods for the treatment of high strength wastewater (Moletta, 2005; Pant & Adholeya, 2007). There are many different technologies within AD that can treat high strength wastewater (Mohana *et al.,* 2009) and when operated at optimal conditions these systems can handle high organic

loading rates, achieve an 80-90% COD reduction and produce biogas which can in turn be used as an energy source (Rajeshwari *et al.,* 2000; Pant & Adholeya, 2007; Karthikeyan & Kandasamy, 2009).

The upflow anaerobic sludge blanket (UASB) reactor has become one of the most widely used technologies worldwide (Lettinga *et al.*, 1980; Habeeb *et al.*, 2010). The success of the UASB reactor is in the self-immobilisation of the anaerobic sludge into aggregates called granules (Schmidt & Ahring, 1996). The formation of these granules is a very complex process and cannot be ascribed to one single mechanism or reaction. Many different models have been investigated to define granulation from a specific viewpoint.

From this discussion it can be concluded that the treatment of high strength wastewater is of critical importance for the recycling of water. The treatment options that are available have been thoroughly researched and anaerobic treatment is one of the best treatments available at this point. The use of the UASB reactor has been successful in treating waste with high OLR and has an adequate biogas yield; however, the loss in the biomass is still a matter of concern.

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CHAPTER 3

INVESTIGATING THE EFFECT OF COMBINED MULTIPLE ALCOHOLIC BEVERAGES PRODUCT WASTE STREAMS ON THE EFFICACY OF AN UPFLOW ANAEROBIC SLUDGE BLANKET (UASB) AND THE EFFECT OF ADDED MAGNETISABLE GLASS FOAM PARTICLES (MP) ON THE OPERATION OF A UASB REACTOR

Summary

A combination of winery wastewater (WWW), brandy and marula wastewater was treated with a 2 L lab-scale upflow anaerobic sludge blanket (UASB) reactor for a period of 371 days. The substrate consisted of a combination of the wine and distillery wastewater and varied throughout the trial as the respective waste streams became available in their season.

Phase 1 represented the start-up season where the substrate consisted mainly of synthetic glucose substrate (SGS) and WWW of an organic loading rate (OLR) maintained at 1 kg $COD.m^{-3}.d^{-1}$. The COD reduction during this phase increased gradually from 64% to 91%.

The second phase of this trial was to monitor the reactor performance when the substrate consisted mainly of WWW. The OLR of the substrate during this phase was gradually increased from 1 kg $COD.m^{-3}.d^{-1}$ to 4 kg $COD.m^{-3}.d^{-1}$. The COD reduction was 97% with a stable pH and alkalinity.

Marula wastewater was introduced during the third phase, where the OLR of the substrate increased to 6 kg $COD.m^{-3}.d^{-1}$. The COD reduction during this phase was between 80 and 94%. The alkalinity within the reactor increased as the reactor became more stable and biogas production increased and as the OLR increased.

In the fourth phase the OLR reached a peak of 10 kg $COD.m^{-3}.d^{-1}$; this was due to all four waste streams (wine, marula and Brandy) being used to generate the substrate. The COD reduction during this phase was 82% and the biogas production was 13.4 $L.d^{-1}$.

During the last phase of the trial (5th) the substrate consisted of diluted WWW and Brandy stillage. The OLR during this phase was lowered to 4.7 kg $COD.m^{-3}.d^{-1}$. The COD reduction was 95% and a biogas production of 4 $L.d^{-1}$. A stable alkalinity and pH was maintained throughout this phase.

The stable performance of the reactor during this trial showed that the digestion of the combined multiple alcoholic beverage waste streams was successful, with good COD reduction, biogas production and methane percentage.

Introduction

The agricultural industry, mainly the winery and distillery industry, is one of the largest contributors to wastewater production worldwide (Melamane *et al.*, 2007; Rajagopal *et al.*, 2013). These industries produce on average 8 – 15 L of wastewater for every litre of alcohol produced (Van Schoor, 2005; Melamane *et al.*, 2007; Mohana *et al.*, 2009).

The effluent generated from wineries is produced in various washing operations when rinsing the equipment, during crushing and pressing of the grapes, rinsing the fermentation barrels, tanks and other equipment, for general cleaning and when the product is bottled (Malandra *et al.*, 2003; Rajagopal *et al.*, 2013). Winery wastewater (WWW) generally has a pH range of 3 - 4 and a chemical oxygen demand (COD) of 800 – 21 000 mg.L^{-1} (Driessen *et al.*, 1994; Malandra *et al.*, 2003; Gao *et al.*, 2006). Furthermore the COD of WWW fluctuates throughout the year and can be significantly higher during harvesting season than throughout the rest of the year (Malandra *et al.*, 2003).

Distillery spent wash refers to the effluent generated from distilling off alcohol from fermented products (Melamane *et al.*, 2007; Mohana *et al.*, 2009). The composition and characteristics of these effluents vary depending on the raw materials used for fermentation. The effluents generated from these processes are a thick mass due to their high organic solids and have a deep brown colour. They have a pH of between 3 and 4 and a COD of between 40 000 and 100 000 mg.L^{-1} (Wolmarans & De Villiers, 2004; Musee *et al.*, 2007; Ansari *et al.*, 2012). Wine and distillery spent wash of this nature is classified as high strength wastewater and does not comply with local regulations (Republic of South Africa, 2004), therefore pre-treatment of these effluents is mandatory if it is to be reused for irrigation or other processes.

Anaerobic digestion (AD) is a process that is widely applied for the treatment of high strength effluents (Wolmarans & De Villiers, 2004; Pant & Adholeya, 2007; Mohana *et al.*, 2009). This process is characterised by the degradation of organic pollutants by means of active anaerobic bacteria (Tiwari *et al.*, 2006; Molina *et al.*, 2009). There are many different technologies available, but many studies have found that the upflow anaerobic sludge blanket (UASB) is one of the most commonly used biological treatment systems used (Fang *et al.*, 1994; Schmidt & Ahring, 1996; Tiwari *et al.*, 2006; Nuntakumjorn *et al.*,

2008). There are several advantages when using the UASB reactor as a treatment system. The UASB reactor has been found to be very successful in digesting various types of high strength wastes, particularly wine and distillery wastewater (Moletta, 2005). Furthermore, the UASB reactor produces biogas which in turn can be used as an alternative energy source; a COD reduction of 80 - 90% can be achieved and as this is an anaerobic system, it produces very little sludge (Mailleret *et al.*, 2003). The success of the UASB reactor lies in the formation of microbial aggregates, referred to as granules (Nuntakumjorn *et al.*, 2008). The formation of these granules results in self-immobilisation of the microbial sludge and with their increased density they can withstand, to some degree, the upflow force of the incoming wastewater. The retention of biomass within a UASB reactor is essential for proper digestion and washout has been reported as a common problem found in industrial plants (Rajeshwari *et al.*, 2000). The UASB reactor has become one of the most widely used reactors worldwide (Habeeb *et al.*, 2010); however, this process has been found to be sensitive to a low pH often caused by organic shock loads (Mohana *et al.*, 2009). Organic shock loads often occur due to the variable nature of industrial wastes (Nachaiyasit & Stuckey, 1997). An industry that produces more than one product that is seasonally bound will result in the production of wastewater in different intervals throughout the year and thus organic shock loads may occur as the production of the different products overlap.

Therefore, the first objective of this study was to investigate the effect of combined multiple alcoholic beverage waste streams (each with varying characteristics (pH, COD and TSS) and volumes and each product is produced in different seasons of the year, often overlapping) on the operation and effectiveness of a UASB reactor. The second objective of this study was to investigate the effect of added magnetisable foam glass particles, for biomass retention, on the overall performance of the UASB reactor. Two reactors were operated in parallel to investigate the differences.

Materials and Methods

Wastewater

Wine, marula and Brandy wastewater were used as substrate during the course of this study. Each product is produced in different seasons throughout the year and their waste is therefore produced during that corresponding season (Fig. 1). The production of wastewater occurs throughout the year due to processes such as bottling and cleaning; however, the majority of the factors contributing to polluting the water used in wineries and

distilleries occur during the harvest season (in the case of wine production) and during the distilling process (in the case of Brandy and marula liquor production).

The wine, marula and Brandy wastewater were sampled from January to March 2014 from a local winery and distillery (Distell in Stellenbosch, South Africa). The majority of the solids in the WWW were removed using a 1 mm sieve. The marula and Brandy wastewater was centrifuged at 1 300 g to remove the majority of the solids as is done in the wine and distillery industry. Standard methods (APHA, 2005) were used to determine the pH, COD and total suspended solids (TSS) of the raw wastewater. The wastewater was stored in 25 L drums at -18°C until required. Once required, a 25 L drum was thawed and kept at 4°C while in use.

Figure 1 Representation of the annual wine and distillery wastewater production periods.

UASB reactor setup

Two UASB reactors (Fig. 2) were set up in parallel as described by Gie (2007) and Van der Westhuizen (2014). The UASB reactors had an operational volume of 2.3 L and a hydraulic retention time (HRT) of 24h. The substrate was fed into the bottom of the reactors semi-continuously with the aid of an electronic timer and a peristaltic pump (Watson-Marlow 323). A second peristaltic pump was used for recirculation purposes at an upflow velocity of 0.75 m.h^{-1}. The outflow of the effluent, located at the top of the reactor, was collected in 2 L Schott bottles. The biogas volume was measured by a manometric unit equipped with an electronic controlled counter. The outlets of both the effluent as well as the biogas were equipped with a U-shaped tube to ensure that anaerobic conditions were maintained inside the reactor. The UASB was operated at a temperature of 35°C by means of an electronic control unit and heating tape.
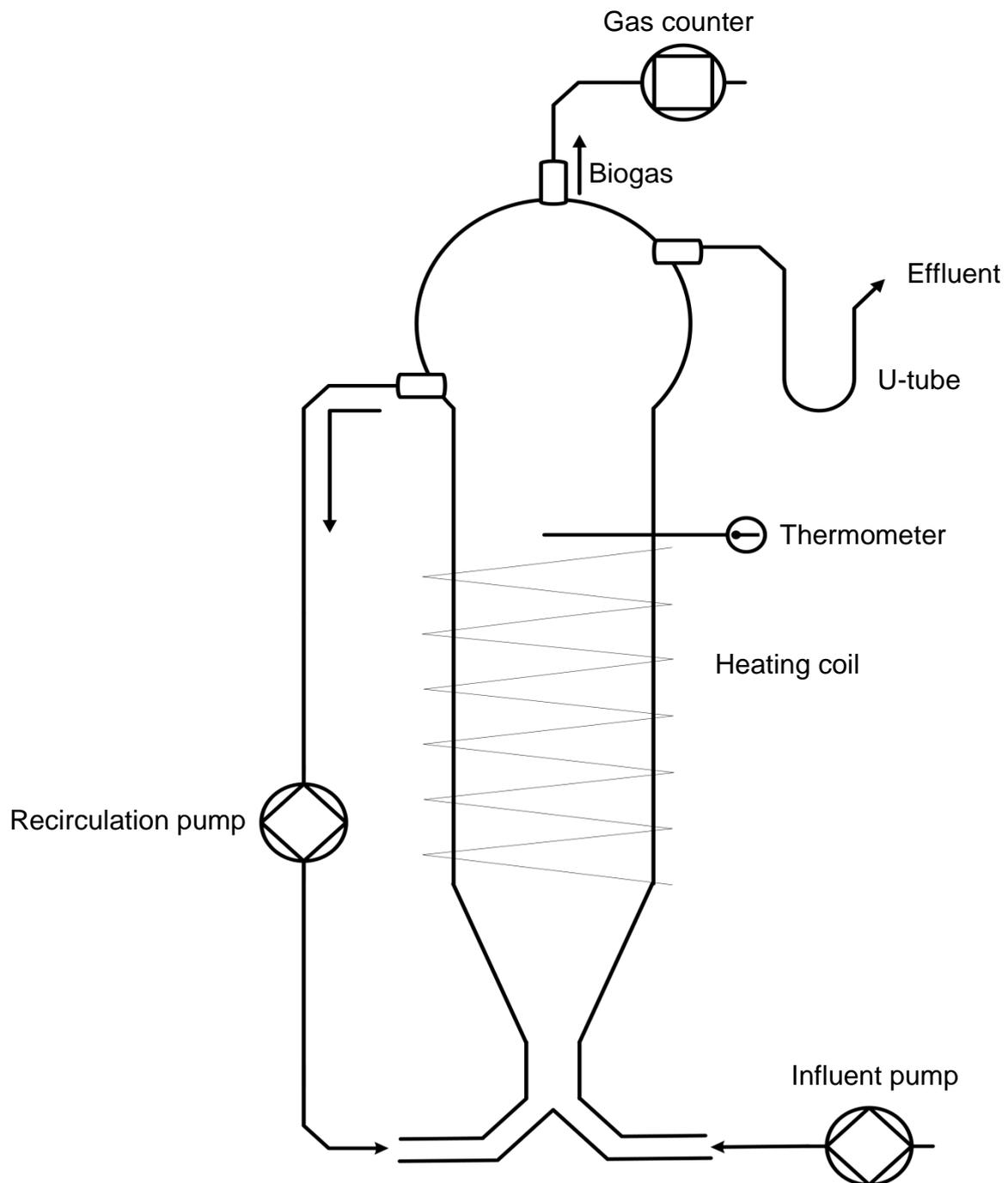
Figure 2 Schematic diagram of a laboratory scale UASB reactor.

UASB start up

Both laboratory-scale reactors were inoculated with 350 g of anaerobic granules (volatile suspended solids (VSS) content = 100 $mg.g^{-1}$ granules) supplied by The James Sedgwick Distillery in Wellington, South Africa. Within the first 24h the substrate fed to the reactors, for stabilisation, was tap water containing 500 $mg.L^{-1}$ urea $((NH_2)_2CO)$ and 500 $mg.L^{-1}$ di-potassium hydrogen orthophosphate (K_2HPO_4). At the beginning of the start-up period (after 24h) the reactors were fed a sterilised synthetic glucose substrate (SGS) (Table 1). The SGS was diluted to a COD value of 1 000 (± 100) $mg.L^{-1}$. As the COD reduction reached approximately 80%, the substrate for the reactors were adjusted to a 9:1 ratio SGS:WWW. The SGS was thereafter gradually decreased in 10%, volume to volume, ratios until the influent consisted of 100% WWW. The pH of the substrate was maintained at 7.5 with 10% acetic acid during the start-up, and thereafter the pH was maintained at 7.0 (± 0.2) with 2M potassium hydroxide (KOH). A trace element solution (1$mL.L^{-1}$) was added to the influent every second week (Nel *et al.*, 1985).

Table 1 Synthetic glucose substrate composition modified from (Show *et al.*, 2004).

Substance	Concentrate ($g.L^{-1}$ unless stated otherwise)
Glucose	1
Yeast extract	20
Urea	0.5
di-Potassium hydrogen orthophosphate	0.5
Trace element solution	1 mL

UASB substrate

Brandy, marula and winery wastewater were used to compose the substrate of both the control reactor ($R_{control}$) and the reactor with added MP (R_{MP}). Due to the seasonal variation in which the wastewater for each product was produced (Fig. 1) the composition of the reactor substrate was adjusted proportionally to simulate the compositional changes that would occur at the winery and distillery treatment plant. The substrate feed was therefore divided into five phases, where each phase represented a change in production as different wastewater was being generated from a new product (Table 2). The organic loading rate (OLR) was increased step-wise, from 1 $kgCOD.m^{-3}d^{-1}$ to 10 $kgCOD.m^{-3}d^{-1}$ throughout the five phases. Phase 1 represented the start-up season (from day 1 - 100),

see above. Phase 2 represented the harvest season of the wine production, where the substrate consisted of bottling wastewater, wash water and WWW. Phase 3 represented the start of the marula production season, the substrate consisted of bottling wastewater, wash water, WWW and marula wastewater. Phase 4 represented the start of Brandy production. During this phase all three products were in production, therefore the substrate consisted of all three products' wastewater as well as bottling wastewater and wash water. Furthermore, phase four represented the peak in OLR during the trial (10 kgCOD.m^{-3}d^{-1}). Phase five represents the end of the harvest season as well as the end of marula production, therefore the substrate consists of bottling wastewater, wash water and Brandy wastewater.

Table 2 Composition of reactor substrate during the five phases of the trial.

Substrate	Composition				
	Phase 1 (day 1-100)	Phase 2 (day 101-170)	Phase 3 (day 171-290	Phase 4 (day 291-319)	Phase 5 (day 320 -371)
SGS	X				
Bottling and wash water	X	X	X	X	X
Winery wastewater		X	X	X	
Marula wastewater			X	X	
Brandy wastewater				X	X

Magnetisable foam glass particles (MP)

The particles used in this study were recently developed and supplied by Poraver GmbH (Postbauer-Heng, Germany). These particles are made from soda-lime silica glass with added magnetic iron. During the drying process these particles expand, leading to a finely pored surface. The particles are not permanently magnetic, a strong magnetic field brought close to the particles will induce magnetism. Three different densities (370 g.L^{-1}, 394 g.L^{-1} and 463 g.L^{-1}) of these particles were supplied, within each density the sizes of the particles differ, the largest being > 1,6 mm and the smallest < 106 µm.

The MPs were soaked in water for 24 h before being added to the UASB reactor on day 197. Two particle sizes of 394 g.L^{-1} density glass foam particles were added: 7 g of 1.6 mm < Particles > 850 µm and 3 g of 500 µm < particles > 300 µm.

Granule analysis

Trace element analysis

Before the reactors were seeded, a portion of the granules were stored at 4°C to be used as a control. Granules from both reactors ($R_{control}$ and R_{MP}) at the end of the trial, as well as control granules, were frozen at −80°C after which they were transferred to the cold stage of the freeze dryer (held at −71°C). The samples were kept in the freeze dryer for *ca.* 48 h until dry. The dried samples were ground to a powder with a pestle and mortar. 0.3g of each sample was weighed out into microwave vessels, 6.5 mL of HNO_3 and 0.5 mL of HCl were added to each vessel. The samples were left for 20 min to predigest before sealing. MARS microwave digester was used as the instrument for analyses. Parameters for digestion were as follows: power level of digester at 800 W, ramp time for 25 min, holding time for 15 min at 210°C and 800 psi. The samples were cooled for 25 min after digestion. Deionised water (43g) was weighed out and added to the microwave vessels to make up 50 mL of each sample.

The digested samples were transferred into sample bottles for the trace element analysis. The trace elements were analysed on an Agilent 7700 quadrupole ICP-MS. The instrument was calibrated using National Institute of Standards and Technology (NIST) traceable standards to quantify selected elements. Dilution factors were corrected resulting from the digestion procedure.

Scanning electron microscopy (SEM)

Granules were separated from the biomass in $R_{control}$ as well as from R_{MP}, after the trial, and frozen at –80°C after which they were transferred to the cold stage of the freeze dryer (held at –71°C). The samples were kept in the freeze dryer for *ca.* 18 h until dry. The dried samples were mounted on a 10 mm aluminium pin stub that was coated with carbon glue. Thereafter the stub was sputter coated with gold palladium alloy and placed in SEM for examination.

Analytical methods

The pH, alkalinity (as $mg.L^{-1}$ $CaCO_3$), total suspended solids (TSS) and COD of both the UASB substrate and effluent were monitored (APHA, 2005).

Spectroquant® Cell Test kits were used to measure the nitrogen (N), phosphorus (P) and COD (Merck, South Africa). The polyphenolic content was determined using the Folin-Ciocalteau method (Singleton & Rossi, 1965).

The biogas composition (CH_4 and CO_2) was determined by injecting 0.2 mL sample biogas into a Varian 3300 gas chromatograph (Varian Inc., Palo Alto, CA) (Sigge, 2005).

The gas chromatograph was equipped with a thermal conductivity detector and a 2.0 x 3.0 mm i.d. Hayecep Q (Supelco, Bellefonte, PA) 80/100 mesh packed column. Helium was used as a carrier gas at a flow rate of 30 mL.min^{-1} and an oven temperature of 55˚C.

Experimental design

1st objective

The performance of the reactors was monitored according to their feeding strategy which was divided into the five phases. Each phase represented a change in the substrate composition as to simulate industrial production for each product (wine, Brandy and marula liquor) (Table 2). The OLR was increased stepwise throughout the phases to reach a peak of 10 kg COD.m^{-3}.d^{-1} at the end of phase 4 (day 319).

2nd objective

The performance of the UASB with added MPs was monitored and compared to the control UASB reactor to determine whether the added MPs affected the efficacy of the reactor.

Results and Discussion

Wastewater composition

The composition and production volume of wine, marula and Brandy wastewater varies and is summarised in Table 3.

Operation and efficiency of the UASB reactor

The performance of the UASB reactors was evaluated by monitoring several different parameters throughout the 371 day trial.

Phase 1 (day 1 – 100)

The aim of the first phase was to achieve a successful start-up. The OLR of the substrates was maintained at *ca.* 1 kg COD.m^{-3}.d^{-1} and the pH at 7.5. The composition of the substrate consisted of SGS and WWW; this ratio was adjusted as the reactors reached a COD reduction of 80% and above. The COD reduction within the first 30 days of operation varied significantly for both reactors (Fig. 4); this was most likely due to the biomass adjusting to the environment.

Table 3 Composition of raw winery (February to November 2013 and February to March 2014), marula (February 2014) and Brandy (March 2014) wastewater.

Constituent	Wastewater		
	Wine	Marula	Brandy
COD (mg.L^{-1})	1 200 – 8 000	40 000 – 47 000	72 000 – 90 000
pH	3.3 – 4.0	3.0 – 5.0	5.0 – 6.0
Phosphorus (mg.L^{-1})	9.35	12.2	16.6
Nitrogen (mg.L^{-1})	5	67	68
Total suspended solids (mg.L^{-1})	20 − 200	100 − 210	300 − 420

The pH of the effluent remained relatively high (7.8 - 8.0) at the initial stage of the start-up; however, as the reactor efficiencies increased the pH of the effluents stabilised (*ca.* 7.2 to 7.5) within the optimal pH ranges for operation (6.5 to 7.6) (Gerardi, 2003). The alkalinity during start-up averaged at about 800 mgCaCO$_3$.L^{-1} (Fig. 3), which is below the optimal range of anaerobic reactors (Anderson *et al.*, 2003). The biogas production was low during start-up (an average of 0.57 L.d^{-1} for R$_{contol}$ and 0.61 L.d^{-1} for R$_{MP}$) (Fig. 5), most likely due to the low OLR that was maintained during this phase.

Phase 2 (day 101 - 170)

The aim of the second phase was to successfully increase the OLR from 1 kg COD.m^{-3}.d^{-1} to 4 kg COD.m^{-3}.d^{-1}. A combination of WWW, bottling wastewater and wash water was used to make up the substrate. The pH of the reactor substrates was maintained at *ca.* 7.0 throughout Phase 2. The alkalinity of both reactors increased gradually (from *ca.* 1 000 – 2 000 mgCaCO$_3$.L^{-1}) at the start of Phase 2 (day 100) until day 130 where a gradual decrease was seen until the end of Phase 2 (day 170) (Fig. 3). A corresponding decrease in pH was found during the same time as the alkalinity decreased (day 130 - 170) where R$_{contol}$ and R$_{MP}$ reached a low of 6.46 and 6.80, respectively. The OLR of the substrate for both reactors was increased at this particular time during Phase 2, and a possible build-up of volatile fatty acids could have occurred, which could have been the reason for the decreasing pH and alkalinity.

There was a slight drop in the COD reduction (%) for both reactors on day 148 and day 170 (Fig. 4) and this is possibly due to the reduced pH as seen for both reactors during that time. A reduced pH will inhibit the activity of the methanogens, and therefore affect the digestion efficiency of the reactor (Tiwari *et al.*, 2006). However, the COD

reduction (%) was still above 80% for both reactors. The biogas gradually increased from day 120 for both reactors and this is likely due to the increase in COD concentration of the substrate (from an average of 0.57 – 3.00 $L.d^{-1}$ for R_{contol} and from 0.63 – 3.22 $L.d^{-1}$ for R_{MP}) (Fig. 5). The methane percentage of the biogas for $R_{control}$ and R_{MP}, measured at the end of the second phase, showed a slight difference: 67% and 63% respectively.

Phase 3 (day 171 - 290)

The substrate in Phase 3 consisted of WWW, bottling wastewater, wash water and marula wastewater (Table 2); the ratio of winery to marula wastewater represented the same ratio as that of industrial production during that season. The initial OLR of the substrate was *ca.* 4.5 kg $COD.m^{-3}.d^{-1}$ and was gradually increased to 6 kg $COD.m^{-3}.d^{-1}$ to represent the OLR similar to that of the industry during that season. The MPs were added to one of the reactors (R_{MP}) on day 197. On day 233 the OLR had just reached 6 kg $COD.m^{-3}.d^{-1}$ for both $R_{control}$ and R_{MP}; however, the pH of $R_{control}$ started to drop gradually while the pH of R_{MP} remained stable. On day 238 the pH of the effluent for $R_{control}$ had reached 5.01 and the internal pH of the reactor was 4.9. A pH below 6.0 can be toxic to the methane forming bacteria (Tiwari *et al.*, 2006); therefore an immediate change had to be made to the substrate inside $R_{control}$. $R_{control}$ was therefore flushed with 500 $mg.L^{-1}$ urea ($(NH_2)_2CO$), and 500 $mg.L^{-1}$ di-potassium hydrogen orthophosphate (K_2HPO_4) dissolved in tap water. On day 244 the pH stabilised and the reactor substrate feed resumed as normal. The pH remained stable for the remainder of Phase 3. A decrease in the alkalinity was seen for both reactors from day 200 to 220, which was possibly the precursor for the drop in pH for $R_{control}$. Alkalinity is a measure of the buffering capacity within a reactor and therefore serves as a precursor for pH changes (Anderson & Yang, 1992). This was however the first difference that was seen between $R_{control}$ and R_{MP}. The pH for R_{MP} only dropped slightly (6.8 on day 235) whereas the pH for $R_{control}$ decreased from 5.4 – 5.0 from day 235 – 240.

There was a slight decrease in the COD reduction (%) for $R_{control}$ from day 238 – 244 (OLR: 6 kg $COD.m^{-3}.d^{-1}$); this decrease in the COD reduction percentage corresponds to the same time that a decrease in pH occurred. The COD reduction percentage for R_{MP} remained above 90% throughout Phase 3. The biogas of both reactors showed a gradual increase during this phase (from an average of 3.00 – 7.00 $L.d^{-1}$ for R_{contol} and from 3.21 – 6.62 $L.d^{-1}$ for R_{MP}) (Fig. 5). The methane (%) production for both reactors was similar during Phase 3; a gradual increase was seen from day 162 (66%) to day 196 (70%) for both reactors. A decrease in the methane production (%) was seen from day 196 (OLR: 5 550 kg $COD.m^{-3}.d^{-1}$) up until the end of Phase 3 (OLR: 6 400 kg $COD.m^{-3}.d^{-1}$) for both

reactors (Fig. 5). This decrease in methane production has a direct correlate with an increase seen in the alkalinity during that same period (Fig. 3). Alkalinity serves as a buffer for the anaerobic system; however, high ammonia levels could result in free ammonia that is toxic specifically to acetogenic bacteria and the methane forming bacteria (Gerardi, 2003; Calli *et al.*, 2005). The source of the high ammonia levels could possibly be from the relatively high nitrogen concentrations within both the Brandy and marula wastewater (Table 3).

Phase 4 (day 291 - 320)

This phase represents the peak OLR (10 kg $COD.m^{-3}.d^{-1}$) of the entire trial. The substrate in this phase consisted of all three waste streams: winery, marula and Brandy wastewater as well as bottling wastewater and wash water. The ratios of winery: marula: Brandy wastewater represented the same ratio as that of industrial production during that season. The initial OLR of the substrate was *ca.* 6.0 kg $COD.m^{-3}.d^{-1}$ and was gradually increased to 10.0 kg $COD.m^{-3}.d^{-1}$ as to represent the OLR similar to that of the industry during that season. On day 293 and day 302 (OLR *ca.* 6.4 kg $COD.m^{-3}.d^{-1}$) the pH of the effluent from $R_{control}$ was 5.43 and 5.08, respectively. This was unexpected as the alkalinity in $R_{control}$ increased during that period (Fig. 3). This was the second time during the trial that a difference occurred between $R_{control}$ and R_{MP}; the pH of R_{MP} remained stable (OLR *ca.* 6.4 kg $COD.m^{-3}.d^{-1}$). It has been found that the addition of iron to anaerobic sludge improves the granulation process and results in a more stable granule (Shen *et al.,* 1993a; Shen *et al.*, 1993b; Gerardi, 2003). It has also been found that the addition of iron markedly increases the conversion of acetate to methane (Hoban & Van den Berg, 1979; Yu *et al.*, 2000), which could possibly be the reason for the increased stability in R_{MP}.

On both day 293 and day 302 $R_{control}$ was flushed and the OLR of the substrate was decreased to *ca.* 5 kg $COD.m^{-3}.d^{-1}$ until the reactor pH stabilised (7.5 on day 308), where the OLR was again increased gradually to *ca.* 9 kg $COD.m^{-3}.d^{-1}$. A decrease in the COD reduction percentage for $R_{control}$ was seen during this period (from 90% on day 290 to 78% on day 301, OLR: 6.4 kg $COD.m^{-3}.d^{-1}$) (Fig. 4). The OLR for R_{MP} was gradually increased to *ca.* 10 kg $COD.m^{-3}.d^{-1}$ (day 301) and the COD reduction (%) remained above 90%.

The biogas for both reactors increased steadily from an average of 7.00 – 10.6 $L.d^{-1}$ for R_{contol} and from 6.62 – 8.72 $L.d^{-1}$ for R_{MP} (Fig. 5). The methane percentage however decreased (Fig. 5), again this could possibly be due to the high levels of alkalinity found during this period (Fig. 3).

Phase 5 (day 321 - 371)

Phase five was the last phase of the trial and the substrates consisted of bottling wastewater, wash water and Brandy wastewater. The substrate during this part of the trial represented the effluent generated during the 'off peak' season of the wine and distillery production season. The OLR of the substrate during this phase was maintained at *ca.* 4.7 kg COD.m^{-3}.d^{-1}.

Both reactors showed very similar results during this phase. The reduction percentage for both reactors remained above 90% for both $R_{control}$ and R_{MP} and the pH of the effluent was above 7 (OLR: 4.7 kg COD.m^{-3}.d^{-1}). There was a decrease in the alkalinity for both reactors, which could possibly be due to the marula wastewater no longer being part of the substrate. The marula wastewater has high levels of nitrogen which can serve as a buffer; this could possibly account for the decrease in alkalinity. An increase in methane production was seen in both reactors that directly correlate with the alkalinity that decreased (Fig. 3). The biogas production decreased from an average of 10.6 – 3.8 L.d^{-1} for R_{contol} and from 8.72 – 3.3 L.d^{-1} for R_{MP} (Fig. 5). The decrease in the biogas can most likely be ascribed to the decrease in OLR from *ca.* 10 kg COD.m^{-3}.d^{-1} to 4.7 kg COD.m^{-3}.d^{-1}

Granule analysis

Trace element analysis

Granules from both $R_{control}$ and R_{MP} were analysed for trace elements to investigate whether granules from R_{MP} had a higher level of iron due to the added MPs that contain iron (Table 4). It is clear from the results displayed in Table 4 that aluminium, iron, copper and zinc are present in higher quantities in all three samples than the rest of the elements. The control granules displayed a large difference in concentration in aluminium, titanium, chromium, manganese, iron, nickel, zinc, tin and lead compared to the granules from $R_{control}$ and R_{MP}. The granules from $R_{control}$ had the highest Cu concentration of all three samples and displayed higher concentration in all the elements when compared to the granules from R_{MP}.

It was hypothesised that the performance of R_{MP} was more effective during the treatment of wine and distillery wastewater due to the added MPs which supplied an additional iron source to the biomass. Iron displays the highest quantities in all three samples of all the elements tested. However, the iron content of the control granules is the highest (7 791 mg.kg^{-1}), second is that of the granules from $R_{control}$ (4 753 mg.kg^{-1}) and the lowest is that of the granules from R_{MP} (4419 mg.kg^{-1}). According to these results the

hypothesis was incorrect and therefore there must be another reason for the enhanced performance of R_{MP}.

Table 4 Trace element analysis of granules extracted from both UASB reactors ($R_{control}$ and R_{MP}) treating wine and distillery wastewater.

Element	Control granules (mg.kg^{-1})	Reactor control granules (mg.kg^{-1})	Reactor MPs granules (mg.kg^{-1})
Al	5489	2644	2090
Ti	94	51	42
V	11	8	5
Cr	109	29	22
Mn	250	91	78
Fe	7791	4753	4419
Co	2	6	5
Ni	33	20	18
Cu	255	913	624
Zn	2134	1304	883
As	4	6	4
Se	3	6	7
Mo	11	12	9
Cd	1	0.3	0.2
Sn	18	5	6
Sb	1	2	1
Pb	31	17	15
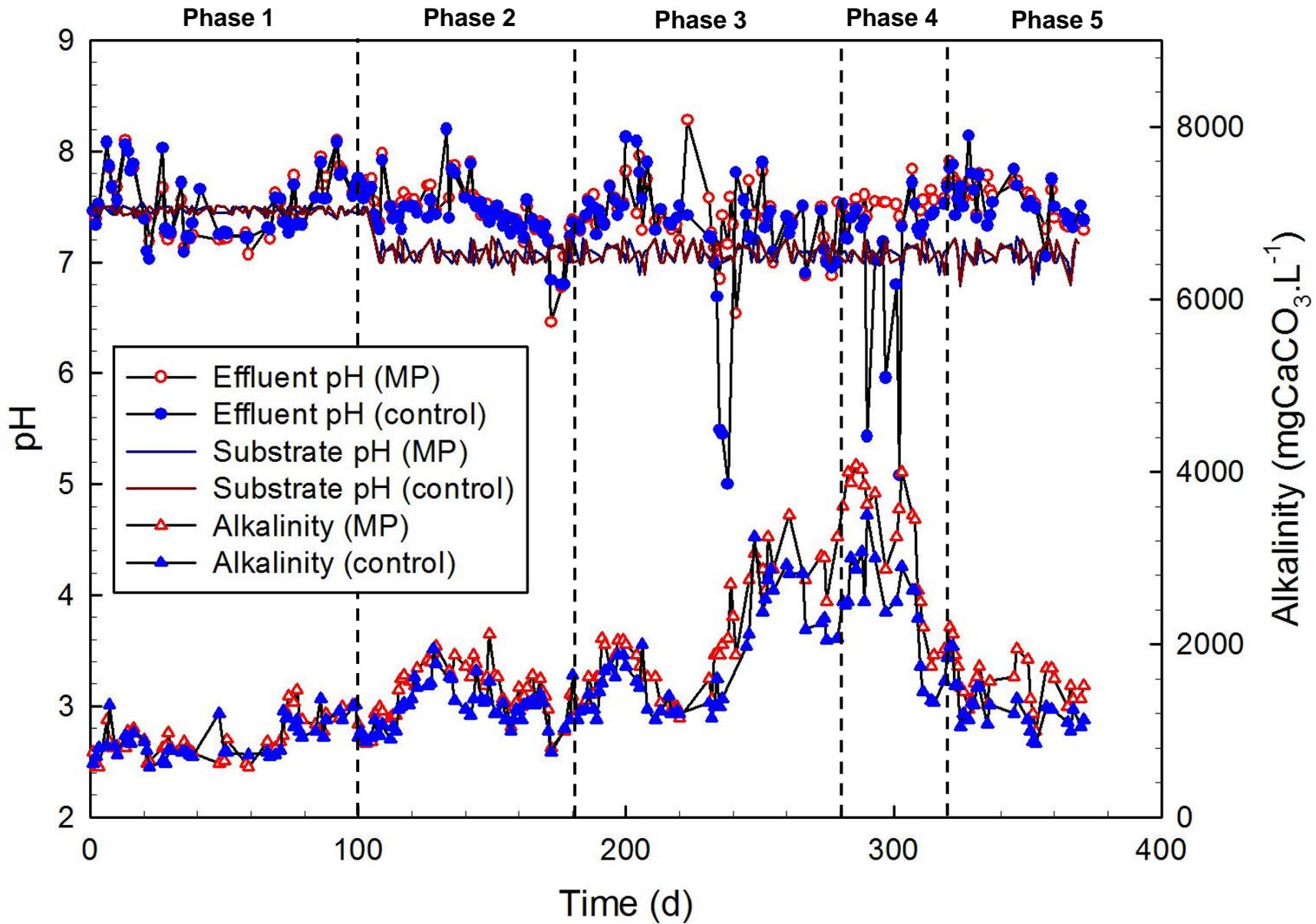
Figure 3 Alkalinity and pH of the substrate and effluent of both UASB reactors treating wine and distillery wastewater.
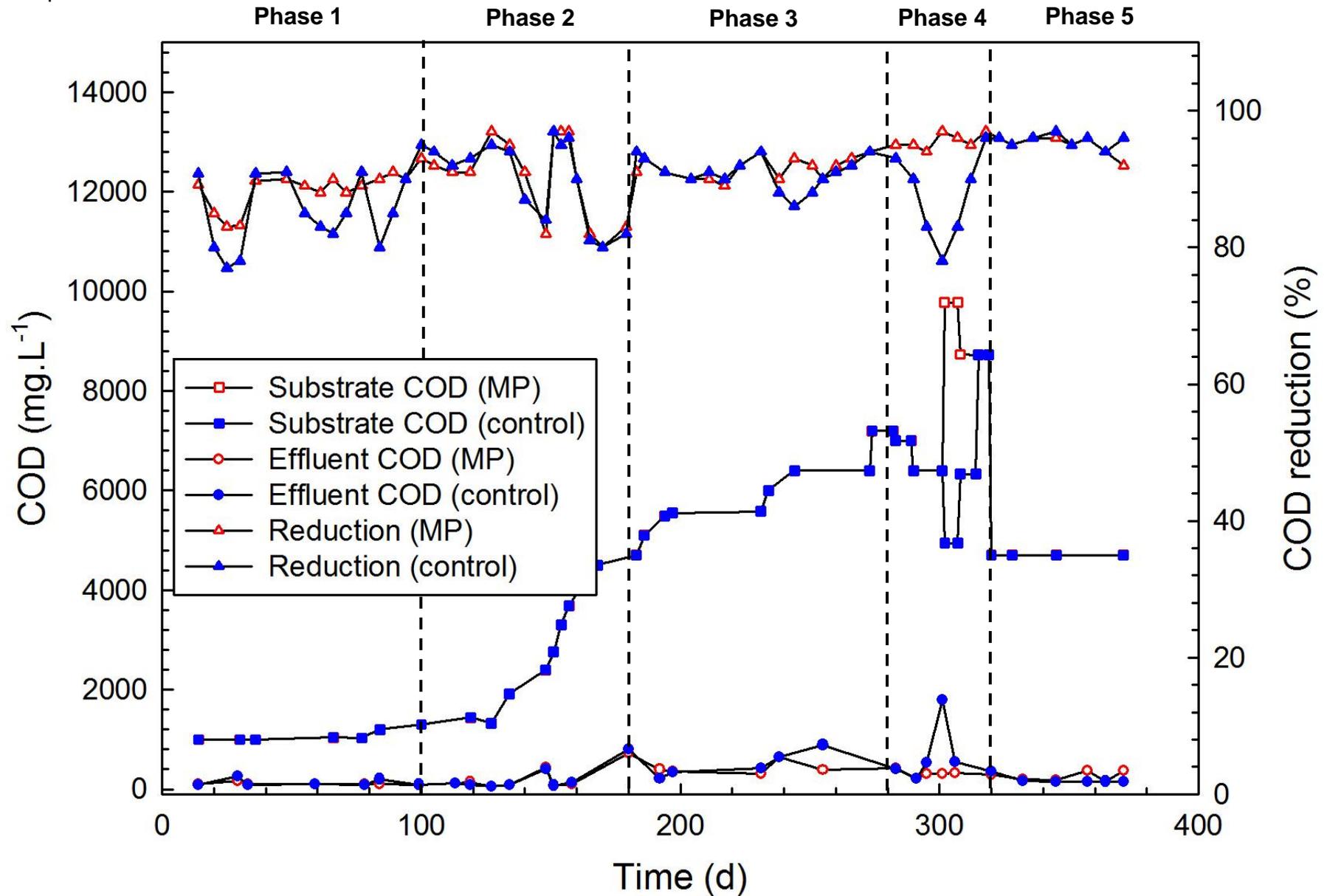
Figure 4 The CODs and COD reduction (%) of the substrate and effluent of both UASB reactors treating wine and distillery wastewater.
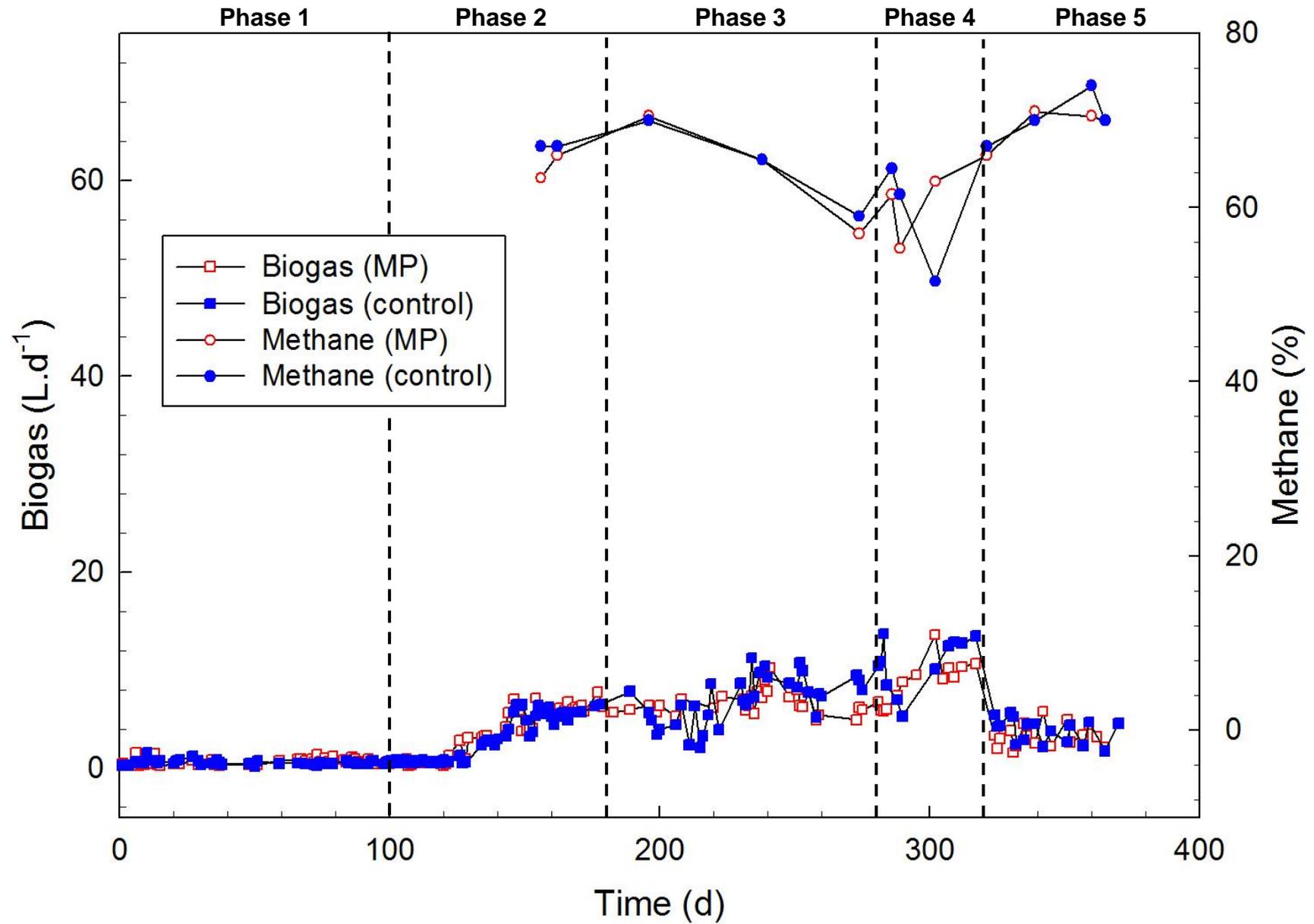
Figure 5 The biogas production and methane content (%) of both UASB reactors treating wine and distillery wastewater.

Scanning electron microscopy (SEM)

Granules from both $R_{control}$ and R_{MP} were extracted at the end of the trial and analysed using scanning electron microscopy to investigate any physical differences, which might have resulted from the addition of MPs to R_{MP} on day 197. A granule from R_{MP} is shown in Figure 6 (a) while the same granule split open is shown in Figure 6 (b). Figure 7 (a) is a granule from $R_{control}$ while Figure 7 (b) is the same granule split open. When comparing Figure 6 (a) and Figure 7 (a), it appears that the surfaces of these two granules differ. The surface of the granule from R_{MP} (Figure 6 (a)) is much coarser than the granule from $R_{control}$ (Figure 7 (a)). This is possibly due to the added MPs to R_{MP} that caused friction between the MPs and the surface of the granules.

Granules from both reactors were also split open as to investigate whether the added MPs had an influence on the internal structure of the granules. However, a significant difference between the internal structures of the granules was not observed (Figure 6 (b) and Figure 7 (b)). It seems that the granules that had been split open displayed a layered internal structure as described by Liu *et al.* (2003) and Els *et al.* (2005) as the multi-layered structure.


(a)   (b)
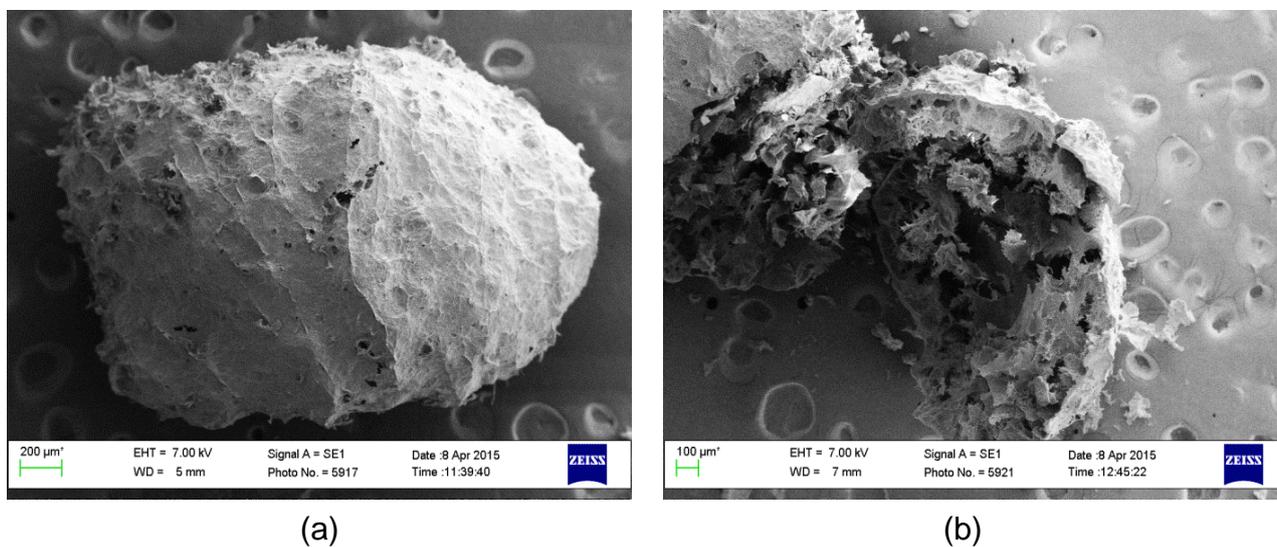
Figure 6 Granular sample extracted from a UASB reactor (R_{MP}) treating wine and distillery wastewater. (a) is a whole granule and (b) is the same granule that has been split open.

(a)   (b)
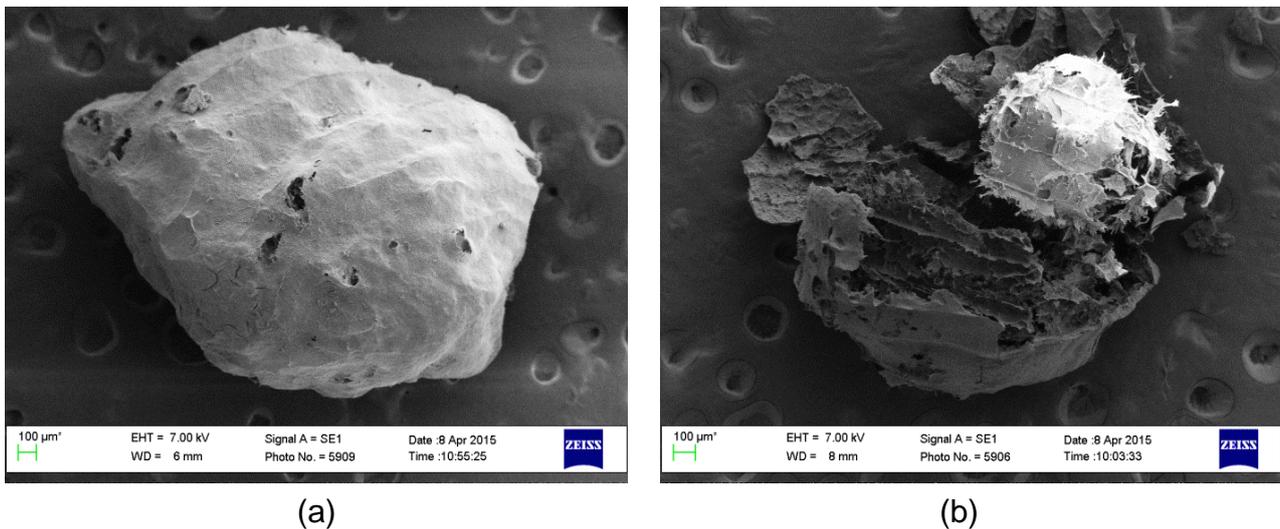
Figure 7 A granular sample extracted from a UASB reactor ($R_{control}$) treating wine and distillery wastewater. (c) is a whole granule and (d) is the same granule split open.

Conclusion

The wine and distillery industry produces large volumes of high strength wastewater annually that does not comply with local regulations. These waste streams have to be treated before they can be discharged. In this study two 2.3 L laboratory scale UASB reactors were used to treat a combination of wine, Brandy and marula wastewater.

Brandy, marula and winery wastewater were used to compose the substrate feed of both $R_{control}$ and R_{MP}. The production of these three different waste streams varied according to the season; therefore to simulate the production season of the wine and distillery treatment plant, the composition of the substrate feed for the UASB reactors varied accordingly.

The pH of the substrates was maintained at 7 and a hydraulic retention time (HRT) of 24 h was maintained throughout the trial. The organic loading rate (OLR) of the substrates was increased gradually throughout the trial from 1 kg $COD.m^{-3}.d^{-1}$ to 10 kg $COD.m^{-3}.d^{-1}$ (day 0 – day 320); the substrates were then decreased and maintained at 4.5 kg $COD.m^{-3}.d^{-1}$ throughout the last phase of the trial (day 321 - 371). Magnetisable foam glass particles were added to one reactor (R_{MP}) on day 197.

The treatment of the combined substrates was successful throughout the trial where the COD removal increased from 64% to a maximum of 97%. The biogas production increased as the OLR increased from an average of 0.6 – 10.6 $L.d^{-1}$ for R_{contol} and from 0.63 – 8.72 $L.d^{-1}$ for R_{MP}. The alkalinity gradually increased throughout Phases 1 and 2,

and a stable pH was also maintained for both reactors throughout these phases. The pH for R_{contol} did however decrease on three different occasions during the trial, to whereas this was not found in R_{MP}. The difference in the performance between the two reactors are unknown, this should however be investigated further.

The methane production for both reactors increased to *ca.* 70% (day 196) and then gradually decreased to 51% and 55% for R_{contol} and R_{MP}, respectively (day 302). The decrease in methane corresponded directly to the increase in alkalinity during that same period of the trial (day 196 – day 302). Ammonia aids as a buffer for anaerobic systems; however, increased levels can cause toxicity especially to the methane forming bacteria.

Therefore it can be concluded that the digestion of combined waste streams in a UASB reactor is possible and the added MPs did not affect the digestion process negatively; on the contrary, it seems that the addition of the MPs had a beneficiary effect on the digestion process, especially during seasons of high organic loads. It is recommended that the MPs be analysed as to investigate their composition in order to determine whether any other elements could have been the reason for the performance difference.

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CHAPTER 4

INVESTIGATING THE VIABILITY OF ADDED MAGNETISABLE FOAM GLASS PARTICLES TO ENHANCE BIOMASS IMMOBOLISATION IN AN UPFLOW ANAEROBIC SLUDGE BLANKET (UASB) REACTOR

Summary

Magnetisable foam glass particles (MPs) were added to an UASB reactor to investigate the suitability as a good medium for biomass attachment. Two different particle sizes of density 394 g.L^{-1} were added to the UASB reactor on day 197 and the particles remained in the reactor for a period of 174 days (*ca.* 6 months). Scanning electron microscopy (SEM), fluorescence microscopy and activity tests were done on the extracted MPs. The SEM results showed microbial attachment on the surface of the MPs, furthermore similarities were seen between the surface properties of an anaerobic granule and a colonised MP. Fluorescence microscopy confirmed that the colonisation on the MPs contained methanogenic activity as the Archaea group had autofluorescent properties. Activity tests were done on the MPs, granules from both reactors ($R_{control}$ and R_{MP}) and control granules using three different test media. These tests were done in order to identify whether the microbial attachments on the MPs were active as well as to investigate whether the amount of gas produced by the granules in R_{MP} differed from the amount of gas produced by the granules from $R_{control}$. It was seen that the granules from $R_{control}$ produced the highest amount of gas in all three mediums; there was no distinct difference between the gas produced from R_{MP} when compared to $R_{control}$. There was gas production from the MP samples in all three mediums, which confirms that the biomass attachment on the MPs were active.

Introduction

Due to the ability to digest high strength wastewater the upflow anaerobic sludge blanket (UASB) reactor process has become one of the most commonly used anaerobic reactors worldwide (Nuntakumjorn *et al.*, 2008; Karthikeyan & Kandasamy, 2009; Rajagopal *et al.*, 2013). The main principle of the UASB reactor is found in the breakdown of organic matter into simpler compounds, by a consortium of anaerobic bacteria. The success of the UASB reactor is in the retention of an adequate amount of this consortium (Gao *et al.*,

2006; Ofoefule *et al.*, 2011). This is achieved by a process called granulation (Fang *et al.*, 1994; Karthikeyan & Kandasamy, 2009) where the consortium of anaerobic bacteria form conglomerates called granules (Tiwari *et al.*, 2006). There are many sources in literature that discuss the granulation process and the amount of time it takes to form these aggregates; it seems that the general conclusion is between 2 – 8 months (Zhou *et al.*, 2006; Vlyssides *et al.*, 2008; Ramm *et al.*, 2014).

A granule can be described as a near-spherical biofilm possessing different layers of bacterial groups (Tiwari *et al.*, 2006). The degradation of organic matter is therefore a multi-step process where the product of the one bacterial group is the substrate for the next (Moletta, 2005). The first step in the degradation process is the hydrolysis of complex organic molecules such as lipids, proteins and carbohydrates into amino acids and simple sugars. The second and third steps are the transformation of volatile fatty acids and alcohols into carbon dioxide, acetate and hydrogen by acidogenic and acetogenic bacteria. The last step in the process is the formation of methane by the methanogenic bacteria (Schmidt & Ahring, 1996; Moletta, 2005; Tiwari *et al.*, 2006; Ofoefule *et al.*, 2011).

The key feature of the UASB process lies in the granulation process; however, loss in biomass still occurs especially during seasons of high organic loading rates (OLR) and high volumetric loading rates (Rajeshwari *et al.*, 2000). Washout also occurs when the gas that is formed by the granules does not detach from the granule; it then decreases the density of the granules and they become more prone to washout. The loss in granular biomass could result in complete performance failure of the treatment process. The accumulation of organic load within the reactor and the decreased digestion activity due to washout will result in the build-up of volatile fatty acids, solids and COD, and a decrease in the pH and alkalinity, which then result in reactor failure and possibly inactive biomass (Wu *et al.*, 2012).

There are some techniques that can be applied to compensate for the loss in biomass, like sedimentation tanks and filters; however, there still are certain disadvantages and limitations (Rajeshwari *et al.*, 2000). A relatively new technology for the immobilisation of biomass has recently been developed. This technology is a magnetisable foam glass particle (MP) that has many properties to be a viable medium for bacterial attachment, as these particles can be magnetically removed.

Therefore the aim of this study was to investigate whether MPs would be a viable medium for biomass attachment to aid in the immobilisation of granular biomass in a UASB reactor.

Materials and Methods

Granular and MP analysis

Recovery of MPs from UASB reactor

The MPs were extracted from the biomass within a UASB reactor treating wine and distillery wastewater after spending 174 days within the UASB reactor. The MPs were analysed by different methods to determine whether they had been colonised, specifically by methanogenic bacteria, and whether the colonised MPs were capable of producing biogas, specifically methane. The MPs were prepared differently depending on the specific analysis.

Scanning electron microscopy (SEM)

Some MPs were frozen at –80°C after which they were transferred to the cold stage of the freeze dryer (held at –71°C), and were kept in the freeze dryer for *ca.* 18 h until dried. The dried samples were mounted on a 10 mm aluminium pin stub that was coated with carbon glue.   Thereafter the stub was sputter coated with gold palladium alloy and placed in the Zeiss EVO® MA15 scanning electron microscope for examination. The magnifications that were used included: 200 µm; 100 µm; 10 µm and 2 µm.

Fluorescent microscopic analysis

The development of biomass and methanogens on the MPs was identified by means of fluorescence microscopy. The MPs were separated from the granular biomass after the reactor trial was complete by means of a magnetic separator bar. These particles were stored at 4°C in a glass container filled with BTM (pH adjusted to 7) (Table 1) until needed for analysis. After 24 h the BTM was decanted and replaced with fresh BTM.

Fluorescence of the entire bacterial consortium was achieved by adding the nuclear dye Syto9 (Stock solution of 5 mM in DMSO, diluted 1:1000 with phosphate buffer saline) (34854, Molecular probes, Oregon, USA) to a small sample, followed by incubation for 10 min at room temperature. Samples were then put into the chambers of an 8-well coverglass system (155411, Lab-Tek, Nunc, NY, USA) and imaged using a Carl Zeiss LSM780 confocal microscope. The samples were stimulated with a 488 nm laser and emission was detected in the range 516 – 665 nm.

The autofluorescence of methanogenic bacteria was stimulated with a 405 nm laser and emission was detected in the range 476-535 nm (no dye was added for autofluorescence).

Granule activity

Activity tests are performed on the granular biomass of anaerobic reactors to determine their metabolic activity. Different mediums can be used to determine the metabolic activity of specific species. Activity tests were performed as described by O'Kennedy (2000) and tests were performed on the granules from both reactors separately (reactor control and reactor with added MPs). Granules that had been stored at 4˚C were used as control granules (to indicate initial granule activity), as well as on MPs that had been isolated from the UASB reactor.

The granular activity of granules from both reactors and the MPs was compared to the initial granular activity (control granules) and all tests were expressed as cumulative biogas. The methane percentage of all four samples was measured over 25 h incubation time. Three different test media were used, each being specific to a certain microbial group. A basic test medium (BTM) (Table 2) was used as control, a glucose test medium (GTM) (Table 3) was used to determine the activity of the acidogens and active methanogens and an acetic acid medium (ATM) (Table 3) was used to measure the activity of the acetoclastic methanogens.

The granules of both $R_{control}$ and R_{MP}, MPs and the control granules were incubated at 35˚C for 48 h in 1 L activation media, respectively (Table 1). After 24 h the media was decanted and replaced with fresh media. After the incubation period, 3 g of each sample (granules from control reactor, granules from reactor with added MPs, granules stored at 4˚C and MPs) was placed in 20 mL glass vials, to which 13 mL of the specific test medium (BTM, GTM or ATM) was added, leaving 6 mL headspace. The vials were sealed with butyl septa, capped with aluminium caps and incubated at 35˚C for 25 h. The biogas volume was measured after 5, 10 and 25 h using a free moving 10 mL syringe with a 12 gauge needle. The needle was inserted through the butyl septa of the vial and the biogas volume was determined once the piston stopped moving. All granular samples were analysed in triplicate and the MP samples were done in duplicate.

Table 1 Composition of activation media

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

Table 2 Composition of the basic test medium (BTM)

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 3 Composition of the three different test media used to determine the activity of different microbial groups

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

Results and Discussion

Analysis on magnetisable particles (MP)

Scanning electron microscopy (SEM)

The MPs remained in the UASB reactor for a period of 174 days (*ca.* 6 months), SEM analyses were done on the MPs to confirm whether there was any microbial attachment and colonisation onto the surface of these particles. As can be seen in Figures 1 (a), (b) and Figure 2 (c), colonisation onto the surface of the MPs was initiated in protected areas such as cracks and cavities; furthermore an assortment of organisms such as cocci as well as rod shaped microorganisms are seen in one of the cavities of a MP (Fig. 1 (a)).

Methanosaeta (formerly known as *Methanothrix*) and *Methanosarcina* have been found to be the two most predominant methanogenic organisms in granulated sludge (Kosaric *et al.*, 1990; Forster, 1991; Shen *et al.*, 1993). Furthermore, *Methanosaeta* is also found to play a crucial role in initiating granulation (Veiga *et al.*, 1997; Lamprecht, 2009). *Methanosaeta* are straight filamentous rods with flat ends and their dimensions are between 0.8 – 1.3 µm by 2 – 6 µm (Kamagata *et al.*, 1992). *Methanosarcina* are cocci with an irregular surface typically occurring in aggregates and a single cell can be between 1-3 µm in diameter (Singh *et al.,* 2005). Due to these two species being the most significant in initial granular formation it is speculated that they would be present in the initial attachment onto the MPs. The magnification of Figure 1 (a) is 2 µm, therefore it is highly likely that the single cells observed could possibly be the above mentioned species. Figure 2 (b) displays filamentous/fibril like structures that could possibly be an indication of extracellular polymeric substances (EPS) growth, which aids in the granulation attachment or biofilm formation process.

SEM images were also taken of fully colonised MPs. In Figure 3, a fully colonised MP is seen lying next to a control MP. There is a clear difference in the characteristics of the surface between the colonised and the control MP. An uneven surface area with many cavities is seen on the control MP, whereas on the colonised MP no cavities and a much rounder, even surface of biomass are seen. Figures 4 (b) and (d) are images of a granule taken from $R_{control}$ to allow comparison between a colonised MP (Fig. 4 (a) and (c)). The surface properties appear to be similar, as there are small cracks and holes (possibly where biogas is released by the bacterial consortium). Figures 5 (a - d) are images taken of the surface of the colonised MP in Figure 4 (c) at higher magnifications. It is clear that there are many different organisms attached to the surface of the MP as well as structured matrix that could possibly have been formed by EPS. Figures 5 (c) and (d) are taken at 10 µm and 2 µm, respectively; these magnifications are high enough to observe single celled organisms. The microorganisms seen in these images are similar in appearance to *Methanosarcina* (cocci of irregular shape). Figure 6 is an image taken of another colonised MP. Here again, a large consortium of organisms can be observed, namely different sized bacilli as well as cocci.

Figures 7 (a) and (b) are SEM images taken of two different colonised MP that have been cut in half. The image was taken specifically at the edge of the MP to observe the thickness of the bacterial attachment. In both images there is bacterial colonisation on the surface of the MP. There is also some growth on the inside of the MP.
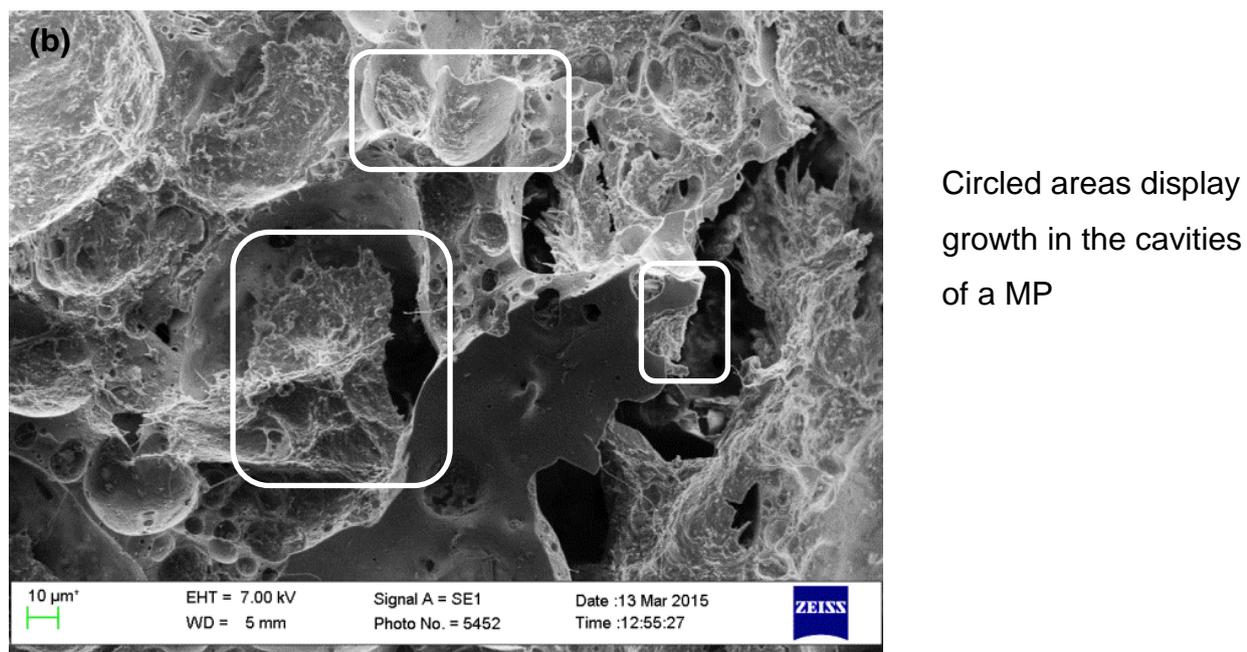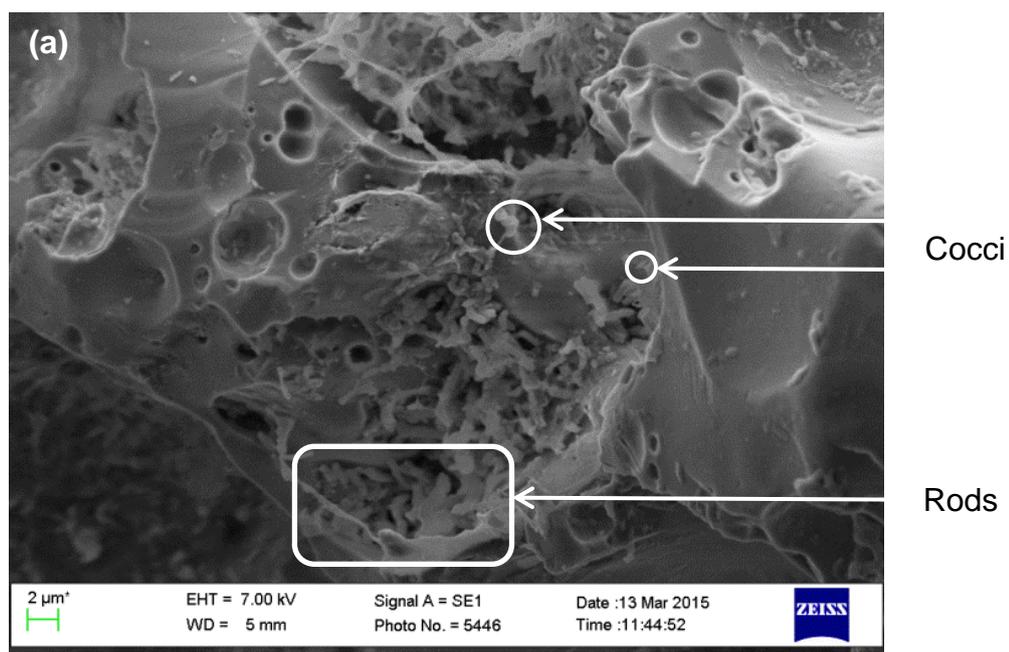
Figure 1 Scanning electron microscopy (SEM) images of a partially colonised magnetisable foam glass particle (MP) where colonisation is seen to initiate in cracks and cavities. Image (b) is a closer magnification of (a) where initial colonisation is observed in a cavity of a MP.

Circled areas display growth in the cavities of a MP
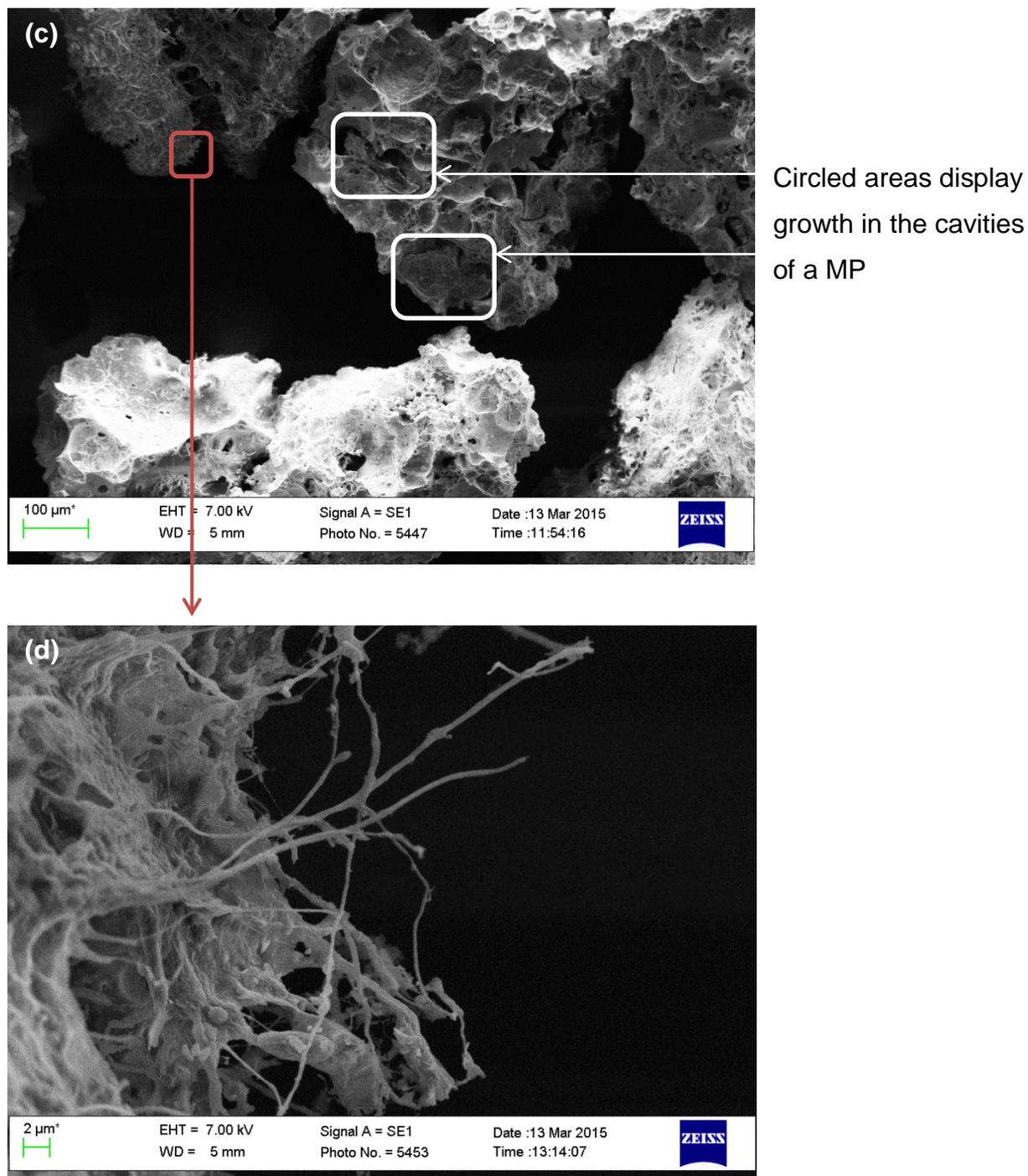
Figure 2 Scanning electron microscopy (SEM) images of the surface of the magnetisable particles (MPs) (c) where colonisation is seen to initiate in cracks and cavities. (d) is a closer magnification of (c) where filamentous growth is observed.
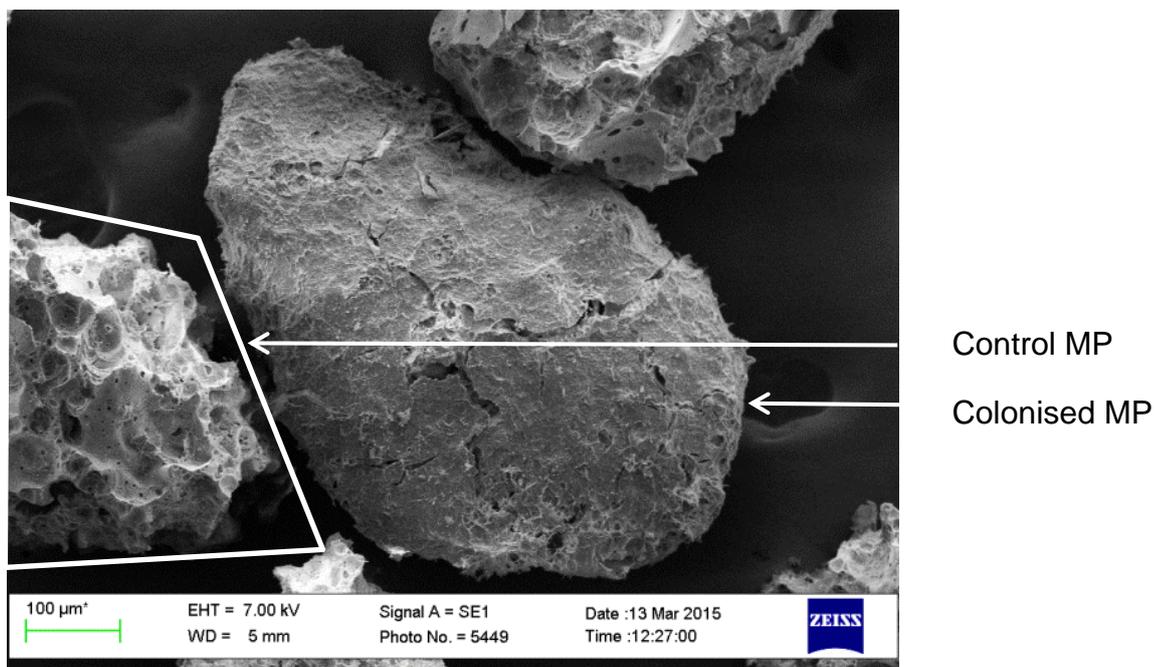
Figure 3 Scanning electron microscopy (SEM) images of a colonised MP next to an un-colonised MP (control).
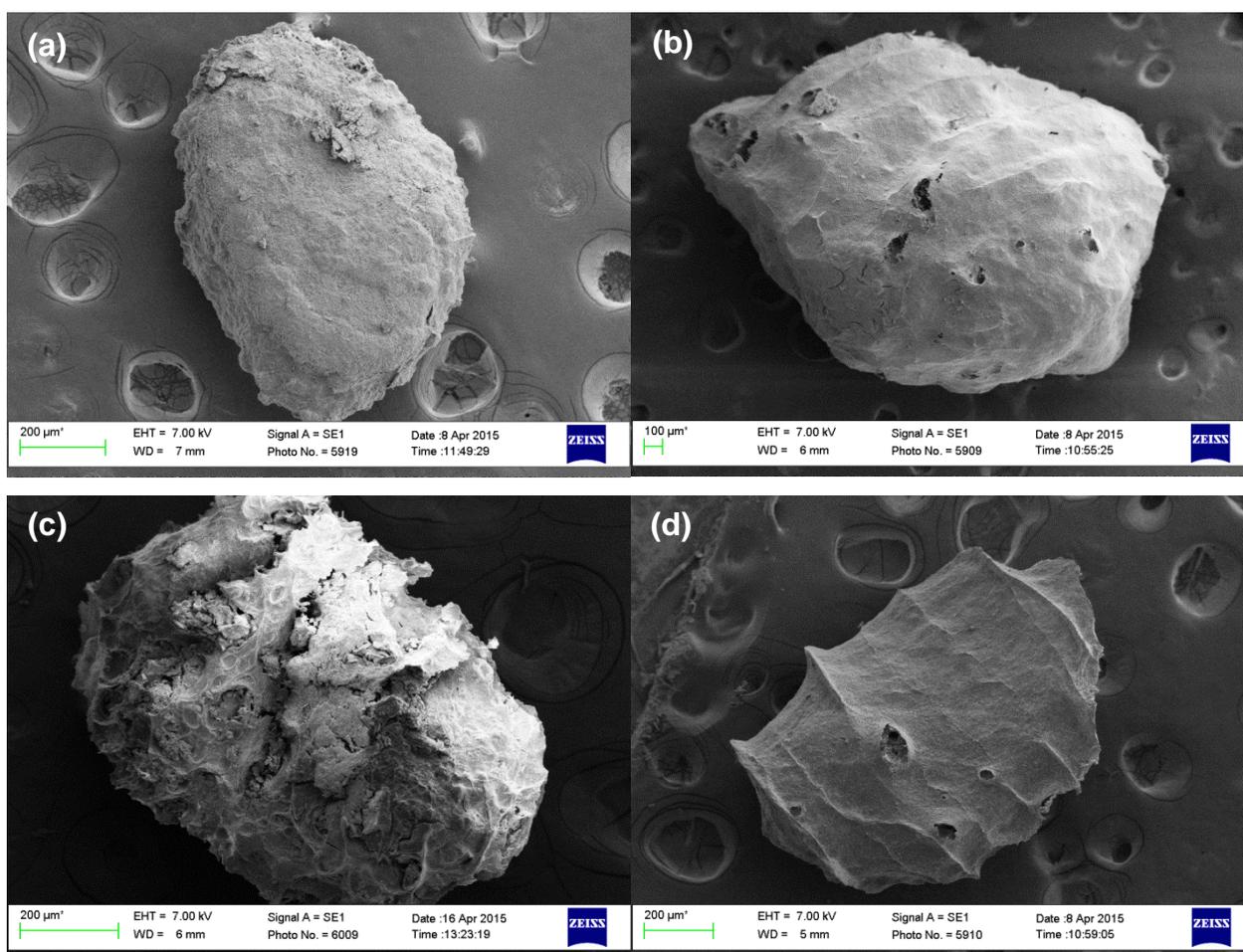
Figure 4 Scanning electron microscopy (SEM) images of a colonised MP (a and c) as well as a whole granule from the control reactor (b and d).
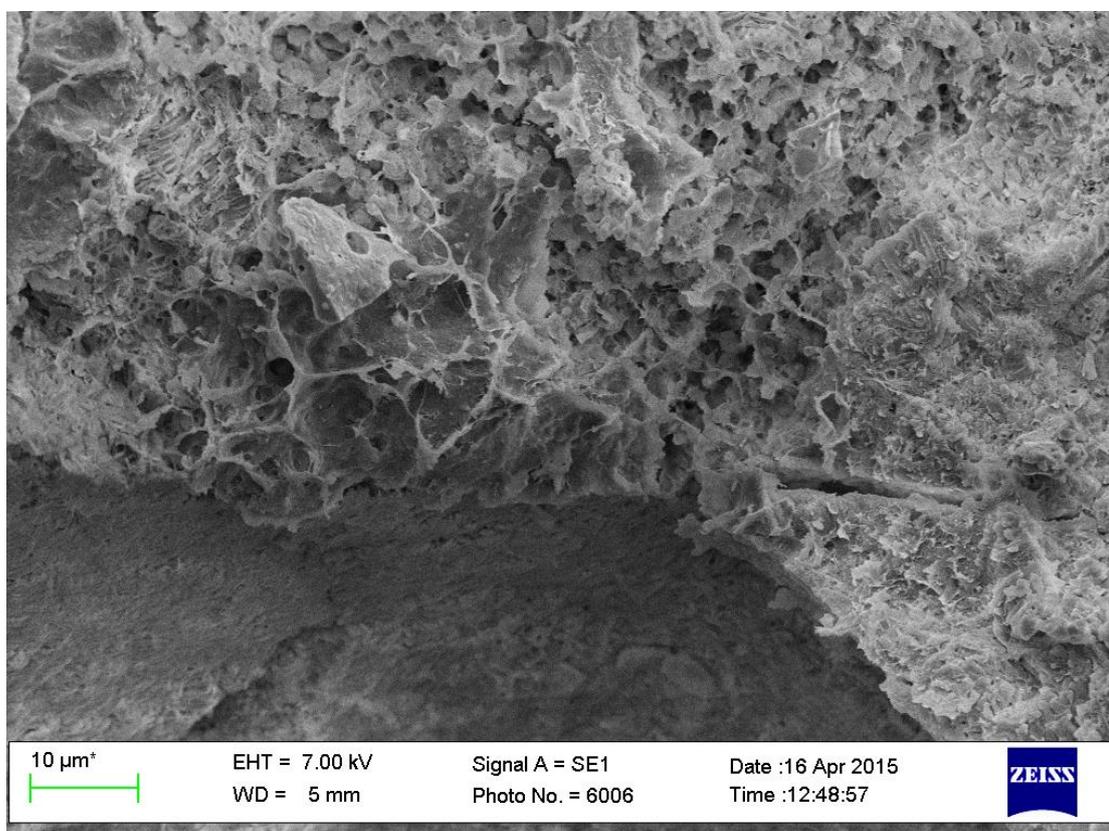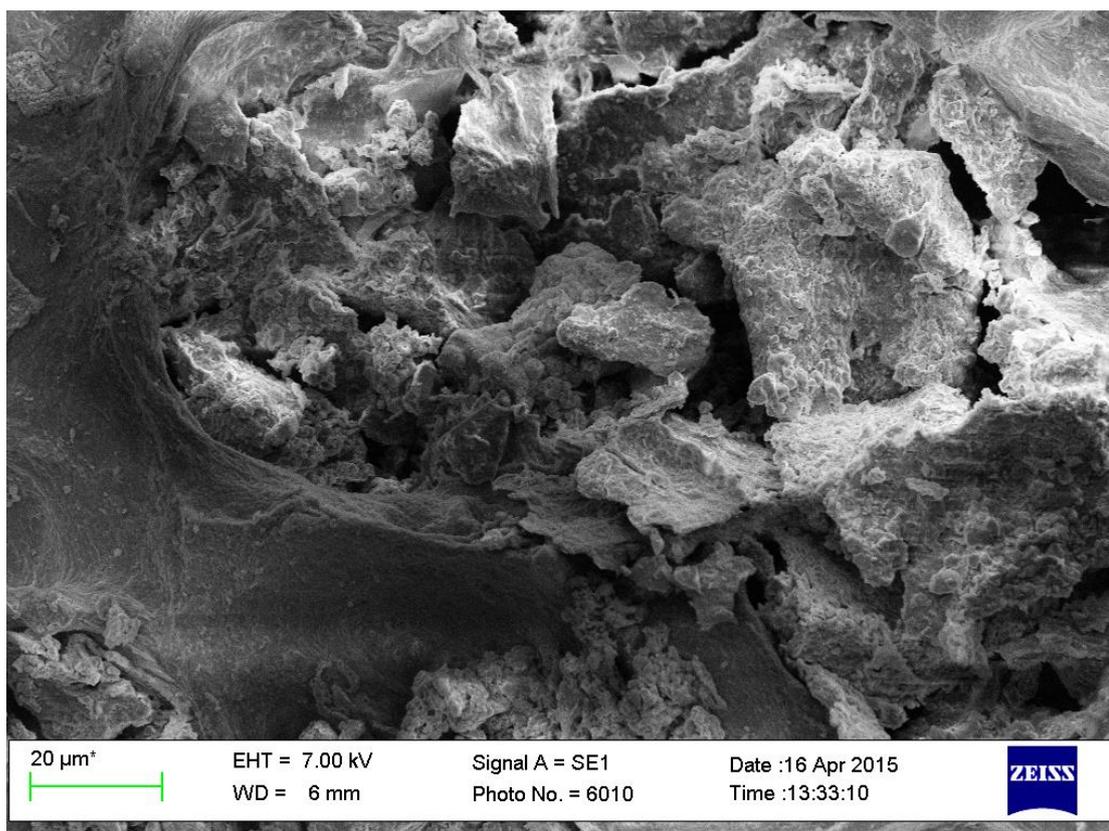
Figure 5 (a) and (b) are scanning electron microscopy (SEM) images of the colonised MP in Figure 4 (c) at a higher magnification.
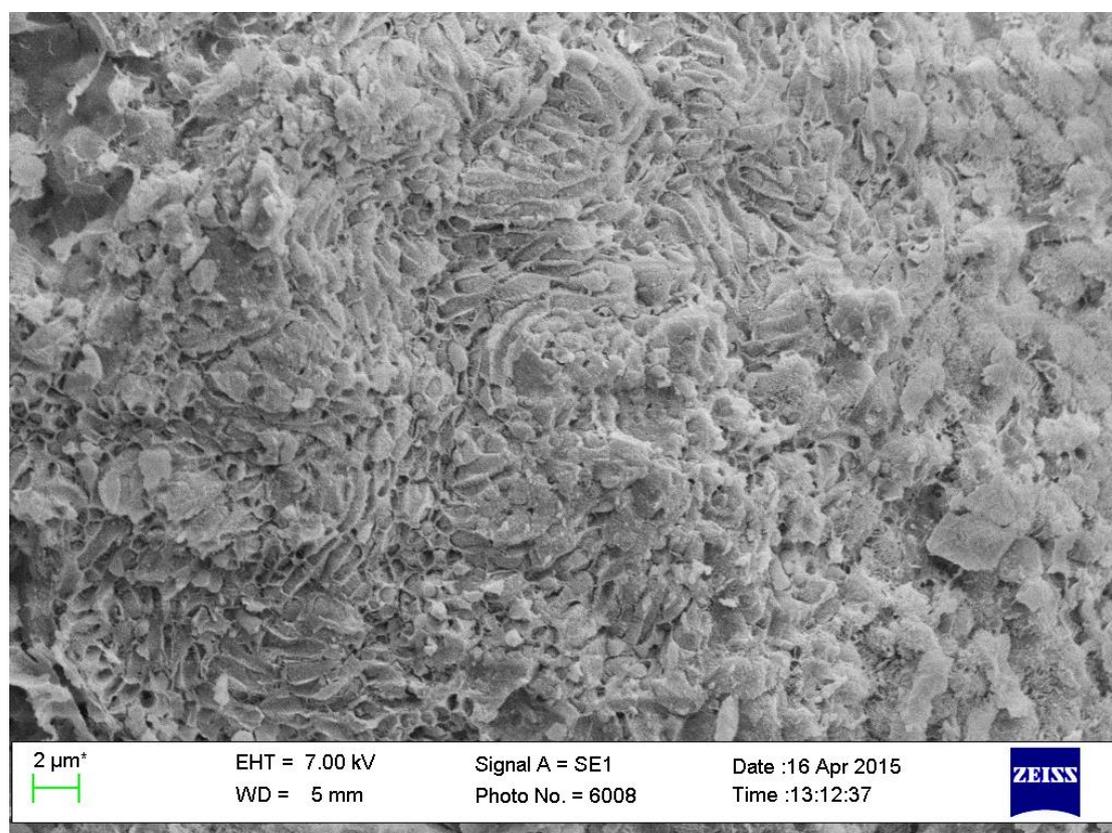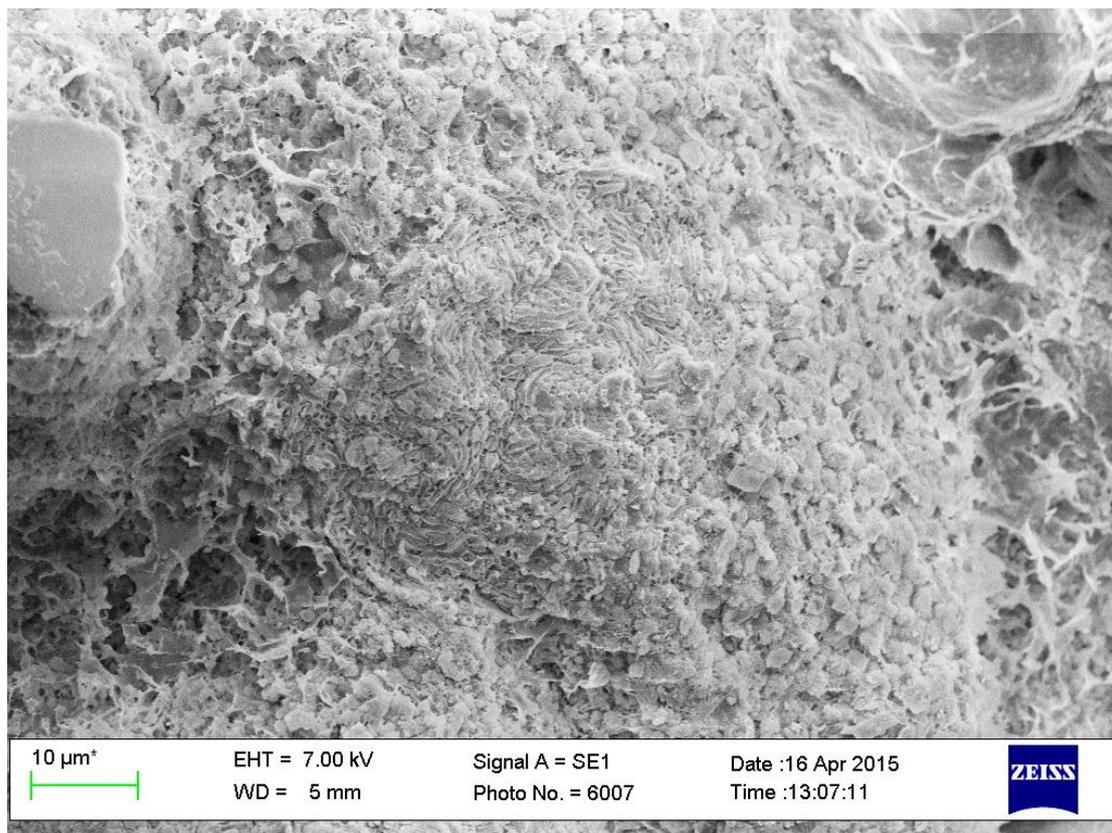
Figure 5 (c) and (d) are scanning electron microscopy (SEM) images of the colonised MP

in Figure 4 (c) at a higher magnification.
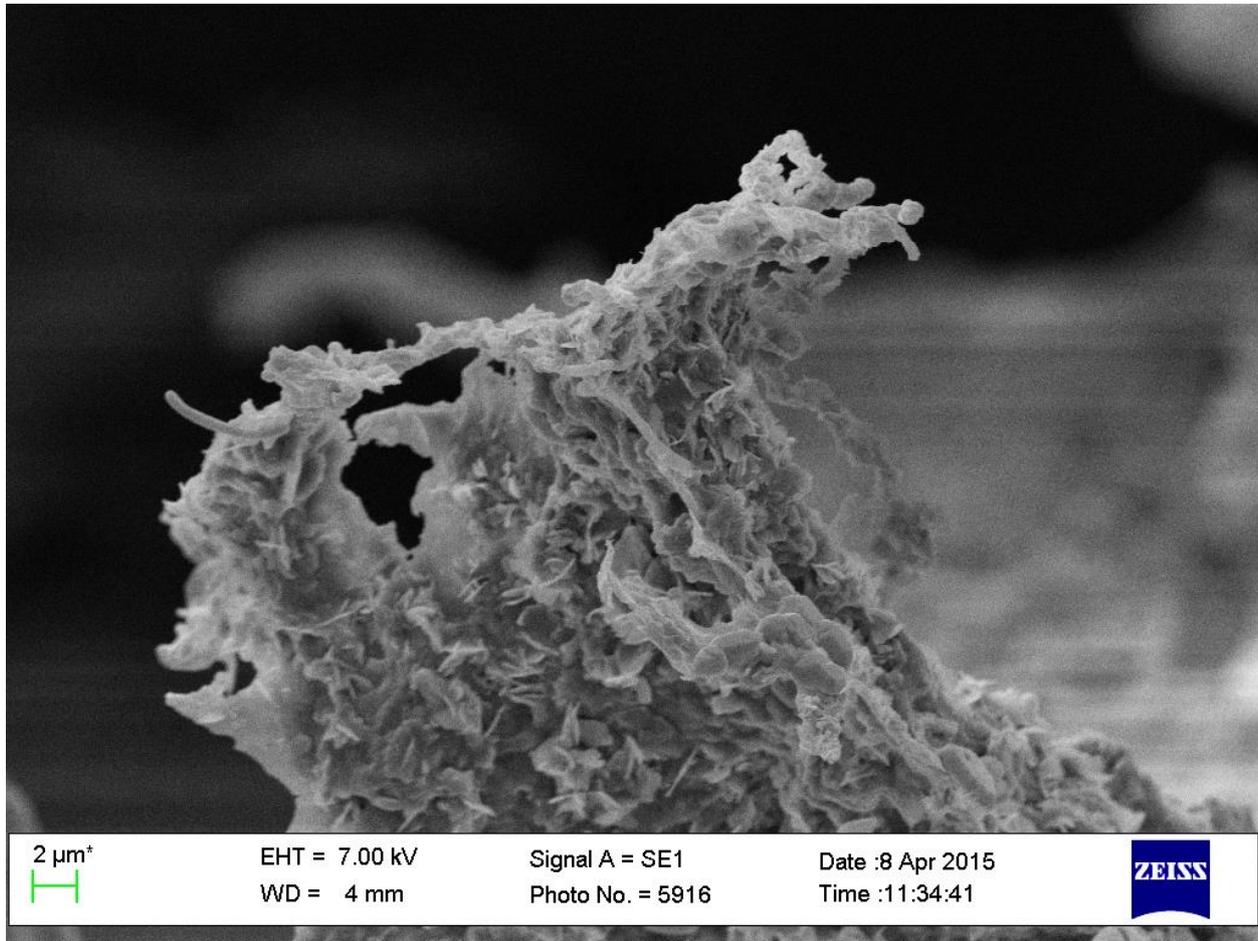
Figure 6 The surface of another MP that was colonised, various different microorganisms can be observed.

Bacterial growth on the side of the MP

Part of the MP

Bacterial growth

Bacterial growth

Bacterial growth on the side of the MP

Part of the MP
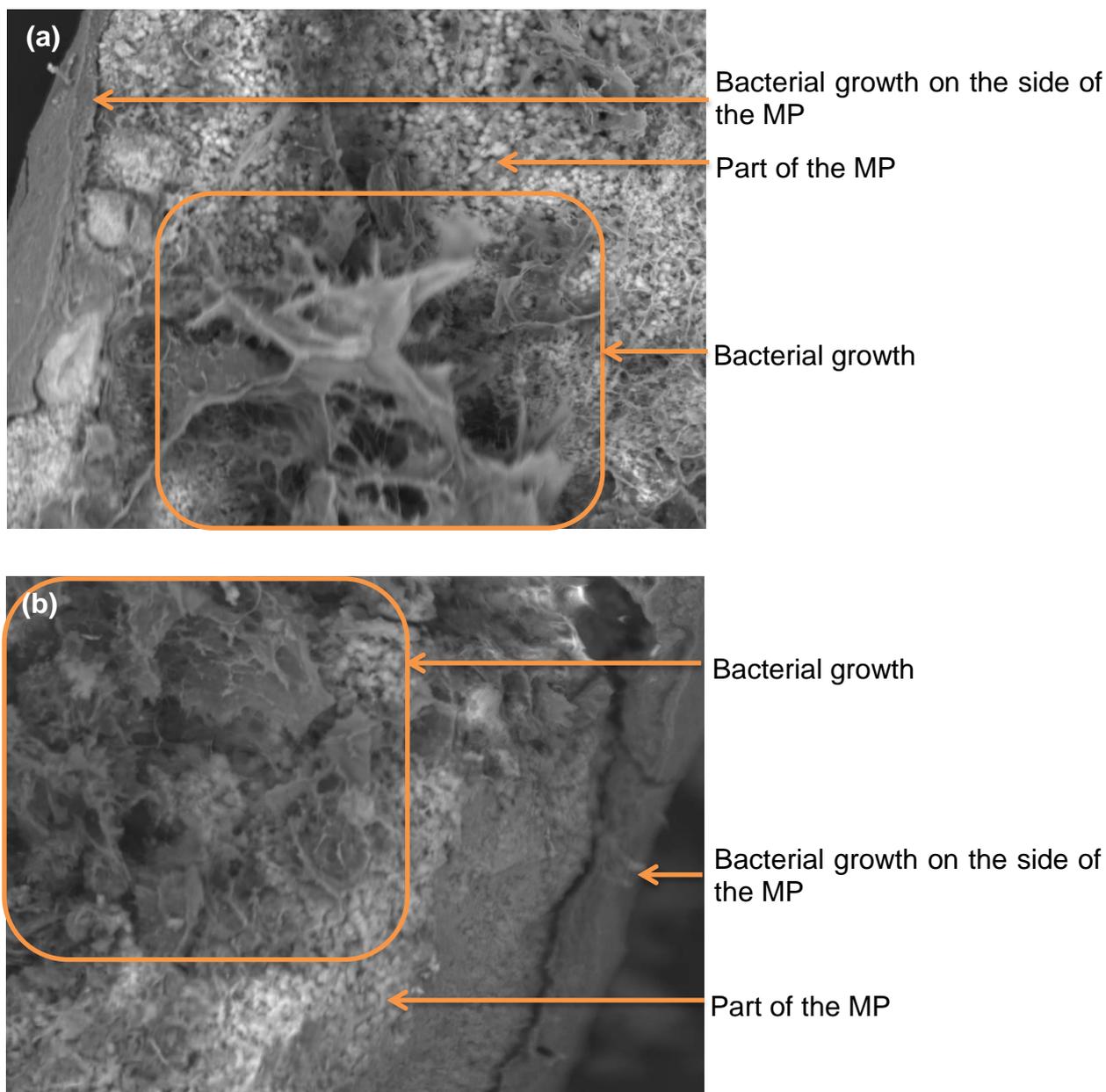
Figure 7 Scanning electron microscopy (SEM) images of a colonised MP split in half (magnification 100 μm).

*Fluorescence microscopic analysis*

Microscopy analysis was done on the MPs that had been recovered from the UASB reactor (Chapter 3). SYTO 9 dye was initially used to stain the MPs to observe any/all biological attachment. After excitation of the stained samples green fluorescence was observed (Fig. 8 (a) and (b)). The green fluorescence, as seen in Figures 8 (a) and (b), is a clear indication of the presence of biological attachment on the surface of a MP.

Figure 8 (b) is taken at a higher magnification and single cells of both rod shaped and cocci shaped organisms can be observed.

Autofluorescence of methanogenic bacteria is observed as seen in Figure 8 (c). Methanogenic bacteria are autofluorescent, which means that they emit energy at a certain wavelength, without being stained, when exposed to UV light (405 nm). Therefore, a comparison can be made between a sample which has been stained and samples that autofluoresce.   In Figure 8 (c) the autofluorescence confirms the presence of methanogenic bacteria; furthermore, differences in the morphology of the cells are also observed: long and short rods can be seen as well as a variety of cocci. *Methanobacterium* and *Methanosaeta* are long filamentous type bacteria that could easily be confused; however, *Methanosaeta* is much larger than *Methanobacterium* and can grow up to 200 µm (Mink & Dugan, 1977; Zinder *et al.*, 1984; Janssen, 2003). Furthermore, Janssen (2003) found that there was no autofluorescence displayed by *Methanosaeta;* however, *Methanobacterium* does display autofluorescence as confirmed by Mink & Dugan (1977) and Hattori *et al.* (2000). *Methanosarcina* is found to be the larger cocci of the methanogens (1 – 3 µm); and they have an irregular shape display autofluorescence (Singh *et al.*, 2005). The images displayed in Figures 8 (d) and (e) are magnified up to 2 µm, therefore it is possible that the autofluorescence displayed by the cocci shaped organisms in these images are *Methanosarcina.* *Methanobrevibacter* has a coccobacilli morphology (intermediate between a cocci and bacilli), with rounded ends and is between 0.5 – 1.2 µm (Rea *et al.*, 2007); furthermore, Dridi *et al.* (2012) confirmed autofluorescence of *Methanobrevibacter.*

Granule activity

Activity tests are usually performed on the biomass of a reactor, such as granules of a UASB reactor, to determine the biodegradability and the degradation potential of the wastewater. They are also used to determine the microbial activity of the different species within the microbial consortium of the biomass used to seed the reactor (Van der Westhuizen, 2014). There were two main aims of the activity tests performed in this study. The first aim was to determine whether the biogas production of the two reactors differed due to the addition of MPs to one of the reactors, R_{MP}. The second aim was to determine whether the biomass attachment on the MPs was enough to produce significant amounts of biogas, specifically methane production.
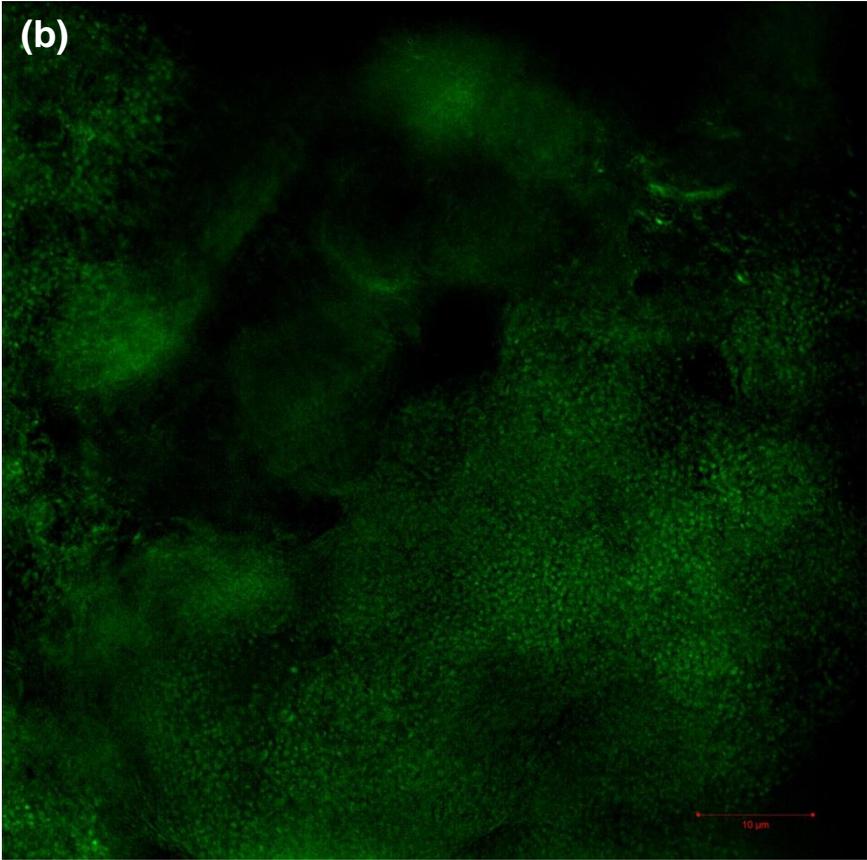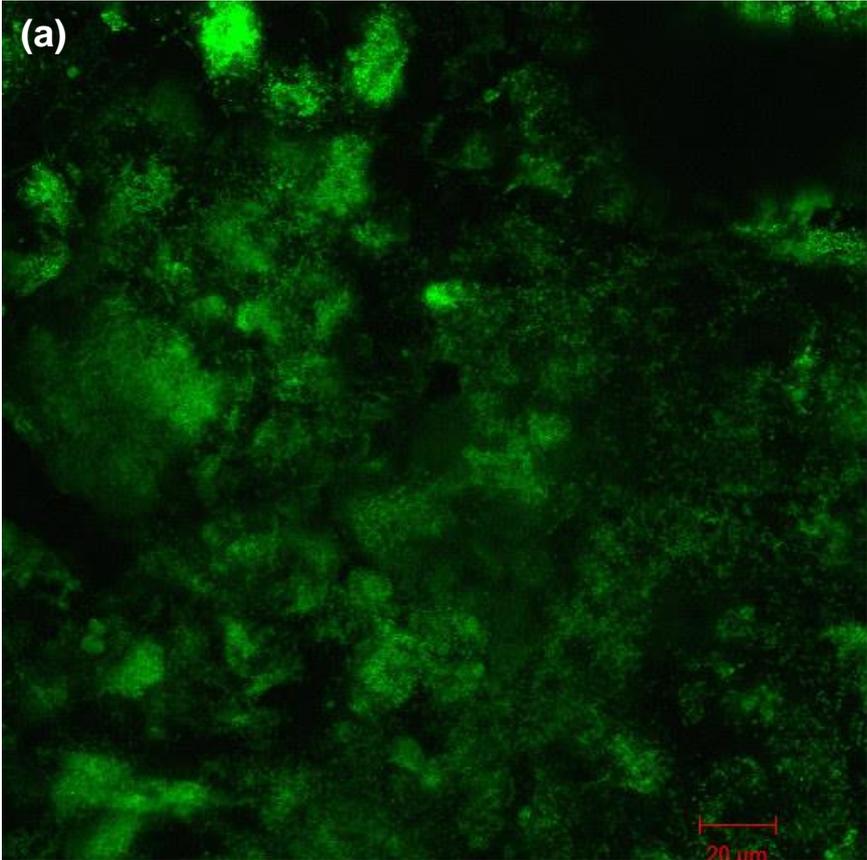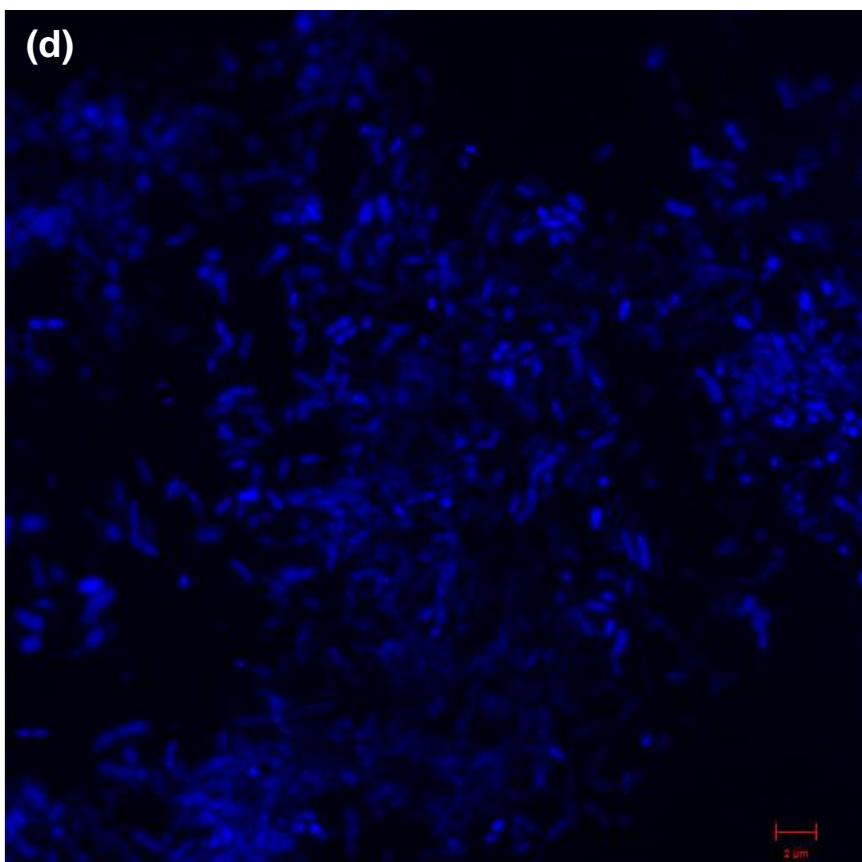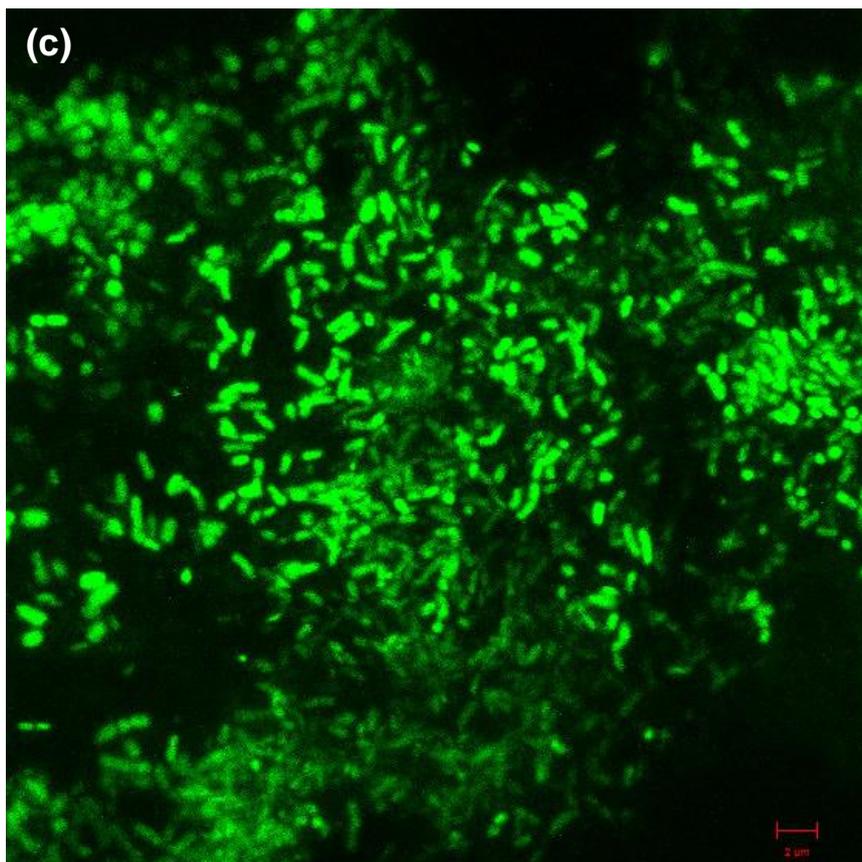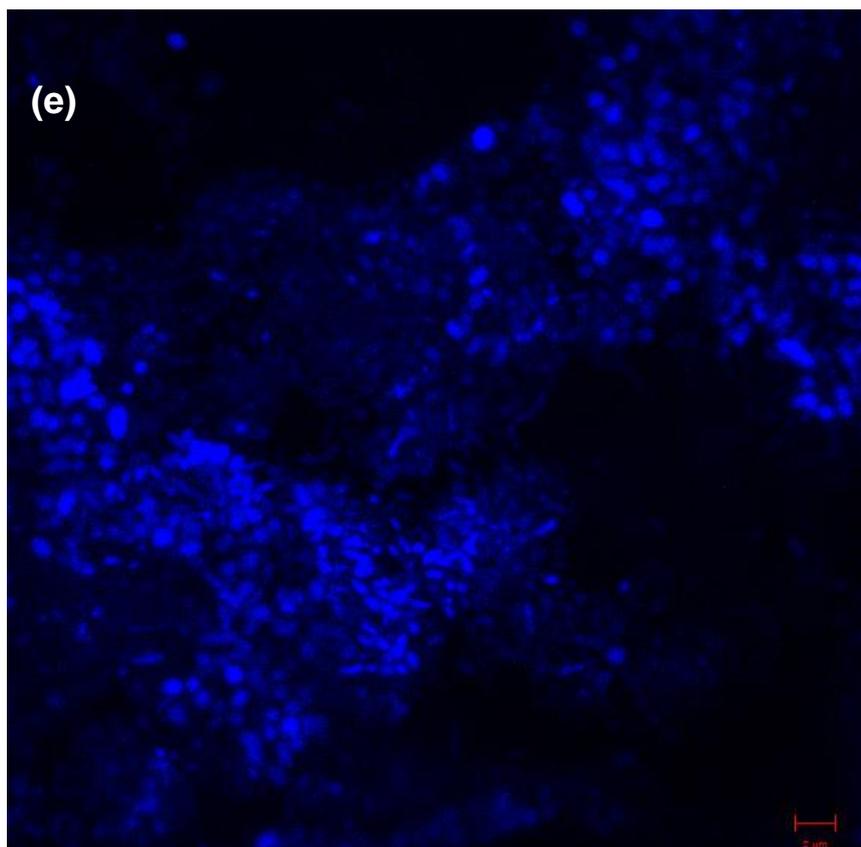
Figure 8 (a) (b) and (c) represent MP that were stained with SYTO 9 - the green fluorescence shows all microbial attachment. Images (d) and (e) are MP that display blue autofluorescence where methanogens have colonised.

Granule activity, measured as cumulative biogas, was determined at 5, 10 and 25 h incubation time using BTM, GTM and ATM as test media (Fig. 9). The different media used for the activity test are specific for certain microbial groups within the biomass consortium.

The activity of the samples incubated for 25 h in BTM increased over time (Fig. 9). The granules from $R_{control}$ presented an overall higher production of biogas compared to the other three samples (R_{MP}, MPs and control granules). It was expected that $R_{control}$ would produce higher volumes of biogas compared to the control granules and the MPs alone, due to the former not being as acclimatised as those granules that had been in a digester for 371 days and the latter not containing as much microbial biomass (the biomass of 3 g MPs is not equivalent to 3 g of granules). A large difference in the overall activity between R_{MP} and $R_{control}$ in all three mediums was not observed (BTM: 1.7 mL; GTM: 1.7 mL; ATM: 1.4 mL). Therefore the addition of MPs to an anaerobic UASB reactor

did not have a large effect on the amount of biogas produced. However, a small difference was observed; and this could possibly be due to a scouring effect between the MPs and the granules within R_{MP} causing granules to lose biomass and therefore affecting the activity. The overall activity of all the samples showed lower gas production in BTM when compared to GTM and ATM.

The activity of the samples incubated for 25 h in GTM increased over time (Fig. 9). The added glucose to the test media favours the activity of the acidogens; these are the largest group within the consortium of granular sludge (Gerardi, 2003). The biogas production for $R_{control}$ again showed the highest volume, followed by R_{MP}, MPs and lastly the control granules. The activity for all four samples showed a much higher biogas production compared to the BTM test. This gives an indication of favourable acidogenic activity in both reactors and more importantly this show that there is acidogenic attachment on the MPs.

The activity of the granules incubated for 25 h in ATM increased over time (Fig. 9). The added acetic acid to the basic test media favours the activity of the acetoclastic methanogens. This group is responsible for the conversion of acetic acid to methane (Anderson *et al.*, 2003). The biogas production for $R_{control}$ again showed the highest in volume, followed by R_{MP}, MPs and lastly the control granules. The overall biogas production of the ATM compared to the BTM showed a significant increase in the volume of gas produced; this gives a positive indication of the methanogenic activity present in all the samples, specifically on the MPs, indicating that there was methanogenic attachment onto the particles.

The control granules showed the lowest overall gas production in all the mediums used; but this was expected as these granules were not acclimatised as the rest, for they were not exposed to wastewater in a UASB reactor for 371 days. Gas production on the MPs showed positive results for all three test mediums, especially the ATM used; thus indicating that there was methanogenic attachment. Furthermore it must be considered that the amount of acidogenic activity on 3 g MPs is much less than the amount of acidogenic activity within a granule due to the MP's 3 g not being 100% biomass.
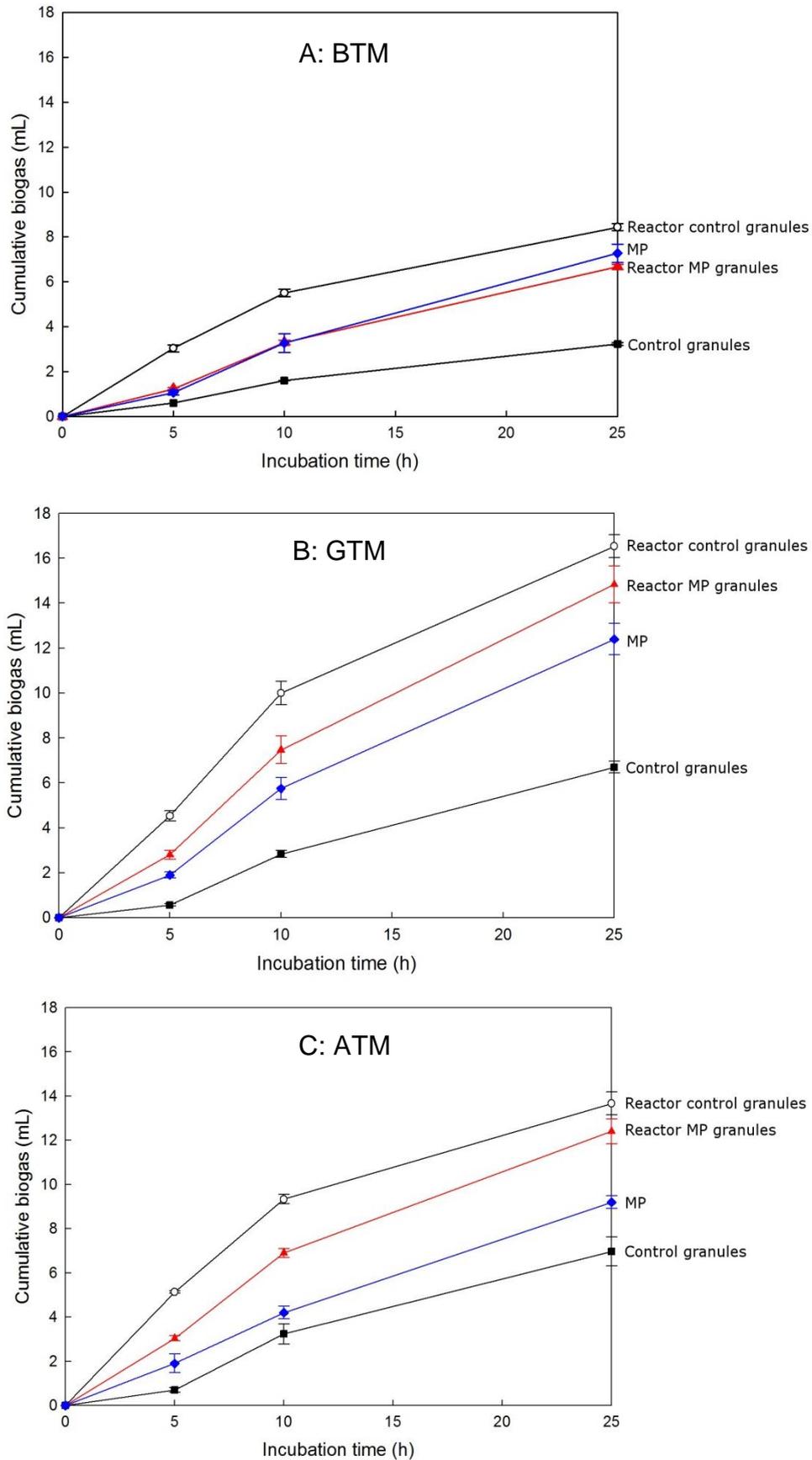
Figure 9 The biogas production of control granules, MP, granules from reactor with added MP (R_{MP}) and granules from reactor control ($R_{control}$) was measured using three different test mediums.

Percentage methane produced for all four samples was measured at 25 h incubation time in each medium (Fig. 10). The methane produced (%) of the control granules was the lowest in all three mediums compared to the rest of the samples (BTM: 53.5%; GTM: 42%; ATM: 46%). This was expected due to these granules not being acclimatised as they were not exposed to wastewater in a UASB reactor for 371 days. It can be seen that there is not a large difference in methane production between the samples within each medium. The ATM showed the highest methane percentage when compared to BTM and GTM. This was expected as the addition of acetic acid to the medium stimulates methane production. The MPs sample displayed the highest methane percentage compared to the other samples. These results were considered positive as this confirms active methanogenic activity attached onto the MPs. The MP samples also displayed the highest methane percentage in the GTM, which confirms the attachment of active acetogenic bacteria.
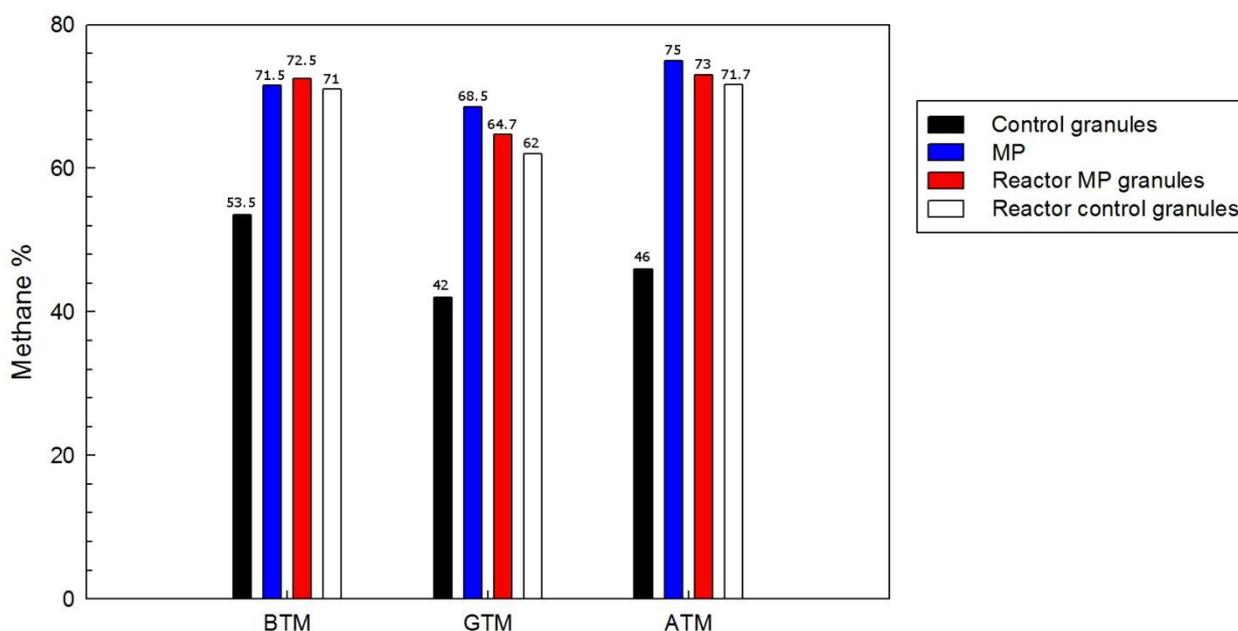

Figure 10 The methane production (%) of control granules, MP, granules from reactor with added MP and granules from reactor control after 25 h incubation in various test mediums.

Conclusion

The aim of this study was to determine whether added magnetisable foam glass particles (MPs) would display anaerobic microbial attachment after 174 days within an UASB reactor. After the MPs were extracted from the reactor and separated from the biomass several different analyses were performed to identify colonisation (SEM and fluorescent microscopy) and whether the attached biomass was active (activity tests). The SEM analyses displayed initial attachment to occur within the holes and cavities of the magnetisable particles' surface. The course and porous surface of the MPs was therefore found to be a suitable platform for biofilm attachment. Furthermore, a fully colonised particle displayed a large variety of organisms attached to its surface. The morphology of these organisms gave an indication that *Methanobacterium*, *Methanoplanus*, *Methanosaeta*, *Methanobrevibacterium* and *Methanosarcine* were possibly present on the surface of the MPs.

The green fluorescent microscopy images of the stained MPs confirmed the presence of a large consortium of bacterium. Furthermore, colonisation of methanogenic bacteria was confirmed by unstained samples that displayed blue autofluorescence as the MPs were exposed to UV light. These observations confirm microbial attachment of various anaerobic bacteria onto the surface of the MPs.

Additionally, the results from the activity tests indicated that the biomass attachment was active (i.e. biogas production). The MPs produced methane in all three test mediums, confirming active acetogenic and methanogenic attachment.

In conclusion, attachment and colonisation of different species found in anaerobic granular sludge onto the surface of MPs were successful. The attachment of active acetogenic and methanogenic species was confirmed by fluorescent microscopy as well as from the biogas and methane production from the activity tests. Furthermore, the MPs did not influence the UASB reactor negatively; on the contrary, the magnetisable properties of the particles allow the retrieval of lost biomass, should washout of colonised particles occur.

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CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

One of the fastest growing industries in South Africa is the wine and distillery industry (SAWIS, 2014). As a result of the increasing demand on these products the water use as well as the waste production increases. The wine and distillery industry produces on average between 8 – 20 L of wastewater for every 1 L of product produced (Wilkie *et al.*, 2000; Dillon, 2011). The wastewater produced by this industry is not suitable to be reused or discharged into the environment unless it is treated (Republic of South Africa, 2004). Therefore it is essential for wine and distillery wastewater to be treated before it is discharged, as to conserve the rapidly decreasing fresh water availability.

Anaerobic digestion is widely applied wordwide to treat high strength industrial waste like wine and distillery wastewater (Pant & Adholeya, 2007). Various treatment processes have been developed to suite industrial needs; one of these technologies is the upflow anaerobic sludge blanket (UASB) reactor. The UASB process has become a widely applied process that successfully treats high strength effluent. It has been found that the UASB process can reduce high chemical oxygen demands by up to 90%. However, operational problems have been found during seasons of high volumetric loading rates as well as high organic loading rates.

The first objective of this study was to investigate the operational efficacy of two UASB reactors when treating a combination of alcoholic beverage waste streams each with varying characteristics (pH, COD and TSS), volumes and production seasons. The trial was divided into five phases, each phase representing an added waste stream to the substrate of both reactors. The performance of the reactors were determined by monitoring the pH, alkalinity, COD reduction (%) and biogas production of both UASB reactors while the COD of the substrate (combination of wine, marula and Brandy wastewater) was increased up to 10 $kgCOD.m^{-3}.d^{-1}$. The pH for both substrates after the start-up period was maintained at 7 and a HRT of 24 h was maintained throughout the trial.

The initial start-up (phase 1) for both reactors was successful, where both UASB reactors displayed 80% COD reduction with an OLR of 1 $kgCOD.m^{-3}d^{-1}$ as well as a stable pH and alkalinity. Phase 2 represented the addition of WWW to the substrate of both reactors. During this phase the OLR was successfully increased from 1 $kgCOD.m^{-3}.d^{-1}$ to

4 kgCOD.m^{-3}.d^{-1} and both reactors maintained a good COD reduction (%) of above 80%. The alkalinity increased to 2 000 mgCaCO$_3$.L^{-1} and the pH of the effluent was maintained at *ca.* 7.5 for both reactors. Phase 3 represented the addition of marula wastewater to the substrate of both UASB reactors. During this phase the OLR was gradually increased from 4 kgCOD.m^{-3}.d^{-1} to 6 kgCOD.m^{-3}.d^{-1}. The overall COD reduction for both reactors during this phase was maintained at above 80% as well as an overall increase in the alkalinity. The pH in R$_{control}$ did however drop to 4.9 near the end of phase three; this was rectified immediately and the trial continued as normal. At the end of phase three a good COD reduction (%), a stable alkalinity and a stable pH was found for both reactors. Phase 4 represented the addition of Brandy wastewater to the substrate of both UASB reactors. During this phase the OLR was gradually increased from 6 kgCOD.m^{-3}.d^{-1} to 10 kgCOD.m^{-3}.d^{-1}, representing the peak in OLR for the entire trial. The COD reduction, pH and alkalinity of both reactors were stable during the initial stage of this phase. However, the pH of R$_{control}$ dropped on day 293 and day 302 (OLR *ca.* 6.4 kgCOD.m^{-3}.d^{-1}) to 5.43 and 5.08 respectively, whereas the pH for R$_{MP}$ remained stable. On both days 293 and 302 R$_{control}$ was flushed with 500 mg.L^{-1} urea ($(NH_2)_2CO$), and 500 mg.L^{-1} di-potassium hydrogen orthophosphate (K_2HPO_4) dissolved in tap water. After which the OLR was decreased to 5 kg COD.m^{-3}.d^{-1} until the reactor pH stabilised. R$_{control}$ stabilised on day 308 (pH 7.5 and alkalinity 2600 mgCaCO$_3$.L^{-1}) whereafter the OLR was again gradually increased until it reached *ca.* 9 kgCOD.m^{-3}.d^{-1}. At the end of phase four the COD reduction of both reactors was above 90%, the pH of the effluent above 7 and the alkalinity *ca.* 1 900 mgCaCO$_3$.L^{-1}. Phase five represented the end of the marula season as well as the end of the wine production season, the substrate for both reactors therefore consisted of bottling wastewater, wash water and Brandy wastewater only. The OLR during this season therefore reduced to *ca.* 4.7 kgCOD.m^{-3}.d^{-1} due to marula and winery wastewater being absent from the substrate. Both reactors displayed similar results during this phase. The COD reduction percentage for both reactors remained above 90% and the pH of the effluent above 7 for the remainder of the trial. The alkalinity; however, decreased in both reactors; possibly due to the absence of the marula wastewater from the substrate, which contained high levels of nitrogen which could have served as a buffer. An increase in the methane production for both reactors was observed during this phase, possibly due to the decrease in alkalinity, resulting in a decrease of free ammonia which is toxic for the methane forming bacteria.

The treatment of combined wine and distillery wastewater for both reactors during the entire trial was successful. A maximum COD reduction percentage of *ca.* 97% was

achieved for both reactors, an increase in biogas and methane production was found as well as a stable pH and alkalinity reached. Therefore it can be concluded that the UASB reactor is a viable treatment option for the treatment of combined alcoholic beverage effluents.

The second objective of this study was to investigate whether the added MPs affected the performance of the UASB reactor. Two UASB reactors were operated in parallel; where the first reactor served as a control ($R_{control}$) and the second reactor was seeded with magnetisable glass foam particles (MPs) (R_{MP}).

A difference in performance between $R_{control}$ and R_{MP} was observed; specifically during phases 3 and 4. The pH and alkalinity, in $R_{control}$, were unstable when compared to R_{MP}. It was therefore hypothesised that the added MPs to the UASB reactor could have served as an added iron source, which would account for the more stable performance observed in R_{MP} compared to $R_{control}$. However, trace element analyses were performed on the granules from both reactors and a higher iron quantity in the granules from R_{MP} was not found. The reason for the performance difference between the two reactors is unknown at this stage; however, future research as to why there is a difference is recommended. The added MPs did not have a negative influence on the operation of the UASB reactor, on the contrary, the magnetisable properties of the particles allow retrieval of biomass should washout occur of colonised MPs.

Scanning electron microscopy images were taken of the granules from both reactors and were compared and a difference was observed. The surface of the granules from R_{MP} was much coarser than when compared to the surface of the granules from $R_{control}$. This could possibly be due to a scouring effect caused by the added MPs. This did not; however, result in a negative effect on the performance of the UASB reactor.

The third objective of this study was to investigate whether the added MPs were a viable medium for biomass attachment to enhance self-immobilisation of anaerobic granular biomass.

Scanning electron microscopy images (SEM) were taken of the surfaces of different MPs after extraction from the biomass in R_{MP}. Initial microbial attachment was confirmed in the cavities of the MP's surface and filamentous growth was observed, which could possibly be EPS formation. Furthermore, surface images of a fully colonised MP next to an uncolonised MP (control) were taken. A clear difference between the colonised MP and the control MP was seen, the surface of the control MP was uneven and many cavities can be seen, whereas the surface of the colonised MP had no cavities and a much rounder, smoother surface was seen. Colonised MPs were also compared to whole

granules taken from the control reactor. Clear similarities were observed, as there were cracks and holes in both the colonised MPs as well as the granules.

Higher magnification images were taken of the surface of a colonised MP and a large consortium of microorganisms was observed. The high magnification of 10 µm and 2 µm, respectively were close enough to observe single celled organisms and organisms similar to those of *Methanosarcina* were observed. Furthermore, a clear attachment layer of biomass was observed on the surface of the MP as well as some growth inside the MP.

Fluorescent microscopy images were taken of colonised MPs to confirm attachment that had been observed in the SEM images. Stained samples as well as unstained samples were tested. The stained samples emitted green fluorescence confirming all microbial growth on the surface of the MPs. The unstained samples emitted blue fluorescence that confirmed the presence of autofluorescent methanogens. Images were taken at a magnification of 2 µm; here single celled organisms of both cocci and bacilli were observed from the green fluorescence as well as the blue autofluorescence.

Activity tests were performed to confirm whether the microbial attachment onto the MPs were active. Three different test mediums were used and granular activity was determined at 5, 10 and 25 h incubation time. Biogas production was observed from the MPs in all three mediums; this does not only confirm biomass attachment, as seen in the SEM and autofluorescent images, but it confirms attachment of active biomass. The methane production (%) was measured at 25 h incubation time. This again confirmed that the biomass attachment onto the MPs was active, as the MPs produced methane in all three mediums. The production of methane in the acetic acid medium confirms that there was active methanogenic attachment and the methane production in the glucose test medium confirmed that there was active acetogenic attachment.

Therefore it can be concluded that the viability of using magnetisable glass foam particles as a medium for anaerobic microbial attachment to immobilise UASB granular biomass was successful. The attachment found was of a large variety of anaerobic organisms from granular biomass and the attachment was active, producing biogas, specifically methane.

FUTURE RESEARCH AND RECOMMENDATIONS

During the course of this study, various points were identified that could be addressed in future. During the treatment of combined wastewater, the performance of the UASB reactor was unstable as the OLR reached 6 $kgCOD.m^{-3}.d^{-1}$ and higher. It is therefore recommended that the treatment system be carefully monitored during seasons of high OLR.

It was also found that during the treatment of wine and distillery wastewater the performance of the UASB with added magnetisable particles (MP) (R_{MP}) was better than the performance of the control UASB reactor ($R_{control}$), especially during phases of high OLR. A better understanding of the composition of the MPs is necessary to understand their influence on the UASB anaerobic biomass. The mineral composition of the MPs can possibly serve as a micro- and/or macronutrient to the biomass.

The investigation of the attachment of anaerobic biomass onto the MPs was successful; however, further investigation as to the specific genus of the species attached would be advised as to improve the understanding and mechanism of attachment. DNA sequencing can be done on the colonised MPs; this will give a clear indication as to what has attached.

Furthermore, it is recommended that when evaluating the activity of the colonised particles, volatile suspended solids (VSS) of the samples are compared as the results are analysed.

It was also found that the suspension properties of the MPs within the UASB reactor were not consistent. As these particles are porous and not identical, the diffusion rate of the liquid into the particles will differ. Therefore, resulting in an uneven distribution of particles throughout the reactor. It is recommended that the particles be analysed with regards to their suspension properties before added to the UASB reactor.

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