ENHANCEMENT OF THE BIODEGRADABILITY OF GRAIN DISTILLERY WASTEWATER TO IMPROVE UPFLOW ANAEROBIC SLUDGE BLANKET REACTOR EFFICIENCY

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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any other university for a degree.

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ABSTRACT

The distillery industry generates large volumes of heavily polluted wastewater and thus effective wastewater treatment is essential. It has been reported that a chemical oxygen demand (COD) reduction of more than 90% can be achieved when wine distillery wastewater (WDWW) is treated in an upflow anaerobic sludge blanket (UASB) reactor. The first objective of this study was to investigate UASB treatment of WDWW and to try to enhance the efficiency by using ozonation treatments. Secondly, the impact of grain distillery wastewater (GDWW) on UASB granules was determined. The third objective was to determine whether ozonation and enzymatic treatment combinations might improve the biodegradability of GDWW and thus make GDWW more amenable to UASB treatment.

It was found that UASB treatment combined with ozonation improved the WDWW treatment efficiency. When diluted WDWW (chemical oxygen demand COD = 4 000 $mg.L^{-1}$) was ozonated (dose = 47 $mg.L^{-1}$) in a 50 L venturi circulating contactor system, the COD reduction was 7%. When WDWW was treated in a laboratory-scale UASB reactor (substrate pH = 7.0, COD = 4 000 $mg.L^{-1}$ and organic loading rate (OLR) = 4.0 kg $COD.m^{-3}.d^{-1}$), the COD reduction was 92%. When the UASB treatment was combined with either pre- or post-ozonation, the COD reduction was 94 and 96%, respectively. When UASB treatment was combined with pre- and post-ozonation, a COD reduction of 98% was achieved. The activity of the UASB granules was also found to improve over time, despite the addition of the ozonation treatment.

It has been reported that operational problems occur when GDWW is treated in an UASB reactor as a result of the encapsulation of the granules. This was confirmed when granules from a full-scale UASB treating WDWW became encapsulated in a layer after being exposed to GDWW (COD = 4 000 $mg.L^{-1}$) for 24 d. The results showed that the lipid content of the granules increased from 1.25 to 60.35 mg $lipid.g^{-1}$ granule over the 24 d exposure period. Therefore, granules exposed to GDWW were encapsulated in a lipid-rich layer and as a result the contact between the GDWW and microbial consortium in the granules was reduced. The operational problems found during the industrial UASB treatment of GDWW were ascribed to the encapsulation of the granules.

Combinations of ozonation (dose = 1 476 $mg.L^{-1}$) generated in a 2 L bubble column and enzymatic treatments (1% FogFree^{TM} (FF) dosage and 2 d incubation at 35°C) were

found to improve the biodegradability of GDWW. This improvement was in terms of lipid reduction in GDWW, granule activity and visual appearance of the encapsulating layer of the granules. The highest lipid reduction (90%), highest granule activity, lowest lipid content of the granules (3.74 ± 0.10 $mg.g^{-1}$ granule) and best visual appearance were achieved in ozonated GDWW treated with 1% FF, followed by just ozonation. The higher lipid reduction and subsequent higher granule activity were ascribed to the reduction in lipids which resulted in the fact that fewer lipids were available to encapsulate the granules. As a result of the lipid reduction, the granule activity improved and the GDWW was made more amenable to UASB treatment.

This study proved that UASB treatment combined with ozonation led to an enhancement of the treatment efficiency of WDWW. It was also found that the cause of the operational problems during UASB treatment of GDWW was as a result of the granules being encapsulated in a lipid-rich layer. It was established that treating GDWW prior to UASB treatment improved the biodegradability of GDWW. The data from the study showed that high lipid reduction in the GDWW directly led to better granule activity, lower granule lipid content and a thinner encapsulating layer. Based on the data from this study, it is recommended that GDWW be ozonated prior to other treatments because it can be done in-line and the costs would be lower than that of enzymatic treatments.

OPSOMMING

Die distilleringsbedryf genereer groot volumes erg besoedelde afloopwater en gevolglik is die doeltreffende behandeling van afloopwater noodsaaklik. Daar is gerapporteer dat 'n reduksie van meer as 90% aan chemiese suurstofbehoefte (CSB) haalbaar is wanneer wyndistillering se afloopwater (WDAW) in 'n opvloei-anaërobieseslykkombers- (OAS-)reaktor behandel word. Die eerste doelwit van hierdie studie was om OAS-behandeling van WDAW te ondersoek en die doeltreffendheid daarvan met behulp van osoniseringsbehandelings te probeer verhoog. Tweedens is die impak van graandistillering se afloopwater (GDAW) op OAS-korrels vasgestel. Die derde doelwit was om te bepaal of kombinasies van osoniserings- en ensiematiese behandelings die bioafbreekbaarheid van GDAW sou verbeter, en sodoende GDAW meer geskik vir OAS-behandeling sou maak.

Daar is vasgestel dat OAS-behandeling in kombinasie met osonisering die doeltreffendheid van WDAW-behandeling verhoog het. Met die osonisering (dosis = 47 $mg.L^{-1}$) van verdunde WDAW (chemiese suurstofbehoefte CSB = 4 000 $mg.L^{-1}$) in 'n 50 L venturi-sirkulerende kontakstelsel was die CSB-reduksie 7%. Toe WDAW in 'n laboratoriumskaal-OAS-reaktor (substraat pH = 7.0, CSB = 4 000 $mg.L^{-1}$ en organiese ladingstempo (OLT) = 4.0 kg $CSB.m^{-3}.d^{-1}$) behandel is, was die CSB-reduksie 92%. Deur die OAS-behandeling met óf voor- óf na-osonering te kombineer, is 'n CSB-reduksie van onderskeidelik 94% en 96% bereik. Waar die OAS-behandeling met voor- én na-osonering gekombineer is, was die CSB-reduksie 98%. Daar is gevind dat die aktiwiteit van die OAS-korrels ook met verloop van tyd verbeter het, ten spyte van die bykomende osoneringsbehandeling.

Daar is gerapporteer dat bedryfsprobleme ontstaan wanneer GDAW in 'n OAS-reaktor behandel word, as gevolg van die enkapsulering van die korrels. Dit is bevestig toe korrels van 'n volskaalse OAS tydens die behandeling van WDAW in 'n laag geënkapsuleer is ná blootstelling aan GDAW (CSB = 4 000 $mg.L^{-1}$) oor 'n tydperk van 24 d. Die resultate het getoon dat die lipied-inhoud van die korrels toegeneem het van 1.25 tot 60.35 mg $lipied.g^{-1}$ korrel oor die blootstellingstydperk van 24 d. Dus is korrels wat aan GDAW blootgestel is in 'n lipied-ryke laag geënkapsuleer, wat gevolglik die kontak tussen die GDAW en die korrels verminder het. Die bedryfsprobleme wat tydens die industriële OAS-behandeling van GDAW voorgekom het, is aan die enkapsulering van die korrels toegeskryf.

Daar is gevind dat kombinasies van osonisering (dosering = 1 476 mg.L^{-1}) wat in ’n 2 L-borrelbuis gegenereer is, tesame met ensiematiese behandelings (1% FogFree^{TM} (FF) dosering en inkubasie van 2 d teen 35°C) die bioafbreekbaarheid van GDAW verhoog het. Hierdie verbetering het op die lipied-reduksie in GDAW, korrel-aktiwiteit en visuele voorkoms van die enkapsulerende laag van die korrels betrekking gehad. Die hoogste lipied-reduksie (90%), hoogste korrel-aktiwiteit, laagste lipied-inhoud van die korrels (3.74 ± 0.10 mg.g^{-1} korrel) en beste visuele voorkoms is bereik in geosoniseerde GDAW wat met 1% FF behandel is, gevolg deur slegs osonisering. Die hoër lipied-reduksie en gevolglike hoër korrel-aktiwiteit is toegeskryf aan die reduksie van lipiede, wat veroorsaak het dat daar minder lipiede beskikbaar was om die korrels te enkapsuleer. As gevolg van die lipied-reduksie het die korrel-aktiwiteit verbeter, waardeur die GDAW meer geskik vir OAS-behandeling gemaak is.

Hierdie studie het bewys dat OAS-behandeling in kombinasie met osonisering tot die doeltreffender behandeling van WDAW gelei het. Daar is ook vasgestel dat bedryfsprobleme tydens OAS-behandeling van GDAW die gevolg was van die korrels wat in ’n lipiedryke laag geënkapsuleer was. Daar is gevind dat die behandeling van GDAW voor OAS-behandeling die bioafbreekbaarheid van GDAW verbeter het. Die data van hierdie studie dui daarop dat hoë lipied-reduksie in die GDAW direk gelei het tot beter korrel-aktiwiteit, ’n laer korrel-lipiedinhoud en ’n dunner enkapsulerende laag. Op grond van die data in hierdie studie word daar aanbeveel dat die osonisering van GDAW voor ander behandelings moet plaasvind, omdat dit direk gedoen kan word en die koste laer as dié van ensiematiese behandelings sal wees.

Dedicated to my parents, Robert and Nulda Gie

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God the Almighty, for the glory and honour is His alone.

CONTENTS

The language and style used in this thesis are in accordance with the requirements of the *International Journal of Food Science and Technology*. This dissertation represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

CHAPTER 1

INTRODUCTION

In South Africa, of the 5.1 x 10^7 m^3 of potentially available annual rainwater, 74% is used for the production of crops, trees and livestock, while the remaining 26% is run-off to the sea or used for industrial, domestic and other purposes (Bennie & Hensley, 2001). Furthermore, as the population continues to grow, the availability of fresh water per capita is decreasing rapidly (Kivaisi, 2001). Since 1970, the global water demand has increased annually at an average rate of 2.4% (Kamara & Sally, 2003). It is predicted that by 2025 two thirds of the world's population will be living in physically water scarce countries (including South Africa) (Arnell, 2004). The scarcity of water is intensified by the decline in the quality of fresh water due to industrialisation (Kivaisi, 2001).

In South Africa, wineries and distilleries are considered to be of the most polluting industries (Bezuidenhout *et al.*, 2000). Alcohol is produced at distilleries using various substrates that usually undergo malting (if malted alcohol is produced), mashing, fermentation and distillation (Wilkie *et al*., 2000). This process generates large amounts of wastewater – up to 20 L of wastewater for each litre of ethanol manufactured (Beltrán *et al.*, 2001; Uzal *et al.*, 2003). For example, at a distillery in Wellington, Western Cape, South Africa, 72 to 118 $m^3.d^{-1}$ wine distillery wastewater (WDWW) is generated specifically during the grape harvest season, while an additional 30 to 50 $m^3.d^{-1}$ of grain distillery wastewater (GDWW) is generated during the remainder of the year (Laubscher, 2000). The amount and composition of distillery wastewater may exhibit major daily and seasonal variations due to the nature of the alcohol-producing industry, the substrate used, the specific production process utilised and the location of the industry (Bustamante *et al.*, 2005; Sangave & Pandit, 2006). It is generally characterised by a high chemical oxygen demand (COD) (10 000 – 60 000 $mg.L^{-1}$), low pH (3.5 – 4.0), high total solids (TS) content (20 500 – 52 000 $mg.L^{-1}$) and high total suspended solids (TSS) content (± 10 000 $mg.L^{-1}$) (Uzal *et al.*, 2003). GDWW is also rich in proteins (280 – 340 g crude $protein.kg^{-1}$ dry matter) and lipids (± 125 g $oil.kg^{-1}$ dry matter) (Hill, 2002). Therefore, this heavily polluted wastewater can lead to significant environmental and ecological problems (Bezuidenhout *et al.*, 2000; Bustamante *et al.*, 2005).

Effective treatment of distillery wastewater is imperative (Wilkie *et al*., 2000). Anaerobic biological digestion is a suitable distillery wastewater treatment due to the high

acidity, COD and temperature of the wastewater (Uzal *et al.*, 2003). The upflow anaerobic sludge blanket (UASB) reactor has effectively been used to treat distillery wastewater because of its high biomass concentration and rich microbial diversity (Liu *et al*., 2003). Goodwin and Stuart (1994) and Laubscher (2000) reported that when malt GDWW and WDWW, respectively, were treated in UASB reactors, COD reductions of more than 90% were achieved at organic loading rates (OLR) of approximately 15 kg $COD.m^{-3}.d^{-1}$.

Distillery wastewater often contains toxic compounds that may restrict the overall UASB reactor efficiency (Benitez *et al.*, 1999). When GDWW is treated in an UASB reactor, the following operational problems may occur: scum layer formation; foaming; hampered biogas release; a high degree of COD and organic or long chain fatty acid accumulation; extensive clogging; biomass or sludge washout and scaling (Lettinga & Hulshoff Pol, 1991; Laubscher, 2000; Zeeman & Sanders, 2001). These problems occur because the amount of contact between the bacteria and wastewater is reduced by grain particles that become entrapped in the UASB sludge bed and/or lipid molecules that enclose the sludge particles (Nadais *et al.*, 2001).

Additional wastewater treatments may be needed to improve the overall UASB treatment efficiency (Delgenès *et al.*, 2003). Pre- and/or post-ozonation has been used to improve the biodegradability of distillery wastewater (Beltrán *et al.*, 2000). Ozone (O_3) is selective towards the double bonds found in toxic, recalcitrant or refractory compounds such as lipids, volatile fatty acids (VFA) and polyphenols (Andreozzi *et al.*, 1999). Subsequently, these compounds are degraded into simpler, more biodegradable fragments that are readily used by anaerobic populations (Martín *et al.*, 2002). Benitez *et al*. (2003) found when ozonated WDWW was treated in an activated sludge system, the COD reduction increased from 28 to 40%.

Enzymatic treatments have also been used to enhance the biodegradability of distillery wastewater (Sangave & Pandit, 2006). The addition of enzymes may be used to reduce or remove the lipids or proteins in the wastewater by increasing the hydrolysis of these compounds prior to or during anaerobic digestion (Cammarota & Freire, 2006). Sangave and Pandit (2006) reported that when distillery wastewater was treated in an aerobic bioreactor, the COD reduction increased from 18 to 29% when a 12 h enzymatic pre-treatment was included.

In this study it was determined whether the UASB treatment efficiency of distillery wastewater could be improved. The first objective was to enhance the UASB treatment efficiency of WDWW by combining UASB treatment with pre- and/or post-ozonation steps. The compositional change of the wastewater, the UASB reactor performance and the

granule activity were all monitored. The second objective was to determine the impact of GDWW on UASB granules. The visual appearance, composition and activity of the granules before and after exposure to GDWW were determined. The third objective was to investigate whether ozonation, enzymatic treatment or combinations thereof would make GDWW more amendable for UASB treatment. The compositional change and degree of foaming of the treated GDWW were investigated. The visual appearance, composition and activity of the granules before and after exposure to the treated GDWW were also determined.

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CHAPTER 2

LITERATURE REVIEW

A. BACKGROUND

Water is the most vital component of the earth, making up 70% of the world's surface (Stikker, 1998). All humans require clean water for survival and there is no substitute for this commodity (MacKay & Ashton, 2004). The primary renewable source of fresh water is continental rainfall, which supplies 4.0 x 10^{13} to 4.5 x 10^{13} m^3 fresh water annually (Kivaisi, 2001). This more or less constant supply of fresh water must support the entire world population, which is growing at about 85 million people per year. Thus the availability of fresh water per capita is decreasing rapidly. Since 1970 the global water demand has increased annually at an average rate of 2.4%, with higher trends in developing countries (Kamara & Sally, 2003). It was estimated that due to rapid population growth and climate changes, in 1997 a third of the world's population was living in physically water scarce countries, and that by 2025 this figure will double, including sub-Saharan African countries such as South Africa (Arnell, 2004).

The natural scarcity of water is intensified by the fact that the quality of fresh water is also decreasing due to industrialisation (Kivaisi, 2001). Rapid industrialisation has led to the generation of vast quantities of wastewater with high organic loads and serious pollution potentials (Rajeshwari *et al.*, 2000). Thus as the population and industrialisation continue to grow and increasing pressure is put on natural resources, the wastewater produced must be disposed of, treated or re-used correctly and economically (Lata *et al.*, 2002). As a result, purification of industrial wastewaters is a worldwide dilemma of increasing significance (Lalov *et al*., 2000). Water can no longer only be allocated to meet the increasing demand from industry, agriculture and other productive sectors, but must also meet the requirements of stringent environmental regulations and legislations (Lévite *et al.*, 2003).

Water in South Africa

Approximately two thirds of South Africa is arid or semi-arid with an average rainfall of 450 mm per year – about half of the global average (Kamara & Sally, 2003). Of the 5.1 x 10^7 m^3 of potentially available rainwater, 74% is used for the production of

crops, trees and livestock and 26% is runoff to the sea or used for industrial, domestic and other purposes (Bennie & Hensley, 2001).

In South Africa water is of utmost importance to all development and its scarcity may limit future economic growth (Anon., 2003). Furthermore, South African wastewater regulations stipulate the maximum volume of wastewater that may be used daily for irrigation or released into certain water resources, provided that certain conditions are met (Table 1) (Anon., 1998a & b; Anon., 2004).

B. THE SOUTH AFRICAN DISTILLERY INDUSTRY

Alcohol may be produced using sugar crops, starch crops, dairy or cellulose-rich materials (Wilkie *et al*., 2000). After malting, mashing and fermentation, the solution (containing 2 – 12 % ($^{v}/_{v}$) alcohol) is distilled (Lata *et al.*, 2002). Effective control over the distillation process, which ensures that the wastewater contains less than 0.2% ($^{v}/_{v}$) ethanol, is imperative, because each percent of ethanol remaining in the wastewater increases the chemical oxygen demand (COD) by 20 000 mg.L^{-1} (Wilkie *et al*., 2000). During the production of alcohol, large volumes of water (25 – 240 L water.L^{-1} alcohol produced) are required, mainly for cleaning and cooling purposes, and large amounts of heavily polluted distillery wastewater are generated (Driessen *et al*., 1994; Martín *et al.*, 2002; Nataraj *et al.*, 2006).

Distilleries are considered to be one of the most polluting industries (Chandra *et al.*, 2002). In the spirit industry up to 20 L of wastewater may be generated for each litre of ethanol manufactured (Beltrán *et al.*, 2001a). The amount and composition of the wastewater may exhibit major daily and seasonal variations due to the nature of the alcohol-producing industry, the substrate used, the specific production process utilised and the location of the industry (Beltrán de Heredia *et al*., 2005b; Bustamante *et al.*, 2005; Sangave & Pandit, 2006).

Distillery wastewater is chemically complex, containing large amounts of total suspended solids (TSS) in the form of organic compounds, such as acids, soluble proteins, glycerol and carbohydrates, phenolic compounds as well as inorganic compounds and possibly even cleaning agents (Moosbrugger *et al.*, 1993; Nandy *et al.*, 2002). Some of these compounds have been found to resist biodegradation, thus slowing the wastewater treatment process. These high-strength wastewaters are characterised by a high COD, low pH and relatively high salinity level (Table 2)

Table 1 Specific maximum COD values and volumes of wastewater that can be used for certain applications, according to the South African wastewater regulations (Anon., 2004)

Application	**COD ($mg.L^{-1}$)**	**Volume ($m^3.d^{-1}$)**
Irrigation	5 000	50
	400	500
	75	2 000
Release into a non-listed water resource	75	2 000
Release into a listed water resource	30	2 000

Table 2 Composition of wine distillery wastewater (WDWW) and grain distillery wastewater (GDWW) (Driessen *et al.*, 1994; Kim *et al.*, 1997; Laubscher, 2000; Uzal *et al.*, 2003; Bustamante *et al.*, 2005; Vlyssides *et al*., 2005)

Constituent ($mg.L^{-1}$)*	**WDWW**	**GDWW**
COD	14 900 – 58 220	10 000 – 60 000
Biochemical oxygen demand (BOD)	11 150 – 16 500	15 000 – 34 000
Total solids (TS)	620 – 113 600	20 500 – 52 000
Total suspended solids (TSS)	150 – 49 760	10 000 – 11 400
Volatile suspended solids (VSS)	100 – 3 250	–
Total nitrogen (N)	20 – 650	200 – 1 900
Total phosphorus (P)	59 – 200	–
Total polyphenols (as gallic acid equivalents)	0 – 862	–
Salinity (%)	± 3.5	–
pH	2.4 – 5.3	3.5 – 4.0

* Unless indicated otherwise

(Benitez *et al*., 2003; Shayegan *et al.*, 2005). The dry matter of distillery wastewater generated when grain is used as the substrate (known hereafter as grain distillery wastewater [GDWW]) has a high lipid (± 125 g oil.kg^{-1} dry matter) and protein content (280 – 340 g crude protein.kg^{-1} dry matter) (Arosemena *et al*., 1995; Hill, 2002).

Distillery wastewater can, thus, lead to significant environmental and ecological problems (Bezuidenhout *et al.*, 2000; Bustamante *et al.*, 2005). Effective treatment of distillery wastewater is imperative (Akunna & Clark, 2000; Wilkie *et al*., 2000). In the past, distillery wastewater was either released into evaporation ponds and public sewerages, disposed of directly using methods like river/sea disposal and irrigation, or used as a fodder or fertiliser (Kida *et al*., 1995; Benitez *et al.*, 2003).

C. POSSIBLE TREATMENT OPTIONS

Current treatment options used to treat wastewater include: physical; chemical; physicochemical and/or biological treatment methods. The selection of a treatment method depends on the treatment efficiency, treatment cost, local geography, climate, land use, regulatory constraints and public acceptance of the treatment.

Physical wastewater treatment methods

Sedimentation

Settleable solids or TSS in wastewater may be removed inexpensively via sedimentation by using the force of gravity to separate suspended material, oil and grease from the wastewater (Jayanti & Narayanan, 2004). It may be used to control particulate pollutants and serve as a pre-treatment or post-treatment for wastewater (White & Verdone, 2000). Ripley (1979) found that distillery wastewater was treated effectively in rectangular sedimentation tanks. Beltrán *et al.* (2001a) also reported that when spirits distillery wastewater was treated in a system consisting of an aeration system, a 4 L mixed tank, feed and effluent reservoirs and a sedimentation tank, the COD and polyphenol reductions achieved were 82 and 35%, respectively.

The wastewater enters at the inlet section of the sedimentation basin (also known as a settling tank or clarifier) and flows through the settling zone towards the outlet (Nazaroff & Alvarez-Cohen, 2001). In the settling zone suspended materials rise or fall, depending on their density. It is possible to remove coarse solids that settle at the bottom of the settling tank and to skim off greases, oils or other materials

that float to the top. Sedimentation is ineffective at improving water clarity and removing bacteria or viruses.

Granular media filters

Granular media filters have been used since the 19th century as polishing steps, after sedimentation or chemical precipitation, to remove particles smaller than 10 µm (Boller & Kavanaugh, 1995). These filters may remove particulate, suspended or colloidal matter, unwanted ions, colour, taste, odour and pathogens from wastewater.

Filtration is accomplished by passing wastewater through a fine granular media such as sand (Hamoda *et al.*, 2004). As the wastewater passes through the filter, particles are captured in the fine pores and sorbed on the surfaces of the granular media. The correct balance between filter efficiency and hydraulic throughput should be obtained. Particle removal efficiency depends on the concentration of particles in the suspension, filtration velocity and characteristics of the particles and filter media. Over time the pores become blocked and the filter must be cleaned via backwashing. Ripley (1979) found that granular media filters could be used as a relatively cheap and simple polishing step during the wastewater treatment process of distillery wastewater. These filters are disadvantageous because the performance is insufficient and regular cleaning is needed.

Membrane separation techniques

Membranes are described as thin layers of material that allow the transmission of water at a different rate relative to impurities (Stephenson *et al.*, 2000). Membrane separation techniques can be applied to ensure that effective liquid-solids separation, water reclamation or fractionation of macromolecular and colloidal substances occurs (Sonune & Ghate, 2004). Wilkie *et al*. (2000) reported these techniques could be used successfully to treat distillery wastewater. Nataraj *et al*. (2006) also reported that membrane-based nanofiltration and reverse osmosis processes can be used to reduce the total dissolved solids (TDS), COD and potassium (K^+) content of distillery wastewater by 99.80, 99.90 and 99.99%, respectively.

Membrane separation techniques are not commonly used because the solids retention time (SRT) is low and fouling often occurs due to an intolerance towards inert solids (Gerardi, 2003). Thus, operation, maintenance and replacement costs are high (Kentish & Stevens, 2001). These techniques are also often ineffective

because low molecular weight organic molecules may pass through the membrane and problems associated with limited aeration may occur (Stephenson *et al.*, 2000).

Membrane-coupled anaerobic bioreactor (MCAB) systems, which incorporate microfiltration or ultrafiltration after anaerobic digestion, have also been developed to completely retain biosolids in the reactor and produce relatively clear wastewater (Choo & Lee, 1996). When MCAB systems are used, the COD and TSS in the wastewater are decreased significantly, while large quantities of methane (CH_4) are generated. Nagano *et al.* (1992) found that when distillery wastewater was treated in a MCAB system at an organic loading rate (OLR) of 7 kg $COD.m^{-3}.d^{-1}$, the COD reduction was 98% and the CH_4 production was 0.28 to 0.34 $m^3.kg^{-1}$ feed.

Chemical and physicochemical wastewater treatment methods

Chemical treatment methods

During chemical wastewater treatment methods, compounds like chlorine (Cl_2), oxygen (O_2), ozone (O_3) and permanganate (MnO_4), are added to the wastewater to oxidise the wastewater components into carbon dioxide (CO_2), water, inorganic matter and other harmless products (Andreozzi *et al.*, 1999; Benitez *et al.*, 2003). Chemical treatment methods, leading to partial degradation of organic matter and inactivation of microorganisms, may eliminate mineral compounds, colour, turbidity, TSS or foul odours (Camel & Bermond, 1998).

Coagulation, flocculation and precipitation

Coagulation and flocculation are important treatment processes that are used for rapid and economical removal of suspended, inert or undesirable colloidal materials in industrial wastewaters (Tatsi *et al*., 2003). Coagulation and flocculation are generally combined in a two-staged transformation process (Nazaroff & Alvarez-Cohen, 2001). This process works by reversing electrostatic charges on small colloidal particles, which are allowed to aggregate or floc together and are removed via sedimentation or filtration (Tatsi *et al*., 2003). Chemical precipitation can also be employed to remove undesirable dissolved ions, which are converted to solids and then removed by sedimentation (Veeken *et al*., 2003; Dąbrowski *et al.*, 2004).

Pikaev *et al.* (2001) found that when iron sulphate ($Fe_2(SO_4)_3$) was added to distillery wastewater as a coagulant, 40% of the wastewater pollutants were removed. Beltrán de Heredia *et al*. (2005a) also reported that when an integrated

Fenton-coagulation/flocculation process was used to treat wine distillery wastewater (WDWW), COD reductions of more than 55% were obtained.

Sorption of organic molecules

Sorption of undesirable organic molecules can be achieved by using powdered activated carbon (PAC) or granular activated carbon (GAC) (Guo *et al*., 2004). PAC is generally added to the wastewater as a slurry upstream of a granular filter while GAC is used in a fixed-bed (Meidl, 1997). Sorption of organic molecules may be used to remove trihalomethanes, chlorinated organics, pesticides, non-biodegradable residual organics and compounds that cause odour or flavour problems. Seth *et al.* (1995) reported that a COD reduction of 67% was achieved when GAC was used to treat distillery wastewater, at an OLR of 21.3 kg $COD.m^{-3}.d^{-1}$ and a hydraulic retention time (HRT) of 4 d. The process is based on the filtering action of the activated carbon (AC) bed, which removes TSS and colloidal particles (Meidl, 1997). The performance is influenced by the equilibrium and kinetic characteristics of the system (Nazaroff & Alvarez-Cohen, 2001). AC is advantageous to use, as it is relatively inexpensive and has a large specific surface area of about 1 000 $m^2.g^{-1}$. Thus small amounts of AC can be used to capture large quantities of pollutants. AC is also desirable to use as it is non-polar and highly porous. Regeneration of the spent AC is possible, making the process more economically viable (Meidl, 1997).

Ion-exchange

The main objective of ion exchange is to remove ions and replace them with less harmful or more desirable ions from a charged resin (Sonune & Ghate, 2004). It may be used for nitrogen (N) or phosphate (PO_4^{3-}) reduction, demineralisation, COD reduction, colour removal and wastewater purification (Green & Kramer, 1979). Lalov *et al*. (2000) found that when 10 $g.L^{-1}$ chitosan was added to distillery wastewater as an anion-exchanger for 30 min, the COD reduction was 93%. Ion-exchange may also be applied for water softening, de-ionisation, disposal of concentrated brines and the reduction of heavy metals and anions such as cyanide (CN^-), fluoride (F^-), nitrate (NO_3^-) and sulphate (SO_4^{2-}) (Dąbrowski *et al.*, 2004).

Biological wastewater treatment methods

All biological wastewater treatment processes depend upon the natural growth and selection of microorganisms in a suspended culture or fixed-film (Droste, 1997;

Lettinga *et al.*, 1997). During these wastewater treatments, microorganisms utilise pollutants for growth and convert the organic substrate in the wastewater into simpler substances such as CO_2 and water, in the presence (aerobic) or absence (anaerobic) of O_2 (Henry & Heinke, 1996). Apart from converting soluble and colloidal wastes into solid or gaseous material, the main aims of biological wastewater treatment processes are to reduce the volume of solids present, and to aid the separation of organic and inorganic compounds from the aqueous stream (Droste, 1997; Gerardi, 2003). However, biological processes are much more complex than the other methods because mass transfer, hydrodynamics and microorganism kinetics must all be considered in biological reactors (Martínez *et al.*, 2001).

Lagoon technology

Treatment of wastewater in stabilisation or oxidation ponds or lagoons is most probably the oldest wastewater treatment method used by man (Bitton, 1999). Stabilisation ponds, which are shallow with a large surface area, are dynamic waste recycling ecosystems (Hosetti & Frost, 1995). Microorganisms degrade the organic matter in the wastewater to produce treated wastewater with reduced health risks and a lower organic content. Furthermore, stabilisation ponds are classified as anaerobic lagoons, facultative lagoons or tertiary lagoons (also described as aerobic or maturation ponds) (Maynard *et al.*, 1999). Stabilisation ponds may also be used for secondary treatment or as polishing ponds.

When stabilisation ponds are well-designed and well-operated, good reductions of biochemical oxygen demand (BOD), N, PO_4^{3-}, TSS, indicator or enteric bacteria, helminthes, and viruses can be achieved (Maynard *et al.*, 1999; Kivaisi, 2001). Ramana *et al.* (2002a) reported that when distillery wastewater was treated in a stabilisation lagoon, the organic carbon, N and PO_4^{3-} content of the wastewater was reduced by 63, 16 and 6%, respectively. During treatment the pH increased to 8.2, which is important because if the stabilisation lagoon pH is greater than 8.0, the production of foul odours is controlled.

Lagoon technology is advantageous because it is flexible, easy to operate and simple to construct and maintain (Pearson, 1996; Maynard *et al.*, 1999). Lagoon technology can also be used to treat wastewater economically due to the low capital investment, energy requirements and operation costs involved (Zhao & Wang, 1996; Bories *et al.*, 2005). Sludges, liquids and gases from stabilisation ponds may be used as by-products such as fertiliser, irrigation water or building material (Hosetti &

Frost, 1995). Lagoon sludge from a lagoon treating distillery wastewater can successfully be mixed with other fertilisers in order to improve the yield of groundnuts (Ramana *et al.*, 2002a) and maize (Ramana *et al.*, 2002b).

However, the long HRTs and large land area requirements are disadvantageous (Ripley, 1979; Zhao & Wang, 1996). Residual nutrients in the treated wastewater may also contaminate other water bodies if they are released into the environment without further treatment (Kivaisi, 2001). The long-term storage of WDWW may lead to the formation of malodorous compounds such as butyric acid (Beltrán de Heredia *et al*., 2005a). However, Bories *et al.* (2005) reported that it was possible to eliminate this problem by adding nitric acid (HNO_3) to the wastewater.

Land application

Wastewater treatment via land application involves the controlled application of wastewater onto the land surface (Crites *et al.*, 2001). The soil acts as a filter because the microorganisms and plants present use the nutrients in the wastewater to form cellular matter or to oxidise compounds into gases such as CO_2, water vapour and N_2 gas (Nazaroff & Alvarez-Cohen, 2001). Three types of land application systems are available, namely overland flow, infiltration percolation and irrigation systems (Pardue *et al.*, 1988; Van Cuyk *et al.*, 2001; Rynk & Goldstein, 2003). The type of soil, topography, geology and weather conditions at the available site determine which system should be used (Ghosn & Al-Muzani, 2004).

Overland flow land application, used as secondary treatment processes, traps dissolved and TSS in the soil or vegetation before biodegradation (Cameron *et al.*, 1997). During the process, pre-treated wastewater is discharged down a 2 to 8% slope of clay soil and a large portion of the wastewater is collected as run-off in a basin or ditch (Henry & Heinke, 1996). It has been found that the major treatment mechanisms involved are sedimentation and biological conversion.

Infiltration percolation systems act like ‘natural’ sand filters by using sandy soil as the sand bed and groundwater as the underdrain (Crites *et al.*, 2001; Van Cuyk *et al.*, 2001). The wastewater is applied at a high rate by sprinklers or by flooding the basins for a certain period of time. Next a rest period occurs to allow the soil to drain and enable the microorganisms and plants to absorb the nutrients. Compared to the other systems, infiltration percolation systems require the least amount of land per unit of wastewater treated but have the lowest dissolved pollutants reduction.

Irrigation allows wastewater to infiltrate and percolate into the soil without run-off and at low application rates (Rynk & Goldstein, 2003). The main aims are to grow crops that utilise the nutrients and to remove most of the water by evapotranspiration (Bond, 1998).

Wilkie *et al*. (2000) reported that land application systems are suitable for treating distillery wastewater if it is controlled correctly, because nutrients that have been removed from soils are returned. Soil conditions and crop production are also often improved after land application methods (Bond, 1998; Rynk & Goldstein, 2003). Nutrients, BOD, TSS and toxins are removed from wastewater as effectively as in other systems but often at a lower cost (Pardue *et al.*, 1988, Cameron *et al.*, 1997).

The main disadvantages associated with these systems, if effective management does not occur, include: groundwater contamination; interference with soil mechanics by specific minerals and increased uptake by plants of potentially toxic compounds (Cameron *et al.*, 1997; Chen *et al.*, 2004). Wilkie *et al*. (2000) reported when distillery wastewater was applied to croplands, environmental degradation, such as phytotoxicity or damage by sodium (Na^+) salts, occurred. Tano *et al*. (2005) also found that if large quantities of undiluted WDWW were used, the high doses of N and polyphenols present may detrimentally affect the soil microbiological activity. The production of foul-smelling odours and the disposal of unsanitary wastes or aerosol produced by sprinkler systems may cause problems associated with the safety of public health (Rynk & Goldstein, 2003).

Strict regulations are vital to ensure that the damage from land applications is minimised (Mahmood *et al.*, 2003). In South Africa, irrigation of wastewater is only permitted if it does not impact on a water resource or any other person's water use, property or land and if is not detrimental to public health and safety (Anon., 2004). South African wastewater regulations also specify the amount of wastewater that may be used daily for irrigation and the conditions that must be met (Table 3).

Wetlands

A popular, cost-effective and environmentally-friendly biological treatment option for industrial or agricultural wastewaters is the use of natural or artificially constructed wetlands (Hench *et al*., 2003). Wetlands are defined as transitional areas between land and water (Kivaisi, 2001). These systems are mostly or completely covered with water-tolerant plants, such as reeds (*Phragmites australis*), cattails (*Typha* spp.) and

Table 3 Maximum volume of wastewater permitted for irrigation and conditions that must be met, according to South African wastewater regulations (Anon. 2004)

Constituent ($mg.L^{-1}$)*	**Maximum volume of wastewater**		
	50 $m^3.d^{-1}$	**500 $m^3.d^{-1}$**	**2 000 $m^3.d^{-1}$**
COD	< 5 000	< 400	< 75
pH	6.0 – 9.0	6.0 – 9.0	5.5 – 9.5
Faecal coliforms (per 100 mL)	< 100 000	< 100 000	<1 000
Ammonia (NH_3) as nitrogen (N)	–	–	< 3
Nitrate/Nitrite (NO_3^{2-}/NO_2^{2-}) as N	–	–	< 15
Chlorine (Cl_2) as free chlorine (Cl^-)	–	–	< 0.25
Suspended solids (SS)	–	–	< 25
Electrical conductivity (mS)	–	–	< 200
Phosphate (PO_4^{3-}) as phosphorus (P)	–	–	< 10
Fluorine (F_2)	–	–	< 1.0
Soap, oil and grease	–	–	< 2.5
Sodium adsorption ration	5	5	–

* Unless indicated otherwise

bulrushes (*Scirpus* spp.) that are rooted in the wet soil but emerge above the water surface (Rousseau *et al.*, 2004).

In principle, pre-treated water flows through the wetland, which consists of a matrix of plant roots, rhizomes, sand and gravel (Rousseau *et al.*, 2004). This matrix is colonised by a layer of attached microorganisms that form a biofilm. Wetlands are also characterised by high organic matter accumulated due to anaerobic conditions that are created in the system (Kivaisi, 2001). Wetlands are able to reduce or remove wastewater contaminants, such as organic matter, inorganic matter, viruses and pathogens (Foresti, 2001). As a result substantial reduction of TSS, COD, total Kjeldahl nitrogen (TKN), total PO_4^{3-} and ammonia (NH_3) can occur (Hench *et al*., 2003). Physical, chemical and biological processes including sedimentation, filtration, precipitation, sorption, plant uptake, microbial decomposition or interaction and N transformations probably accomplish these reductions (Kivaisi, 2001). Billore *et al.* (2001) reported that molasses distillery wastewater could be treated in a field-scale 4-celled constructed wetland. It was found that 49% TSS, 64% COD, 59% TKN and 79% phosphorous (P) were removed.

Wetlands are advantageous for wastewater treatment because they are cheap, easy to operate and require no or minimal fossil fuels and chemicals for operation (Kivaisi, 2001). The systems may also be used for additional purposes such as swamp fisheries, biomass production, seasonal agriculture water supply, public recreation, wild life conservation and scientific studies (Knight, 1997). However, wetlands are disadvantageous due to the possible harmful effects of toxic materials or pathogens in the wastewater and the long-term efficiency and stability of the system (Hench *et al*., 2003). The presence of pollutants in wastewaters may negatively affect the environment or lower the overall treatment efficiency of the wetland system by inhibiting the microbial population (Kivaisi, 2001). Wetlands may also be associated with bad odours and the presence of mosquitoes, which may be a public health concern, as they are vectors of malaria.

Activated sludge systems

Activated sludge systems have been used since 1916 to treat wastewater on a large scale (Nazaroff & Alvarez-Cohen, 2001). A conventional activated sludge system consists of an aeration tank and sedimentation tank – connected in series. This system can be defined as an aerobic flocculated suspended-growth process, in which organic matter is oxidised by microorganisms suspended in flocs within the

reactor. As a result CO_2, NO_3^-, SO_4^{2-}, PO_4^{3-} and cell biomass are produced (Bitton, 1999). After treatment in the aeration tank, an effective reduction of BOD, soluble solids, P, pathogens and parasites occurs. The treated wastewater has a high floc concentration and thus solid and liquid separation must occur in the sedimentation tank (Foresti, 2001; Contreras *et al.*, 2002).

Most organic wastewaters can effectively be treated in an activated sludge system. Benitez *et al*. (2003) found that when WDWW was treated in an aerobic activated sludge system at 25°C, the COD reductions at an HRT of 24 h and 72 h were 31 and 85%, respectively. Disadvantages of the system include: high costs; foaming; production of turbid effluents or unpleasant odours; generation of large amounts of sludge that must be disposed of; dispersed growth of the activated sludge and bulking that produces an undesirable, low density floc (Sponza, 2002).

Trickling filters

Wastewater treatment using a biofilm reactor is a relatively old practice and was first introduced in England in 1893 (Nazaroff & Alvarez-Cohen, 2001; Schubert & Günthert, 2001). Trickling filters are based on biofilms, which contain high concentrations of microorganisms attached to inert material found in the reactor (Biesterfeld *et al.*, 2003). Bacteria, fungi, protozoa and higher organisms are involved in the process (Schubert & Günthert, 2001). Exopolymeric substances produced from microorganisms are also involved. Furthermore, aeration is required to maintain aerobic conditions (Nazaroff & Alvarez-Cohen, 2001).

Unlike the name suggests, trickling filters do not separate solids from wastewater physically (Foresti, 2001). In a trickling filter biological oxidations occur as the wastewater passes in thin layers over the inert material, placed above an overdrain (Biesterfeld *et al.*, 2003). Various biofilms develop on the inert material, depending on the wastewater load and composition (Schubert & Günthert, 2001). Therefore, during the wastewater treatment, surplus sludge and excess biomass in the treated wastewater must be removed (Biesterfeld *et al.*, 2003).

Trickling filters are often used for wastewater treatment because they can be used in series with other filters or biological systems, need little operator attention, require less land than lagoon systems and have low to moderate operating and maintenance costs (Hammer & Hammer, 1996; Nazaroff & Alvarez-Cohen, 2001). These filters also produce satisfactory BOD and TSS reductions, allow for PO_4^{3-} reduction by chemical treatment and permit for nitrification (Biesterfeld *et al.*, 2003).

Although overall costs are generally lower than activated sludge systems, the initial capital investment is still high (Biesterfeld *et al.*, 2003). Other disadvantages include: lack of operator control over the system; poor effluent quality; a slow start-up time; odour or fly problems and clogging of the system by dislodged filter sludge (Hammer & Hammer, 1996). Trickling filters are also very large in size, which is unfavourable compared to other wastewater treatment systems (Henze *et al.*, 1997).

Rotating biological contactors

The rotating biological contactor (RBC) process was first used to treat municipal wastewater in Europe in the mid-1950s and adapted in the late 1960s in North America to treat industrial wastewater (Green & Kramer, 1979; Henry & Heinke, 1996). Rotating biological contactors are described as fixed-film systems, consisting of partly submerged rotating discs on which biofilms form (Hammer & Hammer, 1996). When the shaft is rotated, aerobic conditions are created when the discs come into contact with the wastewater and air (Malandra *et al.*, 2003). Microorganisms are able to assimilate compounds from the wastewater, thus decreasing the organic load. As a result satisfactory BOD, carbon (C), TSS, phenol and ammonia-nitrogen (NH_3-N) reductions at low hydraulic loading rates can be achieved (Alemzadeh *et al.*, 2002). If certain chemicals are added, PO_4^{3-} removal may also be possible. Although primary sedimentation may be eliminated, the RBC process is regarded as a secondary treatment, as biological oxidation reactions of complete oxidation, synthesis and endogenous respiration occur (Hiras *et al.*, 2004).

Advantages of the system include: operation at a high biomass concentration without using settlers or recirculation; minimal operator attention; small space requirements; low operation costs; rapid start-up and no odour or fly problems (Malandra *et al.*, 2003). Disadvantages include: high hydraulic loads that negatively affect BOD reduction; PO_4^{3-} removal that decreases BOD or TSS reduction; relatively high capital costs; little or no denitrification and the need for housing to protect against weather damage (Green & Kramer, 1979; Henry & Heinke, 1996).

Sequencing batch reactors

The sequencing batch reactor (SBR) is a popular treatment option used to treat winery and distillery wastewaters (Andreottola *et al.*, 2002). SBRs operate in a four-step cycle: wastewater is fed into the reactor with settled biomass; wastewater and biomass are mixed; biomass is settled and treated wastewater is removed (Angenent

et al., 2004). These reactors can operate aerobically or anaerobically (Foresti, 2001). Moletta (2005) reported that an anaerobic SBR may be used to effectively treat distillery wastewater.

SBRs can be used to effectively treat wastewaters with vast quantities of TSS (Angenent *et al*., 2004). Denitrification, nitrification, COD reduction, decolouration and PO_4^{3-} removal are also possible using a SBR (Foresti, 2001). Shayegan *et al*. (2005) found that when distillery wastewater was treated in a SBR, 70% decolourisation was achieved after 12 h.

SBRs are advantageous because these systems have minimal space requirements and cycle modifications during plant operation are possible (Andreottola *et al.*, 2002). Plant configuration and management are also simpler than in activated sludge systems because sludge recycling is unnecessary.

Enzymatic treatment

Enzymatic treatments may be used to enhance the biodegradability of distillery wastewater (Sangave & Pandit, 2006). Dharmsthiti and Kuhasuntisuk (1998) also found that when lipid-rich wastewater was treated with lipase, the lipid content decreased by 70% within 24 h. Enzymatic treatments may also be used to reduce or remove the lipids or proteins in the wastewater by increasing the hydrolysis of these compounds prior to or during other anaerobic or aerobic wastewater treatments (Cammarota & Freire, 2006). Sangave and Pandit (2006) reported that when distillery wastewater was treated in an aerobic bioreactor, the COD reduction increased from 18 to 29% when a 12 h enzymatic pre-treatment was included. The advantages of enzymatic treatments include: applicability to biorefractory compounds; mineralization; decolourisation; operation over a wide range of contaminant concentrations, pH, temperature or salinity; absence of shock loading effects; absence of acclimatisation period and reduction in sludge volume as no biomass is generated (Sangave & Pandit, 2006). However, costs associated with enzymatic treatments are often considerable.

Anaerobic digestion

Anaerobic digestion is one of the oldest known technologies for stabilising wastewaters and is suitable for treating most wastewaters (Angenent *et al.*, 2004). Examples of early treatment methods are septic tanks, slurries in digesters and anaerobic filters (McCarty, 2001). Due to the need for cost-effective treatments in

the growing food industry and the international oil crisis, treatment systems using anaerobic reactors have been developed (Van Lier *et al*., 2001a).

In the 1960s, in Bellville, South Africa, the first full-scale reversed flow Dorr-Oliver clarigester was developed to treat concentrated glucose-starch wastewater (Wood, 1992; Lettinga, 2001). From the 1970s onwards, the discovery of high rate reactors (which separates the HRT from the SRT) increased the popularity of anaerobic systems as a cost effective alternative for wastewater treatments (Akunna & Clark, 2000). These high rate reactors include: upflow anaerobic sludge blanket (UASB) reactors; contact reactors with bio-physical filters; microbial film expanded bed (MFEB) reactors and biofilm reactors (Lettinga *et al.*, 1997; McCarty, 2001).

Furthermore, exciting developments that incorporate membrane systems into the anaerobic process have also occurred in South Africa (Wood, 1992). Today, anaerobic digestion in South Africa ranges from simple pit latrines, septic tanks and household digesters to the more sophisticated systems found at Prospecton Brewery in Durban and Mossgas oil-from-gas project in Mossel Bay.

Anaerobic Digestion Process Microbiology

Anaerobic digestion (AD) is a natural process in which various microbial species work together, in the absence of O_2, to transform organic wastes through a variety of intermediates into biogas (McCarty, 2001; Mata-Alvarez, 2003). During anaerobic digestion, biomass and biogas are produced, while pathogenic microorganisms and offensive organic matter are reduced. Therefore, anaerobic digestion can be used both as a depollution tool and to produce energy (Moletta, 2005).

Anaerobic digestion of high-strength wastewater usually occurs in successive steps and is accomplished by four trophic groups of bacteria (Ranade *et al*., 1999). These bacteria groups function in a synergistic relationship and form a food chain, in which the final products are CH_4 and CO_2 (Fig. 1). The interactions between the various bacteria are complex because of the competition for substrates occurring between these bacteria. Intermediate products produced may also inhibit the growth, change the metabolism or alter the end products of other bacteria (Bitton, 1999).

In the first step, hydrolytic and/or acidogenic bacteria, such as *Clostridium*, *Acetobacterium* and *Sporomusa* ferment organic polymers (including carbohydrates, lipids and proteins) into sugars, fatty acids and amino acids (Mata-Alvarez, 2003). These monomeric or dimeric substances are then fermented into volatile fatty acids

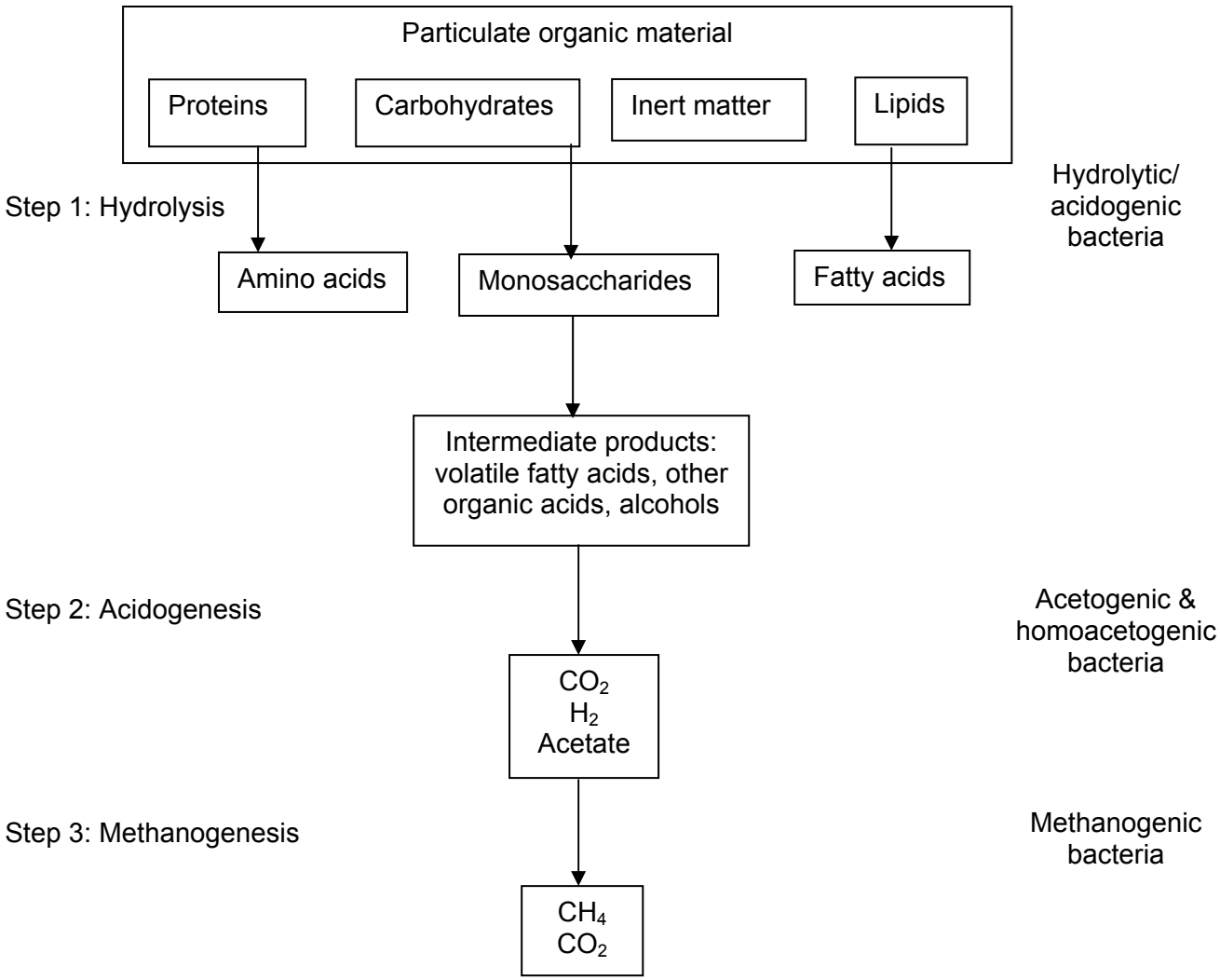

Figure 1 Schematic diagram of anaerobic digestion, indicating the three steps and the four bacteria groups involved in the process on the left and right, respectively (adapted from Batstone *et al.*, 2002; García-Heras, 2003).

(VFA), other organic acids, alcohols, CO_2 and hydrogen (H_2) (Moletta, 2005). These bacteria are obligate or facultative anaerobes and have large substrate ranges and short generation times. In the presence of H_2 consuming bacteria, acidogenic bacteria may produce H_2 for the disposal of excess electrons. However, during reactor failure, H_2 accumulation due to the inhibition of the H_2 consuming bacteria results in conditions that are unfavourable for electron disposal as molecular H_2. Consequently, the accumulation of alternative electron sink products is an indication of reactor failure. Excessive acidogenic bacteria activity can also lead to reactor failure because the unionised organic acids and H_2 are inhibitory to the other bacteria present in the reactor. Although enzymes catalyse this step, it proceeds rather slowly and may be restricted by the digestion of certain wastes, such as raw cellulolytic wastewaters containing lignin (Bitton, 1999).

The intermediate substances generated are catabolised to acetate, formate, H_2, and CO_2 by acetogenic bacteria, which obtain their energy from the oxidation process without additional electron donors or acceptors (Ranade *et al*., 1999). These bacteria also have a low growth rate and an obligate requirement for the disposal of electrons such as H_2. Thus, they are very sensitive to H_2 and grow only at partial pressures of H_2 less than 10^{-5} atmospheres.

During the second step, acetate is also produced by homoacetogenic bacteria that catabolise bicarbonates or degrade carbohydrates, using H_2 and CO_2 (García-Heras, 2003). Some species are also able to donate H_2 to the methanogenic bacteria via interspecies H_2 transfer, while *Acetobacterium woodii* can degrade aromatic compounds. Although the significance of homoacetogenic bacteria as H_2 consumers under normal conditions is minor, they are important in maintaining low H_2 partial pressure under conditions that inhibit the methanogens.

In the third step, the diverse group of methanogens generate biogas containing CH_4 and CO_2 gas. This insoluble biogas should be released from the system to prevent it from building up to an inhibitory concentration (Fang, 2000). About 72% of the CH_4 gas is produced when acetate is degraded by acetoclastic methanogens, from the genera *Methanosarcina* and *Methanoseata* (previously known as *Methanothrix*), as follows (Pérez *et al.*, 2001):

$$CH_3COOH \rightarrow CH_4 + CO_2 \qquad \Delta G = -31.0 \text{ kJ (Mata-Alvarez, 2003)}$$

The remainder of the gas is generated by hydrogenotrophic methanogens that utilise H_2 and CO_2 as follows (Bitton, 1999; Pérez *et al.*, 2001):

$$4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O \qquad \Delta G = -135.6\ kJ$$ (Mata-Alvarez, 2003).

All methanogens are strict anaerobes and require a low oxidation-reduction potential of at least -300 mV for growth. This step is also the rate-limiting step of the anaerobic degradation process because methanogenic bacteria grow more slowly than the others (Nazaroff & Alvarez-Cohen, 2001). They are also very sensitive to pH values below 6.0 or above 7.5 and are inhibited by unionised VFAs. Furthermore, all the species can grow autotrophically on H_2 and CO_2 as their sole C source to produce CH_4, while a few species can utilise formate and *Methanosarcina barkeri*, the predominant species in anaerobic reactors, can utilise acetate. Methanogenic bacteria also have a high H_2 affinity and are considered to be the major sink for H_2 in anaerobic systems (Bitton, 1999). Thus the biogas, which leaves the reactor, is usually composed of 60 to 65% CH_4, 30 to 35% CO_2 and small amounts of H_2, hydrogen sulphide (H_2S), N_2 and water (Nazaroff & Alvarez-Cohen, 2001).

Nitrate and SO_4^{2-} reducing bacteria may also be involved in anaerobic digestion, reducing NO_3^- and SO_4^{2-} to ammonium (NH_4^+) and sulphide (S^{2-}), respectively (García-Heras, 2003). During these processes, oxidation of alcohols, butyric acid and propionic acid to acetate and CO_2, as well as oxidation of formate, acetate or H_2 occurs. Nitrate reduction can also affect the C and electron flow, gas composition and microbial competition (Batstone *et al.*, 2002).

Types of anaerobic reactors

Various types of anaerobic reactors have been developed and are used to treat wastewater (Gerardi, 2003). Types of reactors can be classified according to temperature, configuration and bacterial growth.

Psychrophilic reactors operate between 5 and 20°C, while mesophilic and thermophilic reactors usually operate at 30 to 40°C and at 50 to 60°C, respectively (Droste, 1997; Gerardi, 2003). Distillery wastewater is treated successfully with a COD reduction of 70% or more under mesophilic conditions (Laubscher, 2000; Wolmarans & De Villers, 2002; Moletta, 2005). Pérez *et al.* (2001) found that a COD reduction of 81.5% was achieved when WDWW was treated in an upflow thermophilic fluidised bed reactor operated at an OLR of 32 kg $COD.m^{-3}.d^{-1}$. Pérez-

García *et al*. (2005) stated that under thermophilic conditions, when WDWW was treated in either an upflow anaerobic fixed film reactor or a anaerobic fluidised bed reactor, COD reductions of 76% (at an OLR of 6.29 kg $COD.m^{-3}.d^{-1}$) and 96% (at an OLR of 5.88 kg $COD.m^{-3}.d^{-1}$) respectively were obtained. Romero *et al.* (1990) reported that a COD reduction of 90% was achieved when WDWW was treated under anaerobic conditions in a 1.8 L semicontinuous flow digester, under both mesophilic and thermophilic conditions. The optimum HRT for the mesophilic system was 6 d, while that of the thermophilic system was 4 d.

Traditionally, an anaerobic digester is a single-stage system that consists of a large fermentation tank with mechanical mixing, heating, gas removal and supernatant and sludge addition and withdrawal (Bitton, 1999; Moletta, 2005). The digestion and settling of the sludge occur simultaneously in the tank, thus the sludge is stratified, from bottom to top, into layers consisting of: grit; active digested sludge; active digester sludge; supernatant; scum and gas (Gerardi, 2003).

Higher OLRs and lower HRTs are achieved by using a two-stage anaerobic digester (Bitton, 1999; Moletta, 2005). As the name suggests, these systems consist of two consecutive tanks in which different processes occur (Gerardi, 2003). Processes that may be separated include: CH_4 and acid production; mesophilic or thermophilic digestion and sludge storage and the production of sludge and CH_4. Blonskaja *et al*. (2003) found that a mesophilic two-stage anaerobic digestion system could be effectively used to treat alcohol distillery wastewater. The system used consisted of an anaerobic filter and an UASB reactor, in which the fermentation and methanogenesis stages were separated. Optimal system stability was achieved when the OLR for the acidogenic stage was 2 to 4 kg $COD.m^{-3}.d^{-1}$ at pH 6.0 and that for the methanogenic stage was 1 to 2 kg $COD.m^{-3}.d^{-1}$ at pH 7.6. During the first stage the COD reduction was low (54%) because high loadings and the accumulation of VFAs inhibited digestion. This stimulated the methanogenic activity in the second stage and thus COD reductions of 93% were obtained.

Suspended growth systems are defined as systems in which the bacteria are evenly suspended in the digester through a mixing action (Henry & Heinke, 1996). In these mixed systems, the SRT is usually the same as the HRT because biomass is not retained and is concentrated in the system (Gerardi, 2003). Suspended growth systems, such as completely mixed reactors or anaerobic sludge beds, have been used to treat distillery wastewater at OLRs of 1 to 5 kg $COD.m^{-3}.d^{-1}$ (Moletta, 2005).

In fixed-film systems the wastewater passes from the bottom (upflow) or top (downflow) of the reactor over the fixed-film of microorganisms (Gerardi, 2003). Microorganisms absorb soluble organic compounds in the wastewater while insoluble organic compounds are adsorbed to the surface of the microorganisms. These systems are used to treat a variety of wastewaters because they provide good contact between the wastewater and bacteria and can operate over a large temperature range. Systems used to treat distillery wastewater include: anaerobic filters (85 – 98% COD reduction at OLRs of 5 – 20 kg $COD.m^{-3}.d^{-1}$) (Moletta, 2005); fluidised or expanded bed (AFB) reactors (75 – 97% COD reduction at OLRs of 15 – 30 kg $COD.m^{-3}.d^{-1}$) (García-Bernet *et al.*, 1998; García-Calderon *et al.*, 1998; Pérez *et al.*, 2001; Moletta, 2005); granular bed anaerobic baffled reactors (GRABBR) (96% COD reduction at OLRs of 2.73 kg $COD.m^{-3}.d^{-1}$) (Akunna & Clark, 2000); hybrid digesters (70% COD reduction at OLRs of 48 - 50 kg $COD.m^{-3}.d^{-1}$) (Lata *et al.*, 2002; Moletta, 2005); fixed film reactors (60 – 70% COD reduction at OLRs of 25 kg $COD.m^{-3}.d^{-1}$) (Rajeshwari *et al.*, 2000; Lata *et al.*, 2002) and UASB reactors (>90% COD reduction at OLRs of 10 – 18 kg $COD.m^{-3}.d^{-1}$) (Laubscher, 2000).

Akunna and Clark (2000) used a GRABBR to treat malt whisky distillery wastewater successfully. The COD reductions achieved varied from 90 to 96%. The best performance occurred at an OLR of 2.73 kg $COD.m^{-3}.d^{-1}$ and at an HRT of 4 d. The GRABBR was also found to be effective at retaining biomass, with the effluent TSS concentration being relatively constant at about 80 $mg.L^{-1}$ for all the HRTs tested. Throughout the experiments the CH_4 content of the biogas also remained constant between 60 and 70%.

Lata *et al.* (2002) also showed that distillery wastewater could be treated using a 100 L fixed film reactor operating at an OLR of 25 kg $COD.m^{-3}.d^{-1}$. A COD reduction of 60 to 70% and a CH_4 yield of 0.4 $m^{-3}.kg^{-1}$ COD reduced were obtained.

D. THE UASB PROCESS

Dutch scientist Lettinga and his co-workers designed the UASB reactor in the 1970s to treat beet sugar wastewater (Lettinga, 2001). Since then, the system has been used to successfully treat a large variety of wastewaters. Today it is the most popular anaerobic design used worldwide for wastewater treatment (Britz *et al.*, 2002; Liu *et al.*, 2003). UASB reactors have been used to treat a variety of wastewaters from distilleries producing alcohol (Harada *et al.*, 1996; Lata *et al.*,

2002), brandy (Laubscher *et al.*, 2001; Wolmarans & De Villers, 2002), spirits or whisky (Goodwin & Stuart, 1994; Uzal *et al.*, 2003).

Working principle and process design

UASB reactors are used to convert organic compounds in wastewater to CH_4 and CO_2 (Angenent *et al.*, 2004). The process relies on the upward movement of the wastewater through a blanket of active anaerobic sludge granules. These granules are retained as immobilised biomass and form when bacteria attach to the sludge ingredients (Lettinga *et al*., 1997; Bitton, 1999). The granulation success depends on the self-immobilisation of active biomass and ensures that the mean cell residence time is greater than the HRT (Akunna & Clark, 2000; Angenent *et al.*, 2004). The operation advantage is that no carrier material is required (Schmidt & Ahring, 1996).

The UASB reactor is composed of four major parts – the granular sludge bed, sludge blanket or fluidised zone, gas-solids separator (GSS) and settlement compartment (Fig. 2) (Schmidt & Ahring, 1996). The sludge bed consists of a granular layer of biomass settled at the bottom of the reactor (Lin & Yang, 1991). The sludge blanket is a suspension of sludge particles mixed with gases produced in the reactor. Influent wastewater enters at the bottom of the reactor before being degraded in the sludge bed and blanket (Schmidt & Ahring, 1996; Bitton, 1999). The GSS device separates biogas from the liquid and settleable granular sludge (Rajeshwari *et al.*, 2000). Some sludge particles may enter the settlement compartment, in which an inactive zone is created, and these can either settle back to the reactor or wash out with the effluent (Lin & Yang, 1991). In this way, biomass that is not flocculated is washed out with the effluent (Schmidt & Ahring, 1996).

Operational efficiency factors

In order for the UASB design to operate efficiently, the bacteria in the reactor must be in a state of equilibrium (Jhung & Choi, 1995). Therefore, the efficiency of UASB reactors is influenced by several factors (Lettinga *et al*., 1997).

Temperature

Anaerobic digestion can occur at temperatures ranging from psychrophilic temperatures (± 10°C) to extreme thermophilic temperatures (> 80°C) (Van Lier *et al*., 2001a). Generally, UASB reactors are operated at mesophilic temperatures of

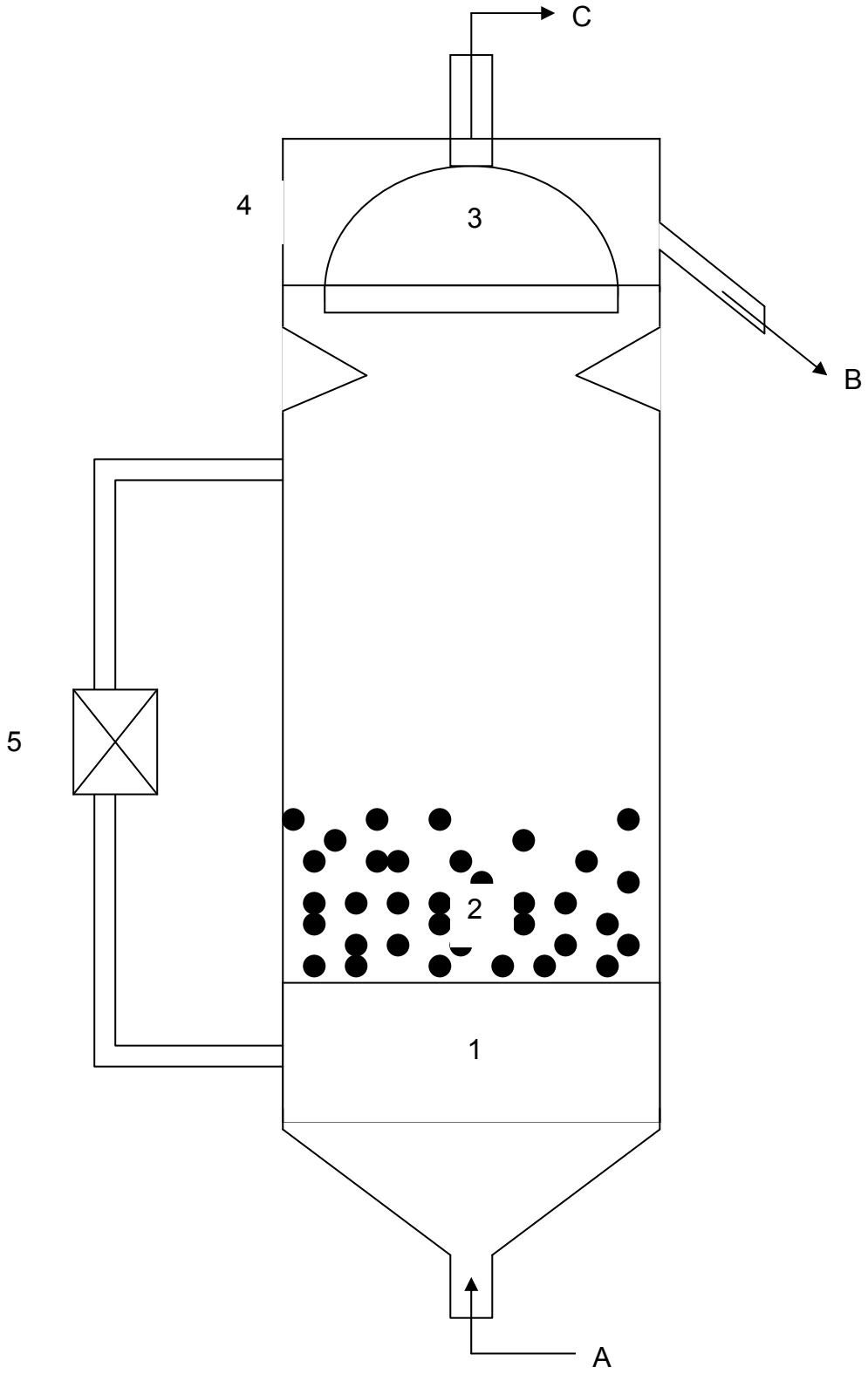

Figure 2 Schematic representation of an UASB reactor: (1) granular sludge bed; (2) sludge blanket; (3) GSS; (4) settlement compartment; (5) recirculation pump; (A) influent; (B) effluent and (C) gas outlet (Schmidt & Ahring, 1996).

35°C or moderate thermophilic temperatures of 55°C (Romero *et al.*, 1990). The optimum temperature is the best compromise between the rate of microbial activity and inactivation. Temperature deviations from the optimum temperature range are undesirable as it negatively affects the activity of the microbial population (Lin & Yang, 1991; Bitton, 1999).

pH

UASB reactors are normally operated at a neutral pH (6.5 – 7.6) (Rajeshwari *et al.*, 2000). Above a VFA concentration of 400 $mg.L^{-1}$, acidity is more inhibitory to the methanogenic bacteria than to the acidogenic bacteria (Mata-Alvarez, 2003; McLachlan, 2004). Therefore, the more acid-tolerant acidogenic bacteria continue to produce VFAs that accumulate in the system and unless corrected, the VFA concentration will continue to increase while the pH will continue to decrease – possibly leading to reactor failure (Segretain & Moletta, 1987; Jantsch & Mattiasson, 2004). Reactor instability occurs when the propionic acid to acetic acid ratio is bigger than 1.4 (Buyukkamaci & Filibeli, 2004).

Alkalinity

Sufficient alkalinity (1 000 – 3 000 $mg.L^{-1}$) is required for digestion to occur effectively and is needed to serve as a buffer preventing sudden pH changes (Droste, 1997; Henze *et al.*, 1997; Gerardi, 2003). A decrease in alkalinity below normal operating conditions generally indicates expected process failure and is followed by a pH change. A significant change must be corrected by chemical addition or by altering the operational conditions. For a stable and well-buffered process, the VFA:alkalinity ratio should range from 0.1 to 0.2 (Blonskaja *et al.*, 2003).

Wastewater characteristics

The microorganisms in the UASB reactor will utilise the carbohydrates, lipids, proteins and aromatic compounds in the wastewater during anaerobic digestion (Jhung & Choi, 1995; Bitton, 1999). Therefore, characteristics of the wastewater partly determine and affect the OLR of UASB reactors (Lettinga *et al*., 1997; Van Lier *et al*., 2001a). Organic underloading and overloading should be avoided because underloading may negatively affect granule settleability and overloading causes imbalances that negatively affect the slow-growing methanogenic bacteria (Lin & Yang, 1991; Mata-Alvarez, 2003). Sharma and Singh (2001) and Moletta (2005)

both found that the average OLR for UASB reactors treating distillery wastewater should range between 5 and 15 kg COD.m^{-3}.d^{-1}.

Wastewater must also be nutritionally balanced to ensure that satisfactory anaerobic digestion occurs (Bitton, 1999). Therefore, the presence of nutrients in wastewater is essential for microbial growth and performance (Gerardi, 2003; Mata-Alvarez, 2003). The wastewater should contain relatively large quantities of macronutrients, such as N and PO_4^{3-}, and smaller quantities of micronutrients or trace elements, such as iron (Fe^{3+}), cobalt (Co^{3+}), nickel (Ni^{2+}), sulphur (S^{2-}), molybdenum (Mo^{2+}), K^+, calcium (Ca^{2+}), magnesium (Mg^{3+}), zinc (Zn^{2+}), manganese (Mn^{2+}) and copper (Cu^{2+}) (Rajeshwari *et al.*, 2000). If the wastewater contains low concentrations of these nutrients, chemical supplementation will be necessary. Sharma and Singh (2001) suggested that when distillery wastewater is treated in an UASB reactor, 10 mg.L^{-1} Fe^{3+}, 0.1 mg.L^{-1} Ni^{2+} and 0,5 mg.L^{-1} Co^{3+} should be added to improve the sludge volume index and methanogenic activity.

The COD:N:P ratio should be 1000:7:1 for UASB reactors treating high-strength wastewaters and 350:7:1 for UASB reactors with low OLR (Gerardi, 2003). Since distillery wastewater is generally deficient in both N and PO_4^{3-}, appropriate chemical substitutes should be added (Ripley, 1979; Rajeshwari *et al.*, 2000). The COD:SO_4^{2-} ratio should be 1.7 to 2.7 as SO_4^{2-}-reducing bacteria are very competitive at this range. An increase in this ratio favours the methanogenic bacteria, while a decrease favours SO_4^{2-}-reducing bacteria. When this ratio is below 0.5, the amount of SO_4^{2-} that is converted into more toxic S^{2-} is inhibitory (Wilkie *et al.*, 2000).

A maximum nutrient level exits and in excess of that, nutrients may be inhibitory rather than stimulatory (Singh *et al.*, 1999). Furthermore, as methanogens are obligate anaerobes, the presence of O_2 may be detrimental (Bitton, 1999). Anaerobic bacteria, especially methanogens, may be negatively affected toxicants, such as: chlorinated hydrocarbons; benzene ring compounds; formaldehyde; phenolic wastes; VFAs; long chain fatty acids; heavy metals; CN^-; alternate electron acceptors; alkaline cations; S^{2-}; NO_3^-; tannins and recalcitrant compounds (Lin & Yang, 1991; Gerardi, 2003). When wastewaters that are rich in lipids and/or proteins are treated in UASB reactors, problems may also occur (Zeeman & Sanders, 2001).

Retention time

The average SRT of an UASB reactor should be in excess of 10 d at 35°C to compensate for the slow generation time of methanogens and to ensure that bacteria

washout is prevented and guarantee sufficient CH_4 is generated (Gerardi, 2003). High SRT values are used to maximise the removal capacity, reduce the reactor volume, permit biological acclimation against toxic substances and provide buffering capacity for protection against shock loadings and toxic compounds.

The HRT should be long enough to ensure that sufficient anaerobic digestion occurs (Bitton, 1999). Normally, an HRT of 24 h or less is adequate when distillery wastewater is treated in an UASB reactor (Droste, 1997).

Biomass availability and immobilisation

The amount of viable biomass in the system influences the overall success because at high substrate levels substrate inhibition will occur (Lettinga *et al*., 1997; Van Lier *et al*., 2001a). Immobilisation of the proper balanced syntrophic anaerobic bacterial association is essential as it produces enhanced degradation kinetics and influences the temperature susceptibility (Lettinga *et al.*, 1997). It guarantees that a long SRT is achieved and that low levels of specific inhibitory intermediates are maintained. The amount of biomass that develops in the reactor also influences the performance because the higher the biomass concentration is, the more microorganisms are available to participate in the anaerobic digestion process (Cho *et al*., 1996).

Mixing and upflow velocity

Without adequate mixing in an UASB reactor, unfavourable microenvironments may develop and local build-up of high concentrations of inhibitory intermediate metabolic products may occur (Droste, 1997). Adequate mixing also distributes buffering agents throughout the reactor. To ensure that adequate mixing occurs, the upflow velocity must be sufficient (Mahmoud *et al*., 2003). However, the upflow velocity should not exceed 1 $m.h^{-1}$ as above this limit biomass washout generally occurs and thus the overall reactor efficiency decreases (Lettinga & Hulshoff Pol, 1991).

Control parameters

Since many factors influence UASB reactor efficiency, monitoring of operational parameters is essential to measure progress and indicate disturbances within the reactor (Van Lier *et al.*, 2001b). The ease of operating, controlling and monitoring anaerobic digestion processes is determined by the type of effluent, plant design, monitoring and control facilities, loading rates and operator experience (Gerardi, 2003). No single control parameter is sensitive enough to reliably determine whether

the reactor is operating effectively (Mata-Alvarez, 2003). Thus a combination of the following physical, visual, biological and chemical variables must be used: alkalinity; pH; biogas production rate and content; COD; temperature; solids present; VFA concentration and the VFA concentration:alkalinity ratio (Gerardi, 2003). If any of the variables are not within the acceptable range, they should be corrected using appropriate methods.

Advantages of UASB reactors

Approximately 60% of all the anaerobic full-scale treatment facilities in the world are based on the UASB design concept because the process is associated with many advantages (Angenent *et al.*, 2004). Like all other anaerobic wastewater treatment options, the energy production rate in an UASB reactor exceeds that of aerobic treatments and energy for aeration is not required (Droste, 1997; Wilkie *et al*., 2000). During anaerobic digestion, minimal (if any) heat is generated (Pérez *et al.*, 1998).

When wastewater is treated in an UASB reactor, the biogas produced can be captured and used as fuel (Beltrán *et al.*, 1999b). Biogas production generally ranges from 500 to 600 $L.kg^{-1}$ COD removed with 60 to 80% methane (Moletta, 2005). Lata *et al.* (2002) reported that when 375 $m^3.d^{-1}$ alcohol distillery wastewater was treated in a full-scale reactor, 13 100 $m^3.d^{-1}$ biogas was produced and when 700 $m^3.d^{-1}$ alcohol distillery wastewater was treated in another full-scale reactor, 24 000 $m^3.d^{-1}$ biogas was produced. For both reactors, COD reductions of 65 to 70% and BOD reductions of 90 to 92% were obtained. Furthermore, when anaerobic treatments, such as UASB reactors, are compared to aerobic treatments, they are regarded as “cleaner” wastewater treatment options because the biogas produced can replace fossil fuel sources and thus decrease the negative influence on the greenhouse effect (Batstone *et al.*, 2002; Lata *et al.*, 2002).

The UASB process can also effectively separate the HRT and SRT (Driessen & Yspeert, 1999; Sharma & Singh, 2001). Thus, the slow-growing microorganisms remain inside the reactor, regardless of the water flow or the presence of carrier materials (Puñal & Lema, 1999; Angenent *et al.*, 2004). The reactor can be operated under high OLRs and still achieve good COD reductions (Chrobak & Ryder, 2005). Driessen *et al*. (1994) reported that when WDWW was treated in an UASB reactor, COD reductions of more than 90% were obtained at an OLR of 15 kg $COD.m^{-3}.d^{-1}$. Similarly, Goodwin and Stuart (1994) reported that COD reductions of 90% were achieved when malt whisky distillery wastewater was treated in a 1.05 L lab-scale

UASB reactor, operated at an OLR of 15 kg $COD.m^{-3}.d^{-1}$ and HRT of 2.1 d. Goodwin *et al.* (2001) also found that when diluted malt whisky distillery wastewater was fed into an UASB reactor, COD reductions of more than 80% occurred at an OLR of 5.46 kg $COD.m^{-3}.d^{-1}$. When Uzal *et al.* (2003) used a two-staged UASB reactor, operated at an HRT of 25.8 h and OLR of 19.4 $kg.m^{3}.d^{-1}$, to treat malt whisky distillery wastewater, the COD and BOD reductions were 96 and 99%, respectively.

The HRT in an UASB reactor is relatively short compared to that of other treatments (Akunna & Clark, 2000). Laubscher (2000) reported that when WDWW was treated in a 450 m^3 UASB system, COD reductions of more than 90% was obtained at an HRT of 24 h, pH of 5.8 and OLR of 10 to 18 kg $COD.m^{-3}.d^{-1}$.

Anaerobic reactors, such as the UASB reactor, generate less biomass compared to aerobic reactors but operate at higher OLRs and concentrations (Foresti, 2001; Gerardi, 2003). Wilkie *et al.* (2000) reported that anaerobic treatments for distillery wastewater produce about 10% of the sludge yield of aerobic treatments. Thus, the process may be operated without large energy, land, nutrient or cost requirements (Lettinga, 2001; Lata *et al.*, 2002; Chrobak & Ryder, 2005).

Compared to aerobic treatments, wastewater treatment in UASB reactors has fewer operational problems, is more flexible and provides process stability under the expected fluctuations in wastewater quality (Kim *et al.*, 1997; Chrobak & Ryder, 2005). Therefore, wastewater of different strengths, temperatures and complexities can be successfully treated using anaerobic digestion methods (Gerardi, 2003). Wastewaters containing inhibitory compounds and those with temperatures above 55°C or below 10°C can also be treated more successfully in an UASB reactor than in a suspended sludge system because UASB reactor biogranules are less sensitive in wastewaters as most of the bacteria are located and protected inside the granule (Lettinga *et al*., 1997; Puñal & Lema, 1999; Fang, 2000).

Like all anaerobic wastewater treatment systems, treatment in an UASB reactor is favourable to food industries because it is safe, does not lead to health or environmental hazards and generally does not generate undesirable odours (Pérez *et al.*, 1997; Britz *et al.*, 2000). Furthermore, UASB reactors are also ideal for food and beverage industries that operate seasonally because anaerobic organisms can be preserved unfed for long periods of time, while biomass viability can be maintained due to reduced decay (Bitton, 1999; Lata *et al.*, 2002; Chrobak & Ryder, 2005).

Disadvantages of UASB reactors

The most important disadvantage associated with UASB reactors is the extended start-up period (Britz *et al.*, 2002). The slow-growing bacteria in the granules limit the overall potential of this process. The treatment capacity may also be limited by the occurrence of concentration gradients, mass transfer limitations and maximum loading rates applied before sludge washout occurs (Driessen & Yspeert, 1999; Van Lier *et al*., 2001a).

Furthermore, the overall efficiency of an UASB reactor can be restricted by the presence of certain compounds, such as long chain or volatile fatty acids, excess nutrients and phenolic compounds, which are often found in complex wastewaters and are toxic to sensitive methanogens (Beltrán de Heredia *et al*., 2005b). Harada *et al.* (1996) reported that when a thermophilic UASB reactor treating alcohol distillery wastewater was operated at an OLR of 28 kg $COD.m^{-3}.d^{-1}$, low COD reductions (39 – 67%) were obtained, due to the presence of phenolic compounds in the wastewater.

Laubscher (2000) also reported that when WDWW was treated in an UASB reactor, precipitation and deposition of struvite ($MgNH_4PO_4$) occurred in the pipeworks and effluent overflow launders. Thus the flow through the pipes was restricted and operational problems occurred. The precipitant could not be removed by using high-pressure jet sprays, scrapping equipment or caustic soda.

Lipid-rich wastewaters, like GDWW, may reduce the reactor efficiency because the lipid molecules enclose the sludge particles and decrease the amount of contact between the microbial consortium and wastewater (Nadais *et al.*, 2001). Problems also occur due to the high lipid-water interface of the lipid emulsions and the accumulation of potentially toxic fatty acids (Zeeman & Sanders, 2001). Thus high COD accumulation in the sludge bed occurs, leading to unstable performance, extensive clogging, biomass washout or a high degree of organic matter accumulation (Nadais *et al.*, 2001). The formation of a scum layer, consisting of floating substrates and sludge, may also occur and lead to sludge washout or hampered release of biogas (Lettinga & Hulshoff Pol, 1991). To improve the process, stabilisation of the organic matter is essential and may be achieved by using flocculent sludge and discontinuous feeding (Nadais *et al.*, 2001). The efficiency can also be improved if a lipid pre-treatment step is included or if a skimmer is installed in the reactor (Lettinga & Hulshoff Pol, 1991; Puñal & Lema, 1999).

When wastewaters with high protein levels, such as GDWW, are treated in an UASB reactor, foaming and sludge floatation (leading to sludge washout or scum

formation) may also occur (Lettinga & Hulshoff Pol, 1991). Furthermore, the hydrolysis of proteins to amino acids requires a long HRT and cannot be effectively carried out in an UASB reactor (Fang *et al.*, 1994; Miron *et al.*, 2000). Therefore, protein-rich wastewater should first be treated for protein hydrolysis before being treated in an UASB reactor. Proteinase and peptidase can also be added to convert the proteins into amino acids, which anaerobic bacteria reduce and deaminate into saturated fatty acids and NH_3-N (Junhuang & Weisheng, 1992).

Complex wastewaters, such as GDWW, are often not successfully treated in an UASB reactor because foaming and sludge floatation occur (Barber, 2005). Excessive foaming (caused by potentially foaming substances such as lipids, protein or filamentous organisms) is undesirable, as it leads to a loss in capacity and increases operation costs. Sludge floatation occurs because of the poor segregation between settleable flocculant sludge and the accumulation of biogas bubbles inside or on top of the granules (Kalyuzhnyi *et al.*, 1998; Saiki *et al.*, 2003).

Furthermore, granule disintegration, granule washout, granular sludge floatation, scaling by inorganic precipitates or the development of fluffy granules may negatively affect the stability of granules (Akunna & Clark, 2000; Van Lier *et al*., 2001b). Granule settleability and stability are also affected by the presence of excessive solids, such as TSS, grease, oil, lipids or filamentous bacteria, because the slowly hydrolysing or inert substances accumulate in the reactor (Saiki *et al.*, 2003; Angenent *et al.*, 2004). Since these substances become entrapped in the reactor, the contact between the substances and microbial consortium in the granules is reduced and the digestion process is restricted. Consequently, dilution of the active biomass, reduction of the specific activity, scum layer formation or sludge washout and delay granule formation may all occur (Lettinga & Hulshoff Pol, 1991).

Treating complex wastewaters in UASB reactors may also cause toxicity, scaling or complete reactor failure (Zeeman & Sanders, 2001). These problems may be partly or completely eliminated by temporarily decreasing the OLR, preclarifying the wastewater by adding flocculants, or applying a HRT of less than 24 h via effluent recycling (Kalyuzhnyi *et al.*, 1998). Maintaining good contact between the sludge and wastewater also lessens these problems (Lettinga & Hulshoff Pol, 1991).

Anaerobic reactors, such as UASB reactors, are normally able to achieve COD reductions of 65 to 95% at OLRs of 5 to 15 kg $COD.m^{-3}.d^{-1}$ (Moletta, 2005). However, the quality of the treated wastewater may be poor due to the high concentrations of TSS, soluble organic compounds and nutrients present (Gerardi,

2003). Organic substances in the wastewaters can also inhibit the UASB process because they may be toxic, stable in aqueous solutions or resistant to biodegradation (Beltrán-Heredia *et al.*, 2001; Martín *et al.*, 2002). Therefore, wastewater treatment prior to or after UASB treatment may be required to purify toxic compounds, increase wastewater biodegradability, facilitate the anaerobic process and decrease treatment costs (Boncz *et al.*, 2003; Delgenès *et al.*, 2003). Junhuang and Weisheng (1992) found that when distillery wastewater treated in an UASB reactor was subjected to an aerobic biological post-treatment, the COD reduction improved from 95 to 99%. Uzal *et al.* (2003) also reported that when malt whisky distillery wastewater was treated aerobically after being treated in a two-staged UASB reactor, the COD reduction increased from 96 to 99.5%.

E. OZONATION

Ozonation has been used in Europe for many years to: treat wastewaters; prevent tainting of cooling towers; disinfect water and remove flavours, colour or odours (Guzel-Seydim *et al.*, 2004). In 1857 Von Siemans constructed the first O_3 generator that was developed into the dielectric system of today (Stedman, 2005). The first drinking water plant to use O_3 was constructed in Oudshoorn, Holland, in 1893. Ozonation was first used commercially to treat municipal water in 1906 in Nice, France, and in 1910 in St. Petersburg, Russia (Bitton, 1999).

In the USA the ozonation is not popular, although the United States Food and Drug Administration assigned “generally recognised as safe” (GRAS) status to the use of O_3 in bottled water in 1982, and to the use of O_3 as a disinfectant in 1997 (Baig & Liechti, 2001). In 1997 the use of O_3 for reconditioning of recycled poultry chilling water was approved by the US Department of Agriculture (Guzel-Seydim *et al.*, 2004).

Characteristics of ozone

Apart from fluorine (F_2), O_3 is the most powerful common oxidising agent and thus has a high oxidation potential (Camel & Bermond, 1998; Guzel-Seydim *et al.*, 2004). This unstable, triatomic atom consists of a central oxygen (O) atom attached to two equidistant O atoms. Based on its physical properties, pure O_3 condenses to a dark blue liquid at 112°C and is a blue gas at room temperature. Although at concentrations at which it is generally produced, it is a nearly colourless gas with a

pungent odour at room temperature. Furthermore, O_3, which occurs at low concentrations in nature, is readily detectable between 0.01 and 0.05 $mg.L^{-1}$.

Ozone spontaneously degrades back to O_2 and decomposes rapidly at room temperature (Guzel-Seydim *et al.*, 2004). The type and kinetics of O_3 decomposition affect the mechanism of disinfection and wastewater treatment (Gehr *et al*., 2003). If O_3 decomposition is slow, the chemicals and microorganisms will undergo direct O_3 attack, which is gradual and selective. During rapid O_3 decomposition, occurring when the alkalinity is low or when the organic concentration is high, non-selective and very reactive oxidation occurs by means of the hydroxyl (OH) radical.

Ozone has a longer half-life in the gaseous state than in an aqueous solution and degrades more rapidly in impure solutions than in pure water (Guzel-Seydim *et al.*, 2004). Ozone is 13 times more soluble than O_2 in water at 0 to 30°C. The solubility of O_3 also increases as the temperature decreases, while it degrades more rapidly as the temperature increases.

Ozone generation and oxidation

Since O_3 is an unstable and reactive gas, it does not accumulate without continual O_3 generation (Guzel-Seydim *et al.*, 2004). Therefore, it must be produced at the point of use. Various generation methods, based on different O_3 sources and working principles, are available, including: electrical, electrochemical, photochemical, radiation chemical and thermal methods (Table 4) (Gottschalk *et al.*, 2000). Ozone can oxidise wastewater via selective, direct reactions or can undergo decomposition indirectly through faster, free radical reactions (Kasprzyk-Hordern *et al.*, 2003). Biological oxidation is negatively affected by changes in the organic load and the presence of recalcitrant or refractory compounds, such as polyphenols or unsaturated fatty acids (Beltrán *et al.*, 2001b). Electrophilic compounds such as O_3 are selective towards the double bonds found in these compounds (Andreozzi *et al.*, 1998). Theoretically, O_3 will attack the double bonds and leave intact the sugars and proteins, which are in any case biodegradable. These complex compounds are then degraded, via chemical oxidation, into simpler, more biodegradable fragments that are readily used by anaerobic populations (Beltrán *et al.*, 1999a; Martín *et al.*, 2002).

When O_3 reacts with phenolic compounds, various oxidised intermediates, which can be degraded biologically, are formed (Amat *et al.*, 2003). Huang and Shu (1995) and Benitez *et al.* (1997) identified the final products produced during this

Table 4 Summary of methods available for O_3 generation (Gottschalk *et al.*, 2000)

O_3 generation	**Working principle**	**O_3 source**	**Field of application**
Electrical	Electrical discharge	Air or O_2	Standard – laboratory to full-scale
Electrochemical	Electrolysis	Highly purified water	Pure water applications – laboratory to small industries
Photochemical	Irradiation ($\lambda < 185$ nm)	Water O_2	New technology – laboratory to full-scale
Radiation chemistry	X-rays Radioactive γ-rays	Highly purified water	Very seldom – solely experimental
Thermal	Light arc ionisation	Water	Very seldom – solely experimental

process as acids and aldehydes that were less toxic than the substrate. However, Beltrán *et al.* (1993) and Andreozzi *et al.* (1998) found that ozonation of aromatic compounds generated more toxic intermediates.

The oxidising power of O_3 can be improved through advanced oxidation processes (AOP) (Beltrán *et al.*, 1997; Sigge *et al.*, 2002). AOPs are defined as processes in which the maximum amounts of highly reactive radical intermediates, such as OH radicals, are generated to aid wastewater treatment processes (Gottschalk *et al.*, 2000). Radicals are usually generated by using a combination of oxidation agents (such as O_3 and H_2O_2), irradiation (such as UV light and ultrasound) and catalysts (Kasprzyk-Hordern *et al.*, 2003). Martín *et al.* (2002) reported that when samples were pre-treated with 34 $g.m^{-3}$ O_3, O_3 and UV light or O_3, UV light and 2 $g.L^{-1}$ titanium dioxide (TiO_2), the COD and TOC decreased regardless of pre-treatment used. When no TiO_2 was added, the yield coefficient for CH_4 increased but the specific rate of anaerobic digestion did not improve. Treatments including TiO_2 increased the yield coefficient and the specific rate of anaerobic digestion by 25%.

Advanced oxidation processes offer an alternative method for catalysing the formation of reactive radicals and accelerating the destruction of organic compounds. The radicals produced are non-selective in their mode of attack, which occurs by adding to the double bonds or by abstracting H atoms (Gottschalk *et al.*, 2000). Thus all reduced materials are oxidised and some organic molecules are mineralised to non-toxic compounds, such as water or CO_2 (Huang & Shu, 1995). Other organic compounds are only partly oxidised and filtration on activated carbon can be used to remove these molecules (Kasprzyk-Hordern *et al.*, 2003).

Efficiency factors

Certain factors influence the overall efficiency of O_3 (Kasprzyk-Hordern *et al.*, 2003). It is negatively affected by molecular O_3-resistant compounds or OH radical scavengers, such as carboxylic acids, aldehydes and carbonates (Beltrán *et al.*, 2001b). The temperature and physical state of O_3 can also influence the decomposition rate and thus the overall efficiency (Guzel-Seydim *et al.*, 2004).

The overall efficiency of the ozonation process is also partly dependent on the pH of the wastewater, as this affects the double action of O_3 on organic matter (Beltrán *et al.*, 2001b). Ozone decomposition is also catalysed by OH ions and proceeds more rapidly as the pH increases (Wu *et al.*, 2000). At an acidic pH, O_3 reacts with compounds with specific functional groups through selective direct

reactions (Beltrán *et al.*, 2001b). At an alkaline pH, indirect oxidation pathways are followed as O_3 decomposes, forming OH radicals that react non-selectively with inorganic or organic compounds. When both pathways are combined during pH-sequential ozonation, the total oxidation rate may increase (Wu *et al.*, 2000; Beltrán *et al.*, 2001b). The latter is true because under acidic conditions direct ozonation reactions occur that remove the carbonates formed under alkaline conditions. Beltrán *et al.* (2001b) found that when WDWW was ozonated, at an O_3 flow rate of 30 $L.h^{-1}$ and at an O_3 concentration ranging from 10 to 20 $mg.L^{-1}$, the most effective results were achieved when pH sequential ozonation, consisting of two pH 4 to pH 10 periods of 10 and 50 min each, was used. It was reported that a COD reduction of 41.4% was obtained, while the O_3 efficiency was 78%.

Advantages of ozonation

Ozone is one of the most effective oxidising agents and disinfectants used (Benitez *et al*., 1999). This powerful oxidant quickly degrades back to harmless O_2 and is soluble and readily available in water (Guzel-Seydim *et al.*, 2004). Ozone also has a higher oxidation potential than Cl_2 and does not normally form by-products that need to be removed (Beltrán-Heredia *et al.*, 2001).

Ozone is capable of degrading a large variety of inorganic and organic compounds in wastewaters (Lin & Wang, 2003). It can be utilised as a primary disinfectant to inactivate pathogens or as an oxidant to oxidise and degrade Fe^{3+}, Mn^{2+}, phenolic compounds, volatile solids, trihalomethanes and refractory organic compounds into simpler fragments (Wu *et al.*, 2000; Goel *et al.*, 2003). Depending on the wastewater ozonated, an increase in biodegradability may lead to a higher decrease in COD and thus the effluent discharge limits may be met (Bitton, 1999, Martín *et al.*, 2002). Alfafara *et al.* (2000) found that when beer distillery wastewater was ozonated, at an O_3 dose of 50 $mg.L^{-1}$, after 40 h, the biodegradability improved by 40% and the BOD to COD ratio increased from 0.3 to 0.5.

Ozonation is effective against viruses, bacteriophages and protozoa cysts – even when low treatment doses and short contact times are used (Lazarova *et al.*, 1998). Ozone may also be used as an algae, taste, colour or odour control as well as for the improvement of coagulation (Kasprzyk-Hordern *et al.*, 2003). Alfafara *et al.* (2000) reported that a total reduction of colour (at an absorbance of 475 nm) of 80% was achieved when beer distillery wastewater was ozonated.

Disadvantages of ozonation

However, using O_3 as a wastewater treatment method is limited (Kasprzyk-Hordern *et al.*, 2003). The industrial applications of O_3 are limited by low O_3 transfer efficiency (Lin & Wang, 2003). Thus, an O_3-water contacting device should be used to adequately transfer O_3 into the liquid phase (Huang & Shu, 1995).

Treating wastewater with O_3 is expensive and using H_2O_2, Cl_2 or UV is more cost effective (Athanasopoulos & Athanasopoulos, 1998; Bitton, 1999). Due to the high cost of ozonation and the partial oxidation of organic compounds that must be removed, ozonation may not be economically feasible as a wastewater treatment method (Kasprzyk-Hordern *et al.*, 2003). The legal wastewater regulations for certain wastewaters can also often only be achieved if ozonation is combined with other methods (Athanasopoulos & Athanasopoulos, 1998).

Although O_3 is not very toxic to humans at low concentrations, it may be fatal at higher concentrations (Guzel-Seydim *et al.*, 2004). At O_3 concentrations higher than 0.2 $mg.L^{-1}$, varying degrees of respiratory tract damage can occur, depending on the exposure time.

Ozone combined treatments

Suitability of wastewater treatment systems may vary depending on the water quality, final requirements and economical aspects (Marco *et al.*, 1997). Compared to biological processes, investment costs and treatment costs for chemical processes are 5 to 20 and 3 to 10 times higher, respectively. Optimal wastewater treatments can also often only be achieved if treatment methods are combined (Athanasopoulos & Athanasopoulos, 1998). Thus ozonation may be combined with other treatments in order to increase the effectiveness or decrease the cost of the wastewater treatment used (Gottschalk *et al.*, 2000). To date, two types of O_3 combined treatments have been used, namely pre- and post-ozonation (Goel *et al.*, 2003).

During pre-ozonation, the wastewater is pre-treated with O_3 prior to other treatments (Goel *et al.*, 2003). Pre-ozonation optimises the overall process by facilitating the second treatment and thus increasing the treatment efficiency (Andreozzi *et al.*, 1998). This two-step process can successfully treat wastewater containing compounds that are recalcitrant, inhibitory or non-biodegradable. Benitez *et al*. (2003) found when WDWW was pre-ozonated before being treated in an aerobic activated sludge system, better COD reductions were obtained compared to those produced during ozonation or treated in an activated sludge system. A COD

reduction of 25.5% was obtained in a single ozonation process at an O_3 flow rate of 30 g $O_3.h^{-1}$ and a HRT of 9 h. When the wastewater was aerobically degraded in an activated sludge system, with HRT of 48 h, the COD reduction was 28%. However, a COD reduction of 39% was obtained when two processes were combined.

Post-ozonation is generally referred to as a closed-loop operation because the conventional step of solids withdrawal is replaced with a recirculation line through ozonation of digested solids (Goel *et al.*, 2003). The retention time for substrate solids is longer. Regulating the amount of solids passing through post-ozonation controls the active biomass retention time. The organic solid degradation efficiency will increase due to higher retention times and a constant supply of ozonated substrate. Beltrán *et al.* (2001a) studied integrated aerobic biological oxidation and post-ozonation of spirits distillery wastewater. After 24 h of aerobic biological oxidation, the COD and polyphenol reductions were 82 and 35%, respectively. When this wastewater was ozonated in a 1.5 L glass bubble column at an O_3 dose of 240 $mg.L^{-1}$ the polyphenol reduction increased to 80%. However, the COD reduction did not improve because of the high alkalinity that accumulated in the wastewater.

Finally, Beltrán *et al.* (2000) compared the effect of pre- and post-ozonation of WDWW to determine which process was more effective. Mixtures of domestic and WDWW were continuously treated using combined ozonation-activated sludge and activated sludge-ozonation systems. When the wastewater was treated using the ozonation-activated sludge system (pre-ozonation) the best COD (59%), TOC (42%) and UV_{254} (78%) reductions were achieved. The TKN (23%) and total phenol reductions (37%) that were obtained in the activated sludge-ozonation system (post-ozonation) were slightly higher than those obtained during pre-ozonation. It was concluded that the best overall results were obtained during pre-ozonation because pre-ozonation increased the biodegradability of the wastewater and thus improved the performance of the activated sludge system.

F. DISCUSSION

Distilleries are one of the most polluting industries (Chandra *et al.*, 2002). Therefore, effective wastewater treatment of distillery wastewater is vital (Akunna & Clark, 2000; Wilkie *et al*., 2000).

UASB reactors have been used successfully to treat distillery wastewater (Laubscher, 2000; Goodwin *et al.*, 2001; Lata *et al.*, 2002). However, when this

complex wastewater is treated in an UASB reactor, operational problems may occur (Beltrán *et al.*, 1999b; Benitez *et al.*, 1999). Treatment of GDWW, which is rich in lipids and proteins, may be problematic because the recalcitrant compounds and grain particles in the wastewater become entrapped in the granular bed, while the lipid molecules enclose the sludge particles (Goodwin *et al.*, 2001; Nadais *et al.*, 2001). This leads to problems including: COD accumulation in the sludge bed; extensive clogging; biomass washout; scum layer formation; hampered release of biogas; foaming; sludge floatation; toxicity and scaling (Lettinga & Hulshoff Pol, 1991; Laubscher, 2000; Zeeman & Sanders, 2001).

Therefore, wastewater may be treated via a process such as ozonation, prior to or after UASB treatment, in order to purify toxic compounds, increase the biodegradability of the wastewater, facilitate the anaerobic process and decrease treatment costs (Gottschalk *et al.*, 2000; Boncz *et al.*, 2003; Delgenès *et al.*, 2003). Pre-ozonation optimises the overall process by facilitating the second treatment and thus increasing the treatment efficiency, especially when the wastewater contains compounds that are recalcitrant, inhibitory or non-biodegradable (Andreozzi *et al.*, 1998). Post-ozonation, increases the organic solid degradation efficiency. Beltrán *et al.* (2000) found that pre-ozonation was more effective than post-ozonation for treating WDWW when O_3 and activated sludge system was used.

This review has highlighted that GDWW, which is rich in lipids and proteins, cannot be treated successfully in an UASB reactor. To date very little research has be conducted in this field. It is necessary to verify which components in the GDWW are responsible for making GDWW unsuitable for UASB treatment. Methods to reduce or eliminate these components and to increase the overall biodegradability of GDWW must also be investigated. Ozone has been used to improve the biodegradability and reduction efficiency of distillery wastewater. Again, little research has been done on the pre- and/or post-treatment of distillery wastewater treated in an UASB reactor. Therefore there is scope to research the UASB treatment of GDWW as well as the pre- and post-ozonation of distillery wastewater.

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CHAPTER 3

OPTIMISATION OF A COMBINED UPFLOW ANAEROBIC SLUDGE BLANKET REACTOR AND AN OZONATION SYSTEM FOR WINE DISTILLERY WASTEWATER TREATMENT

Summary

Wine distillery wastewater (WDWW) was treated in a 2 L upflow anaerobic sludge blanket (UASB) reactor, together with different combinations of pre- and/or post-ozonation. A chemical oxygen demand (COD) reduction of 92% was obtained when the UASB reactor was operated at a substrate pH of 7.0, substrate COD of *ca*. 4 000 $mg.L^{-1}$, organic loading rate (OLR) of 4.0 kg $COD.m^{-3}.d^{-1}$ and hydraulic retention time (HRT) of 24 h. The COD reduction of ozonated (O_3 dose = 47 $mg.L^{-1}$) WDWW was 7%, while that of the UASB treatment was 92%. The COD reductions of UASB treatment combined with a pre- or post-ozonation step were 94 and 96%, respectively. UASB treatment combined with pre- and post-ozonation steps achieved a COD reduction of 98%. The UASB treatment efficiency (measured as microbial activity and energy recovery potential of the granules) improved over time, regardless of the introduction of the pre-ozonation step. Acclimatisation of the granules was also essential. The treatment combinations may have cost-saving implications for the industry. If these results could be obtained on a full-scale basis, up to 500 $m^3.d^{-1}$ of this treated WDWW (COD = 107 – 341 $mg.L^{-1}$) may be used for irrigation, according to South African wastewater discharge standards (COD < 400 $mg.L^{-1}$).

Introduction

Distilleries generate large amounts of polluted wastewater. The amount and composition of the wastewater vary, depending on the nature of the distillery, the substrate used and the production process utilised. Distillery wastewater is normally characterised by a high chemical oxygen demand (COD) (10 000 – 60 000 $mg.L^{-1}$) and a low pH (3.5 – 5.0) (Bustamante *et al*., 2005). If untreated, this wastewater may cause ecological and environmental problems. Effective wastewater treatment is vital, especially in South Africa, where wastewater discharge standards are strict (Bezuidenhout *et al.*, 2002).

The upflow anaerobic sludge blanket (UASB) reactor is the most widely used anaerobic biological reactor technology for treating industrial wastewaters (Chrobak & Ryder, 2005). UASB reactors can be operated under high organic loading rates (OLR) and still achieve good COD reductions. Laubscher (2000) found that the treatment of WDWW in an UASB reactor resulted in COD reductions of more than 90% at a hydraulic retention time (HRT) of 24 h and at OLRs of 10 to 18 kg $COD.m^{-3}.d^{-1}$. The biogas produced by UASB reactors may be converted into energy. UASB reactors are also ideal for industries that operate seasonally, as the granules can be preserved unfed for long periods and biomass viability can be maintained (Chrobak & Ryder, 2005).

However, distillery wastewater can often contain toxic compounds (such as long chain or volatile fatty acids (VFA), excess nutrients and phenolic compounds) that may restrict the overall UASB reactor efficiency (Benitez *et al.*, 1999). Therefore to improve the UASB reactor efficiency, pre- or post-treatments may be necessary.

Ozone (O_3) is an oxidant that is selective towards the double bonds of toxic, recalcitrant or refractory compounds – degrading them into simpler, more biodegradable fragments that are utilised by anaerobic bacteria. When O_3 reacts with phenolic compounds, oxidised intermediates are formed, which may be more or less toxic than the substrates (Huang & Shu, 1995). Beltrán *et al.* (2001b) found that ozonation of WDWW resulted in a COD and polyphenol reduction of 41 and 32%, respectively. Pre- and/or post-ozonation may be included during wastewater treatment to increase the biodegradability or efficiency and to decrease the cost (Gottschalk *et al.*, 2000). Beltrán *et al.* (2000) found that when WDWW was treated in an activated sludge system, pre- and post-ozonation resulted in COD reductions of 59 and 32%, respectively.

The aim of this study was to investigate the efficiency of the UASB treatment of WDWW and to enhance the efficiency by pre- and/or post-ozonation treatments. The activity of the UASB granules was also monitored throughout the study to determine how the UASB and ozonation treatments influenced the granule activity.

Materials and methods

UASB reactor design

The UASB reactor (Fig. 1) was set up as described by McLachlan (2004). The UASB reactor had a height of 880 mm, an internal diameter of 50 mm and an operational volume of 2.3 L. The HRT was set at 24 h. The substrate was fed semi-continuously into the

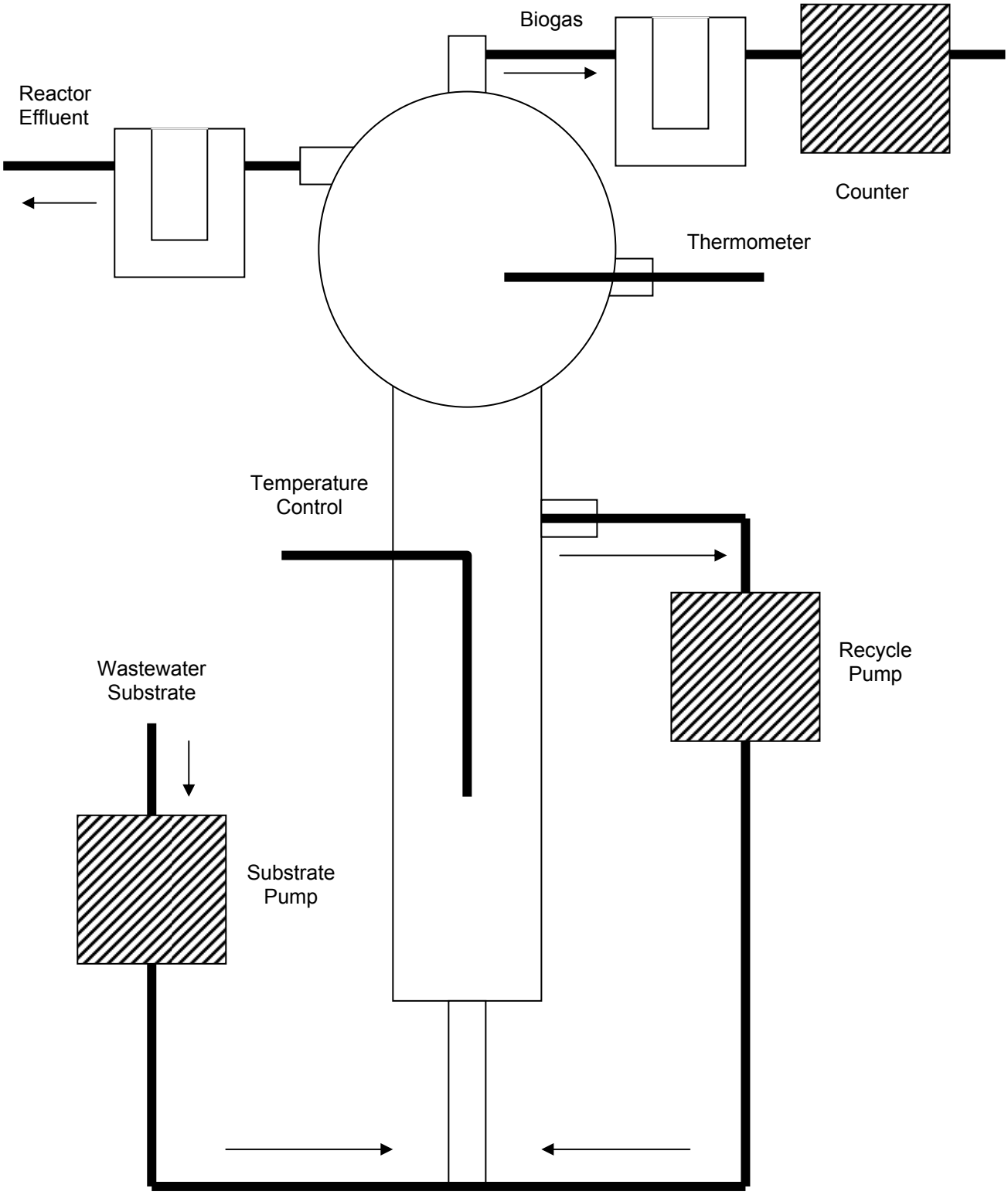

Figure 1 Set-up of laboratory-scale UASB reactor (McLachlan, 2004).

bottom of the UASB reactor with the aid of a peristaltic pump (Watson-Marlow 501) and an electronic timer. The UASB reactor effluent was emptied into a 2 L Schott bottle through a U-shaped tube, which prevented atmospheric oxygen (O_2) from entering the UASB reactor. Recirculation was made possible by using a peristaltic pump (Watson-Marlow 101U), which was set to maintain an upflow velocity of 2.0 $m.h^{-1}$. The temperature of the insulated UASB reactor was maintained at 35°C by using an electronic control unit (solid-state thermostat) and heating tape (Meyer *et al.*, 1983). The biogas produced was released at the top of the UASB reactor and measured using a biogas meter, which consisted of a manometric unit with an electronically controlled counter.

Reactor start-up

UASB granules (500 mL) (volatile suspended solids (VSS) content = 3 750 ± 127 $mg.L^{-1}$) were obtained from a full-scale UASB reactor treating WDWW in Wellington, South Africa and was used to seed the UASB reactor. The UASB reactor was fed, for a stabilisation period of 24 h, with water containing 500 $mg.L^{-1}$ urea ($(NH_2)_2CO$) and 500 $mg.L^{-1}$ di-potassium hydrogen orthophosphate (K_2HPO_4). The UASB reactor was then fed with WDWW diluted to a COD of *ca*. 500 $mg.L^{-1}$. The substrate COD concentration was increased once the system had reached a COD reduction of approximately 90%. These increases were continued until a substrate COD of *ca*. 4 000 $mg.L^{-1}$ was obtained. Additionally, 1 mL trace element solution was added weekly to the UASB reactor (Nel *et al.*, 1985). An identical control UASB reactor was also started and operated in the same manner, but was only fed WDWW as substrate throughout the operational period.

Wastewater

Five batches of WDWW were obtained from local distilleries in Worcester and Wellington, South Africa (February to May of 2004 and 2005). Different batches of WDWW were used as substrate for the first 430 d, while an ozonated batch was used from day 431 to 544. The raw and ozonated WDWW was stored in 25 L drums at -18 °C and defrosted and kept at 4°C when required. The WDWW was diluted with tap water to the desired COD and 500 $mg.L^{-1}$ each of urea and K_2HPO_4 were added. The pH of the substrate was adjusted to a pH between 7.00 and 8.00 with 1 M potassium hydroxide (KOH).

Ozonation of substrate and UASB effluent

Ozonation was accomplished using an O_3 generator (Parc Scientific, Ifafi) that produced 118 mg $O_3.h^{-1}$ (at a gas flow rate of 4 $L.min^{-1}$), as determined by the Iodometric method

(APHA, 1998). Pre-ozonation of diluted WDWW (COD = 4 000 $mg.L^{-1}$) was done in a glass bubble column system and in a venturi circulating contactor system to determine which system was the most effective in terms of COD reduction.

The bubble column (height = 104 cm, width = 10 cm and volume = 2 L) contained a sintered glass disc at its base for bubble formation. The column width widened to 13 cm between 56 and 70 cm, in order to reduce foaming. The column width above 70 cm was again 10 cm, which again widened to 13 cm between a height of 87 and 97 cm. A second sintered glass disc was placed in the column (above the second widening) at a height of 80 cm so as to minimise loss of substrate due to excessive foaming. The column width above the sintered glass disc was 10 cm.

The venturi circulating contactor system had an operational volume of 50 L. It consisted of a sample drum, pump, venturi device, recirculation system and contacting column (height = 103 cm, diameter = 11 cm and volume = 9.8 L) (Sigge, 2005). The contacting column contained stainless steel turnings in order to improve mixing within the system. The wastewater or the UASB effluent was circulated using a stainless steel impeller pump (Calpeda MXH 203E, Vincenza, Italy) with a flow rate of 50 $L.min^{-1}$. O_3 entered the system via the venturi installed on the inlet side under the contacting column. The O_3 residence time was increased by maintaining pressure within the system.

Wastewater samples were ozonated at room temperature in the glass bubble column or the venturi circulating contactor system until an O_3 dose (time) of 1 181 $mg.L^{-1}$ (20 min) and 283 $mg.L^{-1}$ (120 min), respectively, were obtained. The optimal O_3 dose was determined by testing the COD reduction at various time intervals (APHA, 1998).

Analytical methods

WDWW and UASB effluent parameters monitored will include: pH, alkalinity (as calcium carbonate [$CaCO_3$]), total solids (TS), total suspended solids (TSS), phosphorous (PO_4^{3-}) and COD (APHA, 1998). Polyphenol content was determined using the Folin-Ciocalteau method (Singleton & Rossi, 1965). The bed-height of the sludge blanket during recirculation was also measured.

The biogas composition was determined by injecting a 0.2 mL biogas sample into a gas chromatograph (GC) (Varian 3300) (Sigge, 2005). The GC was equipped with a thermal conductivity detector and a 2.0 m x 3.0 mm i.d. column filled with Hayesep Q (Supelco, Bellefonte, PA), 80/100 mesh. Helium was used as carrier gas at a flow rate of 30 $mL.min^{-1}$ and the oven temperature was set at 55 °C.

To determine the VFA concentration, samples were prepared by mixing 1 mL 35% formic acid, 3 mL wastewater sample and 2 µL n-hexanol (internal standard). A standard solution (acetic, propionic, iso-butyric, butyric, iso-valeric and valeric acid) was prepared in a 1 L volumetric flask by diluting 1 mL of each VFA and 0.5 mL n-hexanol in one part 35% formic acid and 3 parts distilled water. Samples (1 µL) were injected into a GC (Varian Model 3700), which was equipped with a flame ionisation detector and a 30 m bonded phase Nukol (Supelco, Inc., Belafonte, PA) fused silica capillary column with a diameter of 0.53 mm and a film thickness of 0.50 µm (Sigge, 2005). The nitrogen carrier gas was used at a flow rate of 6.1 $mL.min^{-1}$. The temperatures of the inlet and detector were set at 130 and 300°C, respectively. For the first two minutes the column temperature was set at 105°C and then increased to 190°C at a rate of $10°C.min^{-1}$, and held for 10 min. VFAs were quantified, using Borwin Version 1.2 integration software (JMBS Developments, Le Fontanil, France). All analyses were done in triplicate.

Granule activity

Activity tests were preformed on UASB granules using the test described by O'Kennedy (2000). The granule activity of the granules used to seed the UASB reactor was determined before the reactor start-up to determine the initial granule activity. Throughout the study granules were sampled from the UASB reactor at various times (before the 2nd start-up, before pre-ozonation and at the end of the study) to determine the granule activity. The measured activity was compared to the initial granule activity. Granule activity and biogas composition were expressed as the cumulative biogas and the methane (CH_4) produced respectively. The test media used were specific for certain microbial groups. The basic test medium (BTM) was used as a control (Tables 1 and 2). The glucose test medium (GTM) was used to determine the activity of the acidogens and the active methanogens (through metabolite formation), while the acetic test medium (ATM) was used to measure acetoclastic methanogen activity.

During all the activity tests, 50 g of the sampled granules were incubated in a 250 mL Schott bottle at 35°C for 48 h in 150 mL activation media (Table 3). This was decanted and replaced with fresh activation medium after 24 h (O'Kennedy, 2000). After the incubation period of 48 h, triplicate granule samples (3 g) were placed in 20 mL glass vials together with 13 mL specific test medium (BTM, GTM and ATM), leaving 6 mL headspace. The vials were sealed with butyl septa, capped with aluminium caps and incubated at 35°C for 25 h. After 5, 10 and 25 h incubation the biogas volume was

Table 1 Composition of the BTM (Valcke & Verstraete, 1993; O'Kennedy, 2000)

Compound	**Concentration ($g.L^{-1}$)**
Glucose	2.0
Di-potassium hydrogen orthophosphate (K_2HPO_4)	1.0
Potassium di-hydrogen orthophosphate (KH_2PO_4)	2.6
Urea	1.1
Ammonium chloride (NH_4Cl)	1.0
Sodium sulphide ($Na_2S \cdot 9H_2O$)	0.1
Magnesium chloride ($MgCl_2 \cdot 6H_2O$)	0.1
Yeast extract	0.2
pH	7.1

Table 2 Composition of the different test media used to determine the activity of certain microbial groups (O'Kennedy, 2000)

Compound	**Microbial group**
Basic test medium (BTM)	Control
Glucose test medium (BTM + 2.0 $g.L^{-1}$ glucose) (GTM)	Acidogens
Acetic acid test medium (BTM + 1.0 $g.L^{-1}$ acetic acid) (ATM)	Acetoclastic methanogens

Table 3 Composition of activation medium used during the activity tests (O'Kennedy, 2000)

Compound	**Concentration ($g.L^{-1}$)**
Glucose	1.0
Di-potassium hydrogen orthophosphate (K_2HPO_4)	0.5
Urea	0.5

measured by using a free moving 10 mL syringe with a 12 gauge needle. The needle was inserted through the butyl septa and the biogas volume was determined once the piston stopped moving. The biogas composition was determined gas chromatographically. Activity tests were prepared in duplicate.

Results and discussion

Wastewater composition

The composition of the WDWW batches is shown in Table 4. The variation in composition was expected, as the composition of distillery wastewater is known to exhibit major daily and seasonal variations.

Operation and efficiency of UASB reactor treating WDWW

Control UASB reactor

A control UASB reactor was operated for 430 days on WDWW at a final substrate COD and OLR of *ca*. 4 000mg.L^{-1} and 4.0 kg COD.m^{-3}.d^{-1}, respectively. When the UASB reactor was operating optimally (day 372 to 430), the COD reduction ranged between 90 and 94%. During this period, the effluent pH, alkalinity and PO_4^{3-} achieved were 7.2, 1 775 mg.L^{-1} and 115 mg.L^{-1}, respectively. Good reductions in TS (24%), TSS (55%) and polyphenols (60%) were also obtained. The biogas production ranged from 1.00 to 2.00 L.d^{-1} (0.55 to 1.10 L CH_4.d^{-1}).

UASB reactor

During the treatment of WDWW in the UASB reactor, various WDWW and UASB effluent parameters were monitored. The substrate COD and COD reduction are shown in Fig. 2, while the substrate pH, effluent pH and effluent alkalinity are shown in Fig. 3.

Phase 1

At start-up diluted WDWW was used as substrate and the reactor bed-height was 31 cm. After the first few days, the substrate COD and OLR were increased gradually to 4 300 mg.L^{-1} and 4.3 kg COD.m^{-3}.d^{-1}, respectively, by day 140 (Fig. 2). During this period, the COD reduction was more than 90% (Fig. 2). The effluent alkalinity increased to 2 300 mg.L^{-1} (Fig. 3), which was within the optimum alkalinity range (1 000 – 3 000 mg.L^{-1}) for UASB reactors, as recommended by Gerardi (2003). As the reactor efficiency increased, the substrate pH was gradually decreased to 7.0 (on day 93) since the reactor

Table 4 Composition of raw WDWW. The range represents 15 samples from five batches (three samples per batch) from February to May of 2004 and 2005

Constituent ($mg.L^{-1}$)	Range for this study	Literature values
COD_{total}	10 938 – 38 637	12 975 – 45 000[a, b, c]
pH	3.4 – 3.7	3 – 4[a, b, d]
Phosphorous (as PO_4^{3-})	642 – 1 274	6 – 1 456[e]
Total solids	13 889 – 31 427	6 685 – 113 615[e]
Total suspended solids	45 – 316	100 – 400[a]
Total polyphenols (gallic acid equivalents)	5 – 46	29 – 766[e]

[a] Laubscher, 2000

[b] Wolmarans & De Villers, 2002

[c] Vlyssides *et al*., 2005

[d] Beltrán de Heredia *et al*., 2005

[e] Bustamante *et al.*, 2005

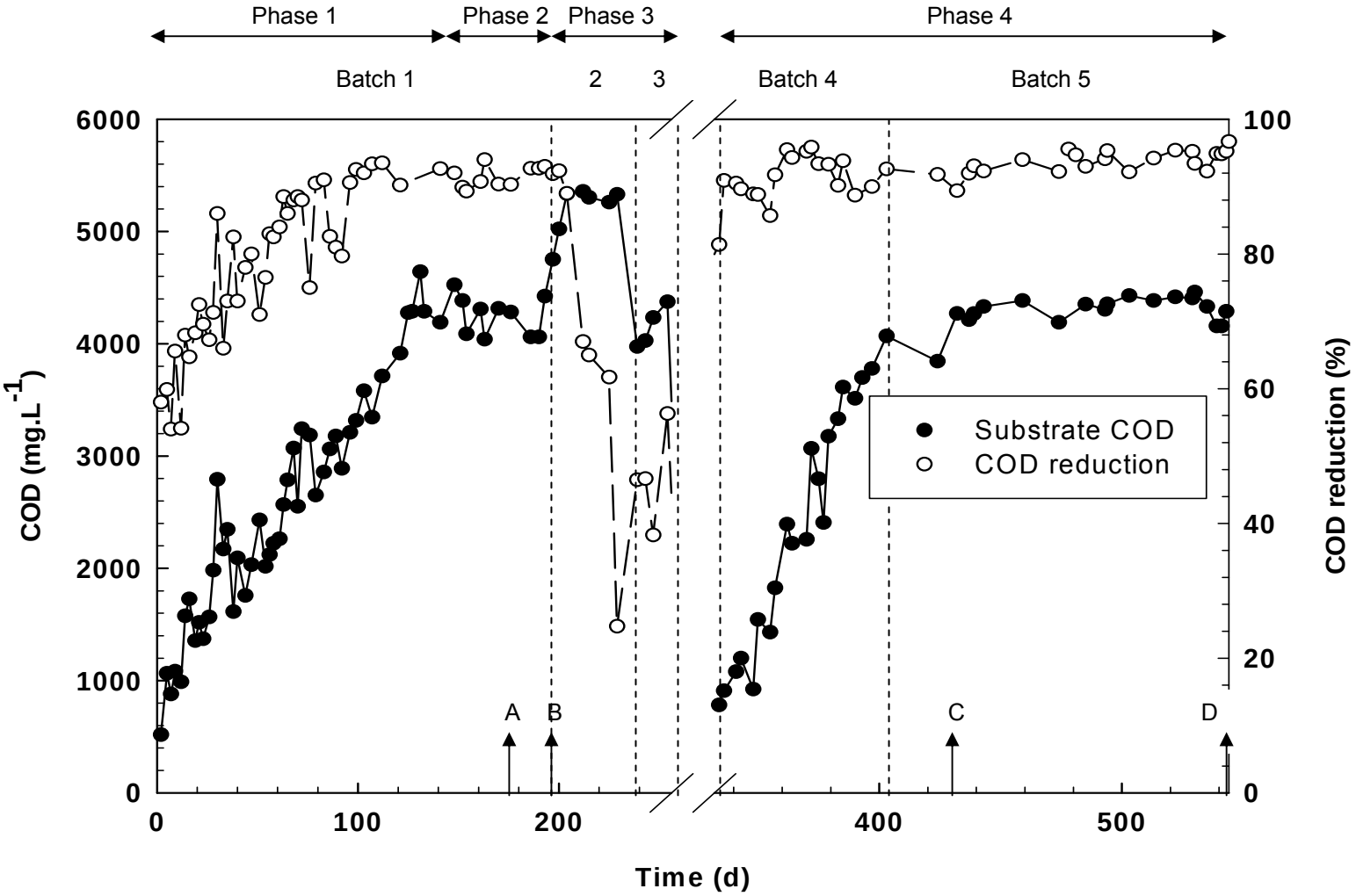

Figure 2 Substrate COD and COD reduction of the UASB reactor treating WDWW. (For the period A to B a post-ozonation step was included and for the period C to D pre- and post-ozonation steps were included).

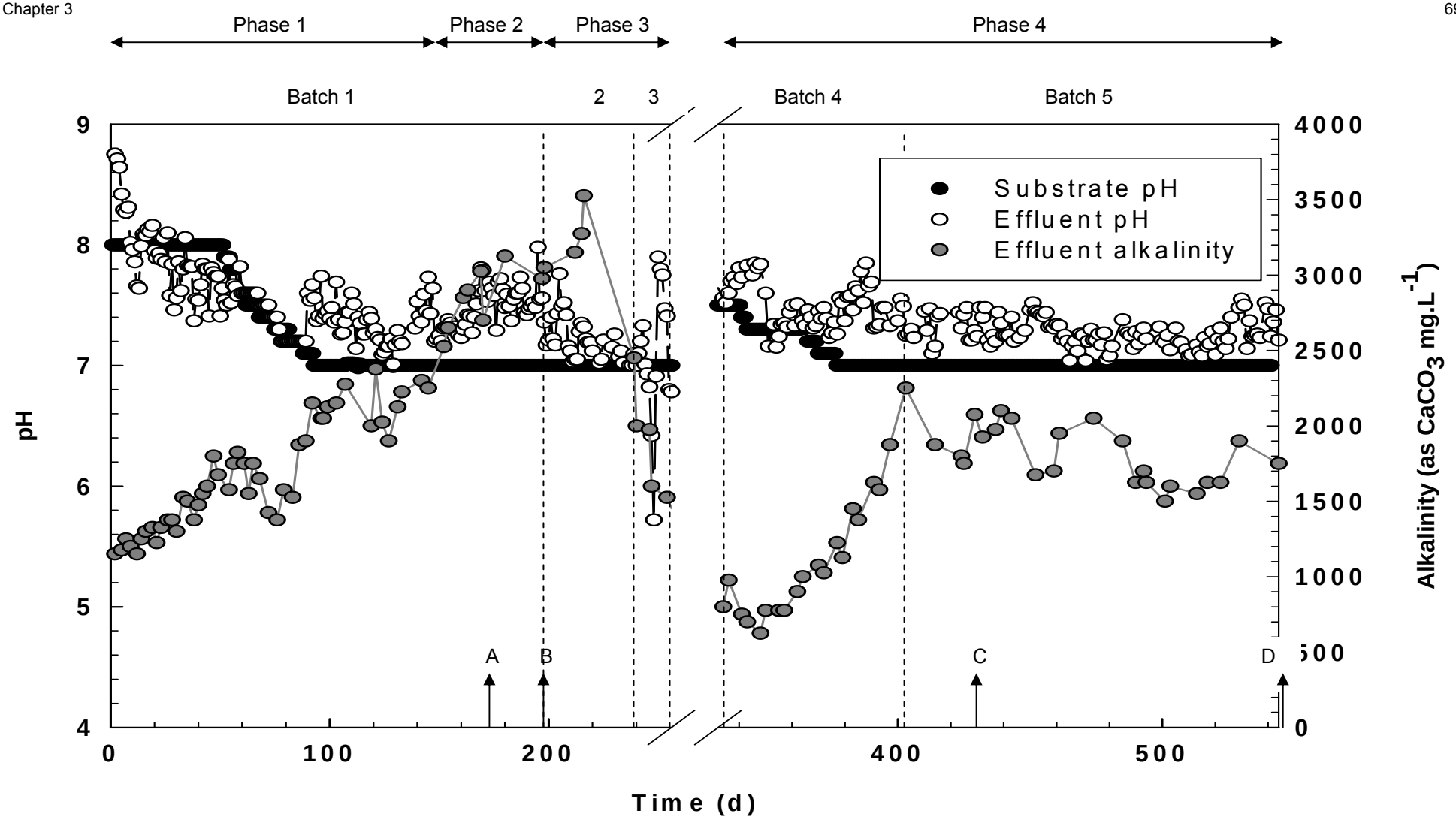

Figure 3 Substrate pH, effluent pH and effluent alkalinity of the UASB reactor treating WDWW. (For the period A to B a post-ozonation step was included and for the period C to D pre- and post-ozonation steps were included).

effluent pH (7.6) was within the optimal pH range (6.5 – 7.6) for UASB reactors, as recommended by Rajeshwari *et al.* (2000). The substrate feed pH was then maintained at 7.0. During this phase, biogas production was low but from day 67 onwards the UASB granules appeared to have adapted to their new environment and the biogas production increased. The biogas production ranged from 1.01 to 2.29 $L.d^{-1}$ (0.50 – 1.15 L $CH_4.d^{-1}$). The bed-height of the UASB reactor was found to increase steadily to 63 cm by day 136. This increase may be ascribed to the increase in the overall UASB reactor activity and subsequently the biogas production increased, lifting the granule bed higher.

Phase 2

From day 141 to 197, the substrate COD of 4 000 $mg.L^{-1}$, OLR of *ca.* 4.0 kg $COD.m^{-3}.d^{-1}$ and COD reduction of 92% were maintained (Fig. 2). The COD reduction achieved during this period was similar to that obtained by Moosbrugger *et al.* (1993), Laubscher (2000) and Wolmarans and De Villers (2002) for WDWW. The COD reduction achieved from day 141 to 197 was better than that obtained by Akarsubasi *et al.* (2006) who treated distillery wastewater in an UASB reactor (COD reduction = 60 to 80% at an OLR of 2.5 to 8.5 kg $COD.m^{-3}.d^{-1}$).

The effluent pH (7.5) (Fig. 3) was within the optimum pH range for anaerobic digestion. The effluent alkalinity increased slightly from 2 525 to 3 050 $mg.L^{-1}$ and was within the marginal alkalinity range (3 000 – 5 000 $mg.L^{-1}$), as reported by Gerardi (2003). The bed-height continued to increase to 69 cm by day 179. The effluent VFA concentration (44 $mg.L^{-1}$) was far below the levels that Mata-Alvarez (2003) reported to be detrimental for methanogenesis (>400 $mg.L^{-1}$). The biogas production on day 196 was 0.50 $L.d^{-1}$ (0.28 L $CH_4.d^{-1}$), which was low.

Post-ozonation

From day 174 to 197, the reactor effluent was ozonated (post-ozonation) at an optimal O_3 dose (time) of 71 $mg.L^{-1}$ (30 min) and additional reductions in COD (50%), alkalinity (3%), TS (11%), TSS (53%), total polyphenols (52%) and VFA (23%) were achieved. The total process efficiency of UASB treatment combined with post-ozonation of WDWW is shown in Table 5. It was found that UASB treatment combined with post-ozonation increased the total process efficiency to: 96% COD reduction, 39% TS reduction, 52% TSS reduction and 88% total polyphenols reduction. The decrease in COD and solids was expected because O_3 is selective towards the double bonds found in wastewater compounds and

Table 5 Efficiency parameters during various UASB reactor-ozonation treatments

Wastewater	COD ($mg.L^{-1}$)	COD reduction (%)	TS ($mg.L^{-1}$)	TS Reduction (%)	TSS ($mg.L^{-1}$)	TSS reduction (%)	Polyphenol ($mg.L^{-1}$)	Polyphenol reduction (%)	VFA ($mg.L^{-1}$)
WDWW*	4 334	–	5 090	–	268	–	8.4	–	75
O_3 (Pre-ozonation)**	4 023	7	3 015	41	125	53	6.5	22	31
UASB **	341	92	3 901	23	134	50	3.9	54	40
O_3+UASB **	246	94	3 430	33	40	85	3.1	63	38
UASB+O_3 **	176	96	3 115	39	128	52	1.0	88	31
O_3+UASB+O_3 **	107	98	2 560	50	34	87	0.2	98	26

* Before treatment

** After treatment

thus degrades them into simpler fragments. The significant polyphenol reduction was expected, as ozonation is selective towards the double bonds in polyphenols. Beltrán *et al.* (2001b) reported that post-ozonation of WDWW at an acidic or neutral pH led to a greatly reduced polyphenol content. A reduction in VFAs (to a final content of 31 $mg.L^{-1}$) was also not surprising because El-Din *et al.* (2006) found that O_3 may degrade VFA into low molecular-weight by-products, which are generally less toxic. During post-ozonation a slight increase in PO_4^{3-} content (from 123 to 127 $mg.L^{-1}$) occurred because PO_4^{3-} is solubilised with ozonation. Better reductions were obtained when UASB treatment was combined with a post-ozonation step compared to UASB treatment.

Phase 3

From day 197 to day 204 (7 days), the substrate COD and OLR were increased step-wise to 5 000 $mg.L^{-1}$ and 5.0 kg $COD.m^{-3}.d^{-1}$, respectively (Fig. 2). On day 204 the COD reduction decreased from 92 to 89%. From day 205 to 236, the substrate COD was maintained at 5 300 $mg.L^{-1}$ but the COD reduction continued to decrease to 25%. On day 230 the propionic to acetic acid ratio was 1.5 – indicating possible reactor instability or failure (propionic to acetic acid ratio > 1.4), as reported by Buyukkamaci and Filibeli (2004). The increase in OLR possibly resulted in an accumulation of VFA, which could not be metabolised at the same rate as which they were produced. The pH decreased and the more acid-tolerant acidogenic bacteria continued to produce VFAs, which accumulated in the system. As a result, the pH would continue to decrease and may lead to reactor failure. Segretain and Moletta (1987) also reported that when WDWW was treated anaerobically, VFA accumulated in the reactor during periods of reactor failure.

As a result of the very low efficiency, it was decided change the WDWW batch and to lower the substrate COD and OLR to 4 000 $mg.L^{-1}$ and 4.0 kg $COD.m^{-3}.d^{-1}$, respectively. However, the UASB reactor efficiency remained low from day 237 to 255, with a substrate COD, OLR and COD reduction of 4 153 $mg.L^{-1}$, 4.2 kg $COD.m^{-3}.d^{-1}$ and 47%, respectively (Fig. 2). Unstable operation of the UASB reactor during this period was emphasised by the drastic decrease in effluent pH that dropped to 5.7 (Fig. 3). The reactor efficiency continued to decrease after the WDWW was changed because batch 3 was obtained from a different source, containing WDWW and winery wastewater, and was found to be high in polyhenols (280 – 1 450 $mg.L^{-1}$) that are toxic for anaerobic bacteria in high concentrations. It was decided to discontinue using batch 3 as substrate until suitable WDWW was available because WDWW is only produced during the grape season and is not generated throughout the year.

Phase 4

When the UASB reactor was started again on day 333, using batch 4 as substrate, the substrate COD, OLR, substrate pH and effluent alkalinity were 783 $mg.L^{-1}$ (Fig. 2), 0.8 kg $COD.m^{-3}.d^{-1}$, 7.5 (Fig. 3) and 800 $mg.L^{-1}$ (Fig. 3), respectively. The initial reactor bed-height was 64 cm and increased steadily to 70 cm by day 430. The substrate pH was decreased gradually from 7.5 to 7.0 by day 379 and then maintained at 7.0. During this period the effluent pH was 7.4, while the effluent alkalinity increased to 1 125 $mg.L^{-1}$ (Fig. 3). The substrate COD was increased to 4 000 $mg.L^{-1}$ by day 397 and the COD reduction increased to more than 90% (Fig. 2).

The substrate COD was kept constant at 4 000 $mg.L^{-1}$ from day 398 to 430. On day 406 the substrate was changed from batch 4 to 5. It was found that the UASB reactor continued to operate optimally with the COD reduction, effluent pH and alkalinity at 92%, 7.3 and 1 950 $mg.L^{-1}$, respectively. From day 398 to 430, a reduction in TS (23%), TSS (50%) and polyphenol (54%) content was also achieved (Table 5).

Ozonation of substrate and UASB effluent

Substrate ozonation

The O_3 dose that produced the maximum COD reduction was chosen as the optimal O_3 dose. When diluted WDWW (COD = 4 000 $mg.L^{-1}$) was ozonated in the bubble column, the maximum COD reduction was 7% at an O_3 dose of 708 $mg.L^{-1}$ (12 min). When diluted WDWW (COD = 4 000 $mg.L^{-1}$) was ozonated in the venturi circulating contactor system, the maximum COD reduction was also 7%, at a much lower O_3 dose of 47 $mg.L^{-1}$ (20 min). This was ascribed to the fact that better mixing and O_3 contacting with the organic components occurred in the venturi circulating contactor system. The efficiency of ozonating WDWW in the venturi circulating contactor is shown in Table 5. Reductions in TS (41%), TSS (53%) and total polyphenols (22%) content were achieved. The VFA (59%) and PO_4^{3-} (3%) content were also reduced. Therefore, ozonations for the pre- and post-ozonation steps were done using the venturi circulating contactor because a lower O_3 dose was required and a larger volume of wastewater could be ozonated.

Pre-ozonation

On day 432, pre-ozonated WDWW was used as substrate (pH = 7.0) and the effluent pH and alkalinity were 7.4 and 1 925 $mg.L^{-1}$, respectively (Fig. 3). Granule and solids washout occurred from day 432 to 435. Therefore, by day 435 the bed-height had decreased from 70 to 60 cm. The bed-height increased again to 69 cm by day 544. By

day 438, the COD reduction had stabilised at 94% (Fig. 2) and the effluent pH and alkalinity ranged from 7.0 to 7.6 and 1 500 to 2 100 $mg.L^{-1}$, respectively (Fig. 3). Reductions in TS (33%), TSS (85%) and polyphenols (63%) content were obtained when pre-ozonated WDWW was treated in the UASB reactor (Table 5).

Pre- and post-ozonation

When effluent from the UASB reactor treating the pre-ozonated substrate was post-ozonated (O_3 dose = 47 $mg.L^{-1}$), a further reduction occurred in the COD (57%), PO_4^{3-} (31%), TS (25%), TSS (14%), polyphenols (95%) and VFA (32%). The total process efficiency of UASB treatment combined with pre- and post-ozonation steps is shown in Table 5. UASB treatment combined with pre- and post-ozonation steps resulted in total reductions in COD (98%), TS (50%), TSS (87%) and polyphenol (98%) content.

UASB treatment combined with pre- and post-ozonation was found to be the most efficient treatment (Table 5). Pre-ozonation probably facilitated the treatment by increasing the biodegradability of the wastewater organics. Beltrán *et al.* (2001a) found that polyphenol reductions in distillery wastewater were greater in an activated sludge-ozonation system (80% polyphenol reduction) than in just an activated sludge system (35% polyphenol reduction). Beltrán *et al*. (2000) also reported that the COD reduction in WDWW treated in an ozonation-activated sludge system (59% COD reduction) was greater than ozonation (4% COD reduction) and treatment in an activated sludge system (30% COD reduction) or activated sludge system-ozonation (32% COD reduction).

Granule activity

Activity tests are generally used to determine the degradation potential and biodegradability of the wastewater as well as the microbial activity of biomass, such as UASB granules. Activity tests can also be used to optimise the start-up process of an UASB reactor, study the potential toxicity of certain substances on biomass, or calculate the energy recovery potential of the biomass in terms of CH_4 yield. The purpose of the activity tests done in this study was to determine the overall performance over the operational period of the UASB granules and the UASB reactor. Therefore the UASB granules were sampled and compared at various times (before the study started, before 2nd start-up, before pre-ozonation and at the end of the study). Granule activity, measured as cumulative biogas, was determined after 5, 10 and 25 h incubation. The results, after 25 h incubation, using the BTM, GTM and ATM, are shown in Fig. 4. The test media used

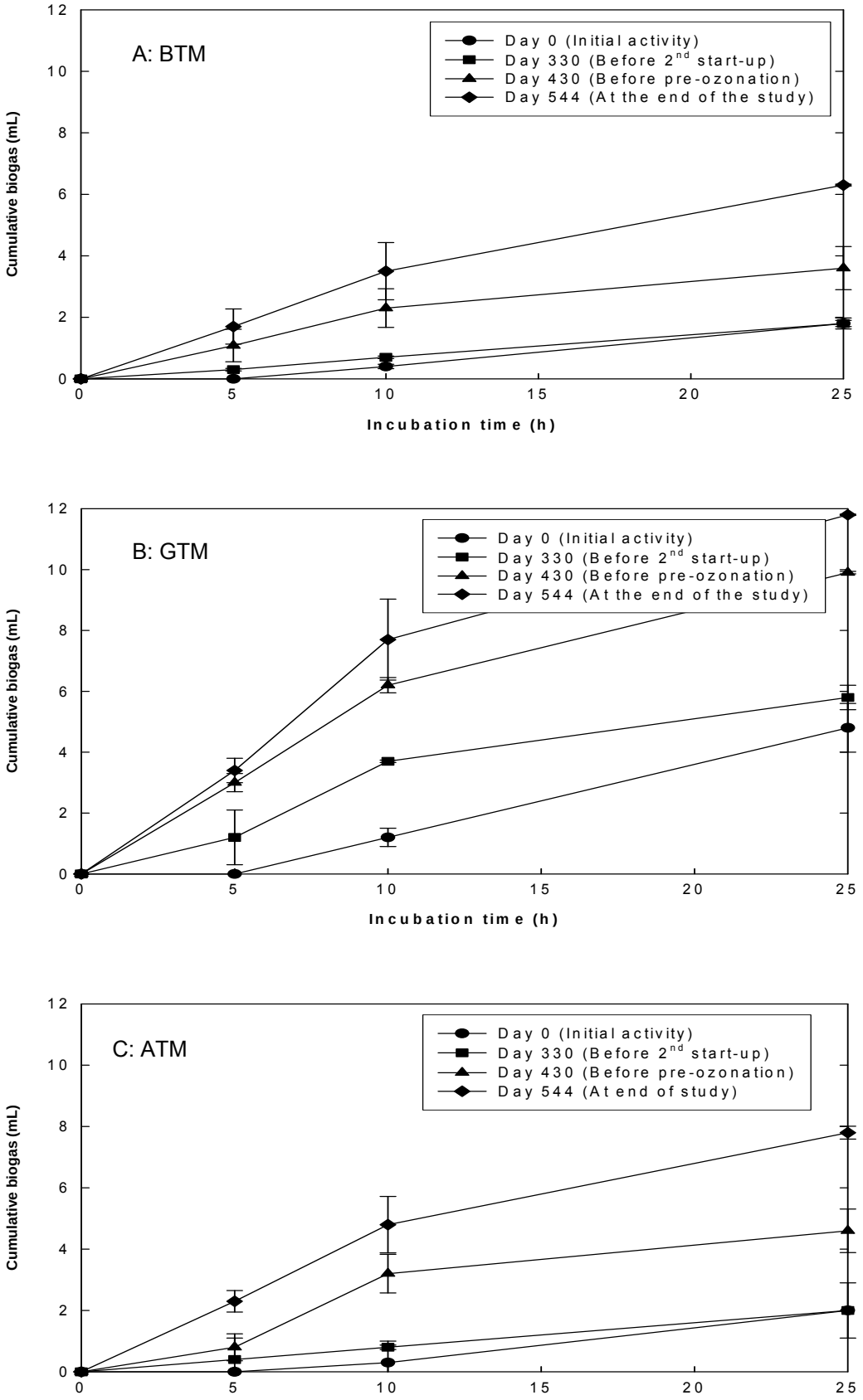

Figure 4 Biogas production of UASB granules used to seed the reactor and of UASB granules removed from the UASB reactor at various periods of operation, after incubation in BTM (A), GTM (B) and ATM (C).

were specific for certain microbial groups. Therefore the results were dependent on the energy and carbon source that the granules were grown on.

When the granules, which were sampled at different times during the operation time, were incubated in BTM for 25 h, the granule activity increased over time (Fig. 4). The granule activity before the addition of a pre-ozonation step (day 430) (3.6 mL cumulative biogas) and at the end of the study (day 544) (6.3 mL cumulative biogas) was higher than that on day 0 and before the 2nd start-up (day 330) (both 1.8 mL cumulative biogas). This increase probably occurred because the microbial population became acclimatised to the conditions in the UASB reactor.

When the sampled granules were incubated in GTM for 25 h, the granule activity increased over time (Fig. 4). The granule activity before the addition of a pre-ozonation step (day 430) (9.9 mL cumulative biogas) and at the end of the study (day 544) (11.8 mL cumulative biogas) was higher than that on day 0 (4.8 mL cumulative biogas) and before the 2nd start-up (day 330) (5.8 mL cumulative biogas). The most biogas was generated by the samples incubated in GTM, as the additional 2g.L^{-1} glucose that GTM contained enhanced the activity of the acidogens. The granule activity in GTM was also higher than that of the other media because acidogens are the largest of the trophic groups involved in anaerobic digestion and have a faster growth rate compared to the other trophic groups.

When the sampled granules were incubated in ATM for 25 h, the granule activity increased over time (Fig. 4). The granule activity before the addition of the pre-ozonation step (day 430) (4.6 mL cumulative biogas) and at the end of the study (day 544) (7.8 mL cumulative biogas) was higher than that on day 0 (2.0 mL cumulative biogas) and before the 2nd start-up (day 330) (2.0 mL cumulative biogas). In general, the granule activity in ATM was higher than that in BTM, as the addition of acetic acid to BTM enhanced the growth of the acetoclastic methanogens, and thus more biogas was produced.

Furthermore, in all the test media, the CH_4 yield increased as both the operation time and the energy recovery potential increased. Since CH_4 is the major final metabolite of methanogens, the activity of the methanogens also increased. This indicates that a possible population shift occurred within the granules. The increase in CH_4 production is of significance for the distillery industry, as CH_4 can be can be captured and used as fuel.

Conclusions

This study showed that ozonation, UASB treatment and combinations of the treatments could successfully be used to treat WDWW. In terms of COD and polyphenol reductions,

UASB treatment was more effective than ozonation alone. UASB treatment combined with a post-ozonation step was also more effective than UASB treatment combined with a pre-ozonation step. However, the advantages of pre-ozonation are more beneficial for the distillery industry. If production volumes were increased, the COD reduction brought about by pre-ozonation could facilitate lowering the HRT of the UASB reactor, thus increasing the daily volumetric throughput. The results of this study also clearly showed that the best efficiency was achieved when UASB treatment combined with pre- and post-ozonation steps. However, this treatment combination may not on full-scale be economical or practical because the wastewater is ozonated twice, increasing the overall cost.

This study also showed that the microbial activity and energy recovery potential of the granules in the UASB reactor increased as the operational period increased. Therefore, as the period of time the granules remained in the UASB reactor increased, the overall UASB reactor performance improved. Due to the improvement in overall UASB reactor performance from day 0 to 330, it is clear that acclimatisation in a new reactor is essential. The overall UASB reactor performance was also better after the treatment of pre-ozonated WDWW than before the treatment. Thus, it was concluded that pre-ozonation of WDWW did not negatively affect the overall UASB reactor performance.

All the treatment combinations used in this study gave COD values that were below 400 $mg.L^{-1}$. If these results could be obtained on a full-scale basis, up to 500 $m^3.d^{-1}$ of this wastewater may be used for irrigation, according to the South African wastewater discharge standards (Anon., 2004). However, none of the treatment combinations were able to reduce the treated wastewater COD to below 75 $mg.L^{-1}$, which is required for wastewater to be released into water resources. Despite this, combining a pre- and/or post-ozonation step with UASB treatment of WDWW may still have important cost-saving implications for the distillery industry, especially in terms of reducing costs needed for additional treatment, or even by first lowering the wastewater penalties. The municipality sets wastewater penalties and the distillery will have to pay fines for every mg $COD.L^{-1}$ wastewater the COD is above a specified value. Reducing the overall COD will also reduce the wastewater penalties paid.

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CHAPTER 4

IMPACT OF DILUTED AND OZONATED GRAIN DISTILLERY WASTEWATER ON UPFLOW ANAEROBIC SLUDGE BLANKET GRANULES

Summary

Operational problems (such as foaming, scum layer formation, clogging and washout) occur when lipid rich (374 – 479 $mg.L^{-1}$) grain distillery wastewater (GDWW) is treated in an upflow anaerobic sludge blanket (UASB) reactor. In this study it was found that granules exposed to diluted GDWW for 24 d became covered in an "encapsulating" layer. It was also shown that these granules contained more lipids (60.04 ± 0.03 $mg.g^{-1}$ granule) than granules from UASB reactors treating wine distillery wastewater (WDWW) (1.31 ± 0.06 $mg.g^{-1}$ granule) or brewery wastewater (1.49 ± 0.02 $mg.g^{-1}$ granule). Ozonation (ozone (O_3) dose = 1 476 $mg.L^{-1}$) was found to reduce the lipid content of GDWW by 74% and this led to a less severe encapsulating layer forming around the granules. Granules were also exposed to different substrates in batch reactors for 24 days and it was found that the most activity was achieved in granules exposed to a synthetic glucose substrate as the substrate contained sufficient nutrients required for microbial growth. The activity of granules exposed to GDWW, ozonated GDWW or WDWW decreased as these substrates contain compounds that inhibit microbial growth, and that are unable to support microbial activity in terms of growth requirements.

Introduction

Whisky manufacturing generates large volumes of grain distillery wastewater (GDWW). GDWW is rich in protein (280 – 340 g crude $protein.kg^{-1}$ dry matter) and lipids (± 125 g $oil.kg^{-1}$ dry matter) (Arosemena *et al*., 1995; Hill, 2002). Since GDWW is heavily polluted, wastewater treatment is imperative to prevent environmental and ecological problems.

Although upflow anaerobic sludge blanket (UASB) reactors have been utilised to treat other distillery wastewaters effectively, operational problems occur during the UASB treatment of GDWW (Laubscher, 2000). These included: scum layer formation; hampered release of biogas; high degree of COD or acid accumulation; extensive clogging; biomass

or sludge washout; foaming and scaling (Lettinga & Hulshoff Pol, 1991; Laubscher; 2000; Nadais *et al.*, 2001; Zeeman & Sanders, 2001). These problems may occur as the grain particles, which do not biodegrade easily, become entrapped in the UASB sludge bed or enclose the granules – reducing the contact between the microbial consortium in the granules and wastewater. Thus, the digestion process is restricted and the granule activity is decreased. Compounds such as long chain or volatile fatty acids (VFA), excess nutrients and phenolic compounds may also negatively influence the digestion efficiency.

Since the composition of the GDWW may be responsible for problems during UASB treatment, GDWW must be pre-treated to make it more amenable to anaerobic digestion. Electrophilic compounds, such as O_3, are selective towards the double bonds found in toxic, recalcitrant or refractory compounds such as lipids, VFAs and polyphenols (Andreozzi *et al.*, 1998). Therefore, these compounds are degraded into simpler, more biodegradable fragments that are used by anaerobic populations (Martín *et al.*, 2002).

The aims of this study were to determine the impact of GDWW on UASB granules and to investigate whether ozonation would make GDWW more amenable for UASB treatment. The composition of both the GDWW and the encapsulating layer that forms around the granules after exposure to GDWW were monitored. The effect of the exposure of diluted or ozonated GDWW on granule activity and layer formation were also evaluated.

Materials and methods

Composition of raw GDWW

Five GDWW batches were obtained from a whisky distillery in Wellington, South Africa between February 2004 and October 2005. The batches that had been ozonated and the unozonated controls were frozen in 25 L drums at -18°C. When needed, a drum was thawed and stored at 4°C.

Composition of layer enclosing granules after exposure to GDWW

To determine whether an encapsulating layer enclosed the UASB granules after exposure to GDWW, granules from a full-scale UASB reactor treating wine distillery wastewater (WDWW) in Wellington, South Africa, were exposed to GDWW. The granules (400 g) were placed in a 2 L batch reactor (2 L Schott bottle) together with 800 mL diluted GDWW (COD = 4 000 $mg.L^{-1}$) and incubated at 35ºC for 24 days. The supernatant was decanted daily and replaced with fresh substrate. At the end of the 24 d exposure period, the physical appearance of the granules was visually monitored (using a Nikon SM2800

stereo-microscope with a camera unit) and their appearance was compared to that of granules from the full-scale UASB reactor. The carbohydrate, protein and lipid contents of the granule samples were determined. Granules obtained from a full-scale UASB reactor treating brewery wastewater (BWW) were also used as an additional reference control.

Determination of optimum O_3 dose

Ozonation was accomplished using a bubble column (length = 90 cm, diameter = 6 cm and operational volume = 2 L) and an O_3 generator (Parc Scientific, Ifafi). The bubble column contained a sintered glass disc at one end for bubble generation. At a flow rate of 4 $L.min^{-1}$ the O_3 concentration was 118 mg $O_3.min^{-1}$, as determined by the Iodometric method (APHA, 1998). Diluted GDWW (COD = 4 000 $mg.L^{-1}$) was divided into three 2 L samples and ozonated at room temperature until an O_3 dose of 5 314 $mg.L^{-1}$ (90 min) was delivered. Samples were taken at various O_3 doses to determine the COD and pH. The GDWW composition before and after ozonation at the optimum O_3 dose was determined.

Impact of different O_3 doses on granule activity

The impact of ozonated GDWW on granule activity was investigated by exposing granules, from a full-scale UASB reactor used for treating wine distillery wastewater (WDWW) in Wellington, South Africa, to ozonated GDWW. To acclimatise the granules to the GDWW, 400 g granules were placed in a 2 L batch reactor (2 L Schott bottle) together with 800 mL GDWW (COD = 4 000 $mg.L^{-1}$) and incubated for 4 d at 35°C. Every 24 h, the supernatant was decanted, replaced with fresh GDWW and then gently mixed.

After the acclimatisation step, the granules were divided into five 50 g samples and each sample was then placed in a separate batch reactor (500 mL Schott bottle). Each batch reactor received 100 mL of GDWW that had been ozonated at different O_3 doses, after which the GDWW was diluted to a COD of 4 000 $mg.L^{-1}$. The O_3 doses (time intervals) were 0 $mg.L^{-1}$ (0 min), 295 $mg.L^{-1}$ (5 min), 886 $mg.L^{-1}$ (15 min), 1181 $mg.L^{-1}$ (20 min) and 1476 $mg.L^{-1}$ (25 min). The batch reactors were incubated at 35°C for 4 d. The supernatant of each batch reactor was decanted daily, replaced with fresh substrate and gently mixed.

After 4 d, the granule activity was determined in basic test medium (BTM), glucose test medium (GTM) and acetic acid test medium (ATM), using the method of O'Kennedy (2000) (Tables 1 and 2). This procedure was repeated twice, using different samples from

Table 1 Composition of the different test media used to determine the activity of certain microbial groups (O'Kennedy, 2000)

Compound	**Microbial group**
Basic test medium (BTM)	Control
Glucose test medium (BTM + 2.0 $g.L^{-1}$ glucose) (GTM)	Acidogens
Acetic acid test medium (BTM + 1.0 $g.L^{-1}$ glucose) (ATM)	Acetoclastic methanogens

Table 2 Composition of the basic test medium (BTM) (Valcke & Verstraete, 1993; O'Kennedy, 2000)

Compound	**Concentration ($g.L^{-1}$)**
Glucose	2.0
Di-potassium hydrogen orthophosphate (K_2HPO_4)	1.0
Potassium di-hydrogen orthophosphate (KH_2PO_4)	2.6
Urea ($(NH_2)_2CO$)	1.1
Ammonium chloride (NH_4Cl)	1.0
Sodium sulphide ($Na_2S \cdot 9H_2O$)	0.1
Magnesium chloride ($MgCl_2 \cdot 6H_2O$)	0.1
Yeast extract	0.2
pH	7.1

the same source.

Impact of different wastewaters on granule activity and layer formation

The impact of GDWW (COD = 3 996 $mg.L^{-1}$ and unadjusted pH = 4.04), ozonated GDWW (O_3 dose = 1 476 $mg.L^{-1}$, COD = 4 018 $mg.L^{-1}$ and unadjusted pH = 3.56) and two control substrates on granule activity, was investigated. The first control was a standard glucose substrate (SGS) (COD = 4 338 $mg.L^{-1}$ and unadjusted pH = 7.99) which was identical to GTM as given in Tables 1 and 2. The term SGS was used instead of GTM to prevent any confusion between the first control and the GTM used in the activity tests. WDWW (COD = 3 694 $mg.L^{-1}$ and unadjusted pH = 3.55) was used as the second control, as the granules used in this study were obtained from a full-scale UASB reactor treating WDWW. It was assumed that these granules were acclimatised to WDWW.

For each of the four substrates, 400 g granules were placed in four separate 2 L batch reactors (2 L Schott bottle). Subsequently, 800 mL of the four above-mentioned substrates was added to a batch reactor and these were then incubated at 35°C for a 24 d period. Every 24 h, the supernatant of each batch reactor was decanted, replaced with the specific fresh substrate and gently mixed. On day 0, 4, 8, 12, 16, 20 and 24, a 30 g granule sample was removed from each batch reactor for granule activity determinations and to visually monitor the physical appearance. The activity of granules from each batch reactor on the four substrates (SGS, WDWW, GDWW and ozonated GDWW) was tested in duplicate, using the method of O'Kennedy (2000) (Tables 1 and 2).

Analytical methods

The composition of the raw or ozonated GDWW was determined by using the following parameters according to Standard Methods (APHA, 1998): pH; fats, oils and greases; alkalinity; total solids (TS); total suspended solids (TSS) and volatile suspended solids (VSS). Orthophosphate phosphorous (PO_4^{3-}) and COD were determined colourimetrically using a DR 2000 spectrophotometer (Hach Co. Loveland, CO) and standardised procedures (APHA, 1998). Total polyphenol content was determined using the Folin-Ciocalteau method (Singleton & Rossi, 1965), while the lipid content was calculated using the method of Bligh and Dyer (1959). The VFA content was determined by gas chromatography (GC) (Sigge, 2005). All analyses were done in triplicate.

The carbohydrate (Dubois *et al.*, 1956), protein (Smith *et al.*, 1985) and lipid (Bligh & Dyer, 1959) contents of the granules samples were measured, in triplicate.

Activity tests were preformed according to the method developed by O'Kennedy (2000) and were used to determine the degradation potential and microbial activity of the granules. In this study the granule activity is expressed as a percentage of the cumulative biogas produced on day 0 after 25 h incubation. This modification of the activity test method was done in order to minimise variations caused by slight variations in granule activity. The day 0 sample refers to the "untreated" granule sample used at the start of each study. The activity test media used were selected to characterise microbial groups, which form part of the anaerobic consortium and included the BTM, which was used as the base control (Tables 1 and 2). The GTM was used to determine the activity of the acidogens and methanogens (indirectly through metabolite formation). The ATM measured the activity of the acetoclastic methanogens.

Activity tests were performed by placing 3 g granules in a 20 mL glass vial together with 13 mL test medium, leaving a 6 mL headspace (Sigge, 2005). All three test media (BTM, GTM and ATM) (Tables 1 and 2) were used for each sample. The vials were sealed with butyl septa, capped with aluminium caps and then incubated at 35°C (Sigge, 2005). After 5, 10 and 25 h incubation, the biogas produced was determined by using a free-moving 10 mL syringe with a 12 gauge needle. The needle was inserted through the butyl septa and the biogas volume was determined once the syringe piston had stopped moving. Activity tests were performed in duplicate.

Results and discussion

Composition of the raw GDWW

The composition of the raw GDWW, representing 15 samples from 5 batches (3 samples per batch), is summarised in Table 3. In this study it was found that the composition of the batches varied considerably, which was ascribed to daily and seasonal variations.

Composition of the layer enclosing granules after exposure to GDWW

In this study it was found that granules exposed to GDWW became covered in an "encapsulating" layer, as can be seen in Fig. 1. The encapsulating layer was beige in colour and in most cases completely covered the granules. In certain cases a smooth layer covered the granules, while others had additional material attached to the encapsulating layer. No encapsulating layer was observed on the control granules obtained from full-scale UASB reactors treating either WDWW or BWW. It was concluded

Table 3 Average composition of the raw GDWW batches used during the study. The range represents 15 samples from 5 batches (3 samples per batch) from February 2004 to October 2005

Parameter (mg.L^{-1})	Range for this study	Literature values
COD_{total}	20 007 – 26 069	20 000 – 31 000[a, b, c]
pH	3.4 – 3.5	3.5 – 4.5[a, b, c, d]
Fats, oils and greases	1 978 – 2 324	125 g oil.kg^{-1} dry matter[e, f]
Lipids	374 – 479	VNA
Phosphorous (as PO_4^{3-})	624 – 880	616 – 862[g]
Total solids	13 915 – 18 395	20 500 – 47 300[h]
Total suspended solids	908 – 1 612	232 – 7 810[a, d]
Volatile suspended solids	812 – 1 560	200 – 7 340[d]
Polyphenol (gallic acid equivalents)	5.80 – 7.29	VNA

[a] Laubscher (2000)
[b] Laubscher *et al.* (2001)
[c] Tokuda *et al.* (1998)
[d] Akunna & Clark (2000)
[e] Arosemena *et al*. (1995)
[f] Hill (2002)
[g] Hati *et al.* (2007)
[h] Kim *et al.* (1997)
VNA – values not available

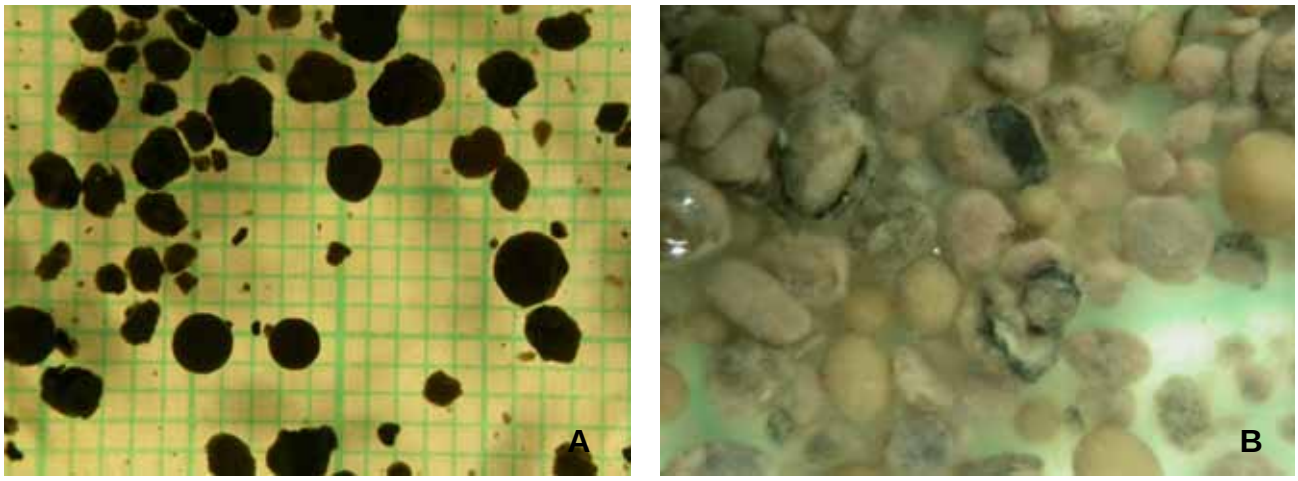

Figure 1 Micrographs of the WDWW granules before the 24 d exposure to GDWW (A) and after 24 d exposure to GDWW (B). The layer enclosing the granules after exposure is clearly visible. The grid in the background measures 1 mm by 1 mm.

that the encapsulating layer consisted of at least one component present in the GDWW that was not present in the WDWW or BWW. The presence of such a layer may restrict the digestion process, decrease the granule activity and lead to operational problems, as the layer limits contact between the wastewater and microbial consortium in the granules.

The compositions of the granules from the WDWW and BWW industrial plants and granules exposed to GDWW for 24 d are shown in Table 4. The granules exposed to GDWW for 24 d and the BWW granules contained similar protein concentrations (2.0 – 2.3 mg protein.g^{-1} granule), while the WDWW granules (4.6 ± 0.6 mg protein.g^{-1} granule) contained twice as much protein compared to the other granules. The BWW granules (4.19 ± 0.03 mg carbohydrate.g^{-1} granule) contained about twice and four times as much carbohydrate compared to WDWW granules (1.29 ± 0.02 mg carbohydrate.g^{-1} granule) and the granules exposed to GDWW for 24 d (2.63 ± 0.01 mg carbohydrate.g^{-1} granule), respectively. The granules exposed to GDWW (60.04 ± 0.03 mg lipid.g^{-1} granule) were much richer in lipids than the WDWW granules (1.31 ± 0.06 mg lipid.g^{-1} granule) or BWW granules (1.49 ± 0.02 mg lipid.g^{-1} granule). Thus it is clear from the data obtained that the exposure to GDWW leads to a drastic increase in the lipid content of the granules.

Determination of optimum O_3 dose

Beltrán *et al.* (1999) and Martín *et al.* (2002) reported that O_3 degrades complex compounds into more biodegradable fragments and this results in increases in the biodegradability. In this study the O_3 dose (time) necessary for the optimum COD reduction of GDWW, as well as the reduction in pH during ozonation, is shown in Fig. 2.

When GDWW was ozonated, it was found that the pH decreased from 3.85 to 3.32 as the O_3 dose was increased (Fig. 2). The initial pH decrease was greater, compared to that at the end of the ozonation period. The decrease in pH was not surprising because acidic products forming during ozonation cause the pH to decrease.

The COD reduction was found to increase steadily, until a COD reduction of 14% was obtained at an O_3 dose of 1 476 mg.L^{-1} (25 min) (Fig. 2). During ozonation, some compounds are degraded and mineralised via chemical oxidation, while others are degraded into simpler, more biodegradable fragments and thus the overall COD decreases. The COD reduction decreased to 10% as the O_3 dose was increased to 2 657 mg.L^{-1} (45 min). This probably occurred as a result of certain recalcitrant compounds being degraded and solubilised, thus increasing the overall COD. From an O_3 dose of 2 657 to 4 428 mg.L^{-1} (45 to 75 min) the COD reduction increased to 19% and

Table 4 Average composition of granules from UASB reactors treating WDWW and BWW as well as granules exposed to GDWW for 24 d. The averages and standard deviations of triplicate determinations are shown

Component (mg.g^{-1} granule)	BWW granules	WDWW granules	GDWW granules
Protein	2.3 ± 0.5	4.6 ± 0.6	2.0 ± 0.5
Lipid	1.49 ± 0.02	1.31 ± 0.06	60.04 ± 0.03
Carbohydrate	4.19 ± 0.03	1.29 ± 0.02	2.63 ± 0.01

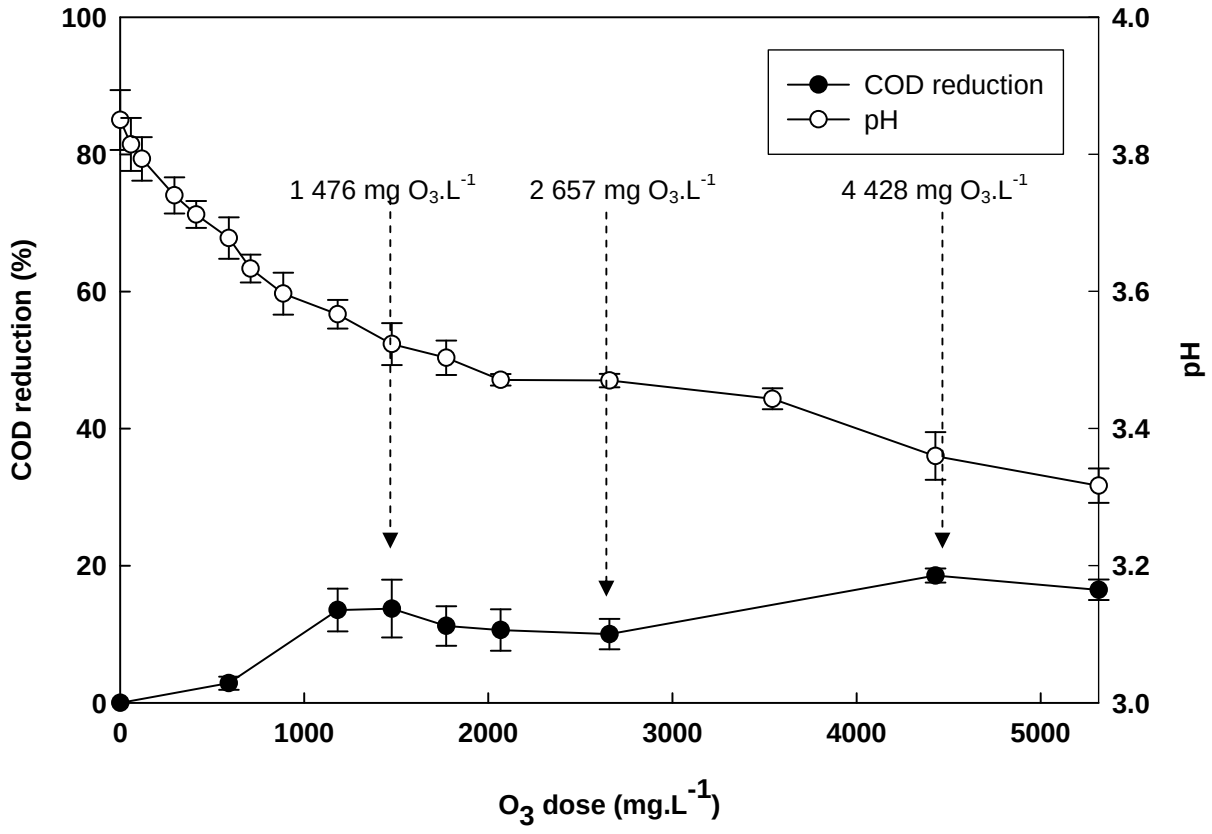

Figure 2 Average COD reduction and average pH after ozonation of GDWW at various O_3 doses. The averages and standard deviations (error bars) of triplicate determinations are shown.

then decreased to 16% at an O_3 dose of 5 314 mg.L^{-1} (90 min). This most likely occurred because at these higher O_3 doses the solubilised recalcitrant compounds were also mineralised to compounds with lower COD values, decreasing the COD. Although the optimum O_3 dose (19% COD reduction) was 4 428 mg.L^{-1}, it was decided, for economical and time reasons, that in this study further ozonations would be done at an O_3 dose of 1 476 mg.L^{-1} (14% COD reduction).

The compositional changes that occurred in GDWW after ozonation at an O_3 dose of 1 476 mg.L^{-1} are shown in Table 5. Reductions in lipid (74%), PO_4^{3-} (18%), TS (0.56%), TSS (62%), VSS (63%) and polyphenol (58%) contents were achieved. The large lipid reduction occurred because O_3 is known to be selective to the double bonds found in fatty acids and thus lipids can be effectively degraded into simpler fragments during ozonation. Although the reduction in TS was small, the reduction in TSS was high because TSS were converted into total dissolved solids (TDS), thus reducing the TSS content but not the TS content (APHA, 1998). The results of this study are similar to the results reported by Rueter and Johnson (1995) who found that ozonation of wastewater could be successfully used to reduce the TSS by more than 50%. The reduction in polyphenols is not surprising, as Hsu *et al*. (2004) reported that O_3 can successfully degrade the double bonds in polyphenols. Beltrán *et al.* (2001) also reported that when a mixture of WDWW and domestic wastewater was ozonated, the polyphenol content was decreased by 32%.

Impact of different O_3 doses on granule activity

The impact of ozonated GDWW on granule activity was investigated by exposing granules to ozonated GDWW and the results are shown in Fig. 3. In this study the granules were exposed to GDWW for 4 d, followed by exposure for 4 d to GDWW that had been ozonated at different doses. The granule activity was expressed as a percentage of the biogas production on day 0 (untreated granules), after 25 h incubation.

When the granules were exposed to GDWW (ozonated at various O_3 doses) and were then incubated in BTM, the biogas production decreased to 65% as the O_3 dose increased to 1 181 mg.L^{-1} (20 min) and then increased to 69% at an O_3 dose of 1 476 mg.L^{-1} (25 min). When the granules were incubated in GTM, the biogas production decreased to 31% as the O_3 dose increased to 1 181 mg.L^{-1} and then increased to 52% at an O_3 dose of 1 476 mg.L^{-1}. When the granules were incubated in ATM, the biogas production decreased to 37% as the O_3 dose increased to 1 181 mg.L^{-1} and then increased to 78% at an O_3 dose of 1 476 mg.L^{-1}.

Table 5 Composition of diluted GDWW before and after ozonation at an O_3 dose of 1 476 mg.L^{-1}. The averages and standard deviations of triplicate determinations are shown

Parameter (mg.L^{-1})	Before ozonation	After ozonation	Reduction (%)
COD_{total}	4 410 ± 237	3 798 ± 217	14
pH	3.85 ± 0.04	3.52 ± 0.03	–
Lipids	72 ± 24	19 ± 6	74
Phosphorous (as PO_4^{3-})	140 ± 2.18	114 ± 1.09	18
Total solids	4 870 ± 18	4 843 ± 28	0.56
Total suspended solids	163 ± 1.41	62 ± 1.77	62
Volatile suspended solids	156 ± 2.12	57 ± 0.78	63
Polyphenol (gallic acid equivalents)	2.65 ± 0.08	1.11 ± 0.03	58

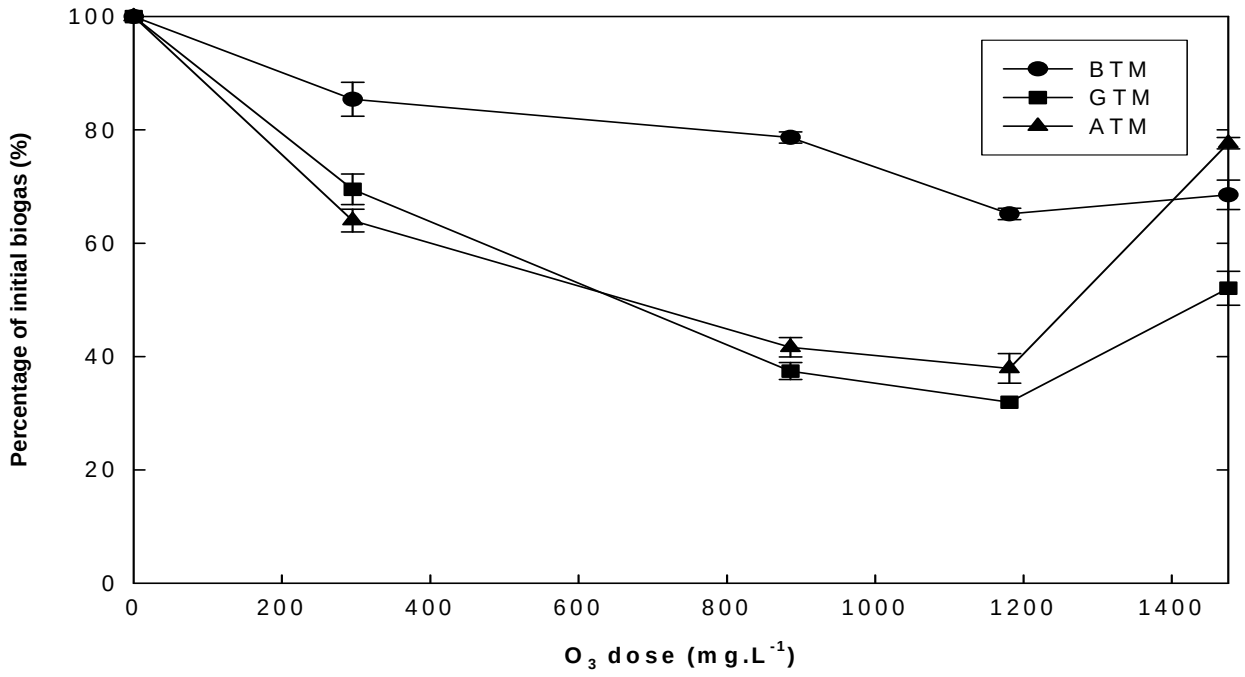

Figure 3 Activity of UASB granules (after 25 h incubation) after exposure to GDWW (ozonated at various O_3 doses). Biogas production is expressed as percentage of biogas production on day 0 (untreated granules). The averages of duplicate determinations of the two granule samples that were tested are shown.

For all these test media (BTM, GTM and ATM), the granule activity decreased as the O_3 dose increased to 1 181 mg.L^{-1} (Fig. 3). This may be attributed to the fact that new compounds were introduced to the granules and acclimatisation to these had not occurred. GDWW is also rich in lipids and the presence of lipids is known to reduce the granule activity. This is of significance for the distillery industry, as it clearly shows that if GDWW (raw or ozonated at or below an O_3 dose of 1 181 mg.L^{-1}) is treated in an UASB reactor, the granule activity will decrease and thus the overall treatment efficiency will also be reduced.

For all these test media (BTM, GTM and ATM), the increase in granule activity at an O_3 dose of 1 476 mg.L^{-1} can be explained by the fact that during ozonation the COD reduction steadily increased up to an O_3 dose of 1 476 mg.L^{-1} (14% COD reduction) (Fig. 2). It should be noted that the COD reduction increased from 3% at an O_3 dose of 590 mg.L^{-1} to 13.50% at O_3 dose of 1 180 mg.L^{-1}. Therefore, the concentration of potentially inhibiting compounds could have gradually been reduced and thus might explain the recovery of the biogas production at O_3 doses above 1 181 mg.L^{-1} (Fig. 3). This is also of significance for the distillery industry, as it indicates that when GDWW is ozonated at an O_3 dose of 1 476 mg.L^{-1} or higher, the granule activity will increase and thus the overall performance of an UASB reactor may also be improved.

The recovery of granule activity was most pronounced in the activity tests where ATM was used (Fig. 3). It should be noted that ATM is specifically designed to indicate the activity of the acetoclastic methanogens, which are sensitive to inhibition compounds. Therefore, ozonation at an O_3 dose of 1 476 mg.L^{-1} might have reduced the compounds in the GDWW that inhibit the methanogens and thus the overall granule activity increased. This is also significant for the distillery industry, as it indicates that the methane content of the biogas, which is important for energy recovery, increased as a result of ozonation of GDWW at an O_3 dose of 1 476 mg.L^{-1}.

Impact of different wastewaters on granule activity

Granule activity may be used to determine both the degradation potential (biodegradability) and microbial activity of UASB granules. In this study activity tests were used to determine the impact different wastewaters would have on granule activity. In this way the impact of these wastewaters on the efficiency of an UASB reactor could be simulated without the timely process of starting-up and operating an UASB reactor.

In this study, the wastewaters tested included WDWW, SGS, GDWW and ozonated

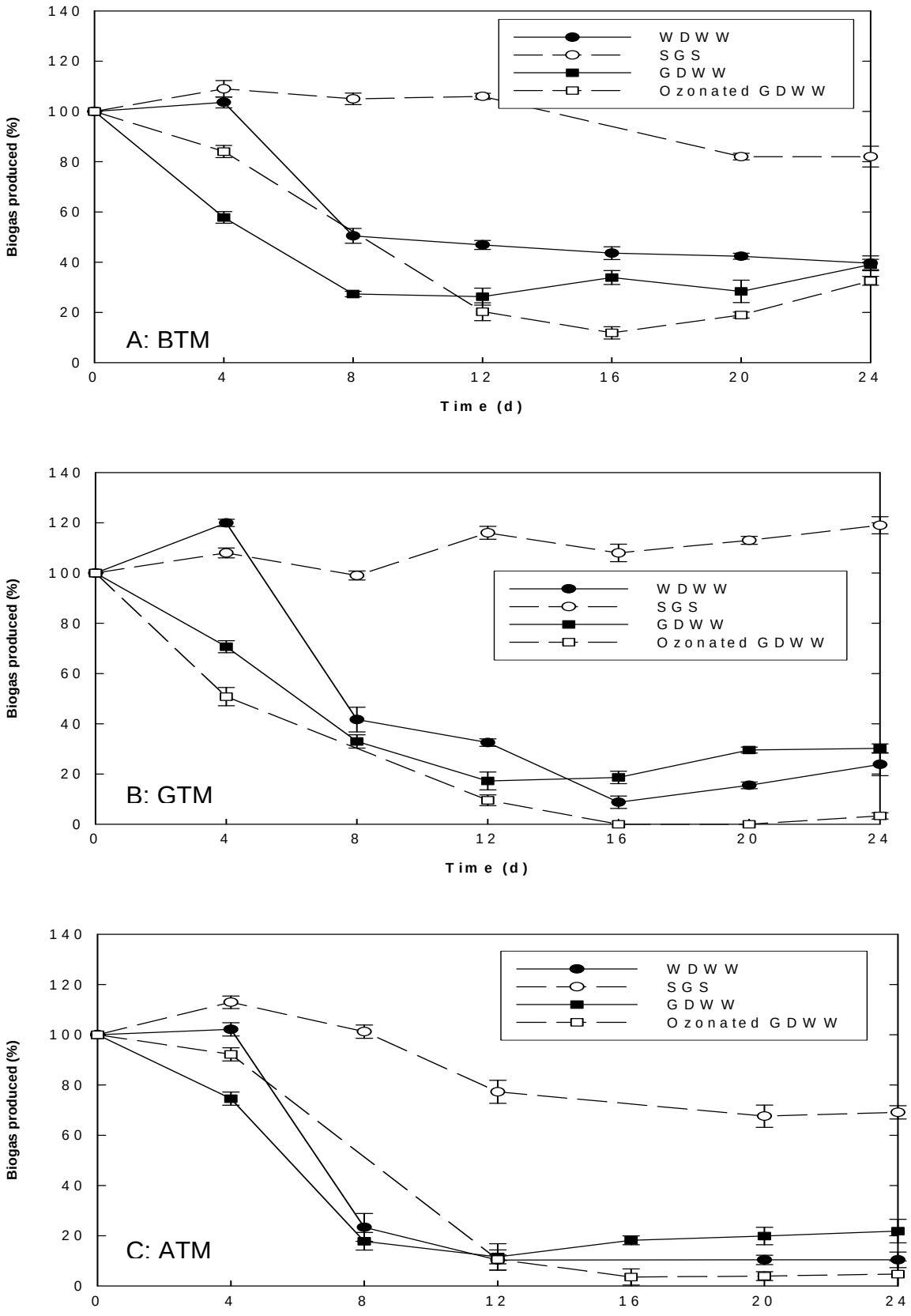

Figure 4 Activity of granules (after 25 h incubation) in BTM (A), GTM (B) and ATM (C) after they had been exposed to WDWW, SGS, GDWW and ozonated GDWW for 24 d. Biogas production is expressed as percentage of biogas production on day 0 (untreated granules). The averages of duplicate determinations are shown.

exposure to the different wastewaters for 24 d is shown in Fig. 4. The values are expressed as a percentage of the biogas production on day 0 (untreated granules).

The biogas production profile of granules incubated in BTM for 25 h is shown in Fig. 4A. The biogas production profile of the granules exposed to SGS decreased to 82% over the 24 d exposure period. Over the 24 d exposure period, the biogas production profile of the granules exposed to WDWW, GDWW and ozonated GDWW decreased to 40%, 39% and 33%, respectively. The biogas production profile of the granules exposed to either GDWW or ozonated GDWW slightly increase by day 24. This could be ascribed to an acclimatisation of the population or even a small population shift.

The biogas production profile of granules incubated in GTM for 25 h is shown in Fig. 4B. The biogas production profile of the granules exposed to SGS slowly increased to 119% over the 24 d exposure period. In contrast, the biogas production profile of the granules exposed to WDWW, GDWW and ozonated GDWW decreased to 24%, 30% and 3%, respectively, over the 24 d exposure period.

The biogas production profile of granules incubated in ATM for 25 h is shown in Fig. 4C. The biogas production of the granules exposed to SGS decreased to 69% by day 24. After the 24 d exposure period, the biogas production of the granules exposed to WDWW, GDWW and ozonated GDWW was 10, 22 and 5%, respectively.

It is clear that for all these test media (BTM, GTM and ATM), the highest activity was achieved when the granules were incubated in SGS. This may be attributed to the fact that SGS contained sufficient nutrients needed for microbial growth. SGS, unlike the other substrates, also contained 4 $g.L^{-1}$ glucose, which is easily digested by certain aerobic and anaerobic bacteria (such as the acidogens) and is essential for their growth. In contrast, the activity of the granules exposed to WDWW, GDWW or ozonated GDWW decreased because these substrates contained compounds that inhibited microbial growth, as reported by Beltrán de Heredia *et al*. (2005). The compounds in these substrates were also unable to support microbial activity in terms of growth requirements.

In this study the volume of cumulative biogas after 25 h incubation was also monitored over the 24 d exposure period. It was found that for all the granule samples, after the 24 d exposure period, the smallest volume of cumulative biogas was produced after incubation in BTM (SGS = 7.0 mL, WDWW = 2.0 mL, GDWW = 1.3 mL and ozonated GDWW = 2.5 mL). This may be attributed to the fact that compared to GTM or ATM, BTM contained fewer nutrients needed for microbial growth (Tables 1 and 2). After the 24 d exposure period, the largest cumulative biogas volume was produced from the granules incubated in GTM (SGS = 8.8 mL, WDWW = 4.4 mL, GDWW = 3.8 mL and ozonated

GDWW = 4.8 mL). This can be ascribed to GTM containing more nutrients (2 $g.L^{-1}$ glucose) than ATM or BTM and thus this additional carbon enhanced microbial growth. GTM was also specifically formulated to support the growth of both the acidogens and methanogens. Acidogens will produce the greatest biogas volume because they are the largest of the trophic groups involved in anaerobic digestion and have the fastest growth rate. After the 24 d exposure period, a smaller volume of cumulative biogas was produced from the granules incubated in ATM (SGS = 7.1 mL, WDWW = 2.1 mL, GDWW = 1.8 mL and ozonated GDWW = 3.5 mL) compared to those in GTM because ATM contained 2 $g.L^{-1}$ acetic acid and not an additional 2 $g.L^{-1}$ glucose, which is most suitable for supporting microbial growth of aerobic and anaerobic bacteria, such as the acidogens. ATM was specifically formulated for the growth of acetoclastic methanogens, which are more sensitive, grow slower and take longer to produce biogas than the other bacteria. Again, for all the test media (BTM, GTM and ATM), the cumulative biogas volume was much higher in SGS than the other substrates because SGS contained sufficient nutrients required for microbial growth. WDWW, GDWW and ozonated GDWW also contained compounds that inhibited microbial growth (Beltrán de Heredia *et al*., 2005).

In this study the granule activity in GDWW could have been reduced by the encapsulating layer (as illustrated in Fig. 1), which reduced the contact between the microbial consortium in the granules and wastewater. The wastewater in this study was not recirculated (as in an UASB reactor), nor was all the substrate decanted off. Therefore, slowly hydrolysing, inert or toxic substances, such as VFAs or lipids, may have accumulated – increasing the overall toxicity, inhibiting the anaerobic bacteria and reducing the granule activity.

Impact of different wastewaters on layer formation

In this study it was found that when granules were exposed to GDWW, the granules became enclosed in an encapsulating layer (Fig. 1). In contrast it was found that granules from full-scale UASB reactors, treating either WDWW or BWW, were not enclosed in an encapsulating layer. Since the encapsulating layer limits contact between the wastewater and microbial consortium in the granules, nutrient transfer, granule activity and overall efficiency are reduced. Therefore, methods to reduce or remove the encapsulating layer were investigated.

Micrographs taken after the granules had been exposed to SGS or WDWW for the

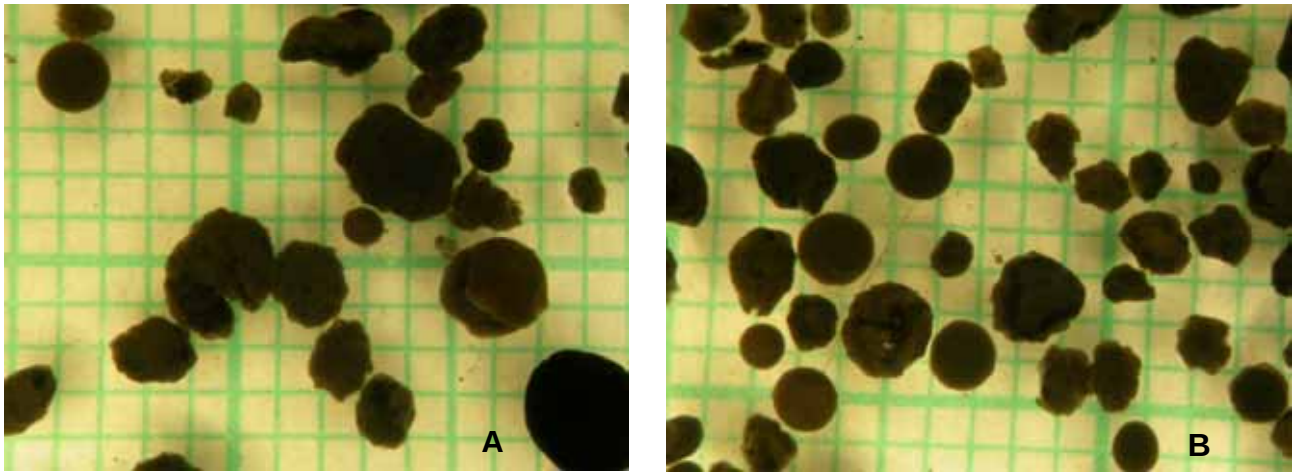

Figure 5 Micrographs of the granules after 24 d exposure to WDWW (A) and SGS (B). The grid in the background measures 1 mm by 1 mm.

24 d exposure period, showed that an encapsulating layer did not form (Fig. 5), supporting the results from the first section of the study. In this study, these specific two granule samples appeared to be unaffected by SGS or WDWW as the colour of the granules did not change through out the 24 d exposure period. This may be attributed to the fact that SGS and WDWW did not contain lipids (1.31 – 1.49 mg lipid.g^{-1} granule) (Table 4).

Micrographs taken after the granules had been exposed to ozonated GDWW for the 24 d exposure period revealed that a layer covered the granules (Fig. 6). However, in certain cases the smooth encapsulating layer covered some of the granules only thinly, making it possible to see the darker original granules underneath. This was ascribed to the impact of ozonation, which possibly decreased the severity of the encapsulating layer. In these cases the encapsulating layers were also beige in colour. The study clearly showed that the encapsulating layer was still visible and based on this it was accepted that complete lipid reduction had not been achieved by ozonation (74% lipid reduction) and that enough lipid (26% lipid) was still available to enclose the granules.

Conclusions

From this study, it is clear that when granules are exposed to GDWW, the granules became enclosed in an encapsulating layer. It was also found that ozonation did not completely prevent this layer from forming but the treatment did visually decrease the encapsulating severity. This knowledge is important for the industry because if an UASB reactor is used to treat GDWW or ozonated GDWW, the granules will become enclosed in an encapsulating layer. Therefore, nutrient transfer and the granule activity are reduced.

Ozonation, at an O_3 dose of 1 181 mg.L^{-1}, was found to reduce the lipid content in GDWW by 74% but an encapsulating layer still formed when granules were exposed to ozonated GDWW. This occurred as complete lipid degradation did not take place and some lipid was still available to enclose the granules. It is thus important in future studies that the effect of ozonation be quantified by monitoring the lipid content of the granules after exposure to ozonated GDWW. Other pre-treatments to reduce the lipid content of the GDWW, so as to enhance the wastewater biodegradability, must also be examined.

It was found in this study that the activity of granules exposed to ozonated GDWW decreased as the O_3 dose was increased to 1 181 mg.L^{-1}. However, when the O_3 dose was increased to 1 476 mg.L^{-1}, the granule activity increased again. This increase is in agreement with the fact that, for the O_3 doses tested, the highest lipid reduction (14%) was

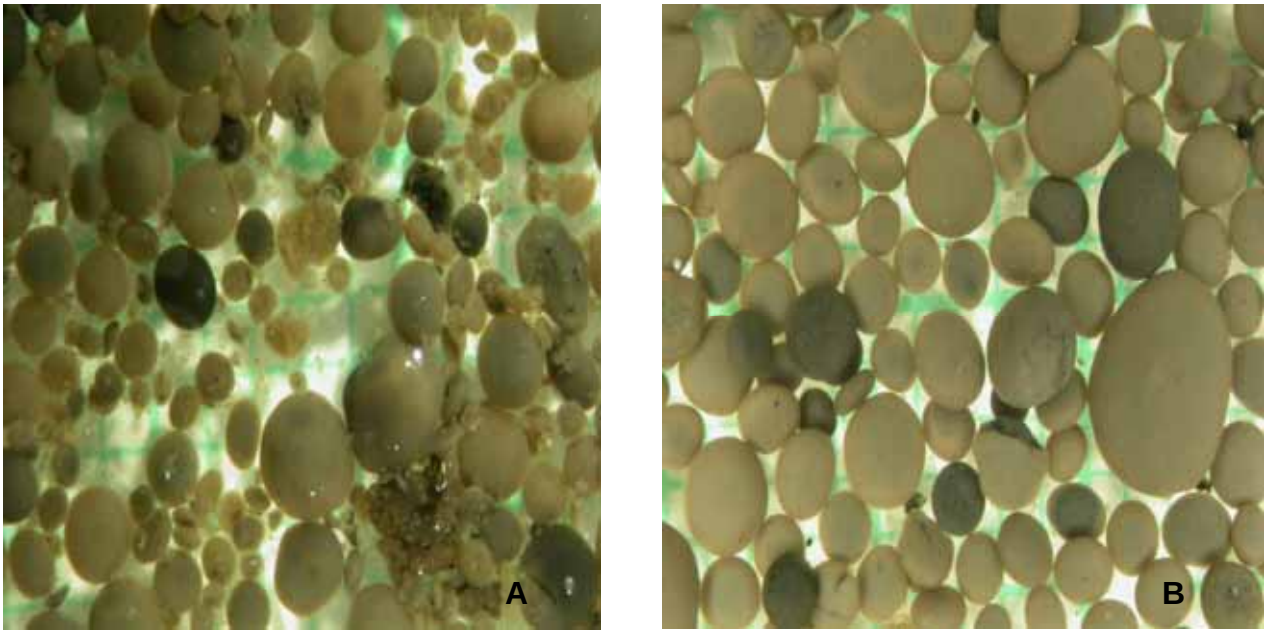

Figure 6 Micrographs of the granules before 24 d exposure to ozonated GDWW (A) and after 24 d exposure to ozonated GDWW (B). The grid in the background measures 1 mm by 1 mm.

obtained at an O_3 dose of 1 476 mg.L^{-1} (Table 5). This is probably because ozonation reduced the lipid content in the GDWW and thus fewer lipids were available to enclose the granules, which caused the granule activity to decrease.

It was also established in this study that when granules were exposed to different substrates for 24 d, the most activity was obtained from those granules exposed to SGS. This may be attributed to the fact that SGS contained sufficient nutrients to support microbial growth. The activity of granules exposed to WDWW, GDWW and ozonated GDWW decreased drastically because these substrates contained compounds that inhibited microbial growth and were not able to support microbial activity, in terms of growth requirements.

In this study the pH of the substrates used in the batch reactors were not adjusted, which may have negatively influenced microbial activity. Therefore, it is recommended that adjustments should be made to stabilise the pH and alkalinity.

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CHAPTER 5

IMPACT OF OZONATION AND ENZYMATIC TREATMENTS ON DILUTED GRAIN DISTILLERY WASTEWATER AND UPFLOW ANAEROBIC SLUDGE BLANKET GRANULES

Summary

In order to improve the biodegradability of grain distillery wastewater (GDWW) pre- and post-treatments were tested. GDWW (COD = 4 000 mg.L^{-1}) dosed with 1% FF (GDWW + 1% FF) and incubated for 2 d had a lipid reduction of 51%. The lipid reduction of ozonated GDWW dosed with 1% FF (ozonated GDWW + 1% FF) was 90%, and that of GDWW dosed with 1% FF and ozonated (GDWW + 1% FF + O_3) was 60%. In the case of the GDWW samples, the highest granule activity was observed in granules exposed to ozonated GDWW + 1% FF, followed by granules exposed to ozonated GDWW, GDWW + 1% FF + O_3, GDWW + 1% FF and GDWW. This was also the order of the highest lipid reduction in GDWW, the thinness of the encapsulating layer and the lowest lipid content in the granules. The treatments resulting in the highest granule activity also resulted in the highest lipid reduction in GDWW, the thinnest encapsulating layer and the lowest lipid content in the granules. This proves that a reduction in the lipid content of the GDWW increases the granule activity and reduces the formation of the encapsulating lipid layer. Thus the efficiency of the UASB reactor may increase.

Introduction

The production and distillation of alcohol generates highly polluted wastewater. Distillery wastewater characteristics differ depending on the substrate, the production process and the nature and location of the industry. Grain distillery wastewater (GDWW) is rich in proteins (280 – 340 g crude protein.kg^{-1} dry matter) and lipids (± 125 g oil.kg^{-1} dry matter), while wine distillery wastewater (WDWW) does not contain significant quantities of proteins and lipids (Hill, 2002; Bustamante *et al.*, 2005).

Upflow anaerobic sludge blanket (UASB) reactors are often used to treat distillery wastewater. However, operational problems can occur during GDWW treatment due to

the presence of lipids in the GDWW (Laubscher, 2000, Akarsubasi *et al.*, 2006). The following problems may occur: scum layer formation; hampered release of biogas; COD accumulation; extensive clogging; washout; foaming; scaling; production of unpleasant odours and sludge floatation (Nadais *et al.*, 2001; Zeeman & Sanders, 2001). In Chapter 4 of this thesis it was also found that when UASB granules are exposed to GDWW, an "encapsulating" lipid layer forms around the granules. Subsequently the granule activity is reduced because the contact between the microbial consortium and GDWW is reduced.

The biodegradability of distillery wastewater may be improved using pre-treatments, which makes the wastewater more amenable to anaerobic digestion. Alfafara *et al.* (2000) found that when beer distillery wastewater was ozonated at a dose of 50 $mg.L^{-1}$, the biodegradability improved by 40%. Sangave and Pandit (2006) reported that when the inclusion of a 12 h enzymatic pre-treatment step was included prior to distillery wastewater being treated in an aerobic bioreactor, the COD reduction increased from 18 to 29%. Dharmsthiti and Kuhasuntisuk (1998) also found that when lipid-rich wastewater was treated with lipase, the lipid content decreased by 70% within 24 h. Single wastewater treatment methods are often insufficient to achieve the desired result, therefore different treatment options are often combined (Gottschalk *et al.*, 2000).

The aim of this study was to determine the effect of ozonation, enzymatic treatments and combinations thereof on the compositional changes and foaming potential of GDWW. The activity, visual appearance and lipid content of UASB granules exposed to the GDWW substrates were monitored.

Materials and methods

Wastewater and substrate

Five GDWW batches were obtained from a whisky distillery in Wellington, South Africa between November 2004 and February 2006. The wastewater was frozen in 25 L drums and stored at -18°C. When needed, a drum was thawed and stored at 4°C.

Impact of enzyme pre-treatments on GDWW lipid content

The effect that enzyme addition had on GDWW was determined by using three commercially available enzyme preparations – namely Microzyme™, FogFree™ (FF) and Lipolase™ (Novozymes SA (Pty) Ltd). Each preparation was specifically chosen for the enzymatic action it produced.

Microzyme™ (Novozymes SA (Pty) Ltd) is a blend of both aerobic bacteria (>8.0 x 10^8 colony forming units (CFU).mL^{-1}) and anaerobic bacteria (7.8 x 10^8 CFU.mL^{-1}) and is available in a powder form on a cereal/salt base. Microzyme™ degrades organic waste, although the exact enzyme pathway used is not known. It operates optimally in a pH range of 5.0 to 8.5 and at a maximum temperature of 50°C. The recommended initial dosage for industrial effluent is 1% (w/w) Microzyme™ and that for the maintenance dosage is 0.3% (w/w) Microzyme™.

FF (Novozymes SA (Pty) Ltd) is a granular powder that contains surfactants, selected enzymes, nutrients, buffering agents, powder base and bacterial cultures of aerobic and anaerobic microorganisms. FF is known to hydrolyse fats, oils and greases, although the exact enzyme pathway used is not known.

Lipolase™ (Novozymes SA (Pty) Ltd) is a lipase used in detergents to aid the removal of fat and oil-containing stains. Lipolase™ hydrolyses fat by cleaving the ester bonds of the triglyceride molecules into monoglycerides, diglycerides, glycerol and fatty acids that are all easily dispersed or dissolved in water. In the granular form it has an activity of 100 kilo lipase units.g^{-1} at a temperature of 30°C and a pH of 7.0.

Samples (100 mL) of diluted GDWW (with a COD of 4 000 mg.L^{-1} and lipid content of 54.78 ± 0.76 mg.L^{-1}) were placed in 250 mL Schott bottles and dosed with the recommended doses of the various enzyme preparations. Dosages of 1% and 10% (w/w) Lipolase™, 1% and 10% (w/w) FF and 1% (w/w) Microzyme™ were tested for an exposure period of 6 days. Different dosages (0.1, 1 and 10%) and exposure periods (after 2, 4 and 6 days) of FF were also investigated. All the samples were incubated on a shaking table (124 rpm) at 35°C. After the exposure period, the lipid content of each sample was determined and compared to the initial lipid content. All the analyses were done in triplicate.

Impact of combining FogFree™ and ozonation treatments on GDWW composition

The effect of combining ozonation treatments with FF treatments was investigated and the combinations are shown in Table 1. The GDWW used during the treatments was diluted to a COD of *ca.* 4 000 mg.L^{-1} and the lipid, pH, COD and polyphenol contents were determined before and after the treatments to determine the treatment efficiency.

Extent of foaming in GDWW samples

To determine the extent of foaming in the GDWW samples, trials were conducted to

Table 1 Substrates used to investigate the effect of combining an ozonation treatment with the FF* treatment

	Abbreviation used	Treatment
Substrate 1	Ozonated GDWW	Diluted GDWW (COD = 4 000 $mg.L^{-1}$) ozonated at a dose of 1 476 $mg.L^{-1}$ (as determined in Chapter 4)
Substrate 2	Ozonated GDWW + 1% FF	Diluted ozonated GDWW dosed with 1% FF and incubated for 2 d on a shaking table (124 rpm) at 35°C
Substrate 3	Ozonated GDWW + 1% FF + O_3	Diluted ozonated GDWW dosed with 1% FF, incubated for 2 d on a shaking table (124 rpm) at 35°C and ozonated at a dose of 1 476 $mg.L^{-1}$
Substrate 4	GDWW + 1% FF	Diluted GDWW dosed with 1% FF and incubated for 2 d on a shaking table (124 rpm) at 35°C
Substrate 5	GDWW + 1% FF + O_3	Diluted GDWW dosed with 1% FF and incubated for 2 d on a shaking table (124 rpm) at 35°C and ozonated at a dose of 1 476 $mg.L^{-1}$

FF* = FogFree™

compare and quantify the foaming potential. Diluted GDWW (COD = 4 000 mg.L^{-1}) and the treated GDWW substrates as given in Table 1 were tested. WDWW was used as the control. Foaming was studied in a bubble column (length = 90 cm, diameter = 6 cm and operational volume = 2 L) that contained a sintered glass disc at one end for bubble generation. Compressed air (Afrox) was passed through the sample (1 L) in the bubble column for 15 min at a flow rate of 4 L.min^{-1}. The height of the sample in the bubble column was measured beforehand and was then monitored at 5 min intervals. If the substrate foamed excessively as a result of the airflow, foam would bubble over the top opening of the bubble column resulting in a decrease in sample volume. Each substrate was tested in triplicate.

Impact of pre-treatments on granule activity, visual appearance and layer content

UASB granules were obtained in February 2006 from a full-scale UASB reactor treating WDWW in Wellington, South Africa. The UASB granules were first washed (60 min) in water containing 200 mg.L^{-1} urea $(NH_2)_2CO$) and 200 mg.L^{-1} di-potassium hydrogen orthophosphate (K_2HPO_4) to stabilise the pH and alkalinity.

The substrates investigated in this section of the study are shown in Table 2. The substrates were prepared in sufficient quantities, frozen and stored at -18°C until needed. A synthetic glucose solution (SGS) (COD = 4 338 mg.L^{-1}) was prepared according to the method used for glucose test media (GTM) (Tables 3 and 4) and used as the first control. The term SGS was used instead of GTM to prevent any confusion between the first control and the GTM used in the activity tests. WDWW was used as the baseline control because the granules used in this study were obtained from a full-scale UASB reactor treating WDWW and it was thus assumed that these granules were acclimatised to WDWW.

The WDWW and GDWW samples were diluted to a COD of 4 000 mg.L^{-1} and the pHs were set at 6.5 with 1 M potassium hydroxide (KOH). Subsequently, the SGS, WDWW and GDWW samples (500 mL) were placed in seven different batch reactors (2 L Schott bottles), each with 250 g UASB granules, and incubated at 35°C for 24 d. Every 24 h the pH of the supernatant in each reactor was measured and decanted. Fresh substrate was then added to each reactor and the pH was measured again.

A 30 g sample of granules was removed from each reactor on days 4, 8, 12, 16, 20 and 24 to determine the granule activity according to the method developed by O'Kennedy (2000). At the same time, the physical appearance of the granule samples was monitored using micrographs (Nikon SM2800 stero-microscope). After the 24 d period, the lipid

Table 2 Substrates investigated to determine the impact of treatment combinations of FF and ozonation on the granule activity, encapsulating layer formation and visual appearance of UASB granules

	Treatment or wastewater used
Substrate 1	Wine distillery wastewater (WDWW)
Substrate 2	Synthetic glucose solution (SGS)
Substrate 3	Grain distillery wastewater (GDWW)
Substrate 4	Ozonated GDWW
Substrate 5	GDWW dosed with 1% FF (GDWW + 1% FF)
Substrate 6	Ozonated GDWW dosed with 1% FF (ozonated GDWW + 1% FF)
Substrate 7	GDWW dosed with 1% FF and ozonated (GDWW + 1% FF + O_3)

Table 3 Composition of the basic test medium (BTM) (Valcke & Verstraete, 1993; O'Kennedy, 2000)

Compound	**Concentration ($g.L^{-1}$)**
Glucose	2.0
Di-potassium hydrogen orthophosphate (K_2HPO_4)	1.0
Potassium di-hydrogen orthophosphate (KH_2PO_4)	2.6
Urea ($(NH_2)_2CO$)	1.1
Ammonium chloride (NH_4Cl)	1.0
Sodium sulphide ($Na_2S \cdot 9H_2O$)	0.1
Magnesium chloride ($MgCl_2 \cdot 6H_2O$)	0.1
Yeast extract	0.2
pH	7.1

Table 4 Composition of the different test media used to determine the activity of certain microbial groups (O'Kennedy, 2000)

Compound	**Microbial group**
Basic test medium (BTM)	Control
Glucose test medium (BTM + 2.0 $g.L^{-1}$ glucose) (GTM)	Acidogens
Acetic acid test medium (BTM + 1.0 $g.L^{-1}$ glucose) (ATM)	Acetoclastic methanogens

content of the granules was determined. All the results obtained were compared to those of the day 0 granules.

Analytical methods

The lipid content was determined according to the method described by Bligh and Dyer (1959). The pH and COD were measured according to APHA (1998). Polyphenol content was determined using the Folin-Ciocalteau method (Singleton & Rossi, 1965). All analyses were performed in triplicate.

Activity tests were performed according to the method developed by O'Kennedy (2000). The activity test media used were selected to characterise microbial groups in the anaerobic consortium (Table 4). The basic test medium (BTM) was used as the control (Table 3). The GTM and ATM were used to determine the activity of the acidogens and acetoclastic methanogens, respectively. In this study the data of the granule activity is expressed as a percentage of the cumulative biogas produced on day 0 after 25 h incubation. This modification of the activity test method was done in order to minimise variations caused by slight deviations in granule activity. The day 0 sample refers to the untreated granule sample used at the start of each study. Triplicate granule samples (3 g) were placed in 20 mL glass vials together with 13 mL test medium (BTM, GTM and ATM), leaving 6 mL headspace. The vials were sealed with butyl septa, capped with aluminium caps and incubated at 35°C. After 25 h incubation, the biogas volume was measured using a free-moving 10 mL syringe. The 12 gauge needle was inserted through the butyl septa and the biogas volume was determined once the needle piston had stopped moving. Activity tests were preformed in duplicate.

Results and discussion

Impact of enzyme pre-treatments on GDWW lipid content

The results of the lipid reduction obtained when GDWW samples were dosed with different enzyme preparations and incubated for 6 d are shown in Table 5. The doses used were those recommended by the manufacturer. The lipid reduction of GDWW dosed with 10% FF (GDWW + 10% FF) was 76%, while that of GDWW dosed with 10% Lipolase^{TM} (GDWW + 10% Lipolase^{TM}) was 74%. The lipid reductions of GDWW dosed with 1% Lipolase^{TM} (GDWW + 1% Lipolase^{TM}), 1% FF (GDWW + 1% FF) or 1% Microzyme^{TM} (GDWW + 1% Microzyme^{TM}) were 40, 70 and 47%, respectively.

Table 5 Lipid reduction of the GDWW (lipid content on 0 d = 54.78 ± 0.76 $mg.L^{-1}$) dosed with the different enzyme preparations for a treatment period of 6 d. The averages and standard deviations of triplicate determinations (n = 3) are shown

Treatment	Lipid content on 6 d ($mg.L^{-1}$)	Lipid reduction (%)
GDWW + 1% Lipolase™	32.71 ± 0.92	40 ± 2.51
GDWW + 10% Lipolase™	14.21 ± 0.61	74 ± 1.47
GDWW + 1% FF*	16.62 ± 0.48	70 ± 1.30
GDWW + 10% FF	13.38 ± 0.20	76 ± 0.70
GDWW + 1% M	28.96 ± 0.74	47 ± 2.11

FF* = Fogfree™

Table 6 Average lipid reduction of the GDWW dosed with different concentrations of FF after a treatment period of 2, 4 or 6 d. The averages and standard deviations of triplicate determinations (n = 3) are shown

Microbial or enzyme preparation	Lipid reduction on 2 d (%)	Lipid reduction on 4 d (%)	Lipid reduction on 6 d (%)
0.1% FF	13 ± 1.40	34 ± 0.81	60 ± 0.34
1% FF	51 ± 1.16	60 ± 1.34	70 ± 0.13
10% FF	47 ± 0.93	73 ± 0.94	76 ± 0.22

It was decided to investigate lipid reduction at lower FF doses and shorter incubation periods because higher lipid reductions were obtained when GDWW was dosed with FF. This was done to identify a more economically feasible combination. The results of the lipid reduction in GDWW that had been dosed with different FF concentrations and incubated for various times are shown in Table 6. After a 2 d incubation, the lipid reduction of GDWW + 1% FF was 51%, while that of GDWW + 10% FF or GDWW dosed with 0.1% FF (GDWW + 0.1% FF) was 47 and 13%, respectively. After a 4 d incubation, the lipid reductions of GDWW + 10% FF was 73%, while that of GDWW + 1% FF and GDWW + 0.1 % FF was 60 and 34%, respectively. Similarly, after a 6 d incubation, the lipid reduction of GDWW + 10% FF was 76%, while that of GDWW + 1% FF and GDWW + 0.1 % FF was 70 and 60%, respectively. The highest lipid reduction rate takes place during the first two days. Furthermore, longer incubation periods are impractical on an industrial scale. Therefore it was decided to further investigate the GDWW + 1% FF treatment after 2 d incubation.

Impact of combining FogFree^{TM} and ozonation treatment on GDWW composition

The results of combining enzyme and ozonation pre-treatments to reduce the lipid, COD and polyphenol content of GDWW are summarised in Table 7. The above-mentioned characteristics of the GDWW were determined before any treatment occurred. The characteristics of the ozonated and FF treatment samples were determined after ozonation and 2 d incubation with 1% FF, respectively.

Ozonation, the FF treatment and combinations thereof were found to affect the lipid content of GDWW, as shown in Table 7. The lipid content of ozonated GDWW decreased from 152.94 ± 1.82 mg.L^{-1} to 44.37 ± 1.61 mg.L^{-1} (71% reduction) before and after ozonation, respectively. The lipid content of pre-ozonated GDWW that was dosed with 1% FF (ozonated GDWW + 1% FF) was 15.44 ± 1.00 mg.L^{-1}. The contribution to lipid reduction of the FF-treatment alone was 65%, while that of the entire treatment process was 90%. The lipid content of pre-ozonated GDWW that was dosed with 1% FF and post-ozonated (ozonated GDWW + 1% FF + O_3) was 3.21 ± 1.27 mg.L^{-1}. The lipid reduction after the post-ozonation step was 79% and that of the entire process was 97%.

The lipid content of GDWW + 1% FF decreased from 92.68 ± 0.90 mg.L^{-1} to 44.95 ± 1.25 mg.L^{-1} (51% reduction) before and after GDWW was dosed with 1% FF and incubated for 2 d respectively. The lipid content in GDWW that was dosed with 1% FF and that was post-ozonated (GDWW + 1% FF + O_3) was 37.07 ± 1.05 mg.L^{-1}. Therefore the

Table 7 Reduction in lipids, COD and polyphenol content after pre- and post-treatments in diluted (COD = 4 000 $mg.L^{-1}$) GDWW. Results for the FF treatment are given after the 2 d treatment period. The averages and standard deviations of triplicate determinations (n = 3) are shown

Substrate	Lipid ($mg.L^{-1}$)	Lipid reduction (%)	COD ($mg.L^{-1}$)	COD reduction (%)	Polyphenol ($mg.L^{-1}$)	Polyphenol reduction (%)
GDWW*	152.94 ± 1.82	–	4 244 ± 122	–	3.02 ± 0.01	–
Ozonated GDWW	44.37 ± 1.61	71	3 980 ± 115	6	1.78 ± 0.07	59
Ozonated GDWW + 1% FF	15.44 ± 1.00	90	3 553 ± 31	16	3.47 ± 0.06	–
Ozonated GDWW + 1% FF + O_3	3.21 ± 1.27	97	3 180 ± 165	25	1.94 ± 0.03	36
GDWW*	92.68 ± 0.90	–	4 487 ± 183	–	3.25 ± 0.02	–
GDWW + 1% FF	44.95 ± 1.25	51	4 331 ± 122	3	5.41 ± 0.10	–
GDWW + 1% FF + O_3	37.07 ± 1.05	60	4 037 ± 53	10	3.28 ± 0.15	–

*Variation due to different GDWW batches that were used

lipid reduction after the post-ozonation step was 18% and that of the entire treatment process was 60%.

The lipid reduction of ozonated GDWW + 1% FF (90%) was higher than that of GDWW + 1% FF (51%). The lipid reduction of ozonated GDWW + 1% FF + O_3 (97%) was also higher than that of GDWW + 1% FF + O_3 (60%). This is probably because pre-ozonation degrades the GDWW compounds into simpler, more biodegradable compounds that are easily degraded enzymatically.

Ozonation, the FF treatment and combinations thereof were also found to affect the COD of the GDWW. Initially, the COD of the samples that were dosed with 1% FF increased by 653 ± 57 mg.L^{-1}. Following the 2 d incubation period, the COD decreased to below that of the COD of the untreated sample. Similarly, Jeganaesan *et al*. (2006) reported that the addition of a lipase preparation to food industrial wastewater increased the overall COD.

The COD obtained when GDWW was ozonated, dosed with 1% FF, or treated with combinations thereof, is shown in Table 7. The COD reduction of ozonated GDWW + 1% FF (16%) was higher than that of ozonated GDWW (6%), which was higher than that of GDWW + 1% FF (3%). The COD reduction of ozonated GDWW + 1% FF + O_3 (25%) was also higher than that of GDWW + 1% FF + O_3 (10%). This may be attributed to pre-ozonation that degrades the GDWW compounds into simpler, more biodegradable compounds that are easily degraded enzymatically.

Similarly, ozonation, the FF treatment and combinations influenced the polyphenol content of the GDWW. It was found that the polyphenol content of ozonated GDWW decreased from 3.02 ± 0.01 to 1.78 ± 0.06 mg gallic acid equivalents.L^{-1} (59% reduction). Similar results were obtained in Chapter 4 of this thesis (Table 5) (58% polyphenol reduction) when GDWW was ozonated. Hsu *et al*. (2004) reported that ozonation can effectively reduce the polyphenol content in wastewater. It was found that the polyphenol content of ozonated GDWW + 1% FF decreased from 8.49 ± 0.24 to 3.47 ± 0.06 mg gallic acid equivalents.L^{-1} after the 2 d incubation period Therefore, the incubation period did result in a polyphenol content decrease. The polyphenol content of ozonated GDWW + 1% FF + O_3 was 1.94 ± 0.03 mg gallic acid equivalents.L^{-1} (36% reduction). In contrast, the polyphenol content of GDWW + 1% FF increased from 3.25 ± 0.02 to 9.96 ± 0.14 mg gallic acid equivalents.L^{-1}, after the addition of 1% FF. After the 2 d incubation period was complete the polyphenol content decreased to 5.41 ± 0.10 mg gallic acid equivalents.L^{-1}. The polyphenol content of GDWW + 1% FF + O_3 was 3.28 ± 0.15 mg gallic acid equivalents.L^{-1}. The addition of 1% FF did increase the polyphenol content of

GDWW. The increase in polyphenols may be attributed to the fact that the FF preparation consisted of a powder base containing surfactants, selected enzymes, nutrients, buffering agents and bacterial cultures of aerobic and anaerobic microorganisms, which seem to also have contained components contributing to the polyphenol content. It was evident, however, that the polyphenol content did decrease during the incubation period. This indicates that polyphenols can be degraded biologically and that an addition of polyphenols during the enzyme dosing step should be avoided.

Extent of foaming in pre-treated GDWW

The impact of foaming in pre-treated GDWW as well as WDWW is shown in Fig. 1. Results are given as the percentage volume loss after 15 min of aeration. The loss of volume due to foaming increased over time in all the wastewater types. The most foaming occurred in GDWW (14.8% volume loss). This is probably because wastewaters containing lipids are known to promote foaming during anaerobic digestion. A relatively large amount of foaming was also observed in WDWW (13.8% volume loss). This may be due to the fact that the WDWW may have contained potentially foaming substances. Steyer *et al*. (1997) also reported that wastewater foaming might occur during the anaerobic treatment of wine distillery.

Foaming was reduced in ozonated GDWW (3.0% volume loss), GDWW + 1% FF (0.6% volume loss), ozonated GDWW + 1% FF (1.0% volume loss) and GDWW + 1% FF + O_3 (1.7% volume loss). Pre-treatments reduced the amount of foaming that occurred because these treatments probably degraded the potentially foaming substances such as lipids, protein and filamentous organisms. Jeganaesan *et al*. (2006) also reported that when a lipase preparation was added to food, industrial wastewater foaming did not occur.

It is clear that there is no linear relationship between lipid reduction and reduction in foaming. However, all the pre-treatments that resulted in lipid reductions also resulted in less foaming. This is of significance for the distillery industry, as foaming occurs when GDWW is treated in an UASB reactor and thus leads to operational problems and possible reactor shut-down (Laubscher, 2000, Akarsubasi *et al.*, 2006). Therefore, any one of the above mentioned treatments might reduce the foaming.

Impact of pre-treatments on granule activity

The impact of various wastewaters and pre-treated GDWW on the activity of UASB granules was determined by granule activity tests. These activity tests were performed to

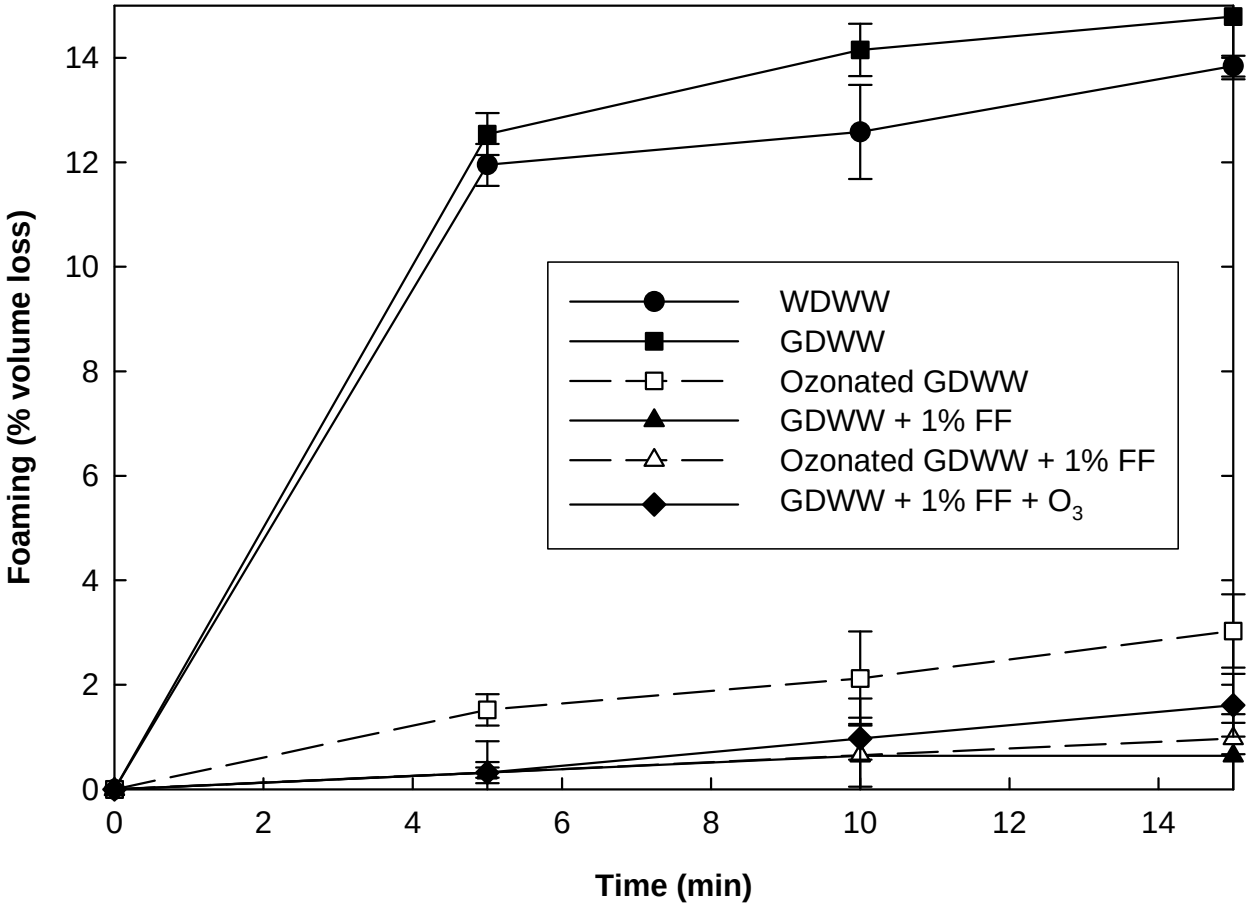

Figure 1 Percentage of volume loss used to indicate the extent of foaming in the WDWW and GDWW samples. The averages and standard deviations (error bars) of triplicate determinations (n = 3) are shown.

establish the granule degradation potential and microbial activity (Schmidt & Ahring, 1995; O'Kennedy, 2000). Granule activity was determined as biogas production (after 25 h incubation activity test) and was expressed as a percentage of biogas produced on day 0 of the granule activity test before exposure to the substrate.

The highest reductions were obtained in ozonated GDWW + 1% FF + O_3 (Table 7). However, in the industry it would not be practical to treat the GDWW using two ozonation steps as double the amount of O_3 is required. A larger ozonator will be needed and the energy needed to operate it will also increase substantially. Therefore ozonated GDWW + 1% FF + O_3 was not investigated in the activity tests.

The activity of granules exposed to different wastewaters for 24 d in terms of the percentage of the biogas production on day 0 in BTM is shown in Fig. 2. The biogas production profile of granules exposed to WDWW on day 24 was 66% of the biogas production on day 0. On day 24, the biogas production profile of granules exposed to SGS was 98% of the biogas production on day 0. The biogas production profile of granules exposed to GDWW and ozonated GDWW on day 24 was 26 and 70% of the biogas production on day 0, respectively. On day 24, the biogas production profile of the granules exposed to GDWW + 1% FF was 40% of the biogas production on day 0. The biogas production profile of granules exposed to ozonated GDWW + 1% FF and GDWW + 1% FF + O_3 on day 24 was 81 and 40% of the biogas production on day 0, respectively.

The activity of granules exposed to different wastewaters for 24 d in terms of the percentage of the biogas production on day 0 in GTM is shown in Fig. 3. The biogas production profile of granules exposed to WDWW and SGS on day 24 was 54% and 103% of the biogas production on day 0, respectively. On day 24, the biogas production profile of granules exposed to GDWW and ozonated GDWW was 37 and 50% of the biogas production on day 0, respectively. By day 24, the biogas production profile of granules exposed to GDWW + 1% FF was 34% of the biogas production on day 0. On day 24, the biogas production profile of granules exposed to ozonated GDWW + 1% FF and GDWW + 1% FF + O_3 was 73 and 32% of the biogas production on day 0, respectively.

The activity of granules exposed to different wastewaters for 24 d in terms of the percentage biogas production on day 0 in ATM is shown in Fig. 4. On day 24, the biogas production profile of granules exposed to WDWW was 43% of the biogas production on day 0. The biogas production profile of granules exposed to SGS on day 24 was 65% of the biogas production on day 0. On day 24, the biogas production profile of granules exposed to GDWW or ozonated GDWW was 23 and 33% of the biogas production on day

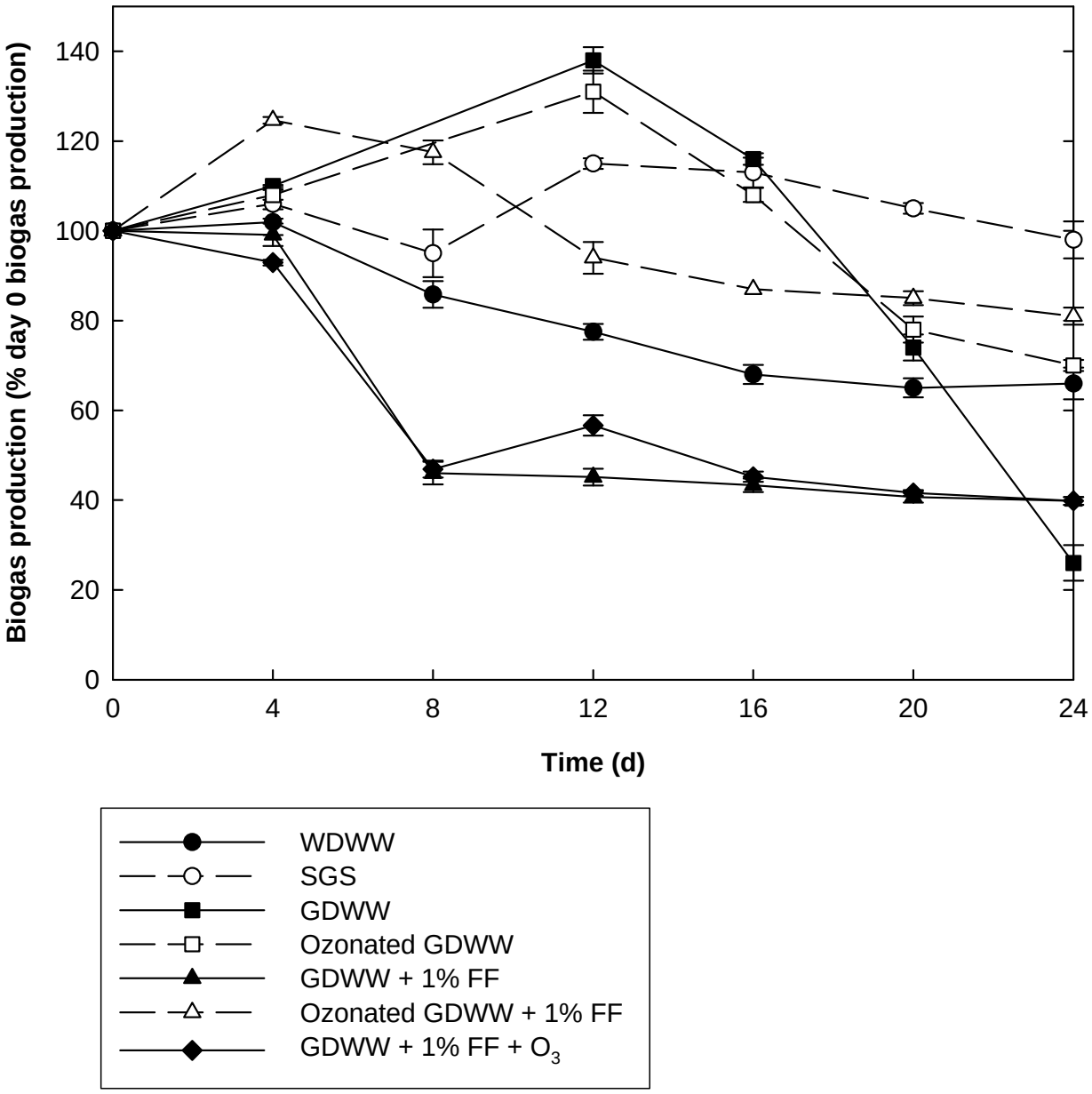

Figure 2 Activity of UASB granules in BTM (25 h incubation) after exposure to various substrates for 24 d. Biogas is expressed as a percentage of the biogas production of the granules on day 0, before exposure to the substrates. The averages and standard deviations (error bars) of duplicate determinations (n = 2) are shown.

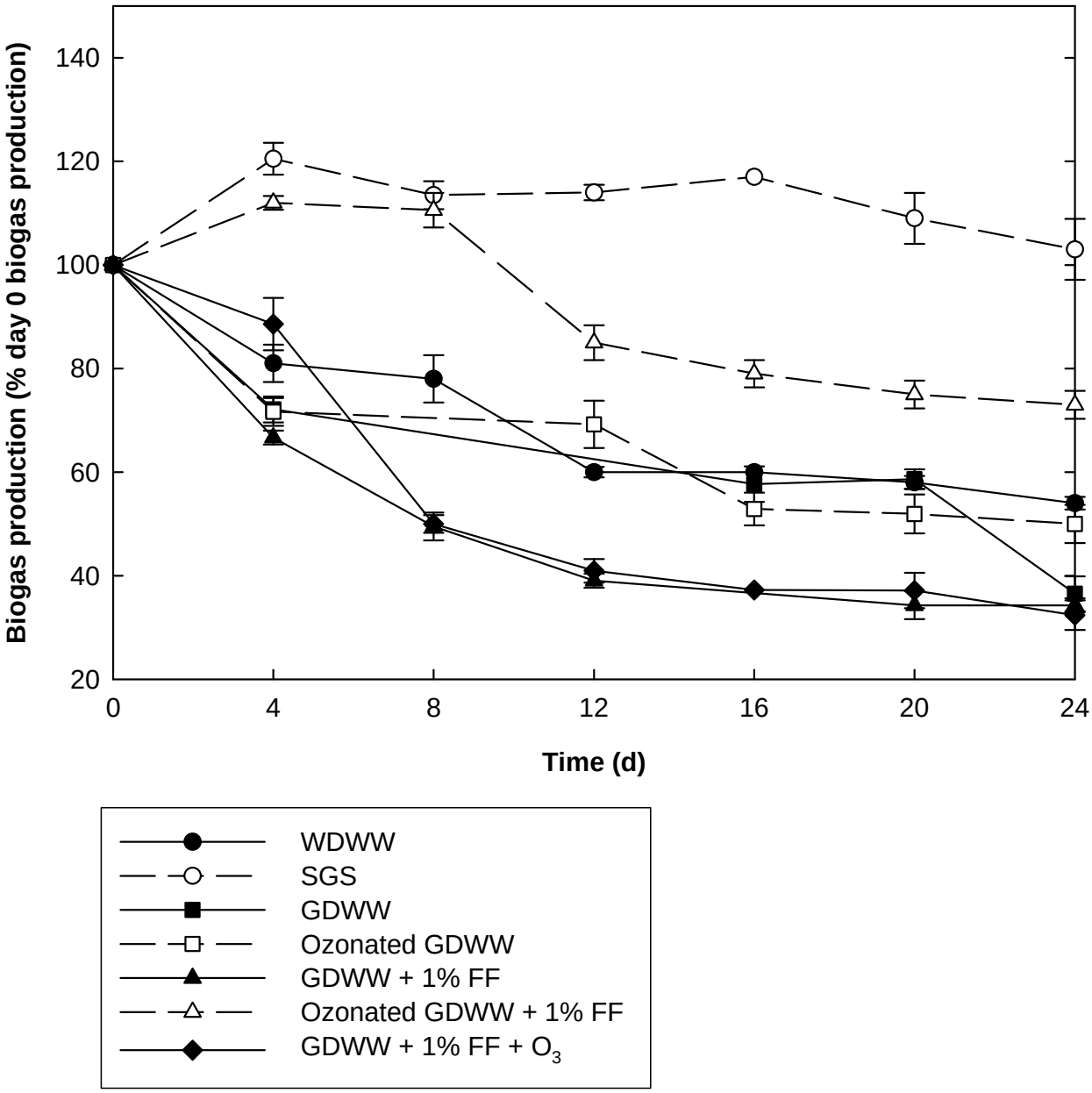

Figure 3 Activity of UASB granules in GTM (25 h incubation) after exposure to various substrates for 24 d. Biogas is expressed as a percentage of the biogas production of the granules on day 0, before exposure to the substrates. The averages and standard deviations (error bars) of duplicate determinations (n = 2) are shown

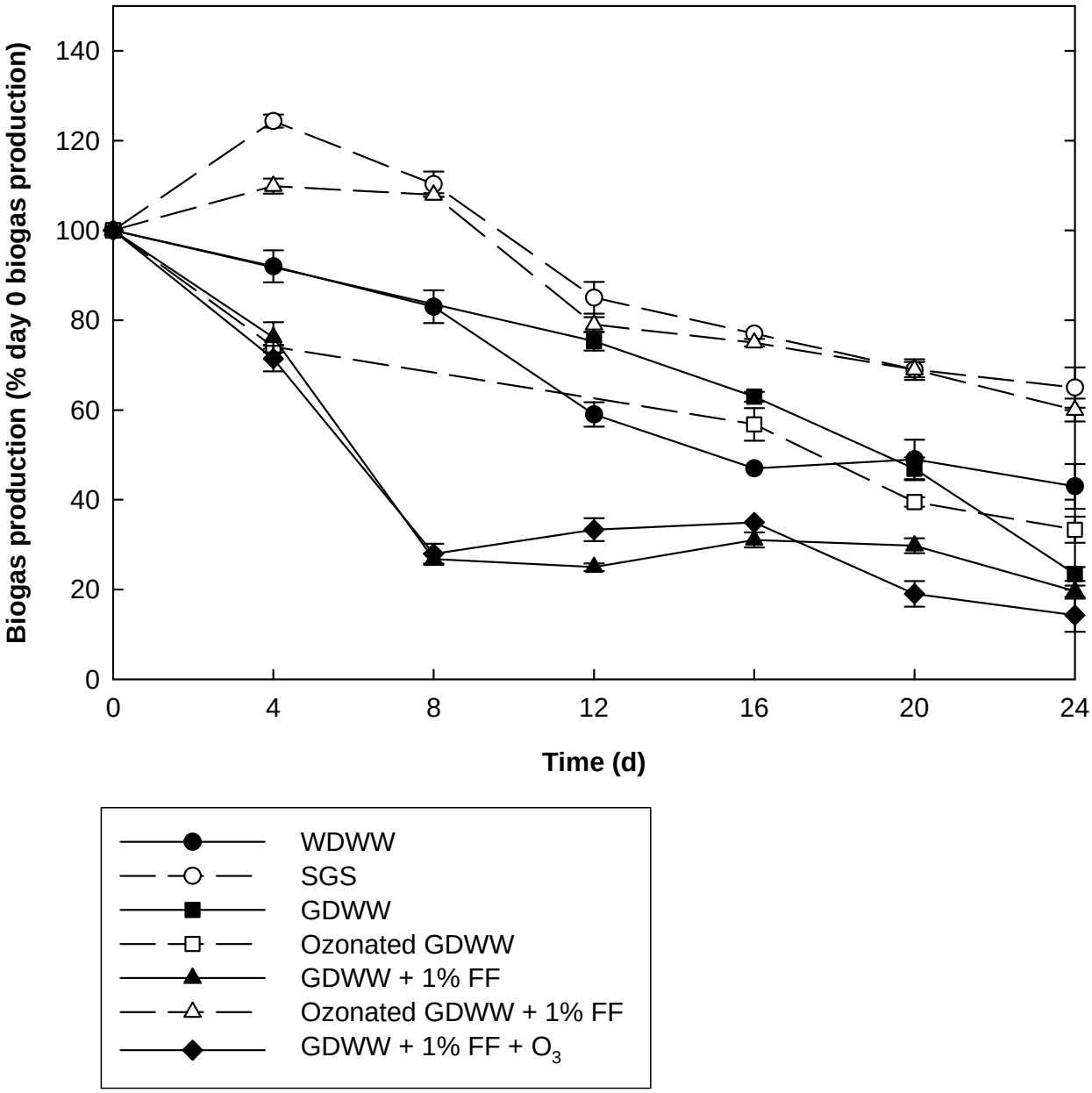

Figure 4 Activity of UASB granules in ATM (25 h incubation) after exposure to various substrates for 24 d. Biogas is expressed as a percentage of the biogas production of the granules on day 0, before exposure to the substrates. The averages and standard deviations (error bars) of duplicate determinations (n = 2) are shown.

0, respectively. The biogas production profile of granules exposed to GDWW + 1% FF on day 24 was 20% of the biogas production on day 0. On day 24, the biogas production profile of granules exposed to ozonated GDWW + 1% FF and GDWW + 1% FF + O_3 was 60 and 14% of the biogas production on day 0, respectively.

When the activity of the granules from the batch reactors was evaluated, certain trends were observed. The highest biogas production profile was always obtained from granules exposed to SGS. This may be explained by the fact that SGS contained sufficient nutrients needed for microbial growth. SGS, unlike the other substrates, also contained 4 $g.L^{-1}$ glucose, which is easily digested by certain aerobic and anaerobic bacteria (such as the acidogens) and is essential for their growth.

In all the test media (BTM, GTM and ATM), the activity of the granules exposed to the distillery wastewaters (WDWW and GDWW samples) decreased over time. This shows that distillery wastewater inhibits activity of the microbial consortium in the granules. Distillery wastewater contains compounds that are unable to support microbial activity in terms of growth requirements, and also contains toxic compounds that inhibit microbial growth, as reported by Beltrán de Heredia *et al*. (2005). Harada *et al.* (1996) also reported that when distillery wastewater was treated in an UASB reactor the efficiency decreased.

In general, the granule activity in all the test media was the same as or higher than that observed in Chapter 4 (Fig. 4) of this thesis. This may be attributed to the fact that the pH of the wastewaters in this chapter was adjusted to pH 6.5, while those in Chapter 4 were not adjusted. Therefore, adjusting the pH to within the optimal pH range for anaerobic digestion (6.2 – 7.6) (Rajeshwari *et al.*, 2000) improved the overall granule activity.

In all the GDWW samples in BTM, there is a clear relationship between lipid reduction in the treated GDWW samples (Table 7) and granule activity (Fig. 2). Granules exposed to ozonated GDWW + 1% FF had the lowest lipid content (90% lipid reduction) and the highest activity, followed by ozonated GDWW (71% lipid reduction), GDWW + 1% FF + O_3 (60% lipid reduction), GDWW + 1% FF (51% lipid reduction) and GDWW (0% lipid reduction). The lower the lipid content of GDWW, the greater the granule activity because the toxic effect of lipids on the microbial consortium decreased as the amount of lipids decreased. Fewer lipids were also available to cover the granules. The contact between the GDWW and microbial consortium in the granules increased.

Similarly, in GTM and ATM for all the GDWW samples in either GTM or ATM, the activity of granules exposed to ozonated GDWW + 1% FF and ozonated GDWW was higher than that of the granules exposed only to GDWW. It is clear that pre-ozonation of

GDWW leads to increased granule activity. The application of this for the industry is that ozonation of GDWW will increase the activity of granules and therefore may improve the efficiency of GDWW treated in an UASB reactor. Again, this may be attributed to the fact that ozonation reduces the toxic effect of the lipids as well as the formation of the encapsulating layer – both of which decrease granule activity.

Impact of pre-treatments on visual appearance

Micrographs were taken of the granules that had been exposed to the different wastewaters for 24 d. Micrographs taken after the granules had been exposed to WDWW and SGS for 24 d are shown in Fig. 5. No encapsulating layer covered either of these granule samples. Similarly, micrographs taken in Chapter 4 (Fig. 5) showed that no encapsulating layer covered the granules exposed to WDWW or SGS. It was also found in Chapter 4 (Table 4) that no encapsulating layer covered these granules because lipids were not present in WDWW or SGS (1.31 – 1.49 mg lipid.g^{-1} granule).

Micrographs taken before and after granules were exposed to GDWW are shown in Fig. 6. After the exposure period, the granules became covered in a clearly visible beige-coloured layer (Fig. 6B). Additional material also adhered to some of the granules, giving the granules a woolly appearance. In Chapter 4 (Fig. 1) of this thesis a similar layer was observed on granules exposed to GDWW. Further analysis in Chapter 4 (Table 4) revealed that granules exposed to GDWW contained more lipids (60.04 ± 0.03 mg.g^{-1} granule) than granules from UASB reactors treating either brewery wastewater (60.04 ± 0.03 mg.g^{-1} granule) or WDWW (1.31 ± 0.06 mg.g^{-1} granule). It was also found that the formation of the encapsulating layer reduced the granule activity because it prevented contact between the microbial consortium in the granules and the GDWW. Lipids are also toxic for many microorganisms and thus reduced the granule activity. Nadais *et al.* (2001), Saiki *et al.* (2003) and Angenent *et al.* (2004) all reported that lipids may reduce the contact between the microbial consortium in the granules and the wastewater, thereby reducing nutrient transfer and thus the granule activity.

Micrographs taken of granules after exposure to ozonated GDWW are shown in Fig. 7A. It was found that the encapsulating layer covered these granules. Ozonation was shown to decrease the lipid content of GDWW (71% lipid reduction) (Table 7), but as the lipids were not completely broken down, a layer still covered the granules. The encapsulating layer was smooth and beige in colour. From the micrograph, it can be seen that ozonation made the encapsulating layer smooth and not woolly in appearance.

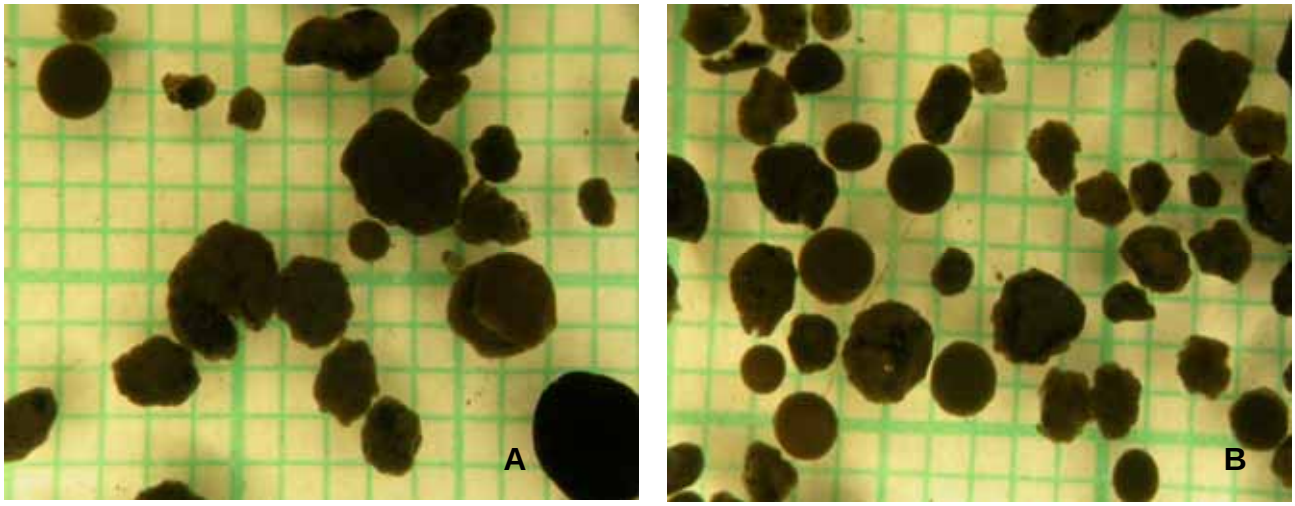

Figure 5 Micrographs of granules after 24 d exposure to WDWW (A) and SGS (B). The grid in the background measures 1 mm by 1 mm.

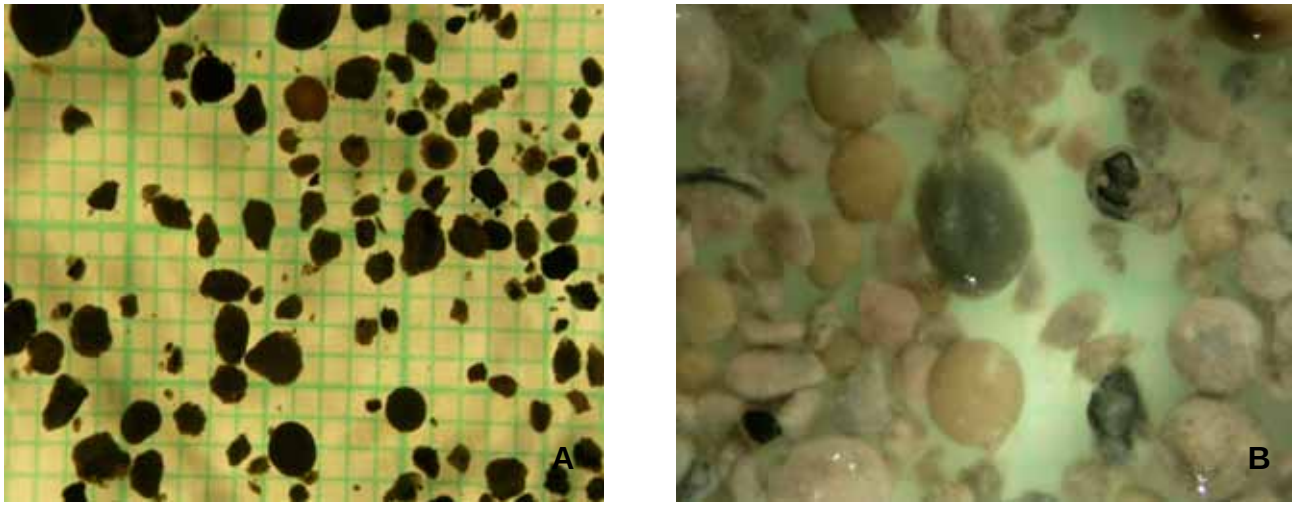

Figure 6 Micrographs taken of granules before (A) and after (B) the 24 d exposure to GDWW. The encapsulating layer covering the granules is visible. The grid in the background measures 1 mm by 1 mm.

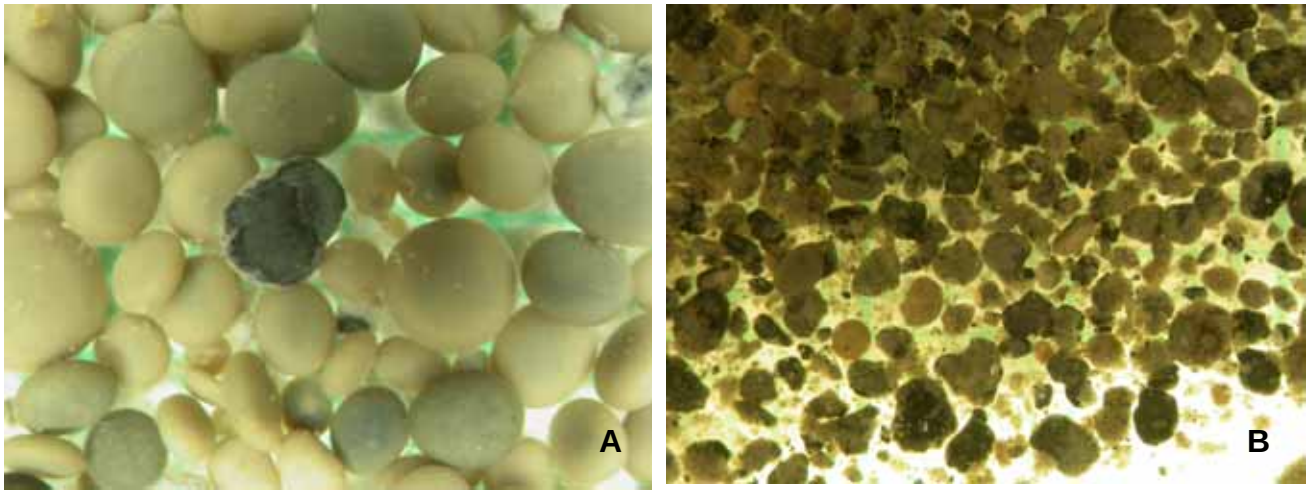

Figure 7 Micrographs of granules after 24 d exposure to ozonated GDWW (A) and GDWW treated with 1% FF (B). The grid in the background measures 1 mm by 1 mm.

Therefore, some of the compounds in the GDWW were most likely degraded.

The micrographs taken after granules were exposed to GDWW + 1% FF are shown in Fig. 7B. An encapsulating layer also covered these granules. The FF dosage altered the colour of the encapsulating layer to brown. The encapsulating layer also did not appear to be smooth and was opaque in appearance. The encapsulating layer did not always cover the entire granule, probably because 51% lipids in the GDWW had been reduced when the GDWW was treated with FF (Table 7). From the micrograph, the FF base powder was visible between the granules.

The micrographs taken after granules were exposed to ozonated GDWW + 1% FF are shown in Fig. 8A. A brown-coloured encapsulating layer covered some of these granules. The FF base powder was visible between the granules. From the micrographs, there is evidence that this treatment reduced the thickness of the encapsulating layer.

Micrographs taken after granules were exposed for 24 d to GDWW + 1% FF + O_3 are shown in Fig. 8B. A clearly visible encapsulating layer also covered these granules. Compared to the encapsulating layer that formed after the UASB granules were exposed to GDWW + 1% FF, the layer was smoother and fewer granules were covered by a layer. The colour of this encapsulating layer was lighter brown in colour than that formed after exposure to GDWW or ozonated GDWW + 1% FF, possibly due to the bleaching effect of the ozonation treatment. The FF base powder was again visible between the granules.

There is a clear relationship between thinness of encapsulating layer, granule activity in BTM and lipid reduction in GDWW. Based on visual appearance, the granules with the thinnest encapsulating layer were those exposed to ozonated GDWW + 1% FF (Fig. 8A), followed by ozonated GDWW (Fig. 7A), GDWW + 1% FF + O_3 (Fig. 8B), GDWW + 1% FF (Fig. 7B) and GDWW (Fig. 6B). This was also the order of the highest granule activity in BTM (Fig. 2) and highest lipid reduction in the GDWW samples (Table 7). The treatments resulting in the visually thinnest encapsulating layer also gave the highest lipid reduction in the GDWW and showed the lowest amount of inhibition in granule activity. This can be ascribed to the fact that the presence of fewer lipids meant a reduction in the toxic effect of lipids on the microbial consortium in the granules. Fewer lipids were also available to cover the granules and thus the contact between the GDWW and microbial consortium in the granules increased. As a result, the granule activity increased.

Impact of pre-treatments on layer lipid content

The lipid content of the granules that were exposed to the different substrates for 24 d is

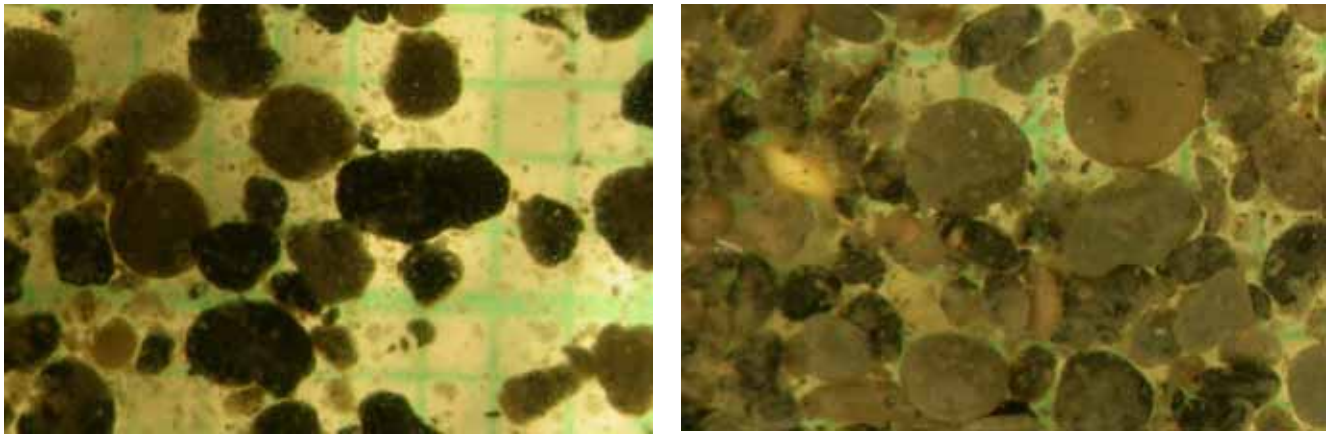

Figure 8 Micrographs of granules after 24 d exposure to pre-ozonated GDWW dosed with 1% FF (A) (ozonated GDWW + 1%FF) and GDWW dosed with 1% FF and post-ozonated (GDWW + 1%FF + O_3) (B). The grid in the background measures 1 mm by 1 mm.

shown in Table 8. The lipid content of the granules obtained from a full-scale UASB reactor treating WDWW (day 0 granule sample) was 1.25 ± 0.01 mg lipid.g^{-1} granule. The lipid content of the granules exposed to WDWW and SGS was 1.21 ± 0.07 and 1.38 ± 0.12 mg lipid.g^{-1} granule, respectively. Similarly, in Chapter 4 (Table 4) of this thesis it was found that the lipid content of granules from full-scale UASB reactors treating WDWW and brewery wastewater was 1.31 ± 0.06 and 1.49 ± 0.02 mg lipid.g^{-1} granule, respectively.

The lipid content of granules exposed was 60.35 ± 0.32 mg lipid.g^{-1} granule. Similar results were obtained in Chapter 4 (Table 4) of this thesis for UASB granules exposed to GDWW for 24 d (60.04 ± 0.03 mg lipid.g^{-1} granule). It can be concluded that when granules were exposed to GDWW, the lipid content in the granules increased.

There was a clear relationship between the lipid content of the granules, thinness of the encapsulating layer, lipid reduction in GDWW and granule activity in BTM. The lowest lipid content in the granules was obtained in granules exposed to ozonated GDWW + 1% FF (3.74 ± 0.10 mg lipid.g^{-1} granule), followed by granules exposed to ozonated GDWW (5.46 ± 0.62 mg lipid.g^{-1} granule), GDWW + 1% FF + O_3 (8.71 ± 0.70 mg lipid.g^{-1} granule) and GDWW + 1% FF (15.47 ± 0.65 mg lipid.g^{-1} granule) (Table 8). This was also the same order as the thinness of the encapsulating layer (Figs. 5 to 8), highest granule activity in BTM (Fig. 2) and highest lipid reduction in the GDWW samples (Table 7). The treatments resulting in the lowest content in the granules also resulted in the thinnest encapsulating layer, the highest lipid reduction in GDWW and the lowest amount of inhibition in granule activity. Again, this can be ascribed to the fact that the presence of fewer lipids reduced the toxic effect of lipids on the microbial consortium in the granules and increased the contact between the GDWW and microbial consortium in the granules. Therefore, the granule activity increased.

Conclusions

Although the highest lipid reduction (97%) was achieved in ozonated GDWW + 1% FF + O_3, this three-step treatment would not be practical for the distillery industry because two ozonators would have to be installed. In the case of the GDWW samples treated with a maximum of two treatments, the highest lipid reduction was achieved in ozonated GDWW + 1% FF (90%), followed by ozonated GDWW (71%), GDWW + 1% FF + O_3 (60%) and GDWW + 1% FF (51%). Foaming was also reduced in all the treated GDWW samples. This is significant for the industry because foaming is known to occur in UASB treatment of

Table 8 Lipid content of UASB granules exposed to various substrates for 24 d. The averages and standard deviations of triplicate determinations (n = 3) are shown

Substrate		**Lipid content (mg.g^{-1} granule)**
Initial		1.25 ± 0.01
After exposure to:	WDWW	1.21 ± 0.07
	SGS	1.38 ± 0.12
	GDWW	60.35 ± 0.32
	Ozonated GDWW	5.47 ± 0.65
	GDWW + 1% FF	15.46 ± 0.62
	Ozonated GDWW + 1% FF	3.74 ± 0.10
	GDWW + 1% FF + O_3	8.71 ± 0.70

GDWW and thus pre-treating the GDWW would reduce or eliminate this problem.

In the case of the GDWW samples, the treatments resulting in the lowest lipid content in the granules also resulted in the thinnest encapsulating layer, the highest lipid reduction in GDWW and the lowest amount of inhibition in granule activity. This is significant for the industry because it proves that a reduction in the lipid content of the GDWW increases the granule activity due to fewer lipids being available to cover the granules in an encapsulating layer, and thus limiting the contact between the microbial consortium in the granules and the GDWW. Therefore the efficiency of the UASB reactor may increase.

Although more effective results were achieved in ozonated GDWW + 1% FF than in ozonated GDWW, ozonated GDWW + 1% FF is not practical for the industry because if 30 to 50 $m^3.d^{-1}$ GDWW is generated (as is the case in a distillery in Wellington, South Africa), 300 to 500 kg FF will be required daily. This will also require superior mixing equipment and a very large mixing tank to store the GDWW throughout the incubation period. The cost and availability of FF may also limit this treatment.

Therefore, it is recommended that GDWW should only be ozonated before treatment in an UASB reactor, which can be done on-line. Even if ozonated GDWW is not treated in an UASB reactor, ozonation may significantly reduce the water penalties paid when GDWW is released into the municipal sewage treatment works.

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CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

Background

The distillery industry generates large quantities of heavily polluted wastewater. At a distillery in Wellington, Western Cape, South Africa, 72 to 118 $m^3.d^{-1}$ wine distillery wastewater (WDWW) is generated specifically during the grape harvest season, while an additional 30 to 50 $m^3.d^{-1}$ grain distillery wastewater (GDWW) is generated during the remainder of the year (Laubscher, 2000). Effective wastewater treatment is, therefore, essential.

In the literature it was found that chemical oxygen demand (COD) reductions of more than 90% were achieved when WDWW was treated in upflow anaerobic sludge blanket (UASB) reactors at organic loading rates (OLRs) of 10 to 18 kg $COD.m^{-3}.d^{-1}$ (Moosbrugger *et al*., 1993; Driessen *et al*., 1994; Laubscher, 2000; Wolmarans & De Villers, 2002). It has also been found that pre- and/or post-ozonation enhanced the overall biodegradability of WDWW (Beltrán *et al.*, 2000; Benitez *et al.*, 2003). Thus, ozonation can be combined with the UASB treatment to enhance the treatment efficiency of WDWW. Little literature is available on the wastewater treatment of GDWW and from the few cases published, it was clear that operational problems occurred during the UASB treatment of GDWW (Laubscher, 2000).

The first objective of this study was to investigate the efficiency of the UASB treatment of WDWW and to enhance the efficiency by using pre- and/or post-ozonation treatments. Secondly, the impact of GDWW on UASB granules was determined in terms of visual appearance and composition of the granules. The third objective was to investigate whether combinations of ozonation and enzymatic treatments may improve the biodegradability of GDWW and thus make GDWW more amenable to UASB treatment.

Treatment of wine distillery wastewater

In this study the UASB treatment efficiency of WDWW was determined and the results showed that WDWW could be treated effectively in a 2 L laboratory-scale UASB reactor. The

COD and polyphenol reductions were 92 and 54%, respectively, at a substrate pH of 7.0, COD of 4 000 $mg.L^{-1}$, OLR of 4.0 kg $COD.m^{-3}.d^{-1}$ and hydraulic retention time (HRT) of 24 h. The results obtained are important for the distillery industry because the successful application of the UASB treatment process will reduce the costs of wastewater penalties. It should be noted that wastewater regulations are set by local municipalities and fines have to be paid for every mg $COD.L^{-1}$ wastewater above a specified COD value.

The inclusion of pre- and/or post-ozonation steps (dose = 47 $mg.L^{-1}$) using a venturi circulating contactor system with an operational volume of 50 L, improved the final UASB efficiency. The highest treatment efficiency (98% reduction for both COD and polyphenol content) was achieved when the UASB treatment was combined with both a pre- and post-ozonation step. The UASB treatment combined with a post-ozonation step (96% COD and 88% polyphenol reduction) was more effective than the UASB treatment combined with a pre-ozonation step (94% COD and 63% polyphenol reduction). The advantages of pre-ozonation may in total be more beneficial for the industry as the efficiency of pre-ozonation is independent of the UASB efficiency, while that of post-ozonation is directly dependent on the UASB efficiency. If production volumes were increased, the COD reduction brought about by pre-ozonation could facilitate lowering the HRT of the UASB reactor, thus increasing the daily volumetric throughput.

It was also found that the activity of the UASB granules increased over time. This is important for the distillery industry, as it proves that acclimatisation of granules in a new reactor is essential for the UASB reactor to operate effectively. It was also found that pre-ozonation of WDWW did not affect the activity of the UASB granules negatively.

It can be assumed that UASB treatment combined with ozonation will have important implications for the industry, especially in terms of reducing the costs required for additional treatments or wastewater penalties. It can also be speculated that if the results of any of the UASB treatment combinations could be obtained on a full-scale basis, up to 500 $m^3.d^{-1}$ of the treated WDWW (COD = 341 $mg.L^{-1}$) may be deposited by irrigation (Anon., 2004).

Treatment of grain distillery wastewater

The South African distillery industry has found that when GDWW is treated in an UASB reactor, several types of operational problems, including granule activity decreases, occur. In

this study the impact of GDWW on UASB granules was determined in terms of visual appearance and composition of the granules. It was found that the GDWW had a COD of 20 007 to 26 069 $mg.L^{-1}$ and a lipid content of 374 to 479 $mg.L^{-1}$. It was also established that UASB granules from a full-scale UASB reactor treating WDWW became encapsulated in a lipid layer after 24 days of exposure to diluted GDWW (COD = 4 000 $mg.L^{-1}$).

When GDWW was treated using combinations of ozonation (dose = 1 476 $mg.L^{-1}$) generated in a 2 L bubble column and enzymatic treatments (dose = 1% FogFree^{TM} (FF) and incubation period = 2 d at 35°C), the lipid content of the GDWW was reduced. This is important because decreasing the lipid content should minimise the occurrence of an encapsulating layer, increase the contact between GDWW and the microbial consortium and improve the anaerobic digestion process. The best lipid reduction (97%) was achieved when GDWW was treated using an enzymatic treatment combined with pre- and post-ozonation (ozonated GDWW + 1% FF + O_3). This was followed by GDWW being treated with an enzymatic treatment combined with pre-ozonation (ozonated GDWW + 1% FF) (90%), when GDWW (71 – 74%) was ozonated, when GDWW was treated using an enzymatic treatment combined with post-ozonation (GDWW + 1% FF + O_3) (60%) and when GDWW was treated using a enzymatic treatment (GDWW + 1% FF) (51%).

In this study it was also found that foaming was reduced when GDWW was treated using any of the above combinations. This is important for the industry because foaming can often be problematic, hampering the UASB treatment process and even interrupting the operation of the UASB reactor due to shutdown and cleaning (Laubscher, 2000).

As part of the study, the activity of granules exposed to different wastewaters was determined. It was found that the composition of the wastewater directly influenced the granule activity. Granules exposed to standard glucose solution (SGS) showed the highest activity. This was expected, as SGS contained no inhibitory compounds and also contained sufficient nutrients to support good microbial growth. The activity of granules that had been exposed to WDWW and GDWW was found to decrease, regardless of the pH. This decrease was ascribed to the fact that these wastewaters contained compounds that inhibited the microbial growth.

The data also showed that the use of pre-treatments improved the biodegradability of GDWW, especially in terms of lipid reduction of the GDWW, and the activity, visual appearance and lipid content of the granules that had been exposed to GDWW. The least inhibition of granule activity was observed when granules had been exposed to ozonated

GDWW + 1% FF followed by ozonated GDWW. The highest inhibition in granule activity was observed when granules were exposed to GDWW followed by GDWW + 1% FF. It was visually found that granules exposed to ozonated GDWW + 1% FF and ozonated GDWW had the thinnest encapsulating layers. The thickest encapsulating layers (determined visually) were observed in granules exposed to GDWW and GDWW + 1% FF. Granules exposed to ozonated GDWW + 1% FF (3.74 ± 0.10 $mg.g^{-1}$ granule) and ozonated GDWW (5.47 ± 0.65 $mg.g^{-1}$ granule) had the lowest lipid content, while those exposed to GDWW (60.35 ± 0.32 $mg.g^{-1}$ granule) and GDWW + 1% FF (15.46 ± 0.62 $mg.g^{-1}$ granule) had the highest lipid content. Based on the above-mentioned data, it is clear that when a high lipid reduction is achieved in GDWW, the inhibition in granule activity is lowered, as fewer lipids are available to encapsulate the granules. The overall anaerobic digestion process will therefore be improved as the contact between the microbial consortium and the GDWW substrate is increased. It can thus be concluded that the application of pre-treatments would make GDWW more amenable to UASB treatment.

The ozonated GDWW + 1% FF treatment may not be practical for the industry to apply, as the treatment requires large quantities of FF. If 30 to 50 $m^3.d^{-1}$ of GDWW is generated, as is the case in a distillery in Wellington (Laubscher, 2000), South Africa, 300 to 500 kg FF will be required daily. Thus GDWW should only be ozonated as it can be done in-line and the initial and operational costs would be lower than when ozonated GDWW + 1% FF is applied. Even if ozonated GDWW is not treated in an UASB reactor, ozonation could still be of value to the industry, as its application would reduce the water penalties paid when GDWW is released into the municipal sewage treatment works.

Future Research

It was concluded that the best wastewater treatment results were obtained when UASB treatment was combined with pre- and post-ozonation steps. However, it must still be determined whether the increase in treatment efficiency is worth the cost of the extra ozonation step. The effectiveness of ozonating WDWW in-line should also be determined. Furthermore, the installation and operational costs of ozonation for treatment with a full-scale UASB reactor should be calculated beforehand and compared to the cost-saving effect of ozonation process.

The data in this study showed that when granules were exposed to GDWW, an encapsulating layer, consisting predominately of lipids, enclosed the granules. Further studies should be conducted to determine the nature of the encapsulating layer and thus determine the most suitable way to prevent the encapsulating layer from forming. It was also found if the lipid reduction in the GDWW was high, the impact on granule activity was low. The inhibition in granule activity also correlated with the thinness and lipid content of the granules. Although treatment combinations using 1% FF and ozonation led to improvements in granule activity, other ozone-enzyme treatments, using lower enzyme concentrations and shorter incubation periods, still need to be examined. The use of liquid enzyme preparations should also be investigated because a higher effective concentration of enzyme can be added in smaller volumes, as no carrier material is required. Methods to completely prevent the formation of the encapsulating layer should also be investigated because when GDWW was treated using combinations of ozonation and FF treatment the encapsulating layer still formed. Total elimination of the encapsulating layer is important for the industry, as it will prevent the granule activity and the overall UASB reactor efficiency from decreasing.

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