INTEGRATION OF ANAEROBIC BIOLOGICAL AND ADVANCED CHEMICAL OXIDATION PROCESSES TO FACILITATE BIODEGRADATION OF FRUIT CANNING AND WINERY WASTEWATERS

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Dissertation presented for the Degree of
Doctor of Philosophy in Food Science
at the University of Stellenbosch.

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April 2005

DECLARATION

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

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ABSTRACT

It has been estimated that unless water consumption and pollution patterns are significantly changed, South Africa will not be able to meet the growing demands for pure and clean water. The Western Cape Province of South Africa has the fastest population growth rate of the nine Provinces. Furthermore, the local fruit canning and winery industries of this province generate large volumes of excessively polluted wastewaters in striving to produce sufficient quality food and beverages. These practices do not contribute to the objectives of lowering water consumption patterns and the stricter regulations governing the disposal of wastewaters to natural water systems, irrigation schemes and municipal treatment plants have obliged these industries to investigate methods of on-site treatment.

With the objectives of producing results that would contribute to a more environmentally friendly and pollution-free water supply, this study was undertaken to assess the applicability of the upflow anaerobic sludge blanket (UASB) process to treat fruit cannery and winery wastewaters and to combine anaerobic digestion (AD) with ozonation as a pre- and post-treatment. Efficiency parameters and scale-up of the processes were also investigated.

UASB treatment of a highly alkaline fruit cannery lye-peeling wastewater was shown to be feasible. Reductions in COD of up to 93% at an organic loading rate (OLR) of 8.1 $kgCOD.m^{-3}.d^{-1}$ were achieved. It was also confirmed that high concentrations of sodium can be inhibitory to anaerobic wastewater treatment and even cause reactor failure in the event of accumulation taking place within the reactor. It was also found that biogas production and methane content can serve as early indicators of pending reactor failure and should be closely monitored when treating wastewaters high in sodium.

Ozone (O_3) and hydrogen peroxide (H_2O_2), singly or in combination with granular activated carbon (GAC) were shown to be effective as post-UASB treatments for AD of alkaline fruit cannery wastewaters. Combinations of O_3 and H_2O_2 gave better results than either oxidant singly, and colour and COD reductions of up to 92 and 91% were achieved, respectively. Final COD levels were reduced to below 75 $mg.L^{-1}$, the "general" legal limit for discharge to a natural water system.

Combinations of pre- and post-ozonation with UASB treatment of fruit cannery and winery wastewaters gave better results than ozonation or UASB alone. Reductions in COD by UASB treatment of 87 – 89% (OLR = 7.5 $kgCOD.m^{-3}.d^{-1}$) for cannery wastewater and 89 – 92% (OLR = 3.7 $kgCOD.m^{-3}.d^{-1}$) for winery wastewater were increased to 95 and 99%, respectively, by pre- and post-UASB ozonation. Final COD

levels were both below the legal limit of 400 $mg.L^{-1}$ for irrigation, while the winery wastewater was below the 75 $mg.L^{-1}$ "general" discharge limit.

Long-term treatment of a pre-ozonated fruit cannery wastewater was shown to be beneficial as the COD reduction efficiency of the UASB process was increased from *ca.* 61% to above 85% at an OLR of 3.4 $kgCOD.m^{-3}.d^{-1}$. Pre-ozonation not only increased the COD reduction efficiency, but also the methane content in the biogas by 10%. Inclusion of a post-UASB ozonation step reduced the effluent COD to 55 $mg.L^{-1}$ (99%), well below the 75 $mg.L^{-1}$ "general" legal discharge limit.

The benefits of pre- and post-UASB ozonation on COD reduction efficiency on laboratory-scale were corroborated in a scaled-up 600 L process. Pre-ozonation of a winery wastewater resulted in the COD reduction efficiency increasing from 81 to 92% at an OLR of 4.3 $kgCOD.m^{-3}.d^{-1}$. The increased biodegradability of the wastewater also resulted in the methane content of the biogas increasing from 43 to 54%. Post-ozonation further increased the total COD reduction efficiency to between 93 and 96%, producing effluents with COD levels below the 400 $mg.L^{-1}$ irrigation limit.

An experimental protocol incorporating an adapted, simple, quick and accurate methanogenic activity test was developed and a distinctive trend in the ozonation time of wastewaters was observed, indicating that larger ozone doses inhibit anaerobic granule activity. This protocol may be used to make fast and accurate decisions about the most efficient pre-ozonation treatment combination in terms of COD reduction, biodegradability and effect on granule activity.

Not only did the results of this study provide the fruit processing and winery industries with proof that AD and ozonation can be combined into an effective and feasible wastewater treatment process capable of lowering the organic load of wastewaters to more manageable levels, but also showed that wastewater treatment costs could be decreased, ensuring a more cost-effective end-product. The implementation of these combined technologies could involve several other benefits. They could claim legal compliance with discharge and irrigation regulations and more importantly could contribute to environmental sustainability.

UITTREKSEL

Daar word geskat, dat tensy waterverbruik- en besoedelingspatrone ingrypend verander, Suid-Afrika nie in die toenemende vraag na suiwer en skoon water sal kan voorsien nie. Die Wes-Kaapprovinsie van Suid Afrika het die vinnigste bevolkingsgroei van die nege provinsies. Verder, produseer die plaaslike vrugte inmaak- en wynbedrywe van hierdie provinsie groot volumes uiters besoedelde uitvloeisel in hul strewe om genoegsame kwaliteit voedsel en drank te produseer. Hierdie aktiwiteite dra nie by tot die doelwitte van laer water verbruikpatrone en die strenger regulasies rakende die wegdoening van afvalwaters na natuurlike waterbronne, besproeiingskemas en munisipale waterbehandelingsaanlegte het hierdie bedrywe genoodsaak om voorbehandelingsmetodes op terrein te ondersoek.

Met die doel om resultate te lewer wat sal bydra tot 'n meer omgewingsvriendelike en besoedelingsvrye watertoevoer, is hierdie studie onderneem om die toepaslikheid van die opvloei anaërobe slykkombersproses (OAS) vir vrugte inmaak- en wynkelderuitvloeisel se behandeling en die kombinasie van anaërobe vertering (AV) met osoon as 'n voor- en nabehandeling te ondersoek. Doeltreffendheidsparameters en opskaal van die prosesse is ook ondersoek.

OAS behandeling van uiters alkaliese vrugte inmaak-loogskil uitvloeisel is as haalbaar bewys. Verminderings in die chemiese suurstof behoefte (CSB) van tot 93% teen 'n organiese ladingstempo (OLT) van 8.1 kgCSB.m^{-3}.d^{-1} is behaal. Dit is ook bevestig dat hoë konsentrasies natrium anaërobe uitvloeiselbehandeling kan strem en selfs kan lei tot reaktor mislukking(die reaktor se ineenstorting/staking) indien dit ophoop binne die reaktor. Dit is ook gevind dat biogasproduksie en metaaninhoud kan dien as vroeë aanwysers van moontlike reaktor mislukking en moet dus noukeurig gemonitor word tydens die behandeling van uitvloeisels met 'n hoë natriuminhoud.

Daar is bewys dat Osoon (O_3) en waterstofperoksied (H_2O_2), apart of in kombinasie met granulêre ge-aktiveerde koolstof (GGC) doeltreffende na-OAS behandelings vir AV van alkaliese vrugte inmaakuitvloeisel is. Kombinasies van O_3 en H_2O_2 het beter resultate gelewer as die oksidante afsonderlik, en kleur en CSB verminderings van 92 en 91% is onderskeidelik behaal. Finale CSB vlakke is verminder na onder 75 mg.L^{-1}, die "algemene" wetlike vereiste vir storting na 'n natuurlike waterbron.

Kombinasies van voor- en na-osonering met OAS-behandeling van vrugte inmaak- en wynkelderuitvloeisels het beter resultate gelewer as osonering of OAS alleen. Verminderings in CSB deur OAS-behandeling van 87 – 89% (OLT = 7.5 kgCSB.m^{-3}.d^{-1}) vir inmaakuitvloeisel en 89 – 92% (OLT = 3.7 kgCSB.m^{-3}.d^{-1}) vir wynkelderuitvloeisel, is verhoog na 95 en 99% onderskeidelik, deur voor- en na-OAS osonering. Finale CSB vlakke was beide laer as die 400 mg.L^{-1} wetlike besproeiingsvereiste, terwyl die wynkelderuitvloeisel laer as die 75 mg.L^{-1} "algemene" stortingsvereiste was.

Daar is bewys dat langtermyn behandeling van 'n vooraf ge-osoneerde vrugte inmaakuitvloeisel voordelig is aangesien die CSB verminderingsdoeltreffendheid van die OAS-proses verhoog is van ongeveer 61% na bo 85% by 'n OLT van 3.4 kgCSB.m^{-3}.d^{-1}. Voor-osonering het nie net CSB vermindering verhoog nie, maar ook die metaaninhoud van die biogas met 10% verhoog. Insluiting van 'n na-OAS osonering het die uitvloeisel CSB verminder na 55 mg.L^{-1} (99%), ver onder die 75 mg.L^{-1} "algemene" wetlike stortingsvereiste.

Die voordele van voor- en na-OAS osonering op die doeltreffendheid van CSB vermindering op laboratorium-skaal is bevestig in die opgeskaalde 600 L proses. Voor-osonering van 'n wynkelderuitvloeisel het daartoe gelei dat die doeltreffendheid van die CSB vermindering verhoog is van 81 na 92% by 'n OLT van 4.3 kgCSB.m^{-3}.d^{-1}. Die verhoogde bio-degradeerbaarheid van die uitvloeisel het ook tot gevolg gehad dat die metaaninhoud van die biogas verhoog is van 43 na 54%. Na-osonering het die totale CSB vermindering doeltreffendheid verder verhoog na tussen 93 en 96%, en uitvloeisels gelaat met CSB vlakke onder die 400 mg.L^{-1} besproeiingsvereiste.

'n Eksperimentele protokol wat 'n aangepaste, eenvoudige en akkurate metanogeniese aktiwiteitstoets insluit is ontwikkel en 'n kenmerkende tendens in die osoneringstye van die uitvloeisels is waargeneem wat aangedui het dat groter osoondosisse anaërobe granule-aktiwiteit beïnvloed. Hierdie protokol mag dus gebruik word om vinnige, akkurate gevolgtrekkings te maak oor die mees doeltreffende voor-osonerings behandelingskombinasie in terme van CSB vermindering, bio-degradeerbaarheid en die effek op granule-aktiwiteit.

Nie alleen het die resultate van hierdie studie die vrugteprosesserings- en wynbedrywe met bewyse verskaf dat AV en osonering gekombineer kan word in 'n effektiewe en uitvoerbare uitvloeiselbehandelingsproses wat in staat is om die organiese lading van die uitvloeisels te verlaag na bestuurbare vlakke, maar ook gewys dat uitvloeiselbehandelingskostes verlaag kan word om sodoende 'n meerkoste-effektiewe eindproduk lewer. Die implementering van sulke gekombineerde tegnologieë kan verskeie ander voordele inhou. Hulle kan aanspraak maak op voldoening aan wetlike vereistes vir storting en besproeiing en meer belangrik, bydra tot omgewingsvolhoubaarheid.

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to the following persons and institutions for their contribution to the successful completion of this study:

Prof. T.J. Britz, as Promoter, for his continued support, initiative, encouragement, guidance and faith in my abilities throughout this study and my career.

Prof. P.C. Fourie, as Co-promoter, for his support, guidance and invaluable critique throughout the study;

The South African Fruit and Vegatable Canner's Association, Harry Crossley Bursary, R & R Fredericksburg and University of Stellenbosch for financial support;

Prof. L. Lorenzen, Centre for Process Engineering, University of Stellenbosch, for interest and engineering advice in the project and financial support;

Neels Barnardt and Rian Strydom for their interest in the project, advice and technical know-how on ozone generation;

Mrs. Marianne Reeves and Mrs. Jenny van Wyk for their help with administrative duties and support;

Mrs. Corné Lamprecht and Mrs. Liezl Maas, colleagues and friends, for their support, encouragement and friendship;

Mr. Eben Brooks, for his support, interest, friendship and help in obtaining wastewaters;

Mr. Arrie Arends, Mr. Theo Valentyn and Ms. Hilda Chandler for laboratory assistance throughout this study;

Mr. Nico van Schalkwyk, Ms. Tania McLachlan, Ms. Lowna-Marie Gie, Ms. Cindy Paulsen and Mr. Jeffrey Green, postgraduate students, for help with laboratory analyses;

Mr. Bruce Hill, RFF Foods, Mr. Clive Radloff and Mr. Schalk-Willem Joubert, R & R Fredercicksberg and Mr. Anton Laubscher, Mr. Koos Steyn, Mr. Robert MacDougall and Mr. Abraham Linde, Distell for their assistance in obtaining wastewaters;

My parents, for their never-ending moral support, encouragement, interest and guidance through life and my "never-ending" studies. Without them I would not have achieved half as much in life;

Karlien, my wife, whose support, encouragement, dedication, faith and endless love gave me the strength and will power to finish this study.

CONTENTS

Language and style used in this dissertation 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 repitition between chapters has, therefore, been unavoidable.

CHAPTER 1

INTRODUCTION

South Africa is a semi-arid country with limited water supplies and an average rainfall well below the world average. Rainfall in Southern Africa is also unevenly distributed and severe, extended droughts occur regularly (Ashton, 2002). It has estimated that unless water consumption patterns are significantly changed, South Africa will be unable to meet the growing demands for water (Republic of South Africa, 1997).

Pollution of rivers, waterways and other water resources is prohibited by the National Water Act, Act 36 of 1998 (Republic of South Africa, 1998). It has also been reported that water pollution is rapidly increasing in South Africa, seriously affecting sustainable economic growth. The most effective means of controlling pollution by industrial wastewaters lies in the re-use of water and reclamation of wastewaters (Republic of South Africa, 1997). In keeping with international practice, the Department of Water Affairs and Forestry has adopted the principle that the polluter pays for the abatement of his own pollution. The production of large volumes of untreated wastewater can thus become a very serious financial burden to any industry. Further, it is a basic requirement of the legislation on pollution control that wastewater purification be an integral part of industrial processes and that the producer of wastewater should provide staff, capital and plant to purify his own effluent to prescribed standards before the purified wastewater is returned to its stream of origin or is re-used (Republic of South Africa, 1998).

The South African fruit and vegetable canning industry is well established and boasted a turnover of close to R3 billion in 2003. With 21 different fruit and vegetable commodities being handled annually, it is estimated that a total of about 650 000 tons of raw fruit and vegetables are processed annually in South Africa (T. Malone, 2003, South African Fruit and Vegetable Canners' Association, Paarl, South Africa, personal communication). Due to the nature of the fruit and vegetable canning process, large volumes of water are used in the transportation of the product, washing and rinsing, blanching, retorting and cooling operations (Wayman, 1996), resulting in water use of up to 7 $m^3.t^{-1}$ of raw product handled (Water Research Commission, 1987).

The South African wine industry is no different, producing large volumes of wastewater throughout the year. The bulk of the wastewater is, however, generated during relatively short periods during and after the vintage season. Most of the water originates from cellar cooling and floor and equipment washdown (Ronquest & Britz, 1999; Bezuidenhout *et al.*, 2002). The amount of water used during wine making varies between 0.7 and 3.8 $m^3.t^{-1}$ of grapes processed, equating to 0.8 to 4.4 $L.L^{-1}$ of wine produced (Water Research Commission, 1993). Cellars have thus had to consider various wastewater treatment options (Hayward *et al.*, 2000).

Both the fruit and vegetable canning industry and the wine industry are thus faced with two major problems. Firstly, maintaining a profitable level of production while reducing the intake of fresh, potable water, and secondly, disposing of the large volumes of wastewater in an environmentally-friendly manner. Disposal of cannery wastewater is often complicated by the presence of suspended solids and particulate organics (Harada *et al.*, 1994), cleaning solutions (most commonly sodium hydroxide), often in formulations with various chelating, softening or surface-active additives, and nitric and phosphoric acids (Mawson, 1997) and sodium hydroxide (used during the peeling of certain fruits and vegetables) in the wastewater. Wastewater from the wine and spirits industry usually has a high organic content (4 000 – 31 000 $mgCOD.L^{-1}$), contains both suspended (200 – 20 000 $mg.L^{-1}$) and dissolved solids (1 300 – 5 300 $mg.L^{-1}$) and the pH (3.5 – 5.3) is acidic (Bezuidenhout *et al.*, 2002). Winery wastewater may also contain caustic soda, various soaps, detergents and surfactants used during cleaning of tanks and process equipment. Sulphide compounds, which can lead to odour problems, and salts originating from anti-corrosion and anti-scaling agents in cooling waters may also be present in the wastewater (Water Research Commission, 1993; Bezuidenhout *et al.*, 2002).

Various methods of disposal of these wastewaters exist, including aerobic treatment by activated sludge, oxidation ditches, biological trickling filters, aerobic and anaerobic ponds and lagoons, spray irrigation, anaerobic digestion and chemical treatment (Austermann-Haun *et al.*, 1997; McLachlan, 2004). Spray irrigation of canning and winery wastewaters is the most commonly used form of disposal in South Africa, mainly due to the availability of cheap land (Water Research Commission, 1987; Bezuidenhout *et al.*, 2002). Although this form of disposal has been proven to be effective in lowering the COD of wastewaters (Murphy, 2000) certain problems, nevertheless, exist. Not only is sufficient land required, but also land with a suitable soil composition and groundcover for handling the wastewater. The presence of organic material, salts, detergents and often high levels of sodium in the wastewater has the potential to disrupt natural processes in the soil, cause unpleasant odours and seriously affect the sodium absorption ratio of the soil. This can lead to irreversible breakdown of the soil structure, rendering the soil impermeable (Murphy, 2000). Alternative treatment and disposal methods resulting in re-use and less environmental pollution hazards, would thus be preferable.

Anaerobic digestion of fruit canning and winery wastewaters has been successfully evaluated (Austermann-Haun *et al.*, 1997; Ronquest & Britz, 1999; Trnovec & Britz, 1998) and has several advantages over conventional disposal methods. Large areas of land are not required (Wood, 1992); bulking of the biomass within the digester is appreciably less than with other treatment methods (Lin & Yang, 1991); bad odours do not occur; the chemical oxygen demand (COD) is substantially lowered; nutrient requirements are low; and water (H_2O), methane (CH_4) and carbon dioxide (CO_2) are obtained as final metabolic end-products (Lettinga, 2001). The methane represents a form of combustible energy and can be used in the factory facilitating considerable savings in energy (Lettinga, 2001). Treatment of these effluents (seasonal fruit cannery and winery) by upflow anaerobic sludge blanket (UASB) reactors has recently been shown to be a feasible option. Chemical oxygen demand reductions of up to 93% at organic loading rates (OLR) of 10.95 $kgCOD.m^{-3}.d^{-1}$ and hydraulic retention times (HRT) of <12 h were achieved while treating a fruit canning wastewater (Trnovec & Britz, 1998). Similarly, COD reductions of 93% at an OLR of 11.05 $kgCOD.m^{-3}.d^{-1}$ and a HRT of 14 h were achieved treating a winery wastewater (Ronquest & Britz, 1999). However, in many instances the COD level of the final effluent was still well above the South African legal limit permitted for wastewaters (75 $mg.L^{-1}$) to be discharged to a water system (Republic of South Africa, 1999) and a further post-treatment was thus deemed necessary.

Ozonation in combination with anaerobic digestion could be useful in increasing the efficiency of biological wastewater treatment processes to such an extent that wastewaters can either be re-used or safely returned to the environment. The use of ozone in the treatment of wastewaters has been shown to be effective in reducing COD, removing colour and increasing the biodegradability of compounds in the wastewater (Gottschalk *et al.*, 2000). The efficiency of ozone in wastewater treatment is mainly due to it's strong oxidising capacity, resulting from the formation of highly reactive hydroxyl radicals. These hydroxyl radicals attack organic molecules by abstracting a hydrogen atom or by adding to the double bonds, thereby mineralising the compounds to non-toxic forms such as carbon dioxide and water

(Gulyas *et al.*, 1995; Beltrán *et al.*, 1999). Ozonation as a post-treatment to reduce the COD content of treated food processing wastewaters has been successful and wastewaters treated include fruit cannery, distillery, poultry, tomato processing, yeast production and table olive debittering wastewaters (Chang & Sheldon, 1989; Filipović-Kovačević & Sipos, 1995; Beltrán *et al.*, 1997, 1999, 2001a and 2001b). Ozonation as a pre-treatment to anaerobic digestion is not as extensively documented as ozonation as a post-treatment. Pre-ozonation was reported to result in reductions (18 – 25%) in COD of vinasse wastewater prior to anaerobic digestion and an increase (25 – 48%) in methane yield during anaerobic digestion (Martin *et al.*, 2002). Benitez *et al.* (1999) reported similar results for the ozonation of winery wastewaters. Reductions in COD of 20 – 30% in the winery wastewater were achieved and an increase of 15% in methane yield for the subsequent anaerobic digestion occurred. The ozonation process may thus possibly be used to improve the biodegradability of wastewaters and thus improve the overall efficiency of wastewater treatment processes, in an attempt to reach final COD levels below the "general" legal limit of 75 mg.L^{-1} for discharge to a natural water resource (Republic of South Africa, 1999).

The major objectives of this study were: to assess the applicability of the UASB process to treat fruit cannery and winery wastewaters; to evaluate combining ozonation, as both pre- and post-treatment steps to anaerobic digestion; to scale up the laboratory UASB design to a 600 L pilot-scale, treating a winery wastewater; and to develop a method using an activity test to predict the optimum ozone concentration/time combination required to improve wastewater biodegradability in the subsequent anaerobic digestion. The scale-up will only be done on winery wastewater as a result of seasonal limitations experienced by the fruit canning industry.

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CHAPTER 2

LITERATURE REVIEW

A. BACKGROUND

South Africa is a semi-arid country with limited water reserves and an average annual rainfall well below the world average (Pybus, 1999). Pollution of rivers, waterways and other water resources is prohibited by the National Water Act, Act 36 of 1998 (Republic of South Africa, 1998). By the year 2020 South Africa's water demand will overtake the water supply, but shortages already exist on a regional basis (Pybus, 1999) and, therefore, proposals have been made to encourage re-use of factory effluents (Republic of South Africa, 1991). It has also been reported that water pollution is rapidly increasing in South Africa, seriously affecting sustainable economic growth. Current disposal practices at canneries and wineries include the disposal of effluents by irrigation onto lands, evaporation in basins or treatment in lagoons or ponds before irrigation onto lands. Some of the larger canneries and wineries, however, dispose of their effluents to municipal sewers. Irrigation of effluents normally leads to ground water pollution problems, odour problems, fly-breeding and an accumulation of toxic contaminants in the soil (J. Visser, 1997, Ashton Canning Company, Ashton, South Africa, personal communication).

The South African fruit and vegetable canning industry is well established and showed a turnover of over R2 billion in 2001 (W. Victor, 2001, South African Fruit and Vegetable Canners' Association, Paarl, South Africa, personal communication). Due to the nature of the fruit and vegetable canning process, large volumes of water are used in the transportation of the product, washing and rinsing, peeling, blanching, retorting and cooling operations (Wayman, 1996). The South African wine industry is no different, producing large volumes of wastewater throughout the year. Most of the water originates from cellar cooling and floor and equipment washdown (Ronquest & Britz, 1999). Both these industries are thus faced with two major problems. Firstly, maintaining a profitable level of production while reducing the intake of fresh, potable water, and secondly, disposing of the large volumes of effluent in an environmentally friendly manner. Disposal of cannery effluent is often complicated by the presence of suspended solids and particulate organics (Harada *et al.*, 1994), cleaning solutions (most commonly sodium hydroxide), often in formulations with various chelating, softening or surface-active additives, and nitric and phosphoric acids (Mawson, 1997) and sodium hydroxide (used during the peeling of certain fruits and vegetables) in the effluent. Wastewater from the wine and spirits industry usually has a high organic content, contains both suspended and dissolved solids and is acidic (Bezuidenhout *et al.*, 2002). Winery wastewater may also contain caustic soda, various soaps, detergents and surfactants used during cleaning of tanks and process equipment. Sulphide compounds, which can lead to odour problems, and salts originating from anti-corrosion and anti-scaling agents in cooling waters may also be present in the wastewater (Water Research Commission, 1993; Bezuidenhout *et al.*, 2002).

Anaerobic digestion of fruit canning and winery wastewaters has been successfully implemented (Austermann-Haun *et al.*, 1997; Ronquest & Britz, 1999; Trnovec & Britz, 1999) and has several advantages over conventional disposal methods. Large areas of land are not required (Wood, 1992); bulking of the biomass within the digester is appreciably less than with other treatment methods (Lin & Yang, 1991); bad odours do not occur; chemical oxygen demand (COD) is substantially lowered; nutrient requirements are low; and water (H_2O), methane (CH_4) and carbon dioxide (CO_2) are obtained as final metabolic products (Lettinga, 2001). Treatment of these effluents (seasonal fruit cannery and winery) by upflow anaerobic sludge blanket (UASB) reactors has recently been shown to be a feasible option. Chemical oxygen demand reductions of up to 93% at organic loading rates (OLR) of 10.95 $kgCOD.m^{-3}.d^{-1}$ and hydraulic retention times (HRT) of <12 h were achieved while treating a fruit canning wastewater (Trnovec & Britz, 1998). Similarly, COD reductions of 93% at an OLR of 11.05 $kgCOD.m^{-3}.d^{-1}$ and a HRT of 14 h were achieved treating a winery wastewater (Ronquest & Britz, 1999). In many instances the COD level of the final effluent was still well above the South African legal limit permitted for wastewaters (75 $mg.L^{-1}$) to be discharged to a water system (Water Research Commission, 1987) and a further post-treatment is thus necessary.

B. TREATMENT OPTIONS

Over the years a wide range of processes have been developed for the treatment of wastewaters. These processes vary in complexity and efficiency from simple land irrigation to complex water treatment systems. All of these processes, however, have one common goal - to minimize the extent of pollution to the environment (Tchobanoglous & Schroeder, 1985).

Lagoon Technology

Lagoon systems or stabilization ponds have been employed for the treatment of wastewaters for many years. The early lagoons were merely holding ponds or evaporation basins created at sites chosen as convenient as possible to the processing plant (Tchobanoglous & Burton, 1991). Lagoon systems are used largely as secondary and tertiary wastewater biological oxidation treatments for small municipalities and isolated rural industries. Their attractions are their low capital investment and low operational costs plus relative ease of operation (Tchobanoglous & Schroeder, 1985). They are effective if properly designed and maintained. However, they require a relatively large amount of flat land in proportion to the amount of waste load being treated and they require prolonged retention time (usually weeks or months) for proper operation. Therefore, they are not well suited where land availability is at a premium or for large municipal and industrial waste loads (Tchobanoglous & Burton, 1991; Bitton, 1999), or where water is in short supply. Lagoons may function both as biological oxidation and solid sedimentation systems. They convert dissolved, suspended and settled solids to volatile gasses (CO_2, CH_4, nitrogen (N_2) and oxygen (O_2)), water and biomass. Lagoons are suitable for seasonal operations and they can also be designed to treat heavy pollution loads (Kilani, 1992). Because of this, lagoons are very popular with small to medium sized food processors (Green & Kramer, 1979; Droste, 1997), and have been used to treat a variety of wastewaters including abattoir and meat industry wastewater, dairy wastes, fruit and canning wastes, brewery wastes, tapioca and maize processing plant wastewaters (Pearson, 1996).

Waste treatment lagoons can be classified into several different types based on their design, function and type of metabolic regime. The organic loading rate is the primary determinant of the metabolic regime. (Green & Kramer, 1979; Droste, 1997; Bitton, 1999). The following designations are used:

Storage Lagoons - Storage lagoons are utilized primarily for operations of a seasonal nature where it is possible to store the waste from an entire seasons production. Prior to discharge into the lagoon, the wastewater is screened to remove the gross solids and most suspended solids. Normally, the wastewater is stored for a period of 90 - 120 d and then discharged during high river flows. The discharge is regulated to avoid substantial increases in the biological oxygen demand (BOD) or reduction of the dissolved oxygen in the receiving stream. During storage, sedimentation of suspended solids and anaerobic decomposition of the organic matter take place (Azad, 1976). The major advantages of storage lagoons are low initial cost, minimal sludge handling problems, and availability of storage for the entire season's operations with controllable discharge timing. However, severe odour problems are usually associated with these lagoons and frequently the reduction of the pollution load is low (Tchobanoglous & Schroeder, 1985). Furthermore, large areas of land are required (Holdsworth, 1970; Sterrit & Lester, 1982). In addition, the enforcement of more strict discharge requirements makes this type of lagoon unsuitable as the only method for treatment of food processing wastes (Azad, 1976).

This type of lagoon was very common for the disposal of wastes from the sugar beet industry (US Environmental Protection Agency, 1971; Green & Kramer, 1979) and some seasonal operations of the canning industry (Canham, 1968).

Anaerobic Lagoons - Anaerobic lagoons have a depth of 2.5 – 9.0 m and a relatively long retention time of 20 - 50 d (Hammer, 1986; Droste, 1997). The anaerobic lagoon's function is the destruction and stabilization of organic matter. It is generally accepted that anaerobic digestion involves three steps: 1) liquefaction of complex organic material, 2) further hydrolysis to a few specific low molecular weight compounds and 3) the conversion of these to CH_4, CO_2 and other gases including hydrogen sulphide (H_2S) (Drysdale, 1981; Bitton, 1999). The heavy organic loads create anaerobic conditions throughout the lagoon (Green & Kramer, 1979). The organic loading level usually accepted as the minimum required to achieve totally anaerobic conditions in a pond or lagoon is 100 g $BOD_5.m^{-3}.d^{-1}$. Anaerobic ponds have considerable advantages over facultative ponds in terms of land use, by a factor of 1 to 10 or more (low surface area to volume ratio). Recent design procedures for anaerobic ponds have greatly reduced the land requirements, as anaerobic ponds can sustain much higher loads than facultative ponds (i.e. 100 – 400 g $COD.m^{-3}.d^{-1}$). This is because their process microbiology relies totally on anaerobic microbes functioning under anaerobic and negative redox conditions (from –200 to –570 mV), and they can thus be built to greater depths (5 m or more) (Gathuo *et al.*, 1991; Pearson, 1996; Pescod, 1996). Modern anaerobic ponds operate with minimum hydraulic retention times of one day and their inclusion in a pond system can give a land area saving of over 75% at design temperatures above 16°C (Pearson, 1996). No expensive mechanical aeration is required and only small amounts of sludge are generated. (Hammer, 1986; Bitton, 1999). Anaerobic lagoon treatment is usually followed by aerobic treatment in facultative ponds, oxidation ditches, aerated lagoons or trickling filters (Azad, 1976; Green & Kramer, 1979). Some problems associated with these lagoons are the production of odorous compounds (e.g., H_2S), sensitivity to toxicants, and the requirement of relatively high temperatures. Anaerobic digestion of wastewater is virtually halted below 10°C (Pescod, 1996; Bitton, 1999).

Anaerobic lagoons have been employed to treat wastewaters arising from sugarbeet processing, fruit and vegetable canning and milk processing (Azad, 1976; Drysdale, 1981; Sterrit & Lester, 1982), coffee production (Gathuo *et al.*, 1991), slaughterhouse effluent, meat and poultry processing wastewater and sugar processing wastewater (Droste, 1997). BOD removals achieved, ranged from 20 - 95% and BOD loads up to 3 360 $kg.ha^{-1}.d^{-1}$ were recorded (Azad, 1976; Droste, 1997).

Oxidation Ponds or Facultative Ponds - These lagoons are the most common and are used to treat wastewaters with intermediate loading rates (45 – 90 kg $BOD_5.ha^{-1}.d^{-1}$ at temperatures above 15°C) and have facultative anaerobic and aerobic zones and the depth may vary from 0.9 - 2.4 m. The greater depth allows for the development of an anaerobic bottom layer and an aerobic surface zone. The oxygen necessary for the aerobic zone is provided by natural aeration and algal photosynthesis (Azad, 1976; Green & Kramer, 1979; Droste, 1997). Large solids settle out to form the anaerobic sludge layer while the soluble and colloidal organic materials are oxidized by the aerobic bacteria using the oxygen produced by the surface growing algae. Carbon dioxide produced in the organic oxidation serves as a carbon source for the algae. Anaerobic breakdown of the solids in the sludge layer results in the production of dissolved organics and gases such as CO_2, H_2S and CH_4, which are either oxidized by the aerobic bacteria or vented to the atmosphere (Tchobanoglous & Schroeder, 1985). BOD removals range from 70 - 95%, with longer retention times resulting in the higher removal rates. Oxidation ponds, however, require large land areas and their performance depends on the climatological conditions. Severe odour problems are sometimes associated with the operation of these lagoons, especially in colder climates (Azad, 1976). In addition, excessive amounts of blue-green algae are often discharged, resulting in effluents of unacceptable quality. The performance of facultative ponds becomes less reliable if the algal biomass drops below 300 g Chlorophyll a.l^{-1}, since below this biomass concentration there can be negative net oxygen production in the pond. At higher temperatures (25° – 35°C) the rate of increase in algal biomass production slows and the solubility of oxygen in ponds also decreases significantly (Pearson, 1996). Subsequent treatment processes may thus be required (Green & Kramer, 1979; Parker, 1978). Oxidation ponds have nevertheless been utilized to a considerable extent for the treatment of food processing and canning wastes (Azad, 1976; Parker, 1978). Yoghurt waste was treated successfully in a laboratory-scale facultative pond, removing 70 and 80% of total and soluble COD respectively, at retention times of 7.9 d and organic loading rates as high as 450 kg COD.ha^{-1}.d^{-1} (Kilani, 1992). A synthetic milk wastewater was also treated in a laboratory-scale oxidation/facultative pond system at loading rates of 300 kg BOD_5.ha^{-1}.d^{-1} and achieving 90% COD removal (Al-Khateeb & Tebbutt, 1992). Corn wet-milling wastewater was also successfully treated in an aerated/facultative lagoon, achieving 95% BOD removal while the system was receiving 3 450 kg COD.d^{-1} (Muirhead, 1990).

Aerobic or Maturation Ponds – Aerobic or maturation ponds are very shallow (30 – 45 cm) and receive effluent from facultative ponds. The organic loading rate is low and aerobic conditions exist throughout the pond depth. There are always some algae present. These ponds are designed to polish the final effluent by settling suspended solids (SS) and stabilizing the low concentration of influent soluble organics (Droste, 1997).

Aerated Lagoons – Aerated lagoons are commonly used as an efficient means of wastewater treatment, relying on little sophisticated technology and minimal, albeit regular, maintenance. Their low capital and operating costs and ability to handle fluctuating organic and hydraulic loads have been valued for years in rural regions and in many tropical countries wherever land is available at reasonable costs (Nameche & Vasel, 1998). Oxygen is supplied to the lagoon by means of a mechanical or diffused aeration unit and a high degree of mixing is provided in order to maintain the solids in suspension (Azad, 1976; Droste, 1997). Aeration is, therefore, not dependent on natural conditions (temperature, winds or sunlight) and the amount of aeration can be controlled (Green & Kramer, 1979; Droste, 1997). The retention time required to attain the desired degree of BOD removal is significantly less than the facultative lagoons because a higher equilibrium level of biological solids is maintained. The aerobic lagoon is analogous to an activated sludge lagoon with extended aeration without sludge return. The effluent from the lagoon is identical to the liquid in the aeration basin and contains the biological solids and the remaining soluble BOD. Therefore, in order to remove these solids and reduce the suspended BOD, a settling tank would be required before final discharge of the effluent (Azad, 1976; Droste, 1997). Aerated lagoons are usually operated at high organic loadings because of the low biological solids maintained in the system. Aerated lagoons have been used to treat a variety of food processing and canning wastes where BOD removals of up to 95% have been obtained (Sterrit & Lester, 1982). However, application of excessively high loadings may result in sludge-bulking problems, and solids separation becomes a serious operational problem. Faulty operation may also give rise to odour problems (Azad, 1976; Bitton, 1999).

Activated Sludge Process

In the activated sludge process, a suspended aerobic microbial consortium is used to treat the incoming wastewater. At the end of the reaction period the microbial biomass is separated from the liquid being treated. Most of the consortium is returned and mixed with incoming wastewater (Tchobanoglous & Schroeder, 1985). The micro-organisms are activated by an input of air (oxygen). The process involves two distinct operations, usually performed in two separate basins: aeration and settling (Droste, 1997; Bitton, 1999).

Micro-organisms are mixed with the sewage in the aeration basin and oxygen is supplied by aeration. The organics in the waste are metabolized to produce end products (CO_2, H_2O, ammonium (NH_4) and other inorganic substances) and new biomass. The contents of the aeration basin are known as the mixed liquor (ML) (Droste 1997; Bitton, 1999).

The second operation is the separation of the biomass and other suspended solids from the wastewater. This separation takes place in the clarifier. The clarified effluent is relatively devoid of any suspended particles compared to the clarifier influent. A portion of the sludge from the clarifier underflow is usually returned to the aeration basin, whereas the remainder is discarded or sent for further processing (Droste, 1997). The settling tanks of an activated sludge process function as powerful selectors for micro-organisms which grow in a form suitable for settling. Organisms which grow as single cells or as small clumps of a few cells do not settle well enough to be separated from the effluent and are carried out of the process. Organisms which grow as larger clumps (floc particles) settle and are returned to the aeration tank, and thus, are able to propagate in the process. This function of the settling tanks is an important mechanism which makes activated sludge processes a feasible method for wastewater treatment (Kim *et al.*, 1998). This is also an area where problems often arise, in that solids are not always sufficiently separated from the treated effluents, and thus, are lost (Contreras *et al.*, 2000).

Oxygen is provided, continuously or semi-continuously, by diffused or mechanical means, and this is the major energy consuming operation in the process. The aerobic conditions result in metabolisms that are efficient in terms of energy recovered by the biomass per unit of substrate processed. This results in a relatively large quantity of sludge production, one of the disadvantages of this process. Sludge processing and disposal are also a major operating expense (Droste, 1997; Bitton, 1999; Garrido *et al.*, 2001). Under the aerobic conditions in the process, the microbial culture grows in clumps or flocs that contain large numbers of bacteria held together by the secreted polymers that accumulate on their capsules (Bitton, 1999).

Several variations of the activated sludge process exist, differing mainly in design, and include: sequencing batch reactors (SBR's), fixed film activated sludge process, extended aeration process, step aeration process, modified aeration process, high rate process, pure oxygen activated sludge process and powdered activated carbon activated sludge process (Droste, 1997; Bitton, 1999).

Application of Activated Sludge Processes to Food and Beverage Processing Wastes - Severe problems can arise when treating high carbohydrate wastes, like those from fruit and vegetable canneries. Although laboratory units successfully run at high loads, in practice bulking is nearly always encountered, due to the excessive growth of filamentous organisms. Bulking sludge has poor settling characteristics and gives rise to a high sludge volume index (SVI) (Plowright, 1976; Nikolavcic & Svardal, 2000). Bulking has been minimized, with considerable success, by installing aerobic selectors in the activated sludge process treating potato-starch factory wastewater and sugar mill effluent (Prendl & Kroiß, 1998; Nikolavcic & Svardal, 2000)

In 1976, Stalzer reported on the successful treatment of a cannery waste on laboratory scale and on a semi-technical scale activated sludge process. Chemical oxygen demand removal efficiencies of 85 - 97% were obtained for low food-to-micro-organism (F/M) ratios (< 0.1 $kg.kg^{-1}.d^{-1}$). For F/M ratios of 0.15 - 0.29 $kg.kg^{-1}.d^{-1}$, the removal efficiency was between 88 and 95%. Influent COD concentrations ranged from 350 – 1 570 $mg.L^{-1}$. Stalzer (1980) also reported COD removals for an activated sludge treatment plant, treating amongst others canning and beet sugar mill effluents. The influent COD varied between 2 000 and 3 500 $kg.d^{-1}$. The COD of the effluent showed COD contents which represented COD removals of up to 95%. It was estimated that 25 - 35% of the original COD entered the cell mass.

In Australia, due to the short seasonal nature of canning operations, the activated sludge process has often been responsible for inadequate treatment leading to odour nuisances and stream pollution. The efficiency of the activated sludge process, treating fruit and vegetable processing waste, is detrimentally affected by bulking as well as by nutrient deficiencies, which often have to be corrected. BOD removal efficiencies for the activated sludge process of between 70 and 96% may be expected, with BOD loadings up to 4 $kg.m^{-3}.d^{-1}$. Typical figures for nutrient additions are 1.4 - 1.8 kg nitrogen and 0.3 kg phosphorous per 100 kg of BOD removal (Sterrit & Lester, 1982).

Bloor *et al.* (1995) used an aerobic Jet Loop Reactor (JLR) activated sludge process to treat a brewery wastewater. At a loading rate of 50 $kgCOD.m^{-3}.d^{-1}$, a COD removal efficiency of 97% was achieved for a period of 5 weeks. Non-flocculating motile bacteria caused the effluent to be cloudy and have a high suspended solids concentration in the order of 200 – 350 $mg.L^{-1}$. An effluent polishing stage would thus be necessary before final discharge.

Garrido *et al.* (2001) investigated the treatment of a dairy laboratory effluent in a coupled anaerobic filter-sequencing batch reactor. The COD of the influent into the SBR varied between 1 000 and 7 000 $mg.L^{-1}$, the OLR between 0.5 and 1.5 $kgCOD.m^{-3}.d^{-1}$. The soluble COD in the effluent exiting the SBR was lower than 200 $mg.L^{-1}$. Thus, in combination with the anaerobic filter, COD removal efficiencies of 98% were reached.

Wastewater from a small cheesemaking dairy has also been successfully treated in a SBR. Torrijos *et al.* (2001) found that at a volumetric loading rate of 0.5 $kgCOD.m^{-3}.d^{-1}$ and 75% of the organic matter being soluble COD, removal efficiencies of 97.7 and 98.8% could be achieved for COD and BOD_5 respectively. In addition, 86% nitrogen and 75% phosphorous removal was achieved.

Winery wastewaters have also been treated in a sequencing batch bioflim reactor. A COD removal efficiency of between 85 and 99% was achieved at an average daily loading of 6.3 kgCOD.m^{-3}.d^{-1}. The wastewater had an average COD of 21 770 mg.L^{-1} of which 50% was soluble. The required retention time varied between 5 and 10.7 h (Andreottola *et al.*, 2002).

Trickling Filters

Trickling filters, also known as percolating filters, were first introduced in the 1890's and consist of four major components (Azad, 1976; Parker, 1978; Tchobanoglous & Schroeder, 1985; Tchobanoglous & Burton, 1991; Barnett *et al.*, 1994; Bitton, 1999). A circular or rectangular tank containing the filter medium (stones (crushed limestone and granite), ceramic material, treated wood, hard coal, or plastic media) with a bed-depth of approximately 1.0 - 2.5 m and providing a large surface area to maximize microbial attachment and growth. It should also provide sufficient void space for air diffusion as well as allowing sloughed microbial biofilm to pass through. Selection of filter media is based on factors such as specific surface area, void space, unit weight, media configuration and size, and cost. The smaller the size, the higher the surface area for microbial attachment and growth, but the lower the percentage of void space. Plastic media are made of polyvinylchloride (PVC) or polypropylene and are mainly used in high rate trickling filters. They have a low bulk density and offer optimum surface area (85 - 140 m^{2}.m^{-3}) and much higher void space (up to 95%) than other filter media. Thus filter clogging is considerably minimized when these media are used. Plastic is also a light material that requires less heavily reinforced concrete tanks than do stone media. Therefore, the biological tower reactors containing these materials can be as high as 6 - 10 m. The wastewater distributor allows an uniform hydraulic load over the filter material. It has one to four arms and its configuration and speed depend on the filter media used. Hydraulic load averages 5 m^{3}.m^{-2}.d^{-1} for low-rate filters. Wastewater is percolated or trickled over the filter and provides nutrients for the growth of micro-organisms on the filter surface.

An underdrain system is normally also included and is necessary for collection of treated wastewater and biological solids (i.e., microbial biomass) that have been sloughed off the biofilm material as well as for the introduction of air. A final clarifier, also called a humus tank, for separation of solids from the treated wastewater is also necessary.

A trickling filter essentially converts soluble organic matter to biomass which is further removed via settling in the final clarifier. Typical organic loading rates are 0.5 kgBOD.m^{-3}.d^{-1} and may vary from 0.1 to 0.4 for low-rate filters to 0.5 to 1.0 kgBOD.m^{-3}.d^{-1} for high-rate filters. This parameter is important to the performance of the trickling filter and may dictate the hydraulic loading onto the filter. BOD removal by trickling filters is approximately 85% for low-rate filters and 65 – 75% for high rate filters (Bitton, 1999). Trickling filters are attractive to small communities because of their easy operation, low maintenance costs, and reliability. They are used to treat toxic industrial effluents and are able to withstand shock loads of toxic inputs. Furthermore, the sloughed biofilms can easily be removed by sedimentation (Tchobanoglous & Schroeder, 1985; Bitton, 1999). High organic loading may lead to filter clogging as a result of excessive growth of slime bacteria in biofilms. Excessive biofilm growth can also cause odour problems in trickling filters. Clogging restricts air circulation, thus resulting in low availability of oxygen to biofilm micro-organisms. However, modifications have helped improve the BOD removal of trickling filters (Azad, 1976; Tchobanoglous & Burton, 1985; Barnett *et al.*, 1994).

Application of Trickling Filters to Treat Food and Beverage Processing Wastes – Biological oxygen demand loadings of up to 11 kg.m^{-3}.d^{-1} from a fruit cannery waste have been tolerated by a trickling filter, with BOD removal as high as 85%. It has however, become clear that some wastes are more amenable than others to treatment by trickling filters. BOD removals were found to be relatively good for peach, carrot and beet wastes, although apricot and pea wastes were only partially treated (Sterrit & Lester, 1982). Similar results were obtained by a pilot-plant operating on a fruit cannery waste (peaches and pears) in the USA, where a BOD removal of 85.5% was obtained (Holdsworth, 1970). Trickling filtration does not, however, lend itself to the seasonal nature of canning factory waste, due to the long start-up time required (Sterrit & Lester, 1982).

Rotating Biological Contactors

Rotating biological contactor (RBC) is another example of fixed a film bioreactor, introduced at the beginning of the 20th century to the United States (Tchobanoglous & Burton, 1991). A RBC consists of a series of disks that are mounted on a horizontal shaft and rotate slowly in the wastewater. The corrugated disks act as a support medium for attached microbial cultures. The disks are approximately 40% submerged in the wastewater (Barnett *et al.*, 1994; Droste, 1997; Bitton, 1999). At any time, the submerged portion of the disk removes BOD as well as dissolved oxygen. The rotation of the disks provides aeration as well as shear force that causes sloughing of the biofilm from the disk surface. Increased rotation improves oxygen transfer and enhances the contact between attached biomass and wastewater. Several RBC stages are usually employed in a staggered arrangement (Bitton, 1999). The advantages offered by RBC are short residence times, low operation and maintenance costs, and production of a readily dewatered sludge that settles rapidly. Operational problems, such as shaft failure, media breakage, bearing failures and odour problems, have been encountered. Odour problems are most frequently caused by excessive organic loadings, particularly in the first stage (Barnett *et al.*, 1994; Droste, 1997; Bitton, 1999).

A RBC process, consisting of six stages and 5 disks per stage, treating a synthetic apple juice waste with a BOD of 900 $mg.L^{-1}$ attained BOD removals of 96%. The first of six stages removed most of the BOD, while the last stage played an important role in handling shock loads (Sterrit & Lester, 1982).

Wilson (1993, 1997) used vegetable pickling waste to study the kinetics and reaction order in a rotating biological contactor. Substrate COD concentrations ranged from 200 to 5 500 $mg.L^{-1}$ and hydraulic loading rates of 0.001 to 0.025 $m^3.m^{-2}.d^{-1}$ were applied. Wilson & Lee (1997) used a RBC to treat a synthetic wastewater containing a mixture of peptone, glucose and meat extract. High removal efficiencies of 90 to 94% in total organic carbon (TOC) were achieved at loadings of 1 to 21 $gTOC.m^{-2}.d^{-1}$.

Natural Treatment Systems (Land Irrigation)

In the natural environment, physical, chemical, and biological processes occur when water, soil, plants, micro-organisms and the atmosphere interact (Bitton, 1999). Natural treatment systems are designed to take advantage of these processes to provide wastewater treatment. The processes involved include many of those used in mechanical or in-plant treatment systems - sedimentation, filtration, gas transfer, adsorption, ion exchange, chemical precipitation, oxidation and reduction, and biological conversion and degradation, plus ones unique to natural systems, like photosynthesis, photo-oxidation and plant uptake (Tchobanoglous & Burton, 1991; Barnett *et al.*, 1994; Bitton, 1999). In natural systems the processes occur at "natural" rates and tend to occur simultaneously in a single "eco-system reactor", as opposed to mechanical systems in which processes occur sequentially in separate reactors or tanks at accelerated rates as a result of energy input (Tchobanoglous & Burton, 1991). Four main types of natural treatment systems can be identified, namely, slow rate irrigation, rapid infiltration, overland flow and wetlands (Tchobanoglous & Burton, 1991; Bitton, 1999).

Slow Rate Irrigation - Slow rate irrigation is the most frequently used land treatment system (Tchobanoglous & Burton, 1991) and involves the application of wastewater to vegetated land to provide treatment and to meet the growth needs of the vegetation (Bitton, 1999). Crop water requirements, soil characteristics and climate factors interact to determine the wastewater application rate. Hydraulic loading rates are the limiting loading rate constraint. The BOD and soluble solids (SS) removals will be excellent after the water has percolated through 1.5 m of soil (Droste, 1997). Sprinkler or ridge-and-furrow systems are usually used for distributing the wastewater. The pre-treatment required is screening and grit removal, but primary sedimentation is desirable. As some storage is usually included as part of slow-rate systems, sedimentation is nearly always provided (Tchobanoglous & Schroeder, 1985; Droste, 1997). The applied wastewater is either reduced by evapotranspiration or percolation vertically and horizontally through the soil profile. Any surface run-off is usually collected and re-applied to the system. In most cases, the percolate will enter the underlying groundwater, but in some cases, the percolate may be intercepted by natural surface waters or recovered by means of underdrains or recovery wells. The relatively low application rates combined with the presence of vegetation and the active soil ecosystem provide slow-rate systems (Tchobanoglous & Burton, 1991). Some limitations of slow-rate irrigation systems are land cost, and high operating cost of transport of wastewater to the treatment site (Bitton, 1999). Average hydraulic application rates are in the order of 0.5 – 6 $m.yr^{-1}$ and average BOD loading is 370 – 1 830 $kgBOD.ha^{-1}.yr^{-1}$ (Droste, 1997).

Rapid Infiltration - Rapid infiltration systems are used where highly permeable sandy soils are available and where it is an objective to replenish groundwater (Droste, 1997). Loading occurs over a period of several days and is followed by a drainage period of 1 - 2 weeks. There is usually no cover crop and the surface is occasionally scarified to break up material plugging the surface layer (Tchobanoglous & Schroeder, 1985; Bitton, 1999). Rapid infiltration is often designed for final polishing after primary and secondary treatment. Nitrification or nitrification-denitrification will be achieved along with phosphorous removal. Application periods range from less than a day to 2 weeks and the drying period is at least as long as the application period, ranging up to 20 times the application period (Droste, 1997). The treatment potential of rapid infiltration systems is somewhat less than slow-rate systems because of the lower retention capacity of permeable soils and the relatively higher hydraulic loading rates (Tchobanoglous & Burton, 1991). Average hydraulic application rates are in the order of 6 - 125 $m.yr^{-1}$ and average BOD loading is 8 000 – 46 000 $kgBOD.ha^{-1}.yr^{-1}$ (Droste, 1997).

Overland Flow - In overland flow, pre-treated wastewater is distributed across the upper portions of carefully graded, vegetated slopes and allowed to flow over the slope surfaces to run-off collection ditches at the bottom of the slopes. Percolation through the soil profile is, therefore, a minor hydraulic pathway. A portion of the applied water will also be lost to evapotranspiration (Tchobanoglous & Burton, 1991). The most suitable soils are clay or loamy-clay soils with low permeability, to limit wastewater percolation through the soil profile. The bacterial community that grows on the thatch that accumulates at the soil surface carries out biological bioxidation, nitrification, and denitrification. Nutrients (N, P) and BOD, suspended solids and pathogens are removed as wastewater flows down the slope. This type of system can achieve 95 - 99% removal of BOD and suspended solids (Tchobanoglous & Schroeder, 1985; Droste, 1997; Bitton, 1999). The two major restrictions to overland flow are difficulty in maintaining consistent quality in the renovated water and site preparation cost (Hammer, 1986; Droste, 1997). Average hydraulic application rates are in the order of 3 – 20 $m.yr^{-1}$ and average BOD loading is 2 000 – 7 500 $kgBOD.ha^{-1}.yr^{-1}$ (Droste, 1997).

Wetlands - Wetlands are inundated land areas with water depths typically less than 0.6 m that support the growth of emergent plants such as cattail, bulrush, reeds, and sedges. The vegetation provides surfaces for the attachment of bacterial films, aids in the filtration and adsorption of wastewater constituents, transfers oxygen into the water column, and controls the growth of algae by restricting the penetration of sunlight. Both natural and constructed wetlands have been used for wastewater treatment (Tchobanoglous & Schroeder, 1985; Tchobanoglous & Burton, 1991). Wetlands are designed to remove conventional pollutants of BOD, SS and nutrients. Heavy metals can also be removed to a significant extent. The most common application of wetlands is for the polishing of secondary effluents (Droste, 1997). Biological oxygen demand removal by wetland systems is higher during the warmer seasons when the metabolic activity of both bacteria and plants is not inhibited by cooler temperatures. Hydraulic loading rate is the primary control variable for wetland systems. Recommended loading rates are 500 $m^3.ha^{-1}.d^{-1}$ or less and should achieve effective (> 60%) removal of BOD and total suspended solids (TSS) (Droste, 1997). BOD_5 removal may be as high as 110 $kg.ha^{-1}.d^{-1}$. The principal nitrogen removal mechanism in wetland systems is bacterial nitrification/denitrification, and thus is also a function of climatic conditions (Tchobanoglous & Schroeder, 1985). Two types of wetland systems have been designed. Surface flow systems are similar to natural wetlands and a free water surface is maintained. The other alternative is a subsurface flow system where the water flows through a permeable medium. Treatment is generally better in the subsurface flow systems and these do not have mosquito problems (Droste, 1997).

Application of Natural Treatment to Food and Beverage Processing Wastes - Spray irrigation of canning factory effluents has been practised since 1947 (Canham, 1956), but several potential problems are apparent. Land requirements, the development of odours, type of cover crop, slope of the land and soil permeability are some of the more apparent problems. Several canneries started out by applying wastewater by means ridge-and-furrow methods, but distribution and control of the waste volumes from large and medium-sized canneries became too difficult. The switch was thus made to spray irrigation, although increased land and equipment requirements were needed. It was believed that spray irrigation was beneficial, as water was being returned to its natural source. Soil permeability and the loss of efficiency during rainy periods were, however, noted as limiting factors (Monson, 1959).

Hanks *et al.* (1968) found the pH, alkalinity and dissolved solids of the peach cannery waste to be significantly reduced, when percolated through a Cecil sandy clay loam soil. Waste percolation through the soil reduced the BOD levels to below 7 $mg.L^{-1}$. Similarly, Gambrell & Peele (1973) reported that the COD of peach cannery waste was significantly reduced by forced aeration and further reduced by the addition of nitrogen from ammonium nitrate, when applied to soil columns in the laboratory simulating disposal by spray irrigation. Sandy clay soil reduced the COD by 90% while sand only removed 70% of the COD. Generally, the reduction of COD was greater for longer soil columns. Therefore, it was concluded that the application of peach cannery waste to soils provides an effective method of disposing of the organic load in the waste. However, the pH of the soil was increased by the sodium hydroxide (NaOH) in the waste and there was some accumulation of salts, both of which offer potential hazards to this method of disposal. Bhuiya & Webber (1963) reported that the sodium saturation percentages and the electrical conductivity values increased with depth and also with the application time, in disposal of sodium rich wastes to soils.

Anderson *et al.* (1966) found in their studies that the percolation of citrus wastes through soil employing a furrow system was sufficient to effect a considerable change in waste-percolate quality. The pH changed from 5 to 7, the alkalinity, calcium and conductivity increased greatly, while COD values dropped by a factor of four. The authors concluded that there was only a slight sodium hazard to the soil, due to the relatively high calcium concentration in the wastewater.

Excellent secondary treatment of potato processing wastewater was also achieved in a series of wetlands. The influent wastewater had a COD of between 2 900 and 3 300 mg.L^{-1} and a TSS content of 530 – 600 mg.L^{-1}. Removal efficiencies of 91 – 95% and 82 – 94% were achieved for COD and TSS respectively, (Kadlec *et al.*, 1997). Wetlands have also been used as secondary treatment for high strength abattoir wastewaters. A treatment system consisting of a settling tank and anaerobic digester for removal of gross solids and primary treatment was followed by an artificially constructed wetland for secondary treatment. The COD of the effluent leaving the anaerobic digester had a COD content of 400 – 2 100 mg.L^{-1}. Removal efficiencies of 62 – 91% were achieved in the wetland at a retention time of 1.7 d at a flow of 35 m^3.d^{-1} (Rivera *et al.*, 1997).

Dairy wastewater has been treated in a treatment process including wetlands. Karpiscak *et al.* (1999) investigated a system comprising a solids separator, anaerobic lagoons, aerobic ponds and a wetland system. The wetland was planted with cattail, soft-stem bulrush and reed. BOD_5 was not significantly reduced but TSS and nitrogen were reduced by 30 and 23%, respectively. Greary & Moore (1999), however, achieved better results from a constructed wetland as part of a treatment system treating dairy parlour wastewater. The wetland had a retention time of 10 – 14 d, average hydraulic load was 25 mm.d^{-1} and average mass loadings were 5.6 g.m^{-2}.d^{-1} for BOD, 2.6 g.m^{-2}.d^{-1} for organic nitrogen, 3.2 g.m^{-2}.d^{-1} for ammonia and 1.5 g.m^{-2}.d^{-1} for total phosphorous. Calculated mean monthly pollutant reductions due to the treatment wetland were 61% for BOD, 43% for organic nitrogen, 26% for ammonia and 28% for total phosphorous. Dairy wastewater has also been treated by using water hyacinths, which grew exceptionally well in the waste (840 mg BOD.L^{-1}) . The BOD was lowered from 840 to 121 mg.L^{-1}, COD from 1160 to 164 mg.L^{-1}, TSS from 359 to 245 mg.L^{-1}, total dissolved solids (TDS) from 848 to 352 mg.L^{-1} and total nitrogen from 26.6 to 8.9 mg.L^{-1} in 4 d (Trivedy & Pattanshetty, 2002).

Billore *et al*. (2001) found removal efficiencies of 64% for COD in a field-scale, four-celled, horizontal sub-surface constructed wetland treating a distillery wastewater. The COD was reduced from 8 420 mg.L^{-1} to 3 000 mg.L^{-1} in 12 d. A 40% reduction in solids, 50 – 60% reduction in nitrogen and 70 – 80% reduction in phosphorous was also achieved. Experimental units of wetlands have also been successfully used for the post-treatment of effluent from an UASB reactor treating domestic sewage. The efficiency of organic material removal during 12 months of operation was between 79 and 83% for the applied COD loads varying between 68.80 and 137.78 kg.ha^{-1}.d^{-1} and was affected neither by macrophytes nor the applied hydraulic load. Phosphorous, ammonia and total Kjeldahl nitrogen (TKN) were reduced by up to 89, 70 and 70%, respectively (De Sousa *et al*., 2001).

Chemical Treatment

Chemical treatment methods rely on chemical reactions to bring about changes in water quality (Tchobanoglous & Schroeder, 1985). This is one of the inherent disadvantages associated with chemical treatment processes (activated carbon adsorption is an exception). In most cases, something is added to the wastewater to achieve the removal of something else (Tchobaoglous & Burton, 1991). The most important chemical treatment methods are those used for disinfection, precipitation of dissolved materials, coagulation of colloids, oxidation and activated-carbon adsorption (Tchobanoglous & Schroeder, 1985; Tchobanoglous & Burton, 1991).

Disinfection is used in the treatment of both domestic and industrial wastewater. Industrially, disinfection is used to control biological slime build-up in piping and bacterial counts in food processing (Bitton, 1999). In contrast, precipitation is used domestically and industrially for water softening and iron removal and for the removal of organics and soluble ions such as PO_4^{3-} from wastewaters. Coagulation is used almost entirely for the destabilization of colloids found in surface waters (Tchobanoglous & Burton, 1991).

Activated carbon, either in granular or powder form, is also used to treat wastewater, often in combination with other processes (i.e. activated sludge process). Activated carbon, due to the large surface area, can absorb large amounts of compounds, mainly refractory organics and inorganic compounds such as nitrogen, sulphides and heavy metals (Tchobanoglous & Burton, 1991). Carbon adsorption led to significant COD removal efficiencies with final levels in the range of 10 - 20 $mg.L^{-1}$. However, high influent suspended solids concentrations (more than 20 $mg.L^{-1}$) will cause a loss in adsorption due to deposits forming on the carbon. This leads to pressure loss, flow channeling or blockages. Lack of consistency in pH, temperature and flow rate may also affect performance of carbon adsorption (Tchobanoglous & Burton, 1991). Thus, the main disadvantage of chemical treatment remains the fact that, normally, costs are incurred in adding other chemicals to wastewaters on a continuous basis, plus in many cases, costs are incurred with the disposal of the final precipitate.

Chemical oxidation, which includes chlorination and ozonation, has also been used in the removal or breakdown of ions such as iron (Fe^{2+}), manganese (Mn^{2+}) and CN^- (cyanide) (Tchobanoglous & Burton, 1991). Iron and manganese are much more soluble in the +2 state than in the +3 state and are easily oxidized with oxygen. Thus, their removal is a combination oxidation-precipitation process. Cyanide removal involves oxidation to the innocuous end products CO_2 and N_2 (Tchobanoglous & Schroeder, 1985). The use of ozone (O_3), hydrogen peroxide (H_2O_2), ultra-violet light (UV) and certain catalysts in wastewater treatment are generally classified as advanced oxidation processes.

Oxidation Technology - Two of the strongest chemical oxidants are ozone and hydroxyl radicals (Mourand *et al.*, 1995; Droste, 1997; Hoigné, 1997). Ozone can react directly with a compound or it can produce hydroxyl radicals which then react with a compound. Owing to the low reactivity of ozone toward the majority of organic compounds, degradation by ozone is rather selective. When a more general oxidant is required, the OH radical is the reagent of choice. It is the most reactive oxidant that can be applied in water treatment (Beschkov *et al.*, 1997; Hoigné, 1997). It reacts with most organic compounds at rates which are close to diffusion-controlled. In water remediation, a number of OH-radical generating systems are currently in use, or under study for potential future use: ozone/hydrogen peroxide (O_3/H_2O_2), ultra-violet light/hydrogen peroxide (UV/H_2O_2), iron (II)/hydrogen peroxide (Fe^{2+}/H_2O_2), ultra-violet-light/ozone (UV/O_3), ultra-violet light/titanium dioxide (UV/TiO_2) and ionizing radiation (electron beam). Hydroxyl radicals can also be produced by the photolysis of aqueous chlorine, nitrate, nitrite, dissolved aqueous iron (III) and in Fenton reactions. Advanced oxidation processes are alternative techniques for catalyzing the production of these radicals. When OH radicals react with the organic substrate, radicals are formed which in the majority of cases react with the dissolved O_2 yielding the corresponding peroxyl radical. Much of the oxidative degradation of the organic matter is effected through the ensuing reactions of the peroxy radicals (Mourand *et al.*, 1995; Hoigné, 1997; Von Sonntag *et al.*, 1997; Gottschalk *et al.*, 2000).

Ozonation - Ozone is an unstable gas and thus needs to be generated at the point of use. The transfer to water is achieved via a variety of ozone contacting systems, where it can react with substances in one of two ways, indirectly or directly. These different reaction pathways are controlled by different types of kinetics and can lead to a variety of oxidation products. The interaction and pathways of these indirect and direct reactions are given in Fig. 1.

Indirect reaction – The indirect pathway involves radicals, the first step being the decay of ozone which is accelerated by initiators, e.g. OH^-, to form secondary oxidants such as hydroxyl radicals (OH°). They react non-selectively and immediately with most organic and many inorganic solutes at very high rate constants that approach diffusion controlled limits. The radical pathway is very complex and is influenced by many factors. The mechanism can be divided into three different parts namely: the initiation step, the radical chain and the termination step (Hoigné, 1997; Gottschalk *et al.*, 2000):

Initiation Step:

The reaction between hydroxide ions and ozone leads to the formation of one superoxide anion radical $O_2^{\circ-}$ and one hydroperoxyl radical HO_2°, which is in an acid-base equilibrium (Gottschalk *et al.*, 2000).

$$O_3 + OH^- \rightarrow O_2^{\circ -} + HO_2^{\circ} \qquad (1)$$

$$HO_2^{\circ} \rightarrow O_2^{\circ -} + H^+ \qquad (2)$$

Radical Chain

The ozonide anion radical ($O_3^{\circ -}$) formed by the reaction between ozone and the superoxide anion radical $O_2^{\circ -}$ decomposes immediately into an OH-radical (Hashem *et al.*, 1997; Gottschalk *et al.*, 2000).

$$O_3 + O_2^{\circ -} \rightarrow O_3^{\circ -} + O_2 \qquad (3)$$

$$HO_3^{\circ} \rightarrow O_3^{\circ -} + H^+ \qquad (4)$$

$$HO_3^{\circ} \rightarrow OH^{\circ} + O_2 \qquad (5)$$

This OH° can react with ozone in the following way (Hoigné, 1982):

$$OH^{\circ} + O_3 \rightarrow HO_4^{\circ} \qquad (6)$$

$$HO_4^{\circ} \rightarrow O_2 + HO_2^{\circ} \qquad (7)$$

With the decay of HO_4° into oxygen and hydroperoxide radical the chain reaction can start again (see reaction 1). Compounds which convert OH° into superoxide radicals $O_2^{\circ -}/HO_2^{\circ}$ promote the chain reaction; they act as chain carriers, the so-called promoters.

Organic molecules, R, can also act as promoters. Some contain functional groups which react with OH° and form organic radicals R°.

$$H_2R + OH^{\circ} \rightarrow HR^{\circ} + H_2O \qquad (8)$$

If oxygen is present, organic peroxy radicals ROO° can be formed. These can further react, eliminating $O_2^{\circ -}/HO_2^{\circ}$ and so enter again into the chain reaction.

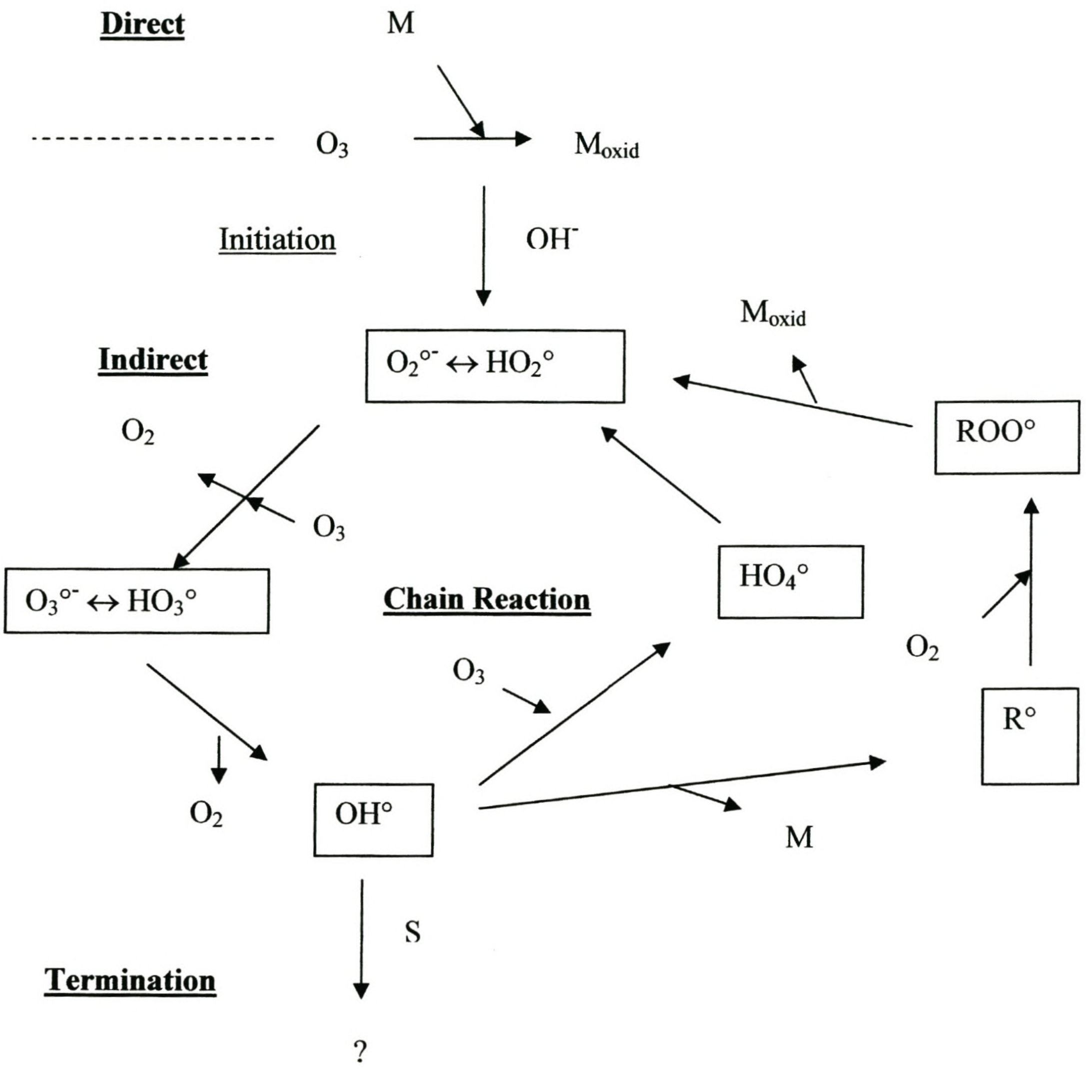

Figure 1. Mechanism of indirect and direct ozonation, S: Scavenger, R: Reaction product, M: Micropollutant (Gottschalk *et al.*, 2000).

$$HR^\circ + O_2 \rightarrow HRO_2^\circ \qquad (9)$$

$$HRO_2^\circ \rightarrow R + HO_2^\circ \qquad (10)$$

$$HRO_2^\circ \rightarrow RO + OH^\circ \qquad (11)$$

The experimental proof of the existence of HO_4°, necessary for the verification of this reaction pathway, is still missing (Gottschalk *et al*., 2000).

The decay of ozone, which can be initiated by the hydroxide ion, leads to a chain reaction and produces fast-reacting and thus non-selective OH-radicals. This also means that OH-radicals have a very short half-life, e.g. less than 10 μs at an initial concentration of 10^{-4} M. Due to its electrophilic properties, the OH-radical reacts at the position with the highest electron density of the largest molecule (Gottschalk *et al*., 2000).

Termination Step

Some organic and inorganic substances react with the OH° to form secondary radicals which do not produce $HO_2^\circ/O_2^{\circ-}$. These inhibitors (or scavengers) generally terminate the chain reaction and inhibit ozone decay.

$$OH^\circ + CO_3^{2-} \rightarrow OH^- \qquad (12)$$

$$OH^\circ + HCO_3^- \rightarrow HCO_3^\circ \qquad (13)$$

Another possibility to terminate the chain reaction is the reaction of two radicals:

$$OH^\circ + HO_2^\circ \rightarrow O_2 + H_2O \qquad (14)$$

The combination of the equations (1 through 7) shows that three ozone molecules produce two OH°.

$$3\ O_3 + OH^- + H^+ \rightarrow 2\ OH^\circ + 4\ O_2 \qquad (15)$$

Many substances exist that initiate, promote or terminate the chain reaction (Table 1).

By adding carbonate to ozonated water, the half-life of ozone can be increased. Even a few µmoles of carbonate decreases the decay rate of ozone by a factor of ten or more. Increasing the concentration of bicarbonate/carbonate up to a concentration of 1.5 nM increases the stability of ozone. Thereafter no further stabilization occurs (Gottschalk *et al.*, 2000).

Direct reaction – The direct oxidation ($M + O_3$) of organic compounds by ozone is a selective reaction with slow reaction rate constants. The ozone molecule reacts with the unsaturated bond due to its dipolar structure and leads to a splitting of the bond.

Ozone's reaction rate with water contaminants varies. Slow reactions are observed with alicyclic taste or odour compounds, such as geosmin or *tri*-halomethanes (THMs) and unactivated aromatics such as chlorinated benzenes. Aromatic compounds with electron supplying substituents, such as the hydroxyl group in phenol will produce faster reactions with ozone. If there is no such substituent the rate of ozonation is much slower. In general, the ionized or dissociated form of organic compounds reacts much faster with ozone than the neutral (non-dissociated) form. Olefins are generally more reactive than aromatic compounds with the same substituents (Gottschalk *et al.*, 2000).

Under acidic conditions ($pH < 4$) the direct pathway dominates, whereas the indirect pathway takes over at $pH > 10$. In ground and surface waters ($pH \cong 7$) both pathways – direct and indirect – can be of importance. In special wastewaters even at $pH = 2$ the indirect oxidation can be of importance, depending much on the contaminants present (Beltrán *et al.*, 1993). Both pathways should always be considered when developing a treatment scheme.

Advanced Oxidation Processes

Advanced oxidation processes (AOP's) have been defined as processes which involve the generation of hydroxyl radicals in sufficient quantity to effect water purification. The most common processes are ozone/hydrogen peroxide (O_3/H_2O_2), ozone/ultra-violet light (O_3/UV) and hydrogen peroxide/ultra-violet light (H_2O_2/UV). It is accepted that a combination of these oxidants results in a more powerful method of generating hydroxyl radicals and thus improves the rate of organic/inorganic

Table 1. Typical initiators, promoters and scavengers for decomposition of ozone in water (Gottschalk *et al.*, 2000).

Initiator	Promoter	Scavenger
OH^-	humic acid	HCO_3^-/CO_3^{2-}
H_2O_2/HO_2^-	*aryl*-R	PO_3^{4-}
Fe^{2+}	primary and secondary alcohols	humic acid
		alkyl-R
		tert-butyl alcohol (TBA)

content degradation (Beltrán *et al.*, 1997). The development of the AOP's is an attempt to produce the non-selective and rapid OH° to oxidize pollutants (Gottschalk *et al.*, 2000). Each of these processes involves chemistry similar to that discussed above.

Oxidation of organic compounds with oxidants such as ozone or OH° radicals usually yields more oxidized ones which are in most cases more easily biodegradable, by biological oxidation, than the former ones. This is the general idea that makes some investigators think of a combination of chemical oxidation technology followed by a biological one (Droste, 1997; Gulyas *et al.*, 1995; Marco *et al.*, 1997).

Ozone/Hydrogen Peroxide – Hydrogen peroxide reacts with ozone when present as the anion, HO_2^-. The reaction rate of the system ozone/hydrogen peroxide is based on the initial concentration of both oxidants:

$$H_2O_2 \rightarrow HO_2^- + H^+ \qquad (16)$$

$$HO_2^- + O_3 \rightarrow HO_2^\circ + O_3^{\circ -} \qquad (17)$$

The reaction of ozone with the undissociated hydrogen peroxide is negligible.

$$H_2O_2 + O_3 \rightarrow H_2O + 2\,O_2 \qquad (18)$$

The reaction continues along the indirect pathway, described previously and OH^- radicals are produced (Gottschalk *et al.*, 2000).

Comparison of the initial reaction with HO_2^- and with OH^- shows that in the O_3/H_2O_2-system the initiation step by OH^- is negligible. Whenever the concentration of hydrogen peroxide is above 10^{-7} M and the pH < 12, HO_2^- has a greater effect than OH^- has on the decomposition rate of ozone in water.

The combination of equations (2 through 7, 17 and 18) shows that two ozone molecules produce two OH° (Gottschalk *et al.*, 2000):

$$2\,O_3 + H_2O_2 \rightarrow 2\,OH^\circ \qquad (19)$$

Ozone/UV-Radiation (O_3/UV) – In this process, the photolysis of ozone is brought about by UV-radiation. The decomposition of ozone leads to hydrogen peroxide. Efficient photolysis of ozone requires UV lamps to have a maximum output at 254 nm (Gottschalk *et al.*, 2000).

$$O_3 + H_2O \xrightarrow{hv} H_2O_2 + O_2 \qquad (20)$$

This system contains three components to produce OH° and/or to oxidize the pollutant for subsequent reactions:

- UV-radiation
- ozone
- hydrogen peroxide

Direct photolysis of the pollutant can occur if it absorbs the wavelength used. Direct oxidation by hydrogen peroxide can be neglected under normal conditions (pH between 5 to 10 and ambient temperature). The role of direct and indirect ozonation depends on the conditions already mentioned. Therefore, the reaction mechanism of ozone/hydrogen peroxide is of importance as well as the combination of UV-radiation/hydrogen peroxide. The extinction coefficient ε of ozone at the wavelength of 254 nm is much higher (ε_{254nm} = 3 300 M^{-1} cm^{-1}) than that of hydrogen peroxide (ε_{254nm} = 18.6 M^{-1} cm^{-1}). The decay rate of ozone is about a factor of 1 000 higher than that of H_2O_2 (Gottschalk *et al.*, 2000).

Hydrogen Peroxide/UV-Radiation (H_2O_2/UV) – The direct photolysis of hydrogen peroxide leads to OH° (Mourand *et al.*, 1995; Braun & Oliveros, 1997; Gonzalez & Mártire, 1997):

$$H_2O_2 \xrightarrow{hv} 2\ OH^\circ \qquad (21)$$

Also HO_2^- which is in an acid-base equilibrium with H_2O_2 (see eq. 16) absorbs the wavelength 254 nm (Gonzalez & Mártire, 1997).

$$HO_2^- \xrightarrow{hv} OH^\circ + O^{\circ -} \qquad (22)$$

$$HO_2^- + O^{\circ -} \rightarrow O_2^{\circ -} + OH^- \qquad (23)$$

The stoichiometric yield of OH° is the greatest from the photolysis of hydrogen peroxide (Table 2). Ozone has a higher molar extinction coefficient than hydrogen peroxide and thus photolysis of ozone yields more OH° than that from H_2O_2 (Table 3). This comparison is only theoretical. In reality a high production of OH° can lead to a low reaction rate because the radicals recombine and are not useful for the oxidation process (Gottschalk *et al.*, 2000).

Application of ozone and AOP's to Food and Beverage Processing Wastes – Advanced oxidation processes involving hydroxyl radicals OH°, represent a treatment concept that has gained increasing interest in the treatment of industrial wastewater and fresh water to remove organic and inorganic contaminants. Ozonation has been used for the treatment of several wastewaters, the removal of substituted phenols, pesticides and other organic and inorganic compounds (Mokrini *et al.*, 1997).

Ozone is usually used as a combination treatment system, but applications of ozone alone have also proved successful. Ozone has been used as a post-treatment step to the aerobic biological treatment of high strength distillery (cherry stillage) wastewater (Beltrán *et al.*, 2001a). The overall conversion of BOD and COD in the biological step were 95% and 82%, respectively. Polyphenol content and absorbance at 254 nm could not be reduced by more than 35% and 15%, respectively. Post-treatment ozonation was found to reduce the COD by 34.0%, TOC by 15.0%, total phenols by 79.5%, absorbance by 59.9% and inorganic carbon by 50.7%. The high concentration of carbonates (which are known to be hydroxyl radical scavengers) present, inhibits wastewater mineralisation since the hydroxyl radicals are primarily consumed by reacting with the carbonates, thus the low COD reduction (Beltrán *et al.*, 2001a). Beltrán *et al.* (2001b) studied the pH sequential ozonation of domestic and wine-distillery wastewaters. The process involves a succession of acidic and alkaline wastewater conditions. The alkaline phases allow oxidation of organic matter by hydroxyl radical and produce carbonates that would eventually inhibit the oxidation. The acidic phases favour the development of direct ozone reactions and strip off carbonates as carbon dioxide from the wastewater. For domestic wastewater a 2 h ozonation at constant pH 2 and 10 resulted in a 25.0 and 60.8% COD reduction, respectively. A sequential ozonation with an acidic period of 20 min followed by an alkaline period of 100 min led to a 75.6% COD removal. For

Table 2. Theoretical values of oxidants and UV required for the formation of hydroxyl radicals in ozone-peroxide-UV systems (Gottschalk *et al.*, 2000).

	Moles of oxidants consumed per mole of OH° formed		
System	**O_3**	**UV[a)]**	**H_2O_2**
Ozone-hydrogen peroxide ion [b)]	1.5	-	-
Ozone-UV	1.5	0.5	(0.5) [c)]
Ozone-hydrogen peroxide	1.0		0.5
Hydrogen peroxide-UV	-	0.5	0.5

[a)] Moles of photons (Einsteins) required for each mole of OH° formed

[b)] Assumes that superoxide $O_2^{\circ-}$ is formed which yields one OH° per $O_2^{\circ-}$, may not be the case in certain waters

[c)] Hydrogen peroxide formed *in situ*

Table 3. Theoretical values of hydroxyl radicals produced by the photolysis of ozone and hydrogen peroxide (Gottschalk *et al.*, 2000).

	ε_{254nm} in M^{-1} cm^{-1}	**Stoichiometry**	**OH° formed per incident photon [a)]**
H_2O_2	20	$H_2O_2 \rightarrow 2\ OH^{\circ}$	0.09
O_3	3 300	$O_3 \rightarrow 2\ OH^{\circ}$	2.00

[a)] assumes 10 cm path length; $c(O_3) = c(H_2O_2) = 10^{-4}M$

wine-distillery wastewater, a pH sequential ozonation with two cycles (two acidic-alkaline periods of 10 and 50 min, respectively) represented optimal conditions. A COD removal and ozone efficiency of 41.4 and 78%, respectively were achieved, compared with 21.4 and 43.0% for ozonation at constant pH 4.

In another study, ozonation of distillery slop waste was investigated to evaluate ozonation in terms of organic matter removal and decolourization efficiencies (Alfafara *et al.*, 2000). The chemical properties of melanoidins, which are known to harbour chromophoric groups in the waste, were also investigated. Ozone had primary effects on the decolourization of the slops and improvement in its biodegradability of 40% was obtained in 40 h. Only 16% of the COD was removed. The melanoidins showed a 10% decrease in molecular weight indicating slight depolymerization. UV spectral studies of the melanoidins showed that olefinic linakages (which are said to be important in the structure of chromophores) decreased (Alfafara *et al.*, 2000). Ozonation of yeast-production industry wastewater has also been used to decolourize the wastewater. Ozonation alone resulted in COD reductions of up to 65% and colour reduction of 90% was achieved after a 60 min ozonation (Filipović-Kovačević & Sipos, 1995).

Ozonation has also been combined with physical treatments in order to achieve COD reductions. Chang & Sheldon (1989) combined ozonation with diatomaceous earth and sand filtration to improve the wastewater characteristics of poultry processing wastewaters. Ozonation alone of overflow prechiller water resulted in COD reductions of between 47 and 62%. Ozonation followed by sand filtration and another ozonation treatment of overflow prechiller water resulted in COD reductions of 92.8%. Diatomaceous earth (DE) filtration followed by ozonation resulted in COD reductions of 79.0 – 92.3%, and it was concluded that a combination of screening, DE filtration and ozonation yielded the highest quality water. With this combination, significant reductions in chemical oxygen demand, total solids and the total microbial load, including coliform bacteria and salmonellae of 87.0, 65.0 and 99.9%, respectively were achieved in reconditioned prechiller water.

Ozone alone and in combination with hydrogen peroxide and UV-light has been used to treat table olive debittering wastewaters. COD reductions of 80 – 90% with ozone doses of 3 – 4 g (per 700 mL) in presence of 10^{-3} M initial H_2O_2 or 254 nm UV radiation were achieved, while moderate reductions of total carbon could be achieved (between 40 and 60%). Both aromatic content and colour nearly completely disappeared with less than 0.5 g ozone applied (per 700 mL). Also, the biodegradability of wastewaters, measured as BOD/COD, increased from 0.16 (untreated wastewaters) to nearly 0.70 or 0.80 when using ozone combined with H_2O_2 or UV radiation as the oxidizing systems, respectively (Beltrán *et al.*, 1999). Degradation of olive mill wastewaters by means of two chemical oxidation processes (Fenton's reagent and ozonation) and their consecutive treatments with aerobic micro-organisms have also been studied by Beltrán-Heredia *et al.* (1999 & 2001). Fenton's reagent treatment moderately reduced COD, and to a greater extent the polyphenolic compounds. Ozonation contributed to low conversion of COD and moderate reduction of polyphenols. The aerobic biological treatments degraded to values higher than 70 and 90% for COD and polyphenolic compounds, respectively.

Ozonation as a treatment method or pre-treatment to anaerobic digestion of winery wastes was studied by Benitez *et al.* (1999). Ozonation in combination with H_2O_2 and UV-light resulted in COD reductions of between 20 and 30% and significant removals of aromatic compounds, in the range of 60 – 67%. Pre-treatment of the winery waste by ozonation led to an enhancement of the methane yield, with a value of 215 ml of $CH_4.g^{-1}$ of COD removed, compared to 187 ml of $CH_4.g^{-1}$ of COD removed during anaerobic digestion alone. Andreozzi *et al.* (1998) studied the integrated treatment of olive oil mill effluents (OME) by ozonation combined with anaerobic digestion. It was found that ozone selectively oxidizes phenols and unsaturated lipids in OME. Ozonated OME have a stronger inhibitory effect than untreated ones on methanogenic bacteria (this effect is not present on acidogenic bacteria). Oleic acid is less inhibitory to methanogenesis than its ozonation products (probably due to the formation of azelaic acid). It was noteworthy that the ozonated OME did not present any additional inhibition effects on acidogenic bacteria. If ozonation products were degradable under acidogenic conditions, a previous separate acidogenesic step could overcome their negative effects on methanogenesis.

C. ANAEROBIC DIGESTION BY UPFLOW ANAEROBIC SLUDGE BLANKET REACTORS

Background

Anaerobic digestion (AD) is a biological process in which organic matter is converted to methane and carbon dioxide. The process involves a bacterial community with complex nutritional requirements and specialized ecological roles (Ianotti *et al.*, 1987 Barnett *et al.*, 1994). In contrast to aerobic waste processing, anaerobic digestion results in much smaller amounts of biomass (Forday & Greenfield, 1983; Barnett *et al.*, 1994).

The AD process is ascribed to the synergistic relationship of four trophic groups of bacteria, namely: hydrolytic, fermentative acidogenic, acetogenic and methanogenic bacteria (Forday & Greenfield, 1983; Ditchfield, 1986; Bitton, 1999). The process is shown schematically in Figure 2.

Hydrolytic Bacteria - Anaerobic bacteria break down complex organic molecules, like proteins, cellulose, lignin, lipids and carbohydrates, by a process of hydrolysis into soluble monomer molecules such as amino acids, glucose, fatty acids and glycerol. The monomers are directly available to the next group of bacteria. Hydrolysis of the complex molecules is catalyzed by extracellular enzymes such as cellulases, proteases and lipases (Forday & Greenfield, 1983; Bitton, 1999; Batstone *et al.*, 2002; Mata-Alvarez, 2003).

Fermentative/Acidogenic Bacteria - Acidogenic bacteria convert sugars, amino acids and fatty acids to organic acids (e.g., acetic, propionic, formic, lactic, butyric or succinic acids), alcohols and ketones (e.g., ethanol, methanol, glycerol and acetone), acetate, CO_2, and H_2. Acetate is the main product of carbohydrate fermentation. The products formed vary with the type of bacteria as well as with culture conditions (temperature, pH, redox potential) (Forday & Greenfield, 1983; Ditchfield, 1986; Ianotti *et al.*, 1987; Bitton, 1999; Batstone *et al.*, 2002; Mata-Alvarez, 2003).

$$\underset{\text{glucose}}{C_6H_{12}O_6} + 4H_2O \rightarrow \underset{\text{acetate}}{2CH_3COO^-} + 2HCO_3^- + 4H^+ + 4H_2 \qquad \Delta G^\circ = -206\text{ kJ}$$

$$\underset{\text{glucose}}{C_6H_{12}O_6} + 2H_2 \rightarrow \underset{\text{propionate}}{2CH_3CH_2COO^-} + 2H_2O + 2H^+ \qquad \Delta G^\circ = -358\text{ kJ}$$

$$\underset{\text{glucose}}{C_6H_{12}O_6} \rightarrow \underset{\text{lactate}}{2CH_3CHOCOO^-} + 2H^+ \qquad \Delta G^\circ = -198\text{ kJ}$$

$$\underset{\text{glucose}}{C_6H_{12}O_6} \rightarrow \underset{\text{ethanol}}{2CH_3CH_2OH} + 2HCO_3^- + 2H^+ \qquad \Delta G^\circ = -226\text{ kJ}$$

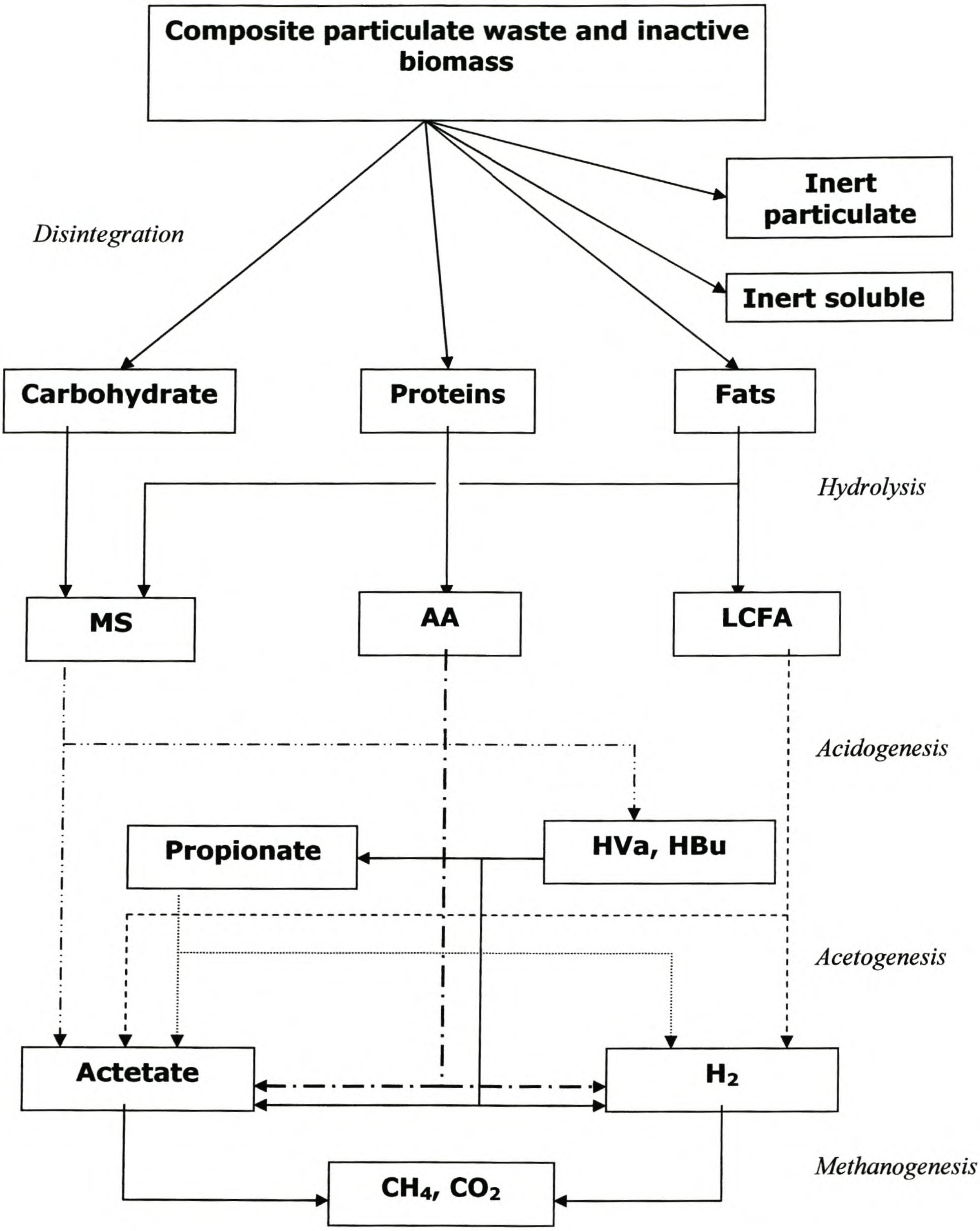

Figure 2. An outline of the conversion processes in anaerobic digestion according to the Anaerobic Digestion Model No. 1 (ADM1) (Batstone et al., 2002). Abbreviations include MS (monosaccharides); AA (amino acids); LCFA (long chain fatty acids); HVa (valeric acid); HBu (butyric acid).

Acetogenic Bacteria - Acetogenic bacteria (acetate and H_2 - producing bacteria) convert fatty acids (e.g., propionic acid, butyric acid and valeric acid) and alcohols into acetate, hydrogen, and carbon dioxide (Ianotti *et al.*, 1987). This group requires low hydrogen tensions for fatty acid conversions. A close monitoring of hydrogen concentration is thus necessary. Under relatively high H_2 partial pressure, acetate formation is reduced and the substrate is converted to propionic acid, butyric acid, and ethanol (Bitton, 1999). There is a symbiotic relationship between acetogenic bacteria and methanogens. Methanogens help achieve the low hydrogen tension required by acetogenic bacteria (Ianotti *et al.*, 1987).

Ethanol, propionate, and butyrate are converted to acetate by acetogenic bacteria according to the following reactions (Forday & Greenfield, 1983; Bitton, 1999; Batstone *et al.*, 2002; Mata-Alvarez, 2003):

$CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + H^+ + 2H_2$ $\Delta G° = +9.6$ kJ
ethanol acetate

$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + H^+ + HCO_3^- + 3H_2$ $\Delta G° = +76.1$ kJ
propionate acetate

$CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + H^+ + 2H_2$ $\Delta G° = +48.1$ kJ
butyrate acetate

$CHCHOHCOO^- + 2H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 2H_2$ $\Delta G° = -4.2$ kJ
lactate acetate

$3CHCHOHCOO^- \rightarrow 2CH_3CH_2COO^- + CH_3COO^- + HCO_3^- + H^+$
lactate propionate + acetate $\Delta G° = -165$ kJ

$CHCHOHCOO^- + 2H_2O \rightarrow CH_3CH_2CH_2COO^- + 2HCO_3^- + 2H_2$
lactate butyrate + acetate $\Delta G° = -56$ kJ

The conversion of these higher volatile fatty acids, caproate, butyrate and propionate to methanogenic substrates, hydrogen and acetate, is an important step in the digestion process as the unionized forms of these acids are particularly toxic to the methanogenic bacteria (Wang *et al.*, 1997; Dhaked *et al.*, 2003).

Methanogens - This group is composed of both Gram-positive and negative bacteria with a large variation in morphology (Bitton, 1999). They are obligatory anaerobes, requiring a lower oxidation reduction potential (-300 mV) for growth than most anaerobic bacteria (Forday & Greenfield, 1983; Ianotti *et al.*, 1987). Methanogens can be subdivided into two sub-categories, namely those utilizing hydrogen and those that split acetate (Ditchfield, 1986; Bitton, 1999). The hydrogenotrophic methanogens convert hydrogen and carbon dioxide to methane (Ianotti *et al.*, 1987; Tchobanoglous & Burton, 1991).

$$HCO_3^- + 4H_2 + H^+ \rightarrow CH_4 + 3H_2O \qquad \Delta G° = -135.6 \text{ kJ}$$

The hydrogen-utilizing methanogens help maintain the very low-level hydrogen partial pressure necessary for the conversion of volatile fatty acids and alcohols to acetate (Speece, 1983).

The acetotrophic methanogens, also known as acetoclastic bacteria, convert acetate into methane and carbon dioxide (Tchobanoglous & Burton, 1991; Bitton, 1999).

$$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^- \qquad \Delta G° = -31 \text{ kJ}$$

About two thirds of methane is derived from acetate conversion by acetotrophic methanogens. The other third is the result of carbon dioxide reduction by hydrogen (Ditchfield, 1986; Mackie & Bryant, 1981).

Operational Factors Contolling Anaerobic Digestion

Anaerobic digestion is affected by temperature, retention time, pH, chemical composition of the wastewater, competition of methanogens with sulphate-reducing bacteria, and the presence of toxicants (Bitton, 1999; Van Lier *et al.*, 2001).

The three optimum temperature ranges at which anaerobic digestion and other biological reactions occur are: thermophilic (50° - 65°C), mesophilic (20° - 45°C) and psychrophilic (< 20°C) (Van Lier *et al.*, 2001). In municipal wastewater treatment plants, anaerobic digestion is carried out in the mesophilic range at temperatures from 25°C to up to 40°C with an optimum at approximately 35°C (Bitton, 1999). Because of their slower growth as compared to acidogenic bacteria, methanogenic bacteria are very sensitive to small changes in temperature. A decrease in temperature leads to a decrease in the maximum specific growth rate (Lawrence & McCarty, 1969). Thus, mesophilic digesters must be designed to operate at temperatures between 30°C and 35°C for their optimal functioning (Bitton, 1999).

The hydraulic retention time (HRT), which depends on wastewater characteristics and environmental conditions, must be long enough to allow metabolism by anaerobic digestion (Bitton, 1999). These hydraulic retention times vary also with the type of digester used, and may vary from 10 h to several days (Van Lier *et al*., 2001).

The optimum pH range of methanogenic bacteria is between 6.7 and 7.4, with an optimum at 7.0 - 7.2. Methanogenesis may fail in the event of the pH dropping to 6.0 (Bitton, 1999). Maintenance of this pH is due to the conversion of acid end products to methane by the combined activities of acetogenic and methanogenic bacteria. The major controlling buffer in this pH range is the carbonate-bicarbonate system, with orthophosphoric acid, hydrosulphuric acid, the volatile acids and ammonia contributing to pH stabilization. At lower pH values the volatile fatty acids regulate buffer capacity. Acidity is more inhibitory to methanogens than to acidogenic bacteria. An increase in volatile acids levels thus serves as an early indicator of system upset. Monitoring the ratio of total volatile acids (as acetic acid) to total alkalinity (as calcium carbonate) has been suggested to ensure stable operation (Sung & Santha, 2003).

Wastewater must be nutritionally balanced (nitrogen, phosphorous, sulphur) to maintain an adequate anaerobic digestion. The C : N : P ratio for anaerobic bacteria is 700 - 1000 : 5 : 1 (Sahm, 1984; Sam-Soon *et al*., 1986). Methanogens use ammonia and sulphide as nitrogen and sulphur sources, respectively. Although unionized sulphide is toxic to methanogens at levels exceeding 150 - 200 $mg.L^{-1}$, it is required by methanogens as a major source of sulphur (Speece, 1983). Trace elements such as iron, cobalt, molybdenum, and nickel are also necessary (Williams *et al*., 1986).

Methanogens and sulphate-reducing bacteria may compete for the same electron donors, acetate and H_2. Sulphate-reducing bacteria have a higher affinity for acetate than methanogens and will outcompete methanogens under low acetate concentrations (Yoda *et al*., 1987).

A wide range of toxicants are responsible for the occasional failure of anaerobic digesters. Inhibition of methanogenesis is generally indicated by reduced methane production and increased concentration of volatile acids (Bitton, 1999). The following are some of the more common toxicants: oxygen (Oremland, 1988), ammonia (at high pH when higher levels of unionized ammonia are formed

(Bhattacharya & Parkin, 1989), chlorinated hydrocarbons (Blum & Speece, 1992), benzene ring compounds (Patel *et al.*, 1991), formaldehyde (Hickey *et al.*, 1987), heavy metals (e.g., Cu^{2+}, Pb^{2+}, Cd^{2+}, Ni^{2+}, Zn^{2+}, Cr^{6+}) (Lin, 1992), sulphide (Anderson *et al.*, 1982), and tannins (Field & Lettinga, 1987). Volatile acids such as acetic and butyric appear to be only slightly toxic to methanogenic bacteria. Propionic acid, however, displays toxicity towards both acid-forming and methanogenic bacteria (Bitton, 1999).

Application of Anaerobic Digestion to Food and Beverage Processing Wastes
Hemming (1981) reported on a full-scale anaerobic digestion plant treating potato processing effluent. The effluent had a BOD of 5 000 $mg.L^{-1}$ which was reduced by 60%, had an HRT of seven days and produced 1 800 $m^3.d^{-1}$ of gas containing 70% methane. The feasibility of the upflow anaerobic sludge bed process for the treatment of crude potato-maize wastewater of a chip processing industry was also investigated (Kalyuzhnyi *et al.*, 1997). The wastewater had a high concentration of suspended solids (up to 7 000 $mg.L^{-1}$), a high content of insoluble fraction of COD (up to 60% of total COD) and significant quantities of potentially foaming substances like proteins and fats. Organic loading rates of up to 14 000 mg $COD.L^{-1}.d^{-1}$ were employed and treatment efficiencies higher than 73 and 63% were achieved for dissolved and total COD, respectively (Kalyuzhnyi *et al.*, 1997). Potato processing wastewaters generally have COD levels between 4 500 and 7 500 $mg.L^{-1}$ and relatively high suspended solids concentrations (800 – 1 500 $mg.L^{-1}$) and thus, different anaerobic configurations have been applied as treatment options (i.e. UASB and expanded granular sludge bed (EGSB)). The advantage of EGSB's is that their smaller footprint and greater height allows COD loads of up to 30 $kgCOD.m^{-3}.d^{-1}$, compared to the 10 $kgCOD.m^{-3}.d^{-1}$ of conventional UASBs treating potato processing wastewater. In both cases COD removal efficiencies of *ca.* 80% were obtained, with excellent stability and all-year efficiency and reliability (Zoutberg & Eker, 1999). An UASB reactor was also adapted to incorporate a denitrification step during the treatment of parboiled rice industry wastewater (Lopes *et al.*, 2001). The top of the UASB reactor (an anoxic zone) was used to facilitate denitrification. Nitrification was then performed in an aerated mixed reactor. A fraction of the nitrification reactor effluent was recycled into the top of the UASB reactor above the sludge blanket for denitrification in the anoxic zone. The maximum removal efficiency of TKN was 80% when using the recycle loop (Lopes *et al.*, 2001).

Nadais *et al.* (2001) investigated the application of shock loadings (organic, hydraulic and fat shocks) on the performance of an UASB reactor treating dairy wastewater. Total influent COD ranged from 5.6 to 11.9 $g.l^{-1}$ and fat concentration between 2.7 and 6.1 $g.l^{-1}$. Total COD removals of 74 – 93% and fat reductions of 78 – 91% were achieved at HRT's of 6 – 12 h and OLR's of 12 $g\ COD.L^{-1}.d^{-1}$. The use of an EGSB reactor with internal circulation was used to treat a low strength dairy industry wastewater, a medium strength food industry wastewater (potato based salads and various vegetables) and a high strength brewery wastewater (Driessen & Yspeert, 1999). The dairy wastewater influent COD averaged 1 550 $mg.L^{-1}$ and a total COD removal of 51% was achieved. The food industry wastewater had an influent COD in the range of 995 to 7 450 $mg.L^{-1}$ (average = 4 550 $mg.L^{-1}$). Volumetric loading rates of up to 42 $kgCOD.m^{-3}.d^{-1}$ at HRT's of 3.6 to 9.1 h were attained with a total COD removal efficiency of 80%. When treating the high strength brewery wastewater with an average COD load of 13 000 $mg.L^{-1}$ (max. 23 000 $mg.L^{-1}$), a total COD removal of 70 – 90% was achieved. Soluble COD removal amounted to 80 – 98%, while the reactor was stable at volumetric loading rates of up to 36 $kgCOD.m^{-3}.d^{-1}$ at HRT's of 8 – 24 h (Driessen & Yspeert, 1999).

The performance of UASB and EGSB reactors have also been compared using ethanol, brewery wastewater and coffee processing wastewater as substrates. During the operation of an EGSB reactor treating ethanol at 500 $mg.L^{-1}$ and a sludge loading rate of 0.8 $g\ COD.gVSS^{-1}.d^{-1}$, COD removal efficiencies of up to 80% were obtained. The UASB's performance was similar at 75%. Operating both reactors at ethanol concentrations of 10 $g.l^{-1}$, removal efficiencies of 98% were achieved. Biogas production was 0.4 $l.g\ COD^{-1}$ removed. Operation of the same reactors with diluted beer as substrate, with organic loadings of 0.8 $g\ COD.g\ VSS^{-1}.d^{-1}$, resulted in removal efficiencies of 85% for the EGSB and 70% for the UASB reactor. With coffee industry wastewater, the COD removal efficiencies were somewhat lower, probably as a result of the complex nature of the wastewater, at around 60%. The EGSB reactor gave a slightly better removal efficiency (ca. 5%) than the UASB reactor at sludge loading rates of 0.7 $g\ COD.g\ VSS^{-1}.d^{-1}$ (Jeison & Chamy, 1999).

Olive mill effluents (OME) are known to contain biorecalcitrant and/or inhibiting substances (essentially phenols and lipids) which hinder the methanogenic process (Andreozzi *et al.*, 1998; Beccari *et al.*, 1998 and 2001). A two reactor system, consisting of an acidogenic phase followed by a methanogenic phase, was used to treat the OME. A maximum of 5% of the total COD was converted to methane in the acidogenic reactor. Complete methanogenic activity was established in the second reactor. Polyphenols were not degraded at all in the acidogenic reactor, while 20 – 30% was removed in the methanogenic reactor. Lipids removal in the acidogenic phase was low (13.6%) but the total two reactor system gave a 77.2% removal. An overall COD removal of 76.5% was achieved (Beccari *et al.*, 1998). Chemical and physical treatments have been employed to remove the biorecalcitrant compounds from OME before anaerobic treatment (Beccari *et al.*, 2001). The addition of $Ca(OH)_2$ to a pH value of 6.5 and bentonite addition at 15 $g.l^{-1}$, followed by centrifugation, resulted in lipid and phenol removals of 99.5 and 45.0%, respectively. A laboratory-scale continuous methanogenic reactor was also fed with the substrate mixture (OME, $Ca(OH)_2$ and bentonite) to evaluate the anaerobic treatability enhancement (Beccari *et al.*, 2001). At the most demanding conditions tested (organic load = 8.2 $kgCOD.m^{-3}.d^{-1}$; dilution ratio = 1:1.5) the reactor performance was excellent (total COD removal = 91%; methane yield = 0.80 g CH_4 (as COD).g^{-1} total COD; lipid removal = 98%; phenol removal = 63%) (Beccari *et al.*, 2001).

Research has also been conducted on the anaerobic treatment of winery and distillery wastewaters (García-Bernet *et al.*, 1998; Ronquest & Britz, 1999; O'Kennedy, 2000; Ruiz *et al.*, 2002). García-Bernet *et al.* (1998) applied a down-flow fluidized bed reactor to treat a wine distillery wastewater anaerobically. The system attained TOC removal efficiency rates between 75 and 95% at an OLR of 17 kg $TOC.m^{-3}.d^{-1}$ and HRT of 0.35 d. The wastewater had a TOC content between 5.2 and 6 $kg.m^{-3}$. Similarly, COD reductions of 93% at an OLR of 11.05 $kgCOD.m^{-3}.d^{-1}$ and a HRT of 14 h were achieved treating a winery wastewater in an UASB (Ronquest & Britz, 1999). Ruiz *et al.* (2002), using an anaerobic sequencing batch reactor (ASBR), treated a winery wastewater. The reactor was operated at an OLR around 8.6 g $COD.l^{-1}.d^{-1}$ with a soluble COD removal efficiency greater than 98%, HRT of 2.2 d and a specific organic loading rate of 0.96 g $COD.gVSS^{-1}.d^{-1}$. The influence of a high strength distillery effluent on the anaerobic digestion process was investigated using a mesophilic lab-scale UASB reactor (O'Kennedy, 2000). It was found that at an OLR of 30 $kgCOD.m^{-3}.d^{-1}$, the pH, alkalinity and biogas production stabilized to values of 7.8, 6 000 $mg.L^{-1}$ and 18.5 $L.d^{-1}$, respectively. An average COD removal > 90% was achieved indicating excellent bioreactor stability (O'Kennedy, 2000).

Anaerobic digestion has been applied to the treatment of cannery effluents for some time. Hills (1982) suggested the use of anaerobic digestion to treat peach and tomato cannery effluents. COD removals of 70 - 88% were achieved at organic loading rates of 1 - 2 kg of volatile solids per digester per day. The gas produced had a methane content of 60%, and a CO_2 content of 40%. Total solids were reduced by 71 - 86%. Trnovec & Britz (1998) applied anaerobic digestion to the treatment of fruit canning effluents. The mesophylic laboratory-scale UASB reactor achieved COD removals of 90 - 93% at an OLR of 10.95 kgCOD.m^{-3}.d^{-1}, at a HRT of 10 h.

Austermann-Haun *et al.* (1994) employed an UASB reactor for treating fruit juice factory effluent. COD removal efficiency of 80 - 90% was achieved with a space loading rate of 3.6 kgCOD.m^{-3}.d^{-1}, a COD influent of 2 337 mg.L^{-1}. The two main problems encountered were washout of granules and decreasing sludge activity. Similar results were obtained in a later study (Austermann-Haun *et al.*, 1997) with COD removal efficiencies still between 82 - 88% at a volumetric loading rate of about 3.5 kgCOD.m^{-3}.d^{-1}. Sam-Soon *et al.* (1986) achieved COD removal efficiencies ranging from 49 - 98% for hydraulic retention times ranging from 3.9 – 20.0 h, respectively. These results were obtained using apple processing wastewater with an influent COD level of 2 500 mg.L^{-1} and an OLR of 7.5 - 12.5 kgCOD.m^{-3}.d^{-1}. More recently, Britz *et al.* (2000) used an UASB reactor to treat a fruit cannery effluent devoid of lye. Organic loading rates of 9.2 kgCOD.m^{-3}.d^{-1} were achieved at HRT of 10 h, with an average COD removal of 81 – 84%. Influent COD levels ranged between 3 800 and 4 300 mg.L^{-1}, and influent pH levels of 5.0 could be successfully treated in the system.

Anaerobic digestion in conjunction with ultrafiltration (ADUF) has been employed to treat fruit processing waste in Ceres, South Africa (Strohwald, 1993). COD reduction of more than 95% was obtained, with final treated effluent COD values as low as 50 mg.L^{-1} at loading rates of not more than 1.5 kgCOD.m^{-3}.d^{-1}. Operation at higher loading rates resulted in the deterioration of the COD reduction potential with a sharp increase in COD levels of the ultrafiltration permeate and volatile acid/alkalinity ratio, both of which are indicative of imminent digester failure. Operation at loading rates of up to 3 kgCOD.m^{-3}.d^{-1} were possible, albeit at lower COD reduction rates of 70 - 85%.

D. DISCUSSION

South Africa being a semi-arid country, with below average annual rainfall, unevenly distributed, faces serious water shortages in the near future if current water consumption levels persist (Odendaal, 1989; Anon, 1997a; Anon, 1997b). The serious nature of this problem has already resulted in tariff increases for water supplied from governmental water schemes (Department of Water Affairs and Forestry, 1997). This leaves the food and beverage industry facing large capital outlays to purchase the large volumes of water required for their effective functioning. To counter these rising costs, the industry has two options: either to minimise the water usage or implement water re-use (Trnovec & Britz, 1998). Another cost saving factor that must be considered is that of waste treatment, as surcharges imposed by municipalities to treat effluent are also likely to increase.

On-site waste treatment of effluents poses several advantages to industries. It offers prospects of water re-use, lower capital outlay for water, lower surcharges for waste disposal, recovery of processing chemicals and generation of useful substances.

As discussed, there are several treatment options available to choose from, each with its own pros and cons. Anaerobic digestion in combination with advanced oxidation technologies appears to offer the most pollution control benefits. COD levels can be significantly reduced, no large land areas are required, no unpleasant odours occur, little biomass is formed, methane gas is produced and can be utilized, process chemicals can be recovered and clean water can be recycled. This contributes to the global effort to conserve water as the valuable resource that it is.

Research into anaerobic digestion in combination with other treatment techniques, especially advanced oxidation technologies holds tremendous potential in providing wastewater treatment solutions for the food and beverage processing industry. The need, however, exists to specifically tailor these technologies to the variety of unique and specific food and beverage processing wastewaters.

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CHAPTER 3

UASB TREATMENT OF A HIGHLY ALKALINE FRUIT CANNERY LYE-PEELING WASTEWATER

Summary

Anaerobic treatment of a highly alkaline fruit cannery lye-peeling wastewater was investigated, using an upflow anaerobic sludge blanket (UASB) reactor. Only a short initialization period was required before COD reduction and OLR had stabilized at 85 – 90% and 2.40 kgCOD.m^{-3}.d^{-1}, respectively. With subsequent increases in OLR to 8.1 kgCOD.m^{-3}.d^{-1}, the COD reduction remained between 85 and 93% and biogas production peaked at 4.1 L.d^{-1} (63% methane). After 111 days, COD reduction and reactor pH started to decrease. Decreases in gas production were observed by day 102, decreasing to 2.48 L.d^{-1} by day 111 and 0.93 L.d^{-1} after 129 days. Subsequent reductions in the OLR, by reducing influent COD, had no effect on reactor stability. Loss of reactor performance was ascribed to the accumulation of sodium (potentially > 20 000 mg.L^{-1}) within the reactor biomass, leading to inhibition of methanogenesis.

Introduction

Food processing industries in South Africa are under increasing pressure to reduce the impact that their wastewater streams have on the environment. With the promulgation of the National Environmental Management Act, Act 107 of 1998 (Republic of South Africa, 1998a) and the National Water Act, Act 36 of 1998 (Republic of South Africa, 1998b) one of the principles that has been adopted is that the polluter pays for the abatement of his pollution. The production of large volumes of untreated wastewater can thus become a very serious financial burden. It is the basic requirement of the legislation on pollution control that wastewater purification be an integral part of industrial processes and that the producer of the wastewater should provide staff, capital and plant to purify the produced wastewater to prescribed standards (Republic of South Africa, 1998a & b; Gray, 1999).

Due to the nature of the fruit canning process, large volumes of water are used in the transportation of the product, washing and rinsing, blanching, retorting and cooling operations (Wayman, 1996). Disposal of cannery wastewaters is often complicated by the seasonal nature of the industry, the presence of suspended solids and particulate organics (Harada *et al.*, 1994), cleaning solutions (most commonly sodium hydroxide), often in formulations with various chelating, softening or surface-active additives, nitric and phosphoric acids (Mawson, 1997) and sodium hydroxide (used during peeling of certain fruits and vegetables) in the wastewater. Treatment of these wastewaters was normally achieved by either irrigation, lagooning or treatment in an activated sludge process (Monson, 1959; Hanks *et al.*, 1968; Gambrell & Peele, 1973; Stalzer, 1976) and irrigation of lye wastewaters was still practiced in South Africa as recently as 2000. Currently, most South African fruit canneries recycle their lye solutions as far as possible and mix the rest with other cannery wastewaters.

Anaerobic digestion of food processing wastewaters has been successfully implemented (Austermann-Haun *et al.*, 1999; Frankin, 2001). The use of UASB technology to treat seasonal fruit cannery wastewaters has recently been shown to be a feasible option, with COD reductions of up to 93% at organic loading rates (OLR) of 10.95 $kgCOD.m^{-3}.d^{-1}$ and hydraulic retention times (HRT) of <12 h (Trnovec & Britz, 1998). The wastewater originating from the lye-peeling operation, however, requires special attention. This highly alkaline wastewater has COD levels of 45 000 – 50 000 $mg.L^{-1}$ and a pH between 12.5 and 13.8, impacting significantly on the overall cannery wastewater.

The aim of this study was to assess the applicability and effectiveness of the UASB process to treat a fruit cannery wastewater. The wastewater used in the study was a highly alkaline lye-peeling fruit wastewater obtained from a local fruit cannery.

Material and methods

Digester design

A laboratory-scale upflow anaerobic sludge blanket (UASB) reactor was used. The digester had an operational volume of 2.3 L (total height of 830 mm and internal diameter of 50 mm) and combined an UASB design with an open gas/solids separator at the top of the reactor (Fig. 1). The biogas exited through the top, while the substrate was introduced into the reactor at the base. The overflow of the reactor emptied through a U-shaped tube to prevent any atmospheric oxygen from entering the system. The re-circulation upflow velocity for the reactor was set at 2 $m.h^{-1}$. The temperature of the insulated reactor was maintained at 35°C using heating

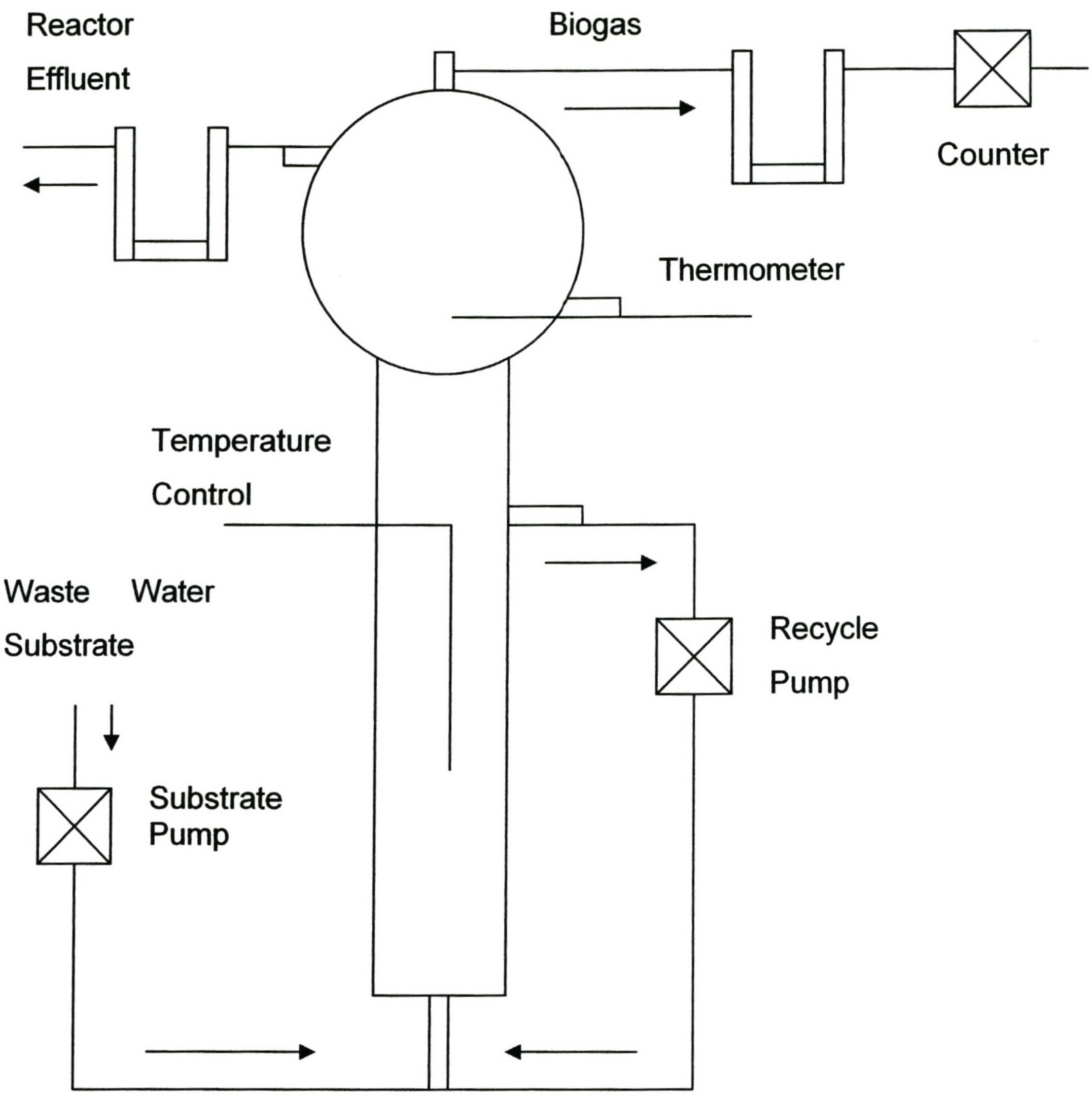

Figure 1. Diagram of the 2.3 L laboratory-scale UASB reactor.

tape and an electronic control unit (Meyer *et al.*, 1985). The volume of the biogas was determined using a manometric unit equipped with an electronically controlled counter and a gas-tight valve and the volumes corrected to standard temperature and pressure. The substrate was fed semi-continuously to the reactor by means of a peristaltic pump (Watson-Marlow 101) controlled by an electronic timer.

Reactor start-up

The reactor was seeded with 500 g of water drained anaerobic granules from another anaerobic digester giving a settled sludge-bed height of approximately 300 mm. The reactor was then allowed to stabilize for 48 h in order to allow the bacterial community to acclimatize and fed with a diluted synthetic substrate and the HRT set at 24 h. After three weeks this was replaced with a high alkaline lye-peeling wastewater from a fruit-canning factory with an average COD of 2 500 mg.L^{-1} (2 030 – 2 630 mg.L^{-1}). Increase in influent COD concentration and subsequent increases in the OLR, were made only once the system had achieved a 85 – 90% COD reduction efficiency. This was considered as a "stable state", during which the variation in reactor performance parameters was less than 10%.

Wastewater and substrate

The full strength high alkaline wastewater had an initial COD of 45 000 – 50 000 mg.L^{-1} and a pH between 12.5 and 13.5. The sodium concentration of the wastewater was *ca*. 40 000 mg.L^{-1}. The full strength wastewater was allowed to undergo natural fermentation until the pH was approximately 7.5. The wastewater was then diluted to 2 500 mg.L^{-1} and the pH poised at 8.5 and used as substrate. The sodium concentration of this diluted substrate was approximately 2 000 mg.L^{-1}.

The composition of the synthetic substrate (in mg.L^{-1}) was: glucose 1 250; sodium lactate 5 000; urea 500; and KH_2PO_4 500. The substrate was also supplemented with 1.0 ml trace element solution (Nel *et al.*, 1985) and the pH poised at 8.5 with calcium hydroxide for maximum granule growth. The substrate was then diluted to the required COD concentration of 2 500 mg.L^{-1}.

Analytical methods

The following parameters were monitored, in duplicate, according to the APHA (*Standard Methods*, 1998): pH; alkalinity; and total solids (TS). COD and orthophosphate phosphorous were determined colorimetrically using a DR2000 spectrophotometer (Hach Co., Loveland, CO) and standardized procedures (*Standard Methods*, 1998). Sodium ion concentration was determined, in duplicate, using a Varian MPX Inductively Coupled Plasma (ICP) Spectrometer at a wavelength of 589 - 592 nm. The emissions were compared to those of standardized sodium solutions.

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

Results and discussion

The UASB reactor efficiency parameters are shown graphically in Figs 2 and 3. The operation of the UASB reactor has been divided into time periods of reactor start-up (not shown), substrate acclimatisation, reactor stabilization, increase in reactor loading and optimum performance:

Reactor start-up

During the start-up period (data not shown) the influent COD of the synthetic substrate was kept at 2 500 $mg.L^{-1}$ (± 150 $mg.L^{-1}$) and the COD reduction increased to 93%, averaging 90% during the third week. The reactor effluent pH varied between 6.84 and 7.48, the average OLR during the three week start-up period was 2.45 kg $COD.m^{-3}.d^{-1}$. The reactor effluent alkalinity remained constant at 1 000 $mg.L^{-1}$ (± 110 $mg.L^{-1}$). After three weeks the synthetic substrate was replaced with a high alkaline lye-peeling wastewater.

Substrate acclimatisation

The average COD load of the high alkaline lye-peeling wastewater was 2 200 $mg.L^{-1}$. Initially the COD reduction decreased to 77% on day 5 before stabilizing and increasing to between 85 and 90% after 14 d. The reactor effluent alkalinity averaged 1 186 $mg.L^{-1}$ (± 447 $mg.L^{-1}$ as $CaCO_3$) (Fig. 2). The reactor pH varied between 7.18 and 7.27, the average OLR during this period was 2.40 kg $COD.m^{-3}.d^{-1}$ (Fig. 3).

Reactor stabilization

During the period day 14 to 35, the influent COD load was kept constant at 2 200 mg.L^{-1} (± 360 mg.L^{-1}) and the COD reduction was found to vary between 85 and 96% (Fig. 2). The reactor effluent pH varied between 6.80 and 7.23, the average OLR for this period was 2.20 kgCOD.m^{-3}.d^{-1} and biogas production remained relatively constant at 1.4 L.d^{-1} (Fig. 3).

Increase in reactor loading

The influent COD was gradually increased, during days 36 – 85, to a maximum of 8 200 mg.L^{-1}, with the OLR reaching 8.10 kgCOD.m^{-3}.d^{-1} on day 85. The COD reduction remained at an average of 90% (83.5 – 93.2%)(Fig. 2) The biogas production was also found to increase from 1.4 to 4.0 L.d^{-1} by day 85. During this period the reactor effluent pH varied between 6.70 and 7.10 (Fig. 3).

Optimum performance

From day 85 to 120 the influent COD was kept constant at 8 300 mg.L^{-1} (8 120 – 8 456 mg.L^{-1}). A COD reduction of between 82 and 90% was achieved (Fig. 2) and the effluent pH was found to vary from 6.78 to 6.98 (Fig. 3) until day 111. After day 111 it was found that the COD reduction and pH decreased to 76% and 6.49, respectively. Methanogens are known to be inhibited at pH levels below 6.5 (Lin & Yang, 1991; Droste, 1997; Bitton, 1999). On day 121 the influent COD concentration was lowered to 6 000 mg.L^{-1} in an attempt to stabilize the reactor system pH and COD reduction by lowering the fermentable carbon source. The COD reduction, however, continued to decrease and two subsequent decreases in influent COD to 4 000 mg.L^{-1} on day 129 and to 3 500 mg.L^{-1} on day 135, were also unsuccessful in stabilizing the COD reduction efficiency or pH, which continued to drop after day 120 to day 143 indicating that acidification of the reactor had taken place. The COD reduction decreased from 76% on day 120 to below 50% on day 129 and to 22% after 143 days. In this same period the reactor effluent pH decreased from 6.49 on day 120 to 5.95 on day 129 and 4.89 after 143 days.

The gas production after day 85 remained relatively constant at *ca*. 4.1 L.d^{-1} (63% methane), until day 102, where after the gas production decreased to 2.48 L.d^{-1} (48% methane) by day 111 and to 0.93 L.d^{-1} (22% methane) by day 129. The data shows that the gas production started to decrease (day 102) before any other negative tendencies were observed in either COD reduction or reactor effluent pH.

This dramatic decrease in the methanisation rate could possibly be as a result of the high levels of sodium (influent Na^+ concentration of *ca.* 7 300 $mg.L^{-1}$) accumulating in the reactor. It has been reported that sodium, at low concentrations, is essential for methanogens, probably because of the role in the formation of adenosine triphosphate, the oxidation of NADH (Feijoo *et al.*, 1995) or in the chemiosmotic coupling mechanism (Rinzema *et al.*, 1988). It has also been shown that the presence of high sodium concentrations (10 000 – 25 000 $mg.L^{-1}$) is inhibitory to anaerobic wastewater treatment in that methanogenesis is inhibited to varying degrees (Feijoo *et al.*, 1995).

In this study the sodium concentration of the initial diluted lye-peeling substrate was *ca.* 2 000 $mg.L^{-1}$ (± 200 $mg.L^{-1}$). When the influent COD concentration was at a maximum of 8 300 $mg.L^{-1}$ (days 85 to 120), the sodium concentration of the substrate was *ca.* 7 300 $mg.L^{-1}$. During this period the sodium concentration of the reactor effluent varied between 6 800 and 7 200 $mg.L^{-1}$. The difference in reactor influent and effluent Na^+ concentrations would suggest an accumulation of sodium within the reactors biomass. Based on the rapid decrease in CH_4 production, it is a possibility that accumulation of sodium within the system led to the inhibition of the methanogenic population and thus the subsequent acidification of the entire system with the accumulation of volatile fatty acids.

The above data are in agreement with reports by De Baere *et al.* (1984) who investigated the effect of NaCl shock treatments on a flow-through anaerobic digester. They reported that initial inhibition and a 50% inhibition were observed at 30 000 and 35 000 $mg.L^{-1}$ sodium, respectively. According to these authors adaptation to increasing amounts of NaCl significantly affected the tolerance of the methanogenic association as compared to the shock treatment. With adaptation, an initial inhibition was observed at 65 000 $mg.L^{-1}$ and a 50% inhibition at 95 000 $mg.L^{-1}$ of NaCl. Feijoo *et al.* (1995) reported that sodium concentrations ranging from 3 000 to 16 000 $mg.L^{-1}$ (in absence of nutrients or other salts) caused a 50% inhibition in the methanisation of volatile fatty acids. Rinzema *et al.* (1988) found that the maximum specific acetoclastic activity of granular sludge was hardly affected at sodium concentrations below 5 000 $mg.L^{-1}$ (10% inhibition), whereas the activity was practically reduced to zero at 14 000 $mg.L^{-1}$ Na^+. In UASB systems, the results were slightly more favourable, with 10% and 100% inhibition at 5 500 and 15 000 $mg.L^{-1}$ Na^+, respectively (Rinzema *et al.*, 1988). Kim *et al.* (2000) also found that methane

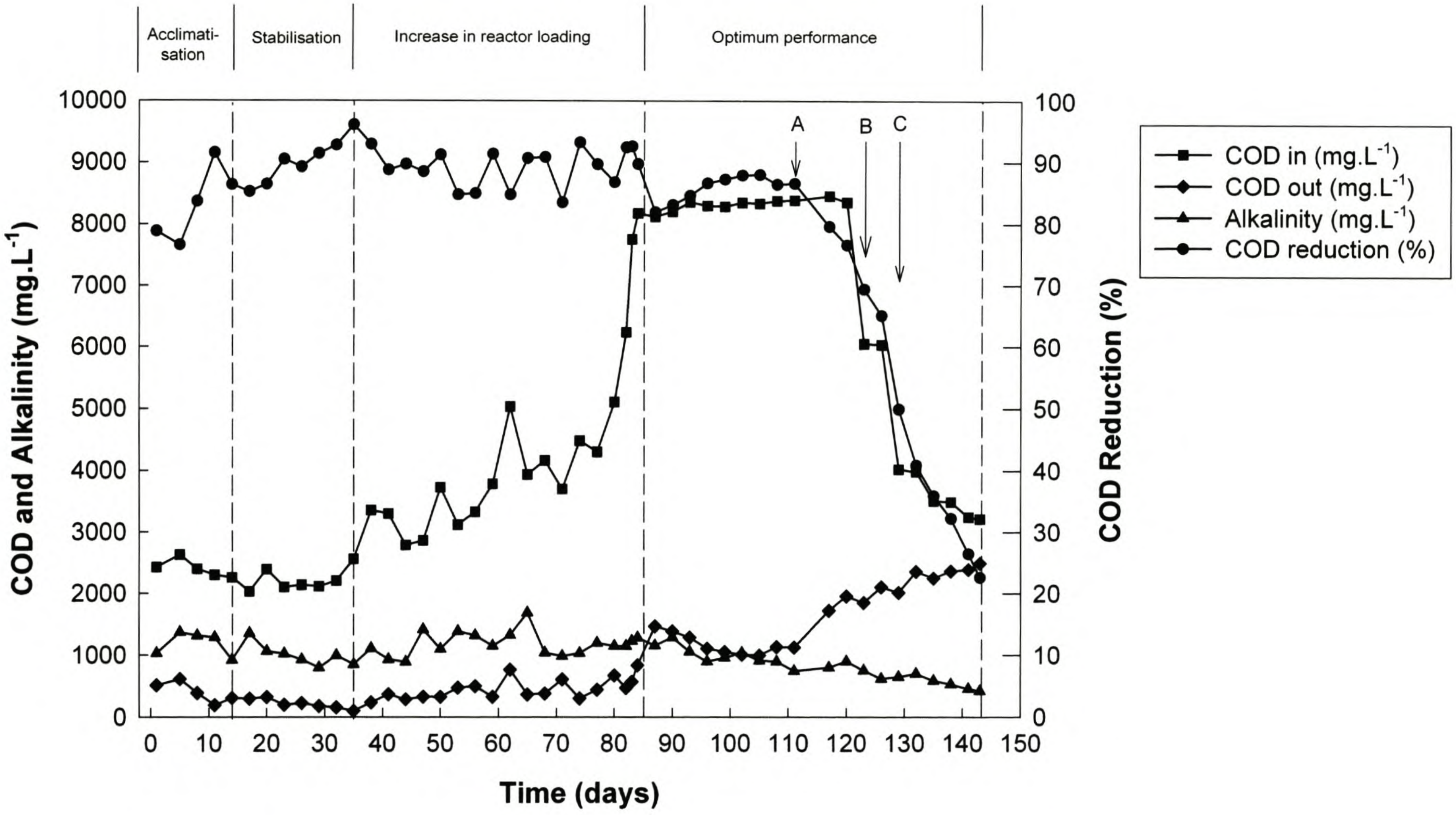

Figure 2. COD reduction efficiency parameters of the UASB reactor treating a high alkaline fruit cannery lye-peeling wastewater (A – COD reduction decreases after day 111; B – Influent COD lowered to 6 000 mg.L^{-1}; C – Influent COD lowered to 4 000 mg.L^{-1}). Alkalinity (mg.L^{-1}) = reactor effluent alkalinity as $CaCO_3$ (mg.L^{-1}).

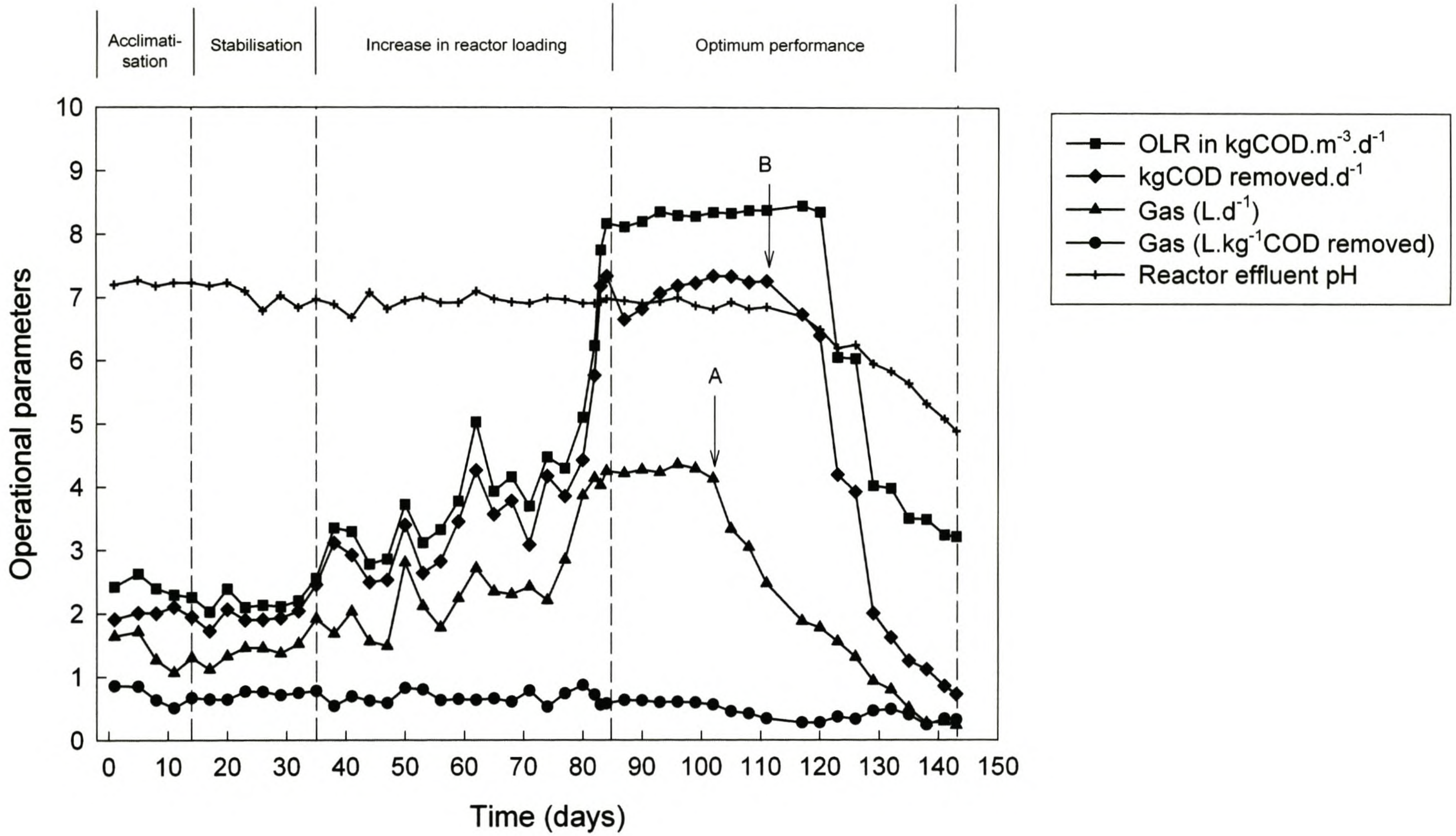

Figure 3. Operational parameters of the UASB reactor treating a high alkaline fruit cannery lye-peeling wastewater (A – Gas production decreased on day 102; B – COD reduction and pH decreased after day 111).

production decreased with increases in sodium concentrations above 5 000 mg.L^{-1} and that at 20 000 mg.L^{-1} a 50% reduction in theoretical gas volume was observed. In this study, it was clear from the concentration of sodium in the reactor effluent that the level of accumulated sodium in the reactor could readily have reached levels of > 20 000 mg.L^{-1}.

Conclusions

In this study, digestion of the organic fraction present in fruit cannery lye-peeling wastewaters was again shown to be a feasible treatment option, with COD reductions of up to 95% being obtained. Furthermore, it appears as if the microbial consortium involved in the anaerobic digestion process can acclimatize to higher sodium ion levels present in the wastewater. This is in agreement with observations in the literature. Accumulation, however, of sodium ions within the reactor biomass to levels above 15 000 mg.L^{-1} can also become detrimental to the overall stability of the reactor, inhibiting the anaerobic digestion process, leading to decreased COD reduction (decreased from 90% to 22%), gas production (decreased from 4.1 to 0.93 L.d^{-1}) and reactor acidification (pH dropped from 6.98 to 4.89). It appears that the methanogens are more sensitive to higher sodium levels, as the biogas and methane percentage were the first parameters to change. Decreases in methane production could thus be an early warning system of sodium accumulation or toxicity and should thus be closely monitored in systems treating wastewaters containing high sodium concentrations. In the case of fruit cannery wastewaters, it would also be advisable to integrate the highly alkaline lye-peeling wastewater with other wastewaters generated in the canning process, in order to dilute the sodium ion concentration, so as to lower the risks of negatively affecting the efficiency of anaerobic digestion processes. From the data obtained in this study it was also considered important to examine the use of additional chemical processes to facilitate the decrease of the organic load of the fruit cannery wastewater after the UASB treatment, so as to try and reach the 75 mg.L^{-1} "general" legal limit for discharge to a natural water system.

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CHAPTER 4

USE OF OZONE AND HYDROGEN PEROXIDE IN THE POST-TREATMENT OF UASB TREATED ALKALINE FRUIT CANNERY WASTEWATER

A condensed version of this chapter has been published in *Water Science and Technology*, **44**(5), 69-74, 2001.

Summary

Upflow anaerobic sludge blanket (UASB) treatment of cannery wastewaters was shown to be feasible. However, the treated reactor effluent still does not allow direct discharge to a water system and a further form of post-treatment is necessary to reduce the chemical oxygen demand (COD) to below the "general" legal limit of 75 mg.L^{-1}. Ozone, hydrogen peroxide (H_2O_2) and granular activated carbon (GAC) were used singly or in combination to assess the effectiveness as post-treatment options for the UASB treated alkaline fruit cannery wastewater. Colour reduction in the reactor effluent ranged from 15 to 92% and COD reductions of 26 - 91% were achieved. Combinations of ozone and H_2O_2 gave better results than either oxidant singly. The best results were achieved by combining ozone, H_2O_2 and granular activated carbon and COD levels were reduced to levels sufficiently below the 75 mg.L^{-1} "general" limit.

Introduction

South Africa is a semi-arid country with an average annual rainfall well below the world average. Emphasis is now being placed on more responsible water usage and the need to reclaim and re-use water, especially in the food industry and agricultural sector where large wastewater volumes are generated (Pybus, 1999). Due to the nature of the fruit and vegetable canning process, large volumes of water are used in product transportation, washing and rinsing, blanching, retorting and cooling operations (Water Research Commission, 1987a; Wayman, 1996). This industry is thus faced with firstly, maintaining a profitable level of production while reducing the intake of fresh, potable water, and secondly, disposing of the large volumes of wastewater in an environmentally responsible manner.

Many methods of treating these wastewaters have been investigated and high-rate anaerobic designs have been developed and successfully applied (Lettinga *et al.*, 1997). The use of the UASB design to treat the seasonal fruit cannery wastewaters has recently been shown to be a feasible option, with COD reductions of up to 93% at organic loading rates (OLR) of 10.95 $kgCOD.m^{-3}.d^{-1}$ and hydraulic retention times (HRT) of <12 h (Trnovec & Britz, 1998). However, the COD level of the final reactor effluent was still well above the South African legal limit permitted for wastewaters (75 $mg.L^{-1}$) to be discharged to a water system (Water Research Commission, 1987b) and a further post-treatment is thus necessary.

The use of advanced oxidation processes (AOP's), including ozone, H_2O_2, ultra-violet (UV) and certain metal catalysts (Benitez *et al.*, 1996) to treat wastewaters has increased (Hunt & Mariñas, 1997; Camel & Bermond, 1998). These involve the generation of the hydroxyl radical that is a reactive intermediate and has a higher oxidation potential (2.80 eV) than either ozone (2.07 eV) or H_2O_2 (1.77 eV). The hydroxyl radical attacks organic molecules by abstracting a hydrogen atom or by adding to the double bonds. Organic molecules are then mineralized to non-toxic forms such as carbon dioxide or water (Mourand *et al.*, 1995; Gulyas *et al.*, 1995; Beltrán *et al.*, 1999). Ozone also has strong reactorcidal characteristics resulting from its high oxidation potential and ability to diffuse through reactorlogical membranes (Hunt & Mariñas, 1997). Hydrogen peroxide, in contrast, is less reactive than ozone (Hughes, 1992), thus a combination of ozone and H_2O_2 is often used. This results in a more efficient generation of hydroxyl radicals, and improves the rate of organic/inorganic degradation (Beltrán *et al.*, 1997; Mokrini *et al.*, 1997). It has been shown that the efficiency of colour reduction by ozone is accelerated by H_2O_2 (Filipovíc-Kovacevic & Sipos, 1995). Some organic compounds react more slowly with ozone and in some cases are only partially oxidized, leading to low molecular weight compounds which would be better adsorbed onto activated carbon and organic solutes are sometimes more susceptible to subsequent reactorlogical oxidation (Gulyas *et al.*, 1995).

The use of GAC (Camel & Bermond, 1998) has also been used in the treatment of drinking water and wastewater. The reasons being that GAC offers a large surface area (500 – 600 $m^2.g^{-1}$) for adsorption and is effective in removing taste and odour compounds, colour, organics, excess chlorine, pesticides and toxic and mutagenic substances (Bitton, 1994).

Ozone, H_2O_2 and/or UV have been successfully applied to lower the COD and polyphenol contents of table olive debittering wastewaters (Beltrán *et al.*, 1999), pesticides (Mokrini *et al.*, 1997), decolour yeast-production industry wastewaters (Filipovíc-Kovacevic & Sipos, 1995), and to degrade and treat distillery, poultry and tomato processing wastewaters (Chang & Sheldon, 1989; Beltrán *et al.*, 1997). Ozone, H_2O_2 and GAC are used extensively in drinking water treatment throughout France (Camel & Bermond, 1998).

The aim of this study was to investigate the possibilities of using oxidation techniques such as ozonation, H_2O_2 and granular activated carbon in single or combined post-treatment steps to further treat UASB treated alkaline cannery wastewater.

Materials and methods

High alkaline cannery wastewater

The high alkaline wastewater was collected from three fruit canneries (Ashton Canning Co Pty Ltd, Langeberg Food Processors Ltd and Rhodes Fruit Farms), specifically at the lye-peeling areas. The COD varied from 12 000 to 45 000 $mg.L^{-1}$ and the pH ranged from 9.8 to 13.5. The various wastewaters were combined, resulting in a wastewater with an average COD of 28 000 $mg.L^{-1}$ and a pH of 12.2. After mixing, the wastewater was left at room temperature for 72 h to facilitate a natural fermentation to lower the pH to *ca*. 8.5. The wastewater was then diluted to a COD of 4 500 $mg.L^{-1}$ and the pH adjusted to 8.5 with 1 M KOH before using as substrate for the anaerobic treatment.

Anaerobic treatment

A 2.3 L laboratory-scale UASB reactor was used (Trnovec & Britz, 1998) and operated at 35°C (Meyer *et al.*, 1985). The volume of the reactorgas was determined using a manometric unit equipped with an electronically controlled counter and a gas-tight valve and the volumes corrected to standard temperature and pressure. The substrate was fed semi-continuously to the reactor by means of a peristaltic pump (Watson-Marlow 302S) controlled by an electronic timer. The reactor was run at a hydraulic retention time (HRT) of 24 h and re-circulation at 2 $m.h^{-1}$ while the substrate

COD was kept constant at 4 500 mg.L^{-1} and the pH adjusted to 8.5. The pH, COD, alkalinity, orthophosphate phosphorous, total solids of the reactor effluent were monitored (Standard Methods, 1998).

Oxidation

Ozonation was carried out in a batch unit connected to an ozone generator (Oz Purification, Ifafi) producing 2.4 g.h^{-1} ozone at a flowrate of 10 L.h^{-1}. The batch type apparatus consisted of a tubular diffusion membrane placed inside a 2 L reaction vessel. The membrane was connected to the O_3 generator via silicone tubing. Ozonation was carried out in the reaction vessel containing 1 800 mL of wastewater. Treatment with H_2O_2 was also carried out in the 2 L reaction vessel. Hydrogen peroxide was added to the wastewater at a concentration of 1, 3 and 5% (v/v) and allowed to react for 24 h. The effect of ozonation, after the 24 h H_2O_2 reaction period and immediately after the addition of H_2O_2 was also investigated. A GAC filter column was also used to facilitate in the COD reduction of the reactor effluent. Filtration took place immediately after the respective treatments with O_3 and H_2O_2 had been completed. The GAC filter column consisted of a glass column (l = 75 cm; ϕ = 6.5 cm) fitted with a fritted disk at one end. The column was filled with 1 kg of GAC to a height of 45.5 cm. The COD of the effluent was determined after each treatment. The different treatment combinations studied are summarised in Table 1.

Analytical methods

The ozone production was measured using an iodiometric titration (Standard Methods, 1998). COD was determined colorimetrically using a DR2000 spectrophotometer (Hach Co. Loveland, CO) and standardised procedures (Standard Methods, 1998). Colour reduction in the effluent was determined by monitoring the absorbance of the effluent at 288 nm using a Spectronic spectrophotometer (Spectronic Instruments, USA).

Results and discussion

Anaerobic digestion

During anaerobic treatment of the alkaline cannery wastewater the COD was reduced by 90 - 93%. The reactor effluent had a pH of 7.0 - 7.2 and a COD

Table 1. Summary of the different treatment conditions studied in the post-treatment of alkaline UASB fruit cannery effluent.

	Treatment Description
1	Reactor effluent + 1% H_2O_2 (v/v) + 24 h reaction period
2	Reactor effluent + 3% H_2O_2 (v/v) + 24 h reaction period
3	Reactor effluent + 5% H_2O_2 (v/v) + 24 h reaction period
4	Reactor effluent + 5 min ozonation
5	Reactor effluent + 10 min ozonation
6	Reactor effluent + 15 min ozonation
7	Reactor effluent + 30 min ozonation
8	Reactor effluent + 60 min ozonation
9	Reactor effluent + 1% H_2O_2 (v/v) + 24 h reaction period + 5 min ozonation
10	Reactor effluent + 1% H_2O_2 (v/v) + 5 min ozonation immediately
11	Reactor effluent + 1% H_2O_2 (v/v) + 5 min ozonation immediately + GAC filter
12	Reactor effluent + GAC filter
13	Reactor effluent + 1% H_2O_2 (v/v) + 15 min ozonation immediately
14	Reactor effluent + 1% H_2O_2 (v/v) + 15 min ozonation immediately + GAC filter
15	Reactor effluent + 5 min ozonation + GAC filter
16	Reactor effluent + 60 min ozonation + GAC filter

concentration of 315 – 450 mg.L^{-1} This reactor effluent was used in the oxidation studies.

Hydrogen peroxide treatment

The addition of H_2O_2 to the reactor effluent resulted in significant colour reduction over the 24 h reaction period, as can be seen from Table 2. The absorbance was decreased by 92% with the 5% H_2O_2 (v/v) treatment. Van der Walt (1993) found that the residual colour decreased by up to 60% when treating drinking water with 20 mg.L^{-1} H_2O_2 (pH = 4), but the extent of colour reduction decreased with increasing pH, to 50% at pH = 10. A COD reduction of 26% was achieved in the case of the 1% H_2O_2 (v/v) treatment of the UASB treated alkaline fruit cannery wastewater (Fig. 1). Beltrán *et al.* (1997; 1999) found that H_2O_2 alone was not an effective oxidizing agent in the treatment of tomato processing wastewaters and distillery wastewaters. The levels of residual H_2O_2 present after the 3 and 5% (v/v) H_2O_2 treatments were, however, too high and interfered with the dichromate COD determination. Hydrogen peroxide acts as a reductor against the dichromate in the COD reagents and increases the value of the determination (Beltrán *et al.*, 1999). Due to the interference with the COD determination, only results from treatments with a 1% H_2O_2 (v/v) are reported.

Ozone treatment

Ozone was found to be effective in reducing the COD of the reactor effluent. Increases in the ozonation time led to increases of 25% after 5 min to 53% after 60 min (Fig. 1) in the COD reduction. This was to be expected, as the ozone dose had been increased, thus increasing the hydroxyl radical formation, which is known to enhance oxidation (Hughes, 1992). The increase in COD reduction with increased reaction time was also found when treating debittering table olive (Beltrán *et al.*, 1999), yeast-production industry wastewaters (Filipovíc-Kovacevic & Sipos, 1995), chlorinated aromatics (Schmitt & Hempel, 1993), tomato and distillery wastewaters (Beltrán *et al.*, 1997). It is also clear from the data in Fig. 1 that the increase in COD reduction over time is not linear (plotted data not shown). It is clear that initially the rate of COD degradation is rapid, as is apparent from the 25% decrease in COD after 5 min. Two further 5 min ozonations only increased the COD reduction to 36 and 43%, respectively. After 30 min ozonation, a 50% reduction was achieved and after 60 min the reduction value was only increased to 53%. This was probably due to the

Table 2. Effect of hydrogen peroxide and ozone on the colour of UASB treated alkaline fruit cannery wastewater.

Treatment	Absorbance (288 nm)	Colour reduction (%)
Reactor effluent	2.716	
1% H_2O_2 (24h)	0.808	70
3% H_2O_2 (24h)	0.393	86
5% H_2O_2 (24h)	0.217	92
Reactor effluent	1.898	
5 min O_3	1.604	15
10 min O_3	1.380	27
15 min O_3	1.229	35
30 min O_3	0.903	52
60 min O_3	0.581	69

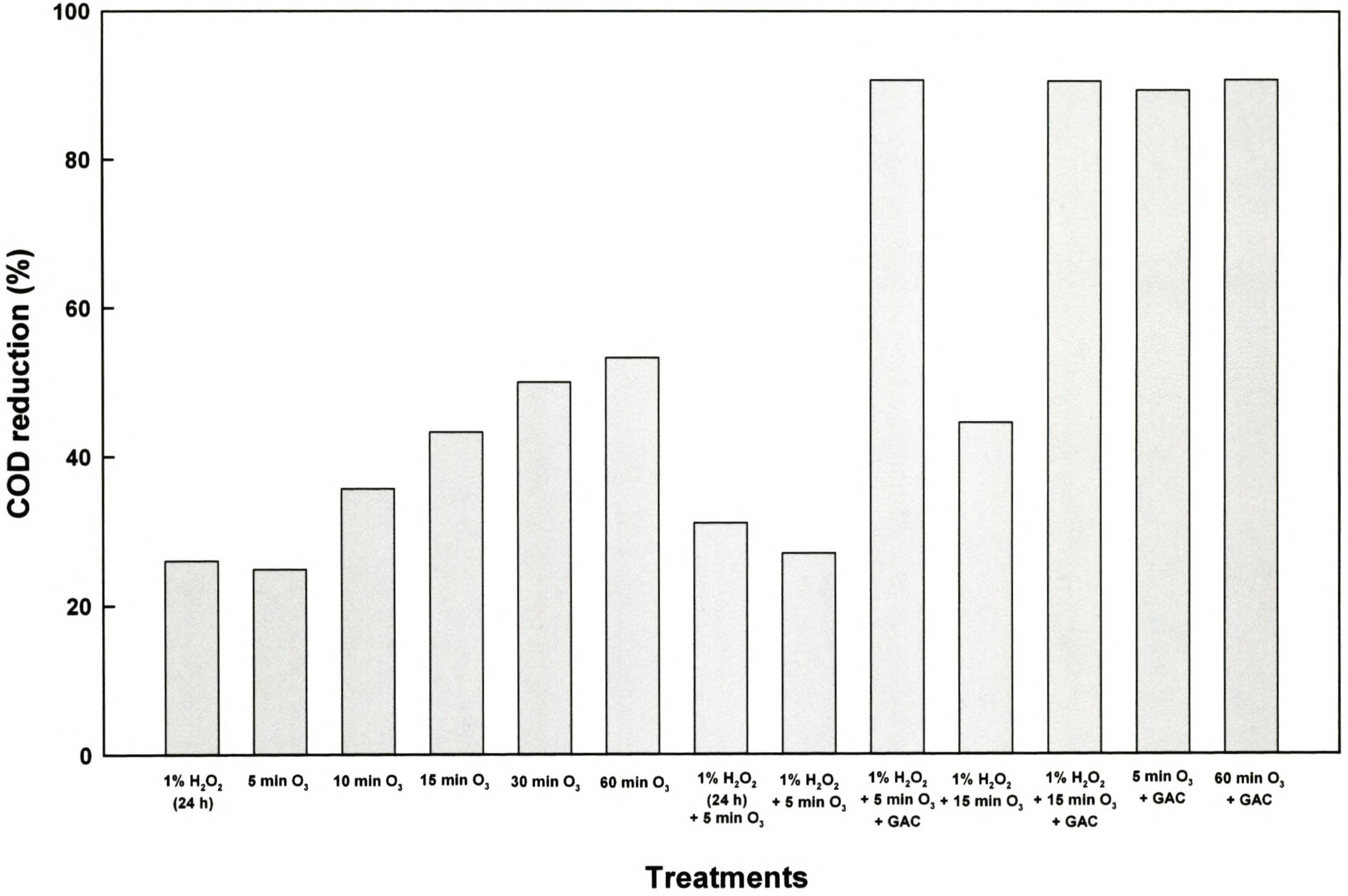

Figure 1. COD reduction during the different treatment conditions used in the post-UASB treatment of alkaline fruit cannery wastewater.

fact that some organic compounds are more susceptible to oxidation than others, while some are only partially oxidized (Camel & Bermond, 1998). This indicates that the UASB effluent contains a considerable amount of compounds that are difficult to oxidize and thus, require a stronger oxidizing system.

Ozone and hydrogen peroxide combined treatment

Ozonation (5 min) of the reactor effluent after a 24 h reaction period with a 1% H_2O_2 (v/v) solution added, resulted in a COD reduction of 31% (Fig. 1). The combination is thus slightly more effective than H_2O_2 on its own (26%). An ozonation treatment (5 min) immediately after the addition of H_2O_2 gave a COD reduction of 27% (Fig. 1). The immediate ozonation after H_2O_2 addition (27%) was slightly less effective than ozonation after a 24 h reaction period (31%), but still more effective than a 24 h reaction alone (26%) or 5 min ozonation (25%). A 15 min ozonation immediately after the addition of 1% H_2O_2 (v/v) (45%) also led to a slightly higher COD reduction than 15 min of ozonation alone (43%). These results are to be expected as O_3 and H_2O_2 in combination lead to the formation of more hydroxyl radicals than produced separately, and thus create a stronger oxidizing capability (Beltrán *et al.*, 1997; Hughes, 1992) with the longer reaction period proving more effective. Hydrogen peroxide on its own is expected to be less effective due to its lower oxidation potential in comparison to ozone and the hydroxyl radical (Hughes, 1992; Mourand *et al.*, 1995). The increase in COD reduction brought about by combining H_2O_2 with ozonation is, however, not very substantial. The fact that the H_2O_2/O_3 ratio is much higher than the optimum of 0.5, probably explains why only marginal increases in efficiency with H_2O_2 over O_3 alone were observed (Van der Walt, 1993; Beltrán *et al.*, 1999). Beltrán *et al.* (1997) found the combination of H_2O_2 and O_3 to work well in lowering the COD of tomato wastewaters, but not for distillery wastewaters, and ascribed the variation to differences in organic compounds present in the wastewater.

Granular activated carbon

Treated waters from four of the treatments (1% H_2O_2 (v/v) + 5 min O_3; 1% H_2O_2 (v/v) + 15 min O_3; 5 min and 60 min O_3) were filtered through the GAC. This filtration resulted in COD reductions between 89 and 91%. Filtering untreated effluent from the UASB through the GAC resulted in a COD reduction of 81% (data not shown). As can be seen in Fig. 1, an increase of between 8 and 10% in COD reduction was thus achieved by combining GAC filtration with the oxidation treatments. This is probably due to the partial oxidation of certain molecules during the ozone or O_3/ H_2O_2 treatment. The partial oxidation leads to the formation of low molecular weight compounds, which are better adsorbed on the GAC (Camel & Bermond, 1998). It appears, however, as though the maximum COD reduction attainable by combining GAC filtration with the oxidation treatments is approximately 90%. Granular activated carbon filtration increased the COD reduction of the 1% H_2O_2 (v/v) + 5 min O_3 treatment from 27 to 91% (Fig. 1), and the 60 min O_3 treatment was increased from 53%, also to 91%. The use of O_3 and H_2O_2 treatments before GAC filtration, thus seems to slightly improve the COD reduction compared to GAC filtration alone. The COD reduction of *ca.* 90% does, however, produce a final COD well within the "general" legal limit of 75 $mg.L^{-1}$.

Conclusions

Advanced oxidation processes and the use of GAC can be successfully used to lower the COD of a UASB treated alkaline cannery wastewater. Combinations of O_3 and H_2O_2 were only slightly more effective than either O_3 or H_2O_2 alone. GAC filtration was also shown to be effective in lowering the COD levels of the UASB treated effluent. Higher COD reductions were, however, achieved when the GAC filtration was preceded by an ozone or O_3/H_2O_2 treatment. O_3, H_2O_2 and GAC can thus be successfully applied to lower the COD of UASB treated alkaline fruit cannery wastewater. However, the data obtained in this study did show that post-ozonation could be successfully applied to reach lower final COD's, but it was also considered essential that the effect of combining pre-ozonation and post-ozonation to the UASB system, be evaluated for increased biodegradability, so as to optimise the treatment combination.

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CHAPTER 5

EFFICACY OF OZONE AS A PRE- AND POST-TREATMENT TO THE LABORATORY-SCALE ANAEROBIC DIGESTION OF FRUIT CANNERY AND WINERY WASTEWATERS

A condensed version of this Chapter has been published in *Water Science and Technology,* **45**(10), 329–334, 2002

Summary

The efficiency of ozone as a pre- and post-treatment to UASB treatment was investigated, followed by a study on UASB reactor performance while utilising an ozonated wastewater as substrate. Combinations of pre- and/or post-ozonation with UASB treatment gave better results than ozonation or UASB alone and colour reductions of 37 – 91% and COD reductions of 53 – 99% were achieved for the apricot canning and winery wastewater treatment. In a separate study an UASB reactor was fed with pre-ozonated peach cannery wastewater for over 70 d. COD reduction improved from between 59 and 64% to between 85 and 92% after pre-ozonated substrate feed was commenced. Subsequent increases in organic loading rate (OLR) from 2.4 to 3.4 kgCOD.m^{-3}.d^{-1} did not affect reactor performance. By including a final post-ozonation treatment to this UASB effluent a total COD reduction of 99% was achieved. The raw cannery wastewater COD of 6 500 mg.L^{-1} was reduced to 55 mg.L^{-1} by a treatment combination consisting of pre-ozonation, UASB treatment and a final post-ozonation.

Introduction

The fruit canning and winery industries are major water users and generate large volumes of polluted wastewater annually (Trnovec & Britz, 1998, Water Research Commission, 1993). Most of the water-intake is used for product transportation, washing, rinsing, blanching, retorting, cooling and floor and equipment washdowns (Wayman, 1996; Ronquest & Britz, 1999). These industries are faced with increasing pressure to reduce the impact their wastewaters have on the environment. New legislation in South Africa is forcing these industries to investigate on-site wastewater treatment facilities.

Anaerobic digestion is a technology that has been shown to be feasible for treating food processing wastewaters (Lettinga *et al.*, 1997). These wastewaters usually have a high organic content, have little or no toxic material present and thus, due to their nature and strength, provide the ideal conditions for anaerobic digestion (Kroyer, 1995). The use of the upflow anaerobic sludge blanket (UASB) design to treat seasonal fruit cannery and winery wastewaters has been shown to be a feasible option. Trnovec & Britz (1998) attained chemical oxygen demand (COD) reductions of up to 93% at organic loading rates (OLR) of 10.95 $kgCOD.m^{-1}.d^{-1}$ and at hydraulic retention times (HRT) of less than 12 h. Similarly, Ronquest & Britz (1999) reported COD reductions of 93% at an OLR of 11.05 $kgCOD.m^{-1}.d^{-1}$ and a HRT of 14 h while treating a winery wastewater. Anaerobic digestion has also been successfully used to treat alkaline cannery wastewaters. Reductions in COD of up to 93% were achieved at an OLR of 8.30 $kgCOD.m^{-1}.d^{-1}$ and a HRT of 24 h (Chapter 3 of this dissertation).

The use of ozone in the treatment of wastewaters has been shown to be effective in reducing COD, removing colour and making compounds more biodegradable (Gottschalk *et al.*, 2000). The success of ozone is dependant on the formation of the hydroxyl radical, a reactive intermediate, which has a higher oxidation potential (2.80 eV) than ozone (2.07 eV). These radicals attack organic molecules by abstracting a hydrogen atom or by adding to the double bonds. In these reactions, the organic molecules are mineralised to non-toxic forms such as carbon dioxide or water (Gulyas *et al.*, 1995; Mourand *et al.*, 1995; Beltrán *et al.*, 1999). Some organic compounds react more slowly with ozone and this sometimes results in organic solutes being formed which are more susceptible to subsequent biological oxidation (Gulyas *et al.*, 1995; Gottschalk *et al.*, 2000). The COD contents of various food processing wastewaters have been successfully treated using post-ozonation treatment methods (ozone, hydrogen peroxide, ultra-violet light or combinations), including fruit cannery, distillery, poultry, tomato processing wastewaters, yeast-production wastewaters and table olive debittering wastewaters (Chang & Sheldon, 1989; Filipović-Kovačević & Sipos, 1995; Beltrán *et al.*, 1997; Beltrán *et al.*, 1999; Beltrán *et al.*, 2001a and b). However, very little research on the use of ozone as a pre-treatment to anaerobic digestion can be found in the literature. Benitez *et al.* (1999) used ozone in combination with hydrogen peroxide (H_2O_2) and ultra-violet (UV)-light to treat winery waste before anaerobic digestion and achieved COD reductions of between 20 and 30%. During anaerobic digestion of these pre-treated wastewaters, an increase in the methane yield was observed. Martín *et al.* (2002) found that anaerobic digestion of vinasse pre-treated with ozone plus ultraviolet light in the presence of titanium dioxide decreased the COD and total organic carbon (TOC), and increased the yield coefficient and the mean specific digestion rate by 25%. Weemaes *et al.* (2000) reported that the anaerobic digestion of ozonized wastewater sludge enhanced methane production and accelerated the digestion rate. The treatment of olive oil mill effluents (OME) with ozone prior to anaerobic digestion was studied by Andreozzi *et al.* (1998). It was found, however, that ozonated OME had a stronger inhibitory effect on methanogenesis than non-ozonated OME.

The aim of this study was firstly, to investigate the use of ozonation, granular activated carbon (GAC) and H_2O_2 in combination as post-treatments to UASB anaerobic digestion of fruit cannery and winery wastewaters. Secondly, to investigate the short-term efficiency of ozonation as a pre- and post-treatment to UASB's treating fruit cannery and winery wastewaters and, thirdly, if short-term ozonation was effective, to investigate the long-term effects of pre-ozonation on the performance of an UASB reactor treating a pre-ozonated fruit cannery wastewater.

Materials and methods

Wastewaters

A fruit cannery wastewater with an average COD of 7 500 mg.L^{-1} (7 430 – 7 612 mg.L^{-1}) and pH of *ca.* 5.0 was collected from a nearby fruit cannery (Rhodes Fruit Farms, Groot Drakenstein) during the apricot canning season. Solid material was removed by sedimentation and the pH of the wastewater was adjusted to 7.5 before use as substrate for anaerobic treatment.

A winery wastewater with an average COD of 3 700 mg.L^{-1} (3 647 – 3 799 mg.L^{-1}) and pH of *ca.* 4.8 was collected from a local winery (Fredericksburg, Simondium). Solid material was removed by sedimentation and the pH of the wastewater was then adjusted to 7.5 before use as substrate for anaerobic treatment.

For the investigation of the long-term effects of pre-ozonation on the performance of an UASB reactor, a fruit cannery wastewater with an average COD of

6 500 mg.L^{-1} (6 360 – 6 725 mg.L^{-1}) and pH of *ca.* 6.0 was collected from a cannery (Rhodes Fruit Farms, Groot Drakenstein) during the peach canning season. Solid material was removed by sedimentation. Batches of the peach canning wastewater were also frozen for later use. The frozen wastewater was thawed and then diluted to the required COD and the pH adjusted to 7.5 before use as substrate for anaerobic treatment.

Ozone pre-treated peach canning wastewater was prepared in a continuous mode, bubble/GAC contacting setup as described below in the Ozonation section. An ozonation time of 10 min was used. After ozonation the pre-treated wastewater was diluted to the required COD and the pH adjusted to 7.5 before use as substrate in the anaerobic treatment.

Anaerobic treatment

A 2.3 L laboratory-scale UASB reactor (Trnovec & Britz, 1998) was operated at 35°C (Meyer *et al.*, 1985). The volume of biogas was determined using a manometric unit equipped with an electronically controlled counter and a gas-tight valve and the volumes corrected to standard temperature and pressure. The fruit canning and winery substrates were fed semi-continuously to the reactors by means of peristaltic pumps (Watson-Marlow 302S) controlled by electronic timers. The reactors were run at a HRT of 24 h. The pH, COD, alkalinity, orthophosphate phosphorous and total solids of the reactor effluents were monitored (Standard Methods, 1998). The reactor effluents were then subjected to the post-treatment processes as described in Table 1.

Once post-treatment trials had been completed the UASB reactors were fed with ozone pre-treated fruit cannery and winery wastewater substrates. The resulting UASB reactor effluents were again treated with ozone and considered the "pre- and post-UASB treatment samples". The different short-term pre- and post-treatment combinations studied are summarized in Table 2.

The 2.3 L laboratory-scale UASB reactor used earlier to treat an apricot canning wastewater was used for the investigation of the long-term effects of pre-ozonation on the performance of an UASB reactor. In order to monitor any possible improvement in efficiency due to pre-ozonation, a quarter of the granules were removed from the UASB reactor, thus lowering the initial reactor efficiency. The reactor was run at a HRT of 24 h. The initial substrate concentration was *ca.* 3 500 mg.L^{-1}, but was later reduced to *ca.* 2 400 mg.L^{-1} (OLR = 2.4 kgCOD.m^{-3}.d^{-1}). The

Table 1. Different post-treatment combinations used for the post-treatment of UASB treated fruit cannery and winery effluents.

Treatment	Description
1	UASB effluent + 5 min ozonation
2	UASB effluent + 10 min ozonation
3	UASB effluent + 15 min ozonation
4	UASB effluent + 30 min ozonation
5	UASB effluent + 1% (v/v) H_2O_2 + 5 min ozonation
6	UASB effluent + 1% (v/v) H_2O_2 + 15 min ozonation

Table 2. Short-term pre- and post-treatment combinations of the fruit cannery and winery wastewaters.

Treatment	Descriptions and combinations
1	10 min ozonation of raw wastewater
2	UASB treatment of raw wastewater
3	UASB treatment of raw wastewater + 10 min ozonation (Post-treatment)
4	10 min ozonation of raw wastewater + UASB treatment (Pre-treatment)
5	10 min ozonation of raw wastewater + UASB treatment + 10 min ozonation (Pre- and post-treatment)

pH, COD, alkalinity, orthophosphate phosphorous and total solids of the reactor effluent were monitored (Standard Methods, 1998). The concentration of short chain volatile fatty acids (VFA) in the reactor effluent was determined gas chromatographically as described below.

Ozonation

Pre-ozonation of fruit cannery and winery wastewaters and post-ozonation of UASB treated fruit cannery and winery effluents were done in a continuous mode bubble/GAC contacting setup. This apparatus consisted of a glass bubbling column connected to a GAC column. Ozone was bubbled upwards through the glass column while the effluent was re-circulated from the bubbling column through a GAC contacting column. The bubbling column (l = 90 cm; ϕ = 6 cm) had a sintered glass disc at one end for bubble generation. The GAC contacting column consisted of a closed stainless steel cartridge (l = 20 cm; ϕ = 6.5 cm) filled with granular activated carbon. An ozone generator (Oz Purification, Ifafi) producing 9.0 g $.h^{-1}$ O_3 at a flowrate of 4 $L.min^{-1}$ was used for the ozonation trials (*ca.* 38 mg $O_3.L^{-1}$).

The effect of H_2O_2 addition to the post-ozonation treatments was also investigated by adding 1% (v/v) H_2O_2 to the reactor effluents before ozonation. The different continuous mode post-treatment combinations studied are summarised in Table 1, while the different short-term pre- and post-treatment combinations studied are summarised in Table 2.

Analytical methods

The ozone production was measured using an iodiometric titration (Standard Methods, 1998). The COD was determined colorimetrically using a DR2000 spectrophotometer (Hach Co. Loveland, CO) and standardised procedures (Standard Methods, 1998). Colour reduction in the effluent was determined by monitoring the absorbance of the effluent at 288 nm using a Spectronic spectrophotometer (Spectronic Instruments, USA). All analyses were done in triplicate.

Short chain volatile fatty acids

A standard solution of short chain fatty acids (acetic, propionic, iso-butyric, butyric, iso-valeric and valeric) was prepared by dissolving 1 mL of each fatty acid and 0.5 mL of n-hexanol in a 1 000 mL volumetric flask with one part 35% formic acid and three parts distilled water.

Samples were prepared by diluting 1 mL of 35% formic acid with 3 mL of aqueous, filtered sample and adding 2 µL of n-hexanol as internal standard. The injection volume used was 1 µL

Short chain volatile fatty acids were determined using a Varian 3700 gas chromatograph (GC) equipped with a flame ionization detector and a 30 m bonded phase Nukol (Supelco, Inc., Belafonte, PA) fused silica capillary column (0.53 mm diameter and 0.50 µm film thickness). The column temperature was initially held at 105°C for 2 min, increased at 10°C.min^{-1} to 190°C and held for 10 min. The injector temperature was set at 150°C, while the detector was set at 300°C. Nitrogen was used as carrier gas at a flowrate of 6.1 mL.min^{-1}. Short chain fatty acids were quantified by means of Borwin Version 1.2 integration software (JMBS Developpements, Le Fontanil, France), operating in the internal standard mode. Results were converted to original sample concentration. All analyses were done in triplicate.

Biogas composition

The biogas composition was determined on a Varian 3300 GC equipped with a thermal conductivity detector and a 2.0 m x 3.0 mm i.d. column packed with Hayesep Q (Supelco, Bellefonte, PA), 80/100 mesh. The oven temperature was set at 55°C and helium was used as carrier gas at a flow rate of 30.0 mL.min^{-1}. The injection volume of biogas was 0.2 mL and percentage composition was determined by integration of peak areas relative to total peak area on a Varian 4290 integrator.

Results and discussion

I. Short-term efficiency of ozone as a pre- and/or post-treatment to UASB anaerobic digestion

Anaerobic digestion

During the anaerobic treatment of the cannery wastewater by UASB, the COD was reduced by 87 – 89%. The reactor effluent had a pH of 7.0 - 7.2 and a COD concentration of 818 – 983 mg.L^{-1}. The effluent from the reactor treating the winery wastewater had a pH of 7.2 – 7.4 and a COD concentration of 303 – 400 mg.L^{-1}, constituting COD reductions of 89 – 92%. These reactor effluents were used in the post-ozonation studies.

Post-ozonation

Oxidation treatments of UASB effluents treating fruit cannery and winery wastewater resulted in pronounced colour reductions, as can be seen in Table 3, from decreases in absorbance at 288 nm. Increases in the oxidation time led to increases in colour reduction of at least 66% after 5 min to greater than 87% after 30 min.

The addition of 1% H_2O_2 (v/v) to the 5 and 15 min ozonation treatments led only to small increases in colour reduction (from 66 to 70% and 87 to 90%, respectively) for the effluent from the reactor treating cannery wastewater. The same tendency was found for the effluent from the reactor treating the winery wastewater. Increases in ozonation time increased the colour reduction from 68% (5 min O_3) to 87% (30 min O_3), with increases from 68 to 71% and 83 to 88% when adding 1% H_2O_2 (v/v) to the 5 and 15 min oxidation treatments, respectively. These results compare favourably with previous studies on the reduction of the UASB reactor effluent colour with O_3 and H_2O_2 (Chapter 4 of this dissertation). The colour reductions achieved in this study were, however, higher. This could possibly be due to the inclusion of a GAC contacting column in the continuous mode oxidation used in this study. Beltrán *et al.* (1997 and 1999) found that H_2O_2 alone was not an effective oxidizing agent in the treatment of tomato processing and distillery wastewaters, as it has a much lower oxidation potential (1.77 eV) than ozone (2.07 eV).

The oxidation treatments were also found to be effective in reducing the total COD of the UASB systems effluents. Longer ozonation times led to increases in total COD reduction of 93 and 94% after 5 min to 96 and 97% after 30 min for effluents from reactors treating cannery and winery wastewaters, respectively (Fig. 1). Similar increases in COD reduction with extended reaction times were also found when treating UASB treated alkaline cannery effluent (Chapter 4 of this dissertation), table olive debittering (Beltrán *et al.*, 1999) and yeast-production wastewaters (Filipović-Kovačević & Sipos, 1995), chlorinated aromatics (Schmitt & Hempel, 1993), tomato and distillery wastewaters (Beltrán *et al.*, 1997). It is clear from the data presented in Fig. 1 that the initial rate of COD reduction is rapid, but that the rate after 5 min is not maintained during the subsequent 5 min ozonation periods. This can probably be ascribed to the presence of organic compounds that are more susceptible to oxidation, with others only being partially oxidized (Camel & Bermond,

Table 3. The effect of oxidation treatments on the colour of effluents from UASB reactors treating fruit cannery and winery wastewaters.

Treatment	Absorbance (288 nm)*	Colour reduction (%)
Reactor effluent (cannery)	1.522	
5 min O_3	0.516	66 ± 1.2
10 min O_3	0.258	83 ± 1.3
15 min O_3	0.196	87 ± 0.9
30 min O_3	0.153	90 ± 1.4
1% (v/v) H_2O_2 + 5 min O_3	0.457	70 ± 1.2
1% (v/v) H_2O_2 + 15 min O_3	0.152	90 ± 1.0
Reactor effluent (winery)	0.520	
5 min O_3	0.166	68 ± 1.5
10 min O_3	0.104	80 ± 1.2
15 min O_3	0.088	83 ± 1.3
30 min O_3	0.068	87 ± 0.8
1% (v/v) H_2O_2 + 5 min O_3	0.151	71 ± 1.1
1% (v/v) H_2O_2 + 15 min O_3	0.062	88 ± 1.2

*Average of triplicate determinations

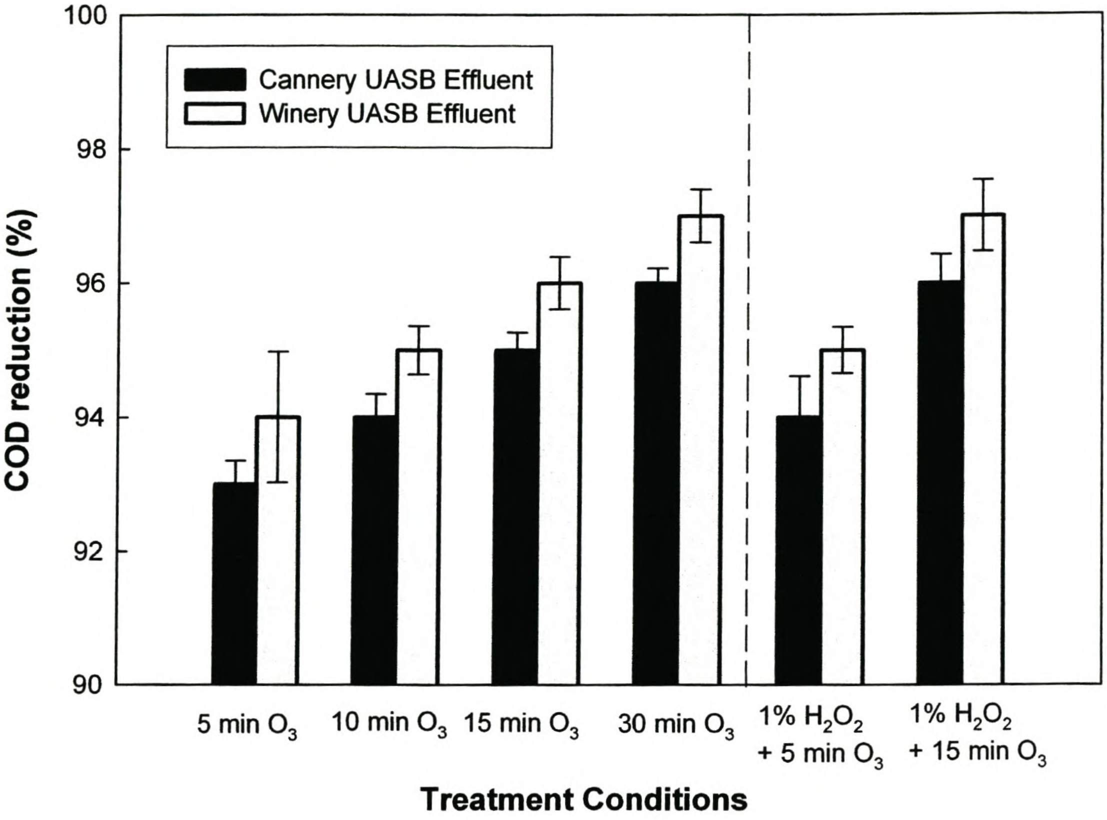

Figure 1. COD reduction (%) after applying the different treatment combinations to effluent from UASB treated fruit cannery and winery wastewaters (COD reduction (%) based on UASB + effluent treatment).

1998). This decrease in COD reduction rate indicates that the UASB effluents contain a considerable amount of difficult to oxidize compounds that require a stronger oxidizing system.

The slightly higher reductions in the reactor effluent COD achieved in this study with the use of ozone in a continuous mode compared to the batch method (Chapter 4 of this dissertation) are probably the result of the GAC contacting column. It is possible that certain molecules are only partially oxidized by the ozone and thus form lower molecular weight compounds, which are better adsorbed on the GAC column (Camel & Bermond, 1998) leading to a lower final effluent COD.

The addition of 1% H_2O_2 (v/v) as part of the oxidation treatments resulted in only small increases in COD reductions compared to the COD reductions found with samples that were ozonated (Fig. 1). A 5 min ozonation of UASB treated cannery and winery effluents resulted in COD reductions of 93 and 94%, respectively. An addition of 1% H_2O_2 (v/v) to the effluents prior to ozonation resulted in the COD reduction increasing to 94 and 95%, respectively. A 15 min ozonation of UASB treated cannery and winery effluents resulted in COD reductions of 95 and 96%, respectively. An addition of 1% H_2O_2 (v/v) to the effluents prior to ozonation resulted in the COD reduction increasing to 96 and 97%, respectively. The increased COD reduction resulting from the longer ozonation time plus the addition of H_2O_2 was expected as both methods lead to the formation of more hydroxyl radicals, and thus create a stronger oxidizing capability (Beltrán *et al.*, 1997; Hughes, 1992). The small increase in COD reduction resulting from the addition of 1% H_2O_2 (v/v) was considered to be insignificant and thus uneconomical. The same tendency was found in Chapter 4 of this dissertation where UASB treated alkaline fruit cannery effluent was ozonated. Hydrogen peroxide on its own is expected to be less effective due to its lower oxidation potential in comparison to ozone and the hydroxyl radical (Hughes, 1992; Mourand *et al.*, 1995).

Combined pre- and post-ozonation system

The application of a 10 min ozonation treatment to the raw cannery and winery wastewaters, resulted in colour reductions of 66 and 62%, respectively (Table 4). A colour reduction of only 37 and 48% was achieved, when using only the UASB process to treat cannery and winery wastewaters, respectively. The low colour reduction resulting from the UASB treatment in this case can probably be ascribed to the fact that the UASB effluent contains considerable amounts of biomass, resulting

Table 4. Pre- and post-UASB treatment of cannery and winery effluents with ozone.

	Treatment description	Colour reduction (%)*	
		Cannery	**Winery**
1	10 min ozonation of raw wastewater	66	62
2	UASB treatment of raw wastewater	37	48
3	UASB treatment + 10 min post-ozonation	80	86
4	10 min pre-ozonation + UASB treatment	69	73
5	10 min pre-ozonation + UASB + 10 min post-ozonation	90	91

* Average of triplicate determinations

in a high degree of turbidity in the effluent. However, the post-ozonation of the UASB effluent (UASB + 10 min) resulted in reductions of 80 and 86% in colour (Table 4). These results are similar to the post-UASB ozonation results obtained in the "post-ozonation section" of this study, where 80 and 83% colour reduction, respectively, were achieved.

Pre-treatment of the cannery and winery wastewaters with ozone plus the UASB treatment (10 min + UASB) resulted in colour reductions in the UASB effluent of 69 and 73%, respectively. Post-ozonation of these UASB effluents further reduced the colour, resulting in a total colour reduction of 90 and 91% (pre- and post-ozonation), as can be seen in Table 4. These results are similar to the colour reductions of up to 90% reported by Filipović-Kovačević & Sipos (1995), while treating a yeast-production wastewater with ozone. Beltrán *et al.* (1999) also found that nearly all colour was removed when treating table olive debittering wastewaters with ozone (*ca.* 40 mg $O_3.L^{-1}$).

Reductions in COD concentration for the various treatment combinations are summarised in Fig. 2. Ozonation of the raw cannery and winery wastewaters for 10 min only brought about reductions of 53 and 62% in the total COD content, respectively. An UASB treatment of the cannery and winery wastewaters resulted in total COD reductions of 88 and 91%, respectively. Post-UASB ozonation (UASB + 10 min) of the reactor effluents achieved total COD reductions of 92 and 94%. This is in agreement with results obtained in the post-ozonation section of this study, where COD reductions of 94 and 95% (Fig. 1) were achieved with a 10 min post-ozonation of UASB effluents.

Pre-treatment of the wastewaters with ozone prior to UASB treatment (10 min + UASB), resulted in the total COD being reduced by 94 and 98%, respectively (Fig. 2). A further 10 min ozonation of these UASB effluents led to a total COD reduction of 95 and 99% being achieved (pre- and post-ozonation), respectively for cannery and winery wastewaters. Final COD values for the pre- and post-treated wastewaters were on average 375 $mg.L^{-1}$ for the cannery wastewater and 41 $mg.L^{-1}$ for the winery wastewater. The latter COD value falls well below the South African legal limit of 75 $mg.L^{-1}$ for discharge to a natural water system (Republic of South Africa, 1999). The COD reductions, for ozone treatment alone (53 – 62%) compare very favourably, and in some cases better than, previous results reported by other authors. Beltrán *et al.* (2001b) achieved a 41.4% COD reduction in wine-distillery wastewaters using a pH sequential ozonation system. Ozonation of a yeast-

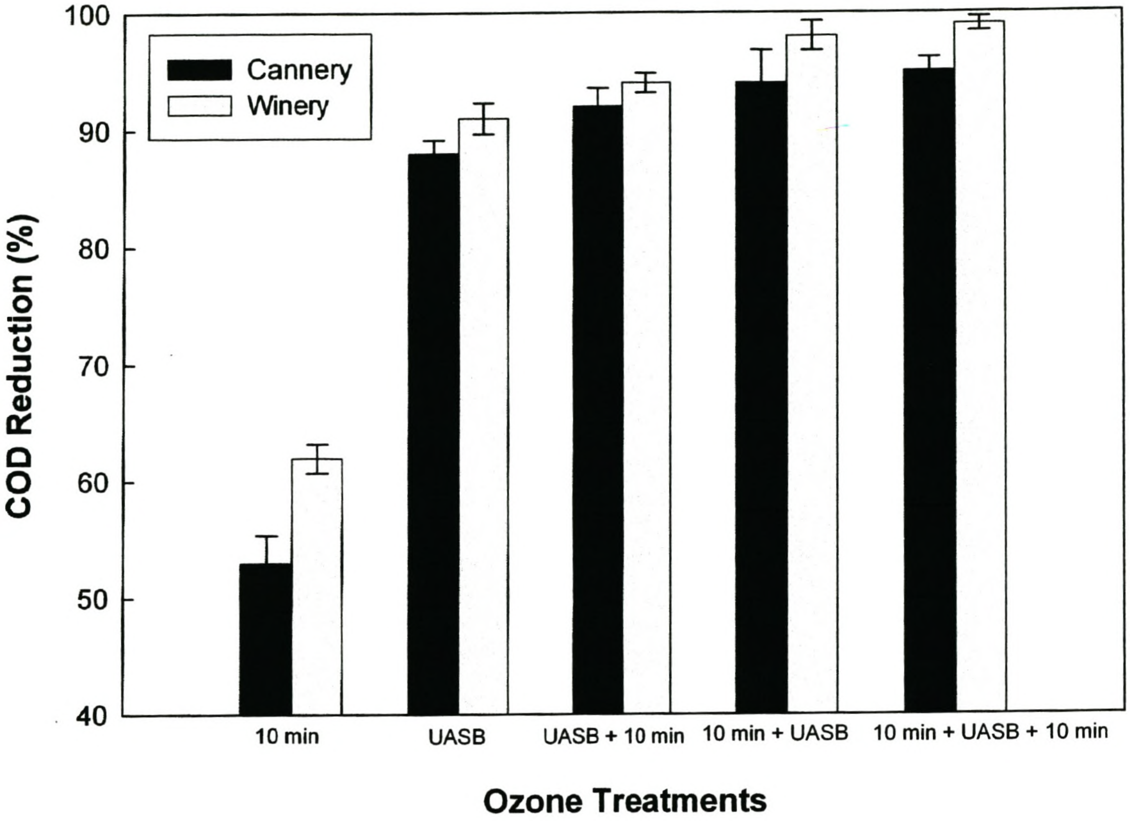

Figure 2. COD reduction (%) after applying different treatment combinations used in the pre- and post-treatment of fruit cannery and winery wastewaters.

production wastewater resulted in a 65% reduction of the COD (Filipović-Kovačević & Sipos, 1995). Chang & Sheldon (1989) achieved COD reductions of 47 – 62%, treating poultry processing wastewaters with ozone. Benitez *et al.* (1999) achieved 20 – 30% COD reduction in winery wastes by ozonation as a pre-treatment to anaerobic digestion, and this was also indicated by the increased methane yield during the anaerobic digestion process.

II. Long-term impact of ozone pre-treatment on the performance of an UASB reactor treating a fruit cannery wastewater

Non-ozonated substrate

The UASB efficiency improved when fed pre-ozonated wastewater over a short period. It is, however, important to determine the effect of pre-ozonating wastewater on anaerobic efficiency over long periods. The UASB reactor efficiency for the complete "long-term study" is presented in Fig. 3. During the initial substrate acclimatization period (days 1 – 40) the substrate COD was *ca.* 3 500 mg.L^{-1}. The COD reduction efficiency did not stabilise during this period (days 1 – 40) and it was decided to lower the substrate COD to *ca.* 2 400 mg.L^{-1}. This resulted in a more stable operation of the UASB, as can be seen from the data in Fig. 3 (days 41 – 70). COD reduction stabilised at between 59 and 64%, which is lower than expected for an optimised UASB. The inability of the UASB reactor to attain a higher COD reduction, could probably be ascribed to the removal of a quarter of the granular biomass resulting in a reduced active microbial capacity. In a previous study, where an UASB process was used to treat fruit cannery and winery wastewaters, COD reductions of 90 – 93% were achieved (Chapter 4 of this dissertation). The concentration of VFA's in the reactor effluent varied considerably during the acclimatization period (days 1 – 40) reaching levels of 668 mg.L^{-1} and led to an unstable reactor pH. Once the substrate COD had been lowered to *ca.* 2 400 mg.L^{-1}, the VFA content of the reactor effluent stabilised, with a average of 327 mg.L^{-1} (day 41 – 72). The biogas produced by the reactor on day 72 contained 28% methane. Once COD reduction had reached a stable-state, not varying by more than 10%, it was decided to proceed with the next section of the study.

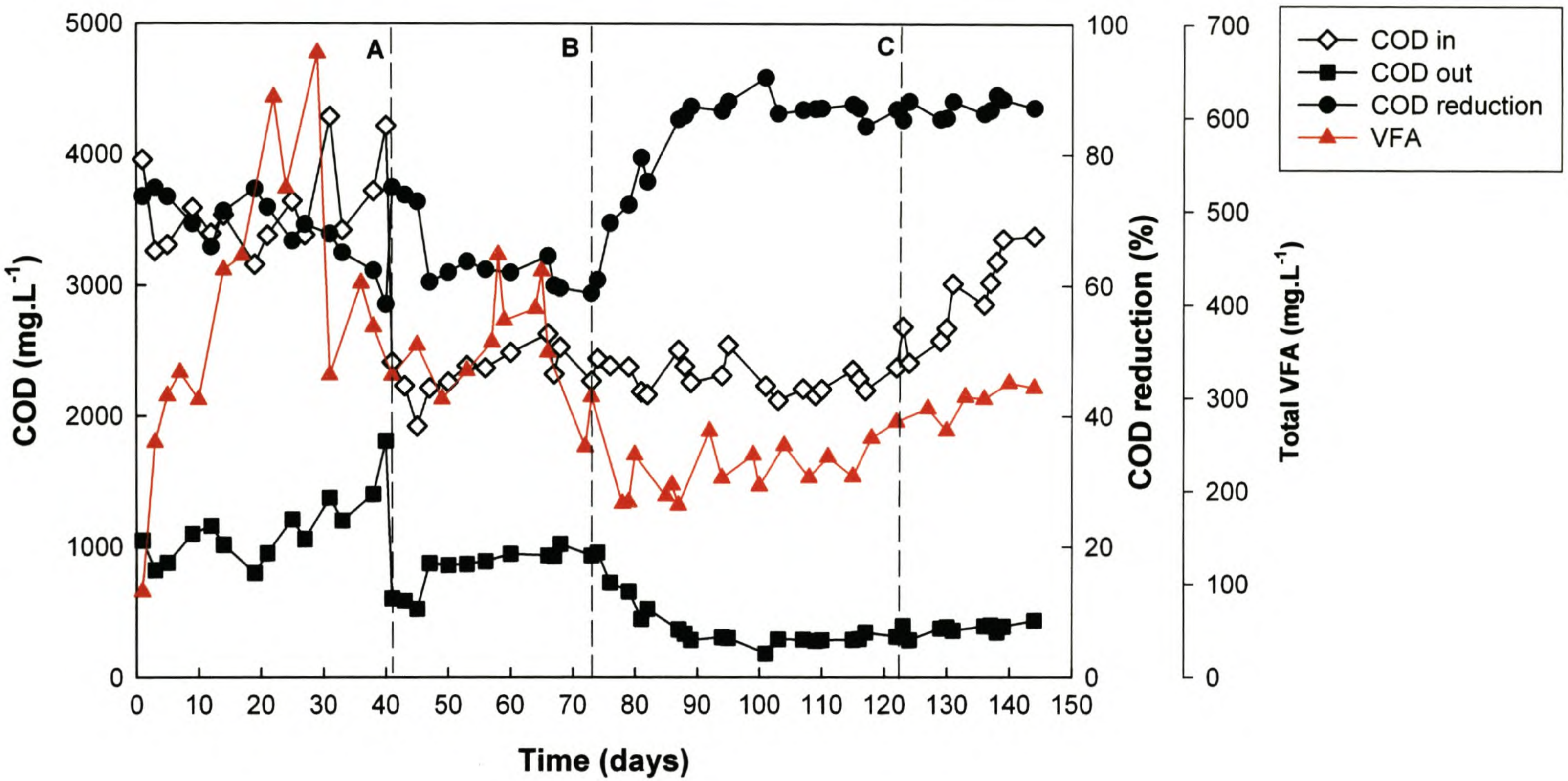

Figure 3. COD reduction efficiency parameters of the UASB reactor treating a fruit cannery wastewater during start-up and a pre-ozonated cannery wastewater for the remainder of the study (A - start-up completed; B - Pre-ozonated substrate feed started; C – increase in OLR).

Pre-ozonated substrate

Pre-ozonated substrate was used to replace the normal substrate on day 73 (Fig. 3 – vertical line B). Pre-ozonation (10 min) of the peach cannery wastewater resulted in a COD reduction of *ca*. 55%, lowering the raw wastewater COD to *ca*. 3 000 mg.L^{-1}. This COD reduction of 55% compares well with the 53 and 62% COD reduction achieved in Part I of this chapter (Fig. 2).

Before feeding the pre-ozonated wastewater to the UASB, the COD was diluted to *ca*. 2 400 mg.L^{-1} for use as reactor substrate (OLR = 2.4 kgCOD.m^{-3}.d^{-1}). The substrate COD concentration was kept constant at *ca*. 2 400 mg.L^{-1} until day 122 (Fig. 3 – vertical line C), after which the dilution of the substrate was gradually reduced. By day 138 the undiluted, pre-ozonated peach canning wastewater, with a COD concentration of 3 400 mg.L^{-1}, was being fed as substrate. The COD reduction of the reactor stabilised at *ca*. 61% by day 73. As can be seen from the COD reduction (Fig. 3), a significant improvement, with the pre-ozonated wastewater being used as substrate, was observed from day 76 onwards. COD reduction reached 80% by day 81 and increased to 85 – 90% by day 87. The COD reduction efficiency remained relatively constant between 85 and 92% until the end of the trial (day 144). Together with the improvement in COD reduction efficiency during this period (days 76 – 122), a decrease in VFA content of the reactor effluent was also observed (Fig. 3). The VFA content during this phase averaged at 228 mg.L^{-1}. This was probably due to the substrate being more readily biodegradable, due to the ozonation treatment. During this time (days 76 – 122) the methane percentage in the biogas gradually increased from 28% to 37%.

The gradual increase in substrate COD from day 122 (as dilution of the pre-ozonated peach canning wastewater was reduced) did not negatively influence the COD reduction efficiency, which remained between 85 and 92%, while the OLR increased to 3.4 kgCOD.m^{-3}.d^{-1}. A slight increase in the reactor effluent VFA content was, however, observed. VFA contents of 50 - 500 mg.L^{-1} are, however, considered to be indicators of an anaerobic digestion process performing efficiently (Shieh & Nguyen, 1997), effectively converting organic matter to methane and carbon dioxide. Methane production increased slightly from 37 to 38% during this final stage (days 122 – 144). The average UASB reactor effluent COD for the last two weeks of the trial was 368 mg.L^{-1}, which represents a 94% reduction of the initial peach canning wastewater COD of *ca*. 6 500 mg.L^{-1} (Table 5). However, this final COD value is not within the South African legal limit of 75 $mg.L^{-1}$ (Republic of South Africa, 1999) and a further treatment is essential to legally discharge to a river or stream.

Post-ozonation of the UASB reactor effluent resulted in a further COD reduction, producing a final effluent with a COD of 55 $mg.L^{-1}$, a total COD reduction of 99% (Table 5). This COD value is well within the South African legal limit (75 $mg.L^{-1}$) permitted for wastewaters to be discharged to a water system (Republic of South Africa, 1999). These results compare favourably to those obtained in Part I of this chapter and also with those of other authors (Benitez *et al.*, 1999; Weemaes *et al.*, 2000; Martín *et al.*, 2002) in that pre-ozonation of substrates resulted in improved biodegradability during anaerobic digestion, and increased the methane production rate.

Conclusions

Advanced oxidation processes and the use of GAC can be successfully used in a pre- and post-treatment combination to lower the COD of UASB treated cannery and winery effluents. The use of a continuous mode of oxidation (inclusion of a GAC contacting column through which the effluent is re-circulated during ozonation) resulted in an improvement in colour and COD reduction.

The use of ozone as a pre- or post-treatment to anaerobic digestion was shown to be successful in that total COD and colour reduction were increased. Ozonation of the wastewaters alone was less effective in reducing the COD than anaerobic digestion by UASB. Ozonation as a post-treatment to UASB seems to be less effective than using ozonation as a pre-treatment to UASB. Ozone as a pre- and post-treatment to UASB, however, achieves the best results in reducing the COD and colour of the wastewaters.

It was also shown that the long-term use of pre-ozonated wastewater as UASB substrate does not inhibit the anaerobic digestion process and improves the total COD reduction efficiency of the UASB reactor, while also increasing the methane production per COD load to the UASB reactor. Furthermore, final COD values of UASB reactor effluent after pre- and post-UASB ozonation, were well below the South African legal limit (75 $mg.L^{-1}$) for discharge of wastewaters to a water system. The use of ozonation, not only as a post-treatment, but also as a pre-treatment to anaerobic digestion is a feasible option in the treatment of cannery and winery wastewaters. By using a combination of pre- and post-treatment, significant improvements in the wastewater treatment efficiency can be achieved, which could be beneficial financially and environmentally. One question that does arise: can the treatment combination used in this study be scaled-up to treat either of the wastewaters evaluated so far.

Table 5. Average COD concentrations at the different stages of treatment.

Treatment stage	COD ($mg.L^{-1}$)	COD reduction (%)
Undiluted peach canning wastewater	6 500	-
Pre-ozonated peach canning wastewater	2 925	55
After UASB treatment	368	94
Post-ozonation	55	99

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CHAPTER 6

PILOT-SCALE (600 L) UASB TREATMENT OF A WINERY WASTEWATER COMBINED WITH PRE- AND POST-OZONATION

Summary

The scale-up of a winery wastewater treatment with pre- and post-UASB ozonation was investigated. Pre-ozonation of the raw winery wastewater (average COD = 4 982 mg.L^{-1}) resulted in an average COD reduction of 17%. UASB treatment of the raw winery wastewater in a pilot-scale 600 L UASB resulted in a maximum COD reduction efficiency of 87%. Treatment of pre-ozonated wastewater in the pilot-scale UASB resulted in the COD reduction efficiency of the UASB increasing from an average of 81% to between 88 and 92%. The increased COD reduction efficiency was ascribed to an increased substrate biodegradability and also resulted in the biogas methane content increasing from *ca*. 43% to 54%. Variations in organic loading rate (OLR) did not influence COD reduction efficiency. Post-ozonation further reduced the UASB effluent COD, increasing the total COD reduction of the combined pre- and post UASB ozonation treatment to between 93 and 96%.

Introduction

Wine production is an agricultural activity of major economic importance to South Africa. Not only are many thousand jobs created but, valuable foreign currency is obtained through export of the wine products. One negative aspect of the processing is that large quantities of wastewater are generated (0.7 – 3.8 m^3.ton^{-1} of grapes processed) mainly by the washing, rinsing, cooling and sanitising operations in the cellar (Water Research Commission, 1993; Bezuidenhout *et al.*, 2002). These wastewaters vary in organic load and pH, depending on the season and activities in the cellar (Ronquest, 1999). Wineries are under increasing legislative pressure to reduce their water intake and dispose of their effluents in an environmentally responsible manner (Hayward *et al.*, 2000).

Various methods of treating food and beverage processing wastewaters have been investigated (Bitton, 1999). The use of anaerobic digestion as a treatment option has been found to be feasible for a variety of wastewaters, including potato processing wastewaters (Kalyuzhnyi *et al.*, 1997; Zoutberg & Eker, 1999), dairy and brewery wastewaters (Driessen & Yspeert, 1999; Nadais *et al.*, 2001), coffee processing wastewaters (Jeison & Chamy, 1999), olive mill effluents (Andreozzi *et al.*, 1998; Beccari *et al.*, 1998 & 2001), winery and distillery wastewaters (García-Bernet *et al.*, 1998; Ronquest & Britz, 1999; Ruiz *et al.*, 2002) and fruit cannery wastewaters (Trnovec & Britz, 1998; Britz *et al.*, 2000).

Ozone has also been shown to be effective in reducing COD, removing colour and making organic compounds more biodegradable (Gottschalk *et al.*, 2000), but very little research on the use of ozone as a pre-treatment to anaerobic digestion may be found in the literature. In one study, the use of ozone in combination with hydrogen peroxide (H_2O_2) and ultra-violet (UV)-light to treat a winery waste before anaerobic digestion was investigated and COD reductions of 20 – 30% were reported with a good methane yield increase (Benitez *et al.*, 1999). Anaerobic digestion of vinasse pre-treated with ozone plus UV light in the presence of titanium dioxide also led to a decrease in COD and total organic carbon and increased the methane yield and rate of anaerobic digestion by 25% (Martín *et al.*, 2002). Weemaes *et al.* (2000) reported that the anaerobic digestion of ozonized wastewater sludge enhanced methane production and accelerated the digestion rate, while in contrast Andreozzi *et al.* (1998) found that ozonated olive oil mill effluents (OME) had a stronger inhibitory effect on methanogenesis than un-ozonated OME when treated with ozone prior to anaerobic digestion. Ozone as a pre- and post-treatment step to the UASB treatment of fruit cannery and winery wastewaters has also been shown to be feasible (Chapter 5 of this dissertation). The COD reduction efficiency of the UASB reactors increased when fed pre-ozonated wastewaters, methane yield increased and no detrimental effects on the microbial population of the reactors was observed. These studies on the use of ozone in combination with anaerobic digestion were were all done on laboratory-scale where variations in organic load, substrate composition and pH are less drastic than in industrial scenarios. Simulation of conditions similar to those encountered at wineries would be beneficial in determining the commercial feasibility of combining ozonation with anaerobic treatment of winery wastewaters.

The aim of this study was firstly to determine the efficiency of a 600 L pilot-scale UASB process while treating winery wastewater, and secondly to investigate the use of ozonation as a pre- and post-treatment option to the pilot-scale UASB process.

Materials and methods

Wastewaters

Wastewater was obtained from a large winery (Bergkelder, Distell, Stellenbosch) producing a variety of red and white wines and some distilled products. This winery was used for the source of winery wastewater for logistical supply and consistency reasons. All wastewaters generated from the various cellar and distillery operations at this winery are channeled to a central "wastewater pit", from where the wastewaters are pumped to the nearby treatment facility. Wastewater used in this study was abstracted from this pit for use in the pilot-scale UASB. Due to the nature of the cellar operations, the wastewater COD varied extensively, from a minimum of 2 225 mg.L^{-1} to a maximum of 7 388 mg.L^{-1}. The average COD content of the wastewater during this study was 4 290 mg.L^{-1} with an average pH of 4.46 (3.39 – 5.46). The average total Kjeldahl nitogen (TKN) and orthophosphate phosphorous (PO_4^{3-}) content of the wastewater were 12.3 mgN.L^{-1} (7.2 – 19.6 mg.L^{-1}) and 24.7 mg.L^{-1} (11.4 – 40.3 mg.L^{-1}), respectively.

Anaerobic treatment

A 600 L pilot-scale UASB reactor (Fig. 1) was used and operated at 35°C and the hydraulic retention time (HRT) was set at 24 h. The reactor was seeded with 100 kg of drained anaerobic granules from an industrial-scale anaerobic digester treating brewery wastewater. The substrate was fed semi-continuously to the reactor by means of an impeller pump (Calpeda PM45, Vincenza, Italy) controlled by an electronic timer. A re-circulation pump (Mono Pumps C32M, Cape Town, South Africa) maintained an upflow velocity of approximately 2 m.h^{-1}. The 600 L reactor was then allowed to stabilise for 6 d in order to allow the bacterial community to acclimatise and was fed a diluted winery wastewater, supplemented with 1 000 mg.L^{-1} sucrose (total COD = 2 000 mg.L^{-1}). The sucrose supplementation was gradually decreased during the start-up to acclimatise the anaerobic granules to the nutrient deficient winery wastewater. The winery wastewater portion was gradually increased over a 6 d start-up period resulting in a diluted winery wastewater with a COD of 2 500 mg.L^{-1}. Feeding of un-diluted winery wastewater was commenced after the start-up period and this was taken as day 1. Substrate pH was not adjusted,

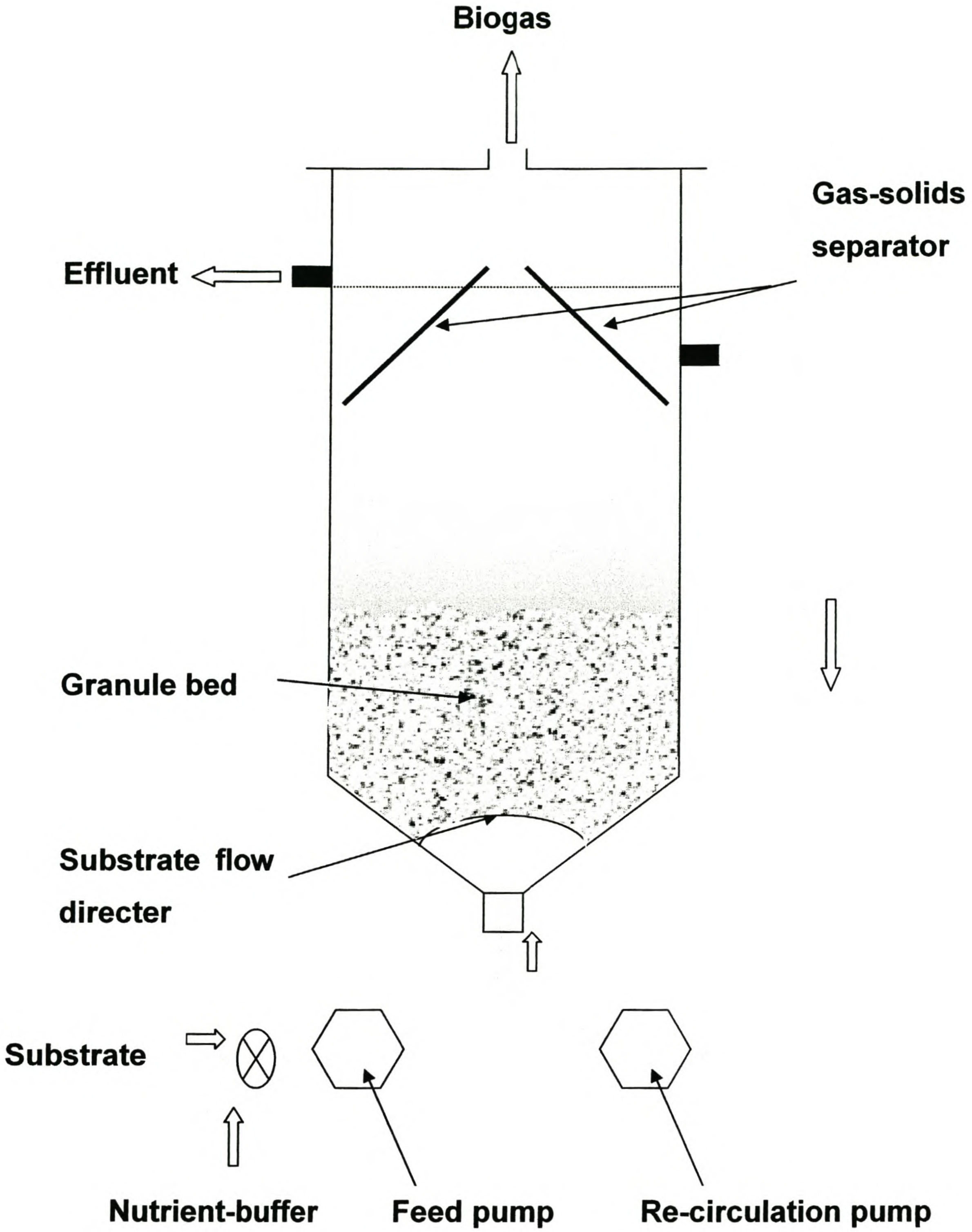

Figure 1. Schematic diagram of the 600 L UASB reactor used to treat the winery wastewater

but the reactor was supplemented with an nutrient-buffer stock solution on a daily basis. The supplementation included the addition of 250 g CaO (lime), 40 g nitrogen (N) in the form of 86 g urea and 4 g phosphorous (P) (18 g KH_2PO_4) per day. The supplementation was done in six equal feedings per day. This supplementation was done in order to maintain reactor alkalinity, as well as supplying a source of N and P. The pH, COD, alkalinity and orthophosphate phosphorous of the reactor effluent were monitored (Standard Methods, 1998). The UASB reactor effluents were subjected to a post-ozonation treatment. Feeding of pre-ozonated winery wastewater was then commenced and after *ca*. 60 days the resulting UASB reactor effluent was again treated with ozone and considered the "pre- and post-UASB treatment" sample. The different treatment combinations studied are summarized in Table 1.

Ozonation

Pre- and post-ozonation was carried out using a venturi, circulating contactor (Fig. 2). The contacting column consisted of a closed PVC tube (l = 103 cm; ϕ = 11 cm) filled with stainless steel turnings to enhance mixing. A stainless steel impeller pump (Calpeda MXH 203E, Vincenza, Italy) was used to circulate (flow rate = 50 $L.min^{-1}$) the winery wastewater or UASB effluent through the contacting system. The ozone was introduced into the system by means of the venturi installed beneath the contactor on the inlet side (Fig. 2). Pressure in the contacting system was regulated at 1 bar to increase the ozone residence time. An ozone generator (Parc Scientific, Ifafi) capable of producing 9.0 g $O_3.h^{-1}$ was used for the ozonation trials. A flow rate of 4 $L.min^{-1}$ was used, resulting in an ozone concentration of 38 $mg.L^{-1}$. Post-ozonation of 25 L batches of UASB effluent was done for 4 min, resulting in an ozone dose of 24.3 $mgO_3.L^{-1}$. Pre-ozonation of the winery wastewater was done in a 5 000 L tank. The ozonation time was 12 h, resulting in an ozone dose of 21.9 $mgO_3.L^{-1}$.

Analytical methods

The pH, COD, alkalinity, orthophosphate phosphorous and TKN of the wastewaters and reactor effluents were monitored (Standard Methods, 1998). The ozone production was measured using an iodiometric titration (Standard Methods, 1998). The COD, orthophosphate phosphorous and TKN were determined colorimetrically using a DR2000 spectrophotometer (Hach Co. Loveland, CO) and standardised procedures (Standard Methods, 1998). Biogas and volatile fatty acids (VFA's) were

Table 1. Pre- and post-UASB ozonation treatment combinations of winery wastewaters and UASB effluents.

	Treatment description
1	UASB treatment of winery wastewater
2	Post-ozonation of UASB effluents
3	Pre-ozonation of winery wastewater
4	Pre-ozonation + UASB treatment of winery wastewater
5	Pre-ozonation + UASB treatment + post-ozonation of UASB effluent

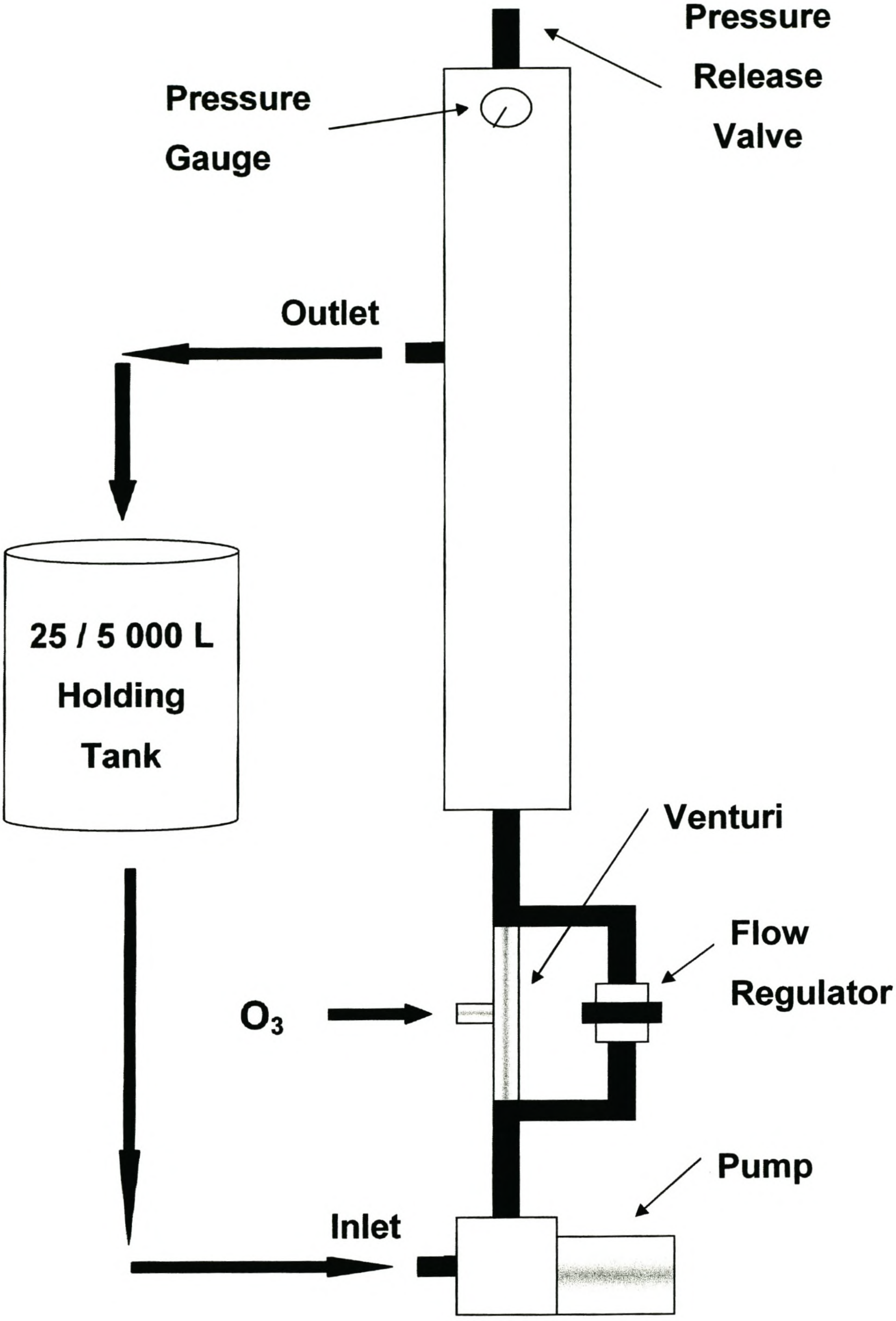

Figure 2. Schematic diagram of the purpose-built venturi, circulating contactor system used for the pre- and post-ozonation of winery wastewater and UASB effluent.

determined gas chromatographically according to the method described in Chapter 5 of this dissertation. All analyses were done in triplicate.

Results and discussion

Anaerobic digestion

The 600 L pilot-scale UASB reactor was fed undiluted winery wastewater for a period of 152 d to determine the maximum operating efficiency of the reactor. During this stage (days 1 – 152) the influent COD averaged 4 202 $mg.L^{-1}$ (2 225 – 6 512 $mg.L^{-1}$) and the average COD reduction was 77% (66 – 88%) producing UASB effluents with final COD contents of 401 – 1 724 $mg.L^{-1}$ (average = 967 $mg.L^{-1}$) (Fig. 3). It can be seen from the data in Fig. 3 that the influent COD varied considerably during this period, especially for days 25 – 120. It can also be seen from the data in Fig. 3 that the VFA content in the UASB effluent varied considerably (89 – 774 $mg.L^{-1}$) during this period. It can also be seen in Fig. 4 that the influent pH for days 1 – 152 varied between 3.39 and 5.46, while the reactor effluent pH varied between 6.60 and 7.18 (average = 6.83). The alkalinity was found to increase steadily during this period from *ca*. 800 to 1 500 $mgCaCO_3.L^{-1}$. The methane content of the biogas produced in the UASB reactor averaged 40% for the period 145 – 152 d.

During the period between days 153 and 189 (Fig. 3 – period A to B) the influent COD varied from 3 544 to 4 431 $mg.L^{-1}$, but the COD reduction remained relatively stable between 75 and 87%, averaging 81% (Fig. 3). The alkalinity remained relatively constant, between 1 400 and 1 500 $mgCaCO_3.L^{-1}$ and the average effluent pH was 6.76, even though the influent pH decreased slightly during this period (Fig. 4). Average methane content of the biogas was 43% during this period. The variation in effluent VFA content was also lower, with levels between 285 and 475 $mg.L^{-1}$ (Fig. 3).

The COD reduction efficiency attained in this study was somewhat lower than in similar studies. Reductions in COD of between 84 and 93% were reported for winery and distillery wastewater UASB treatment (Müller, 1998; Ronquest & Britz, 1999; O'Kennedy, 2000; McLachlan, 2004). Similarly, better COD reductions (89 – 92%) were achieved during UASB treatment of winery wastewater as reported in Chapter 5 of this dissertation. A possible reason for the lower efficiency obtained in the case of this 600 L pilot-scale UASB may be ascribed to the compositional variability of the specific winery wastewater used. For example, the COD varied

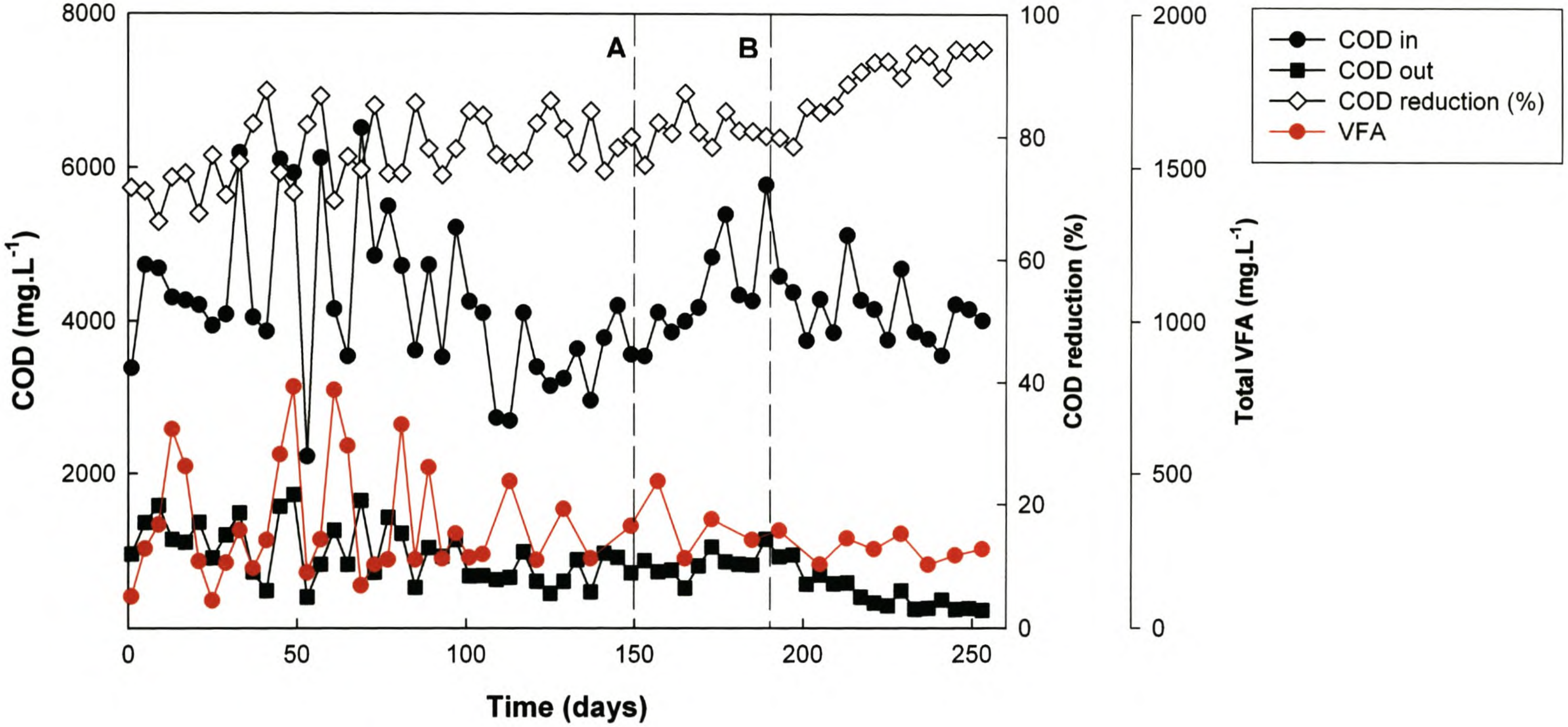

Figure 3. COD reduction efficiency parameters of the UASB reactor initially treating the raw winery wastwater and the pre-ozonated winery wastewater for the remainder of the study (period A to B – post-ozonation of UASB reactor effluent; B - Pre-ozonated substrate feed started).

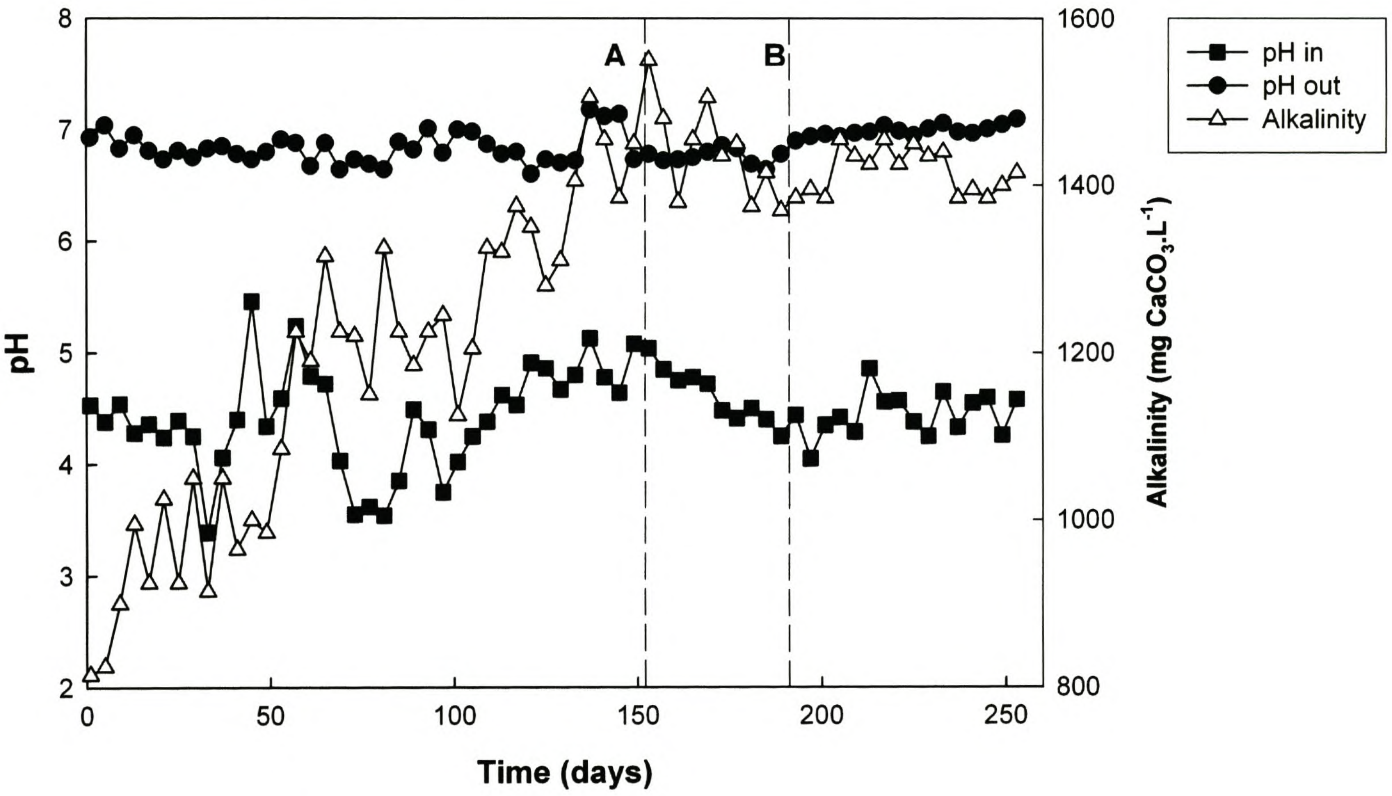

Figure 4. Efficiency parameters of the UASB reactor initially treating the raw winery wastwater and the pre-ozonated winery wastewater for the remainder of the study (period A to B – post-ozonation of UASB reactor effluent; B - Pre-ozonated substrate feed started).

between 2 225 mg.L^{-1} and 7 388 mg.L^{-1}, the pH between 3.39 and 5.46 and the influent VFA's between 301 and 1 199 mg.L^{-1}. The reason for the compositional variation is the diverse nature of activities in the winery and the grape pressing season.

Anaerobic digestion of pre-ozonated winery wastewater

Pre-ozonated winery wastewater was used as substrate from day 190 (Fig. 3 – vertical line B) to day 253 (Fig. 3). The COD of the winery wastewater directly from the cellar varied from 4 265 to 6 144 mg.L^{-1} for the period between days 190 and 253. Pre-ozonation of the raw winery wastewater resulted in an average COD reduction of 17%, and thus the COD of the pre-ozonated substrate ranged from 3 520 to 5 130 mg.L^{-1}. The COD reduction compared favourably with previous results reported in the literature. Benitez *et al.* (1999) reported COD reductions of 20 – 30% after ozonating winery wastes. Reductions in COD of up to 25% were reported during the ozonation of wine vinasses (Martin *et al.*, 2002; Benitez *et al.*, 2003). McLachlan (2004) recently reported COD reductions of 20% when ozonating winery wastewaters prior to anaerobic digestion. Reductions of between 40 and 65% of the COD have also been reported for cannery, yeast production and poultry processing wastewaters (Chang & Sheldon, 1989; Filipović-Kovačević & Sipos, 1995; Chapter 5 of this dissertation). From the data obtained in this study and results reported in the literature it was concluded that the amount of COD reduction achieved, appears to be wastewater dependant.

After the pre-ozonated substrate feed had commenced (Fig. 3 – vertical line B), the COD reduction efficiency of the reactor was found to gradually increase from about 80% on day 190 to 90% by day 217. After day 217 the COD reduction efficiency of the UASB reactor increased slightly and then remained constant at a value of *ca.* 92% (value calculated in terms of the pre-ozonated influent) (Fig. 3) During the period of day 217 to 253, the UASB effluent COD thus varied between 230 and 485 mg.L^{-1}, representing a total (pre-ozonation + UASB) COD reduction of 90 – 95%. As can be seen from the data in Fig. 3, the pre-ozonated influent COD for the period of day 190 to 253 varied from 3 520 to 5 130 mg.L^{-1}. The COD reduction efficiency, however, remained stable between 90 and 92%. The increase in COD reduction efficiency and stability can probably be ascribed to the increased biodegradability of the wastewater due to ozonation (Gottschalk *et al.*, 2000). The VFA content in the UASB effluent was also lower and varied between 205 and 315 mg.L^{-1}. Such low VFA levels are considered to be an indication of an anaerobic digestion process performing efficiently (Shieh & Nguyen, 1997), effectively converting organic matter to methane and carbon dioxide. Alkalinity (*ca.* 1 400 mgCaCO$_3$.L^{-1}), influent pH (4.05 and 4.86) and effluent pH (6.78 and 7.10) remained relatively constant during this period (day 190 – 253) (Fig. 4). An increase in the biogas methane content to 54% was observed for the period day 190 – 253.

The increased efficiency of the UASB reactor in response to pre-ozonation of the substrate compares well with similar studies reported in the literature using ozonation as a pre-treatment to anaerobic digestion. Benitez *et al.* (1999) reported COD reductions of 20 – 30% for ozonated winery wastes, which also subsequently led to an increase in the methane yield during anaerobic digestion. Similarly, Martín *et al.* (2002) reported that vinasse pre-treated with ozone plus UV light in the presence of titanium dioxide, reduced the COD and TOC and resulted in the methane yield coefficient and mean specific rate of digestion to increase by 25% during anaerobic digestion. Pre-ozonation of a fruit cannery wastewater also improved the COD reduction efficiency of an UASB reactor from 61 – 92% (Chapter 5 of this dissertation), while pre-ozonation of a winery wastewater improved COD reduction efficiency of an UASB from 67 – 86% (McLachlan, 2004).

Pre- and post-UASB ozonation of wastewater and UASB effluents

The UASB reactor effluent varied between 517 and 1 150 mg.L^{-1} for the period between day 153 and 189 (Fig. 3 – period A to B). Post-ozonation of the UASB reactor effluent resulted in an average additional COD reduction of 28%, giving a final COD of 370 - 830 mg.L^{-1} (total COD reduction of between 81 and 90%). This reduction is similar to previous results where ozone was used as a post-treatment of UASB reactor effluents. In Chapters 4 and 5 of this dissertation COD reductions of 20 – 40% and 37% were obtained, treating effluents from fruit cannery and winery UASB's, respectively. McLachlan (2004) also reported COD reductions of 22.7% during the post-ozonation of UASB effluents treating winery wastewaters.

In this study it was found that the UASB effluent COD varied between 230 and 485 mg.L^{-1} during the period from day 217 – 253. Post-ozonation of these effluents represented the "pre- and post-UASB treatment" sample. Reductions in COD of the UASB effluent averaged 23%, resulting in effluents with COD's between 160 and 375 mg.L^{-1}. This further 23% COD reduction represents a total COD reduction (from the initial average raw wastewater COD of 4 982 mg.L^{-1}) of 93 – 96%.

Conclusions

The use of ozone as a combined pre- and post-UASB treatment option for winery wastewaters was shown to be feasible at pilot-scale. The 600 L pilot-scale UASB was shown to be effective in that a COD reduction efficiency of between 75 and 87% was maintained at varying organic loading rates (OLR's). Post-ozonation of the UASB reactor effluent was found to be effective in further reducing the COD, with a reduction of at least 28% being achieved. It was shown that at pilot-scale size pre-ozonation increased the biodegradability of the wastewaters, as the COD reduction efficiency of the UASB reactor increased, as did the biogas methane content. The COD reduction efficiency was found to remain stable even though the influent COD levels varied. This indicates a stable process, capable of functioning at conditions below optimum, making it ideal for wineries where activities generating wastewater vary from day to day.

It was found that the COD reduction efficiency of the pilot-scale UASB with pre- and post-ozonation was 93 – 96%. Although the COD was not lowered to below the South African legal limit of 75 $mg.L^{-1}$ for discharge to a natural water resource, the reductions did lower the COD to below the legal limit of 400 $mg.L^{-1}$ for irrigation (Republic of South Africa, 1999).

It has thus been shown, at pilot-scale, that the use of ozone as a pre- and/or post-treatment to anaerobic digestion is a feasible option for use by wineries generating wastewaters from the production of a variety of red and white wines and even some distilled products. Wastewater treatment efficiency can be improved, providing financial benefits to industries involved and contributes to the sustainability of our fresh water resources. One aspect that became clear while doing this specific study was the extended time period required to obtain an answer. Thus, it would be of economic value to have a method that can be used to determine the optimum parameters within a reasonable time period.

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CHAPTER 7

DETERMINATION OF THE OPTIMUM OZONATION PARAMETERS DURING PRE-TREATMENT OF WASTEWATERS FOR ANAEROBIC DIGESTION USING A DIRECT ACTIVITY TEST METHOD

Summary

An experimental protocol incorporating an adapted, simple, quick and accurate methanogenic activity test was developed. The protocol was developed to shorten the time required in order to be able to make predictions about the feasibility of a pre-treatment to anaerobic digestion. Conventional methods of testing a pre-treatment process are time-consuming. A winery wastewater was used in the process of evaluating the protocol. No significant ($p > 0.05$) differences were found between the three ozone concentrations used and no significant differences were found in COD reduction efficiency between the granules subjected to non-ozonated or ozonated substrates. Ozonation time, however, resulted in significantly ($p = 0.003$) lower granule activity, in terms of methane content (%), for granules subjected to a 10.0 min ozonated winery wastewater. The developed protocol can, thus be used to determine the feasibility of pre-ozonation to anaerobic digestion, provide indications as to the optimum ozonation concentration/time combination. This protocol can also be used to determine the effect of a wastewater on specific populations within the anaerobic granules.

Introduction

The treatment of food industry wastewaters has become an important aspect around the world as a result of the increased awareness and need to protect the environment and scarce water resources. Various successful treatment technologies have been investigated and implemented. Two technologies that have found increasing application are the biological anaerobic digestion and advanced oxidation processes, like ozone, often in combination.

The use of anaerobic digestion, specifically the upflow anaerobic sludge blanket (UASB) design, has been shown to be feasible for use in the food industry (Britz *et al.*, 2000). Similarly, the use of ozone to reduce the chemical oxygen demand (COD) content of treated food processing wastewaters has been reported (Beltrán *et al.*, 2001a; Beltrán *et al.*, 2001b), but very little research into the use of ozone as a pre-treatment to anaerobic digestion can be found in the literature.

It has been reported that anaerobic digestion of ozonized wastewater sludge enhanced methane production and accelerated the digestion rate (Weemaes *et al.*, 2000). In contrast, Martín *et al.* (2002) found that the COD and total organic carbon (TOC) of a vinasse wastewater decreased when treated with ozone plus ultraviolet light (in the presence of titanium dioxide) before anaerobic digestion. Ozone has also been used in combination with hydrogen peroxide (H_2O_2) and ultra-violet light (UV) in the treatment of winery waste before anaerobic digestion. COD reductions of 20 – 30% were achieved as was an increase in the methane yield (Benitez *et al.*, 1999). Andreozzi *et al.* (1998) found that ozonated olive oil mill effluents (OME) had a stronger inhibitory effect on methanogenesis than un-ozonated OME when treated with ozone prior to anaerobic digestion. The inhibitory effect was not observed for acidogenic bacteria. The use of ozone as a pre-treatment to anaerobic digestion was also investigated in Chapter 5 of this dissertation. Improvements in the COD reduction, reduction of short chain volatile fatty acids and an increase in the methane production were observed for the anaerobic digestion of a pre-ozonated winery wastewater. The effect of wastewater pre-ozonation over an extended period, on the performance of an UASB reactor, was also investigated and no detrimental effects were observed.

Evaluating the efficiency of a pre-ozonation treatment to anaerobic digestion is a timeous process. The set-up, start-up and stabilisation period required for an UASB reactor can take a few months (Britz *et al.*, 2002). As only one ozonation condition can be evaluated per reactor, this lengthens the time required to evaluate a series of ozonation conditions, even if parallel UASB reactors are used. Also, COD as a parameter of ozonation efficiency does not give an indication of the possible formation of inhibitory compounds. The ideal solution would be to have a rapid, easy to apply test procedure which could simultaneously: i) give an indication of the optimum ozone concentration and ozonation time combinations; ii) give an indication of the effect of pre-ozonation on COD reduction; and iii) give an indication of the effect of pre-ozonation on the activity of the biomass.

The measurement of the methanogenic activity of anaerobic degradation processes is important in order to classify its potential in converting soluble substrate to methane and carbon dioxide (James *et al.*, 1990). Various activity tests have been developed to determine the activity of anaerobic biomass, with most using the amount of biogas produced to characterise the methanogenic activity (Owen *et al.*, 1979; James *et al.*, 1990; Valcke & Verstraete, 1993; Lamb, 1995; Angelidaki *et al.*, 1998; Verstraete & Vandevivere, 1999). Some of these methods are inaccurate and time consuming and thus adaptations have been made to create a simple and accurate method (O'Kennedy, 2000).

The aim of this study was to develop a protocol which could be used to predict which ozonation time and ozone concentration combination would be most suited to be used as a pre-treatment to anaerobic digestion. This will be done by monitoring the COD reduction rate (biodegradability) and effect of ozonation on the direct activity of anaerobic granules.

Materials and methods

Experimental design

The study consisted of a design as given in Fig. 1 to measure the COD reduction achieved by identical masses of granules in wastewaters treated by different ozone concentration/time combinations (four ozonation times and three ozone concentrations). The activity of the granules subjected to the various ozonated wastewaters was compared to the activity of the same granules subjected to un-ozonated wastewater (control). This procedure was done in triplicate for statistical verification.

Wastewaters

Wastewater was obtained from a large winery (Bergkelder, Distell, Stellenbosch) producing a variety of red and white wines. All wastewater generated during the various cellar operations was channeled to a central effluent tank. Wastewater was sampled from this tank for use in the study. The winery wastewater obtained had an average COD of 7 400 $mg.L^{-1}$ (7 290 – 7 550) and a pH of 5.55 – 5.70. The COD of the raw winery wastewater (control substrate), as well as the ozonated wastewater was adjusted to 4 800 $mg.L^{-1}$ by dilution with distilled water, and the pH poised at 6.00 with 1 M KOH. Dilution to 4 800 $mg.L^{-1}$ was done in order to facilitate direct comparisons between ozonated and non-ozonated substrates.

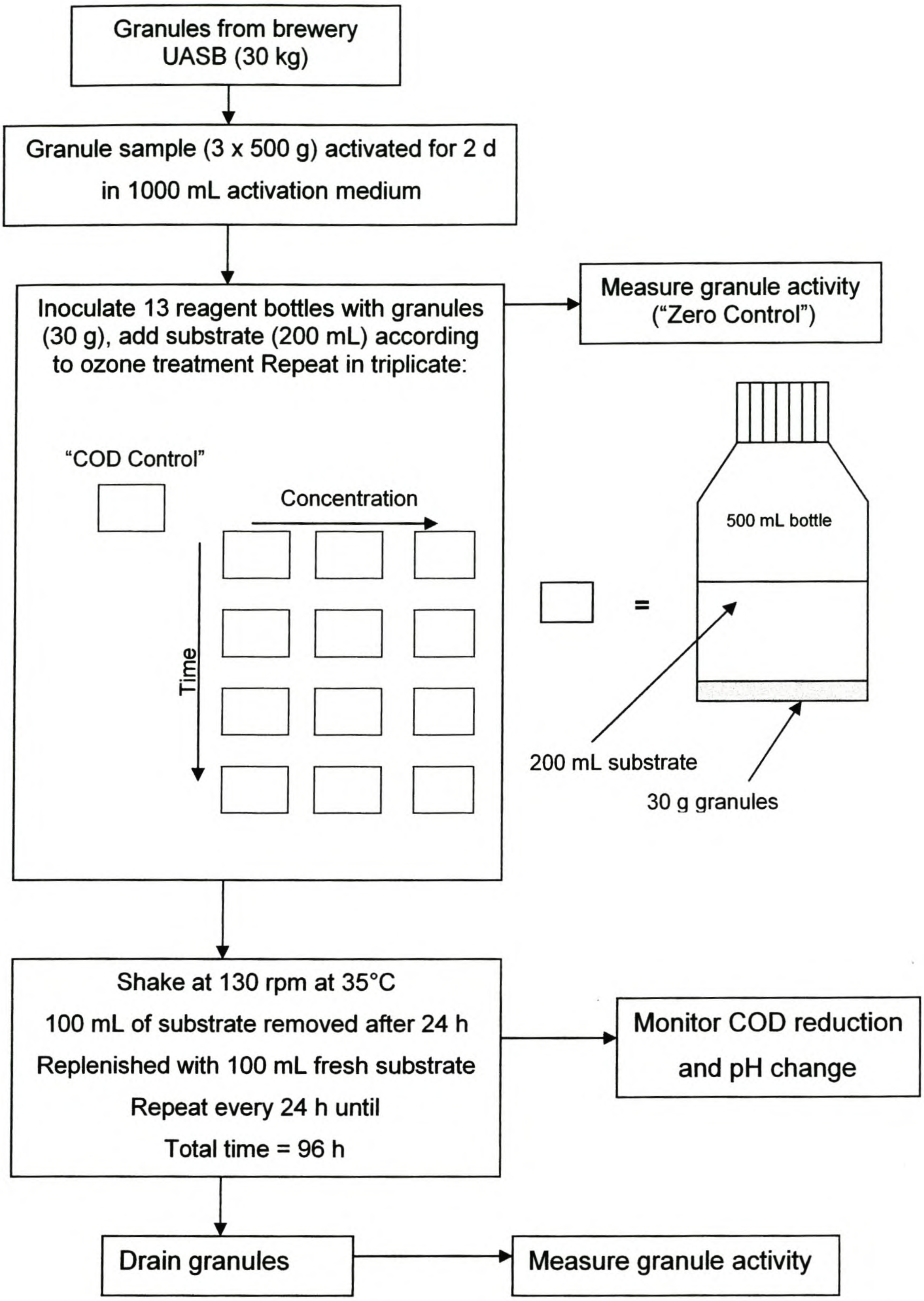

Figure 1. Flow diagram of the experimental procedures for different ozone concentration/time combinations.

Experimental procedures

A flow-diagram of the experimental procedures is shown in Fig. 1. A 1 500 g sample of granules of a mixed 30 kg granule batch from an industrial-scale UASB treating brewery wastewater was used in the study. The 1 500 g granule sample was thoroughly mixed and sub-divided into three equal 500 g batches which were kept at 4°C until required. Each 500 g granule batch was activated for two days in a 1 000 mL activation medium to standardise and acclimatise the granules. The activation medium consisted of ($g.L^{-1}$): 1.0 glucose, 0.5 KH_2PO_4 and 0.5 urea and activation was done at a temperature of 35°C before the trial began. After activation, the 500 g granule sample was thoroughly mixed and 30 g of granules were placed in 13 separate reagent bottles (500 mL). The original granule activity was also determined at this point and was taken as the "zero control" activity. Non-ozonated winery wastewater (200 mL) was added to one of the reagent bottles ("COD control"), while each of the remaining 12 reagent bottles were filled with 200 mL of ozonated winery wastewater, differing only in the ozonation treatment received, as outlined in Table 1. The inoculated bottles were then placed on a laboratory shaker (130 rpm), to facilitate maximum contact between granules and substrate, and incubated at 35°C for a period of 96 h. During this "COD reduction trial", 100 mL of substrate was removed from each reagent bottle at 24 h intervals after settling and replaced with newly prepared substrate (Fig. 1). The COD and pH of the daily removed aliquots were measured. The COD reduction in each bottle was monitored over the 96 h period, taking into account the amount of COD removed in every 24 h cycle as well as the amount of COD added with the 100 mL replenishment every 24 h. On completion of the 96 h COD reduction trial, all granules from the reagent bottles were individually drained. The drained granule sets were then subjected to direct activity tests. The 96 h COD reduction trial and subsequent activity testing was performed in triplicate.

Direct activity tests

Activity tests were performed on each of the 13 granule sets ("COD control" and 12 ozonated substrates) using three different activity media (basic test medium (BTM), glucose medium and an acetic acid medium) according to the method reported by O'Kennedy (2000). The measured activity was taken as the direct activity, without any further acclimatisation or stabilisation period.

The activity of the "COD control" served a dual function. Firstly, it was

Table 1. Different ozone concentration and ozonation time combinations used to make up the different substrates used in the study.

Ozone concentration (mg.L^{-1})	Ozonation time (min)			
13	1.0	2.5	5.0	10.0
19	1.0	2.5	5.0	10.0
38	1.0	2.5	5.0	10.0

Table 2. Composition of the Basic Test Medium (BTM) as used by O'Kennedy (2000).

Compound	Concentration (g.L^{-1})
Glucose	2.0
K_2HPO_4	1.0
KH_2PO_4	2.6
Urea	1.1
NH_4Cl	1.0
Na_2S	0.1
$MgCl_2 \cdot 6H_2O$	0.1
Yeast Extract	0.2
pH	7.1

Table 3. Composition of the different test media used to measure activity of specific microbial groups (O'Kennedy, 2000).

Test medium*	Bacterial group
Only BTM	Control
2 g.L^{-1} Glucose	Acidogens
1 g.L^{-1} Acetic acid	Acetoclastic Methanogens

* The Basic Test medium was used as basic component of each medium

used to compare the granule activity after the 96 h period in the winery wastewater with the "zero control", and secondly, to serve as a reference for the comparison of activities between the granule sets that had been subjected to the various ozonated substrates.

The basic test medium (BTM – Table 2) was used to measure general activity, while the glucose medium (Table 3) was used to measure the specific activity of the acidogens and the acetic acid medium (Table 3) measured the activity specifically of the acetoclastic methanogens.

After the 96 h COD reduction trial (Fig. 1), drained (but not washed) triplicate granule samples (3 g) were placed in 20 mL vials, to which 13 mL of the appropriate medium was added , leaving *ca*. 6 mL headspace. The vials were sealed with butyl septa and capped with aluminium caps and incubated for 10 h at 35°C. The vials were gently mixed at regular intervals during the incubation period to ensure that no substrate limitation occurred due to mass transfer restrictions. The volume of biogas produced was measured after 5 and 10 h by using a free moving gas-tight 10 mL syringe equipped with a 12 gauge needle. The needle was inserted through the butyl septa and the plunger was allowed to move freely until equilibrium had been reached. The composition of the biogas was determined gas chromatographically.

Statistical analysis

The data obtained during the 96 h COD reduction trial and the data obtained from the activity tests (biogas production and methane content) were analysed by a three-way repeated measures analysis of variance. Non-parametric bootstrap techniques were also used to verify some of the results.

Ozonation

Ozonation of the winery wastewater was done in a continuous mode, bubble contacting system. Ozone was bubbled upwards through a glass column for the duration of the ozonation treatment. The different combinations of ozone concentration and ozonation time are given in Table 1. The bubbling column (l = 90 cm; ϕ = 6 cm) had a sintered glass disc at one end for bubble generation. An ozone generator (Parc Scientific, Ifafi) capable of producing 9.0 $g.h^{-1}$ O_3 was used for the ozonation trials. Ozone concentrations of 13, 19 and 38 $mg.L^{-1}$ were used during this study.

Analytical methods

The ozone production was measured in triplicate using an iodiometric titration (Standard Methods, 1998). The COD concentration was determined colorimetrically using a DR2000 spectrophotometer (Hach Co. Loveland, CO) and standardised procedures (Standard Methods, 1998). Biogas was determined gas chromatographically according to the method described in Chapter 5 of this dissertation. All analyses were done in triplicate.

Results and discussion

Effect of pre-ozonation on granule COD reduction efficiency

The data obtained in this study clearly showed that with the ozone concentration/time combinations studied, no significant differences ($p = 0.94$) could be found between the different ozone concentrations. This can be seen from the effect of the different ozone concentrations on the COD reduction efficiency of the granules as shown in Fig. 2. It was found that the COD reduction efficiency for all three ozone concentrations followed the same pattern as that of the COD control. The data for the different ozone concentrations was thus pooled, to enable the effect of the different ozonation times (with pooled ozone concentration data) on the COD reduction efficiency to be determined and compared to the COD control.

The effect of different ozonation times on the COD reduction efficiency of the granules over 96 h is shown in Fig. 3. It can be seen that the COD reduction efficiency did not differ significantly ($p = 0.056$) for the four ozonation times and the COD control over the 96 h period. In fact, the COD reduction efficiency over 96 h for the four ozonation times was very similar to that of the COD control. The COD reduction efficiency for the COD control and ozone treatments did, however, increase over the 96 h period, with COD reduction efficiencies of *ca*. 55% being achieved (Fig. 3).

It can be seen from the data in Figs. 2 and 3 that the COD reduction showed a gradual increase after the first 24% obtained during the 24 h period to *ca*. 55% after 96 h. The data shows that the improvement in COD reduction for the successive 24 h periods, however, decreased. This could be due to a natural process of acclimatisation to the substrates or possibly due to the accumulation of "difficult to degrade" compounds. It is possible that during the 24 hourly replacement of half of

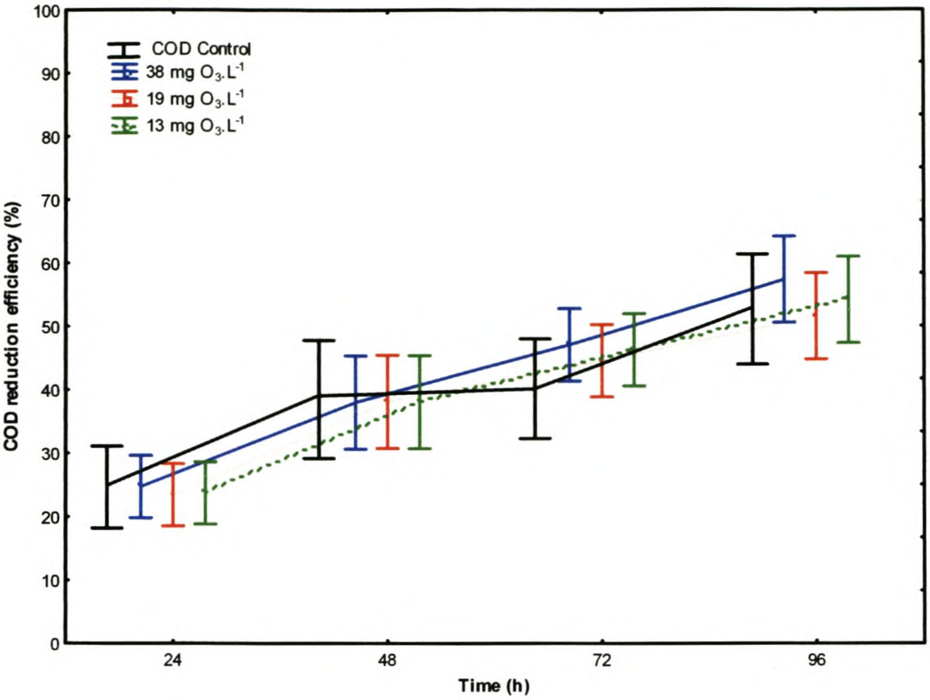

Figure 2. Effect of ozone concentrations on the COD reduction efficiency of anaerobic granules subjected to ozonated winery wastewater (vertical bars indicate 95% confidence intervals).

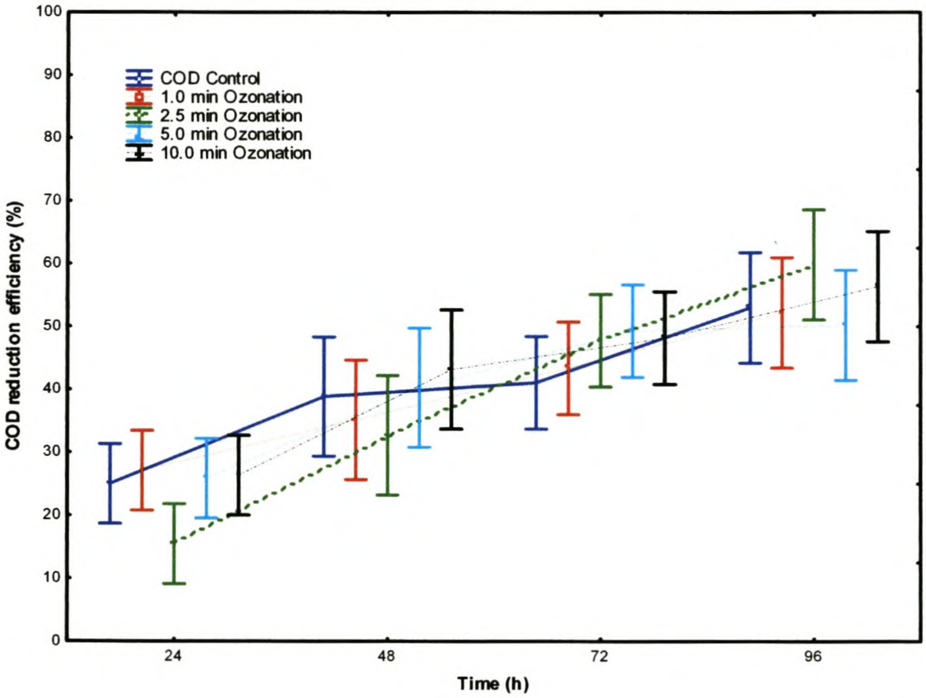

Figure 3. Effect of ozonation times on the COD reduction efficiency of anaerobic granules subjected to ozonated winery wastewater (vertical bars indicate 95% confidence intervals).

the substrate (100 mL), an accumulation of "difficult to degrade" compounds took place in the remaining substrate, thus leading to a reduced COD reduction rate for each successive 24 h period.

The COD reduction efficiency over 96 h thus does not give a clear indication of optimum ozone concentration/ozonation time as no significant differences were found between the ozone concentrations or ozonation times. Positive or negative effects of ozonating substrates may be difficult to detect in such a short period by COD reduction monitoring alone, especially in an already nutrient deficient wastewater. In difficult to degrade wastewaters COD reduction rates may already be slow, and may thus make comparisons difficult. COD reduction alone may not be able to indicate subtle shifts in activity that indicate inhibition or toxicity. Thus, an alternative method of determining optimum ozone concentration/time combination is necessary in which direct granule activity can be compared under more ideal conditions than in a nutrient deficient (like winery wastewater) or recalcitrant substrate.

These findings are in contrast to previous research which showed ozonation to be effective in increasing the COD reduction efficiency during anaerobic digestion of winery wastewaters. Benitez *et al.* (1999) used ozone in combination with hydrogen peroxide (H_2O_2) and ultraviolet light (UV) to treat winery wastewater (COD = 18 500 $mg.L^{-1}$) before anaerobic digestion and achieved COD reductions between 20 and 30%. However, in their studies ozonation times of up to 8 h were used at unknown ozone concentrations. During anaerobic digestion of these wastewaters an increase of 15% in the methane yield was observed. Martín *et al.* (2002) reported that the anaerobic digestion of vinasse (COD = 97 500 $mg.L^{-1}$) pre-treated with ozone (34 mg $O_3.L^{-1}$) and UV-light in the presence of titanium dioxide resulted in an increased methane yield of 25 – 48% and also an increase of 25% in the mean specific rate of methane production. In Chapter 5 of this dissertation, the pre-ozonation of a winery wastewater before anaerobic digestion resulted in the COD reduction efficiency being improved from 91 to 98%. Similarly, pre-ozonation of a peach cannery wastewater before anaerobic digestion resulted in the efficiency of the UASB reactor improving from 61% COD reduction to above 85%, indicating an increase in the biodegradability of the wastewater by pre-ozonation. Pre-ozonation in this case resulted in an enhanced biodegradability of the wastewater being treated by anaerobic digestion. A rapid, easy to apply test procedure to indicate changes in biodegradability would, however, be beneficial.

To determine whether the stabilisation of the COD reduction efficiency observed over the 96 h COD reduction trial was due to natural acclimatisation or due to an accumulation of "difficult to degrade" compounds, a further exploratory COD reduction trial was conducted. In this case granules were subjected to a COD control and to an ozonated substrate for 168 h. The total substrate volume (200 mL) was replenished every 24 h (instead of 100 mL at a time), to try and negate the accumulation of "difficult to degrade" compounds.

A similar increase in COD reduction efficiency (results not shown) was observed over 96 h, with COD reductions of *ca.* 50% again being achieved. Further COD increases were minimal, however, and the COD reduction efficiency after 168 h stabilised at *ca.* 56%. This would suggest that the stabilisation in COD reduction efficiency over time (observed in both trials) was due to a natural acclimatisation to the substrate. This again confirmed the necessity of a method in which direct granule activity can be compared.

Effect of pre-ozonation on granule activity in terms of biogas and methane production

The initial activity of the brewery wastewater UASB granules was determined ("zero control") before the granules were sub-divided and subjected to the 96 h COD reduction trial (Fig. 1). After the trial the direct activity of the granules from the "COD control" and 12 ozonated substrates, was determined. The activity of the "COD control" and ozone treatments were significantly ($p < 0.05$) higher than the "zero control" (results not shown). This was expected, however, as the granules used in the "COD control" and ozone treatments had a period of 96 h to acclimatise to the specific substrates. The "COD control" activity was used hereafter as reference for all further granule activity comparisons.

The data for activity tests showed no significant ($p \geq 0.63$) differences between the three ozone concentrations over time for biogas production or methane content (%), and thus the data for the ozone concentrations was pooled. The data for the four ozonation times (with the pooled ozone concentration data) was used to compare the granule activity. The BTM was used to measure the overall activity of the granules and is not specific for any bacterial group, while the BTM + glucose and BTM + acetic acid were used to measure the activity of specifically the acidogens and acetoclastic methanogens, respectively. No statistically significant differences ($p > 0.05$) in the volume of biogas produced could be observed between the COD

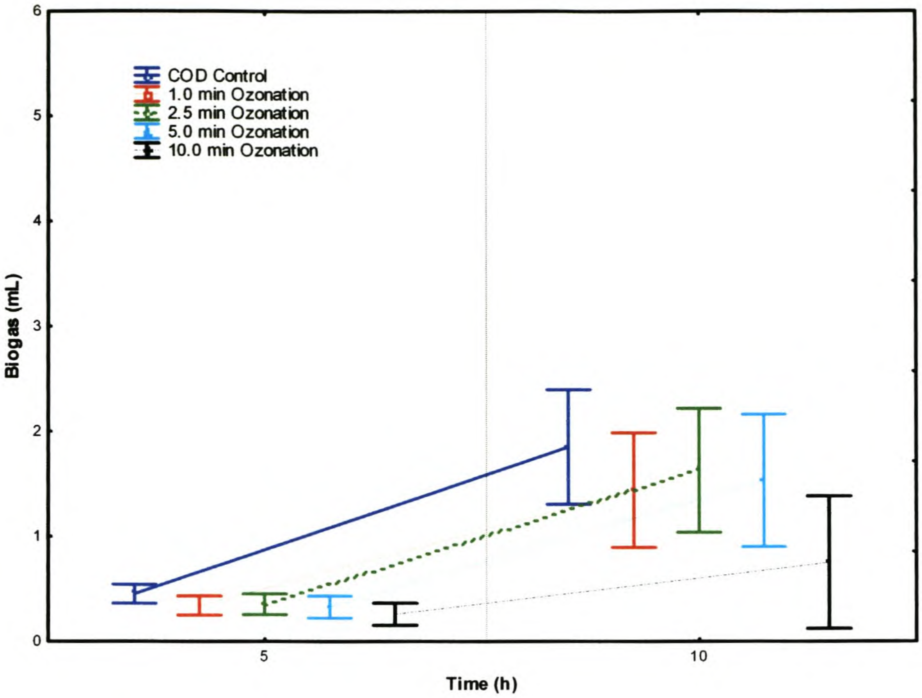

Figure 4. Effect of ozonation time (ozone concentration data pooled), in terms of biogas production, on granule activity in the BTM (vertical bars indicate 95% confidence intervals).

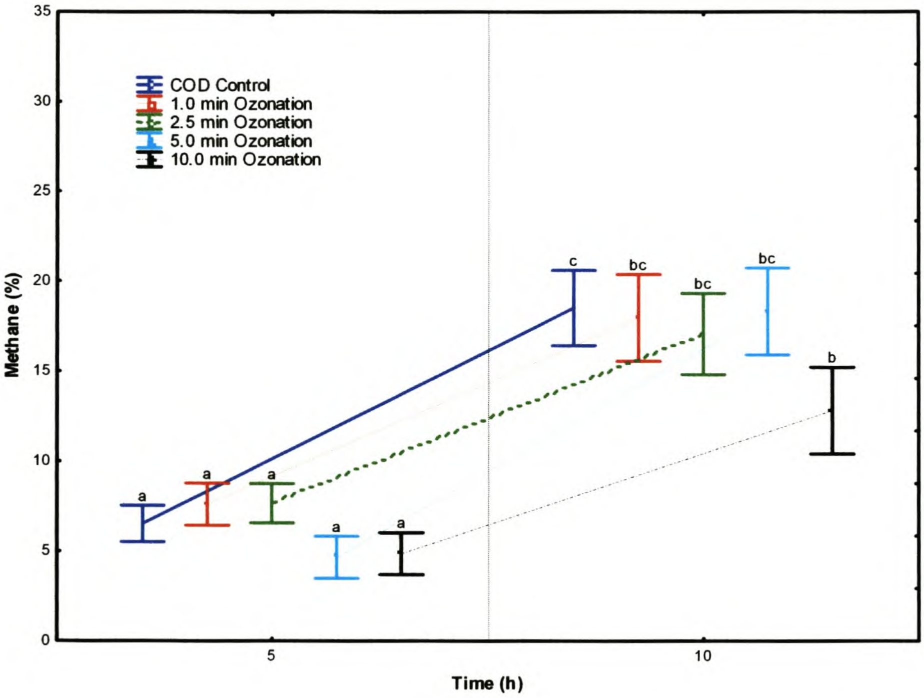

Figure 5. Effect of ozonation time (ozone concentration data pooled), in terms of methane (%), on granule activity in the BTM (vertical bars denote 95% confidence intervals; letters indicate 5% significant differences).

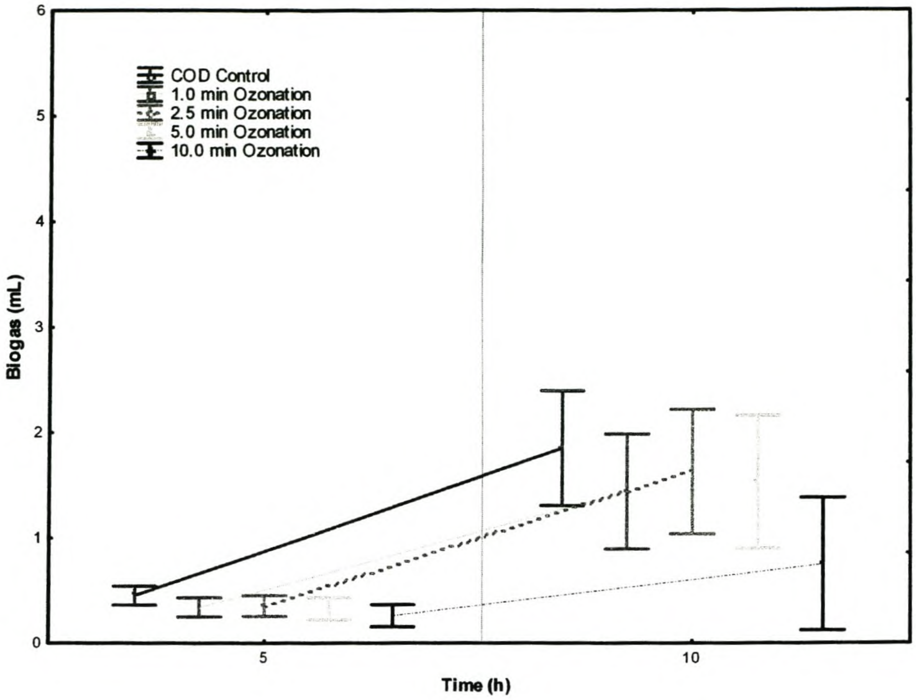

Figure 4. Effect of ozonation time (ozone concentration data pooled), in terms of biogas production, on granule activity in the BTM (vertical bars indicate 95% confidence intervals).

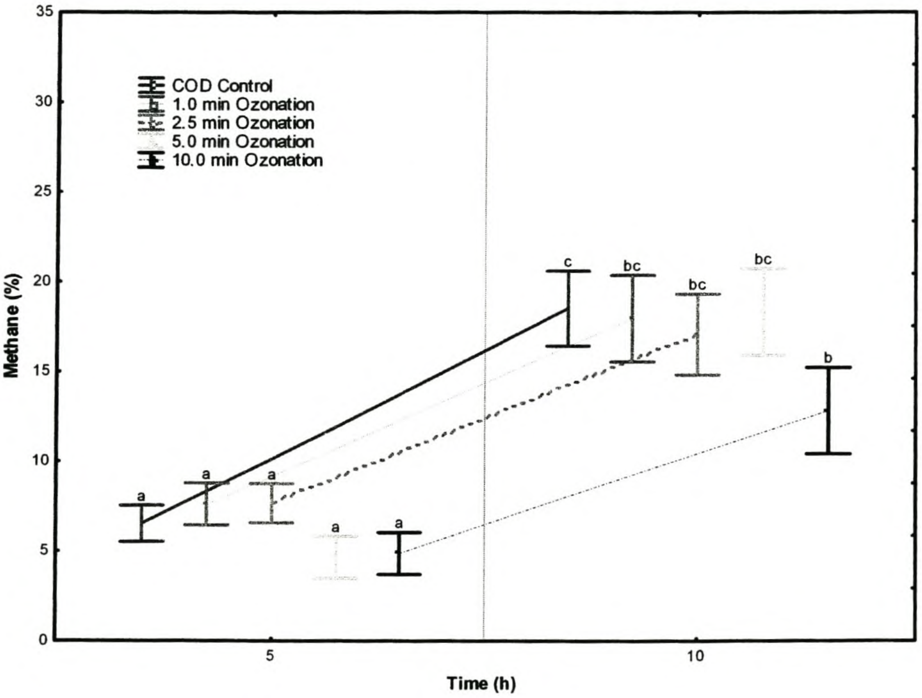

Figure 5. Effect of ozonation time (ozone concentration data pooled), in terms of methane (%), on granule activity in the BTM (vertical bars denote 95% confidence intervals; letters indicate 5% significant differences).

control and the ozonation treatments after 5 and 10 h incubation using BTM (Fig. 4), BTM + glucose or BTM + acetic acid media (results not shown). It was observed, however, that the volume of biogas produced by granules subjected to a 10 min ozonated substrate was lower than the other ozonation times and COD control in all three media, but not statistically significant ($p > 0.05$). Variations in biogas volume between triplicate repititions for each treatment were also reasonably large, as can be seen from the error bars in Fig. 4, possibly negating any statistical differences between different ozonation times. A possible reason for this could be due to the fact that biogas volume was determined by measuring the volume displacement in a free moving syringe inserted into the activity test vial. By increasing the accuracy of the biogas volume measurement (and thus the reproducability/repeatability) the differences in biogas production between the granules subjected to different substrates might be more significant.

The methane content (%) of the biogas produced in the activity test using only BTM for the control and the four ozonation times is shown in Fig. 5. No statistically significant differences ($p > 0.05$) in methane content (%) could be observed after 5 h incubation, although the 5.0 and 10.0 min ozonations resulted in a lower methane content (%). After 10 h incubation the activity of granules that had been subjected to substrates ozonated for 10 min, showed a significantly ($p = 0.003$) lower activity in terms of methane (%) content compared to the COD control, 1.0, 2.5 and 5.0 min ozonation treatments (Fig. 5). This was in accordance with the tendency (not statistically significant) observed during the activity measurement in terms of biogas production, in which the 10.0 min ozonation resulted in lower activity (Fig. 4). The lower activity as a result of the 10.0 min ozonation suggests that longer ozonation times are detrimental to anaerobic activity. The data for the methane content (%) of the biogas produced in the activity test using the BTM + glucose and BTM + acetic acid (specifically for the acidogens and acetoclastic methanogens, respectively) showed no statistically significant differences ($p > 0.05$) between the COD control and four ozonation times (data not shown). The 10.0 min ozonation time did, however, in both media show a slightly lower activity, in terms of methane content (%), than the COD control and other ozonation treatments, after the 10 h incubation, again indicating that longer ozonation times are detrimental to anaerobic activity. This could possibly be the result of a longer ozonation time leading to the formation of inhibitory compunds by reaction with compounds such as polyphenols (Andreozzi *et al.*, 1998).

Conclusions

The BTM was used to measure the overall granule activity and not any specific group of bacteria present in the granules. Thus, it was evident that a 10 min pre-ozonation of the winery wastewater was detrimental to granule activity (Figs. 4 and 5), especially in terms of methane content (%). The methane content (%) was significantly lower when granules had been subjected to a 10.0 min ozonated substrate. When measuring the activity specifically for the acidogens and acetoclastic methanogens in the population (BTM + glucose and BTM + acetic acid, respectively), the activity in terms of biogas production and methane content (%) for the different ozonation times did not differ significantly from one another or the COD control. It was observed, however, that the activity in terms of biogas and methane content (%) tended to be lowest for the 10 min ozonation, suggesting that longer (> 5.0 min) ozonation times should be avoided. Differences in the 1.0, 2.5 and 5.0 min ozonation times were not significant and thus the choice of the lowest ozonation time would be most economically viable.

From the results of the activity tests it is clear that deductions can be made as to the optimum ozonation conditions for winery wastewater prior to anaerobic digestion and the resultant effect on granule activity. The ozone concentrations used (13, 19 and 38 $mg.L^{-1}$) did not differ significantly in the results obtained. Ozonation time did, however, have an effect on the activity of the granules, especially a 10.0 min ozonation. The reaction with compounds such as polyphenols could result in the formation of inhibitory compounds. It has been previously reported that wastewaters containing polyphenol compounds could, during ozonation, give rise to the formation of byproducts that are more inhibitory than the original compounds. Andreozzi *et al.* (1998) treated olive oil mill effluents (OME) with ozone prior to anaerobic digestion. Olive oil mill effluents are mainly composed of sugars, proteins and polyphenols (Andreozzi *et al.*, 1998) and it is mainly lipids and polyphenols that are responsible for the toxicity of the wastewaters (Hamdi, 1993). It was found that the ozonated OME had a stronger inhibitory effect on methanogenesis than un-ozonated OME. This inhibitory effect was, however, not observed for the acidogenic bacteria (Andreozzi *et al.*, 1998). These findings correlate with results obtained in this study, where acidogens were less sensitive than methanogens, in that the most significant differences in activity were found when measuring the methane content (%) of the biogas.

Due to the ozone concentrations (13, 19 and 38 $mgO_3.L^{-1}$) not differing significantly, probably due to the small range of ozone concentrations used, the differences due to different ozonation times were not always significant, but a distinctive trend in the ozonation times was, however, observed. Use of a more accurate and reproducible means of measuring the volume of biogas produced should be investigated further. Improved accuracy in this regard may decrease the variation in the triplicate determinations and may further elucidate differences in activity in terms of biogas produced.

This study has shown that the developed protocol can be used to determine differences in activity of anaerobic granules as a result of different combinations of concentration and time of pre-ozonation and thus identify the optimum combination. This was done by subjecting anaerobic granules to various pre-ozonated winery wastewaters, monitoring COD reduction efficiency over time and measuring the direct activity of the granules. The direct activity tests, however, provide more conclusive indications than the COD reduction trial. The developed protocol has also shown that methanogenic activity is affected more by pre-ozonation of a winery wastewater than the acidogenic activity. The developed protocol can, thus be used to determine the feasibility of pre-ozonation to anaerobic digestion, as well as providing indications as to the optimum ozonation concentration/time combination. This protocol can also be used to determine the effect of a wastewater on specific populations (fermentative and acetogenic methanogens) within the anaerobic granules. It is also possible to make these deductions in a far shorter space of time than with conventional reactor methods.

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CHAPTER 8

GENERAL DISCUSSION AND CONCLUSIONS

Background

Anaerobic digestion as a food and beverage industry wastewater treatment option has been implemented with success on a global scale. This is probably due to the fact that anaerobic digestion has several advantages over conventional treatment methods. The use of ozone in the treatment of wastewaters has also been shown to be effective in reducing COD, removing colour and making compounds more biodegradable. Very little research is to be found in the literature where ozone has been used as pre-treatment to anaerobic digestion. In the few cases studied, pre-ozonation was reported to enhance the efficiency of the anaerobic digestion process (Benitez *et al.*, 1999; Martín *et al.*, 2002).

The objectives of this study were firstly, to assess the applicability of the UASB process to treat fruit cannery and winery wastewaters. Secondly, to investigate the possibility of combining advanced oxidation processes (AOP's) such as ozonation, as pre- and post-treatment steps to anaerobic digestion. Thirdly, to scale up the laboratory UASB design to a 600 L pilot-scale, treating a fruit cannery or winery wastewater and, fourthly, to develop a method using an activity test to predict the optimum ozone concentration/time combination required to improve wastewater biodegradability.

UASB treatment of a highly alkaline fruit cannery lye-peeling wastewater

Fruit canning processes generate large volumes of highly alkaline wastewater. This is the result of the excessive use of caustic soda (NaOH) during the lye-peeling process. Research was thus conducted to determine the feasibility of using anaerobic digestion to treat the highly alkaline, sodium containing cannery wastewater. The raw alkaline wastewater had a COD load of up to 50 000 $mg.L^{-1}$, a sodium (Na^+) concentration of *ca.* 40 000 $mg.L^{-1}$ and an initial pH of between 12.5 and 13.5. An UASB reactor with an operational volume of 2.3 L was used to treat a diluted, pH adjusted alkaline lye-peeling wastewater. The study was phased into an acclimatisation stage, a stabilising stage, a gradually increasing OLR stage and followed by a constant OLR stage.

After the initial acclimatisation and stabilisation stages the UASB reactor achieved COD reductions of between 85 and 96% at an OLR of 2.2 $kgCOD.m^{-3}.d^{-1}$. During gradual increases in the OLR to 8.1 $kgCOD.m^{-3}.d^{-1}$, the COD reduction remained at an average of 90% and biogas production increased to 4.1 $L.d^{-1}$. After 111 d of operation the COD reduction efficiency, effluent pH, biogas and methane content started decreasing. The COD reduction efficiency dropped to 76% by day 120 and to 22% by day 143. The effluent pH dropped to 4.89 by day 143 and the biogas methane content decreased to 22%. Attempts to stabilise the reactor by reducing the OLR were unsuccessful, indicating reactor failure.

The difference between influent (up to 7 300 $mg.L^{-1}$) and effluent Na^+ (between 6 800 and 7 200 $mg.L^{-1}$) concentrations indicated that an accumulation of Na^+ had taken place in the reactor. Although sodium in low concentrations has been reported to be essential for methanogens, high sodium concentrations (>15 000 $mg.L^{-1}$) have been shown to be inhibitory to the anaerobic digestion process, especially to the methanogens (Feijoo *et al.*, 1995; Kim *et al.*, 2000). In this study the level of accumulated sodium in the reactor could readily have reached levels of > 20 000 $mg.L^{-1}$. It is thus possible that the accumulation of sodium within the reactor had led to the inhibition of the methanogenic population leading to a rapid decrease in methane production and subsequent acidification of the system as volatile fatty acids accumulated. This would also explain the decrease in effluent pH.

The results of this phase of the study clearly showed that anaerobic digestion of the organic fraction present in fruit cannery wastewaters is a feasible option, with 95% COD reductions being achieved at an influent COD of 8 200 $mg.L^{-1}$ and HRT of 24 h. It also appears that the microbial consortium involved could acclimatise to the higher sodium levels in the wastewater but accumulation of sodium over time, however, was detrimental, with the methanogens being most sensitive. It is thus recommended that sodium levels be kept as low as possible to minimise accumulation within the UASB reactor and thus minimising the toxic effects. Dilution of the alkaline lye-peeling wastewater by mixing with other cannery wastewaters might thus be advisable, to dilute the continuous high Na^+ concentrations of lye-peeling wastewaters. The presence of lower Na^+ levels in the treated wastewater would also be beneficial when disposing by irrigation. It appears as if the methanogens are more sensitive to higher sodium levels, as the biogas and methane percentage were the first parameters to change. Decreases in methane production could thus be an early warning system of sodium accumulation or toxicity and should thus be closely monitored in systems treating wastewaters containing high sodium concentrations.

Use of O_3 and H_2O_2 in the post-treatment of UASB treated alkaline fruit cannery wastewater

As discussed above anaerobic digestion of the alkaline fruit cannery wastewater was shown to be a feasible option, but final COD levels in the reactor effluent were still above the legal limit of 75 $mg.L^{-1}$ for discharge to a natural water system. The use of ozone (O_3), hydrogen peroxide (H_2O_2) and granular activated carbon (GAC) separately and in combination as post-treatment steps to the anaerobic digestion were thus investigated to try and further reduce the COD content.

During the anaerobic treatment of the alkaline cannery wastewater the COD was reduced by 90 – 93%. The reactor effluent had a pH of 7.0 – 7.2 and a COD concentration of 315 – 450 $mg.L^{-1}$. This reactor effluent was used in the oxidation studies. Treatment of the reactor effluent with only H_2O_2 resulted in colour and COD reductions of 92 and 26%, respectively, in a 24 h reaction period. Ozone was found to be effective in reducing the COD of the effluent and increases in ozonation time led to increases in COD reduction, from 25% (for a 5 min ozonation) to 53% (for a 60 min ozonation).

Combination of ozonation and H_2O_2 treatment resulted in slightly higher COD reductions than either oxidant separately, with improvements of between 1 and 2% for the different treatments. A 24 h reaction with H_2O_2 prior to ozonation again resulted in slightly higher COD reductions. These results were to be expected as O_3 and H_2O_2 in combination lead to the formation of more hydroxyl radicals than produced separately, creating a stronger oxidising capacity.

The use of granular activated carbon was successful in reducing the COD of the UASB reactor effluent by up to 81%. The use of O_3 and H_2O_2 prior to filtration of the effluents resulted in 89 – 91% of the COD in the reactor effluent being adsorbed by the GAC. This improvement in COD adsorption is probably due to the partial oxidation of certain molecules during O_3 or O_3/H_2O_2 treatment. According to Camel & Bermond (1998) the partial oxidation leads to the formation of lower molecular weight compounds which are better adsorbed on the GAC.

Combination post-treatments with O_3, H_2O_2 and GAC were thus successful in reducing COD levels of the UASB reactor effluent by up to 91% with a final COD of 28 – 41 mg.L^{-1} remaining. Combinations of O_3 and H_2O_2 were only slightly more effective than either O_3 or H_2O_2 alone, and adsorption on GAC was increased by prior oxidation treatments.

Reductions in COD of up to 91% of the UASB effluent for this type of wastewater can be instrumental in attaining COD levels close to or below the 75 mg.L^{-1} legal limit (Republic of South Africa, 1999) for discharge to a natural watercourse.

Efficacy of ozone as a pre- and post-treatment to the laboratory-scale anaerobic digestion of fruit cannery and winery wastewaters

Due to the positive results obtained using oxidation treatments, especially with ozone, as a post-treatment to anaerobic digestion, it was decided to further investigate ozonation (with GAC column) as a post-UASB treatment for other food processing wastewaters. It was also decided to investigate the feasibility of using ozonation as a pre-treatment to anaerobic digestion. The main aim of this was to determine the effect of ozonation on the biodegradability of wastewaters in an UASB reactor.

Short-term efficiency of ozone as a pre- and/or post-treatment to anaerobic digestion

During the anaerobic treatment of the cannery wastewater, the average influent COD of 7 500 mg.L^{-1} was reduced by 87 – 89% at a HRT of 24 h, producing effluent with COD concentrations between 818 and 983 mg.L^{-1}. Similarly, anaerobic digestion of winery wastewaters (average COD of 3 700 mg.L^{-1}) resulted in COD reductions of 89 – 92% and effluents with a COD concentration of between 303 and 400 mg.L^{-1}.

Further oxidation post-treatment of effluents from UASB's treating fruit cannery and winery wastewaters resulted in colour reductions. These colour reductions were improved by extended treatment times. The efficiency of colour reduction was slightly improved by the addition of 1% v/v H_2O_2 to the effluent prior to ozonation and a maximum colour reduction of 90% was obtained.

The oxidation post-treatments were also found to be effective in reducing the total COD of the UASB effluents. Longer ozonation times again resulted in increased COD reduction with total COD reductions after 30 min of up to 96 and 97% being achieved in the UASB treated cannery and winery effluents, respectively. The addition of 1% v/v H_2O_2 to the effluents prior to ozonation resulted in slight increases of *ca*. 1% in COD reduction. These small increases in COD reduction are not considered to be economically viable. Further oxidation treatments were done using only ozone as oxidant.

Combining pre- and post-ozonation treatments with the anaerobic digestion of the cannery and winery wastewaters resulted in better colour reduction than was found for either the UASB or the ozonation alone. This was expected and maximum colour reductions of 90 and 91% were obtained for the pre- and post-UASB ozonation of cannery and winery wastewaters, respectively. A similar trend was observed for COD reduction by combinations of ozonation and UASB treatment. A combination of pre- and post-UASB ozonation was found to be most effective in reducing the wastewater COD and reductions of 95 and 99% were achieved for cannery and winery wastewaters, respectively. Furthermore, pre-UASB ozonation was slightly more effective than post-UASB ozonation, which was slightly more effective than UASB and ozonation alone. Final COD values for the pre- and post-treated wastewaters were on average 375 $mg.L^{-1}$ for the cannery wastewater and 41 $mg.L^{-1}$ for the winery wastewater. The latter COD value falls well below the South African legal limit of 75 $mg.L^{-1}$ for discharge to a natural water system (Republic of South Africa, 1999).

Long-term impact of ozone pre-treatment on the performance of an UASB reactor

The results of this study showed that pre-ozonation improved the short-term efficiency of the anaerobic digestion process in UASB reactors treating fruit cannery and winery wastewaters. It was thus decided to investigate the longer-term effects of wastewater pre-ozonation on the efficiency of an UASB reactor treating a fruit cannery wastewater. COD reduction efficiency improved within 3 d of pre-ozonated feed commencing and increased from *ca*. 61% to between 85 and 90% within 14 d. The COD reduction efficiency remained relatively constant between 85 and 92% until the end of the study (60 days later) even though the OLR was increased from 2.4 to 3.4 $kgCOD.m^{-3}.d^{-1}$ for the last 14 d. The VFA concentration in the reactor effluent also decreased slightly after pre-ozonated wastewater was fed to the reactor, decreasing form *ca*. 330 to 230 $mg.L^{-1}$. The VFA concentration did, however, increase slightly when the OLR was increased but this was expected.

The average UASB reactor effluent COD for the last two weeks of the trial was 368 mg.L^{-1}, which represented a 94% reduction of the initial peach canning wastewater COD (6 500 mg.L^{-1}). This final COD value is below the legal limit of 400 mg.L^{-1} for irrigation, but further treatment is necessary to lower the COD to below the 75 mg.L^{-1} limit for discharge to a natural water source (Republic of South Africa, 1999). Post-ozonation of the UASB reactor effluent resulted in a total COD reduction of 99%, producing a final effluent with a COD of 55 mg.L^{-1}, which was well within the legal limit. These results substantiate previous results obtained by other authors (Benitez *et al*., 1999; Martín *et al*., 2002) who found that pre-ozonation of substrates resulted in improved biodegradability during anaerobic digestion.

By using a combination of pre- and post-treatment, significant improvements in the wastewater treatment efficiency can be achieved, which could be beneficial financially and environmentally.

Pilot-scale (600L) UASB treatment of a winery wastewater combined with pre- and post-ozonation

The efficiency of using ozone as a pre- and post-treatment to UASB was shown to be feasible at laboratory-scale and increases in COD reduction efficiency and methane yield were achieved. These studies were, however, done at laboratory-scale where variations in organic load, substrate composition and pH are less drastic and variable than would be found in full-scale industrial plants. Pilot-scale UASB trials would thus better simulate the full-scale industrial situations and provide further elucidation on the feasibility of pre- and post-ozonation.

During the anaerobic treatment of the winery wastewater in the 600 L UASB, COD was reduced by between 75 and 87%, with an average of 81% at a maximum OLR of 4.4 kgCOD.m^{-3}.d^{-1}. Post-ozonation of the UASB reactor effluent resulted in an average COD reduction of 28%, representing total COD reductions of between 81 and 90%.

Pre-ozonation of the winery wastewater resulted in an average COD reduction of 17%. The COD reduction efficiency of the UASB reactor treating the pre-ozonated winery wastewater increased from *ca*. 81% to 92% (of the pre-ozonated influent) and remained relatively stable. The UASB effluent COD during this period varied between 230 and 485 mg.L^{-1}, representing a maximum total COD reduction of up to 95% (of the raw wastewater). The COD reduction efficiency remained stable, even in periods of variable influent COD. Pre-ozonated substrate feed also resulted in the methane content in the biogas to increase from 43 to 54%. These increases in COD reduction efficiency and methane yield may be ascribed to the increased biodegradability of the wastewater due to pre-ozonation.

Post-ozonation of the UASB effluent with COD concentrations varying between 230 and 485 $mg.L^{-1}$ represented the "pre- and post-UASB treatment" sample. Reductions in COD averaged 23%, resulting in effluents with COD's between 160 and 375 $mg.L^{-1}$, representing total COD reductions (from the initial average raw wastewater COD of 4 982 $mg.L^{-1}$) of 93 – 96%.

It was thus shown, at laboratory and pilot-scale, that the use of ozone as a pre- and/or post-treatment to anaerobic digestion is a feasible option in the treatment of winery wastewaters. Wastewater treatment efficiency can be improved, providing financial benefits to industries involved and contributing to the sustainability of fresh water resources.

Determination of the optimum ozonation parameters during pre-treatment of wastewaters for anaerobic digestion using a direct activity test method

Evaluating the efficiency of a pre-ozonation treatment to anaerobic digestion is a timeous process. The set-up, start-up and stabilisation period required for an UASB reactor can take a few months (Britz *et al.*, 2002). As only one ozonation condition can be evaluated per reactor, this lengthens the time required to evaluate a series of ozonation conditions, even if parallel UASB reactors are used. Also, COD as a parameter of ozonation efficiency does not give an indication of the possible formation of inhibitory compounds. The ideal solution would be to have a rapid, easy to apply test procedure which could simultaneously be used to:

1) give an indication of the optimum ozone concentration and ozonation time combinations;
2) give an indication of the effect of pre-ozonation on COD reduction; and
3) give an indication of the effect of pre-ozonation on the activity of the biomass.

It was thus decided to develop a method which could be used to predict which ozonation time and concentration combination would be the most suited, in terms of COD reduction and effect on activity, for ozonation to be used as a pre-treatment to anaerobic digestion.

The developed protocol was an adaptation of an existing activity method which measures the potential of a granular biomass to convert soluble substrate to methane and carbon dioxide (O'Kennedy, 2000). The activity was determined using three distinctive test media. A basic test media (BTM) was used to measure overall activity of the microbial population in the granules. A glucose enriched media and an acetic acid enriched media were used to measure the specific activity of acidogens and acetoclastic methanogens, respectively. The adaptation involved the granular biomass being subjected to an ozonated wastewater (combinations of three concentrations and four ozonation times) for several days, during which the COD reduction was monitored. Thereafter, the activity was again determined and compared to the original or control activity.

The results found in this study showed that the COD reduction efficiency over 96 h did not give a clear indication of the optimum ozone concentration/ozonation time as no significant differences were found between the ozone concentrations or ozonation times. The different ozone concentrations used in this study (13, 19 and 38 $mg.L^{-1}$) did not differ significantly in the results obtained. Ozonation time did, however, have an effect on the activity of the granules, especially at 10.0 min ozonation.

From the activity results obtained in the BTM it was evident that a 10.0 min pre-ozonation of the winery wastewater was detrimental to granule activity, as the methane content (%) was significantly lower than for the shorter ozonation times. When measuring the activity specifically for the acidogens and acetoclastic methanogens in the population (BTM + glucose and BTM + acetic acid, respectively), the activity in terms of biogas production and methane content (%) for the different ozonation times did not differ significantly from the COD control. It was observed, however, that the activity in terms of biogas and methane content (%) tended to be lowest for the 10.0 min ozonation, suggesting that longer (> 5.0 min) ozonation times should be avoided. Differences in the 1.0, 2.5 and 5.0 min ozonation times were not significant and thus the choice of the lowest ozonation time would be most economically viable.

This study showed that the developed protocol can be used to determine differences in activity of anaerobic granules as a result of different combinations of concentration and time of pre-ozonation and thus be used to identify the optimum combination. The developed protocol has also shown that methanogenic activity is affected more by pre-ozonation of a winery wastewater than the acidogenic activity.

The developed protocol can, thus be used to determine the feasibility of pre-ozonation to anaerobic digestion, as well as providing indications as to the optimum ozonation concentration/time combination. This protocol can also be used to determine the effect of a wastewater on specific populations within the anaerobic granules. It is also possible to make these deductions in a far shorter space of time than with conventional reactor methods.

Concluding remarks

This study has re-confirmed that anaerobic digestion, specifically UASB technology, is a feasible treatment option for seasonal wastewaters such as highly alkaline lye-peeling, fruit cannery wastewater, a normal fruit cannery wastewater and a winery wastewater, where excellent COD reduction efficiencies of up to 93%, were found. High sodium levels in the wastewaters originating from the lye-peeling sections of fruit canneries could, however, be problematic. Unless these wastewaters are diluted with other wastewaters generated in the fruit canning process, the high sodium content would inhibit the anaerobic treatment system. High sodium levels in wastewater streams need to be monitored carefully as there are legislative limits on sodium levels allowed in wastewaters if they are to be irrigated.

This study has provided the fruit processing and winery industries with proof that anaerobic digestion and ozonation can be combined into effective and feasible wastewater treatment processes. The use of pre- and post-ozonation steps together with anaerobic digestion, in the form of an UASB reactor, is a feasible option to treat fruit processing and winery wastewaters. The combination of these technologies can improve the efficiency of the wastewater treatment process to such an extent that major financial savings can be achieved in the cost of wastewater discharges. On-site wastewater treatment could produce effluents much lower in COD, possibly below the legal limit of 75 mg.L^{-1}, and therefore reduce the cost of fines payable to municipal wastewater treatment plants. Alternatively, the wastewaters would then conform to levels allowing irrigation of the wastewaters. It is recommended that the efficiency of the ozonation step be further investigated as the use of industrial-scale contacting systems would permit higher flowrates and mixing efficiency of the ozone and wastewater. Increased ozonation efficiency would result in a further cost saving on ozone requirements. A more efficient contacting process could also reduce the time required for ozonation and thus increase the volume capacity of the treatment process. A further recommendation is that a specific cost analysis of available ozonation systems, for full-scale applications, be done so as to determine the specific cost of ozonating particular volumes of wastewater. With the availability of South African made ozone generators incorporating new innovations, the cost of using ozonation in wastewater treatment has been considerably reduced. Commercialisation of this new South African technology will further reduce the cost. Implementation of these wastewater treatment technologies will not only benefit these industries financially, but also enhance their image of being environmentally responsible and ensuring a sustainable and profitable business.

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