THE COMBINATION OF UASB AND OZONE TECHNOLOGY IN THE TREATMENT OF A PECTIN CONTAINING WASTEWATER FROM THE APPLE JUICE PROCESSING INDUSTRY

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DECLARATION

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

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ABSTRACT

The South African apple juice processing industry is growing rapidly and during the harvesting season the wastewater volumes and organic loads increase significantly with a considerable environmental impact. These larger apple juice processing wastewater (AJPWW) volumes and chemical oxygen demand (COD) loads subsequently lead to faster increases in the organic loading rate (OLR) of an upflow anaerobic sludge bed (UASB) wastewater treatment system and it is necessary to know if the treatment system can handle such drastic increases over short periods. The objective of the study were to evaluate the efficiency of the UASB process in the treatment of an AJPWW; to determine what effect a substrate viscosity increase, based on a pectin calcium gel has on the performance of an UASB system, and to determine what impact ozonation has on the pectin content, gelformation ability and biodegradability of the AJPWW.

The ability of the UASB to maintain stability during the apple-processing season was investigated by increasing the OLR from 2.9 to over 14.0 kg $COD.m^{-3}.d^{-1}$ in 131 days. During this time the COD removal remained constant at 85%, while the pH and alkalinity remained at levels indicative of good reactor stability. It was thus concluded that the UASB reactor could operate successfully during the apple-harvesting season when wastewater volumes and organic loads increase significantly.

In the study it was found that the viscosity of the AJPWW, containing 750 $mg.L^{-1}$ pectin, increased from 8.5 to 47.0 cps after a 312 $mg.L^{-1}$ Ca^{2+} addition. This increased viscosity substrate was then fed to an UASB reactor at an OLR of 15.0 kg $COD.m^{-3}.d^{-1}$. During a 12 day increased viscosity (47 cps) feeding stage the COD removal decreased from 94 to 11%, while the reactor pH decreased from 7.5 to 4.9. During this period, pectin accumulated in the UASB and led to biomass washout and rapid UASB failure. The possible elimination of pectin by ozonation was thus investigated, and a 77% decrease in pectin content and 76% decrease in gel formation ability occurred after ozonation.

The effect of pre- and post-ozonation on the efficiency of the UASB system was subsequently investigated. It was found that a 10 min pre-ozonation decreased the AJPWW COD by 19% and the total suspended content by 36%, while the soluble portion of the total COD was increased from 81.7 to 92.4%. This increase in soluble COD content should lead to increased wastewater biodegradability. The ozonated AJPWW was then used to replaced the raw non-ozonated AJPWW as reactor feed. Results showed that the COD removal increased from 78 to 90% within 24 h of starting with the ozonated feed. It was also found that the reactor stability improved after AJPWW pre-ozonation as an OLR increase from 10.0 to 16.6 kg $COD.m^{-3}.d^{-1}$ in 23 days did not detrimentally influence the

stability of the reactor. This reactor effluent (COD = 465 $mg.L^{-1}$) was then post-ozonated which resulted in 64.8% COD and 79.0% colour reductions. The final effluent had a COD of 180 $mg.L^{-1}$ (98% reduction).

The ability of the ozonation/digestion system as described in this study to degrade AJPWW at a higher OLR is of value to the apple industry, as it may lead to larger organic pollutant removals and thus a more effiecient treatment system. Increased reactor performance will directly improve the quality of the final wastewater produced, which in turn will have a significant impact on the treatment ability of the South African apple processing industry currently limited by the production of large wastewater volumes.

UITTREKSEL

Gedurende die oesseisoen word groot volumes afvalwater met 'n hoë organiese lading in die vinnig groeiende Suid-Afrikaanse appelsapprosesseringsbedryf geproduseer. Dit het 'n groot impak op die omgewing. Die groter volumes appelsapprosesseringsafvalwater (ASPAW) met 'n hoë organiese lading het 'n vinniger verhoging in organiese ladingstempo's (OLT) van 'n UASB-waterbehandelingstelsel tot gevolg. Daarom is dit belangrik om te weet of die stelsel die drastiese verhoging oor kort tydperke kan hanteer. Die doel van hierdie studie was die evaluering van die effektiwiteit van die UASB-proses in die behandeling van ASPAW; om te bepaal watter effek 'n substraatviskositeitsverhoging, gebaseer op 'n pektien-kalsium-jel, op die doeltreffendheid van 'n UASB-stelsel het; en om te bepaal watter impak osonering op die pektieninhoud, jelvormingsvermoë en bioafbreekbaarheid van ASPAW het.

Die vermoë van die UASB om stabiliteit te handhaaf gedurende die appelsapprosesseringseisoen is ondersoek deur die OLT van 2,9 tot bo 14,0 kg CSB.m^{-3}.d^{-1} te verhoog oor 131 dae. Gedurende hierdie tyd het die chemiese suurstofbehoefte- (CSB-) verwydering konstant gebly by 85%, terwyl die pH en alkaliniteit ook op vlakke aanduidend van goeie reaktorstabiliteit gebly het. Daar is sodoende bewys dat die UASB-reaktor suksesvol kan presteer tydens die appelsapprosesseringseisoen, wanneer daar 'n beduidende verhoging in OLT plaasvind.

In die studie is daar gevind dat die viskositeit van die ASPAW, wat 750 mg.L^{-1} pektien bevat, van 8,5 tot 47,0 cps toeneem na die byvoeging van 312 mg.L^{-1} Ca^{2+}. Hierdie verhoogde vikositeitsubstraat is tot die UASB-reaktor toegevoeg teen 'n OLT van 15,0 kg CSB.m^{-1}.d^{-1}. Gedurende 'n 12-dae toevoer van verhoogde viskositeit (47 cps), het die CSB-verwydering van die reaktor afgeneem van 94% na 11%, terwyl die pH gedaal het van 7,5 na 4,9. Gedurende hierdie tydperk het pektien in die UASB geakkumuleer, wat gelei het tot die uitspoel van biomassa en vinnige UASB-reaktormislukking. Die moontlike eliminasie van pektien, deur osonering, is daarom ondersoek. 'n Verlaging van 77% in pektieninhoud en 76% in jelvormingsvermoë het na osonering plaasgevind.

Die effek van pre- en post-osonering op die effektiwiteit van 'n UASB-stelsel is gevolglik ondersoek. Daar is gevind dat 'n 10 minute pre-osonering die CSB van die ASPAW met 19% verlaag en die totale inhoud van gesuspendeerde vaste stowwe met 36% verlaag, terwyl die oplosbare gedeelte van die totale CSB van 81,7% tot 92,4% gestyg het. Die verhoging in oplosbare CSB-inhoud behoort tot verhoogde bioafbreekbaarheid van ASPAW te lei. Die geosoneerde ASPAW is gebruik om die rou, ongeosoneerde ASPAW as reaktorsubstraat te vervang. Die resultate het getoon dat die

CSB-verwydering verhoog het van 78% na 90% na 'n 24-uur toevoer van geosoneerde substraat. Daar is ook gevind dat die reaktorstabiliteit toegeneem het na ASPAW osoneering, aangesien 'n OLT-verhoging van 10,0 na 16,6 kg.CSB.m^{-3}.d^{-1} in 23 dae nie die stabiliteit van die reaktor nadelig beïnvloed het nie. Hierdie reaktoruitvloeisel (CSB = 465 mg.L^{-1}) is hierna gepost-osoneer, wat 'n 64,8% CSB- en 79,0% kleurverlaging tot gevolg gehad. Die finale uitvloeisel het 'n CBS-inhoud van 180 mg.L^{-1} gehad (98,1% verwydering).

Die vermoë van die osonering-/verteringstelsel om ASPAW te degradeer teen 'n hoër OLT, soos beskryf in hierdie studie, is van waarde tot die appelsapprosesseringsbedryf, aangesien dit tot groter organiese afvalstofverwydering kan lei en dus 'n meer effektiewe behandelingstelsel tot gevolg kan hê. Verhoogde reaktordoeltreffendheid sal 'n direkte verbetering tot gevolg hê in die gehalte van die finale afvalwater wat geproduseer word, wat op sy beurt 'n beduidende impak sal hê op die behandelingsvermoë van die appelsapprosesseringsbedryf, wat tans beperk word deur die produksie van groot volumes afvalwater.

Dedicated to my parents

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CONTENTS

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

CHAPTER 1

INTRODUCTION

Water is South Africa's most valuable and also most threatened resource. Severe water shortages are expected in the near future due to the rapid population growth and increased industrial and agricultural activities. The responsible use of the available water is thus essential. Increased national environmental protection strategies are forcing South African industries to make large capital investments in order to treat polluted water (Water Research Commission, 1994). The new and stricter environmental regulations are creating new challenges for successful wastewater treatments (Sigge *et al.*, 2002)

The food processing industry is responsible for the pollution of large volumes of wastewaters that have a severe environmental impact (Trnovec & Britz, 1998). Food processing wastewaters usually contain high organic contents and have a much higher pollution level than municipal sewage. The treatment of these food related wastewaters in municipal treatment systems is thus rapidly becoming unacceptable due to system overloadings, and therefore on-site wastewater treatment has became unavoidable for the food industry.

Various physical and chemical treatment options are available to reduce this organic load but these are, however, expensive to install. Other less expensive treatments like landfilling, wetland systems or direct irrigation of wastewater are rapidly becoming unacceptable due to their severe environmental impact. Biological treatment is the most economical and effective treatment option to reduce the large organic content associated with food processing wastewaters (Bitton, 1999). Most organic pollutants are completely degradable by biological treatments, whereas many physical and chemical processes just concentrate the pollutants or transform them from one source to another (Gottschalk *et al.*, 2002).

Of the various anaerobic reactor designs, the UASB system has been found to have the highest loading capacity and best chemical oxygen demand (COD) reductions and is now the most widely used anaerobic treatment system in the world (Field, 2002). The settling ability of the UASB reactor sludge/granules is a vital characteristic of an effective system as the settling design relies on granule formation (Lettinga *et al.*, 1983). Therefore, wastewater characteristics play an important role in the formation and maintenance of the granules and can also influence the settling ability of the sludge/granules (Van Lier *et al.*, 2001). Wastewaters that contain soluble sugars and organic acids as the main portion of its organic content provide an ideal UASB substrate (Lettinga *et al.*, 1980). Finely

dispersed particles and complex organic matter are, however, detrimental to granule formation and reactor performance (Van Lier *et al.*, 2001). Substrate viscosity can also play an important role in the effectiveness of the system (Lin & Yang, 1990). Any increase in substrate viscosity will decrease the settling ability of the sludge/granules which may lead to the washout of valuable biomass. The inability of the decreased anaerobic community to degrade complex organic matter rapidly enough will also limit the hydraulic retention time (HRT) and organic loading rate (OLR) of the system (Bitton, 1999).

The South African apple juice processing industry currently produces 6.8 million m^3 of wastewater annually (D. Murray, Elgin Fruit Juices (Pty) Ltd, Grabouw, South Africa, personal communication, 2002). Most of this wastewater is produced during the months of February to June when the apples are harvested and processed. In the latter part of the processing season (May to July) apples of a riper and softer nature enter the process. The disintegration of these ripe apples during the transport, washing and drying phases occurs and the disintegrated apples end up in the wastewater (F. Mostert, Elgin Fruit Juices (Pty) Ltd, Grabouw, South Africa, personal communication, 2003). The use of overripe apples for juice extraction is, however, very popular since these apples cannot be sold on the fresh market and produce a high juice yield. Larger quantities of softer apples are thus processed during the latter parts of the processing season which subsequently leads to the production of larger volumes of wastewater that contain higher organic loads (F. Mostert, Elgin Fruit Juices (Pty) Ltd, Grabouw, South Africa, personal communication, 2003). These high wastewater volumes and organic loads put considerable stress on water treatment systems (D. Murray, Elgin Fruit Juices (Pty)Ltd, Grabouw, South Africa, personal communication, 2002).

Apples contain between 5 000 and 16 000 $mg.L^{-1}$ pectin (Whitaker, 1990). During the pressing of apples the juice is separated from the pectin fraction (Mannapperuma, 1995). Some of the pectin remains in the pressed cake and can be used as animal feed (Wayman, 1996) but a large portion of the pectin ends up in the wastewater (Mannapperuma *et al.,* 1995). The size and complexity of the pectin molecule makes biological treatment difficult and not many microorganisms have the necessary enzymes required to degrade the pectin molecule (Bitton, 1999). Previous studies have shown that anaerobic systems are incapable of effectively degrading pectin and that wastewaters with high pectin contents interfere with biological treatments (Shivakumer & Nand, 1995). Pectin also has the ability to form a gel under certain circumstances. The hydroxyl group in pectin has the ability to form a link with calcium causing the formation of a pectin network, trapping water and thus producing a gel (Hoejgaard, 2003). This type of gel

formation could possibly occur in an apple juice processing wastewater if the pectin and calcium concentrations are high enough.

Apple juice processing wastewater (AJPWW) has a pH of between 3.5 and 5.0 (Sam-Soon *et al.*, 1986) and a pH adjustment is required before the wastewater can enter a biological treatment system. The use of calcium hydroxide or calcium oxide, in the form of lime, are the most popular pH adjusters due to their cost effectiveness (D. Murray, Elgin Fruit Juices (Pty)Ltd, Grabouw, South Africa, personal communication, 2002). Sodium hydroxide (NaOH) can also be used but is more expensive and the final effluent will contain high sodium levels, which prevents the use of the effluent for irrigation purposes (Steenveld, 1997). The combination of the pectin in the apple juice processing wastewater and the Ca^{2+} from the lime may lead to the formation of a pectin-calcium gel. The increase in viscosity within the UASB reactor due to gel formation may then lead to biomass retention and washout problems (Battimelli *et al.*, 2003; D. Murray, Elgin Fruit Juices (Pty) Ltd, Grabouw, South Africa, personal communication, 2002), which subsequently reduces reactor efficiency and even causes reactor failure.

An effective pre-treatment of wastewater can have a significant impact on the efficiency of the UASB process (Van Lier *et al.*, 2001). The hydrolysis of the pectin molecule should not only reduce gel formation problems associated with apple juice processing wastewaters but should also increase the biodegradability of the wastewater (Tanabe *et al.*, 1988).

One such form of pre-treatment is the use of ozone which has a long history of use in the water treatment industry and consists of many favourable oxidising properties required for effective water treatment (Benitez *et al.*, 1999). The use of ozone to reduce the total COD content of untreated food processing wastewater would in most cases be uneconomical due to the high organic load of the wastewater and the amount of ozone required (Sigge *et al.*, 2002). The conversion of complex organic pollutants by a pre-treatment into more biodegradable substances with subsequent increased biological treatment efficiency is, however, a viable option.

The combination of an ozone treatment with a biological treatment employs the strengths of both systems to increase the organic pollutant removal efficiency. Ozonation is very effective in hydrolysing complex organic polymers into smaller units (Gottshalk *et al.*, 2000), but less effective in the elimination of smaller molecular weight substances like organic acids and sugars (Volk *et al.*, 1993). Biological treatment on the other hand is more effective in reducing the smaller molecular weight substances like organic acids and sugars but less effective in hydrolysing complex organic polymers like pectin (Lin & Yang, 1990; Van Lier *et al.*, 2001). The degradation products of ozonation are, in most cases,

more biodegradable than the original pollutant (Benitez *et al.*, 1999; Weemaes *et al.*, 1999; Martin *et al.*, 2001) and ozonation done as a pre-treatment could thus increase the efficiency of a subsequent biological treatment.

This study was motivated by the problems experienced with an industrial UASB reactor in Grabouw which is treating AJPWW. The treatment plant reported that UASB steady state conditions could not be reached within the four seasons of operation and reactor failure due to gel formation and granule washout occurred regularly during the processing season.

The objectives of this study were firstly, to evaluate the efficiency of the UASB process in the treatment of an AJPWW, secondly, to determine what effect a substrate viscosity increase, based on a pectin calcium gel has on the performance of an UASB system, and thirdly, to determine what impact ozonation has on the pectin content, gel formation ability and biodegradability of the AJPWW. The efficiency of a combined pre-ozonation-UASB-post-ozonation treatment system will also be evaluated.

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CHAPTER 2

LITERATURE REVIEW

A. BACKGROUND

Water is a scarce and unevenly distributed national resource and forms part of a unitary, interdependent cycle. South Africa is a country with a below average rainfall, and a climate that varies significantly from region to region and season to season. This low and irregular rainfall pattern makes South Africa an area with limited fresh water resources and thus prone to the impact of droughts. The supply of fresh water in South Africa has become critical as a result of increased water demand due to the rapidly growing population as well as increased industrial and agricultural activities (Water Research Commission, 1994; Wayman, 1996; Trnovec & Britz, 1998).

The National Water Act (Anon. 1998) specifies through various regulations that the pollution of waterways by wastes should be prevented at all costs and this will be achieved by strict law enforcement and severe penalties. Waste includes any solid material or material that is suspended, dissolved or transported in water, including sediments, and which may be spilt or deposited on land or into a water resource in such volume, composition or manner as to cause, or to be reasonably likely to cause, the water resource to be polluted (Anon. 1998). The South African limits for wastewater irrigation and disposal are summarised in Tables 1 and 2.

These new regulations implemented in the water and environmental (Anon. 1998) acts have heavily impacted the food industry. Food processing plants that were built in rural areas a few decades ago are now surrounded by residential neighbourhoods. Rising standards of living and education have made the population more sensitive to environmental issues related to food processing residues and the pollution of water sources (Murthy, 1998). Apart from a small minority of specialized micro-organisms, all life on earth requires oxygen in order to survive (Wayman, 1996). In a water course, anything that competes for oxygen threatens whatever life is already established there. Dissolved oxygen in the water enables micro-organisms to self-purify the natural water way. Organic matter in food processing effluents creates an additional demand for dissolved oxygen, which far exceeds the natural aeration capacity of any stream or dam (Green & Kramer, 1979).

Table 1. Wastewater limits values for the irrigation of wastewater according to the South African Government Gazette of 8 October 1999 (Anon.1999).

Parameter	Limit
Less than 500 m ^{3} of biodegradable industrial wastewater per day	
Electric conductivity	200 mS.m^{-2}
pH	6 - 9
Chemical Oxygen Demand (COD)	400 mg.L^{-1}
Fecal coliforms	100 000 units per 100 ml wastewater
Sodium adsorption	Less than 5 mg.L^{-1} of the biodegradable waste
Less than 50 m^3 of biodegradable industrial wastewater per day	
Electric conductivity	200 mS.m^{-2}
pH	6 - 9
Chemical Oxygen Demand (COD)	5 000 mg.L^{-1}
Fecal coliforms	100 000 units per 100 ml wastewater
Sodium adsorption	Less than 5 mg.L^{-1} of the biodegradable waste

Table 2. Wastewater limit values for the disposal of wastewater in a natural water source according to the South Africa Government Gazette f 8 October 1999 (Anon.1999).

Parameter	Limit
Less than 500 cubic meters of biodegradable industrial wastewater per day	
Electric conductivity	150 mS.m^{-2}
pH	5.5 – 9.5
Chemical Oxygen Demand (COD)	75 mg.L^{-1}
Fecal coliforms	1000 units per ml wastewater
Temperature of environment	Not raised by more than 3 °C
Suspended solids (TSS)	25 mg.L^{-1}
Orthophosphate	10 mg.L^{-1}
Nitrogen	15 mg.L^{-1}

The suspended matter in food processing effluents increases the natural turbidity of water, which subsequently reduces the ability of sunlight to penetrate into the water. Without sunlight, the life of all natural occurring organisms is inhibited (Schutte & Pretorius, 1997).

The leakage or spillage of fruit juice wastes to the sub-soil will result in contaminated ground-water with potentially grave consequences. For example: Organic acids present in the wastewater may corrode concrete and steel works; fruit juice is acceptable to fermentation and will loosen the soil structure; or solutes may migrate into local aquifers, bore holes or water courses causing further pollution (Holtshausen, 2002). Other organic material present in fruit juices may also form a substrate for sulphate-reducing bacteria. The consequences of such activity is the the formation of hydrogen sulphide gas which is toxic to humans at the parts per million (ppm) level and if the smell is overlooked, collapse and possible death may occur (Wayman, 1996). Food processing wastes thus need to be reduced or disposed of in some way (Wayman, 1996; Trnovec & Britz, 1998).

B. APPLE PROCESSING INDUSTRY

The apple processing industry in South Africa is a large and expanding industry. South Africa has 24 000 hectares of commercially planted apple trees and this figure is growing by 500 hectares per year (Mabiletsa, 2002). An estimated 22 million trees are harvested each year and produce on average 710 000 metric tons of apples. 515 000 metric tons are used for fresh consumption, locally and abroad. The remaining 195 000 metric tons are processed into mainly fresh and concentrated apple juice (Mabiletsa, 2002). Approximately 9.5 m^3 of wastewater is produced for every ton of apples processed (Mnanapperuman, 1995) giving approximately 6.8 million m^3 of undiluted high oxygen demanding wastewater every year (Wayman, 1996).

Effluent characteristics

Apple juice production consists of several stages: Sorting; washing; grinding to produce a mash; enzyme treatment; pressing; juice treatment; and pomace disposal (Mnanapperuman, 1995). A yield of between 70 and 80% would normally be obtained from the pressing of good apples (Rutledge, 1996). In the food processing industry water is used in most of the processing operations (Brand & Martin, 1994; Mnanapperuman, 1995). including: transportation; cleaning; blanching; retorting; cooling; cooking; peeling; washing; and sanitizing operations. A typical flow diagram of an apple juice processing plant is shown in Fig. 1 (Woodroof & Luh, 1975). Wastewater is produced during the

washing, pressing, juice extraction and dewatering stages. During the pressing a solid waste (pomace) is produced, which is usually resold as animal feed (Wayman, 1996). The dewatering operation is responsible for the largest volume of wastewater produced, and the washing and pressing operations are responsible for the highest organic load in the effluent (Grismer *et al.*, 2002).

The characteristics of wastewater from the apple processing industry are extremely variable (Dold *et al.,* 1987) since they are affected by numerous factors, like processing rate, plant capacity utilization, raw material quality, preparation equipment, water reuse and housekeeping practices (Shober, 1989; Ross *et al.,* 1999). Wastewater parameters of major pollutional significance are the biological oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), turbidity, temperature, nitrogen and phosphorus (Mnanapperuman, 1995). Apple juice processing wastewater will usually contain varied COD concentrations (400 – 15 000 $mg.L^{-1}$), 92 – 116 $mg.L^{-1}$ nitrogen (as total Kjeldal nitrogen) and 11 – 27 $mg.L^{-1}$ phosphorus (in the phosphate form). The solid content of the wastewater will vary considerably depending on the process in the plant and the equipment used (Dold *et al.,* 1987). A variety of substances such as antioxidants, clarifying aids, preservatives, reducing agents, firming agents, sequesterants, pH controllers, anti-caking agents, thickening agents, acidulants and nutrients are also present in the wastewater (Somogyi & Kyle, 1978; Broomfield, 1996).

Complex solid and suspended organic polymers like pectin, cellulose and lignin are also present in apple juice processing wastewater with concentrations depending on various factors, like processing methods and type of raw materials used (Morris, 1985). The nature and functional abilities of the complex organic matter present, can interfere with wastewater treatment processes (Van Lier *et al.,* 2001). One of the major problems encountered during apple processing wastewater is the ability of pectin to increase the viscosity of the wastewater. The fact that pectin is not readily biodegradable may lead to serious problems in the treatment of pectin containing apple wastewaters (Tanabe *et al.*, 1987).

C. THE PECTIN PROBLEM

The pectic substances constitute a unique group of polysaccharides that normally are polymers of galacturonic acid (Whitaker, 1990). The term pectic substances includes: protopectin; pectin; pectinic acid; and pectic acid. Pectic substances are important in the cell wall where they combine with hemicelluloses to reinforce the structural contribution of cellulose (Anon., 2003). The chemical structure of pectic substances gradually change

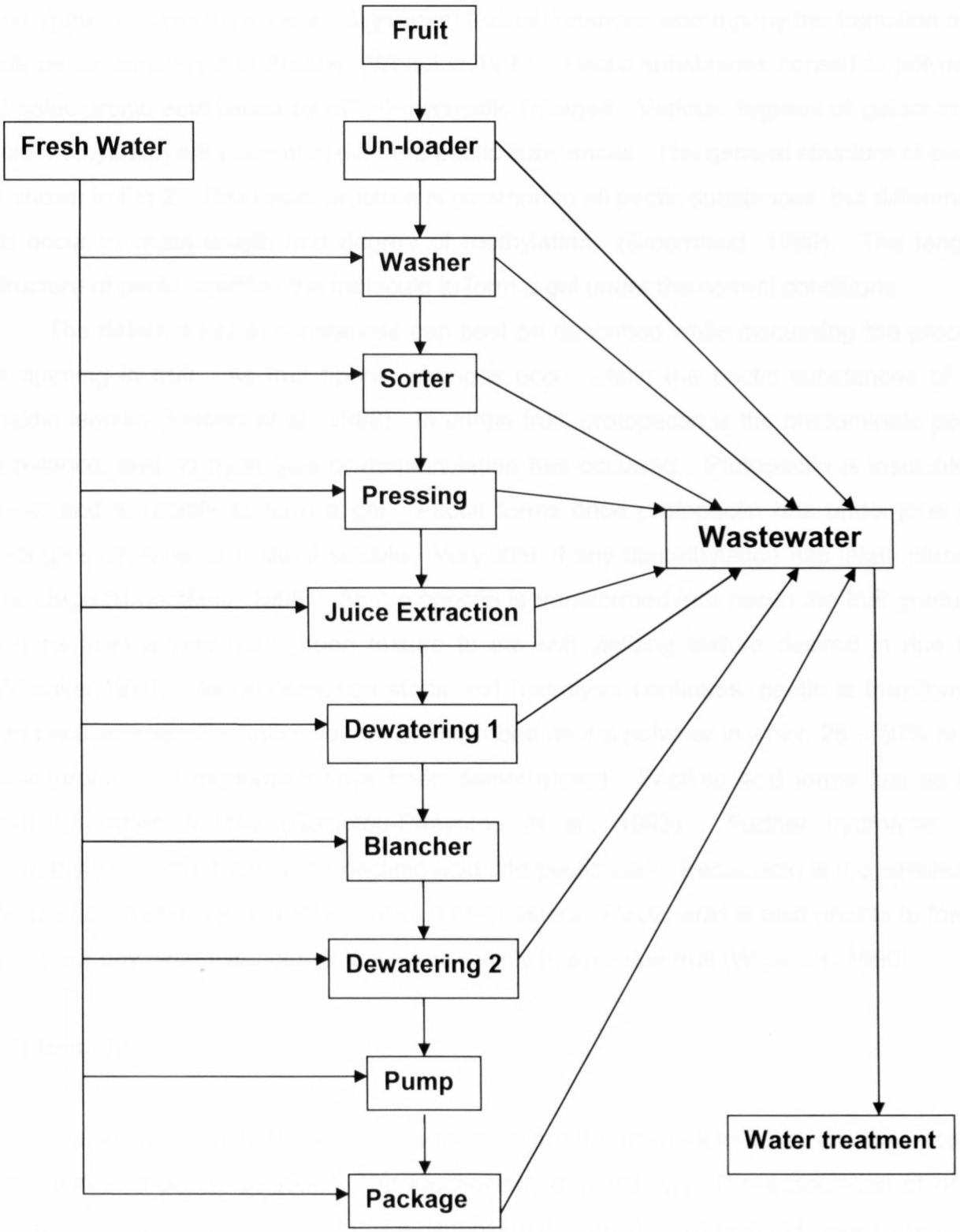

Figure 1. Flow diagram of an apple juice concentrate processing plant (Mannapperuma *etal.*, 1994).

during the maturation process. Significant textural changes accompany the transition from one pectic substance to another (Whitaker,1990). Pectic substances consist of polymers of galacturonic acid linked by α(1,4)-glycosidic linkages. Various degrees of galacturonic acid methylation are present in different pectic substances. The general structure of pectin is shown in Fig 2. This basic structure is common to all pectic substances, but differences do occur in chain length and degree of methylation (Broomfield, 1996). The lengthy structure of pectin enables the molecule to form a gel under the correct conditions.

The different pectic substances can best be described while discussing the process of ripening in fruit. As fruit ripens, changes occur within the pectic substances of the middle lamella (Fedirici *et al.*, 1988). In unripe fruit, protopectin is the predominate pectic substance, and no hydrolysis or demethylation has occurred. Protopectin is insoluble in water and is unable to form a gel. Pectin forms once protopectin has undergone just enough hydrolysis, to make it soluble. Very little, if any demethylation has taken place at this stage (Broomfield, 1996). As protopectin is transformed into pectin the fruit gradually softens from a very hard green texture to the soft yielding texture desired in ripe fruit (Whitaker,1990). As demetylation starts and hydrolysis continues, pectin is transformed into pectinic acid. Pectinic acid can be described as the polymer in which 25 – 50% of the galacturonic acid monomers have been demethylated. Pectinic acid forms just as fruit start to soften a little (Galtiotou-Pnayotou *et al.*, 1993). Further hydrolysis and demethylation would transform pectinic acid into pectic acid. Pectic acid is the smallest of the pectic substances and totally lacks methyl esters. Pectic acid is also unable to form a gel under any circumstances and is found mainly in over-ripe fruit (Whitaker, 1990).

Gel formation

The soluble solids content and pH of a medium are the main factors that will influence gel formation in a pectin solution. The mechanism is as follows: The association of pectin chains leads to the formation of three-dimensional networks; At high pH values the pectin molecules will have a negative charge, repel each other and thus no network or gel formation will occur (Hoejgaard, 2003); As the pH decreases, the pectin molecules become more neutral and the pectin chains develop an affinity for each other; Two or more chain segments bind together and start to interact. Pectin molecules are soluble in solutions with a low soluble solid or sugar content (Shivakumer & Nand, 1995). As the sugar concentration is increased the pectin precipitate to form a network and eventually a gel. Optimum gel formation will occur at a pH of 3.3 and a sugar content of 68 – 70 °Brix (Rutledge, 1996).

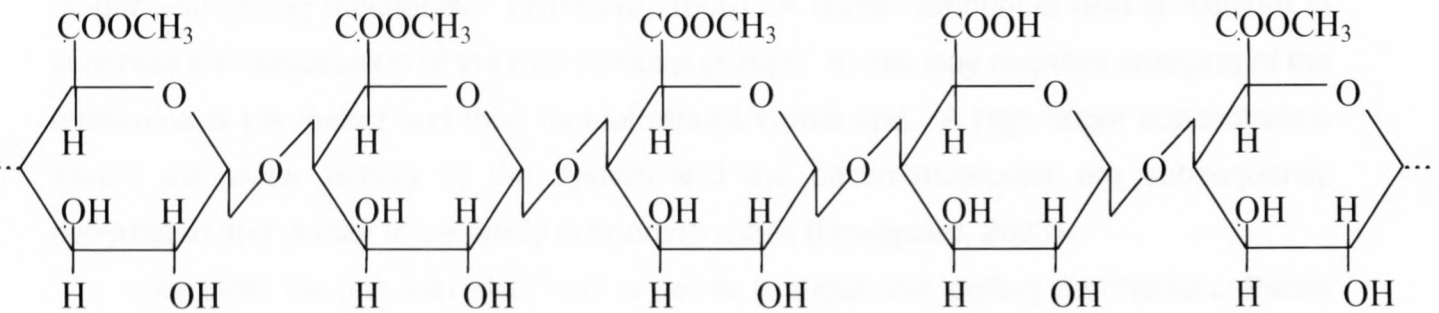

Figure 2. The chemical structure of a pectin fragment (Whitaker, 1990).

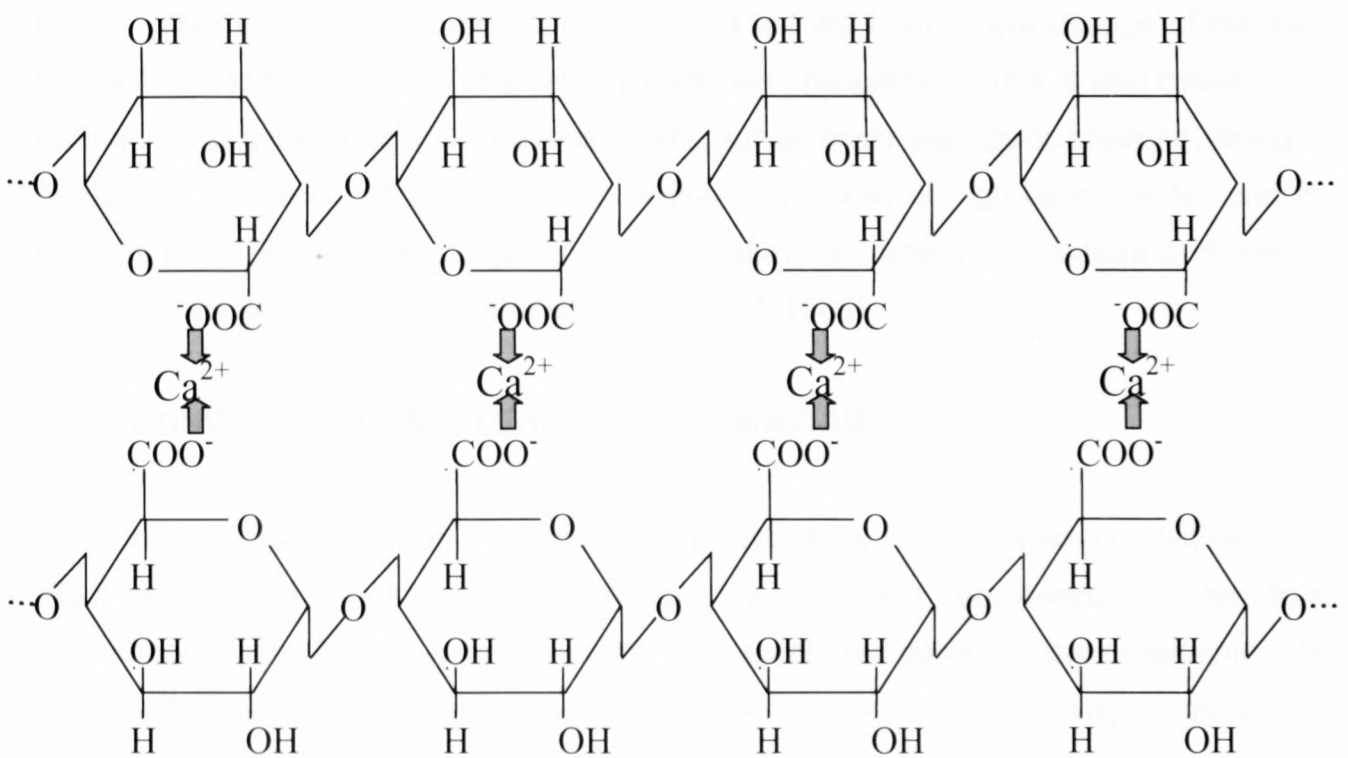

Figure 3. A simplified model of the calcium induced gel formation mechanism that occur in low ester pectins (Whitaker, 1990).

Different types of gelling mechanisms also exist but depend on the degree of estrification in the pectin molecule. High ester pectins will predominantly form a gel by the so-called "sugar-acid gelling mechanism" (Whitaker, 1990). A certain amount of acid is required to suppress the dissociation of the free carboxyl groups. In this way negative charging of the molecules is prevented and their mutual repulsion reduced. A high sugar concentration lowers the water activity of the system and the pectin molecules are subsequently dehydrated and cluster more easily in bonding zones (Hoejgaard, 2003).

Low ester pectins can also form a gel in a sugar-acid gelling mechanism. Pectin molecules with lower ester contents can also form bonding zones with bivalent cations. Low ester pectins may also additionally form gels in relative independence from soluble solids content and pH in the presence of multivalent cations, like calcium ions. Because of the high methoxyl groups (COOH) present in pectinic acid, interactions can form with calcium. Calcium bridges the gap between the negatively charged molecules, water is trapped, and a gel is formed (Whitaker, 1990). This gel formation mechanism is less sugar and pH dependent (Broomfield, 1996). The gel formation mechanism for low ester pectin is illustrated in Fig. 3.

With smaller additions of calcium, pectin chains will start to cluster over calcium bonds. With increased calcium concentration, a gel will form. Over dosage of calcium ions will lead to the formation of a calcium pectinate precipitation. This is also called "pre-gelling". The gel structure will be less elastic, rather pasty with lower breaking strength (Fedirici *et al.,* 1988). This type of gel is also very stable at high temperatures and at extreme pH's. Even when the gel is heated to above its setting temperature and cooled down no destruction will occur (Madamwar & Mithal, 1985).

D. CONVENTIONAL WASTEWATER TECHNOLOGIES

Meeting the challenges of environmental protection and economic competitiveness requires a fresh look at the management of food processing wastewaters (Mnanapperuman, 1995). A large amount of research has gone into the development of new wastewater treatment technologies and the improvement of existing systems and processes (Brand & Marin, 1994). Food processing companies are, however, still experiencing problems with the management and disposal of wastewater (Wayman, 1996). This could be because of the rising cost of fresh water and more strict government regulations and the implementation of quality assurance programs, like Euro-GAP (Trnovec & Britz, 1998).

New problems of a modern society have placed additional burdens upon our waste treatment systems. Today's pollutants are more difficult to remove from the water and an increased demand for fresh water is also added to the problem (Mnanapperuman, 1995). The best intermediate answer to the problem is the wide spread application of existing treatment technologies (Brand & Martin, 1994). But this is only a temporary solution. The discharge of oxygen-consuming wastewaters will increase despite the universal application of existing techniques now available. Conventional treatment processes are already losing the battle against modern day tougher wastes (Schutte & Pretorius, 1997). In periods of drought or areas of low rainfall like experienced in South Africa, the recycling or the further use of water, even if only for irrigation, has became pressing. This increasing need to reuse water now calls for improved wastewater treatment processes (Woodroof & Luh, 1975; Brand & Martin, 1994).

Treatment steps

A complete fruit processing effluent plant should usually consist of four different stages (Wayman, 1996):

1. Recovery of valuable byproducts in the effluent, like the pectin, pomace and fruit sugars;
2. Primary or pretreatment where physical separation processes are used to remove any solid and suspended matter from the water;
3. Secondary treatment, where the dissolved solids are removed through biological degradation techniques;
4. Tertiary treatment, or polishing of the water, are the various physical and chemical processes used to make the effluent meet legal requirement for discharge into a natural waterway, or when water is to be reused.

The effectiveness of each stage depends on the success of the previous stage. The recovery of byproducts would greatly facilitate the primary and secondary treatments. The successful removal of suspended solids would increase the effectiveness of the biological degradation step (Shober, 1998). Cost effective tertiary treatment can only be achieved after an effective secondary treatment process (Volk *et al.*, 1993).

By product recovery

A major portion of the fruit processing residues can be used for different purposes because of their non-toxic nature. Mannapperuma *et al.* (1994) found that there are

enough fruit sugars and organic acids in the effluents of some fruit juice processing plants to produce an additional 52 325 L of fruit juice per day. Removal of these solids would require concentration using membranes and debittering with ion exchange systems, or they would have to be eliminated from the effluent by biological processes, both which are expensive (Baig & Leichti, 2001). By optimising the processing conditions these expenses may be reduced, while the yield of juice obtained would be higher. The recovery of waste for human use, animal feed or ground fertilizer, would not only generate an income but would also facilitate waste treatment because of a reduction in organic material (Shober, 1989). This is frequently done in the apple juice processing industry were the pomace is used for pectin extraction or animal feed. The wastewater from apple processing industries does, however, still contain high amounts of suspended and soluble organic material despite the removal of the pomace. Biological treatment methods would have to be applied to reduce the COD of the effluent to acceptable legal levels (Shivakumer & Nand,1995).

A large reduction in wastewater volume could be achieved through the use of recycled water. Hydraulic transport, heating operations, direct and indirect contact cooling, evaporating condensate, brining, curing and peeling operations all use water that could potentially be re-used (Water Research Commission, 1987). Re-use of water involves treatments to remove accumulated solids and dissolved substances. Solids could be removed through gravity settling and various screening techniques. Removal of dissolved solids is more difficult to control. It can be done by various membrane technologies, like microfiltration, reverse osmosis or nanofiltration. Salt free water could be obtained through electrodialysis, bipolar electrodialysis and capacitive deionization (Mannapperuma *et al.*, 1994). These methods are, however, all very expensive and thus most companies would rather use fresh water than invest in re-use treatments (Ross *et al.,* 1999).

Byproduct recovery for human consumption yields a high return to the producer but it is difficult to meet health and safety regulations (Woodroof & Luh, 1975; Mannapperuma *et al.*, 1994). Some fruit processing effluents contain pesticides from the fruit skin which will makes byproduct recovery for human consumption more difficult and expensive (Walter *et al.,* 1985). The pomace resulting from apple juice processing is, however, frequently used for the production of dietary fiber (Morris, 1985). Pectinase treatment is frequently used to facilitate juice recovery from apple and pear cores and peels (Wayman, 1996).

Solid waste from fruit processing plants has a high nutritional value of: 4.9% protein; 17% crude fat; 26% fiber; 0.13% calcium; and 0.12% phosphorus (on a mass to mass bases) and is frequently used as an animal feed (Shober, 1989). Since ruminant animals have the ability to digest cellulose and pectin, fibrous material, which is of little use to

humans, may be used as an animal feed (Woodroof & Luh, 1975). Feed containing up to 33% apple pomace has been used successfully as a dairy cattle feed. Feeding levels of fresh pomace are about 12 to 18 kg per cow per day. Due to transport costs it is uneconomical to use fresh pomace on farms that are far from processing plants (Bath, 1981).

Wet residues and process waters contain fruit sugars, a potential source of energy which can be utilized via ethanol fermentation and methane production. Ethanol fermentation and distillation is, however, an expensive process and in most cases it was reported to be uneconomical (Sargent *et al.,* 1982; Badger & Border 1989). Wet organic waste and effluents with high concentrations of organic matter, can be digested anaerobically to produce methane gas, which can be used as a boiler fuel (Bitton, 1999). AD is most often used with the primary objective of effluent treatment, and energy extraction as a secondary result (Trotzke, 1988). When wastewater is treated anaerobically under standard conditions about 0.31 m^3 of methane gas with a heating value of 7.0 MJ is obtained per kilogram of COD destroyed (Hills & Roberts, 1982).

Food processing residues can serve as fertilizers and soil conditioners when used in well designed and properly managed land application systems (Wayman, 1996). The application of fertilizers and soil conditioners is, however, a complex task since many nutrients play a role in the growth of plants. Nitrogen, phosphorus and potassium are essential nutrients for plant growth (Brand & Marin, 1994). Excessive salt concentrations, especially sodium, increase the osmotic pressure which makes it difficult for the roots to extract water and this inhibits plant growth. High BOD values (>20 $kg.ha^{-1}.d^{-1}$) will cause the soil to become anaerobic, which lowers the pH and causes odours. A nutrient management plan has to be designed to suit the specific area (Mannapperuma *et al.*, 1994).

Pre-treatment processes

The main aim of pre-treatment technologies is the physical separation of solids from the water or to chemically change the nature of the effluent (Woodroof & Luh, 1975). Physical separation is usually achieved through size or density difference. Static, tangential screens, vibratory screens, rotary screens, centrifugal screens and wedge wire filters are just a few of the devices that are used, based on size, to separate solids from wastewater (Woodroof & Luh, 1975; Martin *et al.*, 2001). Gravity sedimentation, decanter centrifuges, basket centrifuges, hydrocyclones and dissolved air flotation are the main technologies that are used to separate solid waste from effluents through density differences (Green &

Kramer, 1979). More effective separation requires an increase in particle size, or a change in density (Mannapperuma *et al.*, 1994). Coagulants and flocculants are used for this purpose. Colloidal particles are held in emulsion by electrostatic forces, and flocculation or coagulation is achieved by destabilizing the emulsion when flocculating or coagulating agents are added to the effluent (Brand & Marin, 1994).

The bulk of the COD in fruit processing wastewaters is associated with dissolved organic matter, which is not removed during pretreatment. It is, however, crucial that the solid and suspended matter are removed before biological treatments commence (Shober, 1998). Solid and suspended matter are usually not very biodegradable and will interfere with the biological processes (Van Lier *et al.*, 2001). Washout of valuable biomass will occur if bacteria attach to solid and suspended matter in settling reactors. This would contribute to the COD of the effluent and would have to be removed by a tertiary polishing process (Collivignarelli *et al.*, 1990).

Secondary treatment

Secondary treatment of food processing wastewaters involves biochemical reactions, carried out by a variety of micro-organisms, to degrade the dissolved organic matter. Biological wastewater treatment is achieved by many different micro-organisms, mostly bacteria, carrying out a complex, stepwise, continues, sequential attack on the organic compounds found in the wastewater (Grismer *et al.*, 2002). This is done either aerobically in the presence of oxygen or by anaerobic treatment in the absence of oxygen (Mannapperuma *et al.*, 1994). A wide variety of secondary biological wastewater treatment technologies are available like lagoon technology, oxidation ponds, activated sludge processes, trickling filters, rotating biological contactors, slow rate irrigation and wetlands to name just a few (Grismer *et al.*, 2002). Selecting the most appropriate or cost effective technology can be problematic since wastewaters respond different to each treatment process (Hayward *et al.*, 2000).

Aerobic treatment systems vary in sophistication from simple lagoons to high rate activated sludge ponds (Wayman, 1996). All of these aerobic processes, however, work on the same basic principles, but vary in efficiency and implementation cost (Tchobanoglous & Schroeder, 1985). Degradation occurs by bacteria that thrive in oxygen-rich environments, and pollutants are broken down to carbon dioxide, water, nitrates, sulfates and biomass. Aerobic bacteria are very efficient in breaking down waste products (Tofflemire, 1972; Brand & Marin, 1994).

Conventional secondary treatment technologies

The activated sludge process is the most widely used biological wastewater treatment system (Grismer *et al.*, 2002). Sequencing batch reactors, fixed film activated sludge processes, extended aeration processes, step aeration processes, modified aeration processes, pure oxygen activated sludge processes and powdered activated carbon activated sludge processes are all examples of the adapted activated sludge processes (Bitton, 1999). An aerobic jet loop activated sludge process was used to treat a brewery effluent and COD removal efficiencies of up to 97% were achieved at an organic loading rate (OLR) of 50 $kgCOD.m^{-3}.d^{-1}$ (Bloor *et al.,* 1995). It was also reported that the use of a sequencing batch reactor in the treatment of a dairy effluent achieved COD removal efficiencies of up to 97.7% at an OLR of 0.5 $kgCOD.m^{-3}.d^{-1}$(Torrijos *et al.*, 2001). A sequential batch biofilm reactor was also used in the treatment of winery effluents. COD removal efficiencies of between 85 and 99% were achieved at an OLR of 6.3 $kgCOD.m^{-3}.d^{-1}$ (Andreottola *et al.*, 2002).

Oxidation ponds have been used for the degradation of dairy and corn processing wastewaters. Removal efficiencies of between 70 and 90% were achieved at loading rates of 300 to 3 450 $kgCOD.ha^{-1}.d^{-1}$ (Muirhead, 1990; Kilani, 1992). Other aerobic treatment systems include: various lagoon and pond technologies; trickling filters; rotating biological contactors; slow rate irrigation; rapid infiltration; overland flow and wetland systems. These systems vary in efficiency, and implementation and running cost (Bitton, 1999).

Drawbacks of aerobic treatment systems - Even though great efficiencies can be achieved by aerobic treatments, there are concerns (White & Burt, 2002). The main drawback is the production of huge amounts of sludge. The aerobic pathway releases a substantial amount of energy and a significant fraction (50%) thereof is used by micro-organisms for the synthesis and growth of new micro-organisms (Bitton, 1999). This sharp plorification in aerobic biomass allows for fast and efficient pollutant removal but also produces large volumes of biomass which must be disposed of in an environmentally friendly and economical way (Barnett *et al.*, 1994). Biomass is a malodorous mess which may contain volatiles, organic solids, nutrients, disease-causing pathogenic organisms, heavy metals and inorganic ions and toxic organic chemicals. The bio-solids from a wastewater treatment plant consist mainly of water, and only 0.5 – 2% of its weight (on a mass to volume bases) is solid dry matter. Hence, the problem of dissolved organic waste

is transformed into a problem of particulate waste with a high water content (Forday & Greenfield, 1983; Lin & Yang, 1990).

Tertiary treatments

Tertiary processes are used increasingly for the re-use of water to meet regulation requirement for the disposal into natural waterways (Water Research Commission, 1987). Tertiary treatments include polishing lagoons, sand filters, activated carbon filters, ion exchangers, chlorinators, ozonators, electrodialysis, reverse osmosis and crossflow membrane filtration (Wayman, 1996). Activated carbon, chlorinators and reverse osmosis have also been used to remove colour, odour, brine, and taste compounds that are not removed in biological treatment. Filters are used as a tertiary treatment to remove suspended solids (Mannapperuma *et al.*, 1994). Ozonation is frequently used in the treatment of drinking water. The use of ozonation as a tertiary treatment has recently became popular, with many advantages over traditional polishing methods (Sigge *et al.*, 2002).

E. ANAEROBIC DIGESTION

Anaerobic treatment is an attractive alternative, compared to the more popular aerobic system for treating high-strength organic wastewaters from food processing plants (Trotzke, 1988). Anaerobic systems involve lower capital investment, and less energy and chemical consumption compared with aerobic systems (Mannapperuma *et al.*, 1994). Anaerobic digestion (AD) is a complex combination of biochemical reactions carried out in a number of steps by several types of micro-organisms that require little or no oxygen to live. During the process, a biogas composed of methane and carbon dioxide is produced (Barnett *et al.*, 1994). AD of wastes from the food industry normally occurs in four distinct steps and four categories of bacteria are involved in a synergistic relationship in the transformation of complex materials into simple molecules (Forday & Greenfield, 1983; Ditchfield, 1986; Bitton, 1999). Those four steps include:

1. **Hydrolysis by Hydrolytic bacteria -** this group is responsible for the breakdown of complex organic molecules like proteins, lipids, cellulose and lignin, to soluble monomers like amino acids, glucose, fatty acids and glycerol. Extracellular enzymes are used to make these complex molecules directly available for the next bacterial group. The complete methanogenic process is generally limited by the rate of hydrolysis of suspended matter and organic solids driven by hydrolytic bacteria (Van Lier *et al.*, 2001);

2. Fermentation by Fermentative acidogenic bacteria – these bacteria converts soluble monomers like carbohydrates sugars, amino acids and fatty acids to organic acids, alcohols, ketones, acetate, CO_2, and H_2. Acetc acid is the main fermentative product formed from carbohydrate fermentation, and is an essential substrate for the growth of methanogenic bacteria (Forday & Greenfield, 1983).

3. Acetogenesis: Acetogenic bacteria - This group converts ethanol, propionic and butyric acid to acetic acid, H_2 and CO_2 according to the following reactions (Bitton, 1999);

$CH_3CH_2OH + CO_2 \longrightarrow CH_3COOH + 2H_2$
Ethanol acetic acid;

$CH_3CH_2COOH + 2H_2O \longrightarrow CH_3COOH + CO_2 + 3H_2$;
Propionic acid acetic acid;

$CH_3CH_2CH_2COOH + 2H_2O \longrightarrow CH_3COOH + 2H_2$
Butyric acid acetic acid.

The acetogenic bacteria grow in close association with the methanogenic bacteria. The reason for this is that the conversion of the fermentation products by the acetogens is only possible if the hydrogen concentration is kept sufficiently low. Methanogens help to lower the hydrogen tension required by the acetogenic bacteria while consuming the acetate produced by the acetogenic bacteria. This requires a close symbiotic relationship between both groups of bacteria (Speece, 1983; Bitton, 1999); and

4. Methanogenesis by Methanogenic bacteria – the methanogens include both Gram-positive and Gram negative bacteria with a wide variety of morphologies. Methanogenic organisms grow slowly, with a μ_{max} of approximately 0.04 hr^{-1}, compared to acetogenic bacteria with a μ_{max} of around 1 hr^{-1}. (Ditchfield, 1986; Bitton, 1999). Methanogens are subdivided into two categories:

1. Hydtrogenotrophic Methanogens that convert hydrogen and carbon dioxide (CO_2) into methane (CH_4) and water. $CO_2 + 4H_2 \longrightarrow CH_4 + 2H_2O$
2. Acetotrophic Methanogens that split acetate into CH_4 and carbon dioxide. $CH_3COOH \longrightarrow CH_4 + CO_2$

About two thirds of the methane is derived from acetate conversion by acetotrophic methanogens and the other third from carbon dioxide reduction by hytrogenotrophic methanogens (Forday & Greenfield, 1983; Ianotti *et al.*, 1987; Bitton 1999). The conversion processes in the AD pathway are shown in Fig. 4.

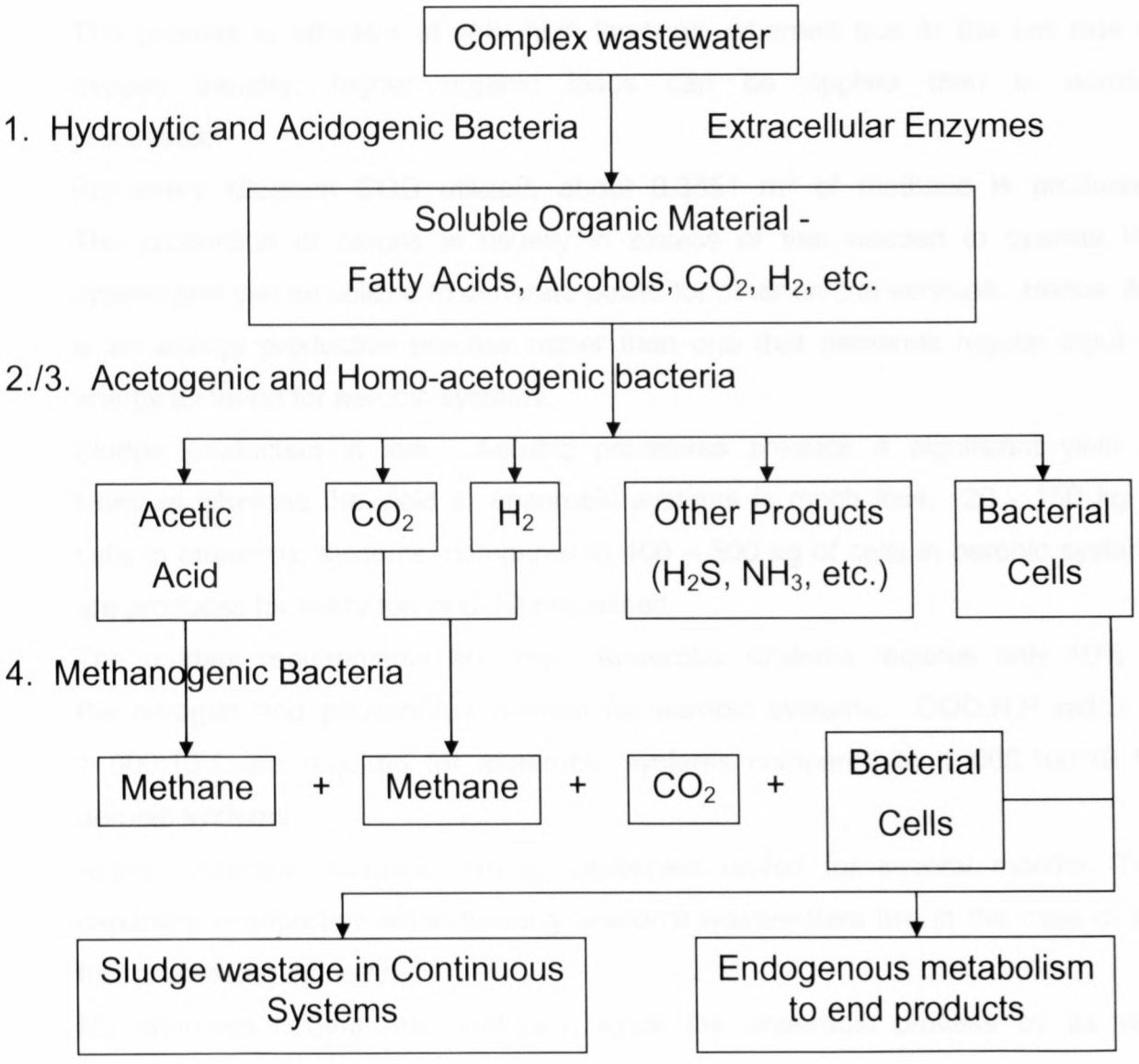

Figure 4. Outline of the overall anaerobic digestion pathway (Murry *et al.*, 1985).

The AD process has advantages and disadvantages (Ditchfield, 1986; Lin & Yang, 1990; Tchobanoglous & Burton, 1991; Anderson, & Saw 1994; Bitton, 1999). Some of the benefits include:

1. The process is effective at very high loadings, whereas due to the low rate of oxygen transfer, higher organic loads can be applied than in aerobic processes;
2. For every kilogram COD utilized, about 0.3551 m^3 of methane is produced. The production of biogas is usually in excess of that needed to operate the system and can be utilized to generate power for other on-site services. Hence, AD is an energy productive process rather than one that demands regular input of energy as found for aerobic systems;
3. Sludge production is low. Aerobic processes produce a significant yield of biomass whereas the yield in anaerobic systems is much less. 20 - 150 kg of cells in anaerobic systems, compared to 400 – 500 kg of cells in aerobic systems are produces for every ton of COD processed.
4. The nutrient requirements are low. Anaerobic systems requires only 10% of the nitrogen and phosphorus needed for aerobic systems. COD:N:P ratios of 1 000:10:1 are required for anaerobic systems compared to 1 000:100:10 for aerobic systems;
5. Active anaerobic biomass can be preserved un-fed for several months. This capability is important when treating seasonal wastewaters like in the case of the fruit processing industry;
6. No offensive odours are produced, since the anaerobic process by its very nature is totally enclosed.

Limitations of AD include:

1. The process is slow at ambient temperatures. Lew *et al.* (2003) reported that COD removals of a UASB reactor treating domestic waste at 10, 14, 20 and 28°C was 48, 70, 72, and 82%;
2. Because of the relatively high polluting strength of the digester effluent, further treatment is usually necessary before discharge into receiving waters;
3. Due to the slow growth rate of anaerobic bacteria, relatively long periods of time are required for the process "start-up".

During the AD process, the pollutants in the wastewater are transformed into methane, carbon dioxide and a small amount of biomass (Tchobanoglous & Burton, 1991). A significant part of the energy originating from pollutant degradation leave the system as biogas, leaving only a fraction of the initial energy for the assimilation of biomass. The

amount of sludge produced in the anaerobic system is therefore much lower than the amount created in aerobic systems (White & Burt, 2002). In anaerobic systems only 5% of the organic matter in the substrate is converted to biomass compared to 50% in aerobic systems (Forday & Greenfield, 1983; Lin & Yang, 1990). Anaerobic sludge is also more compact than aerobic sludge with a total solid content of 2 – 8% compared to only 0.5 – 2.0% (mass to volume basis) solids found in aerobic sludge (White & Burt, 2002). The combination of less but a more compacted sludge results in 7 to 12 times less sludge produced by anaerobic systems compared to aerobic system based on a volume basis (Barnett *et al.*, 1994). A comparison between the conversion processes in aerobic and anaerobic processes is shown in Fig 5. Aerobic systems also need energy consuming aeration whereas anaerobic systems result in a positive energy balance due to the production of biogas (Mannapperuma, 1995). Since the solubility of the methane is low it escapes as methane gas. As a result, a significant part of the energy originating from the system leave the system as biogas, and subsequently less energy is available for the production of biomass (Ditchfield, 1986).

In the current energy and pollution situation, the cost of food processing effluent treatment is becoming more acute every year. AD is economically and ecologically a significant step forward in industrial effluent treatment (Sigge, 2000). The operational drawbacks of an AD process is that the system requires stringent process control and usually reduces the organic pollutant by only 85 – 90% (Ditchfield, 1986). Nutrient removal, in term of organic nitrogen and phosphorus compounds, is low and could cause further eutrophication of a river system to which the effluent is discharged (Tofflemire, 1972; Bitton, 1999). Anaerobic organisms are extremely versatile in degrading compounds that cannot be digested by aerobic processes. However, anaerobic organisms are not invulnerable (Lin & Yang, 1990). Anaerobic systems are also more unstable than aerobic systems and better management is required to run the process. The anaerobic process requires specific adapted biomass and particular processing conditions, which differ considerably from those needed by the aerobic system. A more specific substrate with less daily variations than that of aerobic systems, is also essential (Bitton, 1999).

Furthermore anaerobic organisms are very sensitive to changes in their environment. Even slight modifications can cause the inhibition of certain bacterial groups. If, for instance, the methanogenic bacteria are inhibited, the acid producers continue forming acid. This causes the pH to decrease, which will have a negative effect on all the other bacterial groups (Lettinga & Hulshoff Pol, 1991). For this reason the anaerobic system can be seen as an unstable system. Changes in pH, temperature, essential nutrient

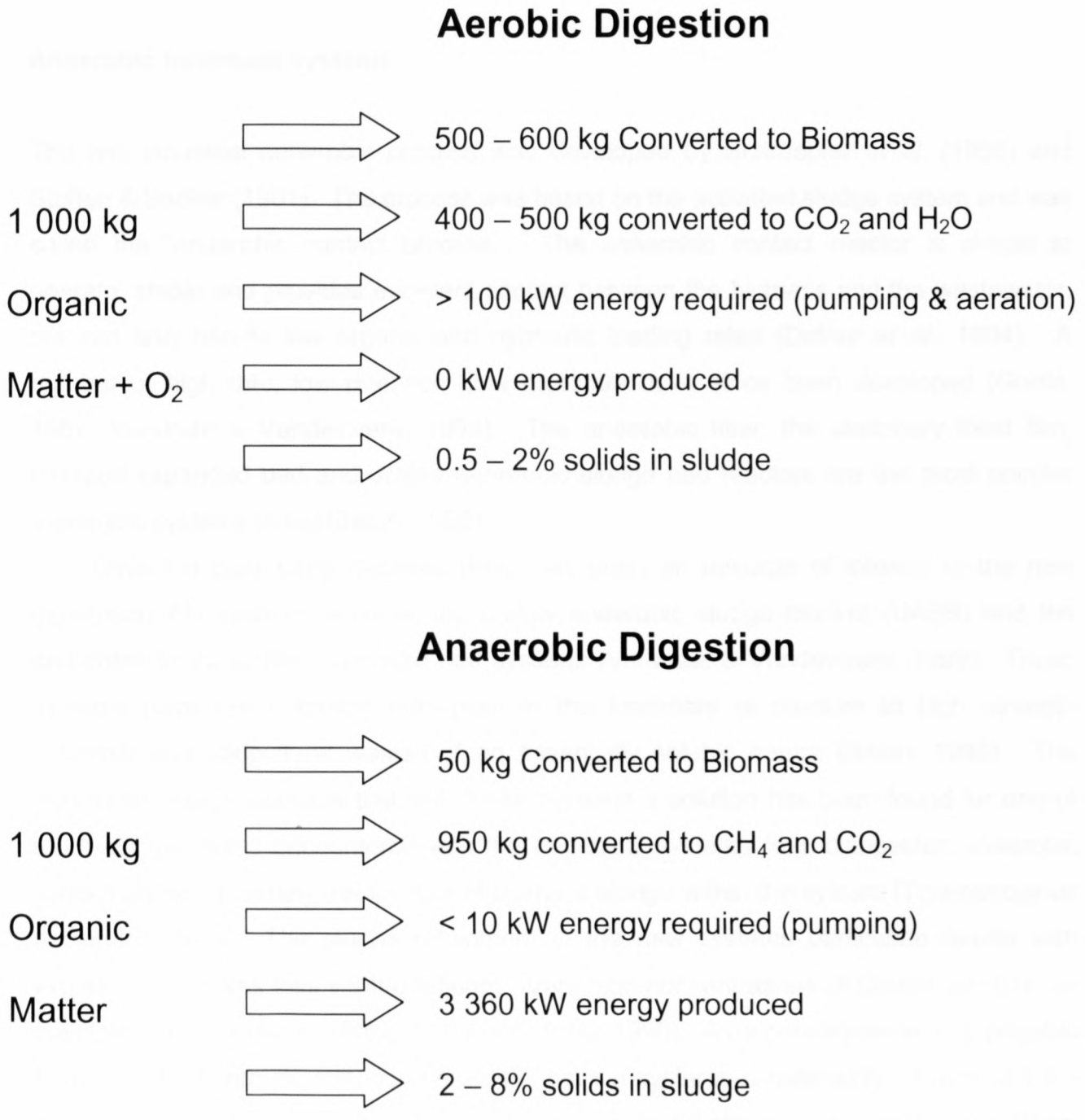

Figure 5: A simplified model of the conversion processes associated with aerobic and anaerobic treatment methods (White & Burt, 2002).

concentration and availability, and degree of mixing are a few of the many changes that affect normal operation of anaerobic bacteria (White & Burt, 2002).

Anaerobic treatment systems

The first industrial anaerobic process was developed by Schroepfer *et al.* (1955) and Steffen & Bedker (1961). The process was based on the activated sludge system and was called the "anaerobic contact process". The anaerobic contact reactor is simple to operate, stable and provides excellent contact between the biomass and the wastewater, but can only handle low organic and hydraulic loading rates (Defour *et al.*, 1994). A number of high rate, low retention time digesters have since been developed (Gorris, 1987; Verstrate & Vandevivere, 1999). The anaerobic filter, the stationary fixed film, fluidized expanded bed and upflow anaerobic sludge bed reactors are the most popular anaerobic systems today (Bitton, 1999).

Over the past three decades there has been an upsurge of interest in the new generation AD systems such as the upflow anaerobic sludge blanket (UASB) and the anaerobic attached film expanded bed systems (Verstrate & Vandevivere, 1999). These systems have been applied principally in the treatment of medium to high strength industrial and agricultural wastes of an essentially soluble nature (Bitton, 1999). The reason for their success is that with these systems a solution has been found for one of the major problems encountered with the traditional systems (e.g. clarigester, anaerobic contact process) namely, retainment of biomass sludge within the system (Tchobanoglous & Burton, 1991). The sludge retainment in the new systems particulate readily with virtually no biomass loss via the effluent. Very high concentrations of sludge can thus be maintained (in excess of 100kg.m^{-3}) (Lin & Yang, 1990). As a consequence, it is possible to reduce the hydraulic retention time (HRT) in the systems substantially. Times of 1.5 – 2.0 hours for low strength effluents have been reported (Lettinga *et al.*, 1983; Lin & Yang, 1990).

The UASB reactor - The UASB concept was developed from a "clarigestor" reactor design, which can be viewed as an ancestor to the UASB reactor. A clarigestor-reactor, situated in the Western Cape (South Africa), was treating wine vinasse when it was recognised that an inert support material for biomass attachment was not necessary to retain high levels of active sludge in the reactor (Field, 2002). Instead, the UASB concept relies on high levels of biomass retention through the formation of sludge granules (Lin & Yang, 1990).

The UASB reactor concept was rapidly developed, and the first pilot plant was installed at a beet sugar refinery in The Netherlands (CSM Suiker). Thereafter, a large number of full-scale plants were installed throughout the Netherlands at sugar refineries, potato starch processing plants and other food industries as well as paper recycling plants (Field, 2002). Since then, full-scale UASB reactors have been installed all over the world for the treatment of a wide range of different wastewaters (Lin & Yang, 1990).

The UASB reactor can be divided into three parts (Lettinga & Hulshoff Pol, 1991):

1. The sludge bed at the bottom of the reactor, where the heaviest fraction (granules) of the biomass is situated.
2. The sludge blanket is situated just above the sludge bed and is a diffuse layer of fluidized sludge flocks and small granules.
3. The gas solid separator at the top of the reactor is where the gas is separated from the sludge by gas baffles. The sludge particles separated from the gas will settle back to the sludge bed in the settling compartment (Hickey *et al.*, 1991).

The wastewater is pumped into the reactor through the influent distribution system at the bottom of the reactor. A distribution system guarantees equal substrate distribution over the entire surface of the reactor. The anaerobic biomass in the reactor metabolizes the dissolved organic compounds and transforms them into biogas and biomass (Lettinga & Hulshoff Pol, 1991). The biogas is separated from the sludge and the clean effluent leave the reactor at the top via the effluent pipe. A schematic illustration of the design of an UASB reactor is shown in Fig. 6. The design of the reactor is such that the development of heavy biomass (granules) is favoured, but that simultaneously the hydrolysis of suspended solids is maximized. The reactor can be designed for low to very high hydraulic capacities and show good

flexibility for wastewater with high COD concentrations (Lin & Yang, 1990). The success of the UASB system is based on a few principles. Optimal contact between the sludge and the wastewater is achieved by natural mixing in the reactor due to the biogas production and a properly designed feed inlet system, resulting in an equal distribution of the wastewater in the reactor (Punal & Lema, 1999).

Of the various "new" systems the UASB system is the least complex, and fairly straightforward to operate (Torkian *et al.*, 2003). In the other "popular" systems, the anaerobic attached film expanded-bed system, the biomass is attached to a support medium (e.g. sand). No such support medium is required in the UASB process (Lin & Yang, 1990). This "lack" of complexity constitutes a significant advantage to the UASB system, particularly in regional areas where technical supervision is lacking (Dold *et al.* 1987). The effectiveness of the UASB reactor can be seen when the loading rates and

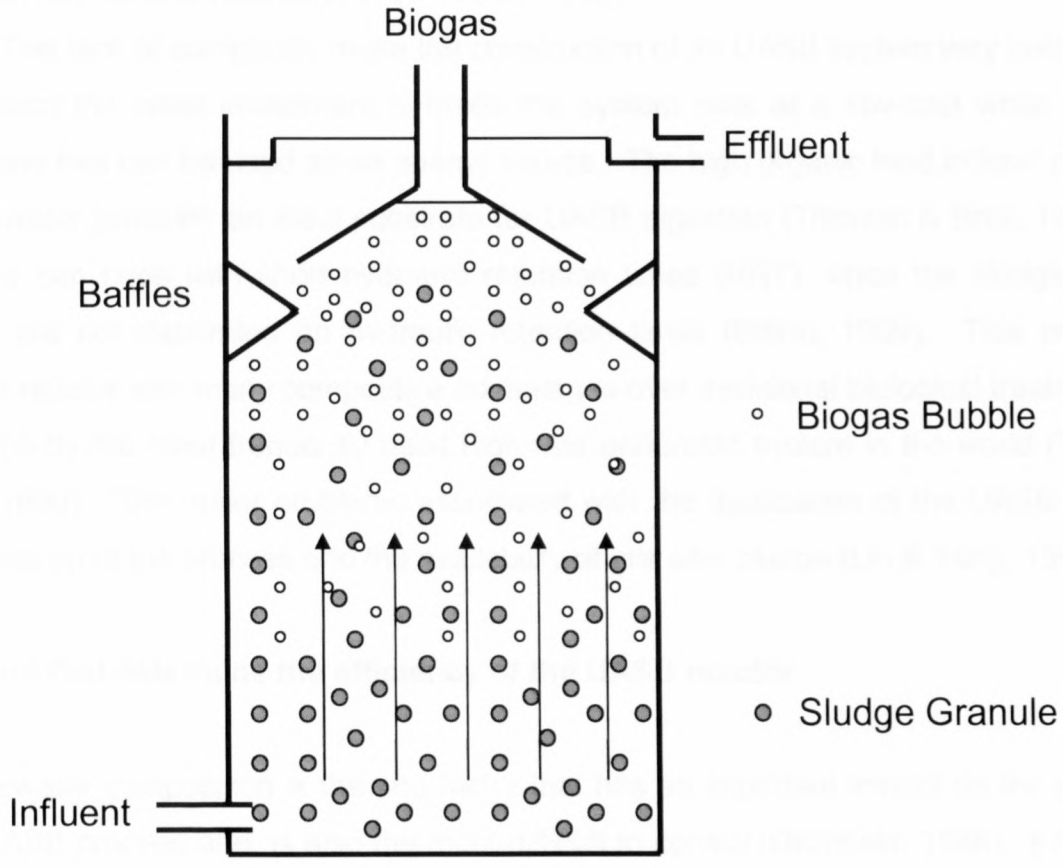

Figure 6: The upward-flow anaerobic sludge bed (UASB) reactor (Field, 2002).

Table 3: Comparison of reactor type, loading rates, and removal efficiencies (Van Den Berg & Kennedy, 1983).

Reactor type	COD loading ($kg.m^{-3}.d^{-1}$)	COD removal (%)
Anaerobic contact	1 – 6	80 - 95
Upflow Filter	1 – 10	80 - 95
Fluidized-bed	1 – 20	80 - 87
Expanded-bed	1 – 20	80 - 87
Downflow Filter	5 - 15	75 - 88
UASB	5 – 30	85 – 95

removal efficiencies of some of the most prevalent reactor designs are compared in (Table 3) (Van Den Berg & Kennedy, 1983: Bitton, 1999).

This lack of complexity make the construction of an UASB system very cost effective, and once the initial investment is made the system runs at a low-cost while producing methane that can be used as an energy source. The high organic load in food processing wastewater provides an ideal substrate for UASB digestion (Trnovec & Britz, 1998). The reactor can cope with short hydraulic retention times (HRT), since the sludge retention times are not depended on hydraulic retention times (Bitton, 1999). This provide the UASB reactor with many competitive advantages over traditional biological treatments and is currently the most frequently used high rate anaerobic system in the world (Trnovec & Britz 1998). The major problems associated with the application of the UASB system is the start-up of the process and the availability of granular sludge (Lin & Yang, 1990).

Factors that determine the efficiency of the UASB reactor

Wastewater composition is the one factor that has an important impact on the success of the UASB process and, is also the most difficult to control (Ditchfield, 1986). For effective AD the wastewater that enters the system must be digestible by the organisms in the reactor, and promote the granulation process (Lettinga, & Hulshoff Pol, 1991). Granulation proceeds faster when the influent is composed of mainly soluble carbohydrates while the presence of finely dispersed non-biodegradable organic and inorganic matter is detrimental to granulation. This is because the attachment of newly formed bacteria to the continuously supplied fresh particles will retard or prevent the further development or growth of existing granules (Lin & Yang, 1990).

Substances with a low biodegradability will build-up in the system and upset the digestion process, or wash out of the system as part of the effluent COD (Van Lier *et al.*, 2001). The ideal would be to ensure that only digestible organic matter enters the system, and to maintain the right microbial consortium and optimum growth conditions to digest this matter. This is, however, not an easy task and very often unwanted matter (toxins or indigestible organic material) will enter the system (Defour *et al.*, 1994).

Many other factors interfere with the digestion process and negatively influence the sensitive suspended biomass. Organic overloading, biocidical substances, complex COD matter, incorrect digestive conditions and to short hydraulic retention times are all factors that could disrupt the system (Ditchfield, 1986). A long recovery process is required for the damaged biomass to regain activity causing the build-up of untreated wastewater.

Good managing skills and technical knowledge are thus essential to effectively operate an UASB system (Bloor *et al.*, 1995).

The metabolic requirements of an UASB system are: the correct growth factors; appropriate temperatures and pH ranges; and a high enough retention time for efficient substrate-biomass contact (Defour *et al.*, 1994). An acceptable balance should be maintained between the nutrients necessary for growth and in general the balance should be BOD:N:P in a ratio of 1000:10:1 for AD (Lin & Yang, 1990; Trnovec & Britz, 1998). The availability of essential nutrients is also of importance, because growth conditions should be optimal. Apart from nitrogen, phosphate and sulfate, trace elements should also be present in sufficient concentrations (Lettinga *et al.*, 1980). Limiting concentrations of cobalt (Co), nickel (Ni) and Iron (Fe) have been reported to be 0.10, 0.25 and 5.00 uM, respectively (Hickey *et al.*, 1991). Monovalent cations such as ammonium (NH_4^+) and sodium (Na^+) may affect the settling ability of sludge. Sodium appears to be essential for the growth of methanogenic bacteria. An optimal concentration of 350 $mg.l^{-1}$ has been reported (Lin & Yang, 1990). However at higher concentrations, Na^+ is inhibitory to growth and flocculation/granulation (Murthy, 1998).

It has been observed that divalent cations such as calcium (Ca^{2+}), magnesium and iorn have a positive effect on the flocculation of sludge (Yu, 2000). A low concentration of Ca^{2+} of up to 150 $mg.l^{-1}$ is beneficial for the retention of sludge and the formation of granules. Higher Ca^{2+} concentrations (> 400$mg.l^{-1}$) can, however, lead to severe scaling problems, as well as being unfavourable for granulation (Mahoney *et al.*, 1986). The reason why calcium is of importance may be explained when observing the flocculation process of bacterial cells (Murthy, 1998). Calcium has the ability to bridge between the electronegative carboxyl and phosphate groups associated with bacterial surfaces. The presence of calcium enhances the adhesion of cells, because they act as a link between negatively charged bacterial cells. In contrast, monovalent cations like sodium will "block" this electronegative charge to prevent the adhesion and flocculation of bacterial cells. Optimum granulation will then occur at a calcium concentration of 150 to 300 $mg.l^{-1}$ (Yu, 2000). Monovalent to divalent cation ratios are also of great importance. Effluents with enough calcium but too much sodium will not promote granulation. It has been observed that the monovalent to divalent cation ratios must be kept lower than 2 for effective flocculation. Effluents with high sodium contents will then prevent flocculation regardless of the calcium content (Murthy, 1998). Biocidical chemicals should be excluded from the system. These include herbicides, insecticides, disinfectants, machinery maintenance materials, solvents, sterilizers, lubricants, timber preservatives, rodent controls and fuel.

Inclusion of these substances in the wastewater will interfere with biological treatment and the system could take weeks to recover (Mannapperuma, 1995).

Fruit processing effluent as substrate and the impact of pectin on the UASB operation

Certain effluents, like wastewater from the sugar beet industry, are an ideal substrate for UASB digestion (Van Lier *et al.*, 2001). Other effluents may contain complex organic matter or other problematic constituents that may influence the UASB process (Lettinga *et al.*, 1980). Effluents from the fruit processing industries usually contain high levels of pectin that will influence the process. The particular nature of pectin in effluents and how they react under different wastewater treatment conditions have not yet been studied. It is, however, clear that an effluent with a high pectin content will cause various problems in the UASB reactor (Fedirici *et al.*, 1988).

Complex carbohydrates are less biodegradable than simple sugars. The UASB process is often limited by the hydrolysis rate of suspended matter and organic solids (Van Lier *et al.*, 2001). Micro-organisms in the suspended biomass do not always have the necessary enzymes to hydrolyze complex molecules (Whitaker, 1990). Pectin is a complex molecule that will interfere with the reactor performance in various ways. Pectin can form a gel at relatively low concentration (0.1% m/v). A gel will usually form at a high soluble organic content (60°Brix) and a low pH (3.3) (Anon., 2003). These conditions are, not present in the UASB reactor and this type of gel formation should not occur. Pectin is, however, able to form a gel by another method. Because of the methoxyl group in pectin, interactions can form with calcium (Shivakumer & Nand, 1995). Calcium can bridge the gap between the two negatively charged molecules to form a complex network that will trap water and form a gel. This type of gel will form at low soluble carbohydrate contents and is not pH dependent (Broomfield, 1996). Under the right circumstances this type of gel formation will occur in the UASB reactor treating a calcium and pectin containing wastewater.

The pH of apple juice processing wastewater varies from 3 to 5, and thus a pH adjustment is required before the effluent can enter the reactor (Wayman, 1996). Traditionally, calcium hydroxide is used for pH adjustments but the combination of pectin and calcium in the reactor leads to an increase in viscosity. Since the success of the UASB process is based on the ability of the biomass to settle (Lettinga, & Hulshoff Pol, 1991) and the settling ability is dependent on the viscosity. An increase in viscosity in the reactor will effectively prevent the gravitational separation of the sludge and the liquid

(Murthy, 1998). This will lead to the overflow of biomass. A constant loss of valuable biomass will increase the amount of substrate relative to the microbial community. Reactor overloading will thus occur, causing reactor failure (Lin & Yang, 1990). .

This problem is greatly increased by using sodium hydroxide instead of calcium hydroxide to eliminate gel formation, since as previously mentioned, calcium is essential for the granulation process, while high sodium levels will prevent granulation (Lin & Yang, 1990). By replacing calcium with sodium, the gel formation problem is reduced, but other problems, like the inability of the sludge to granulate due to the high monovalent to divalent cation ratios consequently occurs (Murthy, 1998). Another problem associated with sodium hydroxide, is that effluents with high sodium contents may not be used for irrigation (Wayman,1996). An excessive salt concentration increases the osmotic pressure, which in turn makes it difficult for the roots to extract water and then reduces the plant growth (Water Research Commission, 1987). High levels of sodium ions relative to calcium and magnesium ions alter the soil structure and reduce soil permeability. In addition to these effects, certain specific ions can have toxic effects on plants (Mannapperuma, 1995).

A solution to the problems associated with calcium and sodium use is urgently needed. The hydrolysis of the pectin molecule would not only prevent gel formation but would also make the pectin molecule more available for biological degradation since not many micro-organisms have the necessary enzymes capable of digesting the pectin (Whitaker,1990). The hydrolysis of pectin would result in the formation of more biodegradable substances, like organic acids, and could be a beneficial pre-treatment to AD (Van Lier *et al.*, 2001).

Pre-treatment of wastewaters prior to AD

Biological degradation is one option to treat wastewater with a high organic loads (Wayman,1996), but complete abatement of wastewater pollutants can hardly be achieved by the use of a single treatment method. A combination of chemical and biological treatment is one way to optimize the overall process. The first step, if wisely chosen, will enhance the second choice, thus leading to more effective treatment solutions (Andreozzi *et al.*, 1997). At the start each waste should be considered separately so as to decide which treatment or combination of options are the most suitable, and in what sequence and intensity they should be applied (Weemaes *et al.*, 1999). Biological inhibitory compounds and less biodegradable compounds will be transformed into biodegradable compounds with the correct chemical pre-treatments (Volk *et al.*, 1993).

The UASB reactor is especially effective in the removal of colloidal and soluble waste fractions, but is less effective in the removal of coarse suspended solids (Van Lier *et al.*, 2001). Molecules of high molecular weight such as soluble polymers are also difficult to degrade owing to their bulky nature or lack of reactive sites. In contrast chemical oxidation may be the answer to these problems as it may break such molecules into smaller, biodegradable fractions (Martin *et al.*, 2001). It would thus be desirable to have a chemical oxidative pre-treatment capable of converting the inhibitory and refractory compounds into simpler molecules of a lower molecular weight that are readily available as substrates for anaerobic populations present in UASB systems (Benitez *et al.*, 1999). A combined chemical oxidation–AD treatment on wastewater with a high refractory compound content should lead to an increase the effectiveness of the overall process (Martin *et al.*, 2001).

F. CHEMICAL OXIDATION

Many chemical processes are available, but oxidizing agents like chlorine, chloroderivatives, ozone, ultra violet (UV) radiation, hydrogen peroxide, are the most effective in reducing the organic matter (Athanasolopoulus & Athanasolopoulus, 1998). The traditional objectives of these oxidations are the transformation of the organic pollutants into non-toxic and less biodegradable substances. The transformation of organic substances into more biodegradable and a more reactor friendly substrates is also possible depending on the intensity of the treatment (Andreozzi *et al.*, 1997).

The use of advanced oxidation processes (AOPs) in the treatment of wastewater has recently become popular (Camel & Bermond, 1998). It has been reported that ozonation as a pre-treatment can improve the biodegradability of an effluent (Weemaes *et al.*, 1999; Baig & Leichti, 2001; Martin *et al.*, 2001). The hydrolysis of complex organic material like pectin and cellulose, found in apple juice processing wastewaters, should improve the suitability of the wastewater for secondary biological treatment by improving biodegradability and reducing the gel formation ability of the pectin in the effluent (Beltran-Heredia *et al.*, 2000; Baig & Leichti, 2001).

Ozonation is one of the methods that have been reported to achieve better results in the oxidation and degradation of waste in general. It is preferred over chlorination for the removal of organic pollutants present in water due to its higher oxidation potential (Braun *et al.*, 1993). In addition, chlorination has been linked to the formation of organohalogens in general, and trihalomethanes in particular, such as chloroform and bromoforms. Ozonation does not leave any by-products that need to be removed from the wastewater (Beltran-Heredia *et al.*, 2000).

Ozonation

Ozone is a pale blue gas with a pungent odour and due to its molecular structure it is highly unstable making it one of the most powerful oxidizing agents (Murray, 1999). It is toxic, but can be detected by smell at levels far below that at which it becomes dangerous. Due to its instability, it breaks down into oxygen very quickly. Commercially, ozone is produced by the passage of a high voltage, alternating electric discharge through a gas stream containing oxygen. (Essop, 1998). This discharge results in the breakdown of diatomic oxygen molecules and the reformation of some into triatomic oxygen (Gottschalk *et al.*, 2000). Ozone is the most powerful chemical oxidant available for water treatment. Once dissolved in water ozone can react with many organic compounds either by direct reaction as molecular ozone or by indirect reaction through the formation of secondary oxidants like free radicals (Sigge *et al.*, 2002).

Ozonation is usually utilised as a method of water disinfection but it also has potential as pre-treatment method before biological degradation processes (Essop, 1998; Gottschalk *et al.*, 2000). Ozonation is a promising technology, as it has many of the oxidizing properties desirable for an efficient wastewater treatment option (Benitez *et al.*, 1999; Gottschalk *et al.*, 2000).

It is well known that ozone can hydrolyze organic polymers into smaller units (Gottschalk *et al.*, 2000; Sigge *et al.*, 2002). Ozonation enhances the biodegradability of organic matter by increasing molecule polarity and by forming smaller molecular weight substances (Volk *et al.*, 1993). The increase in biodegradability after ozonation has already been shown for 28 substituted aromatic compounds under aerobic conditions and for some phenolic compounds under anaerobic conditions (Gottschalk *et al.*, 2000).

Several studies have proven that ozonation increase the methane yield in UASB reactors (Javier Benitez *et al.*, 1999; Martin *et al.*, 2001). It is known that the accumulation of suspended solids within the UASB reactor granule bed hampers the conversion of COD to methane (Harada *et al.*, 1996). The lack of methane production increases the acetic acid concentration in the reactor, causing a pH decrease which has a negative effect on the reactor performance. Weemaes *et al.* (1999) tested the effect of different ozone doses as a pre-treatment for AD and found that 0.05 g O_3.g COD^{-1} resulted in 1.7 times more methane than the untreated substrate. An ozone dose of 0.1 g O_3.g COD^{-1} produced 2.2 times more methane. Hence the biodegradability of the substrate can be significantly enhanced by the pre-treatment. At higher ozone doses (0.2 g O_3/g COD) the effect of the oxidative pre-treatment was less pronounced. The methane production improved by a factor of only 1.3. The reason why lower degradation took place at higher pre-treatment

doses was because ozone increased the redox potential of the substrate. Gottschalk *et al.* (2000) reported that the anaerobic community might not succeed in lowering the redox potential to a level compatible with methanogenesis in wastewater with a high redox potential. At lower levels of ozone the redox potential did not have an effect on the methanogenic population, but at higher ozone doses the higher redox potential outweighed the hydroxylation effect of the ozone and decreased the biodegradability (Gottschalk *et al.*, 2000). A less intense ozone treatment would thus provide a more biodegradable substrate than excessive ozonation.

Ozone is selective towards the oxidation of double bonds (Beltran-Heredia *et al.*, 2000). Theoretically, ozone should not react with proteins and sugars, but would react with double bonds of unsaturated fatty acids, phenols and aromatic compounds found wastewaters with more complex organic material. After ozonation the biomass has not lost its potential as a substrate for AD since the simple sugars and proteins are still present (Andreozzi *et al.*, 1997). A larger portion of the organic constituents in the wastewater will thus be biodegradable after ozonation which will increase the organic removal efficiencies of biological treatments (Volk *et al.*, 1993).

Ozone is very reactive with poly nuclear compounds like pectin. The reaction first involves aromatic ring opening by electrophylic attack and will thereafter form aldehydes and organic acids (Martin *et al.*, 2001). Ozone is extensively used in the paper mill industry to break down lignin and cellulose, substances with a similar polymeric structure as pectin (Van der Walt, 1993).

G. DISCUSSION

The apple industry of South Africa is growing by 2.1% annually. Forty percent of the apples harvested cannot be sold on the fresh market and further processing into an apple juice concentrate is unavoidable. Large volumes of high oxygen demanding wastewater (9.5 m^3.ton of apples processed) are produced during this process. The wastewater has a large impact on the environment, mainly due to its high organic content. Many industries are struggling to reduce the organic content of their wastewater to an acceptable level, and large investments have been made to solve this problem. Biological treatment is the most efficient and economical way to reduce high COD values. Aerobic digestion is the traditional and most widely used method to treat oxygen consuming wastewater. Various problems are, however, associated with aerobic processes like the high energy requirements (>100 $kW.ton^{-1}$ of COD reduced) and the production of large sludge quantities (50% of the COD). The advantages of AD in the processing of high strength

organic waste are well documented. A positive energy balance, due to the production of biogas, and low sludge volume productions are the main advantages of AD. AD can be seen as a mature technology and many successful systems have been installed worldwide. The UASB reactor is the most popular anaerobic reactor design, and has the ability to retain high biomass concentrations which allows a shorter HRT giving rise to an increase in reactor efficiency.

Most fruit processing wastewaters provides an ideal substrate for the UASB process. The successful UASB treatment of many fruit processing wastewaters has convinced the apple industry to invest in this technology. Wastewater characteristics have a large influence on the UASB reactor performance. Finley dispersed and complex organic matter are detrimental to the UASB process. Any increase in wastewater viscosity will also have a negative influence on the reactor performance.

Fresh apples contain 5 000 – 16 000 $mg.L^{-1}$ pectin which are separated from the juice fraction with the other apple solids during the pressing of the apples. It is inevitable that the pectin will end up in the wastewater although some of it are separated during the pressing stage and used as an animal feed. Pectin has the ability to form interactions with calcium causing the formation of a gel. $Ca(OH)_2$ are used to neutralise the pH of the wastewater before UASB treatment. The combination of calcium and pectin in the wastewater cause an increase in the wastewater viscosity. The replacement of $Ca(OH)_2$ with NaOH have been done in the past but this increased sodium concentrations in the reactor effluent. In South Africa most of the treated wastewater is used for irrigation purposes, but high sodium contents in the effluent prevented this use. This forced the treatment plants to use $Ca(OH)_2$ for pH adjustments.

Hydrolysis is the first step in the anaerobic degradation process, and is done by extracellular enzymes produced by a specially adapted microbial consortium. The hydrolysis of more complex material requires specialized enzymes produced by a variety of hydrolytic and acidogenic bacteria. A more complex wastewater would require a larger more diverse microbial consortium to produce the correct enzymes for successful hydrolysis. Long adaptation times are required for this microbial consortium to develop and the hydrolysis process itself is time consuming, and is often the limiting step in the anaerobic degradation process. The hydrolysis of complex organic material into smaller units before biological treatment should shorted the substrate adaptation time and therefore increase the microbial hydrolysis efficiency.

Ozone has the ability to hydrolyse complex wastewater to produce a more biodegradable substrate. AD efficiency can be improved by the application of ozonation as a pre-treatment. Ozone is very reactive with poly-nuclear substances like pectin,

cellulose and lignin. The ability of ozone to form a gel is dependent on the chain length of the pectin fragment. Gel formation will effectively be prevented if sufficient hydrolysis occurs. The hydrolysis of pectin before AD should thus increase the effectiveness of the system due to less gel formation and a shorter microbial hydrolysis processes required. The pre-ozonation of apple juice processing wastewater before AD could be a viable option.

Anaerobic digestion usually reduces organic pollutants by 85 – 90% and further tertiary treatments are often required before discharge or irrigation limits are met. Ozone has the ability to reduce the COD content of a wastewater. Excessive ozonation will extract hydrogen ions to mineralize organic components to CO_2 and water and thereby decreases the COD of the effluent. Total elimination of all organic compounds from raw wastewater would, however, in most cases be uneconomical due to the amount of ozone required. The use of ozone as a post treatment after biological treatment could however be a viable option.

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CHAPTER 3

EVALUATION AND OPTIMISATION OF AN UASB BIOREACTOR TREATING APPLE JUICE PROCESSING WASTEWATER

Summary

A mesophylic laboratory-scale upflow anaerobic sludge bed bioreactor seeded with 600 mL of anaerobic sludge obtained at an apple juice wastewater treatment plant was evaluated for the anaerobic digestion of an apple juice processing wastewater. During the experimental study, a start-up period of 50 days was followed by a reactor optimisation period of 256 days, both at a hydraulic retention time of 27.6 h. During the start-up period an average chemical oxygen demand (COD) removal of 80% was accomplished while the organic loading rate (OLR) was increased to 2.78 kg $COD.m^{-3}.d^{-1}$. Average COD removals of 87% at an OLR of 4.5 kg $COD.m^{-3}.d^{-1}$ were achieved throughout the reactor optimisation period. COD removal efficiencies of 92% at an OLR of 14 kg $COD.m^{-3}.d^{-1}$ and HRT of 14.6 h were achieved during this phase of the study. The reactor effluent COD content during the entire study varied between 300 and 1500 $mg.L^{-1}$ and further treatment will thus be required before the legal limits of 75 $mg.L^{-1}$, for discharge into a natural water system, are achieved.

Introduction

The South African apple industry is growing by 2.1%, in terms of hectares harvested, annually. Even so, a large part of the apple harvest (28.5%) cannot be used for fresh consumption because of the strict quality criteria set by the consumer, and this portion has to be processed (Mostert, 2003). Large volumes of heavily polluted wastewater (9.5 m^3 per ton of apples processed) are subsequently produced, which has a significant environmental impact when discharged (Mannapperuma, 1995).

The nature and strength of apple juice processing wastewater provides an ideal substrate for anaerobic digestion (Dold *et al.*, 1987) as 10 to 16% of the total chemical oxygen demand (COD) consists of organic acids that originate from the fruit itself (Austermann-Haun *et al.*, 1997). The successful use of anaerobic digestion in the treatment of food processing wastes has made this option very attractive to food industries since this technology has many advantages over the traditional aerobic treatment process

(Anderson & Saw, 1994), including the ability to degrade wastewaters at high organic loading rates (OLR) of more than 10 kg $COD.m^{-3}.d^{-1}$.

Many different anaerobic reactor designs are available today (Bitton, 1999), but the up-flow anaerobic sludge blanket (UASB) system has been the most successful in the treatment of a wide variety of wastewaters from many different sectors in the food industry (Lettinga & Hulshoff Pol, 1991). The main advantage of anaerobic digestion is that a significant COD reduction is achieved without the production of excess biomass. Another advantage is energy recovery in the form of biogas, as up to 95% of the organic matter can be converted into biogas (Weber *et al.,* 1984; Bitton, 1999). The advantage of the UASB system lies in the high biomass retainment in the form of granules, despite relatively high upflow velocities due to short hydraulic retention times (HRT) (Trovnec & Britz, 1998). Anaerobic systems have the advantage that active anaerobic biomass can be preserved unfed for several months where after rapid reactivation is possible (Anderson & Saw, 1994).

Austermann-Haun *et al.* (1997) found that the UASB reactor is well suited for the fruit juice industry where high organic loads have to be treated during two to three months of the year while an average wastewater COD of less than 1 000 $mg.L^{-1}$ is produced during the remaining months. Austermann-Haun *et al.* (1997) also evaluated different anaerobic reactor designs in the treatment of fruit juice wastewater and found that the UASB reactor design could reach a maximum OLR of 17.6 kg $COD.m^{-1}.d^{-1}$ compared to that of a fixed-film reactor that only managed effective digestion at an OLR of up to 9.5 kg $COD.m^{-1}.d^{-1}$.

During the South African apple juice processing season (February to June) large volumes of wastewater with a high organic content are produced. Daily and seasonal variations in wastewater volume, composition and concentration make treatment extremely difficult. The aim of this study was firstly, to evaluate the use of a mesophylic UASB bioreactor as a treatment option for apple juice processing wastewater, and secondly, to optimise the efficiency of the bioreactor in terms of COD removal, organic loading rate and hydraulic retention time.

Materials and methods

Bioreactor

A laboratory-scale UASB bioreactor with an operational volume of 2.3 L was used. The substrate was introduced at the bottom of the bioreactor while the gas exited at the top via an open gas/solids separator (Fig. 1). The reactor had an up-flow velocity of 4 $m.h^{-1}$. The overflow of the bioreactor drained through a U-shaped tube to prevent any

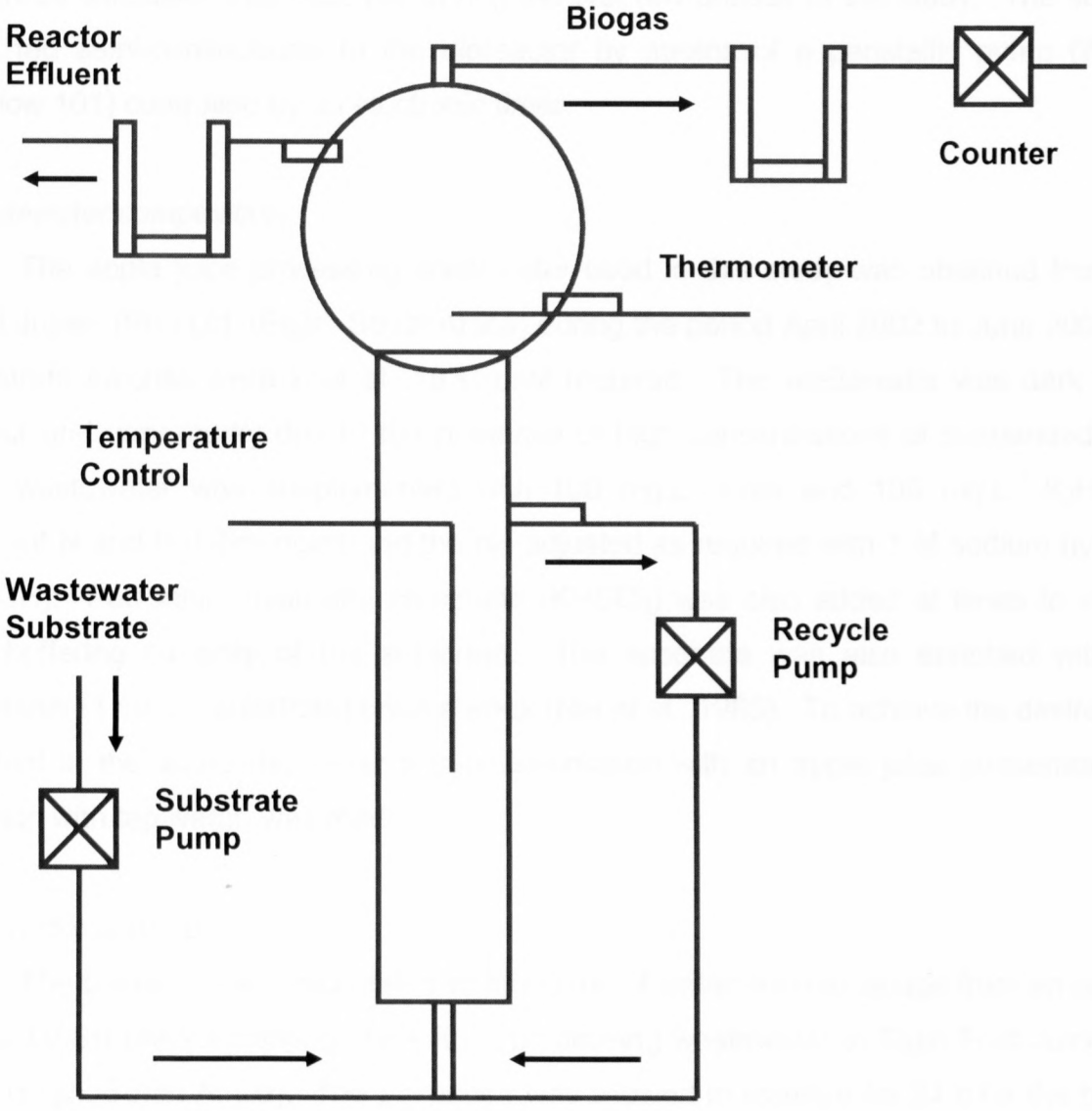

Figure 1. Experimental setup of the laboratory-scale UASB bioreactor.

atmospheric oxygen from entering the system. The bioreactor temperature was set at 35°C and controlled by 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 gas tight valve. Biogas was only measured from day 306 onwards due to technical difficulties that occurred during the first two phases of the study. The substrate was fed semi-continuously to the bioreactor by means of a peristaltic pump (Watson-Marlow 101) controlled by an electronic timer.

Wastewater composition

The apple juice processing wastewater used in this study was obtained from Elgin Fruit Juices (Pty) Ltd. (Elgin, South Africa) during the period April 2002 to June 2003. The substrate batches were kept at -18°C until required. The wastewater was dark grey in colour and very murky due to the presence of high concentrations of suspended solids. The wastewater was supplemented with 100 $mg.L^{-1}$ urea and 100 $mg.L^{-1}$ K_2HPO_4 to prevent N and P deficiencies and the pH adjusted as required with 1 M sodium hydroxide (NaOH). Potassium hydrogen carbonate ($KHCO_3$) was also added at times to increase the buffering capacity of the substrate. The substrate was also enriched with trace elements (1 $mL.L^{-1}$ substrate) once a week (Nel *et al.*, 1985). To achieve the desired COD content in the substrate, either a supplementation with an apple juice concentrate or a dilution with tap water, was made.

Bioreactor start-up

The bioreactor was inoculated with 600 mL of water drained sludge from an industrial scale UASB reactor treating apple juice processing wastewater at Elgin Fruit Juices (Pty) Ltd. (Elgin, South Africa). The bioreactor was allowed to stabilize for 24 h for the bacterial community to acclimatise. The bioreactor was then fed a substrate containing 500 $mg.L^{-1}$ urea and 500 $mg.L^{-1}$ K_2HPO_4 for 24 h. The HRT was set at 27.6 h and a diluted apple juice processing wastewater (COD = 3 000 $mg.L^{-1}$) was then fed for 50 days until a stable state was achieved. For this study stable state was defined as a state, which can be maintained indefinitely without system failure (Cobb & Hill, 1990). System failure occurs when the system environment changes to such an extent as to adversely affect the activity and proper functioning of the anaerobic bacteria present in the system.

Bioreactor operation

The lab-scale UASB bioreactor was run for a period of 444 days to determine the overall long term stability of the system. During this period various changes that reflect the

seasonal variations that occur in a full-scale apple juice processing plant, occurred in the wastewater used as reactor substrate.

The reactor performance was divided into three periods:

1. Start-up (days 1 to 50);
2. Optimisation (days 51 to 305 during the non-processing season); and
3. Increased OLR period (days 306 to 444 during the processing season).

The start-up period occurred during the apple pressing season of 2002 and the wastewater was diluted from a COD of 8 000 $mg.L^{-1}$ to suit reactor start-up conditions. The stabilization period occurred over the winter and spring of 2002 at an OLR of *ca.* 4 kg $COD.m^{-3}.d^{-1}$. The relatively high OLR was due to an apple juice concentrate supplementation and may be considered unrealistically high for the non-processing season when the OLR normally decreases to between 0.4 and 1.3 kg $COD.m^{-3}.d^{-1}$ (Austermann-Haun *et al.*, 1997).

Analytical methods

The following parameters were monitored according to APHA (Standard Methods, 1992): pH; alkalinity; total suspended solids (TSS); total volatile solids (TVS); chemical oxygen demand (COD); ortophosphate phosphorus; total Kjeldal nitrogen (TKN) and sludge settling ability. Mineral analyses were done colometrically according to standard Hach procedures using a DR2000 spectrophotometer (Hach Co. Loveland, CO).

The total volatile fatty acids (TVFA) were determined using a Varian (Model 3700) gas chromatograph, equipped with a flame ionization detector and a 30 m fused-silica capillary column with bonded FFAP stationary phase (Quadrex Co., New Haven). The column temperature was initially held at 105°C for 2 min, then increased at a rate of $8°C.min^{-1}$ to 190°C. The detector and inlet temperature were set at 300°Cand 130°C respectively, and nitrogen gas was used as carrier gas at a flow rate of 6.1 $mL.min^{-1}$.

The biogas composition was determined on a Fisons gas chromatograph equipped with a thermal conductivity detector and 2.0 m × 2.0 mm i.d. column packed with Hayesep Q (Supelco, Bellefonte, PA), 80/100 mesh. The oven temperature was set at 45°C and helium was used as the carrier gas at a flow rate of 40 $mL.min^{-1}$.

To test the sludge/granule activity, the modified method developed by O'Kennedy (2000), was used. Three grams of sludge/granules and 12 mL basic test medium were used. Incubation was done for 10 h at 35 °C and the volume of biogas and the percentage methane were measured after the 10 hours.

Pectin extraction was done according to the method developed by De Giorgi *et al.* (1985). Samples were boiled for 1 h before alcohol extraction. Colometric determinations

and calculations were done according to the carbazole method developed by Dische (1947) and modified by Bitter & Muir (1962). The method is rapid, and accurate when properly used and considered to be the preferred pectin determination method (Kyriakidis & Psoma, 2001).

Results and discussion

Substrate

The average composition of the raw apple juice processing wastewater is given in Table 1. It was found that the wastewater had higher COD, TKN, phosphate and TSS contents and a lower pH than the apple juice processing wastewaters described by Dold *et al.* (1987), Mannapperuma (1995) and Austermann-Haun *et al.* (1997).

Reactor sludge

The characteristics of the sludge, obtained from a full-scale UASB (1 ML) reactor treating apple juice processing wastewater, are summarized in Table 2. Settling ability and sludge density are two of the most important factors that influence the success of an UASB system as denser sludge/granules settle out quickly and this is directly related to sludge retention times. UASB sludge/granules should have a TSS content of at least 40 $g.L^{-1}$. Granules with a TSS of up to 100 $g.L^{-1}$ have been recorded in the past (Lin & Yang, 1990). The low solids content (11.9 $g.L^{-1}$) and long settling time of the apple juice reactor sludge as compared to that of the UASB granules (Table 2) from a brewery UASB suggest a serious settling problem with the apple sludge and its general unsuitability for use in an UASB reactor (Table 2). This apple juice sludge did, however, have better methane and biogas producing activity compared to the brewery granules (Table 2). The pectin content (150 $mg.L^{-1}$) of the apple juice processing wastewater sludge was found to be 12 times higher than that found in the brewery granules. This suggests the possibility of pectin build-up in the full-scale apple juice processing reactor at the Elgin plant.

The data given in Fig. 2 shows the changes that occurred in sludge density and particle size before and after lab-scale UASB treatment. Throughout the entire period of operation of the lab-scale UASB there appeared to be a gradual change in sludge consistency from a very fine (Fig. 2A) to a more grainy flocculated form. During the last treatment period, a more rapid change occurred in sludge morphology and the presence of granules was observed (Fig. 2B). It was also found that sludge overflow gradually decreased as the size of the sludge granular particles in the reactor increased leading to washout of some granules.

Table 1. Composition variations ($mg.L^{-1}$) of apple juice processing wastewater obtained during the period of March 2002 to June 2003 compared to a similar fruit juice processing wastewater.

Parameters	**Concentration ($mg.L^{-1}$)**	**According to Austermann-Haun *et al.* (1997)**
pH	3.6 - 5.4	4.3 - 8.7
COD	1 125 - 9 620	939 - 3 767
BOD_5	n/d	1 000 - 1 661
TSS	1 250 - 2 640	n/d
VSS	770 - 1 550	n/d
Alkalinity	0 - 850	n/d
Kjeldal Nitrogen	27.3 - 37.5	2.9 - 11.7
Total P	8.5 - 50.8	3.1 - 11.8
Pectin	130 - 450	n/d
Conductivity	1 239 - 850	n/d
Ca	13.1	nd
Na	416.9	n/d
Fe	0.6	n/d
K	84.1	nd
Mg	3.3	n/d
VFA	72 - 1 802	187 - 611

n/d Not determined

Table 2. Characteristics of sludge from a full-scale UASB reactor treating apple juice processing wastewater compared to UASB granules from a full-scale brewery UASB reactor.

Characteristics	Elgin sludge	Brewery granules
TSS ($g.L^{-1}$)	11.9	60
VSS ($g.L^{-1}$)	7.3	48
Settling ability (mL) *	925	150
Biogas production (mL)	4.37	3.6
Methane (%)	24.9	19.1
Pectin content ($mg.L^{-1}$)	150	12.5

* Level of 150 mL of sludge after 45 min in a 1 000 mL Imhoff cone.

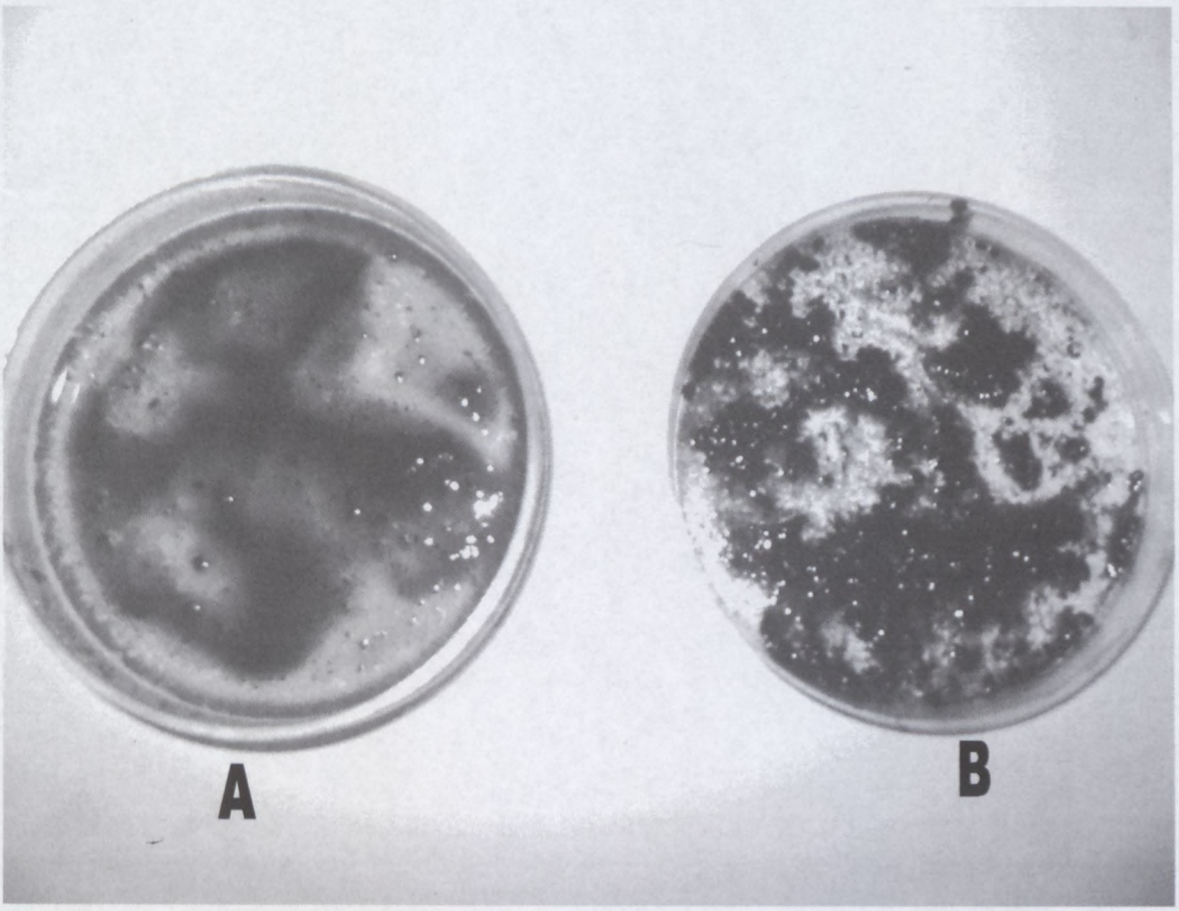

Figure 2. Lab-scale reactor sludge from day 1 (A) and from day 444 (B).

It was also found that the TSS and VSS contents of the reactor sludge increased from 11.9 to 65.3 $g.L^{-1}$ and 7.3 to 56.2 $g.L^{-1}$, respectively. The large subsequent increase in TSS and VSS content of the reactor sludge was ascribed to successful granulation and filling of the reactor with granular biomass. This is in accordance with the report of Sam-Soon *et al.* (1986) who found that slow but long term granulation occurred while treating an apple processing wastewater with final TSS and VSS of 47 and 36 $g.L^{-1}$, respectively.

Overall reactor performance

The reactor performance in terms of OLR, COD removal and HRT for the entire 444 d period is shown in Fig. 3. The COD removal remained relatively constant at between 85 and 90% for most of the study. The HRT of 27.6 h was maintained during the first 2 phases of the study whereafter a stepwise HRT decrease to 14.6 h, which subsequently led to an increased OLR to over 14 kg $COD.m^{-3}.d^{-1}$ (Fig. 3)

Phase 1 - Reactor startup

The reactor performance during the startup period is shown in Fig. 4. Reactor start-up started at an HRT of 27.6 h and was maintained throughout the start-up phase while the OLR was increased from 1.6 to 2.78 kg $COD.m^{-3}.d^{-1}$ during the 50 day period. Initial influent substrate COD concentration was *ca.* 1 800 $mg.L^{-1}$ and this was gradually increased to 3 200 $mg.L^{-1}$ by day 40. Removal efficiencies of over 80% were achieved by day 5 and increased to 85% by day 50. The substrate pH was kept at 8.00 during this period and effluent alkalinity remained between 1 500 and 2 200 $mg.L^{-1}$ (as $CaCO_3$). The slow increase in OLR from 1.75 to 2.78 kg $COD.m^{-3}.d^{-1}$ during start-up insured that reactor overloading did not occur. The drop in COD efficiency on day 24 was due to heater failure and a lower substrate COD concentration (Fig. 4). Efficiency was restored once the optimum temperature was regained. More Stable state conditions were achieved towards the end of this study phase (day 40) at a HRT of 27.6 h and OLR of 2.78 kg $COD.m^{-3}.d^{-1}$ (Fig. 4).

Hickey *et al.* (1991) reported that a period of 60 to 180 days is usually required for sufficient microbial adaptation and granulation to occur when a non-granular sludge is used for reactor start-up purposes. The faster successful reactor start-up found in this study may be ascribed to the fact that the microbial community in the sludge was already acclimatised to the apple juice processing wastewater as the sludge inoculum originated from an UASB reactor treating apple juice processing wastewater. This is also in agreement with the statement made by Hickey *et al.* (1991) that there are normally no

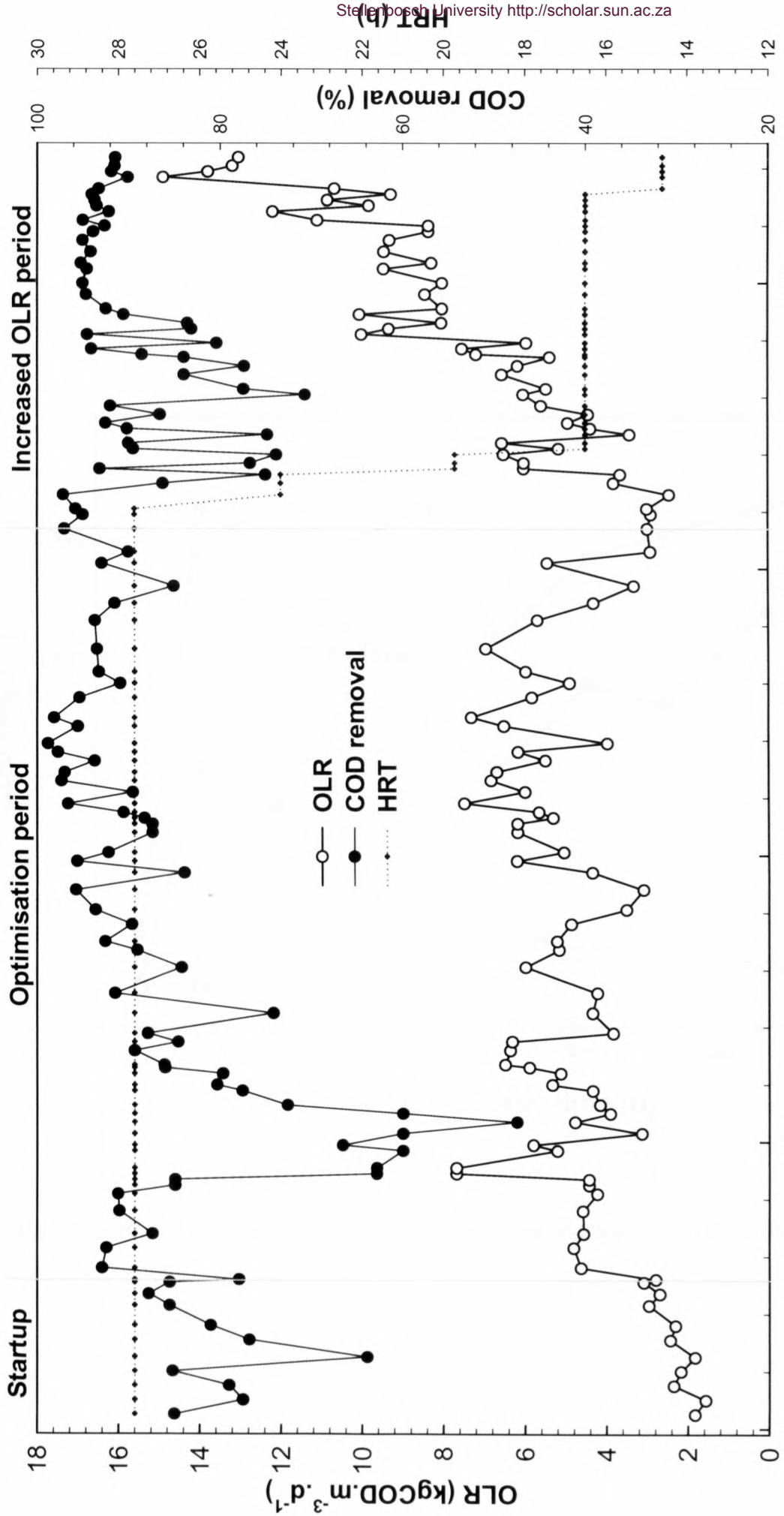

Figure 3. Reactor efficiency parameters for the 444 d treatment period in terms of OLR and COD removal efficiencies and HRT changes.

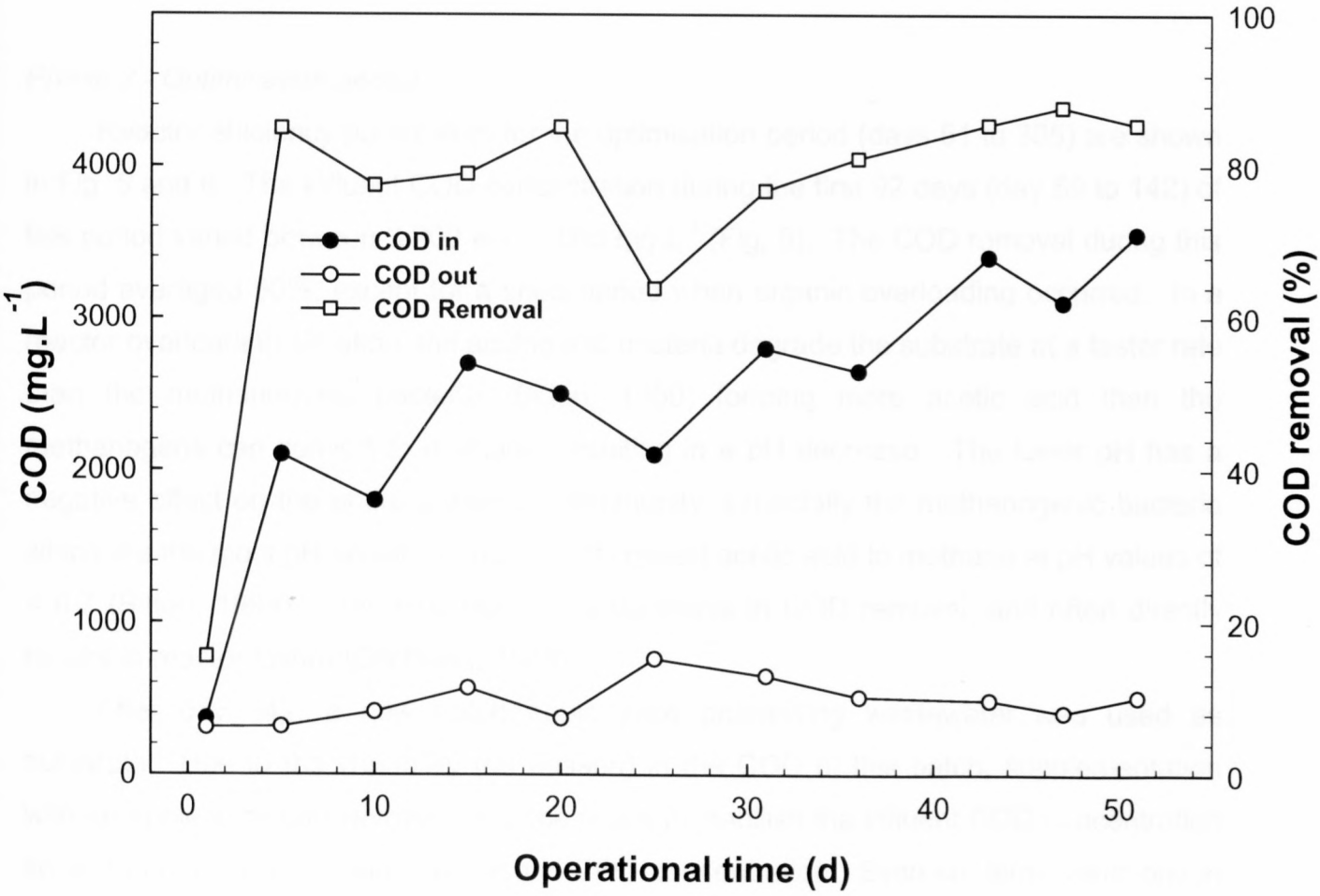

Figure 4. Reactor efficiency parameters during the startup period from day 1 to 50.

problems associated with start-up when sludge inoculums from existing reactors are used to seed reactors receiving a similar type of wastewater. Excessive sludge overflow did, however, occur at times due to poor sludge settling abilities and very active gas production, which then decreased the COD removal efficiencies. This was expected since the full-scale UASB reactor, where the sludge was obtained, also experienced sludge retention problems due to low sludge densities and settling abilities, which did not suit UASB conditions of short HRT's and subsequent high up-flow velocities (4 $m.h^{-1}$). The possible presence of pectin in the wastewater could also have influenced the system's efficiency.

Phase 2 - Optimisation period

Reactor efficiency parameters for the optimisation period (days 51 to 305) are shown in Fig. 5 and 6. The influent COD concentration during the first 92 days (day 50 to 142) of this period varied between 3 200 and 8 800 $mg.L^{-1}$ (Fig. 5). The COD removal during this period averaged 90%, except for a short period when organic overloading occurred. In a reactor overloading situation, the acidogenic bacteria degrade the substrate at a faster rate than the methanogenic bacteria (Bitton, 1999) forming more acetic acid than the methanogens can convert to methane, resulting in a pH decrease. The lower pH has a negative effect on the entire anaerobic community, especially the methanogenic bacteria which are the most pH sensitive and cannot convert acetic acid to methane at pH values of < 6.7 (Bitton, 1999). This thus leads to a decrease in COD removal, and often directly results in reactor failure (Ditchfield, 1986).

After day 142, a new batch apple juice processing wastewater was used as substrate. Due to the variability (off season) in the COD of this batch, supplementation with an apple juice concentrate had to be made to stabilise the influent COD concentration so as to not have a too wide variation in COD concentration. Even so, large variations in influent COD, and thus OLR (3.7 - 7.8 kg $COD.m^{-3}.d^{-1}$), occurred during this period (Fig. 6). Higher COD removal rates were experienced during this time and increases in OLR did not appear to negatively influence the reactor performance efficiency. COD removals of between 83 and 95% were maintained in spite of the variations in influent COD and OLR (Fig. 5 and 6). The apple juice concentrate used for supplementations consisted largely of a less complex organic fraction which is easily degradable and this would explain the high removal efficiencies obtained during this period. Dold *et al.* (1987) reported similar results when using an apple juice concentrate directly as an UASB reactor substrate. Their data indicated that the UASB reactor could successfully handle the large variations in OLR's (8 to 14 kg $COD.m^{-3}.d^{-1}$) (Dold *et al.*, 1987).

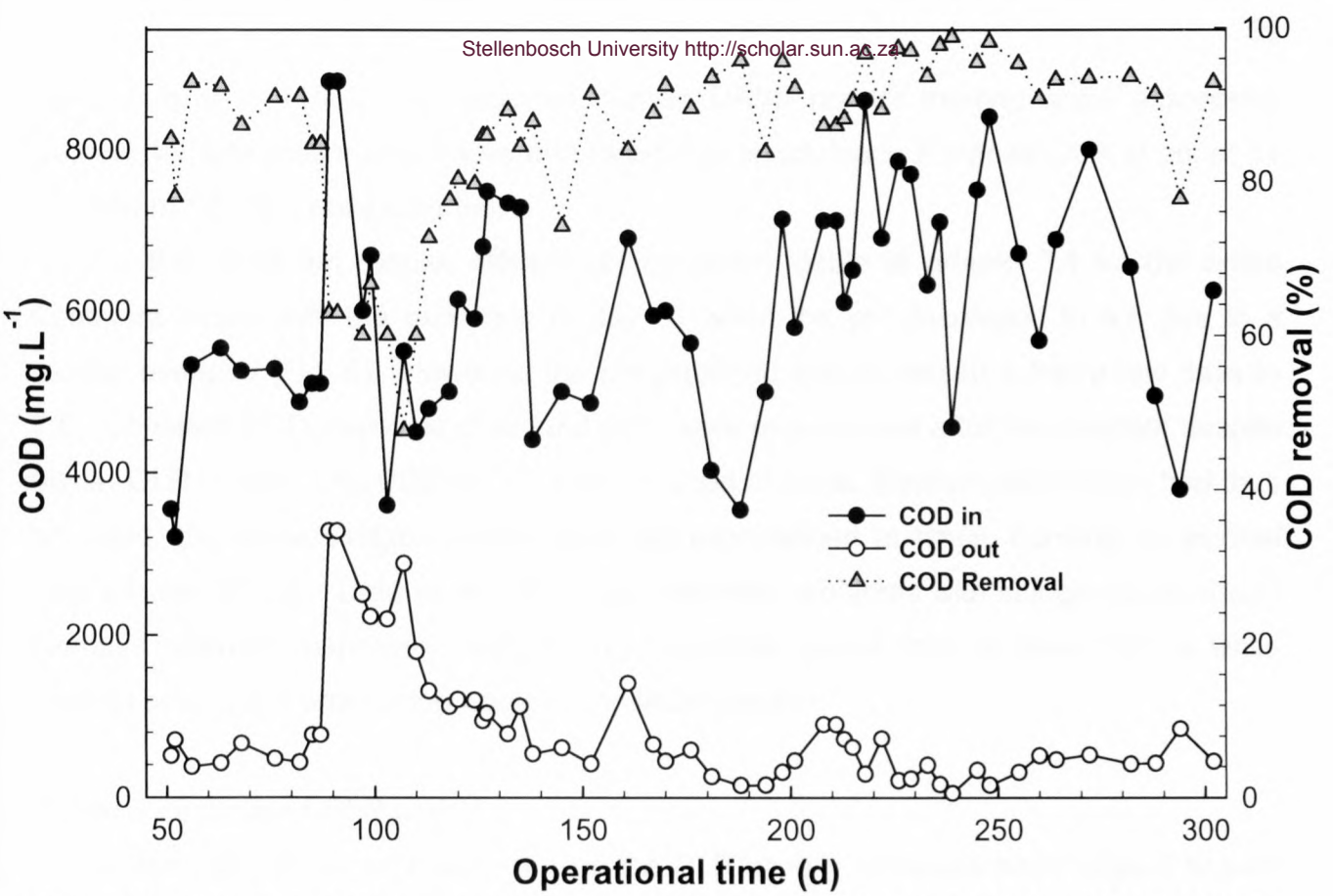

Figure 5. Reactor efficiency parameters for the optimization period (days 50 to 305) in terms of COD loaded and removed.

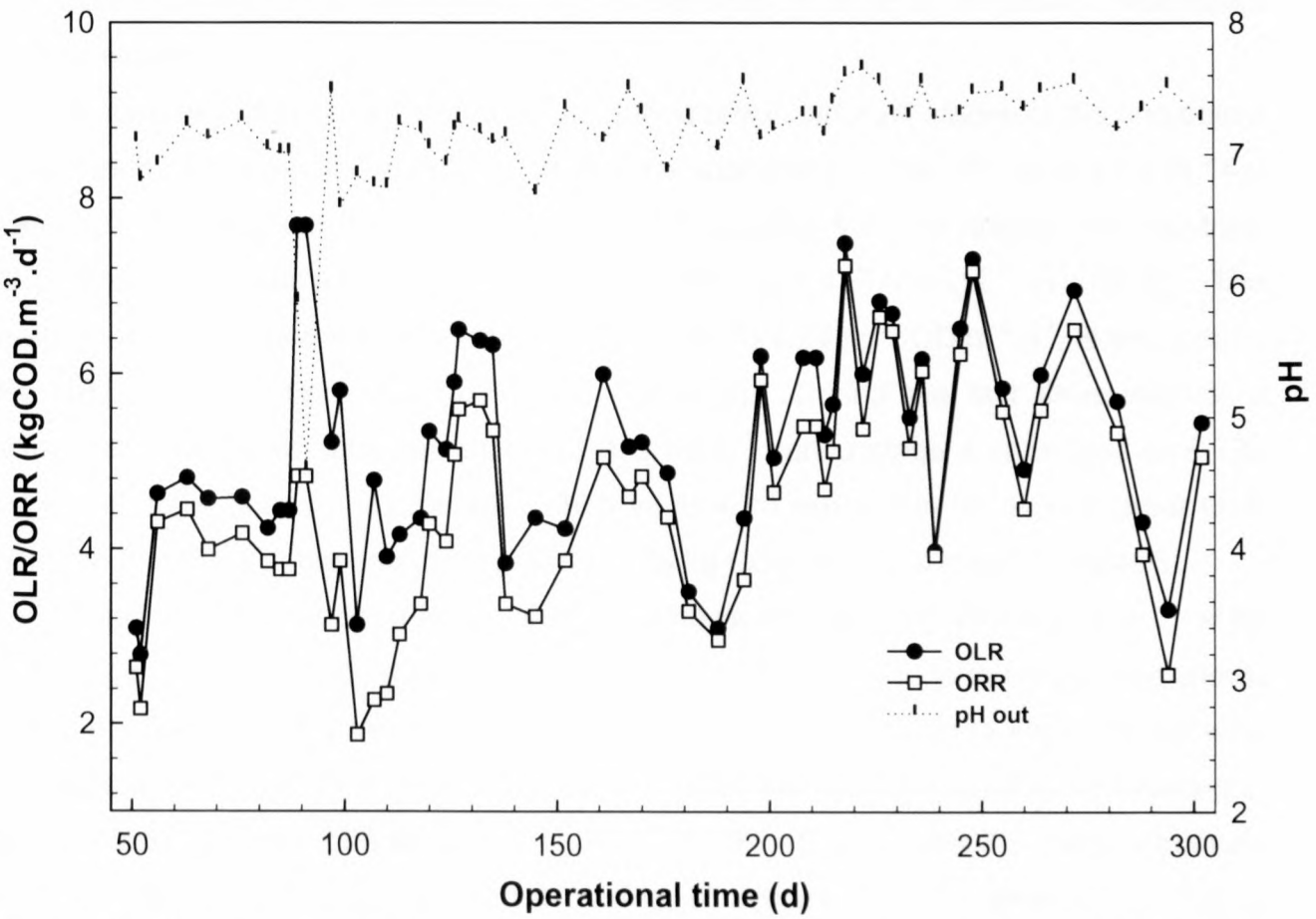

Figure 6. Reactor efficiency parameters for the optimization period (days 50 to 305) in terms of OLR, organic removal rate (ORR) and reactor pH.

Sam-Soon *et al.* (1986) also reported that an UASB reactor treating apple processing wastewater was reasonably stable with respect to shock loads if a peak OLR of about 14 kg.COD.m^{-3}.d^{-1} was not exceeded.

In this study the reactor effluent pH remained stable at around 7.4 for the entire treatment phase with the exception of day 91 when the pH decreased to 4.6 due to a reactor overload (Fig. 6). However, the pH stabilised and increased within a few days to 7.5. Constant COD removals of around 90% were experienced after this incident despite higher OLR of over 7 kg.COD.m^{-3}.d^{-1} that occurred at times. System stabilisation had thus occurred, but some sludge overflow was still experienced at times, causing occasional high effluent COD's. Dold *et al.* (1987) also reported problems with sludge washout and biomass retention especially early in the treatment period and at lower HRT's while treating apple juice processing water in an UASB reactor.

Phase 3 - Increased OLR period

During the apple processing season the OLR usually increases from below 2 to over 14 kg.COD.m^{-3}.d^{-1} (Austermann-Haun *et al.*, 1997). Reactor failure is often the consequence of this large OLR increase. An OLR increase from below 3 to over 14 kg.COD.m^{-3}.d^{-1} was done during this stage of the study in order to simulate a processing season situation.

A summary of the reactor parameters during seven different stages of the "increased OLR period" are given in Table 3. A continuous shortening of the HRT from 27.6 to 14.6 was applied throughout this treatment phase. During the first four stages, the substrate COD was kept relatively constant between 2 958 and 4 777 mg.L^{-1} (Table 3). The increase in OLR during the first 3 stages, from 2.93 to 5.64 kg COD.m^{-3}.d^{-1}, is ascribed to the HRT decrease from 27.6 to 16.5 h (Table 3). During the last three stages, a combination of higher substrate CODs (3 878 mg.L^{-1} during stage 4 compared to 8 728 mg.L^{-1} at stage 7) and a shorter HRT (16.5 vs 14.6 h) resulted in an even sharper OLR increase from 5.64 at the end of stage 4 to 13.76 kg COD.m^{-3}.d^{-1} at stage 7 (Table 3).

Reactor performance in terms of COD removal and HRT, is shown in Fig. 7, while OLR, organic removal rate (ORR), biogas and percentage methane in biogas, are shown in Fig. 8. The pH and alkalinity profiles during this stage are shown in Fig 9. A stepwise decrease in HRT from 27.6 to 16.5 h occurred during the first 36 days (Fig. 7) resulting in an OLR increase from 2.93 to 5.64 kg COD.m^{-3}.d^{-1} (Fig. 8 and Table 3). Large variations in COD removal of between 73 and 97% (Fig. 8) accompanied by alkalinities of below 2 000 mg.L^{-1} (Fig. 9) during this period suggested a lower reactor stability.

Table 3: *UASB bioreactor operating conditions and efficiency during the increased OLR period.*

	Treatment Stage						
Parameters	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Operational time (d)	306 - 321	322 - 333	334 – 340	341 - 379	380 - 420	421 - 431	432 - 444
HRT (h)	27.6	24	19.7	16.5	16.5	16.5	14.6
OLR ($kgCOD.m^3.d^{-1}$)	2.93	3.28	6.17	5.64	8.85	10.65	13.76
Substrate COD ($mg.L^{-1}$)	3 369	2 958	4 777	3 878	6 078	7 328	8 728
Effluent COD ($mg.L^{-1}$)	506	437	886	580	483	571	715
COD removal (%)	84.9	86	81	85	92	92	92
Substrate pH	6.71	6.9	7.81	7.23	6.66	6.52	6.64
Effluent pH	7.37	7.39	7.21	7.26	7.35	7.25	7.38
Substrate alkalinity *	850	733	1 200	2 040	2 219	1 720	2 120
Effluent alkalinity *	2 037	1 866	2 116	2 680	2 889	2 240	2 340
Biogas ($L.d^{-1}$)	2.76	3.14	4.08	5.10	9.19	10.36	13.36
ORR ($kgCOD.m^3.d^{-1}$)	2.48	2.78	5.00	4.79	8.28	9.82	12.53
% methane in biogas	33.8	31.3	40.3	33.9	28.2	34.0	33.4
CH_4 γ ($m^3.kg^{-1}.COD_{removed}$)	0.163	0.153	0.142	0.156	0.136	0.155	0.154

* (as $mg.L^{-1}$ $CaCO_3$)

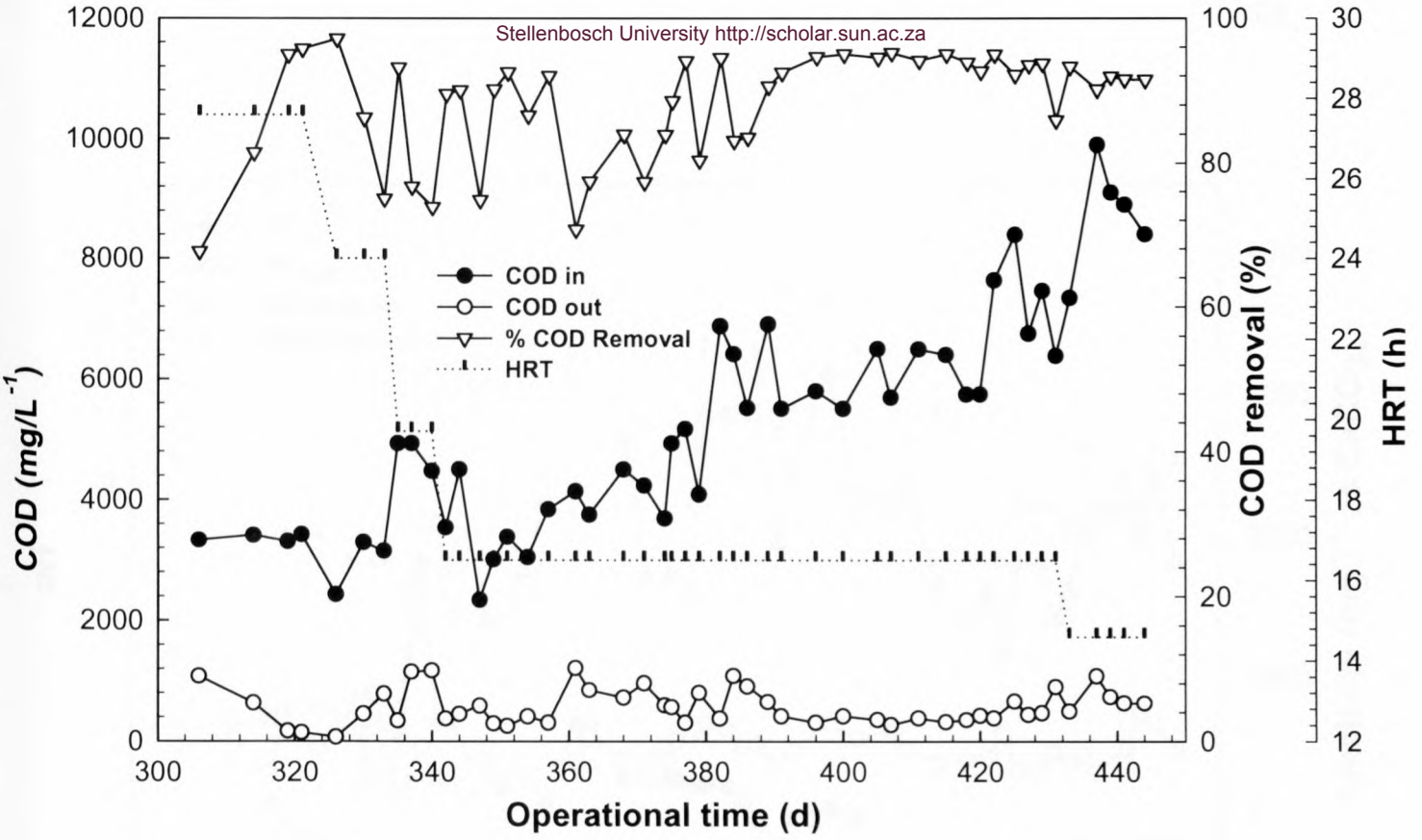

Figure 7. Reactor efficiency as obtained during the increased OLR period (days 306 to 444) in terms of COD loading and removal, and HRT changes.

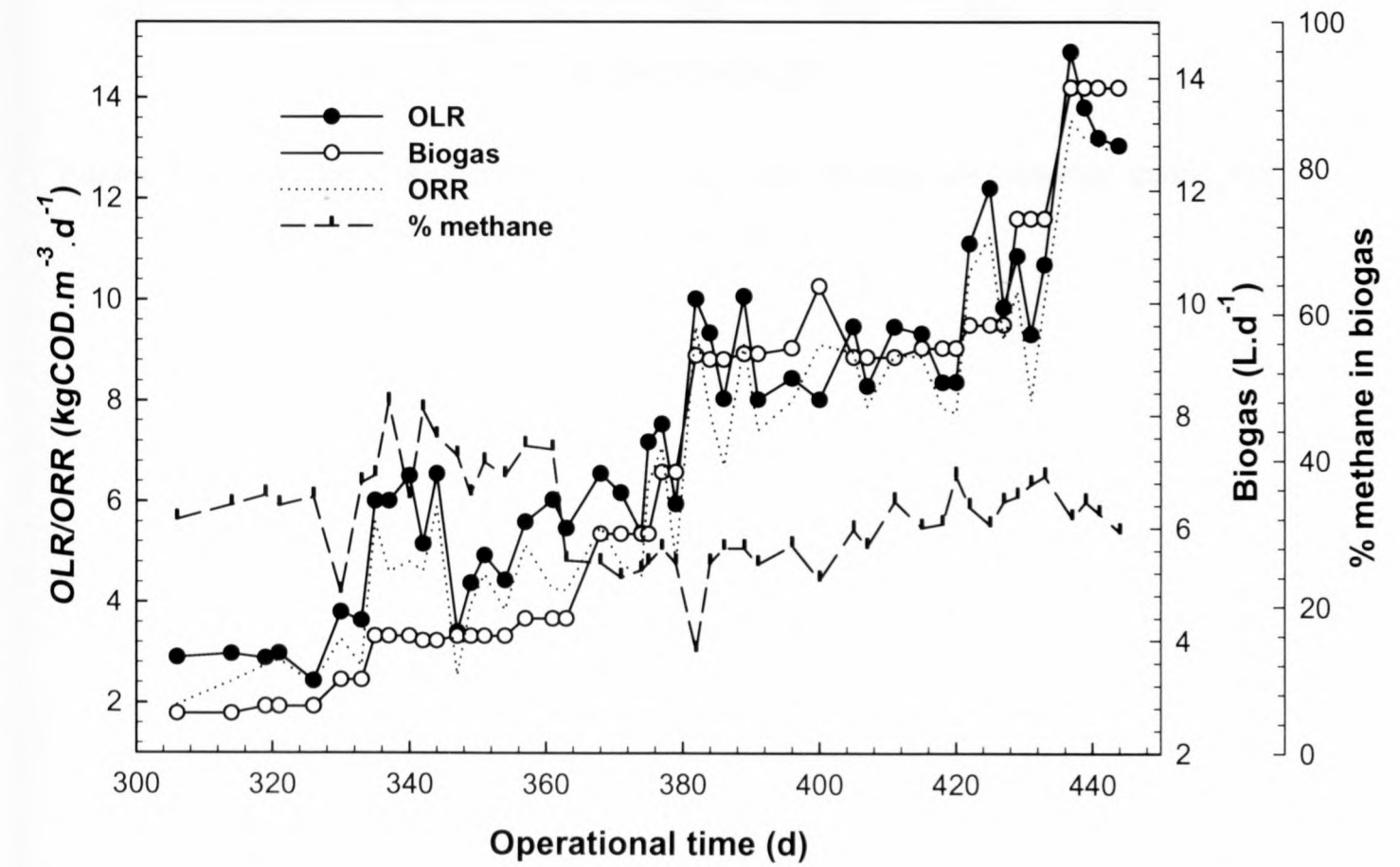

Figure 8. Reactor efficiency as obtained during the changes in OLR (days 306 and 444) in terms of OLR, ORR, biogas production and biogas content.

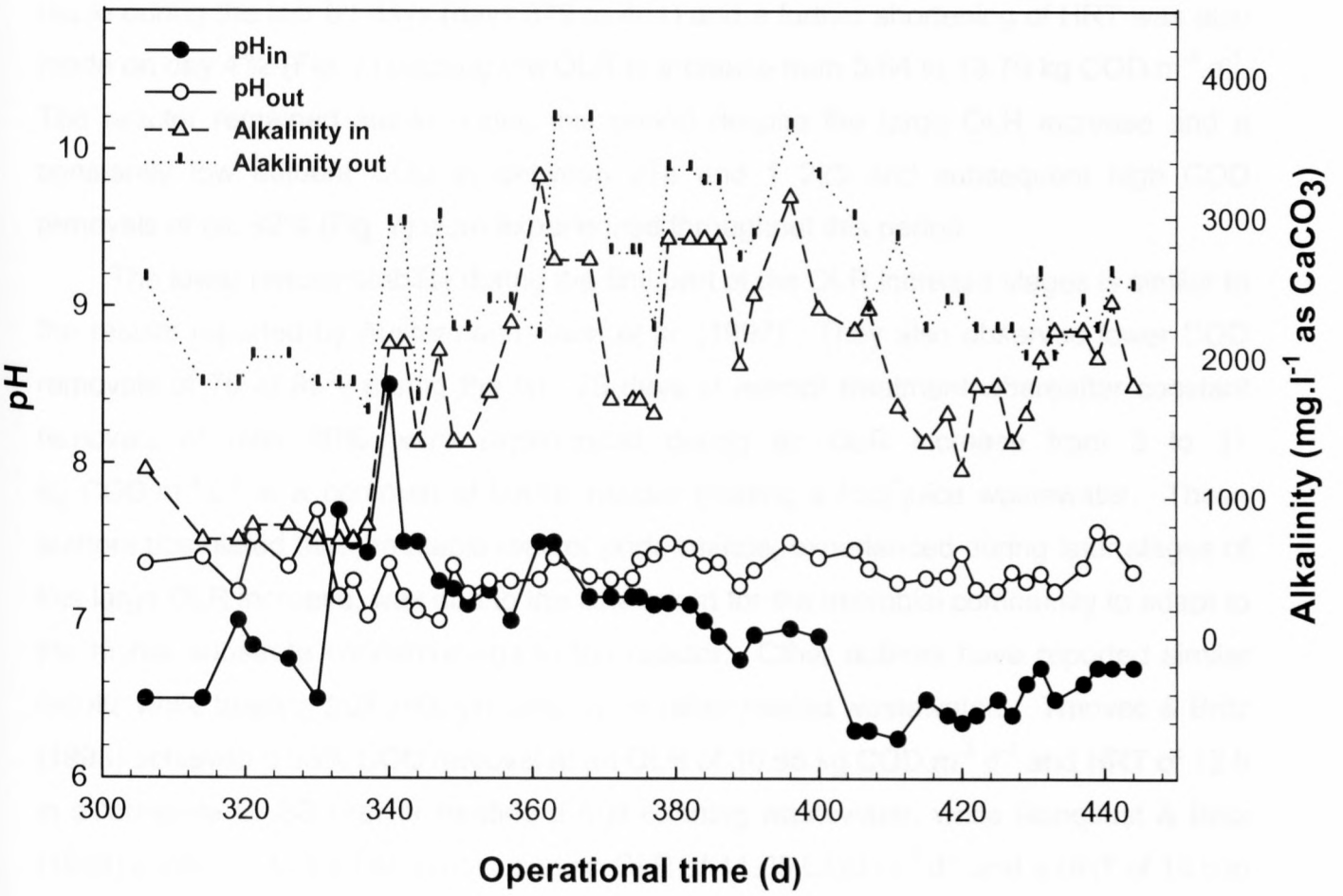

Figure 9. Reactor efficiency in terms of pH and alkalinity as obtained during the increased OLR period (days 306 to 444).

During the next 35 days (days 345 to 380), the OLR remained relatively constant (Fig. 8) at *ca.* 6 kg $COD.m^{-3}.d^{-1}$. An increase in substrate COD from *ca.* 4 000 to over 8 000 was made during the last 60 days (days 379 to 444) and a further shortening of HRT was also made on day 432 (Fig. 7) causing the OLR to increase from 5.64 to 13.76 kg $COD.m^{-3}.d^{-1}$. The reactor remained stable during this period despite the large OLR increase and a constantly low effluent COD of between 274 and 1 225 and subsequent high COD removals of *ca.* 92% (Fig. 7) were experienced throughout this period.

The lower reactor stability during the first part of the OLR increase stages is similar to the results reported by Austermann-Haun *et al.* (1997). They also observed lower COD removals of 70 to 85% during the first 25 days of reactor treatment whereafter constant removals of over 90% were experienced during an OLR increase from 3 to 17 kg $COD.m^{-3}.d^{-1}$ in a commercial UASB reactor treating a fruit juice wastewater. These authors postulated that the stable reactor performance, experienced during later stages of this large OLR increase, was due to the time taken for the microbial community to adapt to the higher substrate concentrations in the reactor. Other authors have reported similar results while treating fruit juice processing, or other related wastewaters. Trnovec & Britz (1998) achieved a 93% COD removal at an OLR of 10.95 kg $COD.m^{-3}.d^{-1}$ and HRT of 12 h in a lab-scale UASB reactor treating a fruit canning wastewater, while Ronquest & Britz (1999) achieved 93% COD removals at an OLR of 11.05 $COD.m^{-3}.d^{-1}$ and a HRT of 14 h in the UASB treatment of a winery wastewater. Sam-Soon *et al.* (1986) found COD removals of between 89 and 95% at OLR's between 5 and 14 kg $COD.m^{-3}.d^{-1}$ in the treatment of an apple juice processing wastewater but found that COD removal started to decrease at an OLR of more than 14 kg $COD.m^{-3}.d^{-1}$ and only 49% removal occurred at an OLR of 16 kg $COD.m^{-3}.d^{-1}$. From the literature it appears that the maximum OLR of apple juice processing wastewater is only about 14 kg $COD.m^{-3}.d^{-1}$ (Sam-Soon *et al.*, 1986), compared to 17 kg $COD.m^{-3}.d^{-1}$ for fruit juice wastewater (Austermann-Haun *et al.*, 1997). The reason why apple processing wastewater has a lower maximum OLR (14 kg $COD.m^{-3}.d^{-1}$) compared to a fruit juice processing wastewater (17 kg $COD.m^{-3}.d^{-1}$) might be due to the presence of more complex compounds, like pectin in the wastewater. Apples have a high pectin content of between 5 000 and 16 000 $mg.L^{-1}$ compared to other fruits that contain less than 3 000 $mg.L^{-1}$ pectin on average (Taylor, 1996). Based on this information no further OLR increases were made after a HRT of 14.6 h (Fig. 8) was achieved, as it was noticed earlier that drastic changes in influent COD and OLR caused reactor instability.

An increase in biogas was observed as the ORR increased (Fig. 8), which is expected in anaerobic systems since most of the organic matter is converted to biogas

and less biomass, as compared to aerobic systems (Weber *et al.,* 1984). The percentage methane in the biogas varied between 22 and 43% and slight decreases occurred after each OLR increase (Fig. 8) probably because of the higher growth rate of carbon dioxide producing bacteria compared to methane producing bacteria (Bitton, 1999). $CH_4\gamma$ was found to vary between 1.36 and 1.63 $m^3.kg^{-1}.COD_{removed}$ during this phase of the study. The maximum possible $CH_4\gamma$ in anaerobic systems is 0.355 $m^3.kg^{-1}.COD_{removed}$.(Lin & Yang, 1990). A relatively small portion (*ca.* 1.5 $m^3.kg^{-1}.COD_{removed}$ or 42%) of the potential methane (0.355 $m^3.kg^{-1}.COD_{removed}$) was thus utilised, possibly due to a lack of methanogenic activity.

A relatively high substrate alkalinity (> 2 000 $mg.L^{-1}$) probably helped prevent drastic decreases in reactor pH during the OLR increases (Fig. 8 and 9). It has been observed that VFA content serves as an indicator of instability or stress for anaerobic systems (Ditchfield, 1986). The effluent VFA content during the period from day 306 to 44 remained between 68 and 149 $mg.L^{-1}$. The fact that the effluent pH remained in the region of 7.4, combined with the low effluent VFA content indicates that sufficient methanogenesis occurred throughout this period despite the large increases in OLR (Bitton, 1999). Less sludge overflow also occurred during this period compared to the start-up and optimisation periods and the particle size of the sludge that washed out, increased. Biomass retention problems were thus reduced due to more effective granulation. This is in accordance with numerous UASB studies where it was found that biomass retention problems seldom occurred after granulation has taken place (Lettinga & Hulshoff Pol, 1991).

Conclusions

Wastewater problems caused by the seasonal processing of apples have yet to be solved (Wayman, 1996). From the results obtained during this study it is clear that the UASB design is feasible for the effective treatment of an apple juice processing wastewater. COD removals were in the region of 85 to 90% throughout the 444 day study period. The high organic loads fed during the increased OLR stage had no drastic effect on the system's efficiency. The study period reflected a typical processing season of an apple juice processing plant where large volumes of wastewater containing a high organic content are produced over a short period of the year. Treatment plants are thus forced to reduce HRTs, which combined with the higher wastewater COD values, puts considerable stress on the treatment system. This study indicated that UASB systems can cope with the large increase in OLR produced during an apple processing season. Excessive sludge

overflow did, however, occur at times, especially during the start of the study, due to the low sludge density and settling ability of the seed sludge. Less sludge overflow, was however, experienced at the end of the study due to granulation having taken place. The effluent COD varied between 300 and 1 500 $mg.L^{-1}$ and further treatment will be required before the legal discharge limits of 75 $mg.L^{-1}$ are met (Water Research Commission, 1987).

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CHAPTER 4

IMPACT OF PECTIN GELLING ON THE EFFICIENCY OF AN UASB REACTOR TREATING APPLE JUICE PROCESSING WASTEWATER

Summary

Apple juice processing wastewaters (AJPWW) are known to contain varying amounts of pectin, which due to its difficult biodegradation and gelling abilities, often leads to treatment problems. In this study it was found that the pectin content of AJPWW increased from 129 to 562 $mg.L^{-1}$ during the 2003 processing season. Pectin has the ability to form interactions with the calcium present in the lime that is used for pH adjustments before biological treatment, and this leads to the formation of a gel. The effect of the gelling viscosity increase on the UASB reactor due to a pectin-calcium gel, was determined. A substrate viscosity increase, from 8.5 to 47 cps, led to a decrease in chemical oxygen demand (COD) removal from 90% to 12%, and a reduction in reactor pH from 7.4 to 4.8 during a 12 d pectin gel treatment period. The effect of ozone on pectin content, gel formation ability and an already formed AJPWW pectin gel was then determined. A 77% pectin degradation and 76% reduction in gel formation ability occurred after a 10 and 20 min ozonation, respectively while the viscosity of an already formed pectin gel was reduced by 62% after a 20 min ozonation. Decreases in pectin content, weaker gel formation ability and decreases in substrate viscosity after gel formation occurred after ozonation and could have a significant impact on the UASB reactor performance. Pectin degradation and a subsequent decrease in gelling ability will decrease the viscosity of the UASB substrate. Less biomass washout problems and better substrate degradation should occur after ozonation, leading to better AJPWW degradation and a more efficient treatment system. The use of ozone as a pre-treatment to improve the suitability of an AJPWW for UASB degradation by breaking the pectin gel could, however, be a viable treatment option for the apple juice processing industry.

Introduction

The apple juice processing industry in SA produces 6.8 million m^3 of wastewater annually (F. Mostert, Elgin Fruit Juices (Pty)Ltd, Grabouw, South Africa, personal communication, 2003). This wastewater is mainly produced during February to June each year when the apples are harvested and processed. The high wastewater volumes and extreme

variations in organic load limit the wastewater treatment options of the apple juice producers (D. Murray, Elgin Fruit Juices (Pty) Ltd, Grabouw, South Africa, personal communication, 2003).

Anaerobic digestion has been shown to be a viable option for the treatment of food processing wastewaters (Lettinga *et al.*, 1997), and especially for fruit processing wastewaters (Sam-Soon *et* al., 1986; Austermann-Haun *et al.*, 1997; Trnovec & Britz 1998; Ronquest & Britz, 1999; Sigge *et al.*, 2002). Various commercial anaerobic reactors are in operation worldwide (Trotzke, 1988; Field, 2002). Anaerobic digestion has the added advantage that it can treat wastewaters that are produced on a seasonal bases, as the microbial community can survive off-season reactor shutdown (Tchobanoglous & Burton, 1991; Anderson & Saw, 1994).

The upflow anaerobic sludge blanket (UASB) system is dependent on granule formation to achieve an efficient biomass settling ability (Dold *et al.*, 1987), which is a vital characteristic of an effective methanogenic system (Hickey *et al.*, 1991). However, an increased substrate viscosity can lead to the reduction in the settling ability of UASB granules leading to biomass retention problems (Lin & Yang, 1990). The presence of finely dispersed particles and complex organic matter such as pectin are detrimental to granule formation and maintenance and the slow hydrolysis rate of complex organic matter also contributes to poor reactor performance (Van Lier *et al.*, 2001).

Apples have a pectin content of between 5 000 and 16 000 $mg.L^{-1}$ (Fedirici *et al.*, 1988). During processing some of the pectin is removed in the pressed cake, but a large portion ends up in the wastewater (Dold *et al.*, 1987). The pectin molecule is a complex organic polymer (Whitaker, 1990) and microbial degradation can only occur through the action of extra-cellular enzymes (Bitton, 1999). Previous studies have shown that most anaerobic systems are incapable of degrading pectin (Madamwar & Mithal, 1985; Tanabe *et al.*, 1988; Shivakumer & Nand, 1995).

The problem that must be taken in consideration when treating apple juice processing wastewater (AJPWW) is that it has a low pH (3.0 – 5.0) and an adjustment must be made before biological treatment can be undertaken (Mnanapperuman, 1995). Calcium oxide (CaO) is usually preferred to sodium hydroxide, because of its cost-effectiveness (D. Murray, Elgin Fruit Juices (Pty) Ltd, Grabouw, South Africa, personal communication, 2003). In contrast, high sodium levels will limit land irrigation as a final disposal option (Steenveld, 1997). With CaO or calcium hydroxide $Ca(OH)_2$ as neutraliser, the calcium ions can interact with the hydroxyl group of pectin molecules to form a gel (Broomfield, 1996; Hoejgaard, 2003), which leads to an increase in raw AJPWW viscosity (Tanabe *et al.*, 1988). This increase in viscosity leads to biomass retention problems that

can cause reactor failure (F. Mostert, Elgin Fruit Juices (Pty)Ltd, Grabouw, South Africa, personal communication, 2003). The pre-treatment of AJPWW is thus essential so as to reduce gel formation in order to prevent biomass washout problems. One treatment option is the use of ozone as it is known that ozonation can decrease the molecular size of complex polymers (Gottshalk *et al.*, 2000) and could, as a pre-treatment, possibly reduce the gelling ability of pectin containing wastewaters which should subsequently improve the suitability of the wastewater for UASB treatment.

The aim of this study was to investigate the effects of pectin containing wastewaters on the performance of an UASB reactor. The effect of ozone on pectin gelling in AJPWW was also evaluated.

Materials and methods

Wastewater characteristics

The apple juice processing wastewater used in this study was obtained from Elgin Fruit Juices (Pty) Ltd. (Elgin, South Africa) during the period February 2003 to June 2003. The substrate batches were kept at -18°C until required. The wastewater generally was dark grey in colour and very murky due to the presence of high concentrations of suspended solids. The wastewater was supplemented with 100 $mg.L^{-1}$ urea and 100 $mg.L^{-1}$ K_2HPO_4 to prevent N and P deficiencies and the pH adjusted as required with either 1 M sodium hydroxide (NaOH) or calcium hydroxide $Ca(OH)_2$. Potassium hydrogen carbonate ($KHCO_3$) was added at times to increase the buffering capacity of the substrate. The substrate was also enriched with trace elements (1 $mL.L^{-1}$ substrate) once a week (Nel *et al.*, 1985).

Bioreactor

A laboratory-scale UASB bioreactor, as described in Chapter 3 of this thesis, was used. The wastewater used as substrate in this study had an average chemical oxygen demand (COD) of 8 400 $mg.L^{-1}$ and a pH of 4.5. The reactor was operating at an efficiency of *ca.* 90% COD removal, organic loading rate (OLR) of *ca.* 15 kg $COD.m^{-3}.d^{-1}$ and HRT of 13.5 h at the start of this study. The HRT and OLR were kept constant for the duration of the study. An AJPWW containing 492 $mg.L^{-1}$ pectin (May, 2003 batch) was fed from days 1 to 7 at an influent pH of 6.5 (adjusted with 2 M NaOH). On day 8, the same batch of AJPWW was supplemented with commercial pectin (Dolcrè Ltd., Durbanville, South-Africa) to a pectin level of 750 $mg.L^{-1}$ and the solids were removed by sedimentation in order to prevent an increase in COD due to the pectin additions. The pH was then

adjusted to 6.5 with 1 M $Ca(OH)_2$. The formation of a gel in the AJPWW substrate could be visually observed after 10 min. Since it was impractical to feed the gel directly to the reactor, the wastewater was stirred for 5 min with a magnetic stirrer. This disrupted the gel structure and the gel was transformed into a "slush" that was used as reactor substrate from day 8 onwards.

Gel formation ability

A synthetic wastewater (COD 12 970 $mg.L^{-1}$) containing 750 $mg.L^{-1}$ commercial pectin (Dolcrè Ltd., Durbanville, South-Africa) and 10 000 $mg.L^{-1}$ sucrose, and the AJPWW May, 2003 batch) supplemented with the commercial pectin to a pectin level of 750 $mg.L^{-1}$, were used to test the gel formation ability of pectin in the presence of calcium (Ca^{2+}). Wastewater viscosity was used as an indication of gel formation ability. A range between 1 and 11 $mL.L^{-1}$ of a 1 M $Ca(OH)_2$ solution was added to the synthetic and the AJPWW. Each mL of the 1 M $Ca(OH)_2$ solution contained 40 mg Ca^{2+}. After Ca^{2+} addition the wastewater was stirred for 1 min and the pH measured. A 20 min gel setting time was then allowed, whereafter viscosity was measured in triplicate.

Ozonation

Ozonation of the pectin containing AJPWW as well as the synthetic wastewater was done in a continuous mode "bubble column" with ozone being bubbled upwards through the glass column. The "bubble column" had a length of 900 mm and a diameter of 65 mm. An ozone generator (Parc Scientific, Ifafi) producing 15.5 $g.h^{-1}$ O_3 at a flow rate of 4 $L.min^{-1}$ was used for all the ozonation trails. All ozonated samples were analysed in triplicate.

Three AJPWW samples, containing 562 $mg.L^{-1}$ pectin (June batch 2003), were adjusted to different pH values (4.5, 6.5 and 8.2) and were subsequently ozonated for 5 min. This was done to determine the effect of ozone on the pectin content at different pH values. The impact of ozone on the pectin content, gelling ability and an already formed pectin gel after progressively longer ozonation times were also investigated in three separate experiments.

The effect of progressively longer ozonations (0, 1, 2.5, 5 and 10 min) on the pectin and COD content of this AJPWW, containing 562 $mg.L^{-1}$ pectin, was evaluated. The effect of ozone on the pectin content was also done on two commercial pectin containing solutions. Firstly, commercial pectin (Dolcrè Ltd., Durbanville, South-Africa) was added to distilled water to a concentration of 1 711 $mg.L^{-1}$ and, secondly, to the AJPWW (June 2003

batch) which gave a final pectin content of 2 085 mg.L^{-1}. Both samples were then ozonated for 0, 1, 2.5, 5 and 10 min whereafter the pectin content was determined.

The effect of ozone on gel formation ability was measured by determining the decrease in viscosity after progressively longer ozonation times. The synthetic wastewater supplemented with the commercial pectin to a level of 750 mg.L^{-1} pectin was ozonated for different periods (0, 2.5, 5, 10 and 20 min) and Ca^{2+} (200 mg.L^{-1}) was added to each ozonated sample whereafter the different viscosities were determined.

The effect of ozone on an already formed pectin gel was also monitored by the change in viscosity. This was done by firstly adding 312 mg.L^{-1} Ca^{2+} to the AJPWW (May, 2003 batch) supplemented to a level of 750 mg.L^{-1} pectin. The formation of a gel could be observed after 10 min. Since it was impractical to ozonate the gel directly, the wastewater was stirred for 5 min with a magnetic stirrer. This disrupted the gel structure and the gel was transformed into a "slush". The pectin gel used for this ozonation was identical to the gel used as a reactor substrate in the previous study. The "broken gel" was then ozonated for different times where after the corresponding viscosity decreases were measured. All ozonations were done in 2 L batches and ozonation times varied between 0 and 20 min.

Analytical methods

Pectin extractions were done according to the method developed by De Giorgi *et al.* (1985). Colometric determinations and calculations were done according to the carbazole method developed by Dische (1947), and modified by Bitter & Muir (1962). The method is rapid and accurate when properly used and is still the preferred pectin determination method (Kyriakidis & Psoma, 2001). All carbazole tests were done in triplicate, using a standard curve to determine the pectin content. Galacturonic acid was used at different concentrations (10 – 90 mg.L^{-1}) to construct a standard curve.

Commercial citrus pectin (Dolcrè Ltd., Durbanville, South-Africa) was used for all pectin supplementations. A 1 000 mg.L^{-1} commercial citrus pectin containing solution corresponded to 765.4 mg.L^{-1} (± 70) pectin as assayed by the carbazole method. This may be because the commercial pectin is not 100% pure or because the pectin extraction and carbazole test does not extract or register all the pectin in the water (Matsuhashi & Hatanaka, 1992).

The pectin content of the AJPWW was determined as the processing season progressed (February to June). The pectin content of different types of UASB granules and the pectin content of the corresponding wastewaters degraded by the particular granules, was also determined. The pectin content of the reactor substrate, effluent and

reactor granules were determined on day 8 (before pectin addition) and day 18 (10 days after the pectin addition).

Viscosity was measured in triplicate with a Brookfield viscosity meter (RVT model, 230 volts, Frequency 50 Hz) using spindles 1, 2 and 3 at 10 to 50 rpm.

The following parameters were monitored according to the APHA (Standard Methods, 1992): pH; alkalinity; total suspended solids (TSS); volatile suspended solids (VSS); and chemical oxygen demand (COD). The ozone production and was measured using an iodiometric titration (Standard Methods, 1992).

The total volatile fatty acids (TVFA) were determined using a Varian 3700 gas chromatograph equipped with a flame ionization detector and a 30 m fused silica capillary column with bonded FFAP stationary phase (Quadrex Co., New Haven). The column temperature was initially held at 105°C for 2 min, and then increased, at a rate of 8 °C per min, to 190°C. The detector and inlet temperature were set at 300 and 130°C, respectively, and nitrogen gas was used as carrier gas at a flow rate of 6.1 $mL.min^{-1}$.

Results and discussion

Wastewater and biomass characteristics

The pectin and COD contents of the AJPWW at different times during the processing season are given in Table 1. It was clear that as the season progressed the pectin and COD content increased. This was expected, since in the latter part of the processing season (May and June), more apples of a riper and softer nature are processed. Damage and subsequent disintegration of some apples during the transport, washing and drying stages occurred, and the disintegrated apples become part of the wastewater and subsequently increased the pectin and COD content (F. Mostert, Elgin Fruit Juices (Pty)Ltd, Grabouw, South Africa, personal communication, 2003). The use of over-ripe apples for juice extraction is, however, very popular, since these apples cannot be sold on the fresh market, and also produce a high juice yield. Large quantities of softer apples are subsequently processed during the latter stages of the harvesting season, producing large volumes of wastewater with high pectin contents (F. Mostert, Elgin Fruit Juices (Pty)Ltd, Grabouw, South Africa, personal communication, 2003).

The pectin content of different wastewaters was determined as well as the pectin content of the granular biomass from the respective UASB reactors treating these wastewaters and the results are shown in Table 2. Wastewater and granules from a brewery (A) were used as "standard reference" to compare three UASB biomass samples treating different pectin containing AJPWWs. Granules B were from an industrial-scale

Table 1. Pectin and COD contents of AJPWW at different times during the 2003 processing season.

Time (Month)*	Pectin ($mg.L^{-1}$)	COD ($mg.L^{-1}$)
February	129 (sd ±8.1)	2 100 (sd ±124)
March	250 (sd ±27.5)	2 850 (sd ±190)
April	339 (sd ±15.5)	5 814 (sd ±227)
May	492 (sd ±14.0)	8 748 (sd ±146)
June	562 (sd ±17.9)	10 716 (sd ±160)

* Values obtained during 2003 processing season

Table 2. Pectin content of different wastewaters and granular biomasses from reactors treating these wastewaters.

Reactor/wastewater treatment	Wastewater pectin ($mg.L^{-1}$)	Granular biomass pectin ($mg.L^{-1}$)
Reactor A	< 10	12.5 (sd ±8.6)
Reactor B	219 (sd ±15.9)	150 (sd ±13.7)
Reactor C	492 (sd ±10.6)	330 (sd ±17.0)
Reactor D	750 (sd ±23.8)	998 (sd ±36.4)

A - Standard reference UASB reactor (brewery wastewater)

B - Industrial-scale UASB reactor (1 ML) as used in the apple industry

C - Laboratory-scale UASB reactor during the first period (AJPWW from May, 2003 batch, Table 1)

D - Laboratory-scale UASB reactor during the second period (AJPWW from May, 2003 batch supplemented with the commercial pectin to a level of 750 $mg.L^{-1}$)

UASB (1 ML) treating AJPWW at Grabouw, South Africa. Granules C and D were from the same laboratory scale UASB, but treated different pectin containing AJPWW during two different periods of this study. During the second period (granules D) the AJPWW (May, 2003 batch, Table 1) was supplemented with the commercial pectin to a level of 750 $mg.L^{-1}$. From the data in Table 2 it is evident that as the pectin content of the wastewater increases, the amount of pectin accumulating in the biomass also increases. Increased reactor pectin levels could lead to biomass gelling and a subsequent decrease in reactor performance.

The hydrolysis of pectin by microorganisms is a time consuming process as not many anaerobic bacteria are able to synthesize the necessary polygalacturonase enzymes required to degrade the pectin molecule (Whitaker, 1990). According to Shivakumer & Nand (1995), the chemical nature of pectin makes pectin biodegradation difficult and results in poor methane generation and reduces reactor efficiency. The accumulation of pectin in reactors treating AJPWW will thus occur. The subsequent higher pectin contents in the reactor would put extra pressure on the systems capacity of degrading the increased pectin content (Bitton, 1999) leading to reduced reactor performance and possible reactor failure.

Gel formation ability

The effect of Ca^{2+} additions on the viscosity and pH of the synthetic wastewater is shown in Fig. 1. It can be seen that relatively small viscosity increases (from 11.2 to 33.6 cps) occurs with Ca^{2+} additions of up to 120 $mg.L^{-1}$. A sharp increase in viscosity (from 33.6 to 796 cps) was, however, observed with the 140 $mg.L^{-1}$ Ca^{2+} concentration. It can also be seen that an addition of only 80 $mg.L^{-1}$ Ca^{2+} was sufficient to increase the pH to >10.0. With the synthetic wastewater gel formation thus only occurred at a pH of well above 7.

The effect of Ca^{2+} additions to the AJPWW (May, 2003 batch) supplemented with commercial pectin to a concentration of 750 $mg.L^{-1}$, is shown in Fig. 2. It is important to take into consideration that in each case where commercial pectin was added to wastewater, or to distilled water, only 70 to 80% of the original pectin is detected as pectin by the carbazole test. The amount of Ca^{2+} added to wastewater before biological treatment depends on the pH of the wastewater. The $Ca(OH)_2$ will usually be added to obtain a neutral pH before biodegradation. Due to the buffering capacity of the AJPWW, larger amounts of Ca^{2+} (between 320 and 360 $mg.L^{-1}$) were required to obtain a neutral pH (Fig. 2), compared to the synthetic wastewater where less than 80 $mg.L^{-1}$ Ca^{2+} was required (Fig. 1). An addition of 360 $mg.L^{-1}$ Ca^{2+} to the AJPWW was, however, sufficient

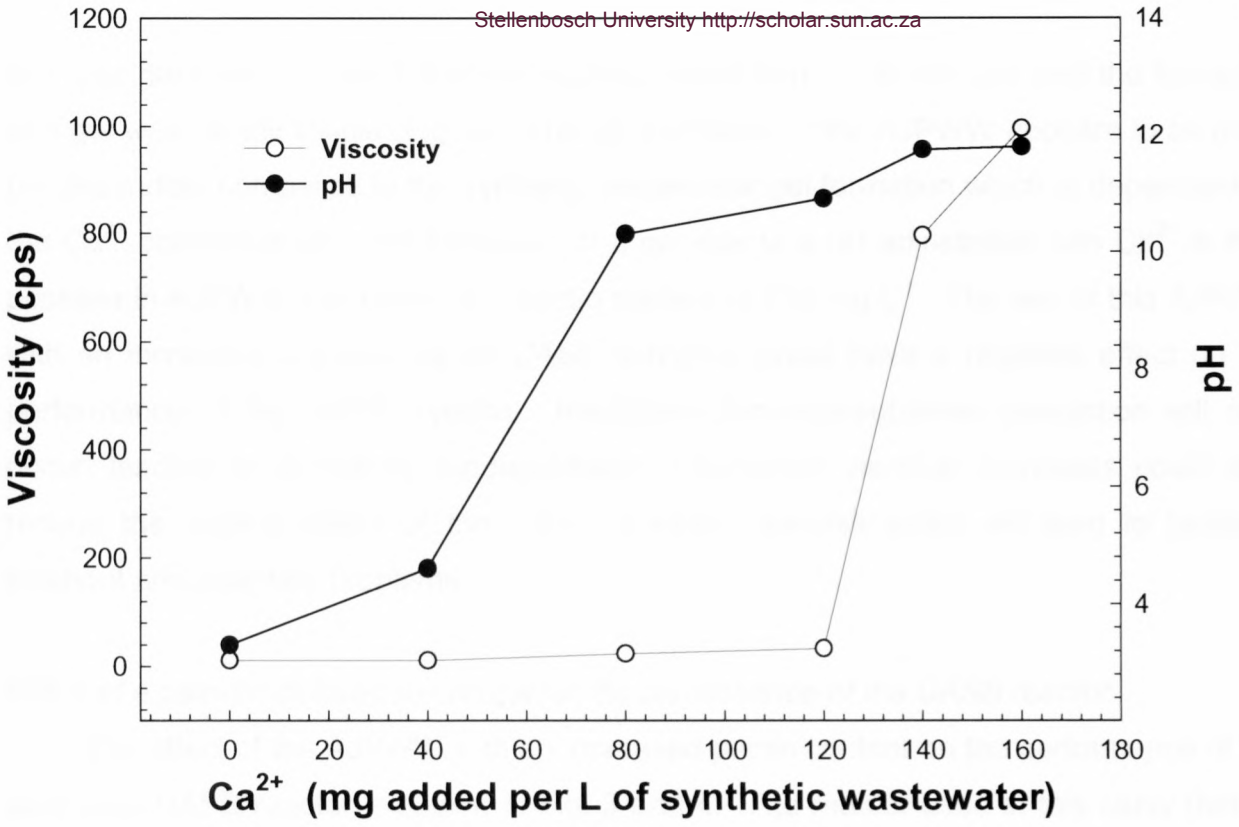

Figure 1. The effect of Ca^{2+} on the viscosity and pH of the synthetic wastewater containing 750 $mg.L^{-1}$ pectin and 10 000 $mg.L^{-1}$ sucrose.

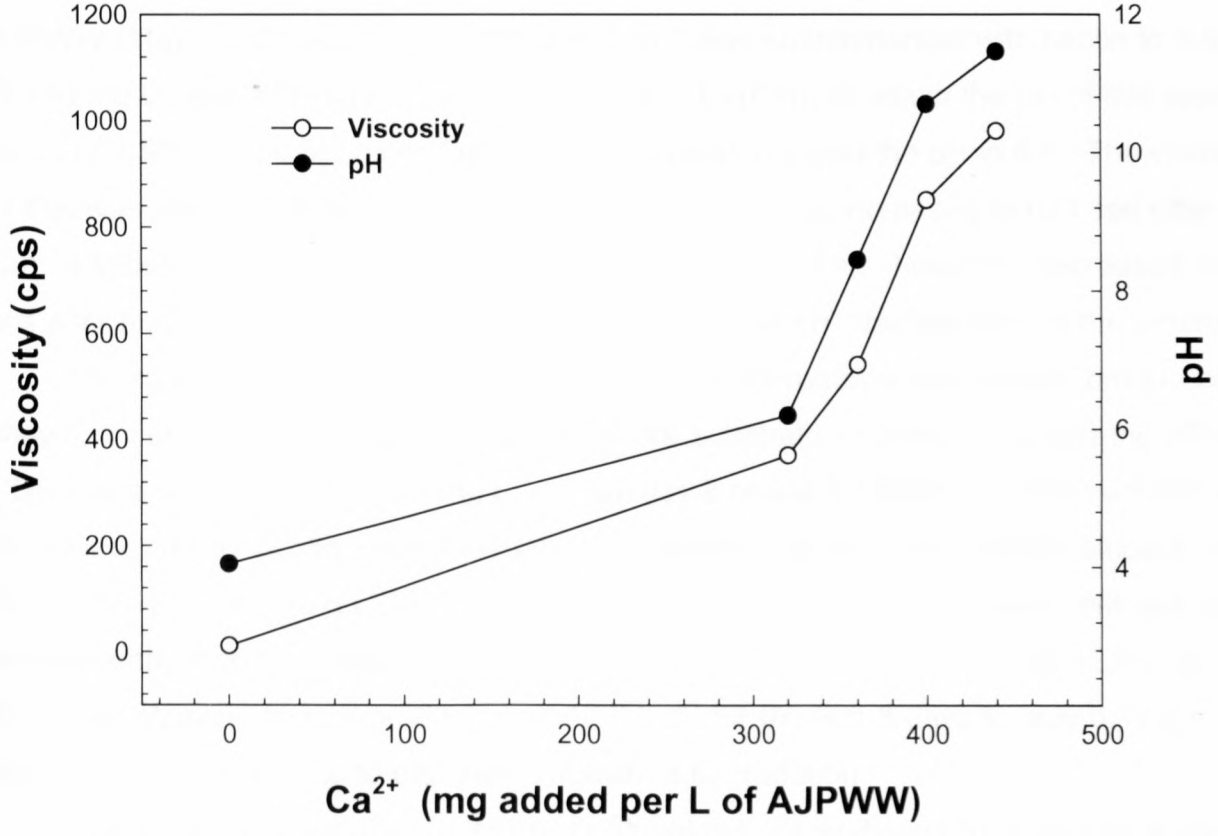

Figure 2. The effect of Ca^{2+} on the viscosity and pH of the AJPWW supplemented with commercial pectin to a pectin level of 750 $mg.L^{-1}$.

to cause gel formation since the viscosity increased from 11 to 540 cps and the formation of a gel was clearly visible (Fig. 2). The gel formation in the AJPWW appears to be more pH dependant compared to the synthetic wastewater gel formation which is dependant on the Ca^{2+} concentration. The formation of a gel due to a pH adjustment with Ca^{2+} is thus possible in AJPWW that contains a pectin content of 750 $mg.L^{-1}$. The use of this AJPWW with an increased viscosity as an UASB substrate could have a negative effect on the performance of the UASB system. Insufficient biomass-substrate interaction will also occur, leading to decreased biodegradation. Substrate viscosity increases could also reduce the settling ability of the UASB granular biomass which will lead to biomass washout and retention problems.

Effect of a calcium induced pectin gel on the performance of the UASB reactor

The effect of the AJPWW with an increased pectin content on the performance of the optimised UASB reactor is shown in Fig. 3 and 4. The reactor used in this study (before gel formation) was operated at a COD removal of 90 – 95%, an OLR of 15.6 kg $COD.m^{-3}.d^{-1}$ while the substrate pH was kept at 6.5 with NaOH, and had been stable for 65 days before the "gel formation study" was started. The HRT and OLR were both kept constant at 13.5 h and 15 kg $COD.m^{-3}.d^{-1}$, respectively for the duration of the study. The stable conditions were maintained for the first 7 days. On day 8, and thereafter, the same AJPWW (May, 2003 batch) used from day 1 to 7 was supplemented with pectin to a level of 750 $mg.L^{-1}$ and 312 $mg.L^{-1}$ Ca^{2+} (in the form of $Ca(OH)_2$ to adjust the pH of this specific batch of AJPWW to 6.5), instead of NaOH, was used to adjust the pH to 6.5. The viscosity of this wastewater before calcium addition was 8.5 cps but increased to 653 cps after the Ca^{2+} addition and the formation of a gel was observed. This, however, decreased to 47 cps after magnetic stirring to facilitate pumping of the "slush" wastewater into the reactor.

The reactor performance in terms of COD, COD removal and biogas production is shown in Fig. 3. During the first 7 days (before substrate viscosity increase) the effluent COD remained constant at 458 $mg.L^{-1}$. From day 8 onwards (after substrate viscosity was increased due to pectin supplementation) a constant increase in reactor effluent COD (from 450 to 7 510 $mg.L^{-1}$) occurred (Fig. 3). This increase in reactor effluent COD subsequently lead to a reduced COD removal from 95% on day 8, to only 12.5% on day 20. A decrease in biogas production of 12.0 $L.d^{-1}$ for the first 8 days to only 0.47 $L.d^{-1}$ on day 20 also occurred due to decreased substrate degradation.

The reactor performance in terms of effluent pH, alkalinity and VFA content is shown in Fig. 4. A decrease in reactor effluent pH, from 7.6 to 4.9, and alkalinity, from 2 600 $mg.L^{-1}$ to 550 $mg.L^{-1}$, occurred between days 8 and 20. A steady increase in VFA content

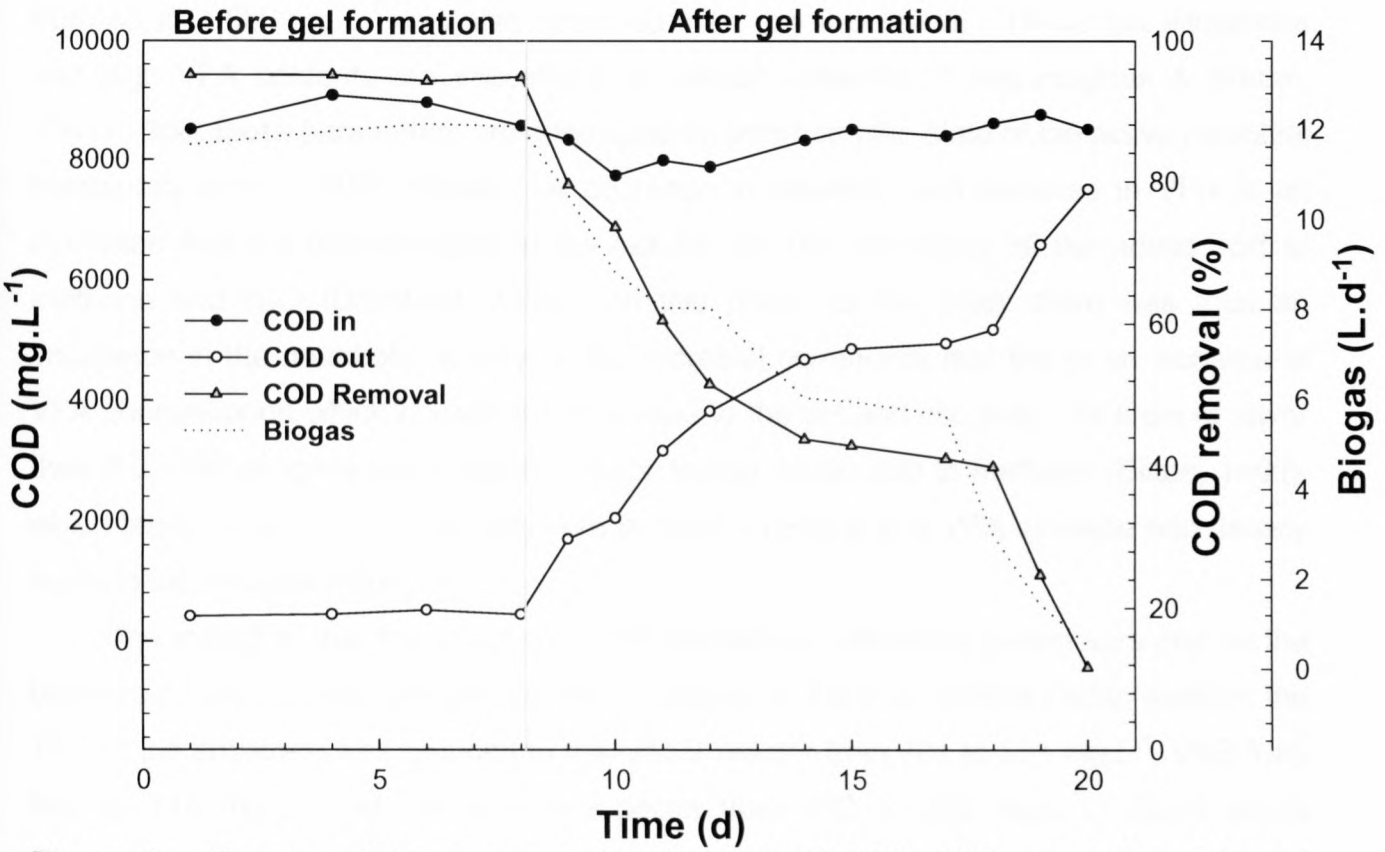

Figure 3. Reactor efficiency parameters in terms of COD, COD removal and biogas production while treating the AJPWW of increased viscosity.

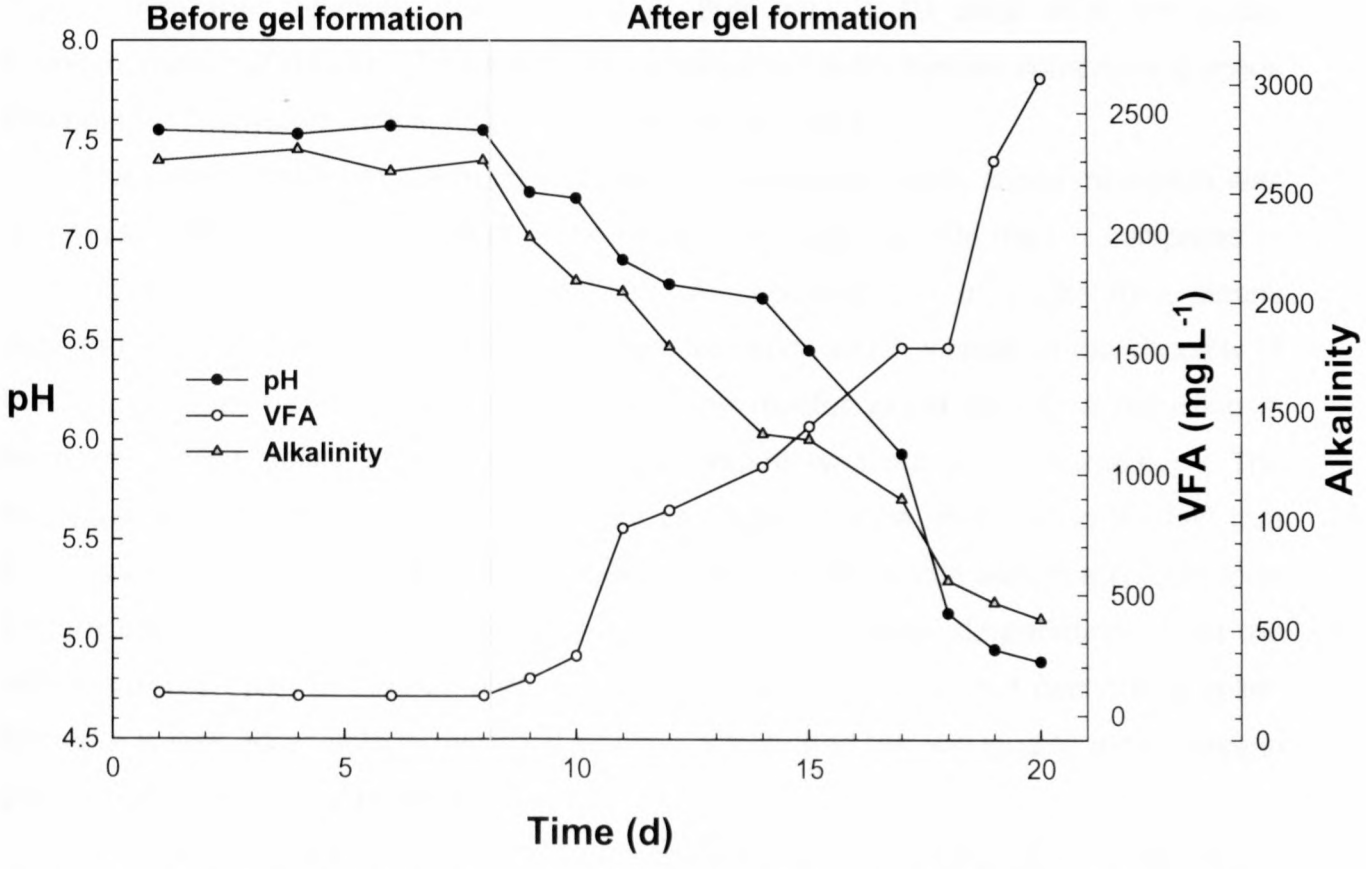

Figure 4. Reactor efficiency parameters in terms of pH, Alkalinity and VFA content of the reactor effluent while treating the AJPWW of increased viscosity.

from 80 to 2 600 mg.L^{-1} was also observed from day 8 onwards. These low alkalinities and high VFA contents are indications of reactor instability (Tchobanoglous & Burton, 1991). Both these parameters are often used to determine the state of the active microbial community in the UASB reactor. A decrease in alkalinity and increase in VFA is an indication that the methanogens in the reactor are not converting all the acetic acid to methane and H_2O (Ditchfield, 1986). In this phase of the study there was thus an imbalance in the metabolic activity of the microbial community that led to an increase in VFA concentration which caused the decrease in the pH and alkalinity. At a pH of lower than 6.0, methanogens are incapable of converting acetic acid to methane (Bitton, 1999), which leads to an even further decrease in reactor pH due to a VFA increase and usually leads to total reactor failure.

The impact of this increased substrate viscosity on efficiency parameters and on the biomass pectin (granular sludge) content, is shown in Table 3. Before pectin addition the TSS of the substrate was reduced in the UASB reactor from 781 to 251 mg.L^{-1}, VSS from 584 to 214 mg.L^{-1} and the substrate pectin from 492 to 333 mg.L^{-1}. Good solids degradation but less pectin degradation thus occurred during this period. After 10 days of substrate pectin supplementation, the reactor effluent TSS content was 3 905 mg.L^{-1} (an increase of 1 556%). The VSS content in the reactor effluent was also higher (3 195 mg.L^{-1}) than the substrate VSS content (2 805 mg.L^{-1}) 10 days after the pectin supplementation (Table 3). More solids thus washed out of the system compared to solids that entered the system indicating possible biomass washout.

The pectin content in the reactor effluent 10 days after pectin supplementation was 603 mg.L^{-1} while the pectin content of the reactor biomass was 998 mg.L^{-1}, compared to 330 mg.L^{-1} before pectin supplementation. An accumulation of pectin thus clearly occurred in the reactor itself and this led to gel formation which increased the viscosity of the reactor content. A higher viscosity within the reactor would lead to a reduction in biomass settling ability and thus cause subsequent washout of the biomass. The excessive pectin accumulation in the reactor could possibly be attributed to the fact that the pectin was in a different form than before. Originally the pectin was in a soluble form (before calcium addition) and thus more accessible to microbial degradation. With the addition of Ca^{2+} part of the AJPWW gelled. It has been reported that pectin in a gelled structure is less accessible to microbial enzyme activity and this will lead to a decrease in pectin biodegradability (Madamwar & Mithal, 1985).

Wastewater characteristics, in terms of composition and biodegradability, are one of the most important factors that determine the formation and maintenance of granules and ultimately the effectiveness of the UASB treatment process (Hulshoff Pol *et al.*, 1983). It is

Table 3. Solids and pectin contents on day 8 (before pectin addition) and on day 18 (10 days after pectin addition started).

Parameter	Before pectin addition ($mg.L^{-1}$)	10 d after pectin addition ($mg.L^{-1}$)
Substrate TSS	781 (sd ±76)	3 550 (sd ±219)
Substrate VSS	584 (sd ±96)	2 805 (sd ±113)
Substrate pectin	492 (sd ±16.4)	750 (sd ±9.2)
Reactor effluent TSS	251 (sd ±21)	3 905 (sd ±197)
Reactor effluent VSS	214 (sd ±16)	3 195 (sd ±91)
Reactor effluent pectin	333 (sd ±12.6)	603 (sd ±16.6)
Biomass pectin	330 (sd ±11)	998 (sd ±33.4)

Table 4. The effect of variation in pH values on pectin degradation after a 5 min ozonation of the AJPWW containing 750 $mg.L^{-1}$ pectin.

pH	Pectin reduction*
4.5	20% (min 18.4 - max 21.8)
6.5	31% (min 29.6 - max 33.7)
8.2	51% (min 48.6 – max 53.1)

* After the ozonation of a 1 000 $mg.L^{-1}$ pectin solution for 5 minutes

well known that the accumulation of solids within the bioreactor granule bed hampers the conversion of COD to methane due to the low biodegradability of the solids (Harada *et al.*, 1996). O'Kennedy (2000) also reported that a high substrate solid content led to the overflow of biomass that decreased the reactor performance. Madamwar & Mithal (1985) reported that no pectin degradation occurred in a laboratory scale batch reactor during the anaerobic digestion of cattle dung. Similarly, the data from this study as given in Table 3 clearly indicates that pectin accumulation due to a gelling in the reactor leads to a decrease in degradability. Thus a suitable pre-treatment, like ozonation, will be required to increase the suitability of the wastewater to UASB treatment.

Effect of ozone on the pectin content, gel formation ability and gel strength

The ozonation of complex wastewaters is influenced by several parameters including the pH, which make it difficult to predict the resultant degradation products that will form (Andreozzi *et al.*, 1997). It is, however, generally known that ozonation decreases the molecular size and increases molecule polarity which usually increases the biodegradability of the wastewater (Gottshalk *et al.*, 2000). The effect of ozone on pectin degradation at different pH levels was investigated and it was found that higher pectin reductions occurred at higher pH's (Table 4). It was found that a 20% reduction in the pectin content occurred at a pH of 4.5 compared to a 51% reduction at a pH of 8.2. The presence of more hydroxyl radicals at a higher pH (Gottshalk *et al.*, 2000) could be responsible for the larger pectin reductions.

The effect of various ozonation times on the pectin and COD contents of an AJPWW (June, 2003 batch), is shown in Fig. 5. The data shows that a steady decrease in pectin content, from 562 to 48 $mg.L^{-1}$ (91%), occurred while a smaller decrease from 10 716 to 9 551 $mg.L^{-1}$ (12%) occurred in the COD content after 10 min of ozonation. The fact that a large pectin reduction occurred while the COD reduction was less drastic (Fig. 5) can be ascribed to the fact that once the pectin chains are broken down into shorter chains, they are no longer detected as a pectic substance. These pectin degradation products will, however, still contribute to the wastewater COD. Ozone and the hydroxyl radicals formed after ozonation, are more reactive with larger polymetric molecules, like pectin, than with the smaller molecules formed after the hydrolysis of pectin (Volk *et al.*, 1993). Pectin is thus partially degraded, but total mineralization to CO_2 and H_2O does not occur (Gottshalk *et al.*, 2000). The nature of wastewater changes after ozonation but its oxygen absorbing properties are not lost.

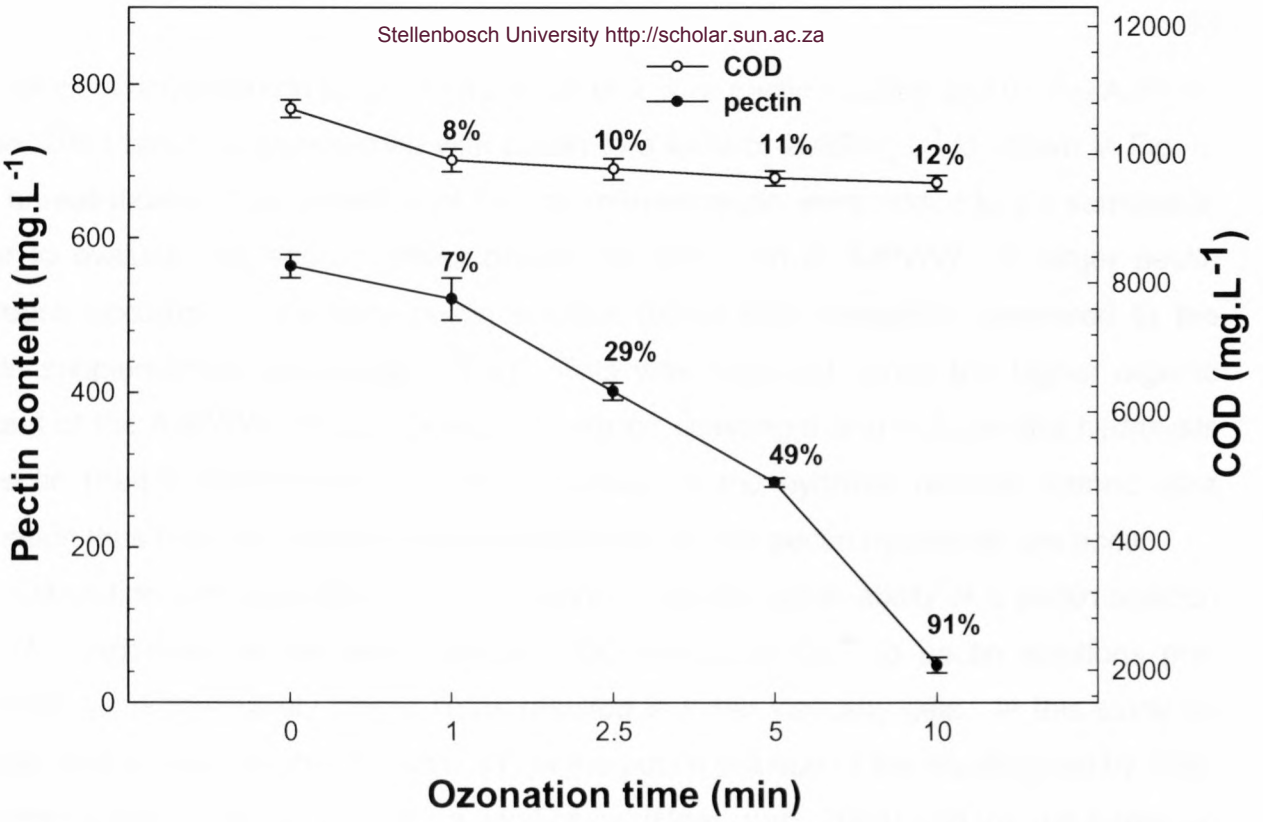

Figure 5. The effect of ozone on the pectin and COD contents of the apple juice processing wastewater. The percentage values indicate the amount of pectin degradation that occurred.

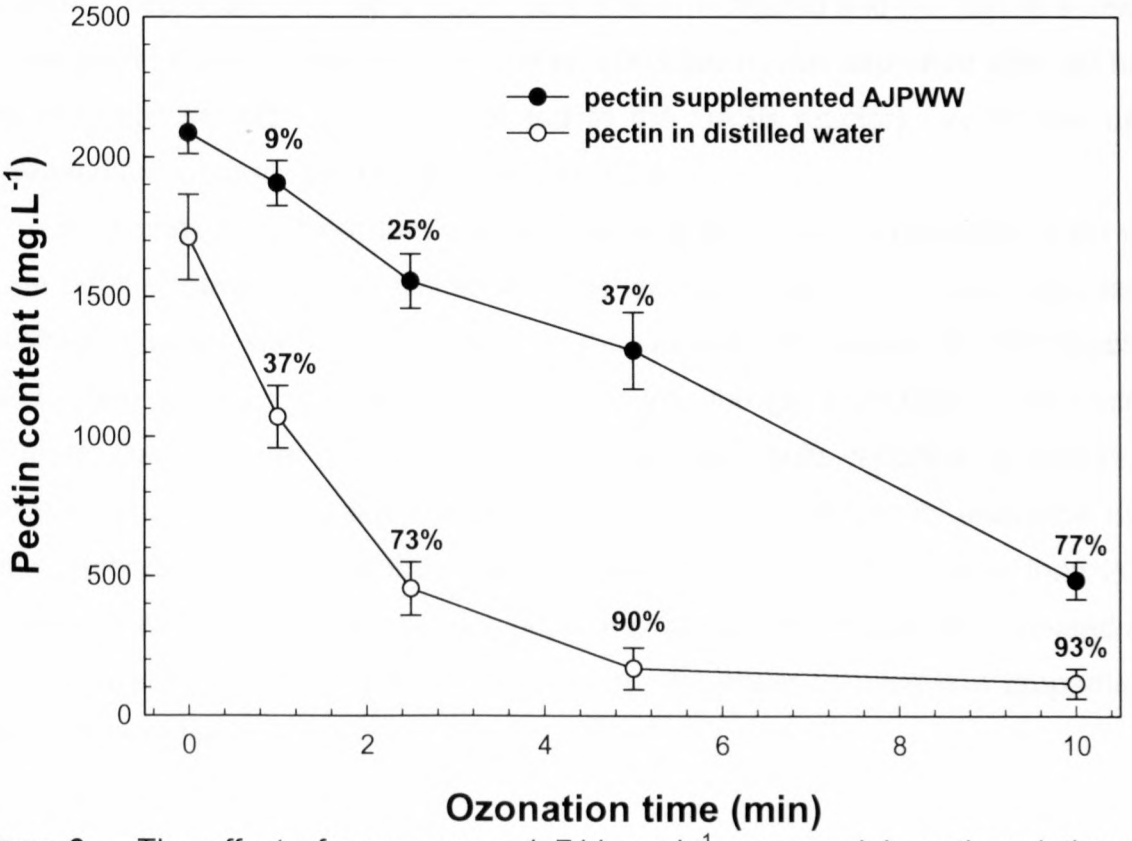

Figure 6. The effect of ozone on a 1 711 mg.L^{-1} commercial pectin solution and the AJPWW supplemented with commercial pectin to a pectin content of 2 085 mg.L^{-1}. The percentage values indicate the amount of pectin degradation that occurred.

The effect of ozonation on pectin degradation of a pure pectin solution and on the AJPWW (June, 2003 batch) supplemented with pectin to a level of 2085mg.L^{-1} is shown in Fig. 6. The unrealistically large quantities of the commercial pectin were added to the samples in order to evaluate the specific effect ozone has on pectin in AJPWW. A larger pectin decrease occurred in the pure pectin solution (93%) after ozonation compared to the pectin supplemented wastewater (77%). This was expected, since the higher organic content of the AJPWW probably acts as an ozone scavenger and reduces the hydrolysis of pectin (Beltrán-Heredia *et al.*, 2000). Ozone, or the hydroxyl radicals formed after ozonation thus react with other organic compounds before pectin hydrolysis can occur.

Ozonation was also effective in reducing the gel formation ability of a pectin solution (Fig. 7). Additions of the same amount (200 mg.L^{-1}) of Ca^{2+} to pectin solutions pre-ozonated for progressively longer times resulted in lower viscosity gels. In this study an ozonation of 20 min reduced the viscosity of the pectin solution of the resulting gel by 76%. A shorter pectin chain will produce a weaker gel (Hoejgaard, 2003) and the gel formation ability of pectin is thus reduced each time the galacturonic acid polymer is hydrolyzed by ozone (Gottshalk *et al.*, 2000).

The effect of a 20 min ozonation on an already formed AJPWW pectin gel, containing 750 mg.L^{-1} pectin and 312 mg.L^{-1} Ca^{2+}, was also investigated and the results shown in Fig. 8. The pectin supplemented AJPWW (May, 2003 batch) was ozonated after gel formation plus magnetic stirring (as was to be fed to the UASB reactor). A 20 min ozonation decreased the viscosity of the wastewater by 62%.

One technical problem that was encountered during these ozonation trails was that ozone bubble formation was hindered at higher viscosities (or gel strengths) leading to insufficient contact with the pectin gel. As the viscosity decreased, smaller bubbles were able to form and ozone contact and diffusion into the gel increased. This decrease in substrate viscosity after ozonation is in line with the results reported by Battimelli *et al.* (2003) who ozonated activated sludge biomass as a pre-treatment for anaerobic digestion. These authors reported that the substrate viscosity decreased by more than 20% after ozonation and that reological changes occurred after ozonation and the ozonated samples showed Newtonian flow properties compared to the shear thinning flow properties of un-ozonated samples.

Conclusions

In this study it was clearly indicated that an increase in substrate viscosity due to a calcium induced pectin gel occurred when $Ca(OH)_2$ was used to adjust the pH of a pectin

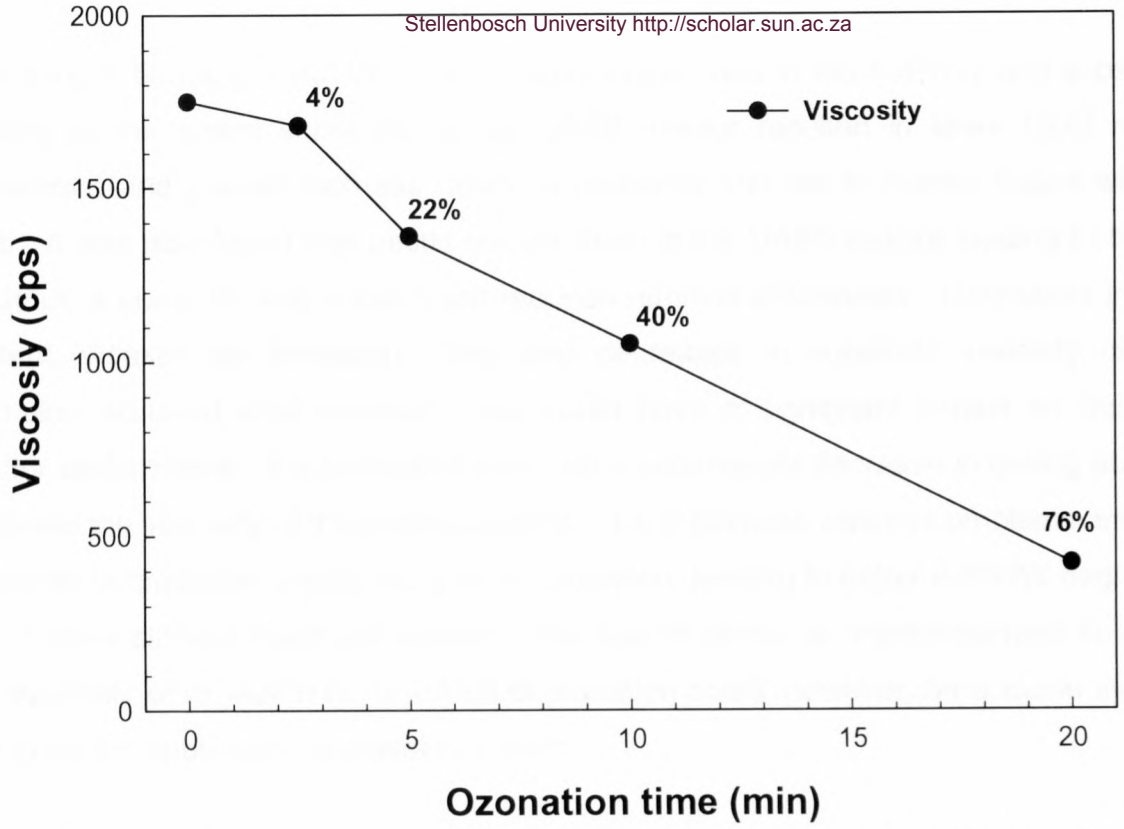

Figure 7. The effect of ozonation (at pH 4.5) on the gel formation ability of a 750 mg.L^{-1} pectin solution (viscosity measured at pH 6.5 after the addition of 200 mg.L^{-1} Ca^{2+}). Percentage values indicate the decrease in viscosity.

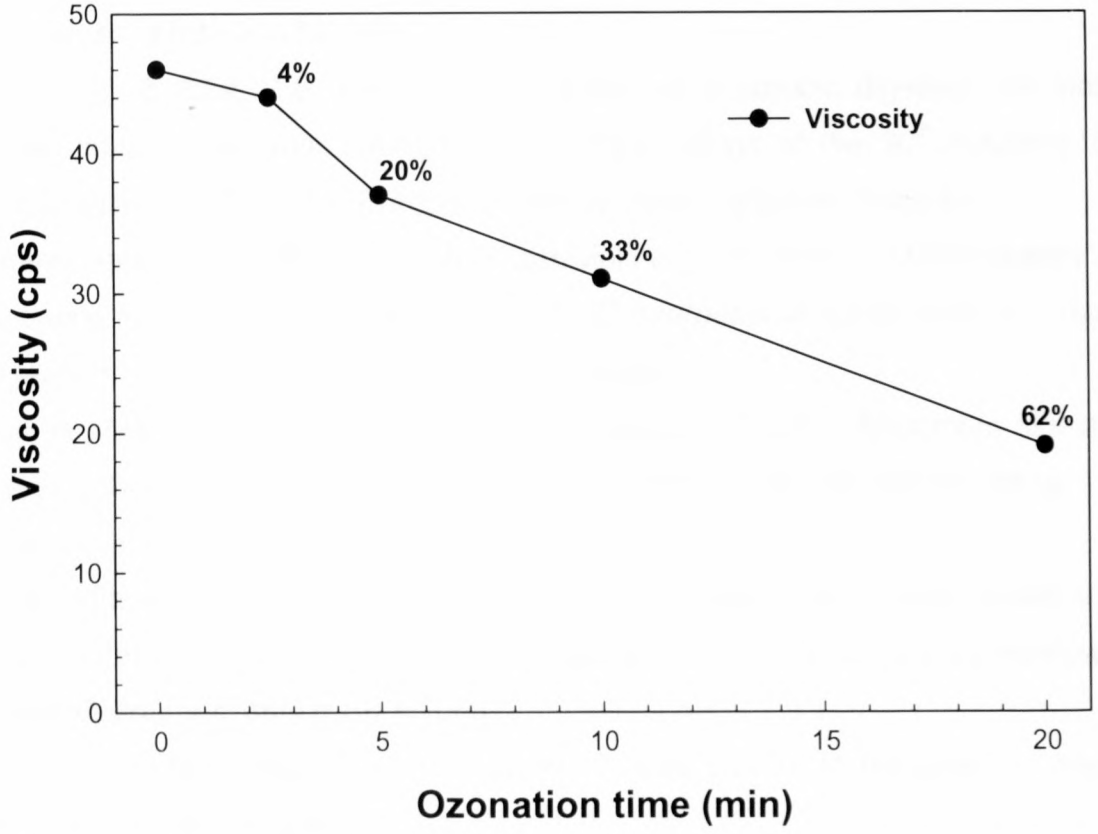

Figure 8. The effect of ozonation (at pH 6.5) on an already formed AJPWW pectin gel (after magnetic stirring) containing 750 mg.L^{-1} pectin and 360 mg.L^{-1} Ca^{2+} (as fed to UASB reactor). Percentage values indicate the decrease in viscosity.

containing (750 mg.L^{-1}) AJPWW. This viscosity increase in the AJPWW and subsequent feeding to the gelled substrate of the UASB reactor resulted in lower COD removal efficiencies and caused biomass retention problems that led to reactor failure within 12 days. It was also found that pectin accumulated in the UASB reactor leading to biomass washout, a lower pH and subsequent reduced removal efficiencies. Decreases in pectin content, weaker gel formation ability and decreases in substrate viscosity after gel formation occurred after ozonation and could have a significant impact on the UASB reactor performance. Pectin degradation and a subsequent decrease in gelling ability will decrease the viscosity of the UASB substrate. Less biomass washout problems and better substrate degradation should occur after ozonation, leading to better AJPWW degradation and a more efficient treatment system. The use of ozone as a pre-treatment to improve the suitability of an AJPWW for UASB degradation could, however, be a viable treatment option for the apple juice processing industry.

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CHAPTER 5

INFLUENCE OF PRE- AND POST-OZONATION ON THE EFFICIENCY OF AN UASB SYSTEM TREATING APPLE JUICE PROCESSING WASTEWATER

Summary

The effects of pre-ozonation on apple juice processing wastewater were determined. A pre-ozonation of 10 min decreased the total chemical oxygen demand (COD) of the wastewater by 19%, while the soluble COD fraction of the total COD increased from 81.7 to 92.4%. The total suspended solids (TSS) in the wastewater also decreased by 36% after a 10 min pre-ozonation. The effects of feeding a pre-ozonated (2.5 min) apple juice processing wastewater on the efficiency of an upflow anaerobic sludge blanket (UASB) reactor was subsequently determined. COD removals increased from 78.0 to 90.0% after the first day of the pre-ozonated substrate feed. After 26 days of feeding ozonated substrate the OLR was increased from 10.0 to 16.6 kg $COD.m^{-3}.d^{-1}$. The COD removal, however, remained constant at between 90 and 95% and the reactor pH and alkalinity increased from 6.9 to 7.65 and 2 600 to 3 200 $mg.L^{-1}$, respectively. The VFA content also decreased from 650 to 78 $mg.L^{-1}$ and the reactor effluent had an average COD of 465 $mg.L^{-1}$ during this period. Post-ozonation was then done on this reactor effluent. A 10 min post-ozonation reduced the COD and colour of the reactor effluent by 64.8 and 79.0%, respectively. A combined pre-ozonation, UASB treatment, and post-ozonation system reduced the COD of the apple juice processing wastewater from 9 653 to 180 $mg.L^{-1}$ (98.1%) compared to UASB treatment alone that only reduced the COD by 78%.

Introduction

Large volumes of wastewater with a high organic content are produced during the washing, pressing, juice extraction and dewatering stages of the apples juice production process (Wayman, 1996; Grismer *et al.*, 2002). The necessity of improved on-site wastewater treatment facilities to degrade this wastewater has become unavoidable due to strict export regulations and government legislation (Trnovec & Britz, 1998).

The effectiveness of the upflow anaerobic sludge blanket (UASB) treatment system in the degradation of food processing wastewaters is well documented (Lettinga *et al.*, 1997). The nature and strength of food processing wastewater provides an ideal UASB substrate (Trnovec & Britz, 1998). It is, however, well known that UASB systems are very

substrate sensitive. The finely dispersed and complex organic matter, present in apple juice processing wastewater (AJPWW) can have a negative effect on the efficiency of an UASB system (Lin & Yang, 1990). The first step of the anaerobic digestion process is the hydrolysis of complex organic matter into more soluble organic fractions (Ditchfield, 1986). Extra-cellular enzymes, produced by the hydrolytic and acidogenic bacteria, are responsible for this degradation process (Murry, 1985; Bitton, 1999). The acclimatisation of these bacteria to the substrate and to the operational environment is time consuming, and often the limiting step in the anaerobic digestion pathway (Van Lier *et al.*, 2001). A chemical pre-treatment that will convert complex organic matter into more biodegradable substances should thus increase the efficiency of the UASB system (Van Lier *et al.*, 2001).

The use of ozonation for the treatment of wastewater is well documented (Gottschalk *et al.*, 2000). It has also been shown that ozonation as a pre-treatment, can improve the biodegradability of wastewaters (Weemaes *et al.*, 1999; Martin *et al.*, 2001). Ozonation enhances the biodegradability of organic matter by increasing molecule polarity and by forming smaller molecular weight substances (Volk *et al.*, 1993; Martin *et al.*, 2001). Several authors (Benitez *et al.*, 1999; Weemaes *et al.*, 1999; Martin *et al.*, 2001) found that a pre-ozonation during anaerobic digestion, increased the methane yield and overall efficiency of UASB systems. Andreozzi *et al.* (1997) did, however, find that the pre-ozonation of phenol containing olive oil mill wastewater, prior to anaerobic digestion, had an inhibitory effect on the methanogenic bacteria due to the formation of azelaic acid.

The higher chemical oxygen demand (COD) level in UASB reactor effluents would in most cases prevent the discharge or irrigation of the effluent and a post-treatment process will be required (Sigge *et al.*, 2002). The ozonation of wastewaters has been shown to be effective in reducing the COD content of the water (Gottschalk *et al.*, 2000). Beltrán *et al.* (2000) and Sigge *et al.* (2002) also reported that the COD of food processing wastewaters can be lowered by ozonation and the ozonation of UASB reactor effluents was especially effective in the reduction of the final COD and colour value of wastewaters.

The aims of this study were to evaluate the effect of ozone as a pre-treatment on AJPWW, the effect of the pre-ozonated AJPWW on the efficiency of an UASB reactor, and as a post-treatment on the effluent from an UASB reactor treating the AJPWW.

Materials and methods

Wastewater

The AJPWW used in this study was obtained from Elgin Fruit Juices (Pty) Ltd. (Elgin, South Africa) during the period April 2002 to June 2003. The substrate batches were kept

at -18°C until required. The wastewater was dark grey in colour and very murky due to the presence of high concentrations of suspended solids, had a COD of 7116.2 (±952) $mg.L^{-1}$ and a pH of *ca.* 4.5. The wastewater was supplemented with 100 $mg.L^{-1}$ urea and 100 $mg.L^{-1}$ K_2HPO_4 to prevent nitrogen and phosphorus deficiencies and the pH adjusted as required with 1 M sodium hydroxide (NaOH) or phosphoric acid (H_3PO_4). Potassium hydrogen carbonate ($KHCO_3$) was added at times to increase the buffering capacity of the substrate. The substrate was also enriched with trace elements (1 $mL.L^{-1}$ substrate) once a week (Nel *et al.*, 1985).

Anaerobic Treatment

A laboratory-scale UASB bioreactor operated at 35°C, as described in Chapter 3 of this thesis, was used. The reactor had been operating at steady state for at least 30 days before this study commenced. During this study the UASB reactor was first fed with just raw AJPWW substrate for the first 34 days, whereafter it was fed with a pre-ozonated (2.5 min) AJPWW substrate for 12 d (days 35 to 47). One quarter (600 mL) of the granular biomass was then removed from the reactor (day 47) and an unozonated substrate was fed to the reactor for the next 24 d (days 48 to 71). During this time (days 48 – 71) post-ozonation tests were done on the reactor effluent to provide results for a combined pre- and post-ozonation UASB system. A pre-ozonated (2.5 min) substrate was then fed, from day 72 onwards and the long term reactor performance was monitored.

Ozonation

Ozonation of the AJPWW was done in a continuous mode, bubble column and ozone was bubbled upwards through the glass column. The bubbling column had a length of 900 mm and diameter of 65 mm. An ozone generator (Parc Scientific, Ifafi) producing 15.5 $g.h^{-1}$ O_3 at a flow rate of 4 $L.min^{-1}$ was used for all ozonation trails. All ozonated samples were analysed in triplicate. The raw reactor substrate at a pH of 6, was ozonated for different times (1, 2.5, 5 and 10 min) during the pre-ozonation study. The UASB reactor effluent was also ozonated for different times (1, 2.5, 5 and 10 min) during the post-ozonation tests.

Analytical methods

The following parameters were monitored according to the APHA (Standard Methods, 1992): pH; alkalinity; total suspended solids (TSS); volatile suspended solids (VSS); and chemical oxygen demand (COD). The ozone production was measured using an iodiometric titration (Standard Methods, 1992).

The total volatile fatty acids (TVFA) were determined using a Varian (Model 3700) gas chromatograph, equipped with a flame ionization detector and a 30 m fused-silica capillary column with bonded FFAP stationary phase (Quadrex Co., New Haven). The column temperature was initially held at 105°C for 2 min, then increased at a rate of 8°C per min to 190°C. The detector and inlet temperature were set at 300 and 130°C respectively, and nitrogen gas was used as carrier gas at a flow rate of 6.1 $mL.min^{-1}$.

The biogas composition was determined on a Fisons gas chromatograph equipped with a thermal conductivity detector and 2.0 m × 2.0 mm i.d. column packed with Hayesep Q (Supelco, Bellefonte, PA), 80/100 mesh. The oven temperature was set at 45°C and helium was used as the carrier gas at a flow rate of 40 $ml.min^{-1}$.

To determine the effect of ozonation on the biodegradability of the AJPWW, the activity test method of O'Kennedy (2000), was used. Biomass from the laboratory-scale UASB reactor treating the unozonated AJPWW (day 10) was used. The apple juice processing wastewaters (12 ml) was ozonated for different times (0, 1, 2.5, and 5 min) and added to 3 g of this reactor biomass, sealed in vials and incubated for 25 h. Biogas and methane production were measured after 5, 10, and 25 h.

Results and discussion

Pre-ozonation

The effect of pre-ozonation on the AJPWW is shown in Fig. 1. The total and soluble COD content, and the percentage soluble COD after each ozonation are shown in Table 1. It can be seen that total COD reduction was enhanced with increasing ozonation times (Fig. 1). The COD reduction of 5% after a 1 min ozonation increased to 19% after 10 min ozonation. The TSS content in the raw AJPWW decreased as the ozonation time increased and a 36% reduction in TSS content was observed after a 10 min pre-ozonation (Fig. 1).

The data also showed that the percentage soluble COD increased from 81.7 to 92.4%, after the 10 min ozonation (Table 1). The lower reduction in the soluble COD content after a 10 min ozonation (Fig. 1) can probably be ascribed to the fact that the percentage of soluble COD had increased (Table 1) as ozonation reduced the TSS (Fig.1) and insoluble organics present in the AJPWW.

An increase in the soluble COD of wastewater will usually result in an increase in biodegradability (Volk *et al.*, 1993). These reductions in COD content, after the ozonation of the untreated AJPWW, are in accordance with results reported by other authors.

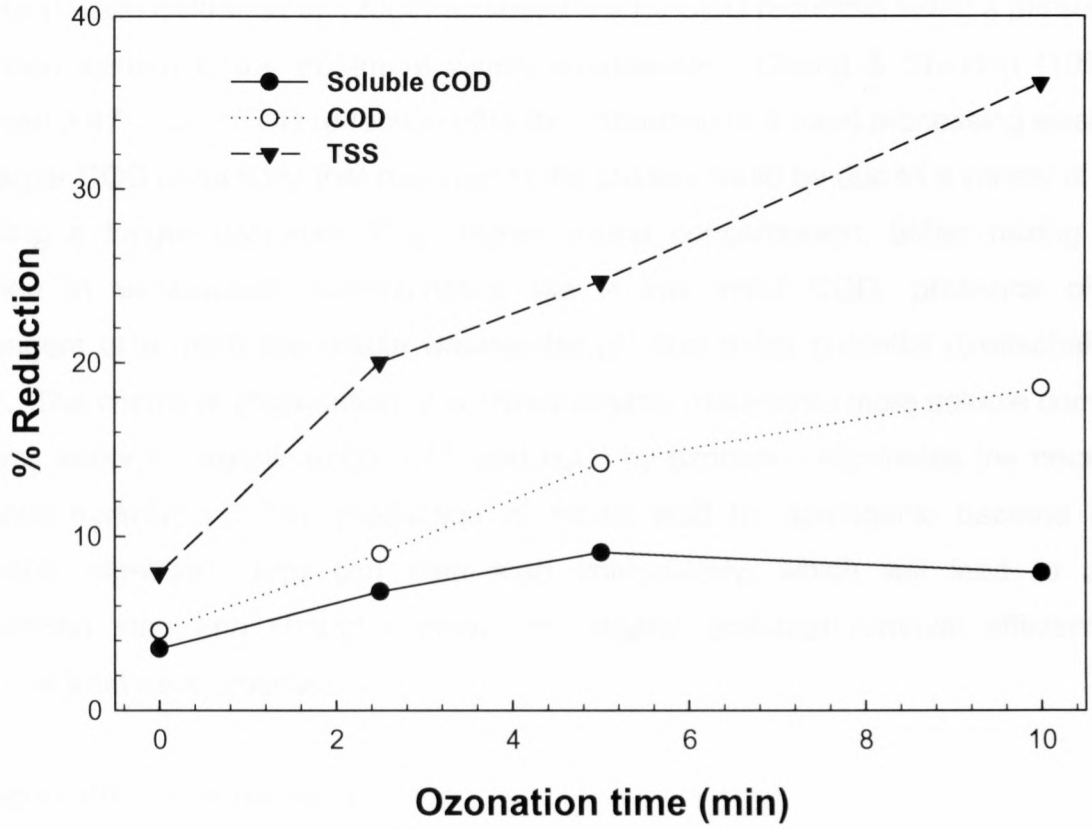

Figure 1. Effects of the raw AJPWW pre-ozonation on the COD, soluble COD and the TSS contents at various ozonation times.

Table 1. Total and soluble COD contents and percentage soluble COD of the raw AJPWW after different pre-ozonation times.

Ozonation time (min)	Total COD ($mg.L^{-1}$)	Soluble COD ($mg.L^{-1}$)	Soluble COD (%)
0	7301	5972	81.7
1	6967	5761	82.7
2.5	6645	5564	83.7
5	6266	5430	86.6
10	5948	5499	92.4

Benitez *et al.* (1999) achieved a 20 to 30% COD reduction in winery wastewater after ozonation while Beltrán *et al.* (2000) achieved a 40% COD reduction using a pH sequential ozonation system in the treatment winery wastewater. Chang & Sheldon (1998) also achieved a 47 – 62% COD reduction after the ozonation of a meat processing wastewater. The larger COD reductions that occurred in the studies could be due to a variety of factors, including a longer ozonation time, higher ozone concentration, better mixing a wide variation in wastewater characteristics like a low initial COD, presence of ozone scavengers or a more favourable wastewater pH and redox potential (Gottschalk *et al.*, 2000). The chemical degradation of complex organic matter into more soluble compounds (sugars, alcohols, organic acids, CO_2 and H_2O) by ozonation eliminates the necessity of microbial hydrolysis. The production of acetic acid by acetogenic bacteria (second anaerobic digestion step) can then start immediately, which will lead to a faster degradation step and should improve the organic pollutant removal efficiency of a biological treatment process.

Biodegradability after ozonation

The use of activity tests to determine the biogas production when identical biomass samples are fed with different substrates can serve as a useful indication of the biodegradability of the different substrates (O'Kennedy, 2000; T.J. Britz, University of Stellenbosch, South Africa, personal communication, 2003). The effect of ozonation on the biodegradability of the AJPWW is shown in Fig. 2 and 3. Activity was monitored in terms of biogas (Fig. 2) and methane (CH_4) (Fig. 3) production. It can be seen that the amount of biogas produced during the activity test decreases with time (Fig. 2). This is to be expected since the amount of substrate is quickly depleted. The amount of methane formed during the activity tests was more constant, indicating that the carbon utilising and methane production is slower. The degradation products of other bacteria are possibly used by the methane producing bacteria (Bitton, 1999) to produce relatively more methane during the latter times of the incubation period.

If the total amount of biogas produced is considered, increased ozonation time resulted in increased biodegradability, as can be seen from the increase of biogas formed after 5 h. After 10 and 25 h of incubation, the amount of biogas produced from substrates ozonated for 2.5 and 5 min, was less than found for the control (0 min). This was ascribed to the depletion of substrate. Ozonation of the raw AJPWW thus resulted in increased biodegradability of the wastewater, resulting in higher total biogas production.

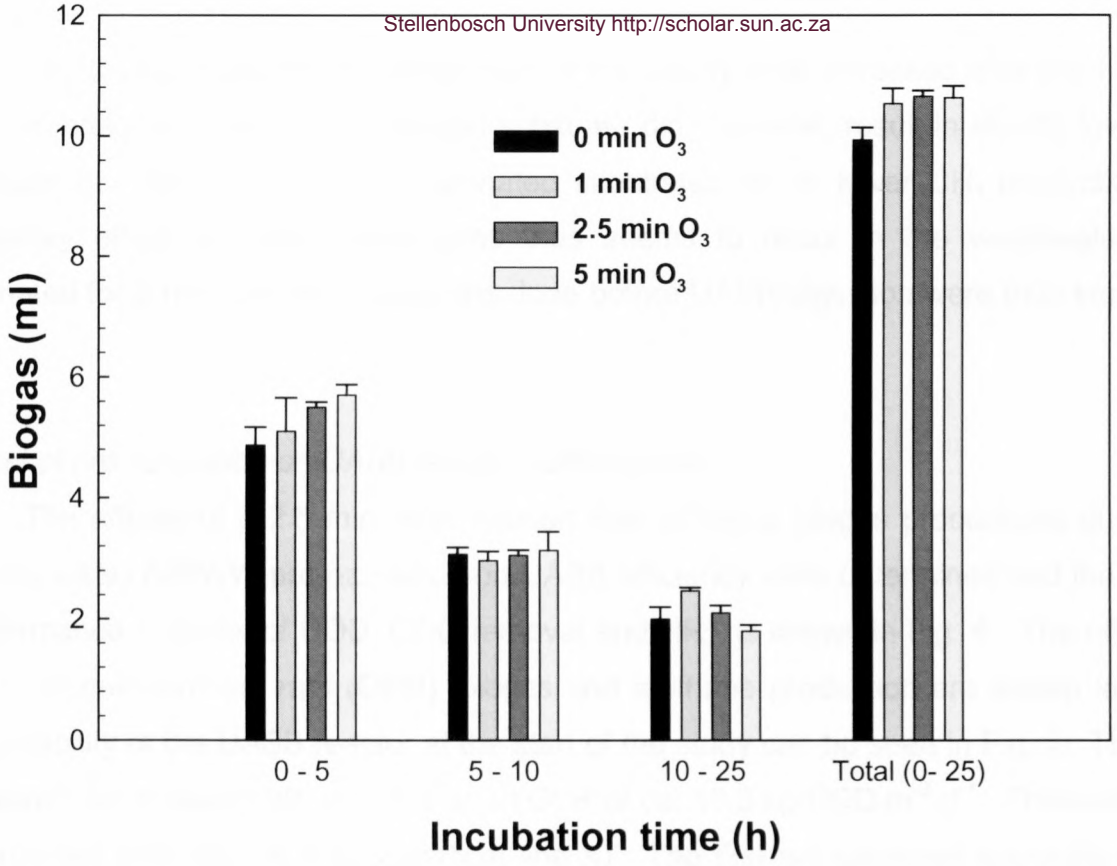

Figure 2. Cumulative biogas production during activity tests done on the UASB biomass and the pre-ozonated AJPWW at different times.

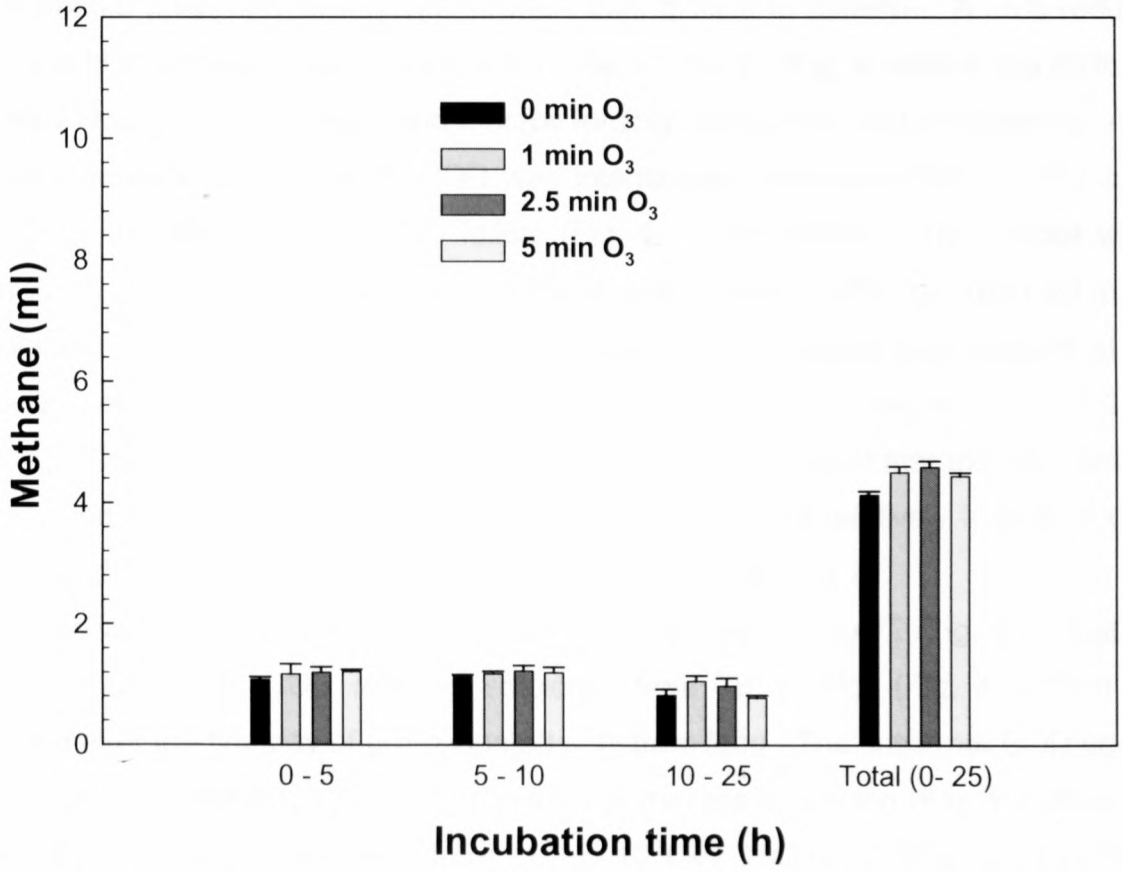

Figure 3. Cumulative methane production during activity tests done on the UASB biomass and the pre-ozonated AJPWW at different times.

The total amount of CH_4 formed during the activity tests increased after the 1 and 2.5 min ozonations. The 5 min ozonated substrate did, however, result in slightly lower CH_4 production. After 25 h, the pre-ozonated substrates led to lower CH_4 production. An inhibitory effect on the methanogens thus seems to occur in the wastewater when ozonated for 5 min and pre-ozonations done before UASB digestion were thus kept at 2.5 min.

Effect of pre-ozonation on UASB reactor performance

The effects of a 2.5 min (time chosen due to higher biogas productions during the activity tests) AJPWW pre-ozonation on UASB efficiency were determined and the reactor performance in terms of COD, COD removal and HRT is shown in Fig. 4. The effects on OLR, organic removal rate (ORR), biogas and methane production are shown in Fig. 5. The stability of the UASB reactor at the start of the study can be seen in Fig. 4. The COD removal was between 90 and 95% at an OLR of *ca.* 10.3 kg $COD.m^{-3}.d^{-1}$. This status was maintained until day 34 (Fig. 4 vertical line A). The first pre-ozonated substrate feeding commenced on day 35 and continued until day 47 (Fig. 4 vertical line B). No significant change in the reactor performance was observed during this time. This could have been due to the fact that the UASB reactor was performing very efficiently before the start of the pre-ozonated feed, and improvements were thus difficult to observe. A 25% reduction in microbial biomass within the reactor was made on day 47 (Fig. 4 vertical line B) to reduce the active microbial population, and thereby forcibly lowers the reactor efficiency. After the biomass removal, the substrate COD was intentionally decreased (from 7 512 to 4 979 $mg.L^{-1}$) to prevent possible reactor failure (Fig. 4, vertical line B). The reactor was then allowed to stabilise and reach its optimum performance with the reduced microbial population, while gradually increasing the influent COD. Stability was reached at a COD reduction of 78% by day 71. The HRT and OLR at this stage were 14.6 h and 11.5 kg $COD.m^{-3}.d^{-1}$, respectively (Fig. 4 and 5) and it appeared that the OLR limit of the system had been reached since the VFA content remained relatively high at 600 $mg.L^{-1}$ (Fig. 6) and no further increases in COD removal occurred (Fig. 4).

A second pre-ozonated substrate feed commenced on day 72 (Fig. 4 vertical line C). A sharp increase in COD removal efficiency from 78 to 90% (Fig. 4 vertical line C) occurred after the first day of pre-ozonated substrate feed. The substrate COD decreased from 7009 to 6 188$mg.L^{-1}$ (12%) due to the 2.5 min pre-ozonation (Fig. 4 vertical line C), resulting in a 12% OLR decrease from 11.5 to 10.1 kg $COD.m^{-3}.d^{-1}$(Fig. 5). The ORR did, however, not decrease after pre-ozonated feed, but stayed constant at 9.1 kg $COD.m^{-3}.d^{-1}$, despite this OLR decrease (12%) (Fig. 5). The same amount of organic degradation

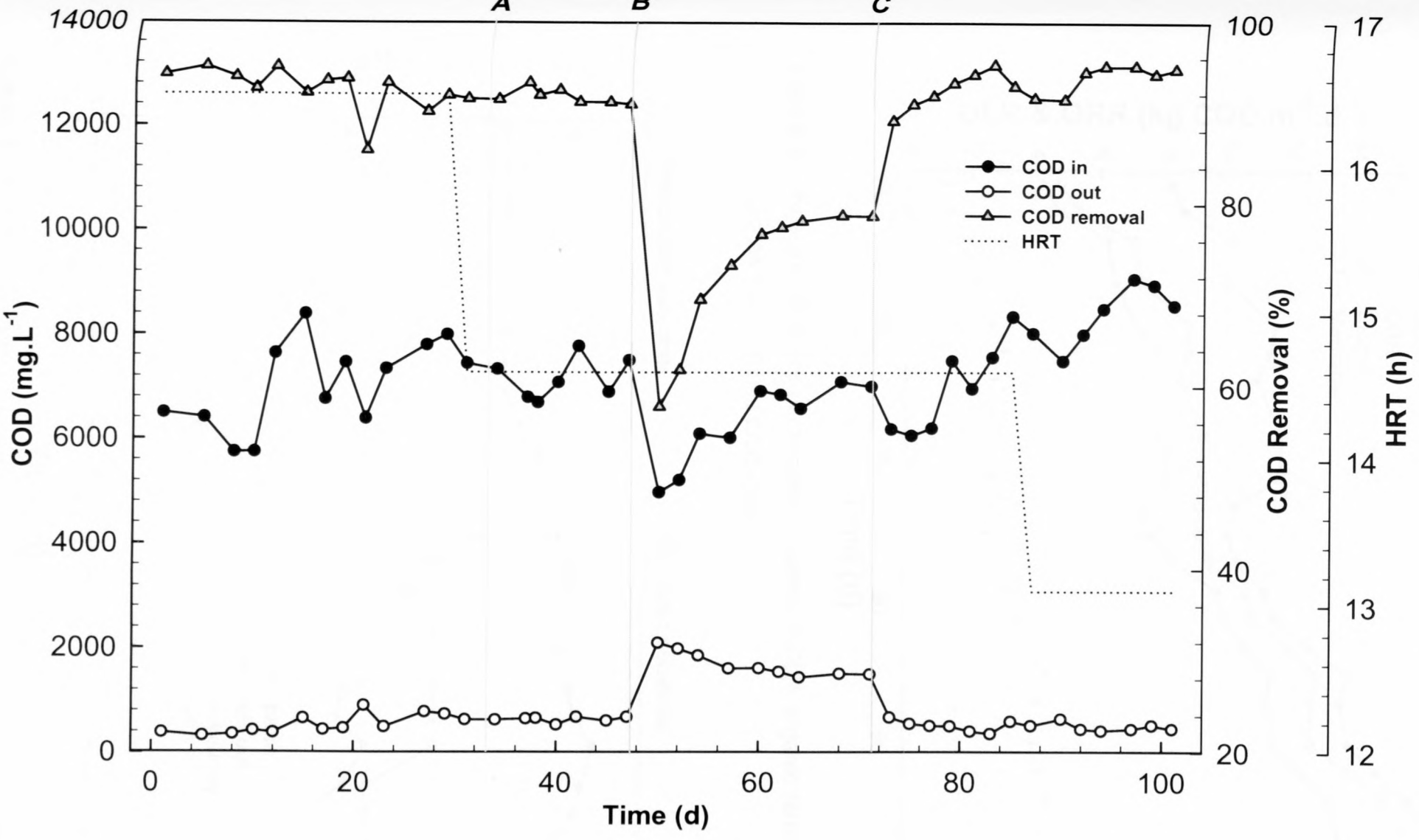

Figure 4. Efficiency parameters of the UASB reactor treating AJPWW and pre-ozonated AJPWW. (A - First Pre-ozonated substrate feed started; B – End of first pre-ozonated substrate feed and removal of 600ml (25%) reactor biomass; C – Second pre-ozonated substrate feed started).

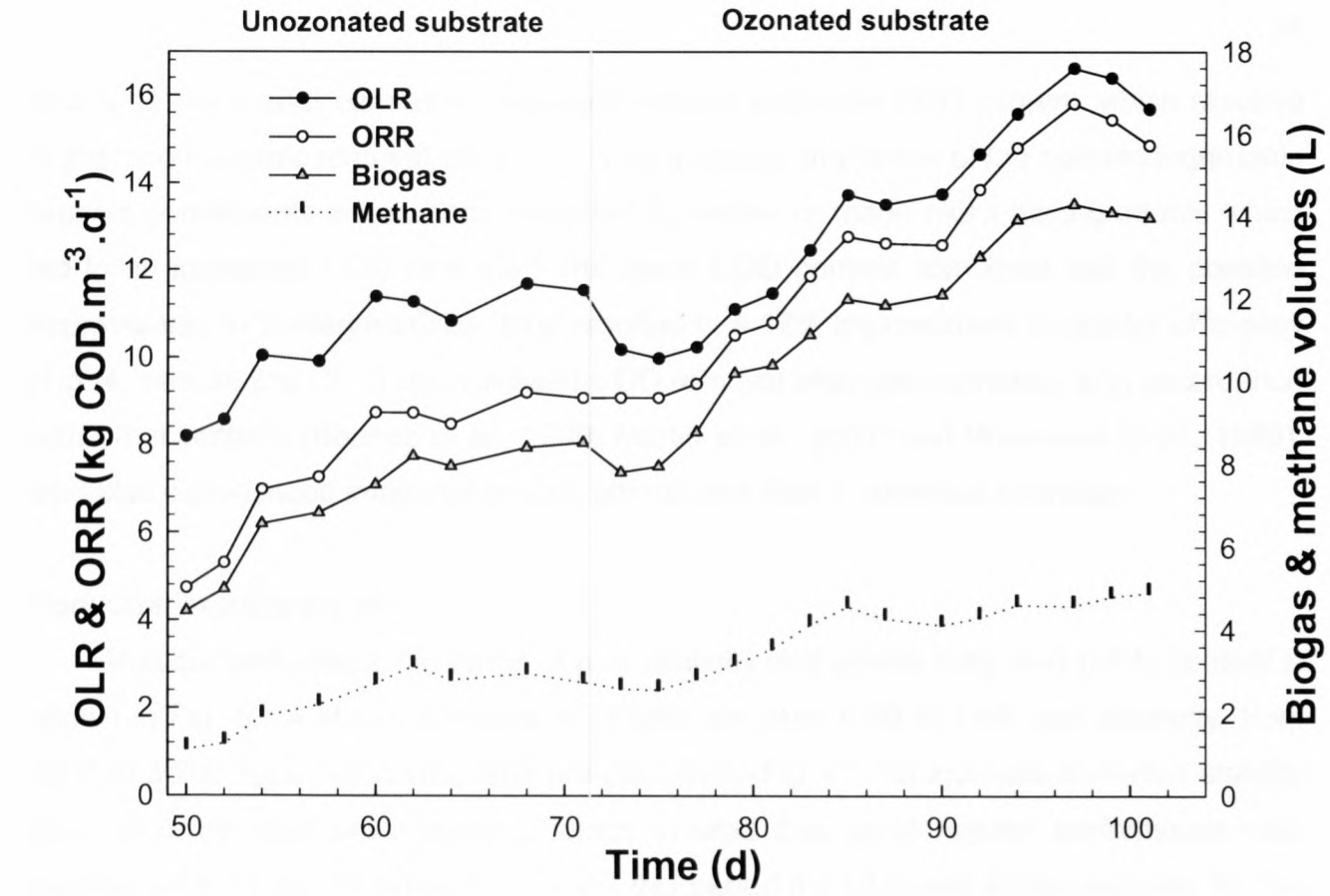

Figure 5. Reactor efficiency parameters in terms of OLR, organic removal rate (ORR), biogas and methane production.

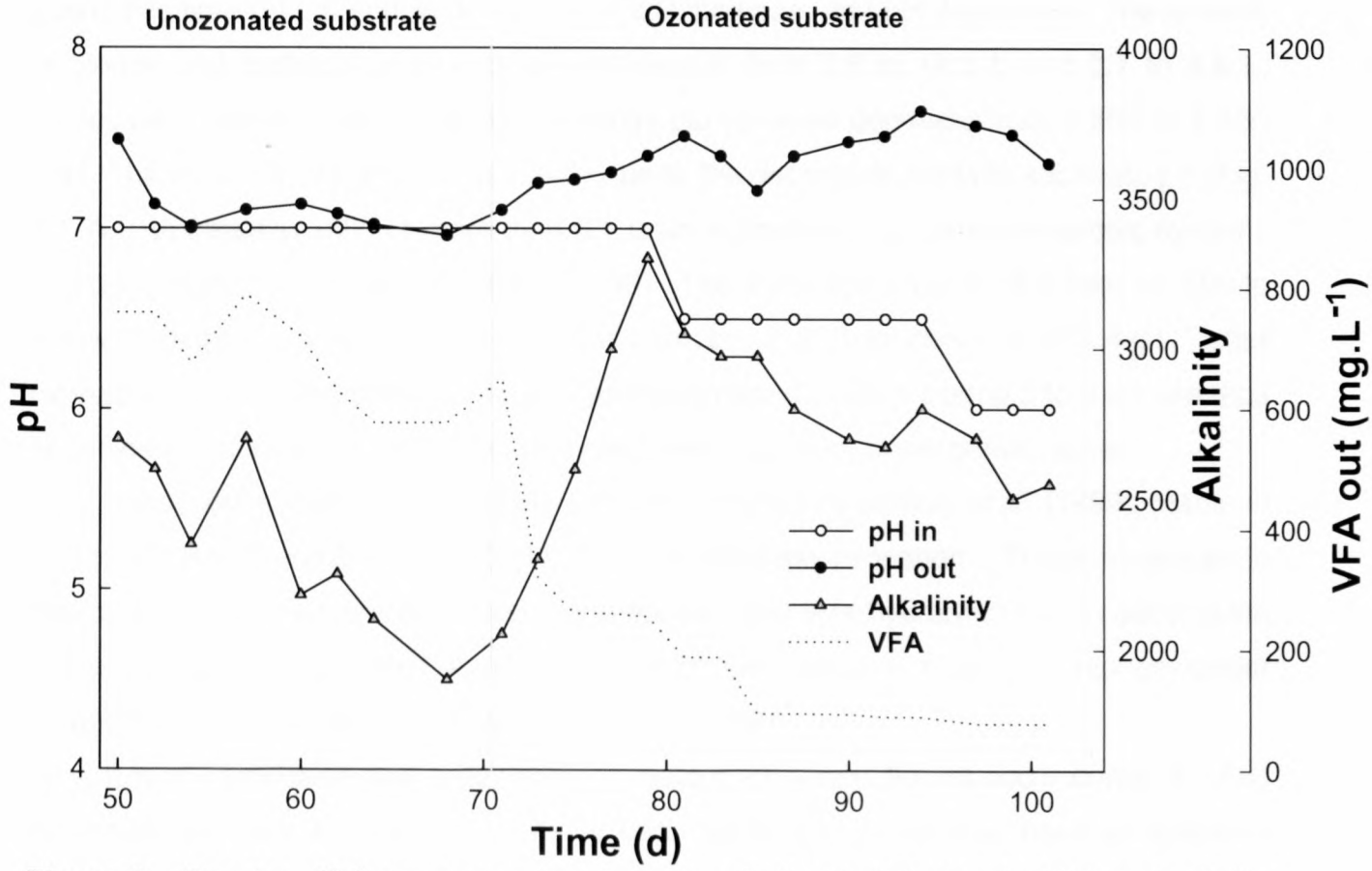

Figure 6. Reactor efficiency parameters in terms of pH, alkalinity (as $CaCO_3$) and volatile fatty acids (VFA) in the reactor effluent.

thus took place after ozonation, despite the lower substrate COD content, which resulted in improved organic removal efficiency. This indicates that some of the non-biodegradable organic constituents were either degraded by ozone or made more biodegradable which led to an increased COD removal. The lower COD content combined with the possible improvement in biodegradability, thus resulted in a 12% improvement in reactor efficiency (Fig. 4, vertical line C). This increased COD removal after pre-ozonation is in accordance with other authors (Benitez *et al.*, 1999; Martin *et al.*, 2001; and Weemaes *et al.*, 1999), who also experienced improved reactor efficiencies after a substrate ozonation.

Reduction in substrate pH

Reactor performance in terms of pH, alkalinity and volatile fatty acid (VFA) content is shown in Fig. 6. A steady increase in reactor pH, from 6.90 to 7.65, and alkalinity, from 2600 to 3 200 $mg.L^{-1}$, occurred after pre-ozonation (Fig. 6). An increase in reactor stability thus occurred after pre-ozonation, which insured that good reactor performance was maintained from day 74 onwards. During this period the OLR was increased from 10.1 to over 16 kg $COD.m^{-3}.d^{-1}$ and the substrate pH was decreased from 7.0 to 6.5 and then to 6.0 on day 79 and 95, respectively (Fig. 5 and 6). High COD removals (over 90%), and alkalinities (over 2 550 $mg.L^{-1}$), combined with low VFA contents was, however, achieved during this time (Fig. 5 and 6) despite this OLR increase, and pH decreases. The amount of biogas and methane produced also increased, from 8.5 to 14.3 L and 2.7 to 4.8 L, respectively, during period (Fig. 5). Alkalinity did however decrease from 3 300 to 2 550 $mg.L^{-1}$ between day 77 and 101 probably due to the decreases made in substrate pH (Fig. 6). A VFA:alkalinity ratio of more than 0.1 is an indication of unstable anaerobic systems and poor methanogenic activity (Bitton, 1999). The sharp decrease in VFA from *ca.* 600 to below 100 $mg.L^{-1}$ and alkalinity increase from *ca.* 2 000 to above 3 000 $mg.L^{-1}$ after ozonation (Fig. 6), and subsequent VFA:alkalinity ratio decrease from 0.3 to 0.03, are thus an indication of improved reactor stability and better methanogenic performance.

Increased methanogenic actively was also reported by Benitez *et al.* (1999), Martin *et al.* (2001) and Weemaes *et al.* (1999) after a substrate ozonation. These increases in reactor performance were also ascribed to the fact that less hydrolysis had to occur since ozonation already degraded a large portion of the complex molecules into an easier biodegradable substrate (Volk *et al.*, 1993; Bitton 1999).

It is of importance that a decrease in reactor efficiency did not occur during the first pre-ozonation (day 35 to 47) since it has been found that ozone may have an inhibitory effect on the microbial populations of anaerobic systems (Andreozzi et al., 1998). Van Lier *et al.* (2001) stated that that the most important objectives for the future of anaerobic

digestion are the improvement of the hydrolysis of complex organic matter since this is the rate limiting step in many anaerobic systems, especially in the treatment of more complex wastewaters. It is also suggested that a mechanical or chemical pre-treatment is the most effective way to achieve this goal. Ozonation done as a pre-treatment clearly achieved this goal since the reactor performance increased drastically after an ozone pre-treatment.

Post-ozonation

The effects of post-ozonation of an UASB reactor effluent, in terms of COD and colour reduction, are shown in Fig. 7. The reactor effluent produced during the pre-ozonated substrate feeding stage (Fig. 4 from day 72 onwards) had an average COD of 469 $mg.L^{-1}$ and a pH of *ca.* 7.5 and was used for the post-ozonation treatments. The effect of a 10 min post-ozonation on the reactor effluent resulted in a 79% colour reduction (Fig. 7). Sigge *et al.* (2002) reported similar results in the post-ozonation of a cannery and winery UASB effluent where a *ca.* 80% colour reduction occurred in both effluents after a 10 min post-ozonation.

Ozonation was also effective in reducing the COD of the reactor effluent. Increases in ozonation time led to increases in COD removals from 31.1% after 1 min of ozonation to 64.8% after a 10 min ozonation. These COD and colour decreases will increase the suitability of the wastewater for irrigation, discharge or reuse purposes. Similar increases in COD reduction with increased ozonation time were also reported by other authors treating food processing effluents (Beltrán *et al.*, 1997 and 1999; Sigge *et al.*, 2001 and 2002). A non-linear COD and colour reduction occurred over time, and was ascribed to the fact that some of the effluent constituents do not react to ozonation (Sigge *et al.*, 2002).

A combined pre-ozonation - UASB treatment - post-ozonation system as used in this study reduced the AJPWW COD by 98.1% (Table 2). The UASB treatment alone resulted in a 78% COD decrease and ozonation alone (10 min) resulted in a 19% COD decrease. The reactor effluent with a COD content of *ca.* 1 700 $mg.L^{-1}$ was produced before the pre- and post-ozonations (Fig. 4, vertical line B to C) compared to an effluent with a COD content of 180 $mg.L^{-1}$ after the application of ozone as a pre- and post-treatment (Table 2). These improved organic removal efficiencies are in accordance with the findings of Benitez *et al.* (1999), Martin *et al.* (2001) and Weemaes *et al.* (1999) who reported that enhanced reactor performance and removal efficiencies are achieved by a combined ozonation, anaerobic digestion system compared to the application of the processes individually.

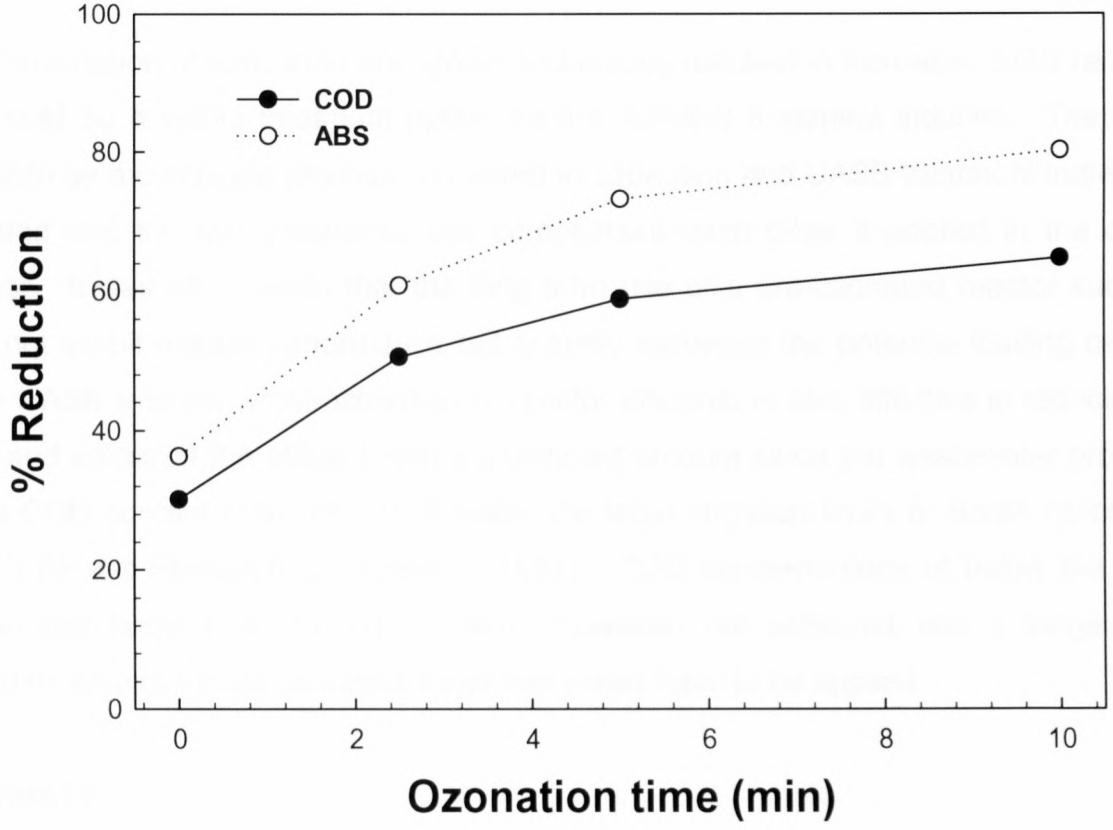

Figure 7. Effects of post-ozonation on the COD and colour content (abs at 450 nm) of an UASB reactor effluent treating an AJPWW.

Table 2. The COD concentrations at the different stages of the treatment.

Treatment stage	COD ($mg.L^{-1}$)	COD removal (%)
Untreated wastewater	9 653	
Pre-ozonated wastewater (2.5 min)	8 321	13.8
UASB reactor effluent	469	95.1
Post-ozonated effluent (10 min)	180	98.1

Conclusions

The combination of ozonation and UASB technology resulted in increased COD removals and could be a viable treatment option for the AJPWW treatment industry. The higher reduction by a combined process compared to ozonation and UASB treatment individually indicated that the two treatments can complement each other if applied in the correct manner. It was also shown that the long term use of a pre-ozonated reactor substrate does not inhibit reactor performance but actually increases the potential loading capacity of the UASB system. Post-ozonation of reactor effluents is also effective in reducing the COD and colour of the effluent with a significant amount since the wastewater produced had a COD content (180 $mg.L^{-1}$) of below the legal irrigation limits of South Africa (400 $mg.L^{-1}$) (Water Research Commission, 1987). COD concentrations of below the South African discharge limit (75 $mg.L^{-1}$) were, however, not achieved and a longer post-ozonation time or further biological treatment would have to be applied.

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CHAPTER 6

GENERAL DISCUSSION AND CONCLUSION

Responsible water use is becoming increasingly important for the South African apple juice processing industry. This industry is growing rapidly and larger volumes of wastewater with a considerable environmental impact are subsequently being produced. Stricter environmental and export regulations have forced the industry to look at alternative treatment technologies since traditional treatment methods like municipal wastewater treatment, wetland systems or direct irrigation of wastewater, have became unacceptable or unpractical.

The use of anaerobic digestion has several advantages as a treatment option and this technology has been shown to be especially effective in the degradation of food processing wastewaters, which may contain high organic loads. Of all the high rate anaerobic reactor designs, the UASB system is the most effective in reducing the chemical oxygen demand (COD) of wastewater and can even operate at low hydraulic retention times (HRT). Anaerobic systems, and especially the UASB design, are also ideally suited for the treatment of fruit processing wastewaters, which are produced on a seasonal basis, as the biomass in the system can survive and stay active during the non-processing season.

During the apple harvesting season the volume and organic load of apple juice processing wastewater (AJPWW) increases significantly. These larger volumes and COD loads subsequently lead to faster increases in the organic loading rate (OLR) of a wastewater treatment system and it is necessary to know if the treatment system can handle such drastic increases. In the first part of this study the ability of the UASB system to successfully degrade higher wastewater volumes and organic loads was shown. An OLR increase from 2.9 to > 14 kg $COD.m^{-3}.d^{-1}$ during a 131 day treatment period did not negatively influence the performance of the UASB reactor and average COD removals of 88.5% were maintained. The pH, alkalinity and volatile fatty acid (VFA) content also remained stable, indicating that the microbial community were not affected by the OLR increase. Based on the data obtained it was concluded that the UASB reactor is a viable option for the successful treatment of fruit processing wastewater.

Wastewater characteristics do, however, play an important role in the effectiveness of the UASB system and complex matter like organic polymers are detrimental to UASB treatment processes and can influence the formation and maintenance of UASB biomass granules. Effective biomass settling ability due to granule formation is essential to prevent

biomass retention problems and their ability to settle depends largely on the viscosity of the wastewater. Any increased viscosity will lead to a decrease in biomass settling ability and cause subsequent loss of biomass through washout. Data from this study showed that AJPWW contains high levels of pectin, which may lead to an increased viscosity. In South Africa calcium is generally used to adjust the pH of AJPWW before biological treatment, and in the presence of pectin viscosity increases cause reactor instabilities. As part of this study on the impact of calcium on pectin in AJPWW it was found that additional calcium levels of 360 $mg.L^{-1}$ in a 750 $mg.L^{-1}$ pectin containing AJPWW led to an increased viscosity from 8.5 to 74 cps (553%) and the formation of a strong gel.

When a lab-scale UASB reactor operated at an OLR of 15.0 kg $COD.m^{-3}.d^{-1}$ and COD removal efficiency of 95% was then fed with this increased viscosity wastewater the COD removal subsequently decreased from 94 to 11% during a 12 day period and to a 95.5% decrease in biogas production. Reactor pH and alkalinity also decreased while the VFA content increased from 89 to 2 643 $mg.L^{-1}$. It was thus concluded that feeding an AJPWW substrate with an increased viscosity leads to rapid reactor failure as a result of biomass washout due to an increased gel formation. With reactor biomass washout, the size of the microbial community responsible for degradation is significantly decreased, resulting in lower degradation rates and reactor efficiency. Fewer microbes were thus available to degrade the substrate to biomass, which in turn led to an increase in VFA formation due to the inability of the now limited methanogenic population to convert all the acetic acid to methane and carbon dioxide.

The degradation process of complex organic polymers like pectin is also time consuming. The production of external enzymes by specially adapted microbes has to occur before sufficient hydrolysis can take place. Previous studies (Madamwar & Mithal, 1985; Tanabe *et al.*, 1988; Shivakumer & Nand, 1995) have shown anaerobic organisms are incapable of sufficient enzyme production and substrate hydrolysis to successfully degrade pectin in high rate anaerobic systems. The hydrolysis of pectin can thus also be seen as the rate-limiting step in anaerobic systems treating a pectin containing wastewater.

From the data obtained it was concluded that the use of a chemical pre-treatment to hydrolyse complex organic pectin containing material is required to make AJPWW more suitable for anaerobic microbial degradation. Various pre-treatments are available, but finding the most efficient and economical method can be problematic. The correct combination and intensity of a combined chemical and biological treatment could, however, improve the efficiency of the total treatment. A chemical oxidation process, like ozonation has been shown to be very effective in the degradation of complex organic

matter, but less effective in the degradation of smaller organic constituents. The influence of ozone on the degradation of pectin was thus evaluated. In this study, a 10 min pre-ozonation treatment of AJPWW that contained 750 $mg.L^{-1}$ pectin was evaluated and a 77% pectin degradation was found. Less COD reduction (12%) occured but the ability of the wastewater to form a gel was largely (76%) eliminated. This increased pectin degradation and decrease in gel formation as found in this study could reduce the problems that occur in UASB reactors treating AJPWW when calcium is used as pH adjuster.

The use of activity tests, where the biogas and methane content was determined, was used to evaluate the effect of ozonation on the anaerobic biodegradation potential of the AJPWW. Apple juice processing wastewater ozonated for progressively longer times was used as substrate and the volumes of biogas and methane produced after each incubation was determined. Higher biogas volumes were produced after a 2.5 and 5 min pre-ozonation compared to the un-ozonated AJPWW samples. Lower methane volumes were observed after a 5 min ozonation, possibly due to inhibitory substances formed after ozonation or depletion of the substrate but this is an aspect that will have to be confirmed by further studies. The above information was then applied by feeding AJPWW that had been ozonated for 2.5 min to a UASB in the place of raw substrate. The COD removal was found to increase from 78 to 90% within 24 h. Based on this efficiency increase the OLR of the reactor was then increased from 10.0 to 16.6 kg $COD.m^{-3}d^{-1}$ in 23 days. Removal efficiencies remained constant at above 90 despite the OLR increase. The overall reactor pH and alkalinity also increased while the VFA content decreased to a low 78 $mg.L^{-1}$. It was concluded that pre-ozonated AJPWW led to better degradation and subsequent better reactor efficiency. The reactor effluent did, however, still contain a COD above the legal irrigation (400 $mg.L^{-1}$) or discharge limit (75 $mg.L^{-1}$) and a further post-treatment or polishing step will thus be required.

As part of a post-treatment step a 10 min post-ozonation of the reactor effluent was implemented and this led to a 64.8% COD and 79% colour reduction with the final UASB effluent having a COD of 180 $mg.L^{-1}$. The data obtained clearly showed that a combined pre-ozonation/UASB/post-ozonation scenario reduced the COD of AJPWW from 9 653 to 180 $mg.l^{-1}$ (98.1%). This final effluent COD content (180 $mg.L^{-1}$) is well within the legal irrigation levels but another form of biological or chemical treatment might be required in order to meet the South African wastewater discharge limit.

It is important to take into consideration that this COD reduction occurred at a reactor OLR of 16.6 kg $COD.m^{-3}.d^{-1}$. This is especially important during the fruit processing season when the AJPWW volumes and COD levels will increase. Sudden increases, even

over a few days, in OLR could have severe impacts on the microbial community of the anaerobic system and this may lead to the over production of acetic acid, due to the inability of the methanogenic bacteria to convert the acid to methane which lead to decreases in pH, and subsequent reactor failure. The ozonation treatment implemented in this study showed an increase in biodegradability of the wastewater, which allowed higher organic removals at higher OLRs, which will prevent reactor overloading problems that occurs during the apple juice processing season.

The ability of the system, described in this study, to degrade wastewater at a higher OLR is of great value, as it may lead to larger organic pollutant removals and thus a more efficient treatment system. Increased reactor performance will directly improve the quality of the final wastewater produced, which in turn will have a significant impact on the treatment ability of the South African apple processing industry currently limited by the production of large wastewater volumes. The use of ozonation to increase the efficiency of an UASB system is thus a viable option for the treatment of AJPWW. Further research is, however, required to establish the success of the system. The installation of a pilot-plant, which will take daily wastewater variations into consideration, is recommended in order to test the viability and practicality of the system on a larger scale. The elimination of pectin and other suspended solids from the wastewater, through improved fruit pressing methods, or a possible screening method should also improve the efficiency of the system. The improvement of the suitability of the AJPWW for UASB treatment is essential. A thorough cost determination of the different pre-treatment alternatives is, however, required in order to find the most economic combination of options.

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