

**Optimising coagulation and ozone pre-treatments and
comparing the efficacy of differently pre-treated grain distillery
wastewaters in an upflow anaerobic sludge
blanket (UASB) reactor**

Louise Robertson

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Supervisor: Dr. G.O. Sigge

Co-supervisor: Prof. T.J. Britz

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Declaration

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Abstract

Large volumes of high strength wastewater are generated annually by distilleries. Treatment of this wastewater is essential to increase its reuse potential. Anaerobic digestion (AD) has been widely applied as high strength wastewater treatment. Additionally, grain distillery wastewater (GDWW) is high in fats, oils and grease (FOG) which can cause problems during AD. Pre-treatment is therefore often required to make the AD process more efficient.

The overall objective of this study was to investigate the operational efficiency of upflow anaerobic sludge blanket (UASB) reactors while treating GDWW as substrate. The first aim was to investigate the pre-treatment of the GDWW specifically for the removal of FOG by evaluating the effect of pH adaption, coagulant concentration and ozone (O_3) dosages on the FOG reduction efficiency. Secondly, the effect of two different pre-treatments (only coagulant and coagulant and ozone) on the subsequent UASB treatment step was investigated.

The pH of raw GDWW (pH 3.4) was adapted to three different pH values (5.0, 6.0, and 7.0) and the coagulant, aluminium chlorohydrate (ACH) ($140 \text{ mg} \cdot \text{L}^{-1}$), was added. To make the process more economically viable, the lowering the coagulant concentration (to $100 \text{ mgACH} \cdot \text{L}^{-1}$) was also investigated. Optimal reductions for chemical oxygen demand (COD) ($33.2\% \pm 4.93$), total suspended solids (TSS) ($91.9\% \pm 1.73$) and FOG ($84.1\% \pm 1.98$) were, however, achieved at a higher coagulant concentration of $140 \text{ mgACH} \cdot \text{L}^{-1}$, and at a wastewater pH of 7.0. The effect of additional ozone treatment was also investigated. Maximum reductions for the ozone treatment were obtained at $100 \text{ mgO}_3 \cdot \text{L}^{-1}$ for COD ($3.6\% \pm 4.08$), and at $900 \text{ mgO}_3 \cdot \text{L}^{-1}$ for TSS ($27.7\% \pm 5.58$) and FOG ($23.9\% \pm 1.83$). The ozone treatment was most efficient for FOG reduction (in terms of mg FOG reduced per mg ozone) at $100 \text{ mgO}_3 \cdot \text{L}^{-1}$. An ozone dosage of $300 \text{ mgO}_3 \cdot \text{L}^{-1}$, was decided on based on economic feasibility, findings in literature on toxicity of ozone and the potential degradation of recalcitrant compounds at this dosage. The final pre-treatment thus included pH adaption to 7.0, coagulant dosage of $140 \text{ mgACH} \cdot \text{L}^{-1}$, and an ozone dosage of $300 \text{ mgO}_3 \cdot \text{L}^{-1}$.

The second part of this study involved the operation of two 2 L laboratory scale UASB reactors for 277 days. The substrate of the first reactor contained GDWW that had only undergone coagulant pre-treatment (R_{control}), while the substrate of the second UASB reactor consisted of GDWW that had undergone coagulant and ozone pre-treatment (R_{ozone}). Both reactors treated the pre-treated GDWW successfully at *ca.* $9 \text{ kgCOD} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$. COD reductions of 96% for R_{control} and 93% for R_{ozone} , were achieved. FOG reductions (%)

showed variations throughout the study and maximum reductions of 88% and 92% were achieved for R_{control} and R_{ozone} , respectively. The R_{ozone} produced more biogas, but the methane content was similar for both reactors. The additional ozone pre-treatment did not show any added benefits to the reactor performance results. UASB granule washout in R_{control} did, however, suggest possible toxicity of unsaturated fatty acids present in the non-ozonated substrate.

The feasibility of FOG removal was shown as both reactors successfully treated pre-treated GDWW. Ozonation, after a coagulant dosage, which resulted in further reduction in the FOG content of the GDWW, is thus not essential to ensure the success of an anaerobic digestion step. Ozonation of the pre-treated GDWW could, however, be beneficial to gas production and the efficiency of a tertiary biological process.

Uittreksel

Groot volumes hoë-sterkte afloopwater word jaarliks deur distilleerderye opgelewer. Die behandeling van hierdie afloopwater is noodsaaklik om die hergebruiksmoontlikheid daarvan te verhoog. Die toepassing van anaërobiese vertering (AV) is wydverspreid in hoë-sterkte afloopwaterbehandeling. Graandistillings-afloopwater (GDAW) is boonop hoog in vette, olies en ghries (VOG), wat probleme kan veroorsaak tydens AV. Voorbehandeling word dus dikwels verlang om die AV meer doeltreffend te maak.

Die oorhoofse doelstelling van hierdie studie was om die doeltreffendheid van opvloei-anaërobiese-slykkomers- (OAS-) reaktore wat GDAW as substraat behandel, te ondersoek. Die eerste mikpunt was om die voorbehandeling van die GDAW, te ondersoek vir die verwydering van VOG. Dit is uitgevoer deur die uitwerking van pH aanpassing, koagulantkonsentrasie en osoon(O_3)dosis op VOG vermindering te evalueer. Tweedens is die uitwerking van twee verskillende voorbehandelings (slegs koagulant asook koagulant en osoonbehandeling) op die opvolgende OAS-behandelingsstap ondersoek.

Die pH van rou GDAW (pH 3.4) is aangepas tot drie verskillende pH waardes (5.0, 6.0 en 7.0) en die koagulant, aluminium-chlorohidraat (ACH), is bygevoeg ($140 \text{ mg} \cdot \text{L}^{-1}$). Om die proses meer ekonomies uitvoerbaar te maak is 'n verlaagde koagulantkonsentrasie (verlaag tot $100 \text{ mgACH} \cdot \text{L}^{-1}$) ook ondersoek. Die optimale afnames vir chemiese suurstofvereiste (CSV) ($33.2\% \pm 4.93$), totale oplosbare vastestowwe (TOV) ($91.9\% \pm 1.73$) en VOG ($84.1\% \pm 1.98$) is egter bereik teen 'n hoër koagulant konsentrasie van $140 \text{ mgACH} \cdot \text{L}^{-1}$, en teen 'n afloopwater-pH van 7.0. Die uitwerking van 'n bykomende osoonbehandeling is ook ondersoek. Die hoogste afnames tydens die osoonbehandeling is bereik teen $100 \text{ mgO}_3 \cdot \text{L}^{-1}$ vir CSV ($3.6\% \pm 4.08$), en teen $900 \text{ mgO}_3 \cdot \text{L}^{-1}$ vir TOV ($27.7\% \pm 5.58$) en VOG ($23.9\% \pm 1.83$). Die osoonbehandeling was mees doeltreffend vir VOG (in terme van mg VOG verwyder per mg osoon) teen $100 \text{ mgO}_3 \cdot \text{L}^{-1}$. Daar is besluit op 'n van $300 \text{ mgO}_3 \cdot \text{L}^{-1}$, as gevolg van die ekonomiese uitvoerbaarheid, bevindinge in literatuur vir die toksisiteit van osoon, en die moontlike afbraak van moeilik-afbreekbare komponente teen hierdie dosis. Die finale voorbehandeling het dus bestaan uit 'n aanpassing van die afloopwater-pH na 7.0, 'n koagulantdosis van $140 \text{ mgACH} \cdot \text{L}^{-1}$, en 'n osoondosis van $300 \text{ mgO}_3 \cdot \text{L}^{-1}$.

Tydens die tweede gedeelte van hierdie studie is twee 2 L laboratoriumskaal OAS reaktore bedryf vir 277 dae. Die substraat van die eerste reaktor het GDAW bevat wat slegs 'n koagulant-voorbehandeling ondergaan het (R_{kontrole}), terwyl die substraat van die tweede OAS-reaktor GDAW bevat het wat koagulant- en osoon-voorbehandeling ondergaan het (R_{osoon}). Beide reaktore het die voorbehandelde-GDAW suksesvol

behandel teen 'n organieseladingstempo van ca. $9 \text{ kg CSV} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$. Afnames in CSV van 96% vir $R_{\text{kontrolle}}$ en 93% vir R_{osoon} , is bereik. Tydens die studie is deurlopende wisseling in VOG verwydering (%) waargeneem, en die hoogste verwyderings wat bereik is, is onderskeidelik 88% en 92% vir $R_{\text{kontrolle}}$ en R_{osoon} . Die R_{osoon} het meer biogas geproduseer, maar die metaanpersentasie was soortgelyk vir beide reaktore. Die osoon-voorbehandeling het nie enige toegevoegde voordele getoon in die reaktorprestasie resultate nie. Die uitwas van die OAS-granules vir die $R_{\text{kontrolle}}$ het egter moontlike toksisiteit van onversadigde vetsure aangedui, wat moontlik teenwoordig kon wees in die nie-geösoneerde substraat.

Die uitvoerbaarheid van VOG verwydering is getoon aangesien beide reaktore voorbehandelde GDAW suksesvol behandel het. Osonering, wat verdere afname in die VOG inhoud van GDAW teweeggebring het (na 'n koagulant dosis), is dus nie noodsaaklik vir die suksesvolle verloop van 'n anaërobiese-verteringsstap nie. Osonering van die voorbehandelde GDAW kan egter voordelig wees vir gasvorming, en kan ook verder die doeltreffendheid van 'n tersiëre biologiese behandeling verhoog.

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

Chapter 1

INTRODUCTION

Water scarcity is becoming an increasingly serious problem faced by several countries across the earth (Nel *et al.*, 2009). Current predictions point towards Africa not having adequate water supply by 2025 as a 30% increase in water demand is expected (Rijsberman, 2006; WWF, 2013). South Africa consists of large arid and semi-arid areas which have a low annual rainfall of 450 mm compared to the global average of 860 mm (Adewumi *et al.*, 2010). South Africa also has a varying rainfall distribution, with 43% falling on 13% of the land (Anon., 2012a). The South African agricultural sector uses 60% of the available rain water and the industrial sector is responsible for approximately 3% of the water usage (DWA, 2010). Of the total water available, 98% has been allocated to various users leaving very little water available to areas or sectors which are under high water stress (Anon., 2012b). Furthermore, the country currently faces vast infrastructure development problems in terms of water supply (SAICE, 2011). This influences the quality of the water, which is often not acceptable in many areas in South Africa (SAICE, 2011). Water has therefore been labelled as an invaluable resource and the careful management thereof cannot be over emphasised (SAICE, 2011).

The food and beverage industry is the largest water consumer when compared to other industries (Mavrov & Bélières, 2000). The distillery industry discharges up to 15 litres of wastewater per litre of alcohol produced during spirit production (Nataraj *et al.*, 2006). Grain distillery wastewater (GDWW), originating from the production of whisky generally has a high chemical oxygen demand load (COD) (10 000 – 60 000 mg.L⁻¹), high total suspended solids (TSS) (45 – 9 600 mg.L⁻¹), high fats oils and grease (FOG) content (1 900 – 2 300 mg.L⁻¹), and a low pH (3.4 – 3.8) (Tokuda *et al.*, 1999; Akunna & Clark, 2000; Laubscher *et al.*, 2001; Gie, 2007; Mallick *et al.*, 2010). It has also been suggested that it requires the addition of alkalinity upon anaerobic digestion (Goodwin & Stuart, 1994). Land application has become common disposal practice for such wastewaters (Gao *et al.*, 2007), but in South Africa the raw GDWW does not comply with the standards specified for land irrigation (Republic of South Africa, 2004). The extended disposal of untreated wastewater will also lead to the lowering of the alkalinity in soil and thus cause ecological damage (Gao *et al.*, 2007).

Due to the water scarcity and pollution caused by industrial wastewater, distilleries should strive towards zero emissions and furthermore consider treatment options that will enable the reuse of wastewater (Ulgiati, 2001). This will also be of economic benefit (Ulgiati, 2001). Anaerobic digestion is one such treatment option (Bitton, 2005). High strength wastewaters have often been treated by making use of this method as it has the ability to handle high organic loading rates at relatively short hydraulic retention times (Bitton, 2005). Further advantages of this treatment are the low sludge production, low maintenance costs, and the production of biogas which can be used as a renewable energy source (Pant & Adholeya, 2007). This creates a possibility for an economically beneficial process and could also make the production of spirits a more environmentally friendly process (Ulgiati, 2001).

The production of spirits entails the use of grain, such as maize, as a substrate (Agu *et al.*, 2006). As maize contains lipids, problems have been encountered with the anaerobic treatment of such wastewaters (Gie, 2007). The long chain fatty acids (LCFAs) found in these wastewaters have been shown to exhibit a toxic effect on the microbes present in anaerobic granules, especially microbes responsible for the production of methane (Pereira *et al.*, 2005). This leads to the formation of a layer composed of lipids around anaerobic granules which then prevents the transfer of nutrients and metabolites (Gie, 2007; Pereira *et al.*, 2005). Ultimately this causes a decrease in the density of granules and subsequent washout and reactor failure (Hwu *et al.*, 1998; Jeganathan *et al.*, 2006). To make the use of anaerobic reactors possible certain pre-treatments are thus suggested to convert the organic content to a more biodegradable state (Gray, 2010). Coagulants have been used to reduce the solid and lipid content in wastewater and therefore serve as a primary pre-treatment in wastewater processes (Gray, 2010). It has also been shown that ozone selectively splits double bonds present in LCFAs making the lipids more biodegradable (Andreozzi *et al.*, 1998). If this was applied it could thus enhance the operational efficiency of UASB reactor systems, and increase the biogas yield and contribute to an effluent suitable for reuse (Sigge, 2005).

The overall objective of this study was to investigate the operational efficiency of UASB reactors while treating GDWW as substrate. This will be achieved by focussing on the pre-treatment of the GDWW and then the operational parameters of the subsequent anaerobic digestion treatment step. The first aim will be to investigate the pre-treatment of the GDWW for the removal of FOG by determining the effect of pH, coagulant concentration and ozone dosage on the FOG removal efficiency. The second aim will be to compare the biodegradability of the pre-treated GDWW (receiving coagulant and ozone

treatment) with GDWW which will only receive coagulant treatment (control) during anaerobic digestion in a UASB reactor.

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Chapter 2

LITERATURE REVIEW

A. BACKGROUND

In recent years water shortages have increased resulting in a worldwide escalation in water demand (Nel *et al.*, 2009). The reasons for the general decline in water availability can be ascribed to population growth, climate change, urbanisation and mechanisation. These all play a role in the change in water use patterns (Ashton, 2002). Several countries face the ever increasing challenge of effective management of water resources (Ashton, 2002). This is a particularly great challenge in arid areas of the world where the scarcity of water is linked to disease, poverty and famine (Ashton, 2002; Mathers *et al.*, 2009).

Of the available water in the world, only 1% is fresh water and the total amount of water easily reachable to human life is equal to 0.007% (Visvanathan & Asano, 2003). This value makes it clear why so many humans suffer as a direct and indirect cause of water scarcity. The amount of available water is sufficient for the demand of the global population, but the distribution of water resources is not uniform and this is the cause of problems that are generally observed across the world (Visvanathan & Asano, 2003). Africa consists of large arid and semi-arid areas (Fischer *et al.*, 2011). Tendencies in population growth and water usage patterns observed on this continent indicate that it will face inadequate water supply by 2025 as a 30% increase in global water demand has been predicted (Rijsberman, 2006; WWF, 2013). The distribution and availability of Africa's water supply varies extensively and this causes certain areas to experience great water scarcity (Ashton, 2002; Fischer *et al.*, 2011). This restriction limits development of many areas ranging from economic and political to social (Ashton, 2002).

Africa's rainfall is highly distributed and very seasonal (Ashton, 2002). It amounts to an average rainfall of approximately 650 mm per annum, which is lower than its equatorial fellows and considerably lower than that of the world, which receives an average of 860 mm per annum (Ashton, 2002). Two thirds of the rainfall evaporates and the remaining part runs off to rivers, lakes and feeding aquifers (Anon., 2011a).

The effect of this water shortage manifests in various ways. According to Black *et al.* (2003) 10 million children die every year and most of these deaths occur in poor

countries of which African countries make up a large proportion. Various risk factors have been identified and these include the lack of water which is safe for ingestion (Ezzati *et al.*, 2002; WHO, 2002). The lack of safe water supply and appropriate sanitation causes up to two million child deaths and is also the cause of 88% of deaths caused by diarrhoea (Ezzati *et al.*, 2002; WHO, 2002; Elimelech, 2006). In 1995 the World Health Organisation estimated the mortality rate, due to diarrhoea, to be 3 300 000 per year (WHO, 1995). The amount of clean water supply has increased in recent years, but potable water supply remains a great challenge (Easterly, 2009; Mathers *et al.*, 2009).

Water scarcity makes the recycling and reclamation of water an obvious alternative to stretch water supplies. This offers numerous advantages to the environment, such as, a reduction in pollution, as effluent would not be released into natural water sources, a decline in the usage of natural freshwaters from sensitive ecosystems, the replacement of soil nutrients and the delay of future expansion of water supply infrastructure (Adewumi *et al.*, 2010a).

Ecological and human disasters are steering the world towards the implementation of sustainable usage of water. If such practices are to be neglected an increase in national and international conflict regarding water resources, deaths as a result of water borne diseases as well the negative altering of eco-systems could increase (Adler *et al.*, 2007). The importance of basic human water supply, maintenance of eco-systems and the sustainability of water supply need to be balanced to ensure long term availability of water (Adler *et al.*, 2007).

The South African agricultural industry (water used for irrigation of crops) consumes the largest amount of water on average (70%) and the industrial sector uses 22% of the water (Anon., 2011b; Anon., 2011c). In low and middle income parts of the world agriculture uses 82% of the available water and 30% in high-income countries (Anon., 2011b). Developing countries dispose of 70% of industrial waste into the clean water supply, although only 8% of the total water usage is ascribed to industrial use (Anon., 2011b). Organic waste originating from the food processing and beverage production industry is the largest contributor to these wastes (54% in low-income countries) (Anon., 2011b). It is therefore clear why there should be an increased drive regarding water purification, reuse and recycling, especially in developing countries.

Water in South Africa

South Africa is a semi-arid country which is under high water stress due to low average rainfall (Adewumi *et al.*, 2010b). Rainfall is estimated at 450 - 500 mm per annum (ranging between 25 – 1 474 mm in different areas) and a large volume is not available as it is lost

due to evaporation (DWAF, 2004; Adewumi *et al.*, 2010b; Anon, 2011b). Of the total water available, 98% is already allocated (Water Wise, 2012). The agricultural industry is responsible for the use of 74% of the available rainwater in South Africa and 59% of the total economic use is taken up by irrigation (Bennie & Hensley, 2001; Van Heerden *et al.*, 2008). The remaining 26% partially returns to the sea and is used for household, industrial and other purposes (Bennie & Hensley, 2001). A large portion of the South African population does not have access to a consistent source of potable water, and this serves as an indicator of water shortages (DWAF, 2004).

Climate, population growth and the nature of the economy are all factors that need to be considered regarding the future water requirements of South Africa (DWAF, 2004). Some areas are more vulnerable to climate change and this has an impact on the distribution and availability of water resources (DWAF, 2004). Tendencies in population growth point towards an increased amount of South African citizens moving to urban settlements (DWAF, 2004). This creates a greater demand for water in these specific areas. As part of the National Water Resource Strategy (NWRS) the future water requirements and the management thereof have been further subdivided into specific regions to make provision for the mentioned trend (DWAF, 2004). Integrated water resource management, as described in the NWRS, aims to gradually improve the management of water sources (Haigh *et al.*, 2010).

The Department of Water Affairs categorises water use into six broad sections namely rural, urban, the mining sector, industry, electricity generation and irrigation (DWAF, 2004). The last mentioned is responsible for 62% of the total water used and therefore shows potential for the usage of recycled wastewater (DWAF, 2004). Water generated from water treatment works makes up 14% of the total water yield and is currently used to generate drinking water, but could also be used for non-drinking purposes (Adewumi *et al.*, 2010b).

Due to water shortages and the allocation of the greater part of South Africa's water resources to different sectors, water usage regulations are bound to become stricter and therefore industries will be forced to decrease water usage. The food and beverage industry has shown to be the greatest consumer of water and the cost of freshwater worldwide is rising steadily (Mavrov & Bélières, 2000). It is in this light that the recycling and reuse of water, and the development and sustainability of such practices should be regarded as highly important in the minds of individuals in the food and beverage industry.

Millions of litres of water are consumed by the food and beverage industry on a daily basis (CIAA, 2002). Fermentation processes require considerably greater volumes of water than soft drink production, for example (UNIDO, 2012). The brewery industry is said

to use 9 000 – 14 500 litres of water per kilogram of product (UNIDO, 2012). In this industry whisky production uses much larger water volumes (54 500 – 72 600 litres per kilogram of product) (UNIDO, 2012). Keeping the looming water crisis in mind such statistics are reason enough to consider the reuse of wastewater resulting from such distillation production processes.

B. THE DISTILLERY INDUSTRY

Cereals are the most common substrate for fermentation purposes producing products such as beer, sake and spirits (Hammes *et al.*, 2005). In South Africa the recent economic downturn has affected consumer spending and the sales of spirits was therefore also negatively impacted (Euromonitor, 2010). A 3% decline in spirit sales has been reported in South Africa (Euromonitor, 2010). The only alcoholic beverage that did experience an increase in sales was that of beer (Euromonitor, 2010). A strong shift in sales to more economic spirit brands was observed (Euromonitor, 2010). The value of whisky increased by 5% in 2010, even though a volume decline of 3% took place during the same time period (Euromonitor, 2010).

The manufacturing of alcohol containing products discussed above requires large volumes of water for the production process (Lucas *et al.*, 2009). As most is not present in the final product the greater water fraction needs to be removed as wastewater. An average distillery is said to produce up to 500 000 litres of spent wash per day (Dahiya & Vimal, 1984). This water originates from the production steps in the manufacturing process, including washing of presses, rinsing of fermentation tanks, bottles and other equipment used, and, liquid residues from wash and spirit stills (Lucas *et al.*, 2009).

Whisky is produced through a five-step process starting with the malting of the grain (Goodwin *et al.*, 2001; Hammes *et al.*, 2005). During this process the grain is steeped (soaked in water and germinated) which allows the starch to be converted to fermentable sugars (Goodwin *et al.*, 2001). Drying occurs next which is followed by mashing leaving the grain in a “grist” form (Goodwin *et al.*, 2001; Hammes *et al.*, 2005). Thereafter three batches of warm water are added (63° - 95°C), each batch being warmer than the previous batch (Anon., 2011b). Fermentation then follows whereby the sugars are converted to alcohol through the action of yeast. Distillation then occurs and the alcohol content is increased from 6 - 8% to 65 - 75% (Whitby, 1992). After this step the spirit enters maturation which lasts between three and 25 years (Goodwin *et al.*, 2001).

The distillery process can produce between eight and 15 litres of wastewater per litre of alcohol (Maiorella *et al.*, 1983; Sekar & Murthy, 1998), but the pollution load varies according to the type of distillery involved (Sheenan & Greenfield, 1980). Wastewater originating from grain distilleries contains large quantities of organic compounds, such as proteins and lipids, and has a high chemical oxygen demand (COD) (Table 1).

Land application of wastewater has become a common practise as it does contain nutrients which could be beneficial to soil (Kaushik *et al.*, 2005). It is also directly discharged into river systems or grass fields damaging the soil (Gao *et al.*, 2007). Although this water could increase the soil fertility, the dissolved oxygen in the soil and water could become exhausted and the soil alkalinity could be reduced causing damage to crops and ecosystems (Kaushik *et al.*, 2005; Gao *et al.*, 2007).

Besides the potential damage of ecosystems, raw wastewater is not suitable for reuse as there are certain legal requirements that need to be met (Table 1). Prior treatment is therefore required to make it suitable for reuse in areas such as irrigation on land or washing of presses in the production area. As legal requirements will become more stringent as water becomes scarcer, distilleries will need to improve treatment and reuse strategies. Different treatment options can be considered and the final choice thereof will be largely dependent on the characteristics of the wastewater and the economic viability thereof.

C. TREATMENT OPTIONS

Various options are available for the treatment of distillery wastewater. Such treatments can be grouped as physical, chemical and physico-chemical and biological methods. Factors contributing to the choice of treatment, or combination of treatments include costs, treatment efficiency, wastewater composition (high, medium, or low strength wastewater), end use of wastewater and regulatory limitations (Republic of South Africa, 2004; Gray 2010). The treatment options are mostly applied as pre-treatments as this increases the biodegradability thereof (Carlsson *et al.*, 2012).

Physical treatment

Sedimentation

This treatment method has been successfully applied in various industries, but not as a sole treatment for distillery wastewater (Sheenan & Greenfield, 1980). It has however been incorporated into several treatment processes (Satyawali & Balakrishnan, 2008).

Table 1 Properties of GDWW and required properties when irrigating on land in South Africa (Republic of South Africa, 2004)

Parameter	Literature values	Required when irrigating on land		
	Untreated GDWW	50 m ³ .d ⁻¹	500 m ³ d ⁻¹	2000 m ³ d ⁻¹
Chemical oxygen demand (mg.L ⁻¹)	10 938 – 62 000 ^{a, c, d, e}	<5000	<400	≤75
Fats, oils and grease (mg.L ⁻¹)	1 978 – 2 324 ^d	n.a.	n.a.	2.5
Total suspended solids (mg.L ⁻¹)	232 – 9 600 ^{a - c, e}	n.a.	n.a.	25
pH	3.4–3.8 ^{b, d, e}	6.0 - 9.0	6.0 - 9.0	5.5 - 9.5
Faecal coliforms (per 100 mL)	n.a.	n.a.	<100 000	≤100 000
Sodium adsorption ratio	n.a.	5	5	n.a.

^a Tokuda *et al.*, 1999

^b Akunna & Clark, 2000

^c Laubscher *et al.*, 2001

^d Gie, 2007

^e Mallick *et al.*, 2010

n.a. = not available

The principle of this treatment is based on the difference in density between the suspended solids and remainder of the liquid which provides a means of unwanted solid removal (Seneviratne, 2007). It is usually applied for the removal of sand and dirt (Seneviratne, 2007). For colloids which have a lower specific gravity, settling tanks are used. Settling tanks implement the formation of a vortex which aids in the settling of suspended solids (Seneviratne, 2007). A disadvantage of this method is the long settling time (1 – 4h) (Seneviratne, 2007). To aid in the settling of solids, coagulants and flocculants are often used, and although this does increase the efficiency of the sedimentation process, this treatment is not successful when applied as sole treatment (Sheenan & Greenfield, 1980).

Filtration

Filtration involves the passing of a liquid media through a fine media filter (sand or gravel or membranes) to mainly reduce the suspended solid content (Gray, 2010). Different types of filters and membranes have been applied as a pre-treatment for alcohol distillery wastewater (Chang *et al.*, 1994; Chang *et al.*, 2001; Nataraj *et al.*, 2006, Boucherie *et al.*, 2010). These filters include ceramic, nano filters and granular activated carbon (GAC) filters (Chang *et al.*, 1994; Nataraj *et al.*, 2006, Boucherie *et al.* 2010). Several authors have reported the success of various filter systems for the treatment of distillery wastewater. Reductions in COD of 50% was observed by Chang *et al.* (1994), 99% by Nataraj *et al.* (2006) (nanofiltration in conjunction with reverse osmosis) and a TSS removal of almost 100% by both mentioned authors.

Filtration systems have shown to have a noticeable effect on secondary treatments. Filters that have been applied in wastewater treatment when a large amount of lipids, and phenolic compounds are present, include sand and activated carbon filtration (Sabbah *et al.*, 2004). Sabbah *et al.* (2004) concluded that the filtration pre-treatments made a positive contribution to the subsequent biological treatment method.

The use of a specific filter system depends greatly on the characteristics of the wastewater which is to be treated (Nataraj *et al.*, 2006). The extent of COD removal is limited as it is dependent on the size of the compounds in the wastewater and the size of the accompanying filter (Chang *et al.*, 1994; Nataraj *et al.*, 2006). Filter systems are not ideal for use in the distillery industry due to high solids content and the subsequent fouling which results in the frequent cleaning of the filter (Chang *et al.*, 1994).

Dissolved Air Flotation

The principle of this method is similar to that of sedimentation, but it is used in the removal of floatable matter, such as fats, oils and grease (FOG) and other low density solid particles (Seneviratne, 2007). It incorporates air into water which increases the difference in density between the water and removable particles (Seneviratne, 2007). The wastewater is saturated with air under pressure, and is then exposed to atmospheric pressure (Seneviratne, 2007). The expansion induces the formation of microscopic air bubbles and air-and-oil bubbles (30 – 120 µm), which have a lower density than other particles creating a more rapid separation of the water and oil phase, in the case of FOG removal (Hanafy & Nabih, 2007; Seneviratne, 2007).

Minimum solubility of the proteins in wastewater can be achieved via pH manipulation, and this enhances removal efficiency (Hansen & Cheong, 2007). A range of optimal pH values has been reported by different authors (pH 1.5 – 8.0) (Al-Shamrani *et al.*, 2002a; Hanafy & Nabih, 2007; Seneviratne, 2007). Lipid reduction of 91 – 99% is achieved at different pH values, but is mostly obtained in combination with a coagulant or flocculant (Al-Shamrani *et al.*, 2002a; Hanafy & Nabih, 2007). The removal rates for TSS in distillery wastewater ranged between 33% without coagulant and 83% with coagulant. The COD removal was negligible without coagulant and 53% with coagulants (Chrobak & Ryder, 2005). Due to the high organic content of distillery wastewater, dissolved air flotation would not be a satisfactory treatment when used as a sole treatment.

Chemical and physico-chemical treatment

Chemical treatments have been proven to be effective in various studies. Ozone, for example, has been utilised for disinfection processes, removal of toxic compounds and the oxidation of colour pigments (Beltrán *et al.*, 1997; Beltrán *et al.*, 1998; Beltran-Heredia *et al.*, 2000; Watanabe *et al.*, 2010). The use of some of these methods can however be expensive in certain cases and should therefore not replace more economic biological methods (Medley & Stover, 1983; Andreozzi *et al.*, 1999). It is advised that such treatments be applied individually only to wastewaters with a low COD content as it would not be economically viable if high strength wastewater is treated only by means of a chemical method (Andreozzi *et al.*, 1999). Chemical oxidation methods can therefore be combined with biological oxidative methods to lower the COD content to satisfactory levels (Beltrán *et al.*, 1997).

Coagulation and flocculation

Sedimentation with the addition of coagulants and flocculants has been widely used in various types of wastewater treatments. These are usually applied when the suspended particles do not settle within a reasonable time (Al-Shamrani *et al.*, 2002a; Doble & Kumar, 2005; Chaudhari *et al.*, 2007; Seneviratne, 2007; Harif & Adin, 2011). This is due to the outer negative charge of colloidal particles which causes repulsion between the individual particles (Seneviratne, 2007). Conventional coagulants and flocculants include aluminium hydroxide, aluminium sulphate, ferric sulphate and ferric chloride. Other modern coagulants and flocculants include aluminium chloride and poly-aluminium chloride (Al-Shamrani *et al.*, 2002a; Doble & Kumar, 2005; Chaudhari *et al.*, 2007; Seneviratne, 2007; Harif & Adin, 2011). Aluminium and iron polymers are charged positively and thus attract negatively charged colloidal particles (Huang & Shiu, 1996; Seneviratne, 2007). The addition of coagulants positively influences the zeta potential (which gives an indication of the electrostatic potential near the surface of the particle) of the colloidal particles decreasing the electrostatic repulsion between the particles causing more rapid and efficient aggregation of particles (Al-Shamrani *et al.*, 2002a).

Aluminium chlorohydrate (ACH) and poly-aluminium chloride (PACl) are two very similar polymers which are used in water treatment (Gabelich *et al.*, 2006). The general chemical formula for PACl is $\text{Al}_2(\text{OH})_x\text{Cl}_y$ (where x and y are dependent on the ratio of Al:Cl), and that of ACH is $\text{Al}_2(\text{OH})_5\text{Cl}$ (Al:Cl ratio of 2:1) (Gabelich *et al.*, 2006). The Al:Cl ratio influences the basicity, or the measure of alkalinity scavenging, which will take place in the water system upon addition of the coagulant (Gabelich *et al.*, 2006; Tzoupanos & Zouboulis, 2008). ACH is a high basicity PACl polymer which means that it will not bring about major changes in alkalinity or pH (Pernitsky, 2003; Tzoupanos & Zouboulis, 2008). For the purpose of this discussion, and to avoid monotonous explanations, high basicity PACl, as found in literature, will therefore be referred to as ACH. The addition of an aluminium (Al) coagulant to wastewater results in the presence of various Al-species (Pernitsky & Edzwald, 2006). The basicity of coagulants influences the prevalence of polymeric Al-species, and it has been shown that a higher basicity would result in a greater abundance in polymeric species (Bottero *et al.*, 1980; Pernitsky & Edzwald, 2006). Besides the coagulant dosage, the presence of such Al-species greatly influences the effectiveness of the flocculant (Pernitsky & Edzwald, 2006). The presence of specific species depends on the pH of the water system (Pernitsky & Edzwald, 2006). Aluminium species include, Al^{3+} , $\text{Al}(\text{OH})^{2+}$, $\text{Al}(\text{OH})_2^{1+}$, Al_{13}^{7+} , $\text{Al}(\text{OH})_4^{1-}$, increasing in prevalence with a rising pH (Pernitsky & Edzwald, 2006). From a pH of 3.0 – 4.0 a larger amount of Al^{3+} ions

are present compared to Al_{13}^{7+} (Pernitsky & Edzwald, 2006). This decreases with an increase in pH and the speciation shifts from pH 7.0 upwards, resulting in an increase in Al_{13}^{7+} (Pernitsky & Edzwald, 2006). Between a pH of 6.0 and 7.0 Al_{13}^{7+} is most soluble and therefore present in its highest concentration (Pernitsky & Edzwald, 2006). Particles in water generally carry a negative charge from pH 6.0 (Pernitsky & Edzwald, 2006; Kosmulski, 2012). As the pH rises the ionisation of carboxyl groups and functional groups of phenolic compounds takes place, which increases the negative charge of natural organic matter (NOM) (Pernitsky & Edzwald, 2006). The affinity of NOM for the positively charged Al-species therefore increases upon an increase in pH (Gregor *et al.*, 1997; Pernitsky, 2003). In the pH range of 6.0 – 7.0 most of the Al-species are charged positively, and therefore these conditions are considered to be favourable for coagulation (Pernitsky & Edzwald, 2006).

Thus, the properties contributing to the efficiency of a coagulant include pH, the nature of natural organic matter, interfacial properties of the system, the properties of the coagulant used, temperature and dosage (Al-Shamrani *et al.*, 2002b; Matilainen *et al.*, 2002; Pernitsky & Edzwald, 2003; Fitzpatrick *et al.*, 2004; Cao *et al.*, 2011). The mentioned factors influence the size and strength of the flocs formed and this determines the degree of solids removal (Fitzpatrick *et al.*, 2004). Authors have observed COD reductions of 29 – 96% and colour reductions of 28 – 94% for molasses distillery wastewater (Chaudhari *et al.*, 2007; Fan *et al.*, 2011).

Electrocoagulation has also been successfully applied in distillery wastewater treatment. This process involves the use of electrodes to bring about coagulation by electrolytic oxidation of an anode (Prasad *et al.*, 2008). The undesirable compounds that are to be removed are allowed to react with ions of an opposite charge and are then removed via coagulation or precipitation (Prasad *et al.*, 2008). COD reductions of 72% were achieved and colour reductions ranged from 80 – 90% for molasses distillery wastewater (Prasad, *et al.*, 2008; Krishna *et al.*, 2010). Such a treatment is effective as a pre-treatment for distillery wastewater and could also be considered as a sole treatment for certain types of distillery wastewaters (Krishna *et al.*, 2010).

Advanced oxidation processes

Advanced oxidation processes (AOPs) are defined as processes that produce sufficient quantities of hydroxyl radicals ($\bullet OH$ s) to induce water purification (Andreozzi *et al.*, 1999). Examples of such processes include the use of ozone in an environment with a pH above seven and combinations of reagents, including peroxone (O_3 and H_2O_2), Fenton's process

(H₂O₂ and iron salts), irradiation (O₃ and UV-radiation) and H₂O₂ and UV-radiation (Hoigné & Bader, 1976; Glaze *et al.*, 1987; Wolfe *et al.*, 1989a; Hager, 1990; Beltran de Heredia *et al.*, 2005; Apollo *et al.*, 2013). The produced •OHs possess high oxidation potential, are non-selective, and thus degrades various compounds (Hoigné & Bader, 1976, Andreozzi *et al.*, 1999; Gottschalk *et al.*, 2000).

These treatments increase the biodegradability of recalcitrant organic compounds making the consequent biological treatments more efficient (Gilbert, 1987). Such processes are applied to remove impurities and toxic compounds from water (Hoigné & Bader, 1976; Lima *et al.*, 2006). Wastewaters originating from the olive oil, winery and fruit processing industries have all been treated successfully with such processes (Andreozzi *et al.*, 1998; Sigge *et al.*, 2005; Lucas *et al.*, 2009).

Biological treatment

Biological treatments all rely on microbes for the degradation of organic matter. The microbial activity of these microbes depends on an acceptable environment and the strict control thereof of utmost importance. These parameters include essential nutrients, the absence or presence of oxygen, moisture, pH, redox potential, retention times, temperature, toxic elements and the organic content of the substrate the organisms feed on (Nel *et al.*, 1985; Hansen & Cheong, 2007).

Trickling filters

This process involves the spraying or trickling of wastewater over a rough carrier medium such as gravel, plastic or stone (Hansen & Cheong, 2007; Simate *et al.*, 2011). The material is covered in microorganisms which oxidise the organic compounds in the wastewater (Hansen & Cheong, 2007; Simate *et al.*, 2011). Trickling filters are generally used in combination with other treatments and dilution of wastewater is required as this method does not allow satisfactory treatment of wastewater at full strength (Travieso *et al.*, 2006; Onodera *et al.*, 2013). These filters do not require extensive maintenance and can absorb shock-loads and shutdowns (Oleszkiewicz, 1981). Travieso *et al.* (2006) observed a COD reduction of 54% when using the trickling filter in conjunction with other treatments for the treatment of cane sugar distillery wastewater. This disqualifies this treatment method as suitable for use as a sole treatment of high strength GDWW.

Rotating biological contactors (RBC)

This system has been applied in several sectors including industrial, dairy and food industries (Hansford *et al.*, 1978). The process involves the rotation of discs in sequence,

containing a biofilm of microorganisms, partially submerged in the passing wastewater (Hansford *et al.*, 1978; Mba *et al.*, 1999; Nahid *et al.*, 2001). The thin biofilm carries a layer of water with it as it rotates, and the organic material present in the water is thus removed by the micro-organisms (Hansford *et al.*, 1978). Advantages include simple construction and low energy consumption (Hansford *et al.*, 1978). The limitations of this aerobic process are the negative implications of sludge production and high capital and maintenance costs (Hansford *et al.*, 1978; Sheenan & Greenfield, 1980).

The RBC showed a decrease in COD of approximately 43% when treating wine distillery wastewater (WDWW) and 94.9% when treating bakers' yeast wastewater (Nahid *et al.*, 2001; Malandra *et al.*, 2003). A decrease in biological oxygen demand (BOD) of 82 - 96% has also been observed upon the treatment of GDWW (Thomas & Koehrsen, 1974). The organic loads of the mentioned cases do, however, not compare to that which anaerobic digestion systems are able to process (Malandra *et al.*, 2003). Furthermore, the efficiency of RBC's depend on retention times, making more efficient anaerobic systems a better choice for the treatment of distillery wastewater.

Enzymatic treatment

Various commercially available enzymes have been used to treat distillery wastewaters (Mallick *et al.*, 2010). Papain, beta-glucanase, protease and lyticase have specifically been applied to digest yeast cells in distillery wash (Mallick *et al.*, 2010). Furthermore, lipase has been applied and identified as a potentially industrial applicable enzyme. It has proven to bring about reductions in lipid content in various types of wastewater including palm oil mill effluent and dairy effluent. Lipid reductions of up to 99% were achieved with the use of *Pseudomonas aeruginosa* (Prasad & Manjunath, 2011). Sangave & Pandit (2006) applied cellulase with the aim of degrading complex polluting molecules to more biodegradable compounds in a study which used enzymatic as a pre-treatment to aerobic biological oxidation (Sangave & Pandit, 2006). The treatment with such enzymes has shown both no and higher COD reduction in the subsequent aerobic and anaerobic treatment stage, respectively (Sangave & Pandit, 2006; Mallick *et al.*, 2010). Sangave & Pandit (2006) concluded that the compounds were more biodegradable as a two-fold increase in degradation rate was observed in the subsequent aerobic treatment step.

A large range of microbial enzymes, have been used to degrade colour compounds in various types of distillery wastewaters (Rajasundari & Murugesan, 2011). These enzymes include manganese peroxidases and other versatile peroxidases (Kuan & Tien, 1993; Galkin *et al.*, 1998; Heinfling *et al.*, 1998; Martínez, 2002).

Aerobic treatment

Aerobic lagoons have been successful in treating WDWW with a COD content of up to 18 000 mg.L⁻¹ (Montalvo *et al.*, 2010). During this study a maximum removal efficiency of 91% was achieved which proves that it can be applied in distillery wastewaters (Montalvo *et al.*, 2010). Aerobic techniques have also been applied as a post-treatment, following anaerobic treatment, to WDWW and increased the COD removal from 88.7% to 96.5% (Musee *et al.*, 2007). It is however not seen as an economic treatment as it has high operational costs regarding energy supply, high oxygen demand and requires sludge disposal (Uzal *et al.*, 2003). The high acidity levels of distillery wastes has also been identified as a problem during this treatment method and additional chemical treatments would therefore be required (Uzal *et al.*, 2003).

Anaerobic treatment

The anaerobic digestion of agricultural and distillery waste involves the degradation of complex organic compounds to small amounts of sludge and a potentially large volume of biogas (Gavrilescu, 2002). A microbial consortium, consisting of several bacterial groups, is responsible for the degradation process (Anderson *et al.*, 2003). There are various different types of anaerobic digestion configurations which include anaerobic filters and anaerobic contact processes which consist of various reactor configurations including the UASB (upflow anaerobic sludge bed), expanded granular sludge bed, baffled reactor, fluidised bed reactor and the hybrid flow reactor (Rinzema *et al.*, 1993; Gerardi, 2003).

This type of treatment method poses various advantages when compared to other methods. These include the production of valuable biogas, low operational costs and little or no sludge production (Gavrilescu, 2002). Other treatments can reduce certain compounds successfully, but the COD and BOD levels of the effluent need to be taken into consideration as high BOD and COD can be reduced successfully by biological methods to comply with statutory limits (Krishna *et al.*, 2010).

Lipid degrading bacteria

Difficulties have been encountered when applying anaerobic digestion if the lipid content of wastewater exceeds 50%. This is due to a low, insufficient mixing rate that is achieved in the anaerobic reactor, as a result of low gas production (Rinzema *et al.*, 1993). Degradation of fatty acids by certain microbes has been proven successful as certain bacteria possess lipolytic activity (Jaeger *et al.*, 1994). The lipase, which is produced by a range of microbial strains, hydrolyses the ester linkages in the long chain fatty acids (LCFAs) converting these to shorter chain fatty acids, glycerol or alcohol which is then

broken down to carbon dioxide, hydrogen and acetate during the process of anaerobic digestion (Jaeger *et al.*, 1994; Ghandi, 1997). This increases the biodegradability of the fatty acids as these can easily be broken down further in the anaerobic reactor (Seghezzeo *et al.*, 1998). The degradation of fatty acids increases the reuse possibilities of the treated wastewater, and the biogas is an excellent source of renewable energy (Hobson & Feilden, 1982; Weiland, 2003; Van Schoor, 2005).

Lipids have frequently been found to be part of various wastewaters and the treatment of such has therefore been investigated (Dharmstihiti & Kuhasuntisuk, 1998; Cavaleiro *et al.*, 2008). This gave rise to the application of lipid degrading bacteria for the treatment of high fat containing wastewaters (Sousa *et al.*, 2009; Cavaleiro *et al.*, 2010). Several bacterial strains were used to degrade fatty acids, including, *Syntrophomonas sapovorans*, *Syntrophomonas curvata* and *Syntrophomonas zehnderi* (Sousa *et al.*, 2009; Cavaleiro *et al.*, 2010).

This conversion of fatty acids to biogas has been considered to be difficult and has induced major challenges in past research (Cavaleiro *et al.*, 2008). However, the optimisation of biogas production could be greatly beneficial. Due to the high biogas potential of lipids, its degradation is of environmental and economic concern (Alves *et al.*, 2009). Kim *et al.* (2004) noted that the theoretic amount of methane which can be produced from one gram of oleic acid is equal to 1.01 L compared to 0.37 L of methane produced from one gram of glucose. This clearly shows that lipids hold a much greater biogas potential making it a beneficial choice as a substrate for anaerobic digestion.

Degradation of lipids in wastewater

Studies regarding the degradation of lipids in lipid rich wastewater have shown that the degradation of LCFAs is possible to a certain extent. This could hold great economic value due to the high methane potential of lipids (Palatsi *et al.*, 2010). Insights regarding the degradation of these fatty acids indicate certain mechanisms of degradation - lipolysis - the hydrolytic process whereby an ester or a lipid is split into a fatty acid component and alcohol or glycerol, respectively (Eq. 1 & 2) (Hoq *et al.*, 1985; Gandhi, 1997).



Many microorganisms produce lipase and these are specifically used for enzyme production on industrial scale. This included *Bacillus licheniformis*, *Bacillus subtilis* and *Pseudomonas fluorescens* (Brooksbank *et al.*, 2007). Combinations of microorganisms in tested commercial supplements produce specific lipases and are therefore able to degrade certain LCFAs and in turn produce specific acids (Brooksbank *et al.*, 2007).

Sun & Wakeham (1994) found that unsaturated fatty acids are broken down more easily than saturated equivalents. Strains that utilise unsaturated fatty acids were identified as *Syntrophomonas sapovorans*, *Syntrophomonas curvata* and *Syntrophomonas zehnderi* (Sousa *et al.*, 2009; Cavaleiro *et al.*, 2010). Hydrolysis of LCFAs also takes place during anaerobic digestion. The main method of degradation is β -oxidation, but the initial steps of the degradation process for unsaturated fatty acids are not clear (Sousa *et al.*, 2009).

Inhibition effect of long chain fatty acids

Adsorption of LCFAs to the surface of bio-granules has been observed to interfere with the transfer of nutrients and metabolites to and from the bio-granule in anaerobic reactors (Pereira *et al.*, 2005). Upon exposure to inhibitory levels of LCFAs it has been observed that the biomass adapts in two ways, namely, population adaption and physiological adaption (Palatsi *et al.*, 2010). Population adaption involves an increase in cell numbers of species which are more efficient LCFA degraders, while physiological adaption involves the phenotypic adaption of the existing microorganisms (Palatsi *et al.*, 2010). Phenotypic adaption could involve an increase in growth rate as observed by Palatsi *et al.* (2010). The degradation of LCFAs is beneficial as the accumulation thereof can lead to the adsorption of a lipid layer onto biomass (Hwu *et al.*, 1998; Gie, 2007). Laubscher *et al.* (2001) and Gie (2007) found that a yellow lipid layer formed around the granules in the UASB system. LCFAs, which mainly consist of stearic, oleic and palmitic acids adsorb to the surface of the biomass present in the reactor and thus prevent the transfer of nutrients and metabolites (including biogas) to and from the granule (Hwu *et al.*, 1998; Pereira *et al.*, 2005). This results in a decreased granule activity, causes sludge disintegration, creates floatation of the granules and results in the washout thereof (Hwu *et al.*, 1998; Amaral *et al.*, 2004; Palatsi *et al.*, 2009).

Hydrogenotrophic methanogens have been observed as more resistant to LCFA inhibition (Palatsi *et al.*, 2010). The methanogenic group is more sensitive than acidogens (Koster & Cramer, 1987; Pereira *et al.*, 2003). Inhibition was found to correlate with the concentration of the fatty acids present (Koster & Cramer, 1987). Furthermore it has been found that certain fatty acids exert greater toxicity than others (Lalman & Bagley, 2002). A

study investigating the effect of LCFAs (linoleic, oleic and stearic acid) on the degradation of glucose, butyrate and hydrogen, by making use of an unacclimated culture found that linoleic acid (18:2) was more toxic than oleic acid (18:1) which was more toxic than stearic acid (18:0) (Lalman & Bagley, 2002). The toxic effect has been shown to be reversible once all the LCFAs associated with the biomass have been converted to methane (Pereira *et al.*, 2005). To resolve the toxic effect and biomass washout biomass could be recycled which then reduces the toxic effect of LCFAs on the methanogens resulting in a greater methane yield and thus increased reactor performance (Pereira *et al.*, 2001).

Bioaugmentation and acclimatisation

The incorporation of LCFA degrading bacteria into the consortium present in anaerobic reactors could greatly influence the degradation of LCFAs. These include assistance during start-up of the reactor as degradation of LCFAs, present in FOG rich effluent, is generally seen as the rate limiting step in the degradation process (Masse *et al.*, 2003; Cavaleiro *et al.*, 2010). Furthermore the recovery from LCFA inhibition, as previously mentioned, can be improved (Cavaleiro *et al.*, 2010). The incorporation of *Syntrophomonas zehnderi* was shown to increase the methane yield to approximately 89% compared to 54% (over 30 days of incubation) with the focus on oleic acid degradation (Cavaleiro *et al.*, 2010). This showed that the degradation of oleic acid was more rapid in the case of the bioaugmented sludge (Cavaleiro *et al.*, 2010).

Acclimatisation of sludge to lipid containing wastewater is another technique that has shown to improve the anaerobic degradation of high lipid content wastewater. This strategy has shown promising results in terms of bioreactor start-up (Conçalves *et al.*, 2011). It has been proven to increase the tolerance of methanogens specifically to palmitic and oleic acid toxicity and thereby a higher degree of biodegradability and shorter lag phase before the initiation of biogas production, was achieved (Cavaleiro *et al.*, 2008; Gonçalves *et al.*, 2011). The organic load and the concentration of phenolic compounds are factors which should be carefully evaluated and considered as this could have a further limiting effect on this approach (Conçalves *et al.*, 2011).

D. OZONE

Ozone characteristics

Ozone (O₃) is a powerful oxidant that is soluble in water and is promptly available as it can be produced on-site (Beltran-Heredia *et al.*, 2000). It is mainly produced by means of

corona discharge (passing of O₂ gas through two electrodes at a high voltage difference), but can also be produced by irradiating O₂ with UV-light (USEPA, 1999). It is a pale blue gas at room temperature and has a strong odour which is detectable at 0.02 - 0.05 ppm (per volume) (Wojtowicz, 2005; USEPA, 1999). It possesses an oxidation potential of 2.07 V which is greater than that of chlorine (1.36 V) but lower than fluorine (3.06 V) (Legrini *et al.*, 1993; Gusel-Seydim *et al.*, 2004). As O₃ is electrophilic in nature it is selective in oxidation and attacks electron dense areas such as double bonds (Andreozzi *et al.*, 1998; Von Gunten, 2003; McMurry, 2004). Ozone is soluble in water although this does depend on the type and concentration of organic content, pH and temperature of the water (USEPA, 1999).

Mechanism of oxidation

There are two main routes of oxidation that ozone follows. In acidic conditions (low hydroxide ion (OH⁻) concentration), the reaction with OH⁻ is not the only initiation step and the direct reaction of O₃ with compounds present in water is favoured (Hoigné & Bader, 1976). It is postulated that the direct reaction also takes place more often in wastewater (Buffle *et al.*, 2006). Ozone is electrophilic and highly selective towards double bonds and therefore the unsaturated fatty acids are largely affected (Hoigné & Bader, 1983; Andreozzi *et al.*, 1998). It also attacks specific functional groups such as -OH, -CH₃, -OCH₃, and atoms that are charged negatively (N, O, P, S) (Mantzavinos & Psillakis, 2004). Fig. 1 illustrates the changes an unsaturated compound would undergo (Criegee, 1975).

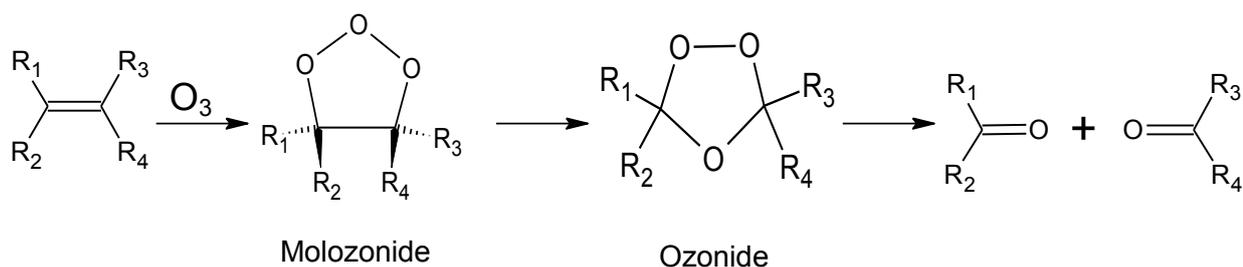
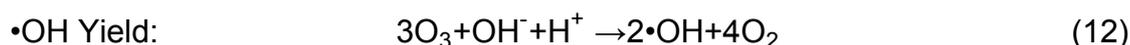
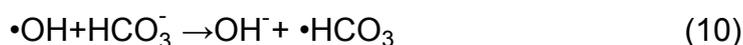
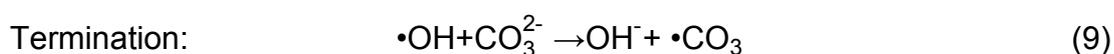
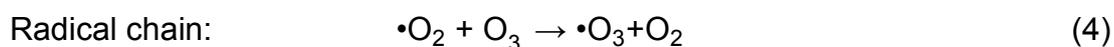
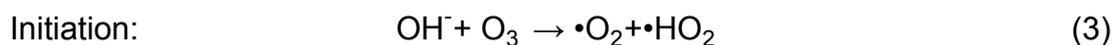


Figure 1 Ozonolysis of a compound containing a double bond (Criegee, 1975; McMurray, 2004)

The second mechanism of oxidation takes place in higher pH environments (higher OH⁻ concentrations) when spontaneous decomposition of O₃ is accelerated resulting in the production of hydroxyl radicals (•OH) (Sotelo *et al.*, 1987; Hsu *et al.*, 2004). The formation of •OH is more beneficial due to the stronger oxidation ability of •OH compared to

molecular O₃ (Hsu *et al.*, 2004). This leads to a chain reaction whereby O₃ decomposes. The rate of this chain reaction is influenced by several factors such as pH, temperature and the solutes present in the system (Hoigné & Bader, 1976). Hydroxide ions promote the decomposition of O₃ as these ions act as initiators in the chain reaction (Eq. 3) and leads to the production of free radicals ($\bullet\text{O}_2$ and $\bullet\text{OH}$) (Eq. 4 - 8) which act as chain carriers for further decomposition reactions (Hoigné & Bader, 1976; Forni *et al.*, 1982; Bühler *et al.*, 1984; Staehelin *et al.*, 1984). Termination of the radical chain takes place in the presence of organic compounds which react with the $\bullet\text{OH}$ to form secondary radicals which do not produce $\bullet\text{HO}_2$ or $\bullet\text{O}_2$ (Eq. 9 & 10) (Staehelin & Hoigné, 1982; Gottschalk *et al.*, 2000). The organic compounds therefore act as scavengers and disrupt the radical chain. Another possibility of radical chain disruption could occur upon the reaction of two radicals with one another (Eq. 11) (Bühler *et al.*, 1984).



Efficiency factors

Various factors contribute to the efficiency of O₃ as the reactivity thereof is strongly dependant on the nature of the surrounding environment. Therefore the conditions of the

treated system need to be analysed and considered before predicting the outcome and effect of ozonation.

The pH of wastewater determines the decomposition of ozone and it has been shown to have a significant effect on the decomposition of organic matter and phenolic compounds in the presence of O_3 (Benitez *et al.*, 1997a; Lucas *et al.*, 2009). The decomposition of the above mentioned two compounds takes place at the highest rates at alkaline conditions (pH 9 and 10, respectively). This creates an optimum environment for the reaction between the compounds and O_3 (Benitez *et al.*, 1997a; Gottschalk, 2000; Lucas *et al.*, 2009). In alkaline conditions the radical species interact with organic compounds, providing an increased reaction time and oxidation efficiency (Eq. 4 – 8) (Hoigné & Bader, 1976; Lucas *et al.*, 2009).

Temperature plays a core role in the reaction rate and solubility of O_3 . An increase in temperature causes an increase in the decomposition rate of O_3 which increases its rate of reaction with compounds in its environment (Sehested *et al.*, 1991). The conversion rate of phenolic compounds has also been reported to increase when temperature increases as the solubility of O_3 decreases with temperature even though the reaction rates increase (Benitez *et al.*, 1997a; Gottschalk, 2000).

Beltrán *et al.*, (1999) observed that the reaction rate of O_3 is influenced by the COD content of the influent. While the reaction rates are faster at higher COD contents, the rate declines at a lower COD concentration (irrespective of direct ozonation or reaction with $\bullet OH$) (Beltrán *et al.*, 1999). They also found that compounds of high molecular weight react at low ozone dosages and those of lower molecular weight react at higher ozone dosages. It was therefore concluded that the amount of COD consumed per gram of ozone ($gCOD.g^{-1}O_3^{-1}$) decreases with an increase in ozone dosage. By controlling all of the mentioned factors the decomposition of ozone can be controlled and this will ultimately determine the oxidative capacity of thereof.

Advantages

The fact that ozone can be generated at the location it is to be used, makes it convenient as this requires no moving or storing of chemicals (USEPA, 1999). Ozone is very effective against a large range of micro-organisms, and its biocidal effect is not affected by pH (Masschelein, 1992). It needs a shorter contact time than chlorine and thus it is an excellent disinfectant (USEPA, 1999; Khadre *et al.*, 2001). It alters membrane permeability and directly attacks intracellular enzymes, nucleic material and viral capsids leading to the destruction of micro-organisms (Khadre *et al.*, 2001). The use of ozone does not bring about the formation of halogenated-substituted compounds, and as ozone

spontaneously decomposes back to O₂, no harmful by-products are normally produced (USEPA, 1999). Ozone can degrade a large variety of compounds making it a versatile oxidant which can be used in many areas (Singer & Zilli, 1975; Wojtowicz, 2005; Van Geluwe *et al.*, 2011). Compounds that are oxidised range from cyanides and sulphides, manganese and iron to colour, odour and taste compounds (Masschelein, 1992).

Ozone has also been shown to improve coagulation pre-treatments (Grasso & Weber, 1988; Asaithambi *et al.*, 2012). It modifies compounds present in wastewater in such a way that destabilisation and adsorption can occur, resulting in polymerisation of natural organic matter (Reckhow *et al.*, 1986). It can also increase the carboxylic acid content resulting in increased binding to metals present in wastewater (Grasso & Weber, 1988; Bose & Reckhow, 2007). This can furthermore increase the binding of organic matter to coagulants, such as alum (Bose & Reckhow, 2007).

Another mechanism of ozone assisted coagulation is the disruption of complexed metal ions, such as Fe³⁺, which consequently allows it to act as a coagulant (Reckhow *et al.*, 1986). Such changes in organic matter have resulted in a decreased coagulant dosage requirement (Orta de Velásquez *et al.*, 1998).

In terms of biodegradability, low O₃ dosages (200 - 400 mg O₃.L⁻¹) have been shown to be more effective compared to higher dosages in WDW treatment (Sigge *et al.*, 2007). This attribute increases the economic viability of such treatment. Ozone has shown to decrease the COD content slightly, but the greater advantage is seen in the increase in biodegradability of wastewaters (Sigge *et al.*, 2007). Ozone treatment has therefore been regarded as a viable treatment method due to its efficiency (while considering specific operational conditions) and the increase in biodegradability as well as increased methane production it brings about during anaerobic digestion (Beltrán *et al.*, 1999; Beltran-Heredia *et al.*, 2000; Sigge *et al.*, 2007; Ak *et al.*, 2013).

Disadvantages

Although ozone is effective to degrade various problematic compounds, it is not an effective agent for the reduction of COD, which is the one of the main parameters regarding environmental pollution and regulatory structures of wastewaters (Wang *et al.*, 1989; Van Schoor, 2005). Unlike chlorine, ozone does not have a residual disinfection effect. Ozone is more expensive than chlorination or UV treatment (Bitton, 2005). Thus the use ozone as sole treatment to decrease the COD content to acceptable levels has proven to be fairly costly and therefore it is not economically viable from a practical point of view (Wang *et al.*, 1989; Hsu *et al.*, 2004; Watanabe *et al.*, 2010). Excessive ozonation

therefore needs to be avoided and the process requires the optimisation of several operational conditions (Hsu *et al.*, 2004).

Lucas *et al.* (2009) reported that O₃ targets simple organic compounds rather than the identified problematic phenolic compounds. The nature of the targeted compound therefore is a factor that should be considered as this would influence the ozonation time, dosage requirements and ultimately the financial cost involved.

Ozone degrades unsaturated organic compounds, such as plastics and rubbers, which limits the materials that are able to be used during ozonation (Huntink, 2003). It is also corrosive to metals and requires equipment of high quality, such as stainless steel. It is also toxic to human mucous membranes and thus causes throat dryness and irritation of respiratory tract (Wojtowicz, 2005). Persons suffering from asthma or other lung conditions are especially susceptible to such experiences (Wojtowicz, 2005). Exposure to ozone could also induce eye irritation (Wojtowicz, 2005). The correct safety procedures should therefore be used when O₃ is to be used in an area where humans work or move.

The formation of by-products, such as hypobromous acid and bromate, has been observed in the presence of bromides or other halogen atoms, although this normally only occurs when these compounds are present in high concentrations (Reckhow & Singer, 2011). Bromate has been said to be potentially carcinogenic and therefore a high concentration in water which could be used for human consumption would be problematic (Magazinovic *et al.*, 2004). Other by-products include aldehydes, acids and aldo- and ketoacids, hydroxylated aromatic compounds and nitrosamines (USEPA, 1999; Wojtowicz, 2005). Ozone characteristically produces larger quantities of simple aldehydes and ketoacids than other major disinfectants due to the Criegee mechanism (Fig. 2) (Reckhow & Singer, 2011). Some of the mentioned products could be toxic to humans, but tests have proven that ozonated water brings about marginally less mutagenicity than water treated with chlorine (Wojtowicz, 2005).

Industrial application

In 1906, a water treatment plant in Nice, France, became the first water treatment facility to make use of ozone to oxidise a range of compounds and the success of the use of ozone has been proven in many studies (Bitton, 2005). The use thereof include the disinfection of water in terms of pathogen reduction, removal of colour and taste compounds, sanitation of equipment and the removal of pollutants such as cyanide and manganese (Rice *et al.*, 1981; Rice, 1997; Beltrán *et al.*, 1999; Gusel-Seydim *et al.*, 2004; Lima *et al.*, 2006; Peter & Von Gunten, 2007; Watanabe *et al.*, 2010).

A large volume of high strength wastewater is produced by the distillery industry. The oxidation capacity of ozone has been investigated by a number of authors for such wastewaters. It has been found that ozonation of wastewater decreased the COD of substituted aromatic compounds and wine distillery wastewater (WDWW) as well as olive mill effluent (OME) (37 - 86%) (Gilbert, 1987; Beltrán *et al.*, 1997; Sigge *et al.*, 2007; Lucas *et al.*, 2009). The degradation of lipids was also found in distillery wastewaters (74%) (Gie, 2007; Sigge *et al.*, 2008). Research regarding OME showed that ozonation decreased the lipid content by 50% with a noticeable effect on unsaturated fatty acid degradation (Andreozzi *et al.*, 1998). Ozone cleaves the double bonds present in unsaturated compounds producing less complex ones which are more biodegradable (Benitez *et al.*, 1997b). Upon direct attack of ozone, carboxylic acids are produced, and these will not be oxidised further by molecular ozone (Mantzavinos & Psillakis, 2004). This selective action is specifically advantageous when long chain unsaturated fatty acids are present, as biodegradability has been observed to decrease with an increase in fatty acid chain length (Loehr & Roth, 1986).

The use of ozone as a sole treatment in GDWW treatment does not bring about a large reduction in COD (6%) (Gie, 2007). Ozone is suggested however as a pre-treatment for wastewater containing recalcitrant compounds as it converts these compounds to ones that are more biodegradable (Hsu *et al.*, 2004; Vimala Ebenezer *et al.*, 2013). Wastewaters containing various types of compounds, such as those of toxic phenolic nature, have been shown to have an increased biodegradability because of the effect of pre-ozonation (Hsu *et al.*, 2004; Bougrier *et al.*, 2006). The increased biodegradability has further been found to have a positive effect during secondary biological treatments such as anaerobic digestion. An increased yield of methane after an increased COD reduction during anaerobic digestion has been observed when making use of ozone as a pre-treatment (Martín *et al.* 2002; Sigge *et al.*, 2005 & 2007). An increased bio-granule activity, indicating an increased biodegradability of the wastewater, was also observed (Sigge *et al.*, 2007). Sigge *et al.* (2005) found the final COD content (55 mg.L⁻¹) of cannery wastewater to be well below the South African limit (75 mg.L⁻¹) for irrigation on land, qualifying this wastewater for reuse (Republic of South Africa, 1998).

E. PEROXONE

In wastewater treatment certain recalcitrant compounds are present that are not degraded with ozone (Paillard *et al.*, 1988). The need for the degradation and optimal reduction of

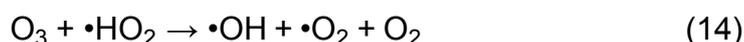
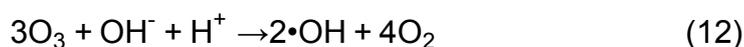
such compounds has thus led to the exploration of ways to improve the ozonation process by means of the addition of reagents or radiation. Peroxone is such an example and can be defined as the combination of ozone with hydrogen peroxide (Wolfe *et al.*, 1989a). This process produces hydroxyl radicals ($\bullet\text{OH}$ s) which react with, and oxidise, organic compounds (Wolfe *et al.*, 1989a; Beltran de Heredia *et al.*, 2005). Hydrogen peroxide (H_2O_2) has been used in combination with ozone, as well as UV-radiation and ultrasound (Wolfe *et al.*, 1989a; Gogate & Pandit, 2004). These combinations lead to the generation of $\bullet\text{OH}$ s which are responsible for the degradation of organic and toxic compounds (Gottschalk *et al.*, 2000; Gusel-Seydim *et al.*, 2004). As $\bullet\text{OH}$ are strong oxidants and react with recalcitrant compounds non-selectively, the oxidative capacity of a water treatment system is increased upon the production of such compounds (Duguet *et al.*, 1985).

Peroxone characteristics

The addition of H_2O_2 causes the decomposition of ozone to increase and subsequently produces more $\bullet\text{OH}$ s than the ozonation process alone (USEPA, 1999). The $\bullet\text{OH}$ s that are produced during the peroxone process are highly potent oxidising agents that are short-lived (Legrini *et al.*, 1993). The $\bullet\text{OH}$ has an oxidation potential of 2.80 V which is greater than that of O_3 (2.07 V) (Legrini *et al.*, 1993). This results in an increased oxidation efficiency in a water system because of the conversion of O_3 molecules to $\bullet\text{OH}$ s (Duguet *et al.*, 1985). Furthermore, the transfer of O_3 from the gas to liquid phase is improved due to increased ozone reaction rates resulting from its increased decomposition upon H_2O_2 addition (Duguet *et al.*, 1985).

Mechanism of oxidation

Hydrogen peroxide only reacts in its deprotonated form (Eq. 13) (Hoigné *et al.*, 1985). During the peroxone process the decomposition of O_3 is highly favoured due to the presence of $\bullet\text{HO}_2$ (Eq. 14) (Staehelin & Hoigné, 1982). When the concentration of H_2O_2 exceeds 10^{-7} M, reaction 14 takes place more rapidly than the reaction of OH^- with O_3 (Eq. 3).



From reaction 12 one can see that the ozonation process requires 1.5 molecules of O₃ to produce one •OH molecule. In the presence of H₂O₂ (Eq. 14) it is clear that one molecule of O₃ produces one •OH molecule, proving the increased oxidative capacity of the peroxone process.

Efficiency factors

In wastewater with a high carbonate ion or bicarbonate content, which is a measure of the alkalinity and buffering capacity of the system, the effect of •OH can be restricted due to the scavenging action of these ions (Eq. 15) (Staehelin & Hoigné, 1982; Buxton & Elliot, 1986; Gerardi, 2003).



Hydrogen peroxide can be formed as part of an interaction with natural organic matter during ozonation of wastewater (Lucas *et al.*, 2009; Pocostales *et al.*, 2010). This formation positively influences the ozonation process as this leads to the formation of •OHs (Legrini *et al.*, 1993).

It should be noted that pH plays an important role in the action of peroxone with higher concentrations of •OH being formed at higher pH values (Glaze *et al.*, 1987). At higher pH conditions an excess amount of OH⁻ is responsible for creating conditions at which the decomposition of O₃ takes place. This results in the production of •OH which competes with the direct oxidation reactions taking place with O₃, resulting in more rapid oxidation (Beltrán *et al.*, 1999).

H₂O₂:O₃ ratio

Authors have reported a wide range of optimal H₂O₂:O₃ ratios that have been used while making use of peroxone (Table 2). This ratio is important due to the promoting and scavenging properties of H₂O₂, the subsequent formation of •HO₂ and thus the optimal oxidation that can result from this ratio (Van Geluwe *et al.*, 2011). Other radicals in the system might interfere with the action of •OH and it might be inhibited by radical-radical coupling (Glaze *et al.*, 1987). The formation of much less potent •HO₂ leads to the scavenging of •OH (Eq. 16 – 18) (Buxton *et al.*, 1988).

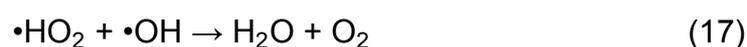


Table 2 Ratios of H₂O₂:O₃ used for the degradation of various wastewaters

H ₂ O ₂ :O ₃ ratio	Wastewater or compound investigated	Authors
0.5	Tetrachloroethylene and perchloroethylene	Glaze & Kang, 1988
0.33	Tetrachloroethylene	Glaze & Kang, 1989
0.5 – 0.6	Tetrachloroethylene and perchloroethylene	Karimi <i>et al.</i> , 1997
0.5	Oxalic acid and organochlorinated products	Paillard <i>et al.</i> , 1988
<0.5	Urban sewage water	Rosal <i>et al.</i> , 2009
0.3; 0.5; 0.8	Indicator organisms in river water	Wolfe <i>et al.</i> , 1989a
0.2	<i>Giardia muris</i> cysts	Wolfe <i>et al.</i> , 1989b



The H₂O₂:O₃ ratio to be used in a specific wastewater is dependent on the alkalinity and composition of the water (Acero & von Gunten, 2001). This ratio cannot be predicted and should therefore be determined experimentally (Acero & von Gunten, 2001; Kosaka *et al.*, 2001; Rosal *et al.*, 2009). At a specific H₂O₂ concentration (which depends on the wastewater system), no or little increase in the performance of the oxidation system is achieved (Acero & von Gunten, 2001). Acero & von Gunten (2001) confirmed that the reaction rates increase above this H₂O₂ concentration, but no increased degradation of the targeted compound is observed. If a certain number of direct O₃ reactions is required this should especially be taken into account due to the decomposition of O₃ being encouraged upon H₂O₂ addition (Eq. 12 – 14) (Acero & von Gunten, 2001). Furthermore, as is seen in Eq. 16, H₂O₂ could lead to the scavenging of the •OH. This occurs when the concentration of H₂O₂ is too great in a system, once again emphasising the importance of the optimal ratio of H₂O₂:O₃ (Kosaka *et al.*, 2001).

Advantages

The production of •OH is advantageous as it leads to the decomposition of unwanted substances ranging from fatty acids to pesticides (Hoigné & Bader, 1976; Beltrán *et al.*, 1999; Catalkaya & Cargi, 2009). The process is especially beneficial when compounds that are not reactive to ozone are present in the treated wastewater due to the non-selectivity of •OHs (Gottschalk *et al.*, 2000; Zhou & Smith, 2001). Beltrán *et al.* (1997) confirmed that the addition of H₂O₂ to O₃ increased the COD reduction when compared to O₃ alone. However, no noticeable effect was observed in distillery wastewaters (Beltrán *et*

al., 1997). Due to the higher oxidation potential of the •OHs this process is much more effective when compared to the sole use of O₃ (Hoigné & Bader, 1976; USEPA, 1999). Furthermore, peroxone has proven to be less expensive than O₃ as lower dosages of O₃ are necessary to obtain the same levels of removed compounds as the rate of decomposition of organic compounds is increased (Aieta *et al.*, 1988; Beltrán *et al.*, 1997).

Disadvantages

Wert *et al.* (2007) found that the combination of H₂O₂ and O₃ produced a greater concentration of assimilable organic carbon (5 – 52%), aldehydes (31 – 47%), and carboxylic acids (12 – 43%) when compared to O₃ alone. This indicates that the formation of by-products is dependent on •OH exposure (Wert *et al.*, 2007). Another disadvantage of the process is that the radical intermediates that are produced could interfere with substances that react with •OHs and this could in turn decrease the positive effect of the process (USEPA, 1999). Peroxone also has been found to be less effective at degradation of iron and manganese compared to O₃ (USEPA, 1999).

F. ANAEROBIC DIGESTION

Background

Anaerobic digestion (AD) has been used since the 1800's and is one of the most common processes used in wastewater treatment (Seghezzi *et al.*, 1998). During this process a consortium of microorganisms degrade organic compounds in the absence of oxygen (Gujer & Zehnder, 1983). Various configuration systems have been applied, including low-rate, one stage high-rate and two-stage high-rate digesters (De Lemos Chernicharo, 2007). High rate systems can be divided into fixed bacterial growth systems and dispersed bacterial growth systems (Gerardi, 2003; De Lemos Chernicharo, 2007). The Upflow Anaerobic Sludge Blanket (UASB) reactor and the expanded granular sludge bed (EGSB) are high-rate examples (Gerardi, 2003; De Lemos Chernicharo, 2007). The anaerobic digestion process lowers the high organic content of wastewaters and offers several advantages when compared to other biological treatments such as aerobic treatment (Gavrilescu, 2002; Gerardi, 2003). The wide application of AD and high success rate has made it the chosen system in the food and beverage industry (Driessen & Yspeert, 1999; Sigge, 2005; Schmidt *et al.*, 2013).

Studies have shown that the UASB system decreases negative constituents (including fats, oils and grease (FOG), biological oxygen demand (BOD) and COD of

GDWW to more acceptable levels (Laubscher *et al.*, 2001; Pant & Adholeya, 2007). It has been reported that the COD and BOD content are reduced by more than 90% and 80 - 90%, respectively (Laubscher *et al.*, 2001; Pant & Adholeya, 2007). The biochemical energy retrieval amounts to approximately 85 - 90% which adds to the renewable energy potential (Pant & Adholeya, 2007).

Applications in food and beverage industry

Several industries have successfully applied this method of wastewater treatment. The agricultural and food and beverage industry have made use of anaerobic digestion in various sectors including wastewaters from cane molasses based vinasse, the dairy industry, vegetable processing and the brewery industry (Driessen *et al.*, 1994; Driessen & Yspeert, 1999; Sigge, 2005; Yamada *et al.*, 2013). The alcohol distillery industry has also made use of the UASB system and other anaerobic digestion systems (granular-bed anaerobic baffled reactor) (Akunna & Clark, 2000; Akarsubasi *et al.*, 2006). It has been reported that a reduction in COD content ranging from 55 to 92% was achieved when treating whisky distillery wastewater and alcohol distillery wastewater, by the respective authors (Akunna & Clark, 2000; Akarsubasi *et al.*, 2006).

Upflow Anaerobic Sludge Blanket (UASB) Reactor

The UASB system (Fig. 2) was developed by Lettinga and his co-workers in the Netherlands and was applied on a larger scale in the 1970's (Lettinga *et al.*, 1980; De Lemos Chernicharo, 2007). This system has been widely applied, particularly in the agricultural industry, and has proven to be the most popular anaerobic digestion configuration applied for the treatment of wastewaters with a high organic load (Driessen & Yspeert, 1999; Laubscher *et al.*, 2001; Pant & Adholeya, 2007). It is widely applied to treat high strength wastewaters and has been reported to handle organic loading rates (OLRs) between 10 and 25 kg.COD.m⁻³.d⁻¹ (Gao *et al.*, 2007). The UASB system is the anaerobic digestion system that produces the largest amount of methane (CH₄) gas (Gerardi, 2003). This gas can be collected during the digestion process and is of great economic value as it can be reused as a renewable energy source (Gerardi, 2003).

Working principle and process design

The fixed film system operates by the upflow of wastewater through the sludge-'bed' created by small granules formed by the microbial consortium (Gerardi, 2003; De Lemos Chernicharo, 2007). The UASB reactor consists of four main parts: the granular sludge bed (A), the fluid area (B), the gas and solid separator (C) and the settlement compartment

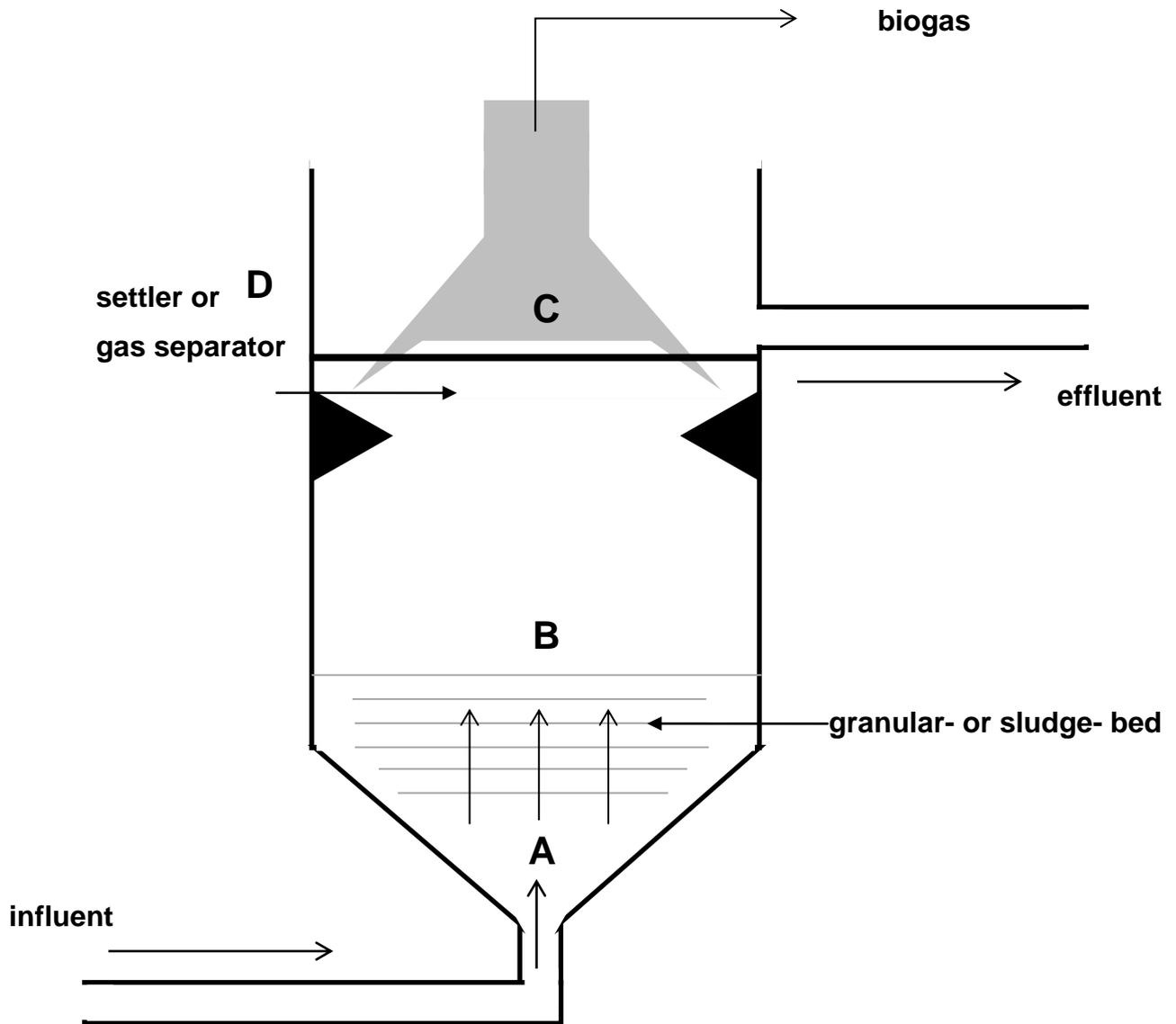


Figure 2 Schematic diagram of Upflow Anaerobic Sludge Bed reactor (Schmidt & Ahring, 1996; Lettinga *et al.*, 1980).

(D) (Fig. 2) (Schmidt & Ahring, 1996). The wastewater enters at the bottom of the reactor, flows upwards, and makes contact with the granular sludge bed. Upon contact the organic compounds present in the wastewater are broken down resulting in the production of biogas which is separated from the liquid phase at the top section of the reactor (C) (Lettinga *et al.*, 1980; Schmidt & Ahring, 1996).

The microbial population present is highly active and degrades organic matter present in the wastewater. This is promoted by agitation of the system through the upflow of the wastewater and the gas bubbles produced by the biomass, as this offers the required contact between biomass and wastewater (De Man *et al.*, 1988; De Lemos Chernicharo, 2007).

The formation of granules improves the maintenance of the biomass inside the reactor as it has good settling abilities (Ross, 1984; Yan & Tay, 1997). The sizes of the granules vary between slightly less than 0.5 and 7.0 mm, and depend on the type of wastewater being treated (Ross, 1984; Fang *et al.*, 1994; Britz *et al.*, 2000; De Lemos Chernicharo, 2007; Li *et al.*, 2008). Granules consist of a densely packed exterior and a loosely packed interior (Fang *et al.*, 1994). Layers can be formed within the granule (Fig. 3) as noted by Fang *et al.*, (1994) while Li *et al.* (2008) observed holes inside the granules and also found specific aggregates of micro-organisms. It has been determined that these micro-organisms consist of facultative acidogenic, acetogenic and methanogenic bacteria (Ross, 1984; Fang *et al.*, 1994). Methanogens have been proven to be the main granule-forming group of bacteria, as these are hydrophobic, and the acidogens have shown to be non-granule forming, and hydrophilic (Akunna & Clark, 2000). The facultative anaerobic genera, which are present on the outside of the granule, remove all the oxygen present and in such a way the methanogenic bacteria present, which are on

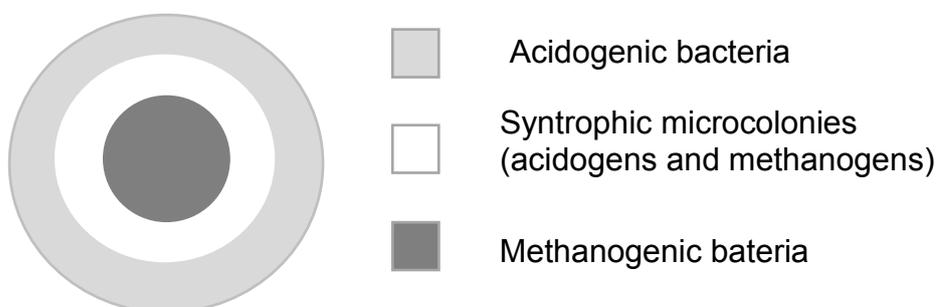


Figure 3 Layered structure of granule treating soluble carbohydrates (adapted from Fang *et al.*, 1994)

the inside of the granule, are protected, being strict anaerobes (Anderson *et al.*, 2003). For the digestion process to take place successfully it is essential that a dense sludge (consisting of the granules) is developed by the biomass (Fang *et al.*, 1994; Yan & Tay, 1997; Seghezzi *et al.*, 1998). When this is achieved the UASB reactor can treat wastewater at high OLRs as this dense sludge would then provide the necessary wastewater-biomass contact, the retention of solids is also encouraged and the wash-out of biomass is prevented (Ross, 1984; Seghezzi *et al.*, 1998; Akunna & Clark, 2000).

Anaerobic digestion microbiology

Gujer & Zehnder (1983) divided the degradation of organic compounds into six steps while others discuss the digestion process in three steps (Gerardi, 2003). Currently a four step process has been identified as there are four main groups of micro-organisms involved resulting in separate mechanisms of degradation of organic compounds (Fig. 4). In this process the COD of the substrate is lowered as the organic compounds are broken down leading to effluent being less harmful to the environment and which also has a larger potential to be reused (Republic of South Africa, 1998; Gerardi, 2003).

Hydrolysis

During the first step of the degradation process the complex biopolymers present in the wastewater influent, consisting of protein, carbohydrates and lipids, are broken down to more simple compounds (Gujer & Zehnder, 1983; Anderson *et al.*, 2003; Gerardi, 2003). These compounds are insoluble in their polymeric form and become soluble when hydrolysed to amino acids, simple sugars and fatty acids (Gujer & Zehnder, 1983; Gerardi, 2003). Some alcohols are also produced (Gujer & Zehnder, 1983). The microbes responsible for this step are anaerobes and facultative anaerobes (Gerardi, 2003). These include genera such as *Bacillus*, *Clostridium*, *Micrococcus*, *Peptococcus* and *Vibrio* (Anderson *et al.*, 2003). These genera of micro-organisms produce extracellular enzymes that are specific towards certain substrates and include protease, lipase, amylase and pectinase, amongst others (Anderson *et al.*, 2003).

Acidogenesis

The second digestion step involves the fermentation of the simple sugars and amino acids to form intermediate fatty acids such as valerate, isovalerate, butyrate and propionate (Gujer & Zehnder, 1983; Anderson *et al.*, 2003). Some of the genera involved in this conversion include *Bacteroides*, *Desulfobacter*, *Lactobacillus* *Propionibacterium* and

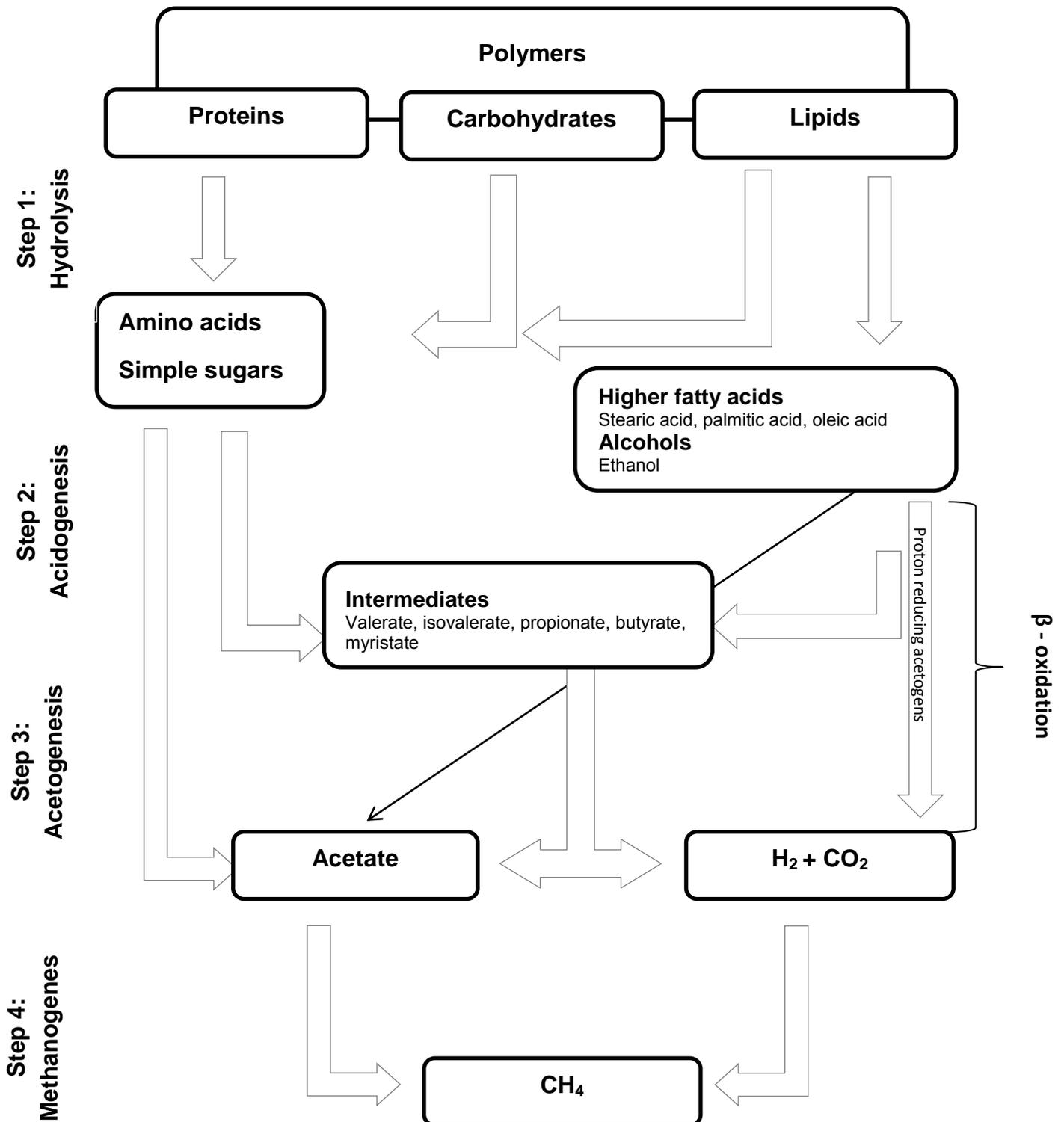


Figure 4 Degradation of organic compounds in the anaerobic digestion process (Gujer & Zehnder, 1983; Anderson *et al.*, 2003)

Streptococcus (Anderson *et al.*, 2003). The population of microbes varies with environmental changes and the composition of the influent (Britz *et al.*, 1994).

Acetogenesis

The main products formed during this step are carbon dioxide, hydrogen and acetate (Anderson *et al.*, 2003). The first group of micro-organisms involved, the obligate hydrogen producing acetogens (OHPA) (proton-reducing acetogens) produce the mentioned compounds from fatty acid intermediates (Fig. 5), alcohols and other higher fatty acids (Anderson *et al.*, 2003; Gerardi, 2003; Palatsi *et al.*, 2010). This group of micro-organisms is mainly involved in the degradation of fatty acids and LCFAs resulting from hydrolysis (Anderson *et al.*, 2003). The degradation of fatty acids takes place via β -oxidation which is known as the rate limiting step in the degradation process (Palatsi *et al.*, 2010). Species involved in this include *Syntrophomonas wolfei* (oxidation of butyrate) and *Syntrophomonas wolinii* (oxidation of propionate) as well as the family *Clostridiaceae* (Anderson *et al.*, 2003; Palatsi *et al.*, 2010). These organisms are only capable of growing in an environment which is low in one of their metabolites, namely H_2 , and therefore a syntrophy with hydrogenotrophic methanogens is necessary, which is generally achieved (Anderson *et al.*, 2003; Palatsi *et al.*, 2010). The group of acetogens responsible for the catalysis of the formation of hydrogen, carbon dioxide and acetate are strictly anaerobic homoacetogens (Anderson *et al.*, 2003). The genera *Acetobacterium*, *Acetoanaerobium*, and *Clostridium* are included in this group of organisms (Anderson *et al.*, 2003).

Methanogenesis

Methanogens are classified as *Archae*, in a different kingdom from hydrolytic- and acid-forming bacteria (Anderson *et al.*, 2003). These organisms differ from the other groups on a structural and metabolic level (Bitton, 2005). The presence and activity of methanogens in the reactor is of great importance as it contributes towards the digester performance, methane yield and also the reuse quality of the effluent (Gao *et al.*, 2007).

These organisms are strictly anaerobic and are responsible for the formation of methane from the compounds produced by the acetogens (CO_2 , H_2 , and acetate) (Anderson *et al.*, 2003). Methane can also be produced from some organic acids other than acetate (Gerardi, 2003). As mentioned, OHPAs need a low concentration of H_2 and this is obtained through the syntrophic relationship between acetogens and methanogens (Anderson *et al.*, 2003). The presence of methanogens is therefore of utmost importance as the degradation of organic compounds would not take place in their absence due to an

accumulation of the produced compounds (hydrogen, organic acids, short-chain fatty acids) which inhibits further degradation of organic compounds (Hwu *et al.*, 1998; Anderson *et al.*, 2003). This is due to a decrease in pH (resulting from the abundance in intermediate organic acids), and, because of the sensitivity of methanogens to a low pH this will provide an environment in which these organisms are unable to function (optimum pH of 6.8 - 7.2) (Anderson *et al.*, 2003).

Two groups of methanogens exist, namely acetoclastic methanogens, which utilise acetate, and hydrogenotrophic methanogens, which utilise hydrogen (Anderson *et al.*, 2003; Palatsi *et al.*, 2010). Acetoclastic methanogens include the genera *Methanosaeta* and *Methanosarcina* (Anderson *et al.*, 2003). Acetate is the primary precursor of methane as 70% of the total amount of CH₄ is produced from acetate (Gujehr & Zehnder, 1983; Gerardi, 2003). The hydrogen utilising group reduces CO₂, formate, methanol and methylamines using the hydrogen produced in the previous steps of the digestion process. This group includes *Methanobacterium* species (Zehnder & Wuhrmann, 1977; Anderson *et al.*, 2003).

It is important for the acidogenic and acetogenic groups to maintain the same rates of conversion as that of the methanogenic groups as this protects the functioning of the methanogenic group (Gerardi, 2003). If this is achieved favourable pH and buffer capacity will be maintained in the reactor (Gerardi, 2003).

Biogas production and the economic value thereof

In recent years interest in biofuels has increased due to ever increasing fuel prices (Ziganshin *et al.*, 2011). As part of sustainable development this practice is encouraged due to potentially large economic benefits (Del Real Olvera & Lopez-Lopez, 2012). Distilleries, along with various other industries, such as dairy plants and slaughter houses, have shown potential for the production of biogas (Rao *et al.*, 2010; Schmidt *et al.*, 2013). The use of biogas as a renewable energy source is an attractive one as anaerobic digestion requires a smaller capital investment when compared to other renewable energy sources (for example solar power) (Rao *et al.*, 2010). The produced energy can be used for the generation of heat and electricity for use in the production process (Kerroum *et al.*, 2012; Van Haandel *et al.*, 2013). This would result in lower operational costs and decrease the environmental impact (Franchetti, 2013). When the produced biogas is used as a fuel in the production process, instead of coal and other fuels, the initial cost of the anaerobic digester can be recovered within a period of two to three years (Doble & Kumar, 2005). Doble and Kumar (2005) calculated the volume of biogas produced by a molasses

distillery to be approximately $560 \times 10^6 \text{ m}^3$ per year resulting in 830 Gigawatt hour per annum. As noted by Kim *et al.* (2004), lipids could be more beneficial in terms of CH_4 production per gram of substrate, because of the fact that the theoretic amount of methane which can be produced from one gram of oleic acid is equal to 1.01 L compared to 0.37 L of biogas produced from one gram of glucose. As GDWW contains considerable amounts of lipids when using maize as the substrate for spirit production, it could be seen as an attractive option for energy generation (Gie, 2007).

Operational efficiency factors

Various factors influence the stability, and subsequently, the efficiency of an anaerobic reactor (Gerardi, 2003). These factors influence the composition and amount of species present in the reactor and the methanogenic activity, which determines the successful degradation of organic matter present in the influent, and the maintenance of the correct conditions in the reactor (Ince *et al.*, 1995). Therefore the following factors should be carefully monitored and controlled.

Temperature

A wide range of temperatures ($0^\circ - 97^\circ\text{C}$) has been used in anaerobic digestion and have all led to the production of methane (Bitton, 2005). However, methane forming bacteria are sensitive to temperature, and are inhibited between 40° and 50°C and usually operate between two temperature ranges ($30^\circ - 35^\circ\text{C}$ and $50^\circ - 60^\circ\text{C}$) (Gerardi, 2003).

Wastewater treatment usually makes use of the mesophilic temperature range as degradation takes place at optimal rates in this temperature range ($25^\circ - 40^\circ\text{C}$) (Bitton, 2005). The maintenance of temperatures, especially during winter months, is essential to avoid decreased reactor efficiency (De Lemos Chernicharo, 2007; Mahmoud, 2008). Low temperatures could further negatively affect the ratio of volatile fatty acids to alkalinity due to the decreased activity of methanogens (Gerardi, 2003; Bitton, 2005). While methane production is inhibited at lower temperatures, volatile fatty acid production continues, and this could therefore result in reactor failure (Gerardi, 2003). Although studies have proven the success of low temperatures, this would not be a viable option for distillery wastewaters due to the often low OLR observed during these studies, and low CH_4 concentration present in the biogas. Low CH_4 concentration in the biogas restricts the reuse thereof and therefore a mesophilic temperature range is recommended (Lettinga *et al.*, 1999; Alvarez & Lidén, 2009).

pH

The pH in the reactor is of high importance due to the sensitivity of specifically the methanogens present (Anderson *et al.*, 2003; Gerardi, 2003). It is affected by a number of compounds of which volatile fatty acids and CO₂ form a part (Gerardi, 2003; De Lemos Chernicharo, 2007). Therefore it is important that fermentation (production of acids) does not take place at a quicker rate than degradation of acetate (De Lemos Chernicharo, 2007). Acid forming bacteria will be active above pH 5.0, but methanogens will only be active above a pH of 6.2 (Zoetemeyer *et al.*, 1982; Gerardi, 2003). However, the most favourable pH is between 7.0 - 7.2 as this creates optimal conditions for the activity of methanogenic bacteria. Operational pH values have been reported to be lower than these optimum values and can differ according to treated substrate (Zoetemeyer *et al.*, 1982; Britz *et al.*, 2000; Gerardi, 2003).

Alkalinity

Alkalinity can be defined as the capacity of wastewater to buffer or neutralise acids (Droste, 1997). It gives an indication of the buffer capacity, and therefore the stability of the reactor (Gerardi, 2003). The buffer capacity is mostly because of salts of weak acids, such as carbonates (Anderson *et al.*, 2003). The alkalinity of established anaerobic reactors should range between 2000 - 3000 mg.L⁻¹ expressed as CaCO₃ (Anderson *et al.*, 2003). Poor reactor performance can be linked directly to low bicarbonate content and an adequate buffer capacity is therefore required to protect the anaerobic system against sudden pH fluctuations (Droste, 1997; Paulo *et al.*, 2003). This could be added to the reactor, but it is desired that the microbial consortium generates alkalinity as compounds are broken down and others are produced (Gerardi, 2003; Paulo *et al.*, 2003). Upon the production of volatile fatty acids, the pH of the reactor decreases. Methanogens use the acids that are produced and produce alkalinity. As a syntrophic relationship is established between the acid-forming and methanogenic micro-organisms, the acids formed should be broken down by the methanogens to achieve a slightly alkaline medium adding to the alkalinity of the reactor and ensuring greater reactor stability (Gerardi, 2003). Thus a rise in pH would take place before it stabilises (Gerardi, 2003; De Lemos Chernicharo, 2007).

The production of ammonia results from the decomposition of proteins and amino acids and forms ammonium carbonate. This compound in turn reacts with volatile acids resulting in the formation of volatile acid salts which acts as a buffer in the digestion system (Gerardi, 2003). Acids are produced during the degradation of the substrates of a bioreactor (Gujer & Zehnder, 1983). As a result alkalinity lowers as it is used to

compensate for acid production (Gerardi, 2003). Alkalinity is restored once methanogens successfully degrade acids to methane (Gerardi, 2003). Certain wastewaters, such as ones containing carbohydrates, lipids and volatile fatty acids, would not generate alkalinity naturally and therefore the only way to maintain suitable pH and alkalinity is by external addition of compounds that adapt pH and enhance buffer capacity (Anderson & Yang, 1992).

Biogas composition

Biogas consists of a variety of gases (CH₄, CO₂, CO, H₂, NH₃, N₂, N₂O) of which methane (CH₄) and carbon dioxide (CO₂) are most important and make up the greatest part thereof (Gerardi, 2003). Biogas typically consists of 65 - 75% methane and carbon dioxide makes up the greater part of the remainder (Droste, 1997). A change in biogas composition, specifically an increase in CO₂ concentration (above 30%), increases the acid concentration in the biomass and causes the pH to drop below seven (Gerardi, 2003). This could indicate the instability of the reactor as increased CO₂ concentration points to the decreased activity of methanogens (De Lemos Chernicharo, 2007). Low gas yield can also be associated with a high C:N ratio (Chen *et al.*, 2008).

The generation of methane and the reuse thereof, is currently of great interest due to the demand in the use of renewable energy sources, especially in Europe (Kratat *et al.*, 2011; Ziganshin *et al.*, 2011). Methane holds potential as a renewable energy source which can be reused in the production plant for generation of heat and electricity (Ziganshin *et al.*, 2011).

Upflow velocity and hydraulic retention time

The approximate time the liquid remains in the reactor is referred to as the hydraulic retention time (HRT) (De Lemos Chernicharo, 2007). This determines the rate of gas-production from volatile solids and needs to be adequate as to allow the sufficient conversion of these components. Treatment of this substrate takes place as contact occurs with the biomass (Gavrilescu, 2002; Gerardi, 2003). As retention time differs, the population levels of different groups of bacteria in the anaerobic reactor also differ (Zhang & Noike, 1994).

Flow rate (Q), upflow velocity (V_{up}) and HRT are closely related to one another (Eq. 21 - 22) (Latif *et al.*, 2011).

$$Q = \frac{V}{\text{HRT}} \quad (21)$$

where Q = flow rate of influent ($L \cdot d^{-1}$), V = volume of reactor (L), HRT = hydraulic retention time.

The upflow velocity is of importance because it is responsible for the mixing of the substrate with biomass, but should be maintained at a rate at which no significant biomass washout occurs (De Lemos Chernicharo, 2007; Latif *et al.*, 2011). The velocity (Eq. 22) is dependent on the density of the present sludge and is generally kept between 0.5 and 1.0 $m \cdot h^{-1}$ (De Lemos Chernicharo, 2007; Mahmoud, 2008).

$$V_{up} = \frac{h}{HRT} \quad (22)$$

where V_{up} = upflow velocity of influent ($m \cdot h^{-1}$), h = height of the reactor, HRT = hydraulic retention time (h).

Wastewater characteristics

The microbes in an anaerobic reactor will feed on the proteins, lipids and carbohydrates present in the substrate that is being digested. Methane will then be produced from these polymers, and the success thereof will depend on the control and influence of the characteristics of the wastewater (Fig. 4) (Akarsubasi *et al.*, 2006). The characteristics of distillery wastewater are largely dependent on the substrate it is produced from. It can however be considered as acidic, with a pH ranging between 3 and 4 (Wolmarans & De Villiers, 2002; Gie, 2007; Mohana *et al.*, 2009). It is normally low in phosphates and nitrogen, but high in COD content as seen in Table 1 (Tokuda *et al.*, 1999; Uzal *et al.*, 2003). The recommended ratio for COD:N:P ratio for high strength wastewaters is 350:7:1 and 1000:7:1 for low strength wastewaters (Gerardi, 2003). If these nutrients are absent they should be added to the wastewater in adequate volumes.

Trace elements are extremely important for efficient reactor performance (Goodwin *et al.*, 2001). These micro nutrients include aluminium, boron, calcium, cobalt, nickel, molybdenum, selenium, tungsten and zinc (Nel *et al.*, 1985; Feng *et al.*, 2010). Such addition has proven to increase the performance of anaerobic reactors significantly (Kida & Sonoda, 1993). Specific micro nutrients influence certain microbial groups and can therefore have positive effects in the anaerobic degradation process, and lead to increases in methane production (Feng *et al.*, 2010; Facchin *et al.*, 2013). Micronutrients are especially necessary when treating waste that are deficient in the necessary nutrients. Such sources often include waste obtained from food sources (Zhang & Jahng, 2012; Facchin *et al.*, 2013).

Goodwin & Stuart (1994) successfully treated whisky distillery wastewater at a maximum OLR of $15 \text{ kgCOD}\cdot\text{d}^{-1}\text{m}^{-3}$ and an HRT of 2.1 days. Goodwin *et al.*, (2001) later found an OLR ranging between three and $10 \text{ kgCOD}\cdot\text{d}^{-1}\text{m}^{-3}$ and in the same study a range in HRT of 2.1 – 7.0 days was observed. Gerardi (2003) recommends a HRT of not less than 10 days, although Goodwin & Stuart (1994) found that 2.1 hours was the shortest viable HRT. The HRT needs to allow a period long enough for acceptable degradation of organic content.

The sensitivity of methanogens to pH needs to be taken into consideration to ensure reactor stability and therefore the volatile fatty acid content is of great importance. The optimal volatile fatty acid content is said to be $50 - 500 \text{ mg}\cdot\text{L}^{-1}$ as acetic acid (Gerardi, 2003).

The concentration of lipids or phenolic compounds should be taken into considered as these could induce a toxic effect if present in too great concentrations (Fedorak & Hrudý, 1984). Volatile fatty acids (VFA) content should be monitored during anaerobic digestion as an increase in certain fatty acids could be an indication of reactor upset (Goodwin *et al.*, 2001). This is due to the termination or decrease in the conversion of fatty acids to methane which indicates a decrease in methanogenic activity (Gujer & Zehnder, 1983). Reactor upset would also be seen in other parameters such as pH and alkalinity (Gerardi, 2003).

Advantages

Anaerobic digestion offers several advantages when compared to other biological treatments. UASB reactors are able to treat high strength wastewater providing a treatment option for many industrial wastewaters (Bitton, 2005). This treatment option does not require oxygen supply which contributes to the low maintenance cost (Bitton, 2005). It does not require large spaces, and this contributes to its viability in a large scale situation. It can however also be easily applied to small scale treatments (Gao *et al.*, 2011).

AD does not require a large supply of nutrients and produces biogas which can be reused as an energy source (Beltran de Heredia & Garcia, 2005; Gao *et al.*, 2007; Latif *et al.*, 2011). Moreover, the UASB system (compared to other AD systems) provides the largest possible biogas production during the AD process (Pant & Adholeya, 2007). This energy can be applied to the heating of digesters to ensure a constant and correct temperature.

Depending on the temperature of operation, pathogen reduction could take place. Temperatures of 50° – 60°C induce a good reduction of pathogens (Droste, 1997). The majority of energy entering the system is converted to biogas, which means that there is very little energy left for cell multiplication, resulting in low sludge production (Bitton, 2005). Low sludge production is promoted by the slow growth of anaerobic bacteria (Gavrilescu, 2002).

The UASB system has also been reported to enclose odours produced and is tolerant toward toxic compounds (Gao *et al.*, 2007). This system has also shown good efficiency of organic content removal (32 - 92%) at high OLR (0.3 - 10 kgCOD.m⁻³d⁻¹) even at low temperatures (4 - 11°C) (Lettinga *et al.*, 2001; Gavrilescu, 2002).

Because of the granule formation of the bacteria present in the system, sludge retention is promoted, and biomass washout is prevented (Gavrilescu, 2002). The activity of these granules is retained, even when reactors are not active over long periods of time (Bitton, 2005). Thanks to the enhanced settling properties of the granules formed, a high measure of biomass retention is made possible at a high upflow velocity (Trnovec & Britz, 1998).

Disadvantages

With regard to the UASB version of AD, the formation of granules, which is extremely beneficial regarding the stability of the reactor, is hard to control due to the complex nature thereof (Hulshoff Pol *et al.*, 2004). Problems that have been associated with the UASB reactor system is the loss of these granules due to the relatively low density thereof (Akunna & Clark, 2000). This problem demands appropriate flow distribution to prevent the loss of granules which will ultimately lead to poor effluent quality (Akunna & Clark, 2000). The sourcing of sludge of a good quality is of vital importance as this contributes to the start-up period of the anaerobic digester which can sometimes take up long periods of time (Bitton, 2005; Latif *et al.*, 2011). The long start-up and acclimatisation period required to full loading capacity is a distinct disadvantage of AD for industries. The microbes in the anaerobic system are extremely sensitive to toxicants and pH which requires careful monitoring and control.

The rate of degradation of organic compounds is remarkably lower when anaerobic digestion is compared to aerobic digestion (Bitton, 2005). During anaerobic treatment pathogens are only partially removed and therefore the removal of these would require pre- or post-treatment of the substrate (Latif *et al.*, 2011). The composition of biogas could

also hold potential problems. A high content of hydrogen sulphide causes corrosion and could therefore damage digester equipment (Latif *et al.*, 2011).

G. GENERAL DISCUSSION

The distillery industry typically produces wastewater with a high organic content. Some compounds can be highly bio-recalcitrant and this affects the biodegradation process negatively (Lucas *et al.*, 2009). Combinations of treatments have proven to be the most reasonable manner of treating distillery wastewaters, especially peroxone pre-treatment in combination with biological treatments such as an UASB reactor, making the degradation process more economically feasible (Medley & Stover, 1983; Andreozzi *et al.*, 1998; Andreozzi *et al.*, 1999; Sigge *et al.*, 2005; Sigge *et al.*, 2007).

Ozone has been widely applied in water treatment and has been a successful pre-treatment in the distillery industry in many instances. The powerful oxidation capability thereof makes it a popular choice for the treatment of lipids as it has demonstrated the degradation of problem causing fatty acids.

Lipase producing isolates degrade lipids and therefore show potential to treat waters containing large amounts of lipids. The presence of such isolates has shown to be beneficial during the start-up of the bioreactor and the presence thereof could aid the biodegradation process (Masse *et al.*, 2003; Cavaleiro *et al.*, 2010).

Anaerobic digestion has been widely applied in the treatment of distillery wastewater. It provides numerous advantages and has been found to be successful in the reduction of COD in wastewater. It has however been proven that GDWW cannot be treated in an UASB reactor without preceding treatments to reduce the FOG content as this has caused numerous problems with regards to reactor stability. The adsorption of fatty acids to granules could result in a toxic effect on the biomass and the washout thereof (Rinzema *et al.*, 1994; Laubscher *et al.*, 2001; Pereira *et al.*, 2005).

From this discussion it can be concluded that a combination of treatments is desirable for the most optimal degradation of the compounds in GDWW. As coagulants and ozone have proven to be successful in the treatment of wastewaters, further research regarding the effect of these on the degradation of GDWW has been suggested. It is desirable for these treatments to increase the efficiency of the UASB process as to produce water which has a greater reuse potential.

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Chapter 3

IMPACT OF PH, COAGULANT CONCENTRATION AND OZONE PRE-TREATMENT ON GRAIN DISTILLERY WASTEWATER

Summary

The pH of grain distillery wastewater (GDWW) was adapted, and two different concentrations of coagulant (aluminium chlorohydrate (ACH)) were used to achieve the optimal reductions of chemical oxygen demand (COD), total suspended solids (TSS) and fats, oils and grease (FOG). An increase in pH brought about increased reductions for the three parameters. Optimal reductions for COD ($33.2\% \pm 4.93$), TSS ($91.9\% \pm 1.73$) and FOG ($84.1\% \pm 1.98$) were achieved at a wastewater pH of 7.0 and an ACH concentration of $140 \text{ mg}\cdot\text{L}^{-1}$. Ozone treatment was used to increase the reductions obtained in the first pre-treatment. An increase in ozone dosage brought about an increasing reduction. Maximum reductions were obtained at $100 \text{ mgO}_3\cdot\text{L}^{-1}$ for COD ($3.6\% \pm 4.08$), and at $900 \text{ mgO}_3\cdot\text{L}^{-1}$ for TSS ($27.7\% \pm 5.58$) and FOG ($23.9\% \pm 1.83$). An optimal ozone dosage of $300 \text{ mgO}_3\cdot\text{L}^{-1}$ was established due to economic reasons, findings in literature for toxicity of ozone and the possible degradation of recalcitrant compounds at this dosage. By obtaining a reduction in the mentioned parameters, the combined pre-treatments rendered GDWW to be a more suitable substrate for a subsequent anaerobic treatment.

Introduction

Whisky production generates large volumes of wastewater that is hazardous to the environment, as it is of high strength and acidic nature (pH 3.4 – 3.8) (Akunna & Clark, 2000; Gie, 2007). Since grain distillery wastewater (GDWW) has a high COD, TSS and FOG content, its reclamation would be greatly beneficial to South Africa as it is a water scarce country (Laubscher *et al.*, 2000; Bennie & Hensley, 2001; Gie, 2007; Adewumi *et al.*, 2010).

There are certain regulatory requirements that need to be met for the reuse of high strength wastewater, and thus pre-treatment is necessary (Republic of South Africa, 2004). Furthermore, GDWW cannot be used for direct land application due to the negative impact it would have on soil properties and the ecological damage it could cause (Gao *et al.*, 2007). Such high strength wastewaters are often treated by using an anaerobic digestion option (Akunna & Clark, 2000; Akarsubasi *et al.*, 2006). However, due to the

high lipid and solid content of GDWW, problems have occurred during the anaerobic treatment process (Hwu *et al.*, 1998; Laubscher *et al.*, 2000). Certain long chain fatty acids (LCFAs) are problematic as they attach to the microbial granules found in anaerobic reactors (Pereira *et al.*, 2005). This creates a barrier between the wastewater and the microorganisms in the granule and prevents the transfer of nutrients and metabolites, which ultimately results in granule floatation and washout (Hwu *et al.*, 1998; Laubscher *et al.*, 2000; Amaral *et al.*, 2004; Palatsi *et al.*, 2009).

Pre-treatment is therefore required to remove or alter the organic content of wastewaters to obtain a substrate that is more suitable for treatment by means of anaerobic digestion (Carlsson *et al.*, 2012). Physico-chemical treatments, such as coagulation, are suitable for the removal of solids and lipids present in wastewater (Doble & Kumar, 2005). Poly-aluminium coagulants have been applied successfully in this regard, and the efficiency thereof is often increased upon subsequent manipulation of the pH (Al-Shamrani *et al.*, 2002; Tzoupanos & Zouboulis, 2008). Advantageous properties of such coagulants include little change in the water's pH and alkalinity upon the addition thereof, as well as better removal efficiencies when compared to conventional aluminium coagulants (aluminium sulphate) (Pernitsky, 2003; Sinha *et al.*, 2004). Literature reports the use of poly-aluminium coagulants in water treatment (Huang & Shiu, 1996; Qin *et al.*, 2006). The studies reportedly use exponentially lower dosages of the coagulant, because of lower organic loads in water. These studies also focus on coagulation of specific individual compounds (Huang & Shiu, 1996; Pernitsky & Edzwald, 2006).

Another advanced oxidation treatment that has been shown to be effective is the degradation of lipids by ozone (O_3) (50 - 74% reduction) (Andreozzi *et al.*, 1998; Gie, 2007). Unsaturated compounds (including phenolic compounds, fatty acids, colour and odour compounds) are prone to the attack by O_3 , which is an electrophilic molecule with a high oxidation potential (2.07 V compared to 1.36 V of chlorine) (Hoigné & Bader, 1983; Masschelein, 1992; Legrini *et al.*, 1993). Upon O_3 application, a COD reduction of 6 - 86% was been found in different wastewaters types, including GDWW (Beltrán *et al.*, 1997; Gie, 2007; Sigge *et al.*, 2007). Ozone increases the biodegradability of substrates and is particularly useful in the case of recalcitrant or toxic substrates (Benitez *et al.*, 1999). It has, however, been found to be unsuitable for the complete degradation of organic compounds due to the large dosage requirement, and should therefore not replace biological treatment methods (Medley & Stover, 1983; Andreozzi *et al.*, 1999).

The objective of this study was to determine the optimal pre-treatment combination of aluminium chlorohydrate (ACH) and O_3 for GDWW. Therefore, the first aim

will be to evaluate the effect of pH manipulation on the efficiency of an ACH blend, and to investigate the possibility of lowering the dosage currently applied by a local distillery (two different coagulant concentrations will be used). The second aim will be to determine the additional effect of ozonation on GDWW, specifically the FOG content and to identify an effective dosage concentration. Changes in COD, FOG and TSS will be monitored to quantify the pre-treatment efficiency.

Materials and Methods

Wastewater

GDWW samples were received from a whisky distillery in Wellington, South Africa between October 2011 and April 2012. This wastewater is centrate from a decanter, and had not undergone any prior treatment except for the removal of approximately 57% solids (Green, J., 2012, Process Manager, The James Sedgwick Distillery, Wellington, South Africa. Personal communication, 28 May.). The raw wastewater had an average COD value of 30 089 mg.L⁻¹ (range: 22 140 - 38 285 mg.L⁻¹), an average FOG value of 3 206 mg.L⁻¹ (range: 2 817 - 3 593 mg.L⁻¹) and an average TSS value of 3.77 g.L⁻¹ (range: 2.79 - 4.56 g.L⁻¹). The wastewater was requested from the local distillery as needed during experiments and stored at 4°C prior to use (unless otherwise stated).

Coagulant pre-treatment of GDWW

Coagulation of the GDWW was investigated using a commercially available coagulant (aluminium chlorohydrate (ACH)), Ultrafloc 3800™ (NCP Chlorochem, 2011), which is used by the distillery. A dosage concentration of 140 mg.L⁻¹ was used as recommended by the supplier, as well as a decreased concentration of 100 mg.L⁻¹. The lower concentration was chosen to determine whether a lower concentration could achieve comparable results resulting in a cost saving.

Treatments included a pH raw (unadjusted, ca. pH 3.4 – 3.5 with addition of ACH at two concentrations), pH 5.0, 6.0 and 7.0 (at each pH, ACH was added at two concentrations). A 2.5 L sample of the raw GDWW was transferred from the 25 L drums (4°C) to a glass beaker. A magnetic stirrer (Heidolph, Germany) was used (1 000 rpm) to maintain a homogenous sample as pH adaption commenced. The pH was adjusted with the use of 2M KOH (Merck, Germany). A 500 mL sample (2 x 250 mL) was taken at each pH value used. Each individual sample (250 mL) was placed in a measuring cylinder and ACH was added. Mixing was achieved by transferring the samples to the centrifuge tubes. Samples were centrifuged at 15°C and 10 000 rpm for 10 mins. After centrifuging, the

supernatant was placed in Schott bottles and stored at 4°C until further analyses. The above trials were done in triplicate for both 100 and 140 mgACH.L⁻¹. The effects of coagulant concentration and pH adjustment were monitored by determining the COD, TSS and FOG.

Ozonation of GDWW

Ozonation was done on the GDWW that had undergone coagulation treatment (i.e. lowered FOG and TSS content) as previously described. The samples were taken from a single batch of wastewater frozen at -18°C until ozonation commenced.

Ozone was generated by a GENESIS™ corona discharge O₃-generator (Del Ozone®) which was able to produce 2 g O₃.h⁻¹ (Anon., 2012). Ozonation was carried out in a glass apparatus as shown in Fig. 1. The apparatus had an operational volume of 6 L. Ozone was introduced into the system with a stainless steel diffuser (A), connected to the ozone generator with Nalgene® tubing. A Calpeda® stainless steel impeller pump (B) (Calpeda® MXH 203E, Vicenza, Italy) was used to circulate the GDWW at a flow rate of ±74 L.h⁻¹. Piping (Polyvinyl chloride) (total length ± 4.5 m) was used to connect the pump to the glass apparatus. This was also used to increase the contact time of the O₃ with the wastewater. Due to excess foam formation, compressed air was introduced at the top of

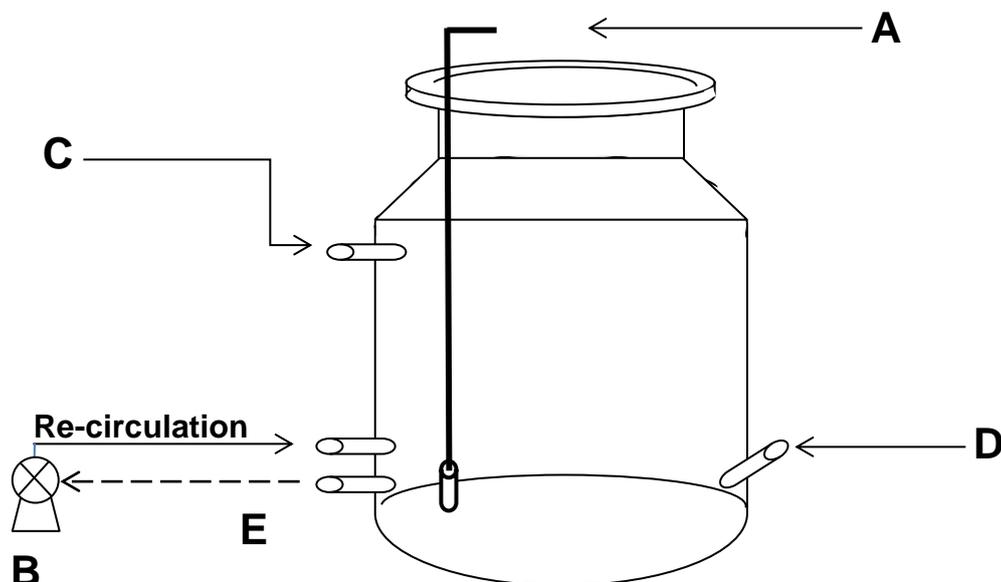


Figure 1 Glass apparatus used during ozonation of GDWW. (A: Ozone supply; B: Recirculation pump; C: Compressed air; D: Thermometer; E: GDWW outflow.)

the glass apparatus (C) to create a vortex-like air flow in the apparatus to break the foam layer. During the process the temperature was monitored (D). The pipe used for the outflow of the GDWW (E) (± 3 m in length) was placed in an ice bath to prevent the water temperature from increasing. The GDWW samples were ozonated at different concentrations (100, 300, 600 and 900 mg O₃.L⁻¹) to determine the optimal ozone dosage. The ozone dosage was calculated by taking ozonation time, GDWW volume and ozone gas production into account. GDWW was sampled at specific time intervals, corresponding to the mentioned concentrations. At the different O₃ concentrations the COD, FOG and TSS were determined. All analyses were performed in duplicate.

Analytical methods

The parameters that were analysed according to the Standard Methods (APHA, 1998) included chemical oxygen demand (COD) and total suspended solids (TSS). The COD was measured colorimetrically using a DR2000 spectrophotometer (Hach Co. Loveland, CO). All determinations were performed in duplicate.

For the FOG analysis a method adapted from APHA (1998) was used. A wastewater sample of 50 g was collected and acidified to pH 2.0 with a mixture of concentrated sulphuric acid (H₂SO₄) and distilled H₂O (1:1). This sample was placed in a separator funnel and 100 mL absolute ethanol ($\geq 99.8\%$) and 40 mL of a 1:1 hexane fraction and diethyl ether solution was added. This was shaken vigorously for 2 mins and the two layers were allowed to separate. The solvent layer, containing the FOG was collected. This procedure was repeated twice. This sample was distilled in a rotary evaporator (Büchi Rotavapor R-114) at 55 - 60°C, and quantified gravimetrically to mgFOG.L⁻¹.

Statistical analysis

Statistical analysis was performed on COD, TSS and FOG data obtained from the coagulation and ozonation trials, which was performed in triplicate. The statistical analysis included the calculation of standard deviation for the trial replications, as well as the calculation of the regression coefficient (R²) for the fitted linear regression lines (coagulation trials). Microsoft Excell 2010 was used to perform the statistical analysis.

Results and Discussion

Impact of coagulant concentration on COD content

The data in Fig. 2 illustrates the percentage COD reductions found in GDWW after pH adaption at two different ACH dosage concentrations (100 and 140 mg.L⁻¹). Linear regression lines were used to obtain an indication of the trend of COD reduction. At 100 mgACH.L⁻¹ the linear regression line ($R^2 = 0.60$) indicated that the percentage reduction increased over the pH range (3.4 – 7.0) as pH increased. The maximum reduction was found at pH 7.0 (24.7% ± 4.60). A total increase in COD reduction of 10.3% points was found over the pH range from 14.4 to 24.7%. The ACH addition at a concentration of 140 mg.L⁻¹ also resulted in an increased COD reduction as pH increased from 3.4 to 7.0 (Fig. 2). The total increase in COD reduction over the pH range was 5.0% points. The maximum percentage reduction for 140 mg.L⁻¹ ACH was also observed at pH 7.0 (33.2% ± 4.93).

The increase in pH showed to exert an increased effect on COD reduction when 100 mg.L⁻¹ was used, as the increase in COD reduction (%) was higher than that of 140 mg.L⁻¹ (10.3% points compared to 5.0% points). However, a lower COD reduction was consistently achieved at the lower coagulant concentration over the pH range (Fig. 1). An increased concentration ACH (140 mg.L⁻¹) resulted in higher reduction efficiency at each pH value, and also displayed less variation (in terms of standard deviation and R^2).

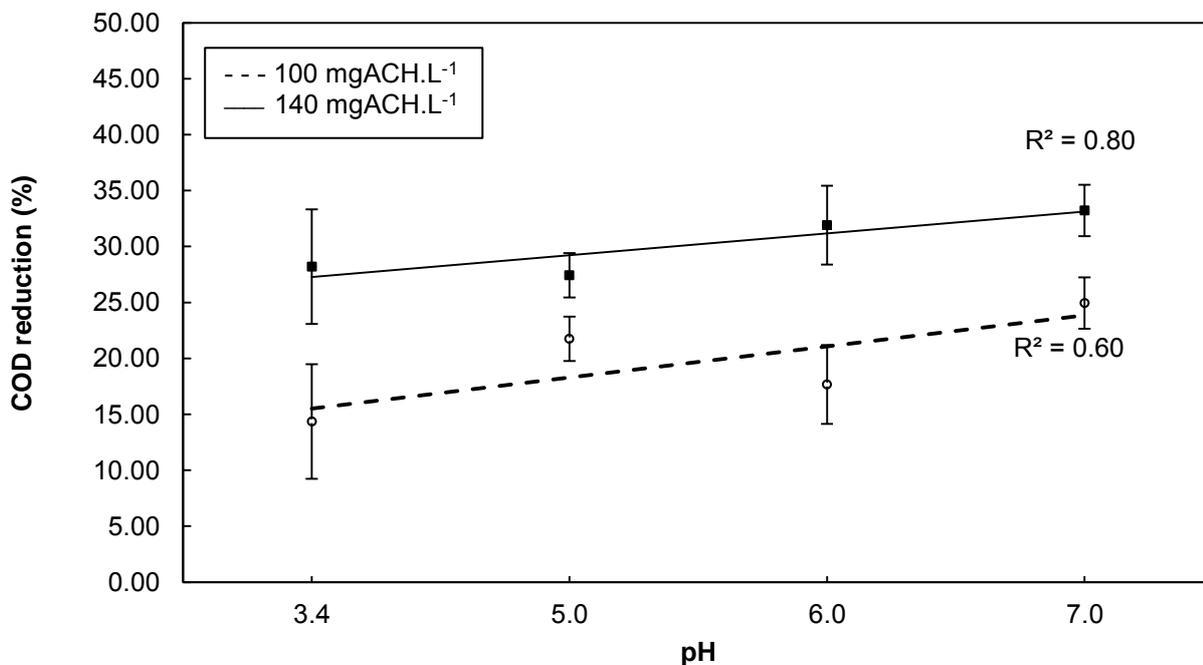


Figure 2 COD reduction (%) obtained for the GDWW after application of the two ACH concentrations (100 and 140 mgACH.L⁻¹) at the four different pH values (error bars represent standard deviations).

The fitted linear regression line obtained for 140 mg.L^{-1} also had a higher R^2 (0.80), compared to that of 100 mg.L^{-1} (0.60), which indicated a better fit.

Impact of coagulant concentration on TSS content

The data for the TSS reduction (%) is illustrated in Fig. 3. At 100 mgACH.L^{-1} , pH adaption led to an increased reduction with an increase in pH from 3.4 to 7.0, as shown by the fitted linear regression line ($R^2 = 0.82$). The highest variation (standard deviation) was found at pH 5.0 (Fig. 3). A total increase in reduction of 25.8% points was found over the pH range from 47.5 to 73.3% points. The optimal reduction was found at pH 7.0 ($73.3\% \pm 3.95$).

At 140 mgACH.L^{-1} the linear regression line ($R^2 = 0.73$) shows that pH adaption resulted in an overall better TSS reduction over the entire pH range from 3.4 to 7.0 (Fig. 3). The total increased reduction over the pH range was 11.1% (from 80.8 to 91.9%). The highest reduction was also found at pH 7.0 ($91.9\% \pm 1.73$).

At the lower ACH concentration (100 mg.L^{-1}) it was clear that pH had a more pronounced effect on the reduction of the TSS (increase in percentage reduction over the pH range = 25.80% points). The pH adaption is therefore greatly advantageous, as an increase in TSS reduction was seen as a result (Fig. 3). The large variation at pH 5.0 could possibly be attributed to the presence of other Al-complexing species in the GDWW, such as F^- , PO_4^{3-} , and SO_4^{2-} , which have been reported to influence the efficiency of an Al-coagulants (Pernitsky & Edzwald, 2006). The variation seen, specifically at pH 5.0 and 6.0 at 100 mgACH.L^{-1} could therefore be ascribed to the influence of such ions.

Coagulant concentration remains a very important factor regarding the efficiency thereof (Pernitsky & Edzwald, 2006). From this data (Fig. 3) it was seen that at pH 7.0 an optimal reduction was achieved at both ACH concentrations. This pH also showed the lowest variation in terms of standard deviation (SD), and therefore the reduction obtained at this pH is more reliable.

Impact of coagulant concentration on FOG content

The results for the reduction of FOG are presented in Fig. 4. At 100 mgACH.L^{-1} , the linear regression line ($R^2 = 0.71$) indicates an increased percentage reduction as the pH increased from 3.4 to 7.0. A large variation (SD) was observed at pH 5.0. A slight increase was found as the pH is increased from 3.4 to 6.0 (from ca. 38 to 42%) (Fig. 4). A larger increase was found from pH 6.0 to 7.0 (from ca. 42 to 65%) and the highest percentage FOG reduction was observed at pH 7.0 ($65.2\% \pm 2.46$).

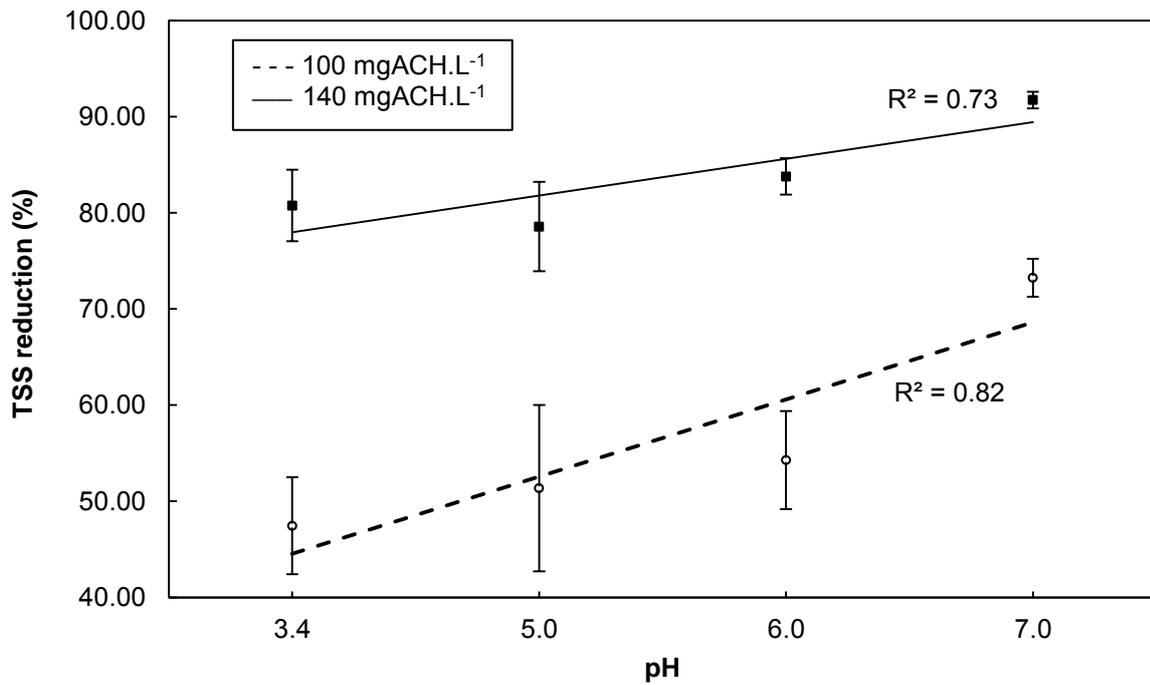


Figure 3 TSS reduction (%) obtained for the GDWW after the application of the two ACH concentrations (100 and 140 mgACH.L⁻¹) at the four different pH values (error bars represent standard deviations).

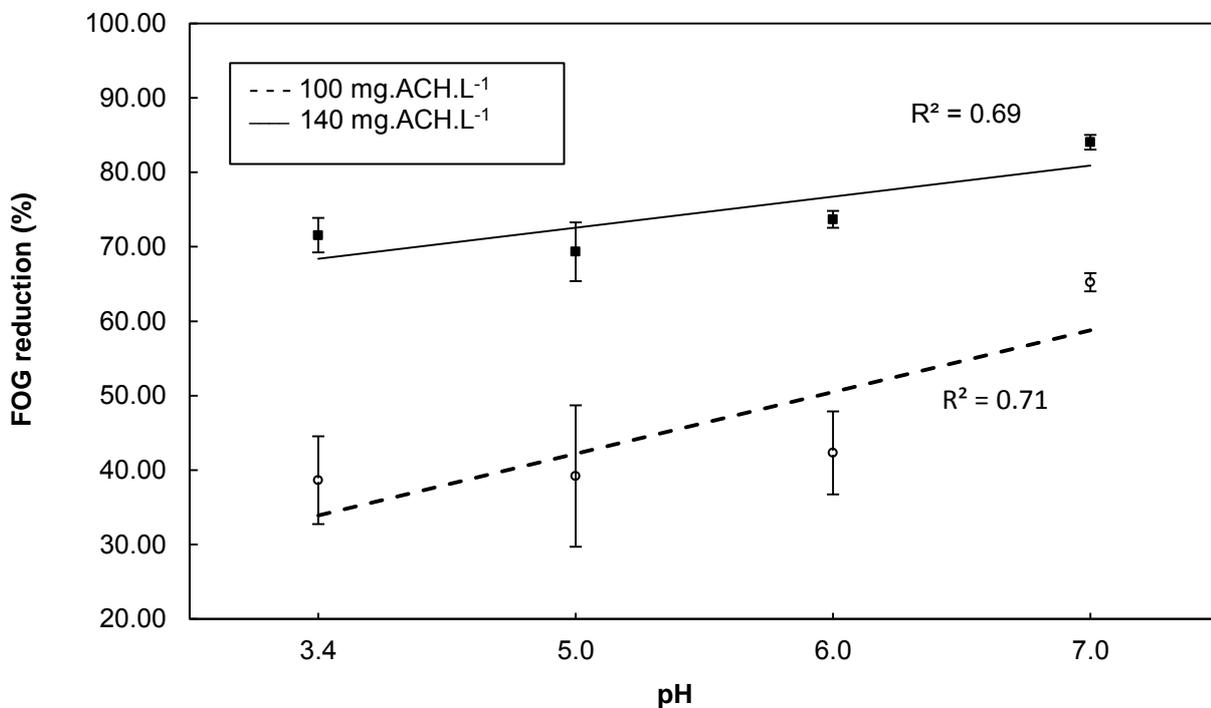


Figure 4 FOG reduction (%) obtained for the GDWW after the application of the two ACH concentrations (100 and 140 mgACH.L⁻¹) at the four different pH values (error bars represent standard deviations).

The higher coagulant concentration of 140 mgACH.L^{-1} shows a similar trend ($R^2 = 0.69$) to that of the 100 mgACH.L^{-1} as an increased FOG reduction was found as the pH was increased from 3.4 to 6.0 (from ca. 71 to 73%) (Fig. 4). A slight increase was found as the pH was increased from 3.4 to 6.0 and a larger increase was found from pH 6.0 to 7.0 (i.e. from ca. 73 to 84%). The highest percentage FOG reduction was observed at pH 7.0 ($84.1\% \pm 1.98$) (Fig. 4).

An increased coagulant efficiency was found at both concentrations as the pH increased. Larger standard deviations were observed at 100 mgACH.L^{-1} . The higher coagulant concentration gave more consistent results as smaller standard deviations were observed overall (Fig. 4). The largest standard deviation was observed at pH 5.0, and the smallest was observed at pH 7.0, for both concentrations. At pH 5.0 large variation could possibly be explained due to a low solubility of three Al-species (Al(OH)^{1+} , Al(OH)^{2+} , and Al^{3+}) (Pernitsky & Edzwald, 2006). This could have caused irregular binding of particles to the mentioned Al-species, and cause the FOG values to fluctuate. Furthermore, Gregor *et al.* (1997) noted that the anionic charge of carboxyl groups will increase only with 20% as pH is increased from 4.7 to 6.0. This slight increase in the negative charge of functional groups of natural organic matter could explain why a slight increased reduction in FOG was found as the pH was increased from pH 5.0 to 6.0 (Fig. 4).

Particles in water generally carry a negative charge from pH 6.0 upwards (Pernitsky & Edzwald, 2006; Kosmulski, 2012). As the pH increases, the ionisation of carboxyl groups and functional groups of phenolic compounds takes place. This increases the negative charge of natural organic matter (Pernitsky & Edzwald, 2006). The affinity of natural organic matter for the positively charged Al-species therefore increases with an increase in pH (Gregor *et al.*, 1997; Pernitsky, 2003). In the pH range of 6.0 to 7.0 most of the Al-species are charged positively, and therefore these conditions are considered to be favourable for coagulation (Pernitsky & Edzwald, 2006). As an increase to pH 7.0 took place, the solubility, and prevalence of Al_{13}^{7+} , increased, providing a possible explanation for decreased variance, and also for increased reduction (Pernitsky & Edzwald, 2006). This observation was also reported by Van Benschoten and Edzwald (1990) and Gregor *et al.*, (1997) who found the maximum reduction of fulvic acid and natural organic matter between pH 6.0 and 7.0. Van Benschoten and Edzwald (1990) did however not find a similar trend of reduction as in this study, but they reported that a reduction often decreased below pH values of 6.0 and above 7.0. Van Benschoten and Edzwald (1990) also concluded that the reduction at higher coagulant dosage levels and pH, was more efficient in the removal of organic matter.

Impact of ozone concentration on COD content

The effect of ozonation on the COD of GDWW is summarised in Fig. 5 and Table 1. The data in Fig. 5 shows an initial COD reduction at 100 mgO₃.L⁻¹. At 300 mgO₃.L⁻¹, however, the COD reduction decreases, resulting in a negative reduction. Thereafter, higher COD reductions were again found at 600 and 900 mgO₃.L⁻¹. The highest COD reduction thus achieved at 100 mgO₃.L⁻¹ (3.6% ± 4.08) (Fig. 5).

Because of ozone's oxidative capacity a general decrease in COD was expected, as this has been reported by numerous authors (Beltrán *et al.*, 2000; Chang *et al.*, 2001; Sigge *et al.*, 2005). The negative percentage reduction found at 300 mgO₃.L⁻¹ was therefore not expected (Fig. 5). However, during COD analysis, K₂Cr₂O₇, possessing an oxidative capacity of 1.23 V (Vanýsek, 1991), was the reagent used to oxidise compounds in the wastewater sample (Merck, 2011). Ozone and the hydroxyl radical possess oxidative capacities of 2.07 V and 2.80 V, respectively (Legrini *et al.*, 1993; Gusel-Seydim *et al.*, 2004). This implies that compounds that could previously not be oxidised during COD analysis and thus consequently not be quantified during analysis, could be quantified after ozone treatment, as ozone could have degraded organic compounds to a state where they were able to be oxidised by K₂Cr₂O₇ during COD analysis.

A negative reduction in COD was also found by Gie (2007) when treating GDWW. This increase was thought to be due to oxidation of recalcitrant compounds, leading to the solubilisation thereof, and thus resulting in an increased COD value being detected. Gie (2007) also found overall low COD reductions of approximately 4% at ± 550 mgO₃.L⁻¹. Upon the ozonation of olive mill wastewaters, Benitez *et al.* (1997) attributed a low COD reduction (6.2%) to the fact that the simplified, less toxic compounds produced as a result of ozonation still require oxygen to be degraded completely, and as a result a low COD reduction is achieved.

Andreozzi *et al.* (1998) and Chang *et al.* (2001) achieved a reduction of 5% and 13%, respectively, of ca. 3 000 mgO₃.L⁻¹ when treating wastewater which was high in lipid content. The main purpose for using ozone is to increase the biodegradability of GDWW and therefore the targeted parameter would be FOG, rather than COD, as anaerobic digestion, which is able to treat substrates with high organic content, will be used in the second treatment phase (Benitez *et al.*, 1997; Bitton, 2005). Sigge *et al.* (2005) found that using ozone as a pre-treatment increased the COD removal in an UASB reactor by up to ca. 25%. Distillery wastewaters are also known to contain refractory compounds which are not easy to degrade, and therefore it is not expected to see a large decrease in COD,

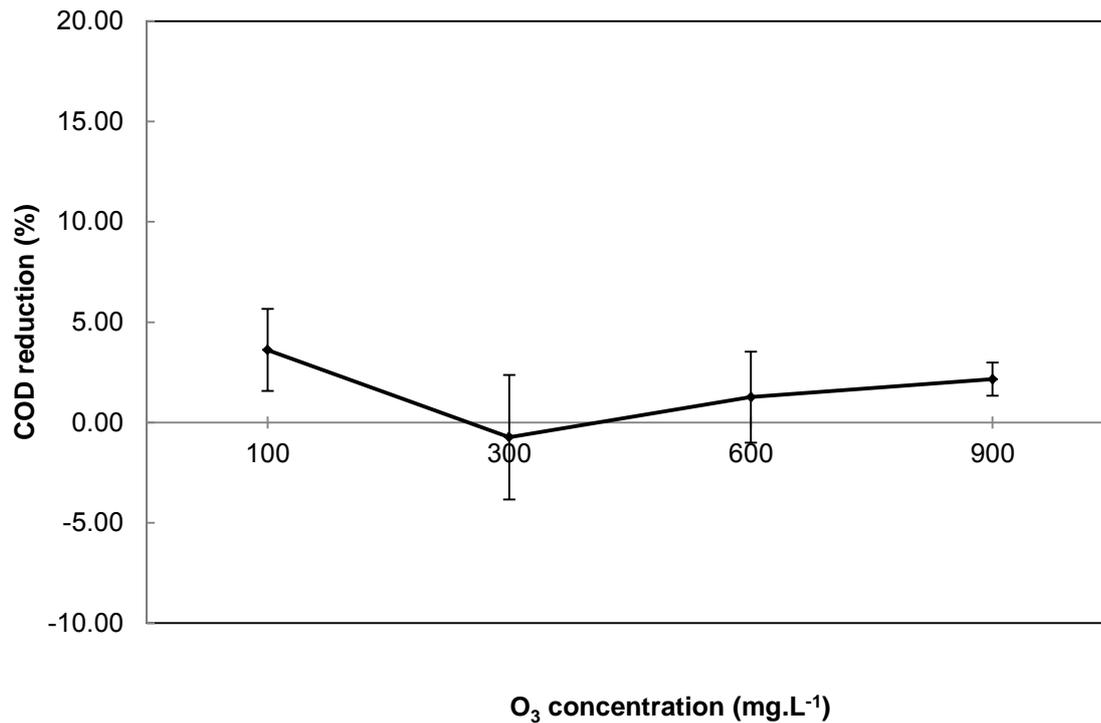


Figure 5 COD reduction (%) obtained in the GDWW after applying the four different ozone concentrations (error bars represent standard deviations).

Table 1 Cumulative effect on COD reduction (%) in GDWW after ACH treatment (140 mg.L⁻¹) and different ozone concentrations

Treatment description	COD reduction (%) ± SD
ACH treatment (pH 7.0; 140 mgACH.L ⁻¹)	30.0 ± 3.56
ACH treatment (pH 7.0; 140 mgACH.L ⁻¹) + 100 mgO ₃ .L ⁻¹	32.5 ± 1.90
ACH treatment (pH 7.0; 140 mgACH.L ⁻¹) + 300 mgO ₃ .L ⁻¹	29.5 ± 3.50
ACH treatment (pH 7.0; 140 mgACH.L ⁻¹) + 600 mgO ₃ .L ⁻¹	30.9 ± 3.13
ACH treatment (pH 7.0; 140 mgACH.L ⁻¹) + 900 mgO ₃ .L ⁻¹	31.5 ± 3.30

but rather that organic compounds are simplified to a more biodegradable state (Beltrán *et al.*, 1999).

The cumulative effect of ACH and ozone pre-treatments is given in Table 1. The data in Table 1 shows the total reduction in COD that had taken place after coagulant addition and also after the different ozone dosages were applied. It thus shows the effect ozone has when seen in relation to that of the coagulant. It was found that the cumulative COD reduction was influenced more by the ACH treatment than by the ozone treatment. The coagulant treatment accounted for the greatest part of COD reduction ($30.0\% \pm 3.56$). The additional ozone treatment ($100 \text{ mgO}_3\cdot\text{L}^{-1}$) increased the total reduction to $32.5\% \pm 1.90$.

Impact of ozone concentration on TSS content

The results for TSS reduction are illustrated in Fig. 6 and Table 2. In Fig. 6 an initial negative reduction (%) was seen at $100 \text{ mgO}_3\cdot\text{L}^{-1}$ ($-9.6\% \pm 5.6$). At $300 \text{ mgO}_3\cdot\text{L}^{-1}$ an increase in TSS reduction (%) was seen (thus a $-6.8\% \pm 7.63$ decrease). At ozone concentrations of 600 and $900 \text{ mgO}_3\cdot\text{L}^{-1}$, TSS reductions of 16.6 and 27.7% were achieved (Fig. 6).

Due to the oxidative effect of ozone it was expected that the degradation of compounds would take place and a decreased TSS value was therefore expected. The negative reduction found at ozone dosages of 100 and $300 \text{ mgO}_3\cdot\text{L}^{-1}$ has also been reported by Reckhow *et al.* (1986), and confirmed and studied by others (Grasso & Weber, 1988; Edwards & Benjamin, 1991; Bose & Reckhow, 2007). According to literature, this occurs due to a polymerising effect ozone has on the solid content of wastewater. One way this could occur is via hydrogen bridging taking place between compounds (Reckhow *et al.*, 1986). This results in destabilisation and the direct precipitation of compounds (Reckhow *et al.*, 1986). Ozone has also been reported to increase the carboxylic acid content, resulting in an increased negative charge of compounds which enhances its binding effect to naturally occurring metals (Grasso & Weber, 1988; Bose & Reckhow, 2007). The ozonation process can also disrupt metal complexes, which results in a similar effect, and thus increasing the TSS value (Reckhow *et al.*, 1986).

Table 2 shows the cumulative effect of ACH and ozonation on TSS. It can be seen that ozone has a notable effect on the overall TSS content, especially at higher dosages. It was found that the cumulative TSS reduction was influenced more by the coagulant treatment than by the ozone treatment. The coagulant treatment accounted for the

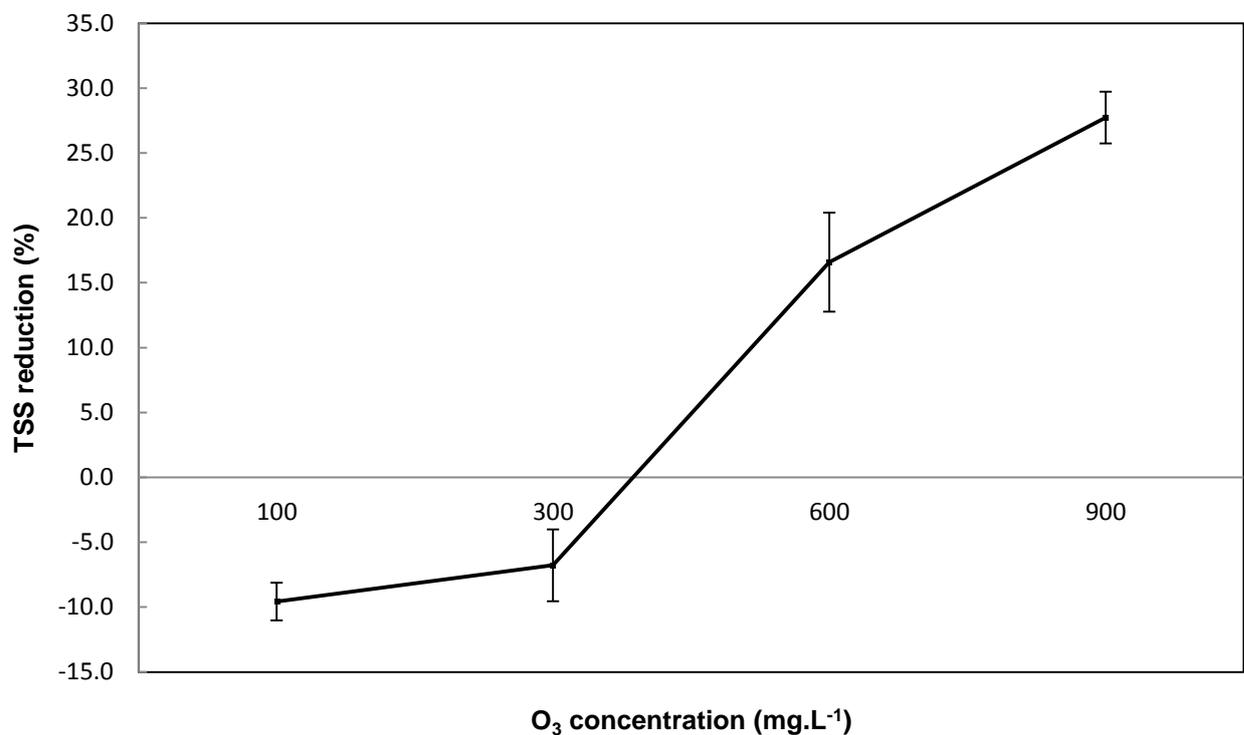


Figure 6 TSS reduction (%) obtained in the GDWW after applying the four different ozone concentrations (error bars represent standard deviations).

Table 2 Cumulative effect on TSS reduction (%) in GDWW after ACH treatment (140 mg.L⁻¹) and different ozone concentrations

Treatment description	TSS reduction (%) ± SD
ACH treatment (pH 7.0; 140 mgACH.L ⁻¹)	63.2 ± 2.92
ACH treatment (pH 7.0; 140 mgACH.L ⁻¹) + 100 mgO ₃ .L ⁻¹	59.7 ± 3.25
ACH treatment (pH 7.0; 140 mgACH.L ⁻¹) + 300 mgO ₃ .L ⁻¹	60.7 ± 4.63
ACH treatment (pH 7.0; 140 mgACH.L ⁻¹) + 600 mgO ₃ .L ⁻¹	69.3 ± 2.25
ACH treatment (pH 7.0; 140 mgACH.L ⁻¹) + 900 mgO ₃ .L ⁻¹	73.4 ± 1.72

greatest part of the TSS reduction and resulted in an average reduction of $63.2\% \pm 2.92$. The additional ozone treatment added up to ca. 10% points reduction at $900 \text{ mgO}_3\cdot\text{L}^{-1}$, and thus the total TSS reduction reached $73.4\% \pm 1.72$. Therefore, if the TSS content is problematic after coagulant application, ozone could be used to decrease the TSS content. Lower ozone dosages were not found to be beneficial for TSS reduction.

Impact of ozone concentration on FOG content

The effect of ozone on FOG reduction is illustrated in Figs. 7 and 8 and Table 3. As expected, an overall positive FOG reduction (%) was found. In Fig. 7 the data shows a FOG reduction of $15.8\% \pm 6.3$ at $100 \text{ mgO}_3\cdot\text{L}^{-1}$. The FOG reduction increased to ca. 16 and 19% at doses of 300 and 600 $\text{mgO}_3\cdot\text{L}^{-1}$. The overall reduction obtained at the highest dosage ($900 \text{ mgO}_3\cdot\text{L}^{-1}$) was $23.9\% \pm 1.8$. Ozone is selective towards double bonds (Criegee, 1975), which are present in unsaturated fatty acids found in maize (oleic-, linoleic- and linolenic acid) (Watson, 1987), and an overall decrease in FOG was therefore expected.

The oxidation rate (in terms of mg FOG reduced per mg ozone) is shown in Fig. 8. At $100 \text{ mgO}_3\cdot\text{L}^{-1}$ the oxidation rate was the highest ($\text{mgFOG}\cdot\text{mgO}_3^{-1}$). At dosages higher than $100 \text{ mgO}_3\cdot\text{L}^{-1}$ a decrease in oxidation efficiency was found (Fig. 8). The lower FOG oxidation efficiency could be due to ozone targeting easily degradable compounds, such as other unsaturated compounds. Unsaturated target compounds, which form part of FOG, could have become exhausted making FOG degradation less favoured and this would therefore result in a decrease in the oxidation rate. These compounds include any compound possessing double bonds such as aromatic and phenolic compounds (Benitez *et al.*, 1997; Hsu *et al.*, 2004; Gül *et al.*, 1999). The reaction of phenolic compounds with molecular O_3 might also have been responsible for the generation of hydroxyl radicals ($\cdot\text{OH}$ s), providing a way of saturated compound oxidation (Buffle *et al.*, 2006). Some recalcitrant compounds are not degraded via direct ozone reactions, and only decompose in reaction to $\cdot\text{OH}$ (Alvares *et al.*, 2001; Pocostales *et al.*, 2010). Although the pH of GDWW was not monitored during the ozonation experiments, a decrease of ca. 0.2 pH units was found at $300 \text{ mgO}_3\cdot\text{L}^{-1}$ by Lucas *et al.* (2009) for winery wastewater. They explained that this is due to the degradation of organic matter and the subsequent formation of carboxylic acids, organic acids and CO_2 . Hoigné & Bader (1976) found that the impact of $\cdot\text{OH}$ can only be considered negligible once the pH had reached a value below pH 5.5. This would therefore mean that, although the pH had decreased, it could be

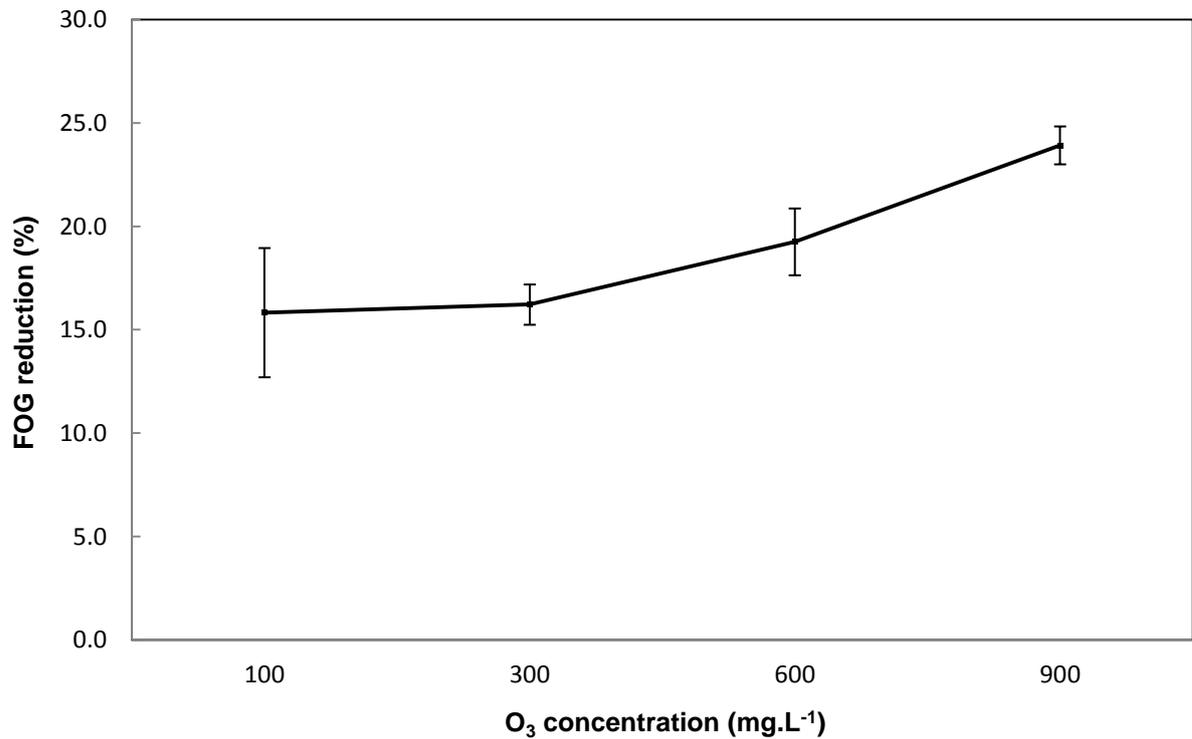


Figure 7 FOG reduction (%) obtained in the GDWW after applying the four different ozone concentrations (error bars represent standard deviations).

Table 3 Cumulative effect on FOG reduction (%) of optimal ACH treatment and different ozone concentrations

Treatment description	COD reduction (%) ± SD
ACH treatment (pH 7.0; 140 mgACH.L ⁻¹)	59.3 ± 4.93
ACH treatment (pH 7.0; 140 mgACH.L ⁻¹) + 100 mgO ₃ .L ⁻¹	65.8 ± 3.58
ACH treatment (pH 7.0; 140 mgACH.L ⁻¹) + 300 mgO ₃ .L ⁻¹	65.9 ± 3.48
ACH treatment (pH 7.0; 140 mgACH.L ⁻¹) + 600 mgO ₃ .L ⁻¹	67.1 ± 3.92
ACH treatment (pH 7.0; 140 mgACH.L ⁻¹) + 900 mgO ₃ .L ⁻¹	69.0 ± 3.63

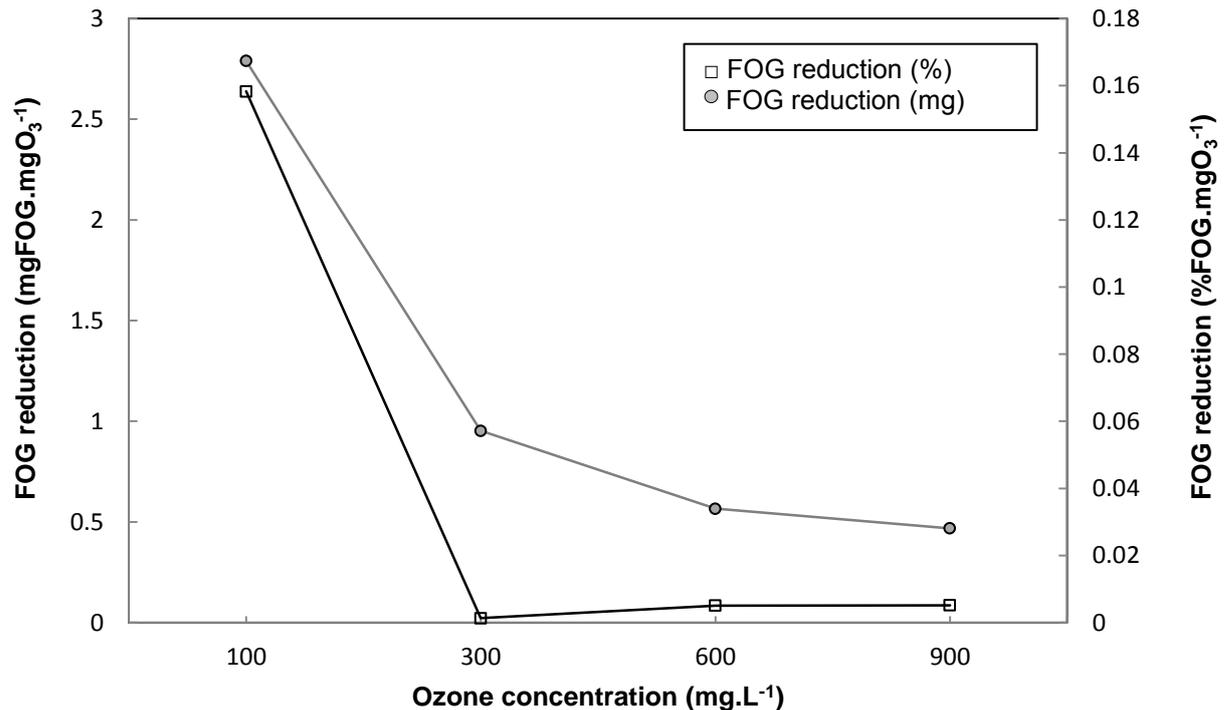


Figure 8 FOG oxidation efficiency in terms of mg FOG degraded per mg ozone applied, and percentage FOG degraded per mg ozone applied, at different ozone dosages.

assumed that the presence of the $\cdot\text{OH}$ would still have a degrading effect, although the effect thereof would decline as the pH decreased (Lucas *et al.*, 2009).

Another reason which could have brought about this decreased oxidation efficiency is the formation of scavenger species, such as carbonates, and the presence of recalcitrant compounds (Hoigné & Bader, 1976; Lucas *et al.*, 2009). Recalcitrant compounds would require a higher dosage of oxidant to be degraded, which would result in a lower oxidation efficiency (Fig. 8) (Hoigné & Bader, 1976; Lucas *et al.*, 2009). Andreozzi *et al.* (1998) (maximum ozone dosage of $16.17 \text{ gO}_3.\text{L}^{-1}$) found a similar trend, but found a constant decrease in oxidation rate.

During ozonation experiments, the wastewater temperature increased (up to 32°C) due to gradual heating of the water pump used to recirculate the GDWW. A decreased solubility of ozone has been reported at higher temperatures, although a simultaneous increase in reaction rate also takes place as temperature increases (USEPA, 1999; Khadre *et al.*, 2001). This could have contributed to the decreased oxidation efficiency found (Fig. 8).

Table 3 shows the cumulative effect of ACH and ozonation on FOG. It was found that even a low ozone dosage ($100 \text{ mg}\cdot\text{L}^{-1}$) increased the FOG reduction (%) after ca. 60% was removed after the ACH treatment. The coagulant treatment accounted for the greatest part of FOG reduction ($59.3\% \pm 4.93$). The additional ozone treatment increased the total reduction by ca. 10% points to $69.0\% \pm 3.63$ at $900 \text{ mgO}_3\cdot\text{L}^{-1}$. The lower dosages of 100 and $300 \text{ mgO}_3\cdot\text{L}^{-1}$ reduced the FOG concentration up to ca. 65%. Thus, ozone proved to be effective in lowering the FOG content in GDWW.

Conclusions

In this study a change in pH of GDWW influenced the performance of ACH positively and an increased reduction in COD, TSS and FOG was the result. Although pH had a more pronounced effect at the lower coagulant dosage ($100 \text{ mgACH}\cdot\text{L}^{-1}$), it is not recommended as the reductions obtained with the higher coagulant dosage ($140 \text{ mgACH}\cdot\text{L}^{-1}$) were much higher. The higher coagulant dosage also showed less variation (lower standard deviation), and thus delivered more consistent results.

The maximum reduction of COD was achieved at $100 \text{ mgO}_3\cdot\text{L}^{-1}$, whereas the highest reduction of TSS and FOG was found at the highest ozone dosage ($900 \text{ mgO}_3\cdot\text{L}^{-1}$). Such high dosages are not recommended due to high operational costs involved. The highest FOG oxidation efficiency ($\text{mgFOG}\cdot\text{mgO}_3^{-1}$) was found at $100 \text{ mgO}_3\cdot\text{L}^{-1}$ and therefore this was the most economical treatment dosage. However, a dosage of $300 \text{ mgO}_3\cdot\text{L}^{-1}$ was decided upon, as the possible degradation of recalcitrant compounds was observed at this dosage. An increased dosage could also make provision for the situation when seasonal fluctuation in COD, TSS and FOG occurs, as found during the coagulant and ozonation experimental trials.

This study concluded that the pre-treatment strategy of $140 \text{ mgACH}\cdot\text{L}^{-1}$ combined with $300 \text{ mgO}_3\cdot\text{L}^{-1}$ resulted in the optimal reduction of COD, and especially TSS and FOG (which at high levels can cause reactor upsets). GDWW with a reduced COD (and possibly higher biodegradability due to the oxidation of recalcitrant compounds), TSS and FOG concentration should provide a more suitable substrate for anaerobic digestion.

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Chapter 4

IMPACT OF OZONE PRE-TREATMENT ON THE PERFORMANCE OF UPFLOW ANAEROBIC SLUDGE BLANKET REACTORS TREATING PRE-TREATED GRAIN DISTILLERY WASTEWATER

Summary

Two 2 L laboratory scale upflow anaerobic sludge blanket (UASB) reactors were operated for a period of 277 days. The substrate of the first reactor contained grain distillery wastewater (GDWW) that had only undergone coagulant pre-treatment (R_{control}) and the substrate of the second UASB reactor consisted of GDWW that had undergone coagulant pre-treatment and ozone pre-treatment (R_{ozone}). The pH of the substrate was set at 7.0 and the hydraulic retention time (HRT) at 24 hours. Both reactors treated pre-treated GDWW successfully at *ca.* $9 \text{ kgCOD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$. Over the trial period COD reductions of *ca.* 96% for R_{control} and 93% for R_{ozone} , were achieved. FOG reductions (%) showed variations throughout the study and maximum reductions of *ca.* 88% and 92% were achieved for R_{control} and R_{ozone} , respectively. R_{ozone} produced more biogas, and the methane percentage was similar in both reactors. The additional ozone pre-treatment did not show an added benefit in the reactor performance results. UASB granule washout in R_{control} did however suggest possible toxicity of unsaturated fatty acids present in non-ozonated substrate. The feasibility of FOG removal was demonstrated as both reactors successfully treated pre-treated GDWW. Post-ozonation was performed on the effluent of both reactors and the effluent of R_{ozone} showed better results. This showed that the ozone pre-treatment possibly led to an easier degradable wastewater and better results could potentially be obtained when other post-treatment steps are applied.

Introduction

High strength wastewater is produced during the whisky production process with volumes exceeding that of other beverage industries (UNIDO, 2012). It is a serious environmental pollution hazard as it is acidic in nature (pH 3.4 – 3.8) and is high in organic content (Akunna & Clark, 2000). Grain distillery wastewater (GDWW) also has a high chemical oxygen demand (COD), total suspended solids (TSS) and fats, oils and grease (FOG) content (Laubscher, 2000; Gie, 2007). South Africa is a water scarce country and

therefore the reuse of such wastewater should be of high priority (Bennie & Hensley, 2001; Adewumi *et al.*, 2010). Direct land application holds a risk of environmental damage (Gao *et al.*, 2007) and local regulations require that certain guidelines are met, which demands prior treatment of GDWW (Republic of South Africa, 2004).

Anaerobic digestion often is the chosen treatment method for this high strength wastewater (Schmidt *et al.*, 2013). This process has, however, often encountered problems when treating the GDWW due to the high lipid and solid content (Laubscher *et al.*, 2000; Dereli *et al.*, 2013). Certain long chain fatty acids (LCFAs) have been pointed out as problematic due to their adsorption to UASB granules (Pereira *et al.*, 2005). It has been shown that when a lipid layer accumulates around granules it prevents the transfer of nutrients and metabolites, ultimately resulting in granule floatation and washout (Hwu *et al.*, 1998; Laubscher *et al.*, 2000; Palatsi *et al.*, 2009). Unsaturated fatty acids have been shown to be more toxic compared to saturated fatty acids (Lalman & Bagley, 2002). The lipids in maize are mostly unsaturated (Watson, 1987) and therefore these need to be removed prior to anaerobic digestion treatment of GDWW.

Coagulants have been widely applied to reduce the organic content, including lipids, in various wastewaters (Gao & Yue, 2005; Bin Banyeng, 2008). Coagulation has been shown to benefit the anaerobic process, especially when high TSS loads are present, which have been linked to foaming problems (Laubscher *et al.*, 2001). Ozone has also been shown to be beneficial in decreasing unsaturated fatty acids, as well as other unsaturated compounds, because of its selectivity towards double bonds (Hoigné & Bader, 1983; Andreozzi *et al.*, 1998). When combining ozone and biological treatments, it has been shown to lead to increases in the biodegradability of the treated substrates (Hsu *et al.*, 2004; Sigge *et al.*, 2005). The increased treatment efficiency is reflected in increased biogas production and subsequent COD reductions (Martín *et al.* 2002; Sigge *et al.*, 2005 & 2007).

It is thus of importance to determine the impact of such pre-treatment methods on the final reactor operational efficiency. The objective of this study was to investigate the effect of ozonation on the performance of anaerobic reactors treating pre-treated GDWW. This will be achieved by monitoring the COD and FOG reductions of two UASB reactors. The substrates of both reactors will be pre-treated with a coagulant. The first reactor will serve as a control reactor, receiving non-ozonated substrate, while the second reactor will receive additional ozone pre-treatment.

Materials and Methods

UASB reactor setup

Two UASB reactors (R_{control} and R_{ozone}) with operational volumes of 2.3 L were operated at an HRT of 24h at 35°C (Meyer *et al.*, 1983; Trnovec & Britz, 1998) as shown in Fig. 1. The temperature was maintained by means of an electronic control unit (Meyer *et al.*, 1983). Peristaltic pumps (Watson Marlow 323) were used for the semi-continuous feeding of the substrate, and for recirculation purposes at an upflow velocity of 0.75 m.h⁻¹. Biogas volume was measured with the use of a manometric unit equipped with an electronically controlled counter. A U-tube was installed before the manometric unit, and also at the outflow of the reactor to ensure anaerobic conditions were maintained inside the reactor.

UASB Operation

Wastewater

GDWW samples were received from a whisky distillery in Wellington, South Africa between April 2012 and February 2013. GDWW was generated as spent wash from the whisky production process, which uses maize as substrate. The wastewater is centrate from a decanter, and had not undergone any prior treatment except for the removal of ca. 57% solids in the decanter (Green, J., 2012, Process Manager, The James Sedgwick Distillery, Wellington, South Africa. Personal communication, 28 May). The wastewater was obtained from the local distillery as needed during experiments and stored at 4°C prior to use.

UASB substrate

Raw GDWW was treated with 140 mg.L⁻¹ aluminium chlorohydrate (ACH) coagulant (Ultrafloc 3800™ (NCP Chlorochem, 2011), centrifuged (10 000 rpm for 10 min at 15 °C) and was treated with ozone (300 mgO₃.L⁻¹) by making use of a GENESIS™ corona discharge O₃-generator (Del Ozone®) which was able to produce 2 g O₃.h⁻¹ (Anon., 2012), as previously described (Chapter 3). As the effluent required was always less than the total volume of substrate, the GDWW was diluted with reactor effluent from the two reactors, to obtain the desired COD values, to prepare the final substrate which was fed to the two reactors. The substrate of the control reactor (R_{control}) contained GDWW which had only undergone coagulation and centrifugation pre-treatments while the GDWW in the substrate of the experimental reactor (R_{ozone}) had been exposed to coagulation, centrifugation and ozone treatments.

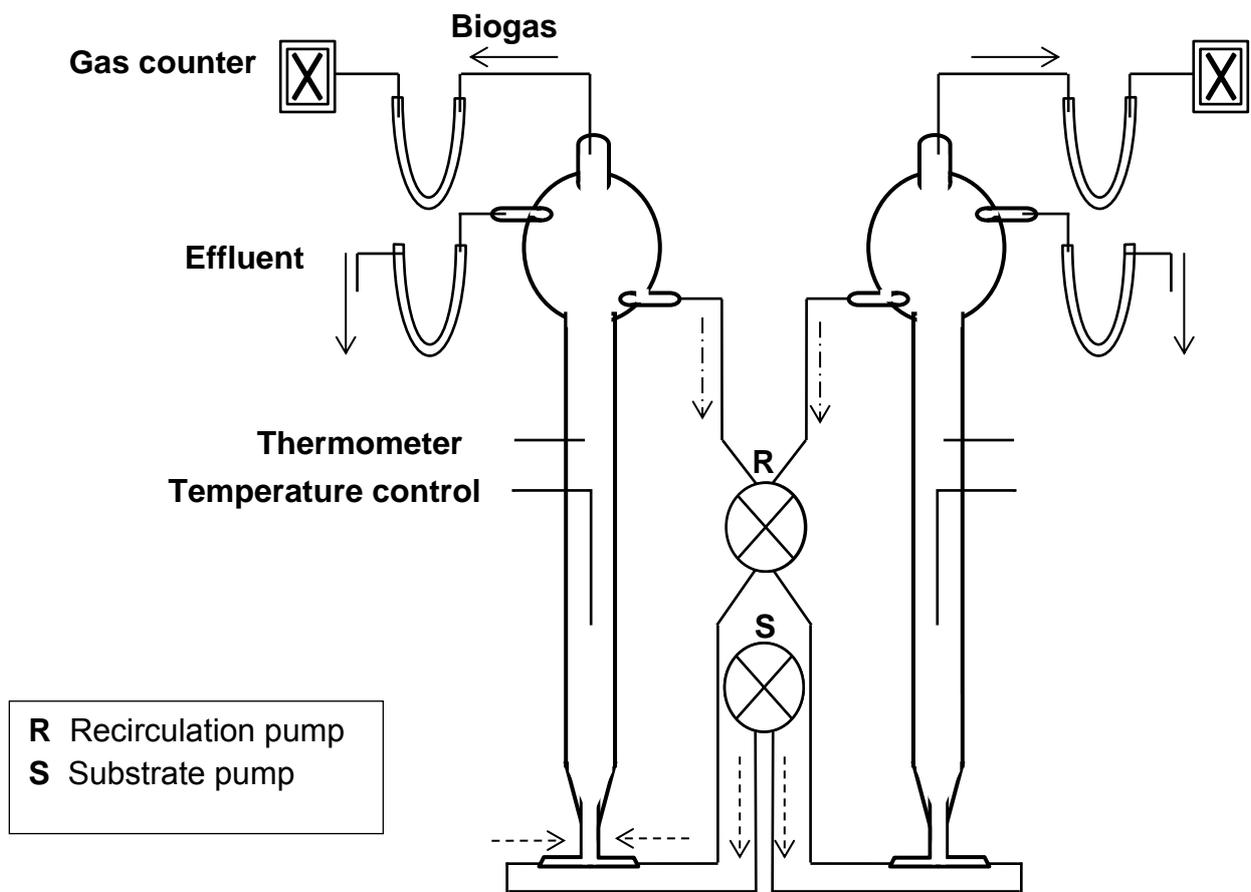


Figure 1 Schematic diagram of the UASB reactors used to treat pre-treated GDWW.

Reactor start-up

Each reactor was seeded with 500 mL of anaerobic granules from the full-scale UASB reactor treating winery distillery wastewater in Wellington, South Africa (The James Sedgwick Distillery, Wellington, South Africa). At start-up, both UASB reactors were fed with a synthetic glucose substrate (SGS) (Table 1). The SGS was diluted to a COD value of 1 250 mg.L⁻¹ and then GDWW (10% v/v) was added to the reactor feed. The ratio of SGS:GDWW (9:1) was decreased until the fed substrate consisted only of diluted GDWW. Initially the pH of the reactor feed was adjusted to 7.5 but was increased to pH 9.2 to facilitate start-up. A trace element solution (Table 2) (1 mL.L⁻¹ of reactor volume), formulated for anaerobic microbes, was also fed to each reactor weekly (Nel *et al.*, 1985).

Granule washout

Granule washout was measured using the volumetric method for settleable solids according to Standard Methods (APHA, 1998). This was measured when the presence of biomass was observed in the bottles which were used to collect the effluent for both reactors. An average value per day was calculated for the time period over which measurements took place (from day 133 onwards).

Effluent pipe clogging took place in both reactors, and this prevented biomass from washing out into the 2 L bottles used to collect the effluent. Therefore, the biomass that collected in the effluent pipes was determined gravimetrically when the pipes were cleaned periodically.

Ozone post-treatment

Ozonation was performed on the effluents of both R_{control} and R_{ozone} (i.e GDWW that had received pre-treatments and had undergone anaerobic digestion). Ozone was generated by a GENESIS™ corona discharge O₃-generator (Del Ozone®) which was able to produce 2 g O₃.h⁻¹ (Anon., 2012), as previously described (Chapter 3). An ozone dosage of 300 mg.L⁻¹ was applied, due to economic reasons, findings in literature for toxicity of ozone and the possible degradation of recalcitrant compounds at this dosage, as mentioned in previous work (Chapter 3).

Analytical methods

Standard Methods were used to measure fats, oils and grease (FOG), total suspended solids (TSS) and volatile suspended solids (VSS) (APHA, 1998) in the raw, coagulant treated and ozonated GDWW substrates, as well as the reactor effluents. The COD was

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

Carbon source/Macro nutrient	Concentration (g.L⁻¹)
Peptone	250
Glucose	850
Beef Extract	175
CaCl ₂ • H ₂ O	12
MgSO ₄ • 7H ₂ O	13.5
NH ₄ Cl	200
FeSO ₄ • 7H ₂ O	20
KH ₂ PO ₄	50

Table 2 Composition of trace element solution (Nel *et al.*, 1985)

Trace element	Concentration (g.L⁻¹)
B (as H ₃ BO ₃)	1.244 x 10 ⁻⁵
Ca (as CaCl ₂)	0.036
Mn (as MnSO ₄ • 5H ₂ O)	2.41 x 10 ⁻⁴
Zn (as ZnCl ₂)	2.015 x 10 ⁻⁴
Mg (as MgCl ₂ • 6H ₂ O)	0.024
Co (as CoCl ₂)	0.91 x 10 ⁻⁴
Mo (as MoO ₃)	6.6 x 10 ⁻⁵
Al (as AlCl ₃)	8.1 x 10 ⁻⁵
Se (as H ₂ SeO)	9.2 x 10 ⁻⁵
Si (as SiO ₂)	3.5 x 10 ⁻⁶
W (as Na ₂ WO ₄ • 2H ₂ O)	1.67 x 10 ⁻⁶
Ni (as NiCl ₂)	6 x 10 ⁻⁶

measured colorimetrically using a DR2000 spectrophotometer (Hach Co. Loveland, CO) and standard methods (APHA, 1998). Biomass washout was measured volumetrically according to Standard Methods (APHA, 1998) and also gravimetrically.

For the FOG analysis a method adapted from APHA (1998) was used. A wastewater sample of 50 g was collected and acidified to pH 2.0 with a mixture of concentrated sulphuric acid (H₂SO₄) and distilled H₂O (1:1). This sample was placed in a separator funnel and 100 mL absolute ethanol ($\geq 99.8\%$) and 40 mL of a 1:1 hexane fraction and diethyl ether solution was added. This was shaken vigorously for 2 mins and the two layers were allowed to separate. The solvent layer, containing the FOG was collected. This procedure was repeated twice. This sample was distilled in a rotary evaporator (Büchi Rotavapor R-114) at 55 - 60°C, and quantified gravimetrically to mgFOG.L⁻¹.

The biogas composition (CH₄ and CO₂) was determined with a Varian 3300 gas chromatograph (Varian Inc., Palo Alto, CA) and a Varian 4290 integrator. 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 carrier gas at a flow rate of 30 mL.min⁻¹ and the oven temperature was set at 55°C. An injection volume of 0.2 mL was used (Sigge, 2005). All analyses were done in duplicate.

Results and Discussion

To monitor the performance of the two UASB reactors, several efficiency parameters were monitored during the 277 day study period, for the substrate and effluent of both reactors. The reactors were operated in different phases (A - D) and the results for the operational efficiency parameters that were monitored are summarised in Table 3. Different feeding strategies were followed during each of the phases, and the OLR was increased stepwise, to reach a final OLR of ca. 9 kgCOD.m⁻³d⁻¹ (day 277) (Table 3).

Phase A (day 0 - 90)

The first phase (A) involved the start-up and stabilisation of both reactors. From day 0 to 90 the COD concentration was increased stepwise from ca. 900 to 2 300 mg.L⁻¹ for R_{control} and from ca. 600 to 2 700 mg.L⁻¹ for R_{ozone}, which corresponds to an organic loading rate (OLR) of 0.9 to 2.3 and 0.6 to 2.7 kgCOD.m⁻³d⁻¹, respectively (Fig. 2; Table 3). At the beginning of phase A, the COD reduction of both reactors was inconsistent (Fig. 2), and the alkalinity of both reactors was below 1 000 mgCaCO₃.L⁻¹ (Fig. 3) which is below the optimal range for anaerobic reactors (2 000 – 3 000 mgCaCO₃.L⁻¹) (Anderson *et al.*, 2003). From day nine the pH of the substrate was then increased for both reactors to pH 9.2

Table 3 Operational parameter ranges for R_{control} and R_{ozone} during the four phases (A - D) of the treatment of pre-treated GDWW

Parameter	Phase A (day 0 - 90)		Phase B (day 91 - 150)		Phase C (day 151 - 230)		Phase D (day 231 - 277)	
	R_{control}	R_{ozone}	R_{control}	R_{ozone}	R_{control}	R_{ozone}	R_{control}	R_{ozone}
OLR ($\text{kgCOD}\cdot\text{m}^{-3}\text{d}^{-1}$)	1.02 - 2.22	0.80 - 2.67	2.34 - 2.92	2.67 - 2.92	3.22 - 8.25	2.93 - 8.74	9.19 - 9.47	8.63 - 9.22
COD reduction (%)	62 - 86	53 - 82	72 - 87	61 - 79	74 - 91	72 - 90	80 - 96	81 - 92
Substrate FOG ($\text{mg}\cdot\text{L}^{-1}$)	-	-	91 - 190	55 - 148	36 - 428	28 - 264	231 - 458	67 - 430
FOG reduction (%)	-	-	23 - 65	25 - 70	26 - 83	33 - 92	65 - 88	45 - 90
Biogas volume ($\text{L}\cdot\text{d}^{-1}$)	0.19 - 0.53	0.59 - 1.64	0.53 - 1.53	1.64 - 3.63	1.72 - 8.09	3.74 - 15.05	8.09 - 11.34	15.05 - 16.31
Methane (%)	-	-	59 - 66	61 - 67	58 - 66	57 - 70	59 - 63	53 - 66
Substrate pH	7.0 - 9.2	7.0 - 9.2	7.0	7.0	7.0	7.0	7.0	7.0
Effluent pH	6.5 - 7.8	6.6 - 7.7	7.0 - 7.4	7.1 - 7.5	6.9 - 7.5	6.8 - 7.7	7.1 - 7.6	7.0 - 7.6
Alkalinity ($\text{mgCaCO}_3\cdot\text{L}^{-1}$)	750 - 3 625	813 - 3 850	2 875 - 3 350	2 600 - 3 300	2 638 - 3 563	2 375 - 4 450	3 600 - 4 338	3 050 - 4 200

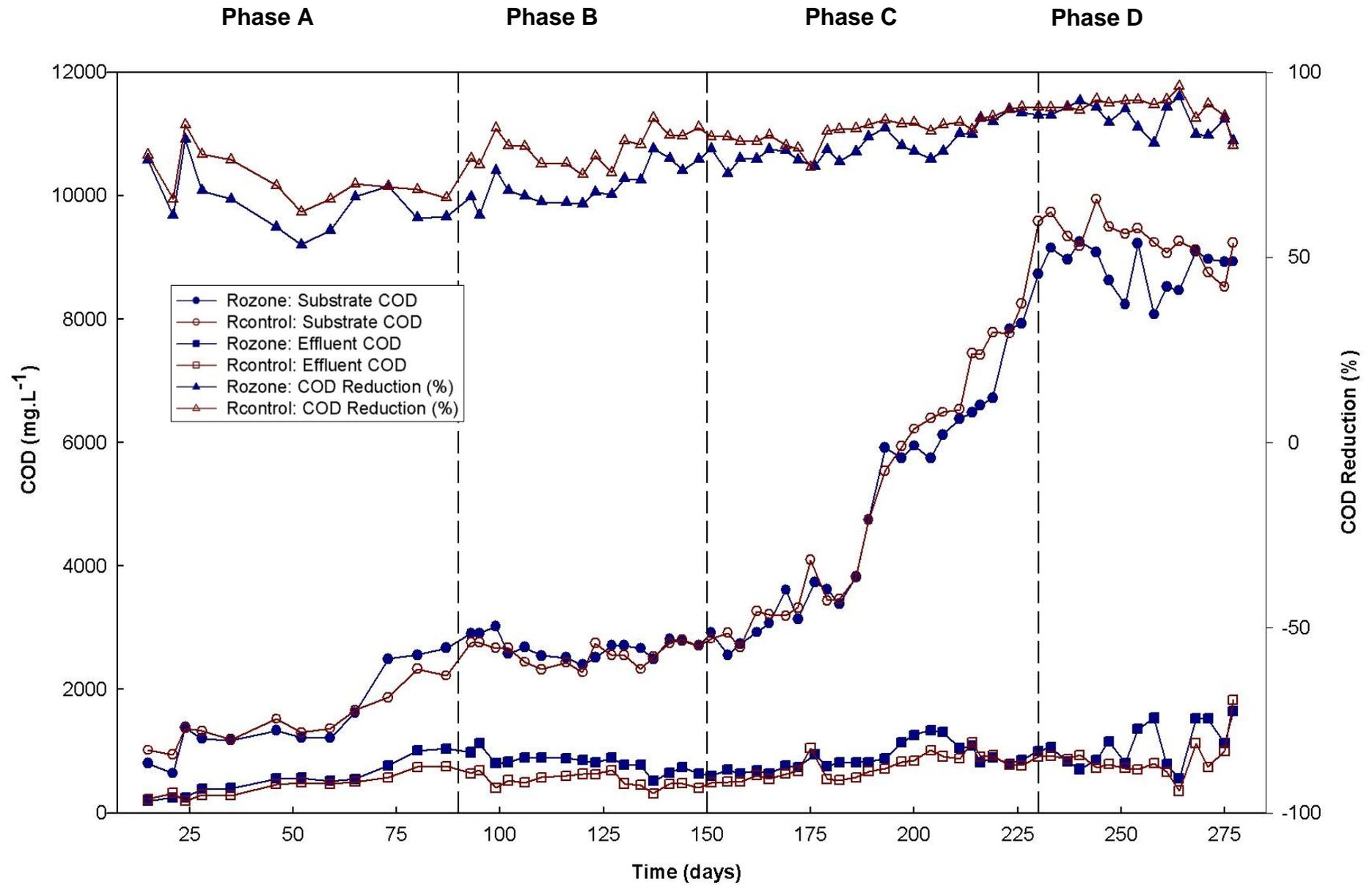


Figure 2 Substrate and effluent CODs and COD reduction (%) of the two UASB reactors treating pre-treated GDWW.

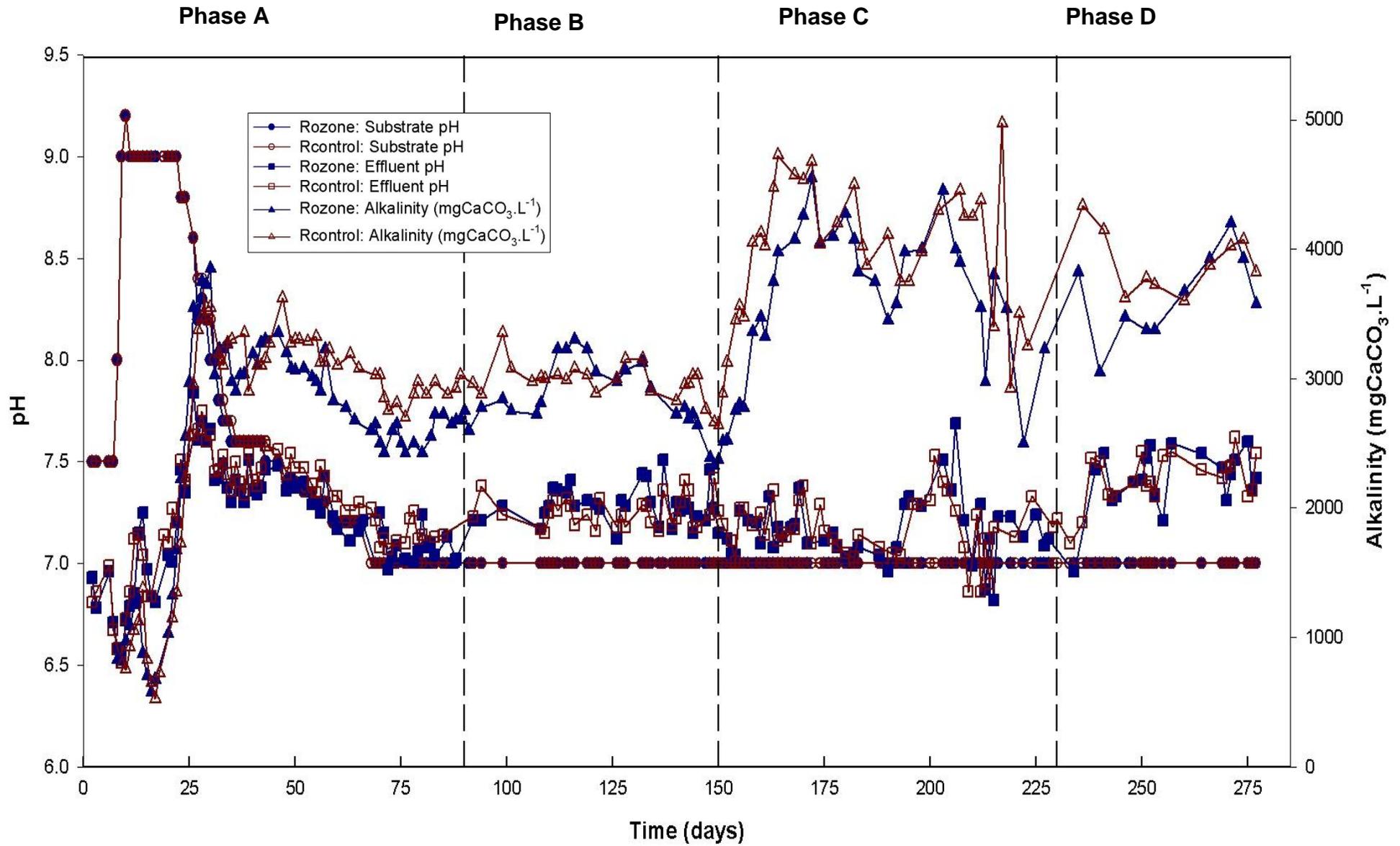


Figure 3 Substrate and effluent pHs and alkalinity of the two UASB reactors treating pre-treated GDWW.

(Fig 3). The pH of the reactor substrates was then decreased gradually from day 23 (Fig. 3). During this time reactor pH in both reactors increased to ca. 7.5 by day 25. After the substrate pH was increased, the alkalinity varied between ca. 500 and 1 000 mgCaCO₃.L⁻¹, but increased steadily from ca. 1 000 to 3 500 mgCaCO₃.L⁻¹ by day 40. After the substrate pH was increased an initial COD reduction (%) increase also occurred for both reactors, but then a COD reduction (%) decrease was seen until day 52 (Fig. 2). Thereafter the COD reduction started to increase again (from day 59), but a decrease was seen at the end of phase A.

The effluent pH decreased gradually until day 67 (substrate pH set at 7.0) to stabilise at pHs between 7.2 and 7.5 (Fig. 3). The COD reduction reached 66 and 61% for R_{control} and R_{ozone}, respectively, by day 87 (Fig. 2). Biogas production (Fig. 4) was low during phase A (day 0 - 90), possibly due to the low OLR (maximum OLR reached = 2.22 and 2.49 for R_{control} and R_{ozone}, respectively (Table 3)), and ranged from 0.19 – 0.53 L.d⁻¹ for R_{control} and from 0.59 – 1.64 L.d⁻¹ for R_{ozone}.

Phase B (day 91 - 150)

During the second phase the OLRs of both reactors were kept within a range as per batch (Table 3) and this was done to acclimatise the biomass to a low FOG value, as acclimated biomass has an increased ability to degrade lipid containing wastewater (Conçalves *et al.*, 2011). The COD concentration which was maintained during this phase was equal to ca. 50% of that of a local whisky distillery (ca. 3 500 mg.L⁻¹). The COD reduction (%) continued to increase and by day 150 both reactors had reached ca. 80% reduction (Table 3). The constant COD concentration of the substrates corresponded to the slow increase in biogas (0.53 - 1.53 L.d⁻¹ for R_{control} and from 1.64 - 3.63 L.d⁻¹ for R_{ozone}) (Fig. 4). The substrate FOG contents decreased slightly during phase B (Fig. 5) possibly due to fluctuations in FOG concentration between different batches of GDWW. In the decanter the FOG layer floats at the top of the GDWW, and thus the sampling time could have caused the FOG concentration to vary between different batches of GDWW. Fluctuating FOG reductions were observed throughout phase B (Fig. 5). The substrate pH was kept at 7.0 throughout phase B, and the effluent pH for both reactors remained between 7.0 and 7.5, which is slightly outside the optimal range for methanogens (6.8 - 7.2) (Gerardi, 2003). The alkalinity remained in a range between 2 600 – 3 200 mgCaCO₃.L⁻¹ throughout phase B and a slight decrease was observed at the end of the phase for both reactors (especially for R_{ozone} from day 145). As a decrease in alkalinity may indicate an increase in volatile fatty acids with a subsequent decrease in the buffer capacity of the anaerobic

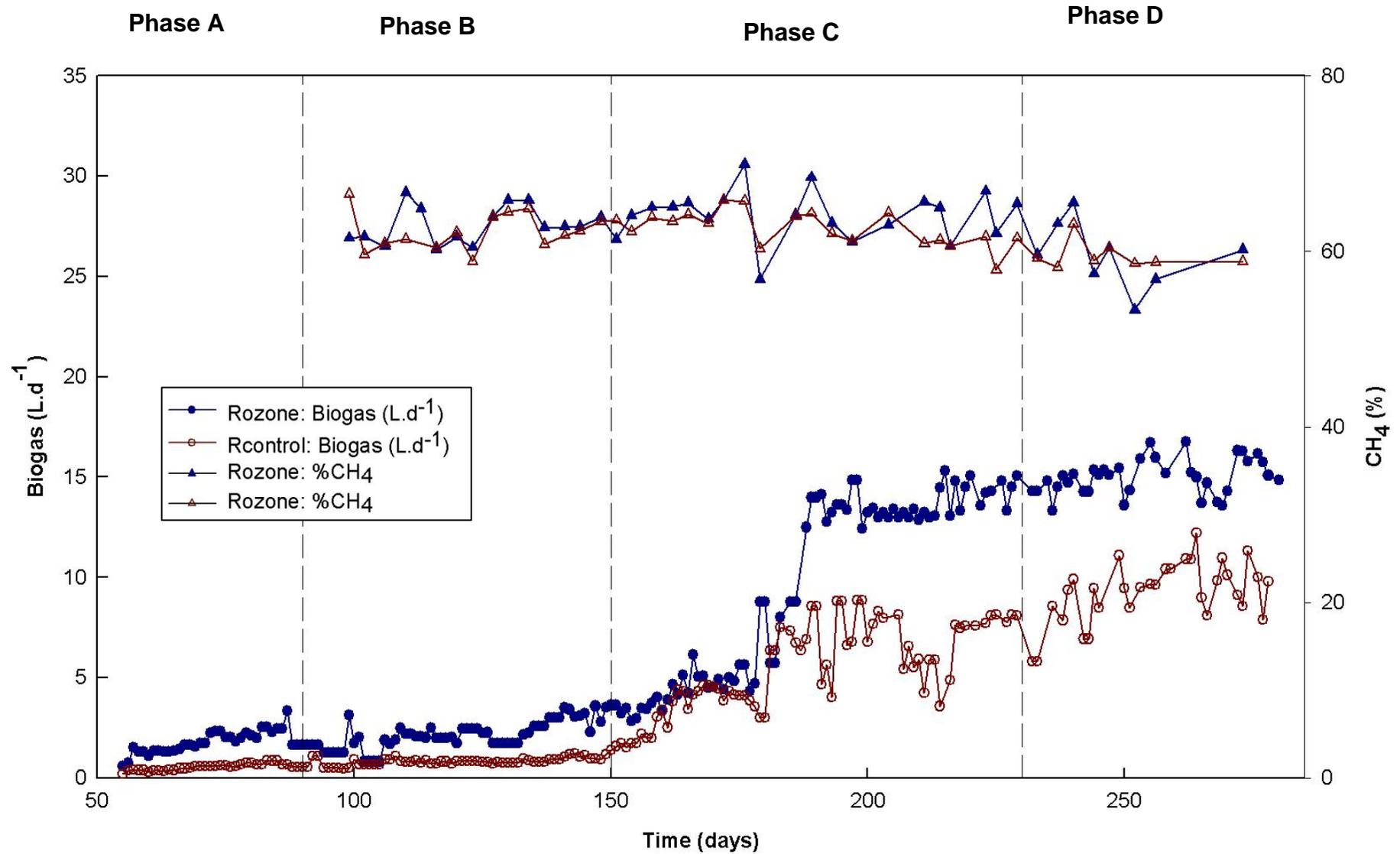


Figure 4 Biogas production and methane content (%) of the two UASB reactors treating pre-treated GDWW.

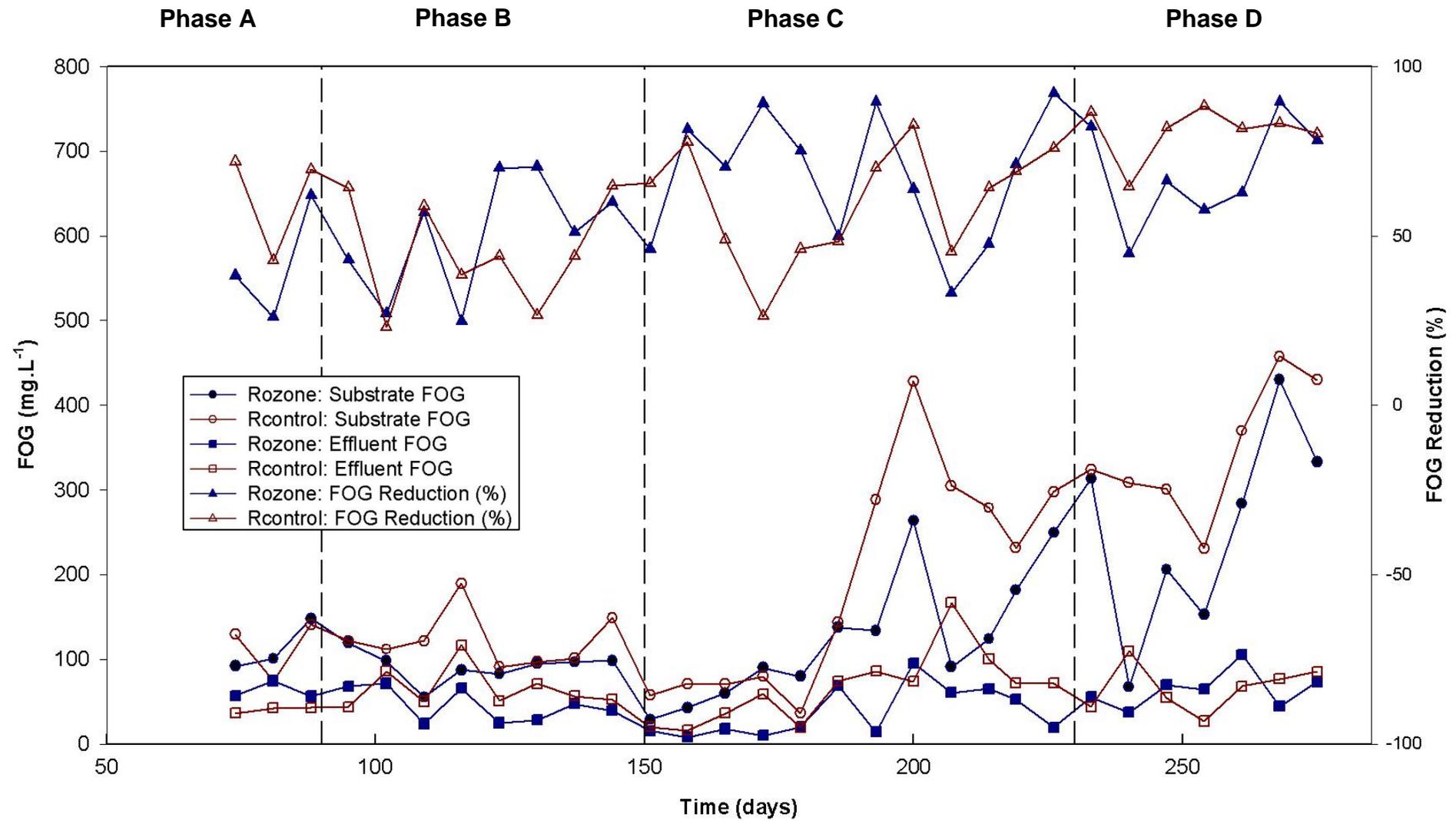


Figure 5 Substrate and effluent FOG values and the FOG reduction (%) of the two UASB reactors treating pre-treated GDWW.

digestion system, a decrease in pH could be expected. However, the pH still remained stable and no detrimental effect followed after the decrease in alkalinity from day 120 (Fig. 3). The R_{control} continued to show a slightly higher alkalinity than the R_{ozone} (Fig. 3).

Phase C (day 151 - 230)

In this phase the substrate COD concentration was increased. The aim was to reach a COD concentration which is in line with that of a local distillery (ca. 7 000 mg.L⁻¹). From day 151 to 230 the COD concentration was increased from ca. 3 000 to 8 250 mg.L⁻¹ for R_{control} and 3 000 to 8 700 mg.L⁻¹ for R_{ozone} (Fig. 2). This corresponds to an OLR of 3.0 to 8.3 and 3.0 to 8.7 kgCOD.m⁻³d⁻¹ for R_{control} and R_{ozone} , respectively (Table 3). During this phase the COD reduction continued to increase from 74 to 91% for R_{control} and from 72 to 90% for R_{ozone} (Fig. 2; Table 3). The COD increase was reflected in the overall increase in the substrate FOG concentration for both reactors, i.e. from ca. 35 and 30 mgFOG.L⁻¹ on day 151 (for R_{control} and R_{ozone} , respectively) to ca. 400 and 250 mgFOG.L⁻¹ on day 230 (for R_{control} and R_{ozone} , respectively) (Fig 5; Table 3). Even though the COD was continuously increased in this phase, the FOG substrate concentration varied (Fig. 5). This was most likely due to seasonal fluctuations in the maize substrate, which led to compositional differences in GDWW batches.

Ozonation leads to a decrease in lipids (Andreozzi *et al.*, 1998) and this is a possible explanation for the higher substrate FOG concentration of R_{control} compared to that of R_{ozone} . This occurrence was discussed in previous work reported in Chapter 3 of this thesis where a reduction of up to 16% was achieved at an ozone dosage of 300 mgO₃.L⁻¹. In this study the FOG reductions varied from week to week, and during phase C, R_{ozone} generally showed a better performance compared to R_{control} (Fig. 5).

An increase in OLR (Table 3) implies an increase in organic compounds which could potentially be converted to biogas and this was confirmed by an increase in biogas produced (Fig. 4). The biogas volume increased up to 8.09 L.d⁻¹ for R_{control} and 15.05 L.d⁻¹ for R_{ozone} during phase C (Table 3). One possible explanation for this could be that R_{control} utilised the available carbon sources for cell multiplication instead of biogas production (Gerardi, 2003). Although the COD was increased (Fig. 2) the pH did not drop below 6.82, and ranged between 6.82 and 7.69 (Fig. 3). However, both reactors showed instability and a decrease in alkalinity and fluctuations in pH from day 190 to 215 (Fig. 3). During this time the FOG concentration was higher in the substrate of both reactors (Fig. 5), and therefore the possible increased production of volatile fatty acids could have occurred, which could have resulted in fluctuations in pH (Fig. 3).

Overall, during phase C, it was expected that the alkalinity in the reactors would decrease as the volatile fatty acids would increase, due to the increase in substrate COD concentration (Figs. 2 & 3). However, the alkalinity increased to 4 700 mgCaCO₃.L⁻¹ for R_{control} and 4 500 mgCaCO₃.L⁻¹ for R_{ozone} by day 230. This was outside the optimal alkalinity range of 2 000 – 3 000 recommended by Anderson (2003) (Fig. 3). This could possibly be accounted for by the increased volume of CO₂ produced (increased biogas production (Fig. 4) from day 180) resulting in an increase in bicarbonate alkalinity (Bitton, 2005).

Phase D (day 231 - 277)

The aim of the last phase was to keep the OLR constant to investigate the differences in reactor performance while the OLR was kept in a range which is slightly higher than that of a local distillery (ca. 7 000 mg.L⁻¹). The COD concentration remained between 9 200 and 9 500 for R_{control} and between 8 600 and 9200 for R_{ozone} (corresponding to an OLR of 9.2 to 9.5 for R_{control} and 8.6 - 9.2 for R_{ozone}). Fluctuations in COD reduction (%) were observed but remained between ca. 80 - 96% for R_{control} and ca. 81 - 93% for R_{ozone} (Table 3). During the trial the maximum COD reductions (%) achieved were ca. 96% for R_{control} and 93% for R_{ozone} (Fig. 2; Table 3). As ozone degrades organic compounds and has been shown to increase the biodegradability of substrates during anaerobic digestion (Sigge *et al.*, 2007), it was expected that R_{ozone} would display a higher COD reduction, however, throughout the trial period, R_{control} displayed a higher reduction (Fig. 2). A possible explanation of this could be that the COD in the substrate of R_{ozone} had been broken down into more simple compounds as a result of the ozone treatment, but was not yet completely oxidised. In agreement with a small increase in COD concentration, the biogas volumes remained within a narrow range as well (Table 3). In phase D, biogas volumes of 11.34 and 16.31 L.d⁻¹ was reached for R_{control} and R_{ozone}, respectively (Fig. 4; Table 3).

The FOG concentration in the substrate did, however, not remain constant. A decrease was seen from day 230 followed by an increase from day 261 to 277 (Fig. 5). No relationship could be seen between substrate FOG concentration and FOG removal. One explanation for this could be that the FOG accumulated in the reactor. This could have led to higher FOG in the effluent at times. Throughout phases A to D variations in the FOG reduction (%) were observed and therefore no conclusive differences were evident in terms of the FOG degradation of the two reactors. However, over the entire trial period the substrate FOG concentration for R_{control} (average = 201.22 mgFOG.L⁻¹) was

higher than that of R_{ozone} (average = 144.65 mgFOG.L⁻¹) (Fig. 5) and this is ascribed to the ozone treatment received by R_{ozone} .

Decreases in alkalinity took place during phase D (from day 231 to 240). The lowest points were 3 600 mgCaCO₃.L⁻¹ for R_{control} and 3 050 mgCaCO₃.L⁻¹ for R_{ozone} . From day 241 the alkalinity started to increase and continued to increase until day 271 after which a decrease was observed again for both reactors (Fig. 3). Throughout the trial R_{ozone} had a lower alkalinity than R_{control} , possibly due to the ozone pre-treatment. Ozone scavenges alkalinity, and therefore it is expected that the substrate that had undergone ozone pre-treatment would have a lower alkalinity (Lucas *et al.*, 2009). The pH increased in the last phase and remained between pH 6.9 and 7.6 for both reactors (Fig. 3).

UASB granule washout

Granule washout occurred in both reactors during phase A to D. Figure 6 shows an example of the granule washout in the U-tubes at the outlets of the reactor. More biomass washout was visually observed for R_{control} . Additionally, R_{control} experienced more pipe clogging compared to R_{ozone} and the biomass washout that collected in the effluent pipes amounted to 75.60 g for R_{control} and 21.50 g for R_{ozone} . This washout is not very high, but possibly indicated a higher amount of LCFAs present in the substrate of R_{control} , which could have caused a decrease in granule density, and have led to washout (Hwu *et al.*, 1998).

Ozone post treatment

The results for the COD and FOG reduction and ozone efficiency, for the ozone post-treatment (300 mgO₃.L⁻¹), are summarised in Table 4. For the COD reduction the ozone efficiency for R_{ozone} (0.95 mgCOD.mg⁻¹O₃⁻¹) was 13% higher than that of R_{control} (0.83 mgCOD.mg⁻¹O₃⁻¹). In the case of FOG the ozone efficiency for R_{ozone} (0.07 mgFOG.mg⁻¹O₃⁻¹) was 43% higher than that of R_{control} (0.03 mgFOG.mg⁻¹O₃⁻¹). From these results it can be said that the effluent of R_{ozone} was more readily oxidisable after the anaerobic digestion process, in terms of COD and FOG. The COD values for both effluents were within the regulatory limit (< 5 000 mg.L⁻¹), but both FOG values were still above the limit (< 2.5 mg.L⁻¹), which makes the effluent unsuitable for irrigation on land for volumes of up to 50 m³.d⁻¹ (Republic of South Africa, 2004). By lowering the COD and FOG content, factories could save on the tariffs charged when discharging to the municipal wastewater treatment works (Bailey, 2004).



Figure 6 Images of granule washout in the U-tubes of R_{ozone} (left) and R_{control} (right).

Table 4 COD, FOG and O₃ efficiency values for 300 mg.L⁻¹ ozone post-treatment performed on the effluents of R_{control} and R_{ozone}

Parameters	Pre O ₃ post-treatment		Post O ₃ post-treatment		O ₃ efficiency	
	COD (mg.L ⁻¹)	FOG (mg.L ⁻¹)	COD (mg.L ⁻¹)	FOG (mg.L ⁻¹)	mg COD degraded (mgCOD.mg ⁻¹ O ₃ ⁻¹)	mg FOG degraded (mgFOG.mg ⁻¹ O ₃ ⁻¹)
R _{control}	1129	85	880	73	0.83	0.04
R _{ozone}	1377	73	1094	52	0.95	0.07

Conclusions

High strength wastewater is produced during the whisky production process with volumes exceeding that of other beverage industries. To prevent environmental pollution, this wastewater (GDWW) must be treated efficiently. The data from this study showed the value of the pre-treatment of FOG containing GDWW prior to the main biological treatment. It is also important to ensure that compounds that will influence the anaerobic digestion process negatively are satisfactorily lowered.

The substrate of the R_{control} reactor received a coagulant pre-treatment, which decreased the FOG content in the GDWW. The substrate of the R_{ozone} reactor also received a coagulant pre-treatment and an additional ozone pre-treatment. It was hypothesised that the ozone pre-treatment would lead to an increase in the biodegradability of the GDWW and that this would be evident during the main anaerobic digestion treatment step. However, the coagulant pre-treatment was found to be satisfactory for the control UASB reactor to successfully treat the GDWW and therefore it was found to remove the inhibiting compounds (FOG in this case) one pre-treatment step is sufficient. This could have operational benefits as it will lead to cost and treatment time reductions.

Another problem is that FOG adsorption to granules has often been found to be toxic, resulting in granule floatation and in many cases, reactor failure. In this study the FOG present in GDWW substrates has on numerous occasions caused granule washout. The R_{control} reactor showed higher granule washout. This was ascribed to the fact that the GDWW substrate fed to the R_{control} contained more unsaturated fatty acids, which has been shown in the literature to be more toxic compared to saturated fatty acids. It is interesting to note that even though granule washout did take place it did not cause reactor failure or even a decrease in reactor performance.

The R_{Ozone} was found to produce more biogas and this could have an important economic benefit as biogas can subsequently be used for energy production. The characteristics of the effluent of the R_{Ozone} reactor also resulted in greater ozone degradation efficiency during the ozone post-treatment step. This showed that the ozone pre-treatment possibly led to an easier degradable wastewater and better results could potentially be obtained (COD and FOG reduction) when other post-treatment steps are applied to treat the effluent of the R_{Ozone} .

The final COD concentrations of the effluents from the R_{control} and R_{Ozone} reactors were lower than $5\,000\text{ mg.L}^{-1}$. This would make it suitable for irrigation on land for volumes of up to $50\text{ m}^3\text{.d}^{-1}$ (Republic of South Africa, 2004). However, the effluent FOG values remain too high ($> 2.5\text{ mg.L}^{-1}$) and this will therefore disqualify land applications as an option for effluent disposal (Republic of South Africa, 2004). Further investigation of technologies to facilitate a decrease in the FOG content is therefore required. It is also recommended that longer trial periods must be evaluated to determine the long term effect of the pre-treatment methods.

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Chapter 5

GENERAL DISCUSSION AND CONCLUSIONS

The tradition of making whisky has been practised and enjoyed in many parts of the world for centuries (Anon., 2010). As this spirit has gained popularity and subsequent increased demand, producers are faced with the consequential challenge of managing its environmental impact (SWA, 2012; Magee, 2013) as the distillery industry generates large volumes of wastewater annually. A local example is a distillery in Wellington, Western Cape, South Africa, which generates 63 – 121 million litres of wastewater annually (Green, J., 2012, Process Manager, The James Sedgwick Distillery, Wellington, South Africa. Personal communication, 28 May). This wastewater cannot be reused for land irrigation, nor can it be disposed of, unless it is treated to reduce the negative environmental impact (Republic of South Africa, 2004). This implies that the successful treatment of grain distillery wastewater (GDWW) is essential.

The UASB process is known to bring about high chemical oxygen demand (COD) reductions (> 90%) making it an efficient treatment option for different types of waste, including distillery wastes. However, operational problems have been reported during the treatment of GDWW, due to the high solids, as well as the high fats, oils and grease (FOG) contents. Even though anaerobic digestion (AD) treatment has numerous benefits and has been shown to be successful in the treatment of distillery waste, the pre-treatment of the substrates is essential, especially if FOG is present, to ensure the success of the AD process.

The overall objective of this study was to investigate the operational efficiency of UASB reactors while treating pre-treated GDWW as substrate. This was achieved by firstly focussing on optimising the pre-treatment of GDWW. Secondly, the effect of different pre-treatments on the subsequent AD treatment was investigated in upflow anaerobic sludge blanket (UASB) reactors.

The high FOG content of GDWW has led to the formation of a lipid layer around the UASB granules in the past (Gie, 2007), which inhibits nutrient and metabolite transfer, leading to the washout of granules. This can ultimately result in reactor failure (Hwu *et al.*, 1998). The first aim therefore was to optimise pre-treatment to decrease the FOG present in GDWW. This was done by determining the effect of pH, coagulant concentration and ozone dose on the FOG reduction. As the pH of GDWW is very low (ca. pH 3.4 – 3.5) it

was decided to optimise the pH for optimal coagulant performance, as coagulants have shown to have increased efficiency at higher pH values (Pernitsky & Edzwald, 2006). It was found that higher pH values increased the efficiency of the coagulant (pH 7.0). Two different coagulant concentrations (100 mgACH.L^{-1} and 140 mgACH.L^{-1}) were also investigated to determine whether pH changes would increase the coagulant efficiency to the extent that a lower concentration could be applied. It was argued that this would result in cost savings and decrease the overall process operational cost. It was found that the coagulant concentration was a greater determining factor compared to wastewater pH, as a higher concentration lead to higher reductions (COD, TSS and FOG).

As an additional pre-treatment, the application of different ozone concentrations was also investigated. Ozone was more efficient for FOG reduction (in terms of in terms of mg FOG reduced per mg ozone) at a low dosage ($100 \text{ mgO}_3.\text{L}^{-1}$), and thus it would be a viable treatment option for the reduction of FOG. This was also found by other researchers (Andreozzi *et al.*, 1998). Polymerisation of solids took place upon ozonation, but this decreased as the ozone dosage was increased. Low COD reductions were observed, and a further biological treatment step is therefore deemed necessary to reduce the COD of GDWW. The final pre-treatment thus included pH adaption to 7.0, coagulant dosage of 140 mgACH.L^{-1} , and an ozone treatment ($300 \text{ mgO}_3.\text{L}^{-1}$).

The second aim of this study was to compare the performance of two UASB reactors, of which the first received GDWW pre-treated with coagulant and was considered the study control (R_{control}). The substrate of the second UASB reactor (R_{ozone}) received the same and an additional ozone pre-treatment. Since literature reports that ozone increases the biodegradability of substrates (Sigge *et al.*, 2007), it was hypothesised that the reactor receiving the ozone pre-treated substrate would show an increased performance compared to the reactor which received only the coagulant pre-treated substrate. The data from this study showed that the COD and FOG reduction results did not indicate increased reactor performance as a result of the additional ozone pre-treatment. The reactor receiving ozone pre-treated substrate did, however, show higher biogas production. The reactor that did not receive an ozone pre-treated substrate showed higher bio-granule washout, possibly due to LCFA adsorption (Hwu *et al.*, 1998). It was interesting to note that this did not cause decreases in reactor performance. It can be speculated that if granule washout increased, the reactor performance could possibly be affected negatively. From this it can be concluded that the FOG levels in GDWW were lowered sufficiently after coagulant pre-treatment to allow satisfactory reactor performance.

During this study the effluent from R_{Ozone} showed better results regarding COD and FOG degradation when compared to that of the R_{Control} , when subjected to the ozone post-treatment. The effluent of the R_{Ozone} could potentially show better results when other post-treatment methods are applied.

Future research and concluding remarks

During this study certain points were identified that could be addressed in future. This includes the investigation of the zeta potential of particles present in GDWW. As zeta potential gives an indication of the electrostatic potential near the surface of the particle (Al-Shamrani *et al.*, 2002), a more in depth investigation thereof could lead to even better reduction efficiencies of the suspended solids content in GDWW.

It was also found that the solid content at times showed coagulation during the ozonation experiments. Future research should thus include the investigation of ozone as an aid during coagulation. This could lead to oxidation and coagulation possibly becoming a one-step pre-treatment process, subsequently leading to a reduction in treatment time and cost. This is also important for process operation, as the behaviour of solids and the impact it would have on the subsequent treatment, would have to be investigated so as to avoid process upsets.

Further tests need to be conducted to evaluate the biodegradability of the two effluents to determine what the effect will be when different post-treatment techniques are used. Even though the granule washout that took place did not negatively influence reactor performance of either of the reactors, extended trials should be conducted to determine the long term effect of the discussed pre-treatments on the efficiency of UASB reactors.

After the ozone post-treatment step the final FOG value was still above the regulatory limits, with the result that the effluents from both reactors cannot be used for direct land application (Republic of South Africa, 2004). Further investigation into FOG lowering treatment steps is therefore required to possibly allow this application, or to lower discharge costs to the municipal discharge system. If the COD value could be lowered (to $< 400 \text{ mg.L}^{-1}$), it would be possible for larger volumes of water (up to $500 \text{ m}^3.\text{d}^{-1}$) to be disposed of in this manner (Republic of South Africa, 2004). The main advantage for the lowering of the COD for a large factory would be the decrease in tariffs charged when discharging to the municipal wastewater treatment works.

This research provided insights into the application of the pre-treatment of GDWW and the impact thereof on the subsequent biological treatment process. This could have

important impacts on the economics of the treatment process. As methane is produced as a by-product during anaerobic digestion, this could be directly applied as energy to the production process (SWA, 2012).

Water reuse and recycling is as important as energy, if not more so, especially in South Africa. As current predictions point towards a water shortage in 2025, water regulations will become stricter (WWF, 2013). Food and beverage producers must therefore strive towards a zero effluent discharge system, which will demand high efficiency treatment systems.

Although pre-treatment is beneficial for the anaerobic digestion process, the effects thereof need to be seen in relation to the overall effect on process time and energy consumption. Factors including bio-granule washout are concerning as it cannot be said that anaerobic reactors will maintain a continuous efficient performance over time.

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