ANAEROBIC DIGESTION APPLICATIONS IN THE TREATMENT OF GELATIN-MANUFACTURING EFFLUENT

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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 submitted the thesis, in its entirety or in part, at any university for a degree.

Magaretha Hester Lloyd Date

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THIS THESIS IS DEDICATED TO MY PARENTS

"..... it is impossible to know the whole
without knowing its parts, as it is to know
the parts without knowing the whole."

B. Pascal, 1995

ABSTRACT

A severely polluted industrial effluent is generated by the local gelatin-manufacturing industry. Due to increasingly stringent restrictions on discharge qualities enforced by the National Water Act of 1998 and National Environmental Management Act of 1998, as well as increasing trade-effluent charges implemented via the Local Municipal Bylaws, the industry is compelled to consider a system to pre-treat the polluted effluent.

A study was undertaken to examine the viability of anaerobic treatment of the gelatin-manufacturing effluent, since the anaerobic digestion technology is well recognised for the high success rate in the treatment of high-strength, complex wastewaters. Various laboratory and pilot-scale studies were done, using different hybrid Upflow Anaerobic Sludge Blanket (UASB) and contact designs.

Two mesophilic laboratory-scale hybrid UASB digester designs, fitted with polyethylene (AD-1) and polyurethane (AD-2), performed well at a hydraulic retention time (HRT) of 1.0 d. Chemical oxygen demand (COD) removal efficiencies of up to 90% (avg. 53%) for AD-1 and 83% (avg. 60%) for AD-2 at organic loading rates (OLR) of 9.56 and 4.62 kg $COD.m^{-3}.d^{-1}$, respectively, were obtained. High sulphate (SO_4) removal efficiencies of up to 96% (avg. 86%) for AD-1 and 98% (avg. 82%) for AD-2 were also achieved, respectively. A maximum total solid (TS) removal of 65% (avg. 25%) for AD-1 and 62% (avg. 28%) for AD-2 was reported. An average methane content of 80% (AD-1) and 79% (AD-2) with average methane yields per COD removed of 2.19 and 1.86 m^3. kg $COD_{removed}.d^{-1}$ for AD-1 and AD-2 were found, respectively.

When the same digesters (AD-1 and AD-2) were combined in a multi-phase series configuration, a total COD removal efficiency of up to 97% (avg. 80%) at an OLR of 8.32 kg $COD.m^{-3}.d^{-1}$, was achieved. Excellent total SO_4 removals of 96% (avg. 69%) were accomplished. Up to 82% TS (avg. 29%) was also removed during this study and the biogas consisted of 89% methane (avg. 79%). For this multi-phase combination up to 92% volatile fatty acids (VFA) (avg. 48%) were removed, indicating possible selective phase separation of the respective fatty acid producing/utilising bacterial populations.

The use of a laboratory-scale UASB bioreactor with recirculation, resulted in COD removal efficiencies of up to 96% (avg. 51%) at an HRT of 3.0 d, and 95% (avg. 54%) at a HRT of 1.0 d. Low performances were generally found, with average SO_4 and TS removals of 59% (max. 97%) and 26% (max. 67%), respectively at an HRT of 1.0 d. The biogas production was very low throughout the study (0.05 - 0.63 $l.d^{-1}$).

A pilot-scale UASB reactor (300 l) was constructed and performed satisfactory with a 58% average COD removal and maximum of 96%. SO_4 and TS removals up to 96% (avg. 44%) and 93% (avg. 63%), respectively, were obtained. The methane content of the biogas was 85%. The pilot-scale studies were conducted under actual field conditions, where various shock and organic loads had to be absorbed by the system.

The pilot-scale contact configuration (300 l) did not perform satisfactory as a result of continuous blockages experienced in the feed and recirculation lines. Maximum COD, SO_4, VFA and TS removal efficiencies of 41% (avg. 27%), 62% (avg. 41%), 64% (avg. 27%) and 39% (avg. 21%), respectively, were obtained.

The results of all the studies indicated acceptable COD removals with increasing OLR's. Indications of the presence of active methanogenic and sulphate-reducing bacterial populations were apparent throughout the studies. One possibility for the successful start-up and commissioning of the anaerobic reactors was the use of a well-adjusted biomass, which consisted of highly selected and adapted microbial consortium for the specific gelatin-manufacturing effluent.

It was clear from this study that gelatin-manufacturing effluent can be treated successfully, especially with the use of the UASB design. A well-defined data base was constructed which could be of great value for further upscaling to a full-scale digester.

UITTREKSEL

'n Hoogs besoedelde industriele uitvloeisel word gegenereer deur die plaaslike gelatien-vervaardigings industrie. As gevolg van toenemende streng beperkings op die kwaliteit van uitvloeisels wat bepaal word deur die Nasionale Water Wet van 1998 en Nasionale Omgewings Bestuurs Wet van 1998, asook toenemende munisipale heffings wat geimplementeer word via Plaaslike Munisipale Wette, word die industrie verplig om die uitvloeisel vooraf te behandel.

'n Studie is onderneem om die lewensvatbaarheid van anaërobe behandeling van gelatien-vervaardigings uitvloeisel te ondersoek, aangesien anaërobe verterings tegnologie alombekend is vir die goeie sukses behaal in die behandeling van hoë-sterkte, komplekse uitvloeisels. Verskeie laboratorium- en loods-skaal studies is gedoen, met verskillende hibried Opvloei Anaërobe Slykkombers (OAS) en kontak ontwerpe.

Goeie werksverrigting was verkry by 'n hidroliese retensie tyd (HRT) van 1.0 d met twee mesofiliese laboratorium-skaal hibried OAS verteerder ontwerpe wat uitgevoer was met poli-etileen (AD-1) en poli-uretaan (AD-2) materiaal. Chemiese suurstof behoefte (CSB) verwyderings van so hoog as 90% (gem. 53%) vir AD-1 en 83% (gem. 60%) vir AD-2 by organiese ladingstempo's (OLT) van 9.56 en 4.62 kg $CSB.m^{-3}.d^{-1}$, was onderskeidelik verkry. Hoë sulfaat (SO_4) verwyderings van tot 96% (gem. 86%) vir AD-1 en 98% (gem. 82%) vir AD-2 was ook onderskeidelik verkry. 'n Maksimum totale vaste stof (TVS) verwydering van 65% (gem. 25%) vir AD-1 en 62% (gem. 28%) vir AD-2 is gerapporteer. 'n Gemiddelde metaan inhoud van 80% (AD-1) en 79% (AD-2) met 'n gemiddelde metaan opbrengs per CSB verwyder van 2.19 en 1.86 $m^3.kg\ CSB_{verwyder}.d^{-1}$ vir AD-1 en AD-2, was onderskeidelik gevind.

Met die aanwending van dieselfde twee verteerders (AD-1 en AD-2) in 'n series gekoppelde multi-fase konfigurasie, is 'n totale CSB verwydering so hoog as 97% (gem. 80%) verkry by 'n OLT van 8.32 kg $CSB.m^{-3}.d^{-1}$. Uitstekende totale SO_4 verwydering van 96% (gem. 69%) is behaal. Tot 82% TVS (gem. 29%) was vewyder gedurende die studie en die biogas het uit 89% metaan (gem. 79%) bestaan. Vir die multi-fase kombinasie is 'n maksimum

van 92% vlugtige vetsure (VVS) (gem. 48%) verwyder, wat dui op die moontlike skeiding van selektiewe fases van die onderskeie vetsuur produserende/verbruiker bakteriële populasies.

CSB verwydering van tot 96% (gem. 51%) by 'n HRT van 3.0 d en 95% (gem. 54%) met 'n HRT van 1.0 d was verkry, tydens die gebruik van 'n laboratorium-skaal OAS bioreaktor met hersirkulasie. Lae werksverrigting was oor die algemeen waargeneem, met gemiddelde SO_4 en TVS verwyderings van 59% (maks. 97%) en 26% (maks. 67%) by 'n HRT van 1.0 d. Die biogas produksie was baie laag gedurende die studie (0.05 - 0.63 $l.d^{-1}$).

'n Loods-skaal OAS verteerder was opgerig en bevredigende resultate was verkry met 'n gemiddeld van 58% CSB verwydering en maksimum van 96%. SO_4 en TVS verwyderings so hoog as 96% (gem. 44%) en 93% (gem. 63%) is onderskeidelik verkry. Die metaan inhoud van die biogas was 85%. Die loods-skaal studie was uitgevoer gedurende ware veld kondisies, waartydens verskeie skok en organiese ladings deur die sisteem geabsorbeer is.

Die loods-skaal kontak konfigurasie (300 l) het nie bevredigende resultate getoon nie, as gevolg van voortdurende blokkasies wat ondervind is in die toevoer en hersirkulasie pype. Maksimum CSB, SO_4, VVS en TVS verwyderings van 41% (gem. 27%), 62% (gem. 41%), 64% (gem. 27%) en 39% (gem. 21%) was onderskeidelik verkry.

Die resultate van al die studies het aanvaarbare CSB verwydering aangedui by toenemende OLT's. Indikasies van aktiewe metanogene en sulfaat-reduserende bakteriële populasies was ook teenwoordig gedurende die studies. Die suksesvolle aansit-prosedure en begin van die anaërobe verteerders kan toegeskryf word aan die gebruik van 'n goed aangepaste biomassa, wat uit hoogs selektiewe en aangepaste mikrobiese populasies vir die spesifieke uitvloeisel bestaan.

Hierdie studie het getoon dat gelatien-vervaardigings uitvloeisel suksesvol met die OAS ontwerp behandel kan word. 'n Goed gedefinieerde data basis kan voorsien word, wat van groot waarde sal wees vir verdere opgradering na 'n volskaalse verteerder.

CONTENTS

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

CHAPTER 1

INTRODUCTION

The conservation of natural water resources enjoys a high priority listing in both developing and first world countries. South Africa is a semi-arid region, which makes it imperative that our water resources should be properly protected, managed and treated, whether to produce water for general consumption, for specific industrial uses, or to limit the discharge of pollution into the environment.

Increasing problems experienced with the disposal of high-strength industrial waste in an environmentally acceptable manner and compliance to local water quality guidelines prompted research and investigations into management strategies and treatment of wastewater (Laubscher *et al.*, 1992; Van Der Merwe & Britz, 1993). Different types of wastewaters are produced daily which must then be accommodated by the wastewater purification works. This may present a problem in itself to local authorities, as the disposal of certain industrial effluents can inhibit the biological treatment processes, such as anaerobic digestion and biological nutrient removal plants, resulting in an inefficient and costly treatment process.

One of the most difficult wastewater types to treat is gelatin-manufacturing effluent. Gelatin has a wide variety of uses, in food processing from wine fining to confectionery, and in the manufacturing industry from matches to photography. The problems associated with the disposal of complex, high-strength wastewaters necessitate industries more and more to consider suitable pre-treatment methods to minimise the detrimental effect of the biological processes used in conventional treatment plants (Maree *et al.*, 1990). There are various treatment options to consider, ranging from physical to chemical to biological methods.

Leiner Davis Gelatin S.A. (Pty.) Limited (LDG) is the only South African manufacturing industry of edible and technical gelatin and is situated in West-Krugersdorp, Gauteng Province. During April 1995, LDG changed their gelatin-manufacturing process by substituting the lime ($Ca(OH)_2$) process step

with a sodium hydroxide (NaOH) process step. This was done for economic and product quality reasons. Following these changes, the local wastewater purification works at Krugersdorp experienced numerous and complex problems with the operational side of the plant. The LDG process produces high effluent volumes, with distinctive peak and low periods in terms of organic, hydraulic and toxic loads. These effluents are typically highly alkaline with a high chemical oxygen demand (COD), suspended solid (SS), sodium (Na), chloride (Cl), ammonia (NH_3-N), electrical conductivity (EC) as well as fats/oils contents (Table 1). High concentrations of sulphate (SO_4^{2-}), peroxide (H_2O_2) and hexavalent chromium (Cr^{6+}) have also been detected. Odours are also a major source of irritation and are associated with reduced sulphur compounds, which are an environmental nuisance rather than a toxic hazard (Lens *et al.*, 1998).

The modification of the gelatin-manufacturing process resulted in an overall decrease in process efficiency and a subsequent increase in operational costs of the local wastewater purification works. It was clear that the 12% (v/v) gelatin-manufacturing effluent fraction of the total inflow volume to the wastewater plant, could be correlated directly to the treatment plant efficiency (Van Der Merwe-Botha, 1998, Personal communication). The gelatin-manufacturing effluent contributes furthermore to 65% (m/m) of the total organic load of the conventional plant. Problems experienced at the local wastewater purification works included a loss in nitrification and denitrification ability, reduced ortho-phosphate (PO_4-P) and COD removal, as well as biosolids carry-over to the secondary clarifiers. The quality of the anaerobic sludge also decreased, resulting in lower digester pH and alkalinity values, decreased biogas production and volatile fatty acid (VFA) removal.

The salinity levels of the gelatin-manufacturing effluent discharged to the local wastewater purification works has a high conductivity (800 - 1 500 $mS.m^{-1}$). This high electrical conductivity suggests the presence of high concentrations of ions leading to high total dissolved solid (TDS) concentrations (Department of Water Affairs and Forestry, 1996). This is unacceptable to the local authorities as it contributes to the mineralisation of the receiving water resources and it also has a definite toxic effect on

Table 1. The composition of typical gelatin-manufacturing effluent before and after modifications to the process (Van Der Merwe-Botha, 1996).

Parameters	LDG effluent (before)	LDG effluent (after)	*Maximum limit allowed
Ammonia ($mg.l^{-1}$ as N)	7.2 - 75.9	3.3 - 920.0	50
Chemical oxygen demand ($mg.l^{-1}$)	1 500 - 9 600	505 - 31 810	5 000
Chlorides ($mg.l^{-1}$)	160 - 1 200	49 - 6 146	600
Chromium ($mg.l^{-1}$)	N/D	0.1 - 34.6	5
Electrical conductivity ($mS.m^{-1}$)	92 - 1 200	74 - 3 870	500
Fats, oils & grease ($mg.l^{-1}$)	N/D	2.0 - 2 134.0	2 000
Iron ($mg.l^{-1}$)	0.0 - 0.7	0.0 - 57.5	5
Lead ($mg.l^{-1}$)	0.0 - 0.1	0.0 - 3.9	5
Manganese ($mg.l^{-1}$)	0.1 - 1.0	0.0 - 5.3	5
pH	7.2 - 12.5	1.8 - 13.4	6 - 10
Sodium ($mg.l^{-1}$)	300 - 500	87 - 19 768	400
Soluble ortho-phosphates ($mg.l^{-1}$ as P)	0.1 - 8.5	0.1 - 23.5	10
Sulphate ($mg.l^{-1}$)	N/D	19 - 2 250	1 800
Sulphide ($mg.l^{-1}$)	N/D	0.10 - 0.30	50
Suspended solids ($mg.l^{-1}$)	300 - 5 900	24 - 12 690	1 000
Total alkalinity ($mg.l^{-1}$ as $CaCO_3$)	100 - 2 800	20 - 16 185	-
Zinc ($mg.l^{-1}$)	0.1 - 0.3	0.0 - 3.9	5

* *Specific bylaw standards according to the requirements of the Water Act (Act 54 of 1956)*

N/D = Not Determined

microbial activity and nutrient removal. Both the high concentrations of Na and Cl cause specific ion toxicity and osmotic stress to the microbial populations of the purification works (Schoeman & Steyn, 1997). It was also found that biomass disintegration of the natural-occurring algae on the secondary clarifier walls occurred, again suggesting severe toxic effects (Schoeman & Steyn, 1997).

It is generally recommended that chromium and sulphide concentration levels in tannery effluents should be less than 40 and 5 $mg.l^{-1}$, respectively, prior to discharge (Schoeman & Steyn, 1997). The presence of chromium and/or heavy metals may possibly inhibit the acidogenic and methanogenic bacteria as well as specific enzymatic processes of the anaerobic digestion process. The high amounts of sulphur compounds in the gelatin-manufacturing effluent also result in an increased growth of filamentous organisms like *Beggiatoa*, *Thiothrix* and Type 021N (Eikelboom, 1975). These organisms can grow on a number of organic compounds, but may also gain energy for growth from the oxidation of reduced sulphur compounds, like hydrogen sulphide (H_2S) (Richard, 1989; Lens *et al.*, 1998). Problems were also experienced with *Micothrix parvicella* and Type 0092 filamentous organisms due to high wastewater grease and fat contents. These filaments result in a high sludge volume index (SVI), poor settling of sludge after the activated sludge treatment process, sludge foaming and carry-over of the biosolids during clarification (Jenkins *et al.*, 1986; Richard, 1989). Furthermore, the presence of these filamentous organisms indicate the conversion of sulphates to H_2S, which then result in the corrosion of equipment and inhibition of specific biological activities (Lens *et al.*, 1998).

It is therefore clear that efficient pre-treatment of this effluent is extremely important to both the manufacturing/producing industry and the receiving local authority. As the direct treatment seems to be a complex and expensive option, the possibility of pre-treating this effluent might provide a suitable option to increase the efficiency of the total process managed by the wastewater purification works and ensure a continual disposal facility to the effluent-producing industry. The idea of such a pre-treatment option has been well received by the industry, which view this option as an attractive

alternative to promote their environmental policy, comply with the stricter pollution control legislation and continue their core business in a suitable manner.

Against this background, the objective of this study was to find a suitable pre-treatment option for the biodegradation of the gelatin-manufacturing effluent, so as to produce an acceptable effluent quality for disposal to the local wastewater purification facilities.

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CHAPTER 2

LITERATURE REVIEW

A. Gelatin-manufacturing process

In South Africa the main source of gelatin is the bovine hide which is not suitable for tanning purposes. The reject hides available to the gelatin industry vary from green masks from local abattoirs through partly processed or dehaired tannery waste (both wet and dry) to hides preserved by drying, salting and treatment with sodium meta-bisulphite for short-term preservation (Maree *et al.*, 1990). Besides the "non-chrome" waste hides (Fig. 1), by-products from the leather tanning industries can also be used to produce gelatin (Cot *et al.*, 1985). These wet or dry by-products embody chrome-tanned leather splits, trimmings, buffing dust and shavings, containing about 90% water-soluble protein and 5 - 6% Cr_2O_3 (chromium (III) oxide) (Cot *et al.*, 1985).

The conversion of the insoluble hide collagen into water-soluble gelatin involves several protein hydrolysis and denaturation processes. Additionally to the "non-chrome" waste hides, the gelatin-manufacturing industry also uses a small amount of chrome-tanned leather shavings for extraction of a good edible food grade gelatin. The use of chrome-tanned leather is undesirable because chromium strongly stabilises the leather structure and remains unaffected by ordinary gelatin manufacturing processing conditions (Cot *et al.*, 1985). The shavings are mixed with magnesium oxide (MgO) after which the shavings undergo the same extraction and primary filtration process as with the "non-chrome" wastes. Carbon treatment is thereafter used to remove the residual amounts of inorganic compounds such as nitrogen, sulphides and heavy metals, especially chromium.

The "non-chrome" waste hides are cut into smaller pieces, after undergoing a mechanical desalting process. To remove all the hair and epidermis, the hides are placed in processors with sodium sulphide (25 - 30 kg 60% Na_2S) and sodium hydroxide (NaOH) at a pH of 12 for several hours.

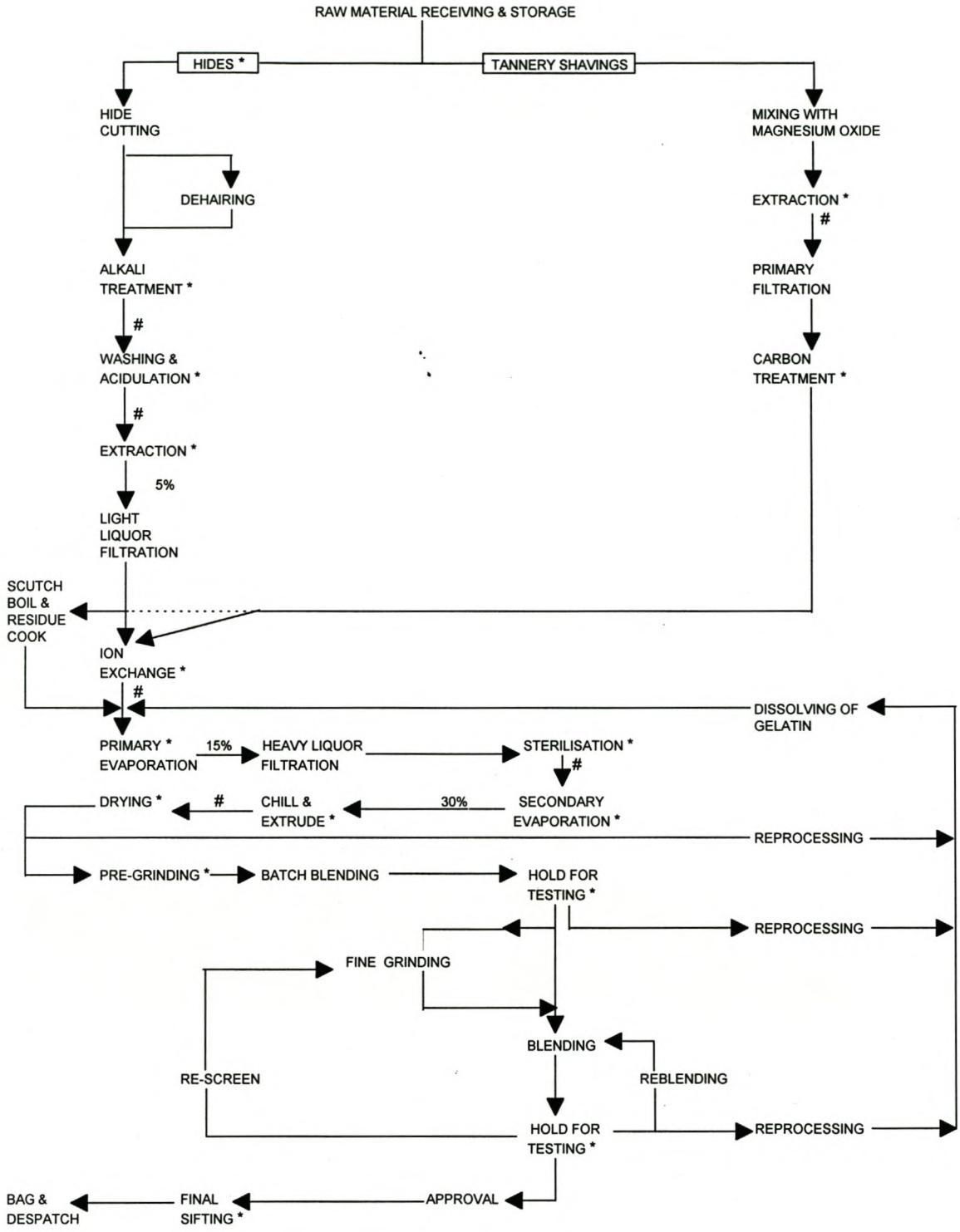

Figure 1. A flow diagram of wastewater generation during the operational conversion of collagen to gelatin (Cole, 1997, Personal communication).

The hides are then chemically conditioned in alkali pits at a pH of 11 - 12 for 9 - 18 days, depending on their condition. The alkali pits contain 1.1 - 1.8% sodium hydroxide (44 kg $NaOH.t^{-1}$), of which 0.6% NaOH can be recovered. During the alkali conditioning the swelling of the hides take place by denaturation of the collagen peptide chains, thus opening the fibre structure. The effluent discharge after the alkali conditioning has high pH, chemical oxygen demand (COD), electrical conductivity (EC) and sodium (Na) values. The hides are then transferred to washers with reclaimed water to remove excess alkali, followed by bleaching with hydrogen peroxide (H_2O_2) for 8 h. Bleaching eliminates the surplus sulphide (S^{2-}) of the dehairing process . The H_2O_2 is reduced with sulphur dioxide (SO_2-gas), since the H_2O_2 may cause corrosion to the metal instruments of the process.

The next step is acidulation with 300 - 350 l sulphuric acid per washer (22 kg $H_2SO_4.t^{-1}$) for 6 - 8 h, resulting in a reduction of pH and inorganic contamination. The hides are once again washed in reclaimed water to change the pH from 1.5 - 3.0. After the acid wash the effluent has a low pH and high EC and total dissolved solids (TDS) values. The water from the alkali and acid-pits are disposed together in a sedimentation tank to neutralise and prevent metal dissociation in the gelatin-manufacturing effluent. This effluent is then discharged into the sewer system and has to comply with the effluent discharge standards as specified by the local authorities.

The final denaturation of the collagen proteins (pH 3.0) is achieved by gently heating (44° - 48°C) the product in fresh water to produce a diluted solution of gelatin. By increasing the temperature (70° - 80°C), more gelatin can be extracted with a recovery rate of 4% gelatin. The undissolved compounds such as fats, fine collagen and suspended solids, are eliminated by primary (light liquor) filtration. The sodium and sulphate ions are removed by adding hydrochloric acid (HCl) and NaOH, respectively. Hydrochloric acid is used as a cation in the ion exchange process and NaOH is used as an anion to remove the contaminating ions. After the extraction and primary filtration process 5% of the gelatin can be recovered. The water used in the ion exchange process is also disposed in the sedimentation tank. This effluent usually has a high TDS concentration, a high pH and low COD

values. The gelatin and water (liquid) mixture is circulated in an evaporator to remove most of the water from the gelatin. Low pressure and high temperature (± 50°C) is maintained in the evaporator to recover a higher concentration of gelatin (15%).

The secondary (heavy liquor) filtration is a more specialised process where cellulose filters and a higher temperature are used to remove unwanted compounds like precipitated albumin. The gelatin is kept at 40° - 45°C at all times to prevent coagulation. The pH of the gelatin is adjusted to 5 or 6 with either ammonia or NaOH to prevent the protein concentrations present in the gelatin to become unstable. Ultra-high temperature (UHT) sterilisation takes place at 140°C for 8 sec to prevent any bacterial growth.

After the secondary evaporation, the gelatin is passed through a barrel (scrape surface heat exchanger votator), with the outer pipe filled with calcium chloride (CaCl) to cool and coagulate the gelatin in the inner pipe (-10°C). The gelatin is then passed through a noodle plater. The CaCl is retained and recirculated continuously. In the final process the coagulated gelatin is passed through a drying belt which is divided into eight compartments to ensure proper drying of the gelatin. As the temperature increases in each compartment from 28° - 60°C, air is constantly blown into the drying belt. At the end, 70% moisture is withdrawn and 30% gelatin powder can be recovered. The dried gelatin then undergo a pre-grinding process and batch blending. The gelatin must be tested for suitability before the final product can be despatched.

The gelatin-manufacturing industry uses approximately 58 000 kl water per month for a 6 day operational week. Up to ± 15% of the water is lost by evaporation. The total volume of water used is between 1 500 - 2 500 $kl.d^{-1}$. The effluent discharge after certain processes, is shown in Fig. 1. The quantity of effluent produced per day after the different processes are: alkali conditioning (70 $kl.d^{-1}$ / 4.5%); ex-alkali washing (300 $kl.d^{-1}$ / 19.2%); acid wash (540 $kl.d^{-1}$ / 34.6%); fluming of washers (160 $kl.d^{-1}$ / 10.3%); ion exchange (70 $kl.d^{-1}$ / 4.5%); secondary filtration (40 $kl.d^{-1}$ / 2.6%); cooling (80 $kl.d^{-1}$ / 5.1%); cleaning of washers (250 $kl.d^{-1}$ / 16.0%) and for washing of chrome shavings (50 $kl.d^{-1}$ / 3.2%).

B. Treatment options

Industrial wastewater treatment requirements are becoming more stringent in terms of limiting concentrations of organic and inorganic substances and suspended solids (Metcalf & Eddy, 1991). Difficulties with the treatment of gelatin-manufacturing effluent is related to the high organic and suspended solid loads, as well as high sodium and chloride concentrations. This results in a complex and highly variable mixture of soluble organic and inorganic compounds, bacteriological constituents and suspended solids in an aqueous medium and, thus, a difficult degradable effluent (Maree *et al.*, 1990). The implementation of effective pre-treatment methods (physical, chemical or biological) in the South African market will lead to better point-source pollution control, water savings, resource recovery and effluent volume reductions. By using these methods to reduce the suspended solid and organic loads, a facilitation of a reduced gelatin-manufacturing effluent strength may become possible (Metcalf & Eddy, 1991). The gelatin-manufacturing industry continuously experiments with different processes to extract the maximum concentration of gelatin from the hides, hence the effluent quality and quantity varies continuously. Subsequently, the local wastewater purification works also have to change processes since the constituents of the gelatin-manufacturing effluent varies periodically. The disposal of gelatin-manufacturing effluent will remain a significant concern, if pre-treatment is not considered. The necessity for pre-treatment and/or other disposal alternatives for the gelatin-manufacturing effluent is therefore well-motivated.

A variety of alternatives for the partial pre-treatment or total treatment of gelatin-manufacturing effluent can be adopted in order to reach environmental quality requirements (Table 1) (Lema *et al.*, 1988; Senior, 1995).

Table 1. Pre-treatment options (Lema *et al.*, 1988; Senior, 1995).

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1. Chemical treatment

- Chemical precipitation
- Chemical oxidation
- Activated carbon adsorption
- Ion exchange

2. Physical treatment

- Sedimentation
- Ultrafiltration
- Reverse osmosis (hyperfiltration)
- Ammonia stripping
- Electrodialysis
- Diffusion dialysis
- Wet-air oxidation

3. Biological processes

- Aerobic treatment
- Anaerobic treatment

4. Combined chemical, physical and/or biological pre-treatment

- Anaerobic digestion combined with ultrafiltration (ADUF)

B.1. Chemical treatment

Chemical precipitation

This treatment involves the addition of chemicals to alter the physical state of the dissolved and suspended solids and to facilitate their removal by sedimentation, as well as the removal of heavy metals (Metcalf & Eddy, 1991). Lime ($Ca(OH)_2$) and alum ($Al_2(SO_4)$) are most commonly used, although the addition of flocculants such as ferric chloride ($FeCl_3$), sodium sulphide (Na_2S) and ferrous sulphate ($FeSO_4$) have also been used successfully (Chian & De Walle, 1976; Saint-Forte,1992). The addition of flocculants will cause sludge and solids to settle faster. The removal of solids will thus reduce the organic loading on a biological system. Due to the reagents added a large quantity of sludge will be generated, which can be potentially hazardous. Good improvements in colour, suspended solids, NH_4^+ and heavy cation elimination are obtained, although the maximum reduction of COD was only 30 - 60% (Harrington & Maris, 1986; Saint-Forte, 1992; Gaydon & De Haas, 1998). High heavy metal concentrations can inhibit biological activity (Senior, 1995). The addition of lime, for instance, results in an increase in pH and this leads to coagulation and formation of insoluble metal hydroxides and calcium carbonate. The resulting flocs then aid in the settling of colloidal material (Kang *et al.*, 1990; Lugowski *et al.*, 1990; Duncan *et al.*, 1995). This treatment option is often the most expensive due to high operating costs and large amounts of sludge generated that have to be either disposed of or further treated (Senior, 1995).

Chemical oxidation

Chemical oxidation can be used to either render several contaminants insoluble, to gasify them or to stabilise them as relatively harmless substances (Metcalf & Eddy, 1991; Saint-Forte, 1992). The effects of several chemicals such as chlorine (Cl_2), ozone (O_3), calcium hypochlorite ($Ca(ClO)_2$), potassium permanganate ($KMnO_4$), hydrogen peroxide (H_2O_2), halogenated oxidants, etc. have been evaluated (Kang *et al.*, 1990; Duncan *et al.*, 1995). The reduction in COD by Cl_2, O_3, $Ca(ClO)_2$ and $KMnO_4$, even at high doses,

was insufficient (< 48%) to give an acceptable treatment efficiency (Lema *et al.*, 1988).

Low COD removals were achieved with Cl_2 (between 20 - 30%) (Loizidou *et al.*, 1993). Chlorine (Cl_2) provides sufficient disinfection of the effluent, but can also combine with organic compounds to form materials which may present health hazards to humans and other life forms (Van Der Walt, 1997). Excellent iron and colour removals were observed with $Ca(ClO)_2$, but it also increased the hardness concentrations of the final effluent.

With H_2O_2, as another option, a 35% COD removal was achieved with the treatment of leachate (Loizidou *et al.*, 1993). However, Van Der Merwe (1994) achieved a COD removal of 57 - 70% with varying H_2O_2 concentrations as post-treatment of baker's yeast effluent. A small percentage of ammonia can also be oxidised with H_2O_2 (Loizidou *et al.*, 1993). It can also be considered as a good option for taste and odour control and treatment (Harrington & Maris, 1986). Chlorine (Cl_2) and H_2O_2 were both however, not effective in the total removal of taste and odour (Van Der Walt, 1997).

Nickel (Ni) concentrations can be reduced (25 - 75%) by chemical oxidation with $KMnO_4$ and H_2O_2. Problems with foaming may occur and this is an unacceptable complication for a full-scale plant (Senior, 1995). In addition to foaming, $KMnO_4$ treatment also generates 10 - 50% (w/v) more sludge (Kang *et al.*, 1990), causing a further disposal problem.

The use of halogenated oxidants as a treatment option leads to the formation of highly dangerous organic halides, which will upset the microbial activity in the system (Senior, 1995).

Good colour removal was found with ozone (O_3) and Cl_2 (Van Der Walt, 1997; Ramlall & Nozaic, 1998). Ozone (O_3) also kills bacteria more rapidly than Cl_2 (Bessarabov & Grimm, 1998). Ozone (O_3) is only applicable to waste streams containing less than 1% oxidisable materials (Enzminger *et al.*, 1987). Oxidation of iron (Fe), manganese (Mn) and organic compounds can also be achieved with O_3 (Bessarabov & Grimm, 1998; Ramlall & Nozaic, 1998). Hydrogen peroxide (H_2O_2) controls bulking during the initial stage of

treatment and O_3 improves sludge settleability consistently and also stabilises nutrient removal (Saayman *et al.*, 1997). A combination, known as peroxone (O_3 and H_2O_2), has a better oxidising capacity than the two separately, and even more important, no harmful by-products are produced (Enzminger *et al.*, 1987; Saayman *et al.*, 1997). The oxidation of the taste and odour compounds in effluents, were enhanced by the addition of peroxone. Chemical oxidation is expensive and involves high treatment costs but depends on the type of unit processes already installed in the treatment plant (Senior, 1995).

Activated carbon adsorption

Carbon adsorption is the most extensively used physical-chemical means of removal of refractory organic compounds, as well as residual amounts of inorganic compounds such as nitrogen, sulphides and heavy metals (Enzminger *et al.*, 1987; Metcalf & Eddy, 1991). This method entails the collection of soluble substances that are in solution, on a suitable carbon interface (Metcalf & Eddy, 1991). The best results reported were obtained when combined with biological methods (Chain & De Walle, 1976; Ying *et al.*, 1987). Better reduction of organic levels (>85%) are achieved by the adsorption of pollutants on activated carbon (Pohland & Kang, 1975). The main disadvantage is the need for frequent regeneration of the carbon columns or an equivalently high consumption of carbon powder (Lema *et al.*, 1988; Senior, 1995). Handling and energy costs are high but this treatment option can be cost effective for the removal of residual organics when the total dissolved solids in solution are lower than 200 $mg.l^{-1}$ (Senior, 1995).

Ion exchange

This is a process where the ions of an insoluble exchange material (resin) are displaced by the ammonia (NH_4^+) ions in the effluent. It comprises of three processes: sorption of heavy metals from the catching bath solution, regeneration of the resin and electro-precipitation of heavy metals in the electrolyser (Grebenyuk *et al.*, 1998). The process may be operated in a batch or continuous mode. The ions which are displaced by NH_4^+ displaces,

vary with the nature of the solution used to regenerate the bed or packed column. This process produces a relatively low TDS effluent and 80 - 90% of the NH_3/NH_4^+ is removed to produce a reclaimable product (aqueous ammonia). High concentrations of other cations will reduce the removal of ammonia (Metcalf & Eddy, 1991).

It was also found that ion exchange is an effective method for the removal of nickel, chromium and copper (Duncan *et al.*, 1995; Schoeman & Steyn, 1995; Grebenyuk *et al.*, 1998). Filtration as pre-treatment option is, however, required to prevent the accumulation of suspended solids. Other problems found are the disposal of the regeneration products and the high operational costs involved (Metcalf & Eddy, 1991).

B.2. Physical treatment

Sedimentation

Readily settleable solids with a higher gravity than the liquid tend to settle when the effluent, which is often high in suspended solids, is placed in a sedimentation tank (Van Der Walt, 1998). This is one of the most commonly used methods in wastewater treatment. Solids with a lower gravity (fats, oils and other floating material) will tend to rise. The aim of sedimentation is thus to remove floating materials and settleable solids. The solids are generally withdrawn from the bottom (Atlas & Bartha, 1993). These solids can then be treated via anaerobic digestion and/or by composting prior to final disposal in landfills or as a soil conditioner (Atlas & Bartha, 1993), thus decreasing the SS and COD concentrations (Metcalf & Eddy, 1991). With the use of a sedimentation tank, the load on biological treatment units can be reduced and the removal of suspended solids is found to be between 50 - 70% (Metcalf & Eddy, 1991). However, initial capital investment and space requirements are high.

Ultrafiltration

Different cross-flow pressure-driven membrane separation processes fulfil different functions. These filtration membranes are mainly fabricated

from poly-ether sulphone or cellulose acetate (Sanderson & Hurndall, 1995; Nell & Kafaar, 1996; Jacobs & Barnard, 1997). Microfiltration membranes remove suspended solids and reduce bacteriological activity, but not the colour and the dissolved organic content of the water. Ultrafiltration, in which a finer membrane is used, does not affect most soluble cations and anions such as alkalinity and hardness concentrations, but is capable of removing medium molecular-mass dissolved organic material and reducing turbidity to levels of 0.1 nephelometric turbidity units (NTU). Ultrafiltration is thus unable to desalinate water (Jacobs *et al.*, 1998). Ultrafiltration can also be used to remove up to 90% of the COD load in effluents (Swart *et al.*, 1996). Nanofiltration membranes, which are even finer, can partly desalinate water (soften it) and can remove substantial quantities of low molecular-mass organic material, as well as viruses (Buckley *et al.*, 1992; Jacobs & Barnard, 1997). A typical nanofiltration membrane has a sodium chloride retention of 50% and a magnesium sulphate retention of 98% (Sanderson & Hurndall, 1995). The membrane technology also includes the removal of fats and oils, chromium, iron and sulphate from tannery effluents, as well as the removal of turbidity from colour colloids (Schoeman & Steyn, 1997). The pH of the effluent must be more or less neutral, as it can cause hydrolysis of the membrane over a period of time (Jacobs & Barnard, 1997). The membranes have a finite life and also show a limited tolerance to the presence of chlorine (Sanderson & Hurndall, 1995). Another major problem with this method is membrane fouling, but this can be cleaned with the correct chemical and enzyme combinations, depending on the type of effluent to be treated (Swart *et al.*, 1996; Maartens *et al.*, 1998). Pressure-driven membrane processes are an ideal treatment option to use together with reverse osmosis to produce a higher quality effluent, but it requires very high running, capital and cleaning costs (Swart *et al.*, 1996; Jacobs & Barnard, 1997). The selection of a suitable filtration technique may pose specific problems due to the variability of industrial effluents (Buckley *et al.*, 1992).

Reverse osmosis (hyperfiltration)

Another pre-treatment possibility includes reverse osmosis, which involves the separation of dissolved solids in a solution. This is a process whereby water is forced, by pressure, through a semi-permeable membrane at a pressure greater than the osmotic pressure caused by dissolved salts in the wastewater (Krug & McDougall, 1988; Metcalf & Eddy, 1991; Sanderson & Hurndall, 1995). The advantage of this process is almost complete desalting or the removal of dissolved organic material, which can not be removed by other demineralisation techniques (Buckley *et al.*, 1992; Juby *et al.*, 1998). The prediction of the precipitation potential of effluents is difficult because of the chemical complexity and variability. However, one major disadvantage of ultrafiltration and reverse osmosis technologies is the precipitation of trace substances and metals (Buckley *et al.*, 1992). The reverse osmosis membrane treatment is an effective physical method for reducing the COD level (80 - 98%) (Chian & De Walle, 1976), the chromium levels from 80% up to 94%, the cadmium levels up to 99%, the zinc concentrations up to 94%, as well as for the recovery of 93 - 99% nickel (Schoeman & Steyn, 1995). However, the zinc recovery in a reverse osmosis plant is not as economical as for nickel recovery (Schoeman & Steyn, 1995).

With reverse osmosis it was shown that it is possible to reduce the electrical conductivity in a heavy metal-rich effluent (Schoeman & Steyn, 1995). Reverse osmosis is usually very effective if it is combined with another pre-treatment method, like ultrafiltration (Schoeman & Steyn, 1997). Due to scale formation, the pH must be adjusted to a range of 4.0 - 7.5 and removal of iron and manganese participates are recommended (Metcalf & Eddy, 1991). It is possible to control membrane fouling with regular chemical cleaning (Schoeman & Steyn, 1995). Prolongation of membrane life necessitates pre-treatment of the effluent to eliminate suspended solids and colloidal material (Juby & Schutte, 2000). The type of membrane, pH, pressure and pre-treatment are important factors in determining the effectiveness of this process (Kettern, 1992). However, the cost of purchasing, installing and cleaning the membranes, can be relatively high.

Ammonia stripping

Ammonia stripping is a possible option to remove excess concentrations of ammonia (Henry, 1985; Prasad *et al.*, 1985; Lema *et al.*, 1988; Smith & Arab, 1988; Kang *et al.*, 1990). The high concentrations of ammonia-nitrogen can be removed from wastewater by increasing the pH, by the addition of lime, to above 9 (Henry, 1985) or 11 (Lema *et al.*, 1988), followed by the formation of NH_3-gas. By bubbling air through the system, ammonia removal for atmospheric discharge can also be accomplished (Smith & Arab, 1988.) Thus, when water contains a volatile gas such as NH_3 in excess of its equilibrium level, the NH_3 will move from the water into the air until equilibrium is reached. Ammonia stripping allows the concentration of NH_3-N to be reduced by an overall removal of 60 - 95%, if it is used in combination with chemical precipitation and biological activated sludge treatment (Keenan *et al.*, 1984; Henry, 1985; Lema *et al.*, 1988). Due to the high operating and maintenance costs, the application of ammonia air stripping is limited (Metcalf & Eddy, 1991). The process is very sensitive to temperature changes, wind speed, aeration rate, lagoon configuration, pH control, surface area and the ammonia solubility increases with a decrease in temperature (Smith & Arab, 1988; Saint-Forte, 1992). The disadvantage of the pH increment, is that carbon dioxide (CO_2) is absorbed from the air and the development of carbonate scaling will occur within towers, lagoons and feed lines (Senior, 1995). Ammonia air stripping can be considered as a cost effective and unsophisticated system, but stripping towers can be expensive to build and operational problems include the formation of an adherent scale in the tower (Smith & Arab, 1988). Lagoons can rather be seen as a lower cost alternative. The high pH is also advantageous in removing heavy metals by precipitation and then air stripping the ammonia from the wastewater, thus minimising ammonia and metal inhibitory effects (Henry, 1985). Air pollution is also a possibility, as a result of the reaction of ammonia with sulphur dioxide (SO_2) (Metcalf & Eddy, 1991).

Electrodialysis

Electrically driven membrane separation processes are very promising technologies in the reclamation of water and chemicals from industrial effluents (Schoeman & Steyn, 1996). These include different methods like, electrodialysis (ED), electrodialysis reversal (EDR), electro-electrodialysis (EED) and bipolar electrodialysis (BED) (Schoeman & Steyn, 1996). Electrodialysis can be effectively applied for the removal of dissolved organic substances, desalination of brackish waters for potable use and for metal recovery in the electroplating industry (Schoeman & Steyn, 1996). The conventional ED also has the potential to be used for cost-effective treatment of chromium (81% removal), cadmium, copper (92% removal), nickel (97% removal), silver (95% removal) and zinc electroplating rinse waters for waste and chemical recovery (Schoeman & Steyn, 1995; Schoeman & Steyn, 1996). The EED process is also effective for the removal of chromium. The BED process is effective for the recovery of acid and caustic soda (NaOH) (Schoeman & Steyn, 1996). The ionic components of a solution are separated through the semi-permeable ion-selective membranes (Metcalf & Eddy, 1991).

Application of an electrical potential between the two electrodes causes an electric current to pass through the solution and a migration of anions to the positive electrode takes place. As a result of the alternate spacing between the cation and anion-permeable membrane, precipitation of salts with low solubility can occur on the membrane surface (Metcalf & Eddy, 1991). Depending on which variant of ED is chosen, the capital costs and electrical energy usage are relatively high (Schoeman & Steyn, 1996). Clogging of the membrane by residual colloidal organic matter and metals is also one of the problems associated with the ED process (Metcalf & Eddy, 1991). To reduce membrane fouling, activated carbon pre-treatment preceded by chemical precipitation, may be necessary. Regular chemical cleaning should also be practised to clean fouled membranes (Schoeman & Steyn, 1995). However, the above methods are expensive to implement and to maintain.

Diffusion dialysis

The gelatin-manufacturing factory uses high volumes of acid (H_2SO_4, HCl) and alkali (NaOH) in the process (Cole, 1997, Personal communication). Diffusion dialysis could effectively be used to recover acid from spent acid (Schoeman & Steyn, 1995) produced during the gelatin-manufacturing process. It was indicated by Schoeman & Steyn (1995), that 74 - 76% HCl could be recovered using this method. Sulphuric acid (H_2SO_4) recovery was approximately 75%. Up to 95% iron, nickel and copper could also be removed from the acid. However, zinc could not be removed effectively by this method as when compared to other available methods (Schoeman & Steyn, 1995). For better recovery of acids and caustic soda, the diffusion dialysis can be combined with a BED process (Schoeman & Steyn, 1996). The capital costs are also high for implementing this treatment option.

Wet-air oxidation

This technology is also suitable for the pre-treatment of high organic and salinity wastewater (Neytzell-de Wilde, 1985). Aqueous wastes can be oxidised into the liquid phase, by using a combination of elevated temperatures and pressures (Neytzell-de Wilde, 1985). Wastewaters with high organic or salinity content are quantitatively mixed with compressed air (Metcalf & Eddy, 1991) and pumped through a heat exchanger where the temperature is increased from 150° to 200°C (Neytzell-de Wilde, 1985). About 5 - 10% of the COD may be oxidised at 150°C and at 320°C complete oxidation would occur in a pressurised reactor (Neytzell-de Wilde, 1985). After oxidation is initiated, the discontinuation of the steam heating occurs, and the oxidation products leave the reactor at temperatures between 220° and 330°C (Neytzell-de Wilde, 1985). These products are re-used to heat the incoming organic liquor and air. Spent air, carbon dioxide and steam are removed from the oxidised effluent in a separator. In wet-air oxidation processes, the organic wastes are used as "free fuel" to heat the incoming wastewater, but the air which must be supplied to the reactor under pressure, can be very costly (Neytzell-de Wilde, 1985).

B.3. Biological processes

One of the most promising treatment options for the removal of organic substances (colloidal or dissolved) in wastewater is biological degradation. The objective of biological treatment is mainly to coagulate and remove the non-settleable colloidal solids and to stabilise the organic matter (Metcalf & Eddy, 1991). The substances can be converted into gas that can escape to the atmosphere and be utilised as carbon in biological cell material that can be removed by settling (Metcalf & Eddy, 1991). Other aims of biological treatment methods are the nitrification, denitrification, phosphorus removal, sulphate reduction and waste stabilisation, as well as the reduction in the volume of biosolids.

Biological processes for treating wastewaters include aerobic, anoxic, anaerobic, combined aerobic, anoxic and anaerobic methods and pond (maturation, oxidation, stabilisation) processes. The biological treatment of industrial wastewater holds several advantages, including: reduced pollution load to the receiving waters; higher stability of the regional treatment plant; lower impact of unexpected changes in industrial processes on discharging to regional treatment plants; less probability of odour nuisance from the sewer system in the drainage area; the microbial transformations of complex organic material and possible adsorption of heavy metals by suitable microbes; reduced overall costs; and better opportunities for the re-use of sludge (Enzminger *et al.*, 1987; Metcalf & Eddy, 1991; Nowak *et al.*, 1996).

Aerobic treatment

Aerobic treatment is one of several alternative biological methods for the treatment of domestic and industrial effluents (Atlas & Bartha, 1993; Lens *et al.*, 1998). This method depends on micro-organisms growing in an oxygen-rich environment, and their ability to oxidise soluble and colloidal organic material to carbon dioxide (CO_2), water (H_2O), sludge (50 to 60% biomass), ammonia (NH_3) and other cellular materials as shown in the following reactions (Gough *et al.*, 1987; Senior, 1995):

$$\underset{\text{(organic matter)}}{COHNS} + O_2 + \text{nutrients} \xrightarrow{\text{bacteria}} CO_2 + NH_3 + \underset{\text{(biomass)}}{C_5H_9O_3N} + \text{other end-products}$$

$$\underset{\text{(biomass)}}{C_5H_9O_3N} + 5O_2 \xrightarrow{\text{bacteria}} 5CO_2 + 2H_2O + NH_3 + \text{energy}$$

The empirical formula $C_5H_9O_3N$ was chosen to represent an bacterial biomass (Kalyuzhnyi, 1997). The micro-organisms use phosphates and ammonia-nitrogen for cell maintenance (Knox, 1985; Metcalf & Eddy, 1991). Only about 75 - 80% of the cell material can be oxidised, the remaining 20 - 25% is composed of inert components and organic compounds that are generally not biodegradable. Aerobic treatment produces a high quality effluent, but the start-up phase requires an acclimation period in order to facilitate the development of a competitive microbial community (Senior, 1995). The disadvantages of this process are the occurrence of foaming, poor solid-liquid separation, high volumes of sludge which may contain high levels of heavy metals and thus need to be disposed off as hazardous waste (Senior, 1995).

There are basically three different types of aerobic treatments (Atlas & Bartha, 1993). A relatively inexpensive film-flow-type aerobic treatment system is the trickling filter, where the wastewater percolates through a bed of porous material covered with a bacterial community (rock or plastic packing). Aeration is supplied by the porous nature of the bed (Atlas & Bartha, 1993). The wastewater filter through the porous bed and the effluent and any biological solids that have became detached from the media, are collected in an underdrain unit (Metcalf & Eddy, 1991) at the bottom of the filter system (Mack *et al.*, 1975). The bacterial community utilises the biodegradable organic substances in the wastewater, thus reducing the COD and SS concentrations of the effluent (Matasci *et al.*, 1986). A drawback of this treatment system is that a nutrient overload can lead to excess microbial slime, which subsequently influences the aeration and filtration rates of the wastewater (Atlas & Bartha, 1993). A relative high incidence of clogging may also occur (Senior, 1995; Van Niekerk & Rudert, 1998) and the efficiency is often reduced by colder winter temperatures (Atlas & Bartha, 1993).

A more advanced aerobic film-flow-type treatment system is the rotating biological contractor or biodisc system (Atlas & Bartha, 1993). Closely spaced circular discs of polystyrene or polyvinyl chloride (Metcalf & Eddy, 1991) are rotated slowly in a trough containing the wastewater. The partially submerged discs become coated with the bacterial community (Atlas & Bartha, 1993) as a slime layer over the wetted surface of the discs. The continuous rotation insures constant aeration and contact with the nutrients in the wastewater. These systems require less space than the trickling filters, are efficient for the removal of heavy metals and NH_3, are stable in operation, no pumping, no aeration or recycling of solids are required and no aerosols are produced (Lugowski *et al.*, 1990; Spengel & Dzombak, 1992; Atlas & Bartha, 1993), but they require a longer initial start-up.

The most popular aerobic treatment system, is the activated sludge process. The wastewater containing the biodegradable organic substances is contained in a mechanically stirred aeration tank (Atlas & Bartha, 1993). A diverse non-pathogenic heterotrophic community of micro-organisms, protozoa and filamentous organisms develop and are maintained in the aerated tank. Well-managed systems are capable of effectively removing phosphate, nitrogen and carbon, with resultant microbial biomass production (Ubisi *et al.*, 1997; Banister & Pretorius, 1998). The activated sludge treatment process is efficient and flexible and can withstand variations in wastewater flow rate and high organic loads (Atlas & Bartha, 1993). A high quality effluent can be produced, with COD removals as high as 99% (Chian & De Walle, 1976; Keenan *et al.*, 1984; Lema *et al.*, 1988; Zhi-rong Hu *et al.*, 2000). The start-up and operation is fairly quick and simple, with few odour problems (Metcalf & Eddy, 1991).

The main disadvantages of the aerobic treatment are the high energy requirements, sludge and foam production, nutrient requirements, precipitation ability of sludge to adsorb specific organic compounds and toxic heavy metals, sludge disposal and the large capital investment needed (Blakey & Maris, 1990; Metcalf & Eddy, 1991; Senior, 1995).

Anaerobic treatment

One of the oldest forms of biological wastewater treatment is the anaerobic digestion method (Metcalf & Eddy, 1991). The simplest anaerobic treatment system is the septic tank, which is popular in rural areas lacking formal sewer systems (Atlas & Bartha, 1993). These tanks serve as a combined settling and skimming tank and as an unheated, mixed anaerobic digester (Metcalf & Eddy, 1991). The residual biosolids settle in the tank, while the clarified effluent can be distributed to a leaching field, where dissolved organic material can undergo further treatment (Atlas & Bartha, 1993).

The first official tank was designed in the 1850's to separate and retain solids and the wastewater plant in Baltimore, Maryland was one of the first to install separate digestion tanks (Metcalf & Eddy, 1991). Thereafter, anaerobic digestion was continuously studied and intensively evaluated, resulting in heat application (Joubert & Britz, 1986; Van Der Merwe & Britz, 1994) and improvement in reactor design, as well as higher removal rates (Britz *et al.*, 1990; Metcalf & Eddy, 1991; Guiot *et al.*, 1997). Progress has been made in understanding the mechanism control of the process, design and size of the digesters (Metcalf & Eddy, 1991; Van Der Merwe & Britz, 1993). In modern practice, large-scale anaerobic reactors are constructed to maintain strict anaerobic conditions, produce utilisable biogas and facilitate the screening and settling of solids.

Anaerobic digestion depends on a microbial consortium for the biological conversion of organic material in the absence of molecular oxygen to a variety of end-products including methane (CH_4), CO_2 and other metabolites, while less than 3% of the organic matter is transformed into biomass (Metcalf & Eddy, 1991). The main disadvantages of anaerobic digestion are: the complexity of the initial start-up process; the long start-up time needed for biomass development; high ammonia concentrations are needed for maximum biomass activity; the anaerobic system is sensitive to heavy metals, solvents and detergents; a large capital lay-out is required; and the need for strict control of many operational parameters is essential (Atlas & Bartha, 1993; Backlund *et al.*, 1998). The pH has to be maintained within the

range of 6.5 - 7.5, with optimal performance at pH 7.0 and 35°C (Atlas & Bartha, 1993; Van Der Merwe, 1994; Senior, 1995). pH extremes and the influx of heavy metals, solvents or other toxic materials can easily upset the anaerobic digestion process (Atlas & Bartha, 1993). Furthermore, heavy metal removal in anaerobic processes are not as efficient as in aerobic processes. Ammonia-nitrogen is not removed and will thus be discharged as part of the digester effluent creating an oxygen demand in the receiving waters (Senior, 1995). The sulphates present in the wastewaters are reduced to hydrogen sulphide which can then result in corrosion, bad odours and the inhibition of the microbial community in the digester. Hydrogen sulphide can also be an efficient precipitant for most metals, which then precipitate and accumulate as inert solids in the sludge blanket or on the filter media.

The main advantages of the anaerobic digestion process are: less space is required than the aerobic process; there is no oxygen demand, thus saving on energy costs since no mixing is required (Senior, 1995; Backlund *et al.*, 1998); only 2 - 3% of the organic matter is transformed into biomass, which is an indirect way to decrease the problem of sludge disposal as found in aerobic processes (Backlund *et al.*, 1998); lower nutrient requirements, since substances like NH_3-N are retained as food for the microbial population, unlike in the aerobic treatment process; and pathogens and viruses are also destroyed during the digestion process.

The anaerobic digestion process can handle high organic loads without previous dilution, bad odours are mainly eliminated since the process takes place in closed reactors (Backlund *et al.*, 1998), the mineralised anaerobic sludge can be used as a fertiliser if heavy metal concentrations are low and, even more important, anaerobic sludge settles more easily than aerobic sludge, which eliminates the use of flocculants (Lema *et al.*, 1988). The most important advantage of anaerobic digestion is the production of high-energy volatile components (biogas) during the transformation of organic matter (Backlund *et al.*, 1998).

The anaerobic digestion of wastewater is an established technology and has already been applied successfully at many municipal sewage works (Augoustinos *et al.*, 1989). Recently, it has become very popular to use

anaerobic digestion for the treatment of high organic loads (Braun & Huss, 1982; Silverio *et al.*, 1986; Augoustinos *et al.*, 1989; Ross, 1989; Lin, 1991; Britz *et al.*, 1992; Çiftçi & Öztürk, 1993; Keenan *et al.*, 1993; Backlund *et al.*, 1998) and high salinity effluents (Shipin *et al.*, 1994).

B.4. Combined chemical, physical and/or biological treatments

Combined processes or "hybrid systems" are becoming more popular for the treatment of industrial wastewater (Dienemann *et al.*, 1990; Abeling & Seyfried, 1992). A small part of an organic load in wastewater can for instance, be removed anaerobically, thereby producing methane. The remaining pollution load can then be removed aerobically. This combined technology is an efficient economical carbon removal solution (Venkataramani *et al.*, 1988; Dienemann *et al.*, 1990; Abeling & Seyfried, 1992).

Biological treatments have furthermore been combined successfully with physical-chemical treatment methods (Ahlert & Kosson, 1990). Better removal efficiencies have been found with combined treatments, than with separate methods (Keenan *et al.*, 1984). Under normal conditions, physical-chemical methods can be used to remove metals and hydrolyse part of the organic material, while the biological methods can be employed to stabilise the degradable organic matter. Reverse osmosis was reported to be a popular and successful post-treatment physical-chemical method following a combined aerobic-anaerobic process (Dienemann *et al.*, 1990). Physical-chemical processes are also ideal final or polishing treatment options, and can remove ammonia-nitrogen successfully before biological treatment is applied (Aynagiotou *et al.*, 1993).

To simplify the presentation, the broad field of combined treatment options is illustrated as one example that is currently successfully applied in South Africa. This is the anaerobic digestion technology being combined with ultrafiltration and generally known as the ADUF process (Ross *et al.*, 1990).

Anaerobic digestion combined with ultrafiltration (ADUF)

This treatment method is a recently developed membrane-assisted process specifically for the treatment of industrial effluents, which, by means of biomass separation, eliminates the sludge concentration and retention problems associated with conventional systems (Ross *et al.*, 1990; Van Der Merwe, 1994; Nell & Kafaar, 1996; Strohwald, 1996). Successful laboratory and pilot-scale ADUF studies were done on wine distillery, malting, egg, brewery, chemical, fruit and maize-processing effluents (Ross *et al.*, 1994; Nell & Kafaar, 1996; Strohwald, 1996). Reduction of organic levels of up to 98% were achieved while operating under stable state conditions, and producing a clear, colloid-free effluent (Sauvegrain *et al.*, 1992; Ahmadun, 1994; Nell & Kafaar, 1996; Strohwald, 1996).

Solvable problems experienced with some of the applications of the ADUF technology were: blocking and deterioration of the ultrafiltration membrane; generation of foam in the digester, which then restricted the space and the organic loading rates which the digester could handle; clogging of gas pipelines, water traps and meters; loss of solids from the ADUF systems; lack of suitable nutrients and alkalinity, which resulted in poor digester capacity and low organic loading rates; flux decline; and difficulty in maintaining the desirable balance between substrate feed and permeate production (Harada *et al.*, 1994; Van Der Merwe, 1994; Nell & Kafaar, 1996; Strohwald, 1996). In total this ADUF combination led to the production of a fairly pure final effluent which could be either reused, recirculated or disposed of into a water system.

C. Microbial populations in anaerobic digesters

The decomposition of organic matter to CH_4 and CO_2 by mixed anaerobic microbial populations has received a great deal of attention and has provided useful insights into anaerobic digester bioconversion technology (Zeikus, 1980; Lettinga *et al.*, 1997). The bioconversion of the organic matter is a stable process when performed under defined environmental conditions, and is based on the catabolic activities of a diverse, yet substrate specific,

population of aerobic and anaerobic bacteria (Zeikus, 1980; Silvey *et al.*, 2000). Certain properties of the microbial populations responsible for the anaerobic decomposition of the organic matter appears constant regardless of whether this process is occurring in man-made digesters or in nature. The properties include a specific population diversity, the effect of certain physical and chemical conditions on species composition and the physiological characteristics of the different active species (Zeikus, 1980). The alteration of several physical and chemical environmental parameters in anaerobic digesters can influence the microbial metabolism and thus also the digester performance (Zeikus, 1980; Britz *et al.*, 1997).

The complex organic material (Fig. 2), including the microbial biomass, serves as a food source for the microbial consortium, and is depolymerised and converted to easier degradable compounds, biomass, energy and other gaseous end-products (Balch *et al.*, 1979). Other end-products of the digestion process include N_2, H_2S and H_2 (Kasan, 1988). The current biological model (Fig. 3) of a methanogenic mixed-culture digestion consists of at least five major consecutive, but metabolic independent, trophic bacterial groups (Gorris *et al.*, 1989; Tursman & Cork, 1989):

1. Hydrolysis of complex organic polymers, such as carbohydrates, proteins, lipids, polysaccharides and aromatic compounds, into smaller subunits for use as a source of energy and cell carbon, which can be easily transported into the bacterial cell. Proteins are then converted to amino acids, polysaccharides and carbohydrates to sugar monomers and lipids to long chain fatty acids;
2. Fermentation (acidogenesis) of the smaller subunits resulting from the hydrolysis process, to identifiable intermediate organic acids, alcohol, H_2 and CO_2. These fermentation products serve as a source for the next trophic group of non-methanogenic bacteria;
3. β-oxidation (acetogenesis) of intermediate and long chain fatty acids (propionate, butyrate, benzoate) and ethanol to acetate, CO_2 and H_2;
4. The homo-acetogenic bacteria can then catabolise one-carbon-compound to acetate as main metabolic product; and

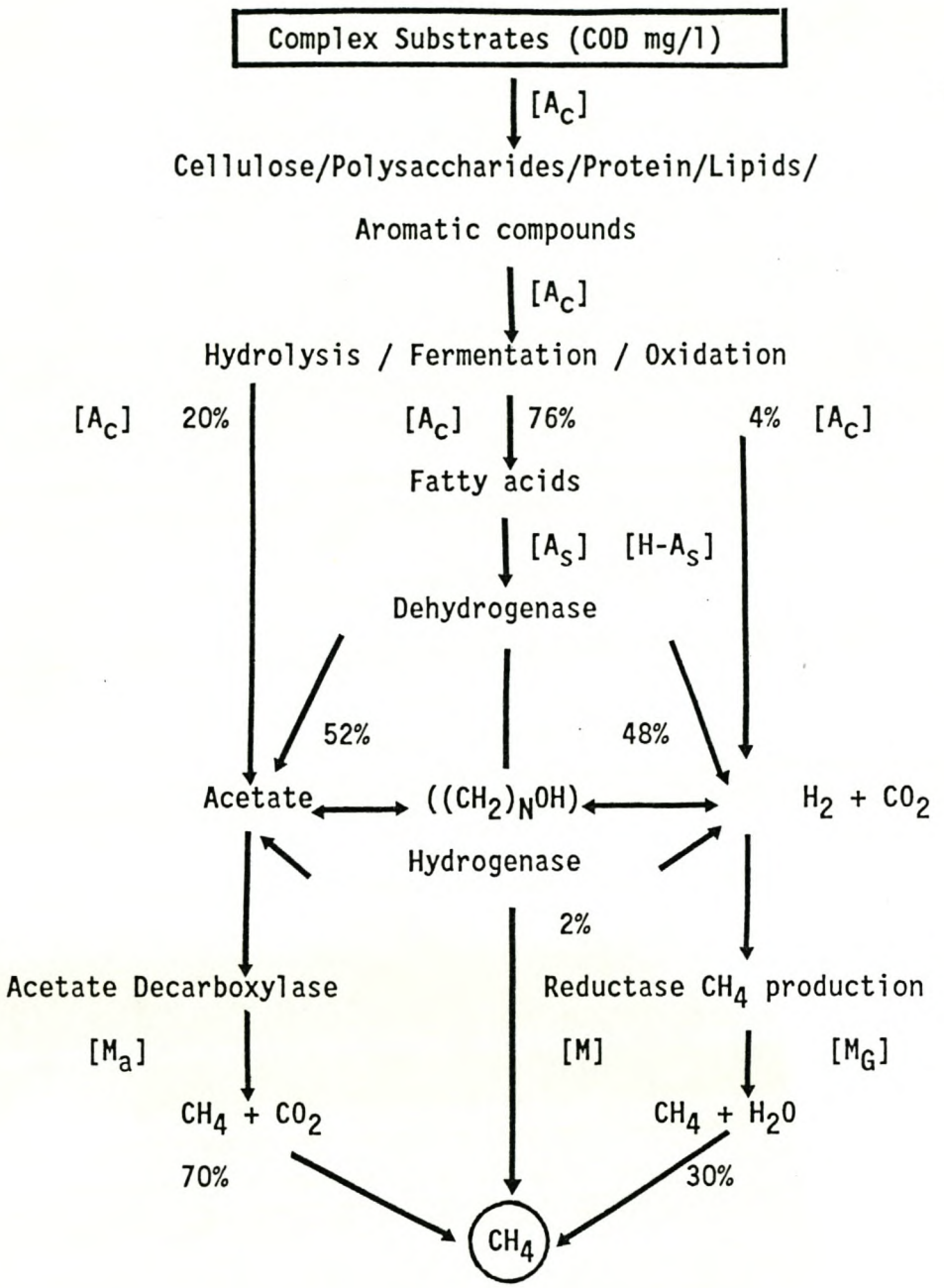

Figure 2. Conversion of complex organic material to different end-products during anaerobic digestion (Britz, 1990; A_c = acidogens, A_s = acetogens, H-A_s = homoacetogens, M_a = acetate-utilising methanogens, M_G = H_2/CO_2-utilising methanogens, % = breakdown of complex substrate to respective metabolites)

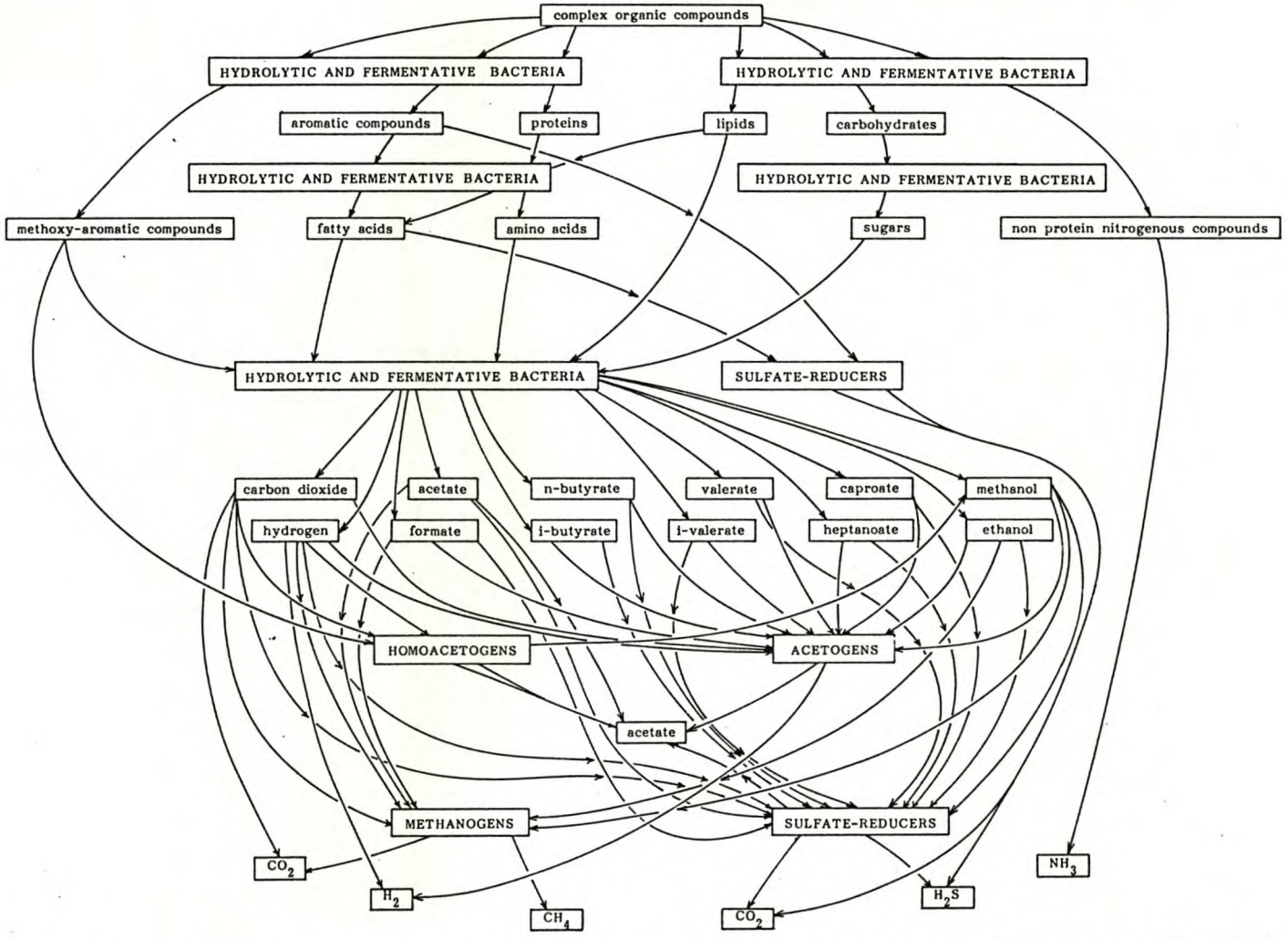

Figure 3. The biological model of the five major consecutive microbiological reactions during the anaerobic digestion process (Britz, 1989).

5. Methane formation is the final stage of anaerobic digestion. Two types of metabolic pathways can be found: acetate is converted to CH_4 and CO_2, and CO_2 and H_2 are converted to CH_4 and H_2O. The efficient metabolism of each trophic group is thus dependent on the activity of the other trophic groups.

Hydrolytic and Acidogenic Bacteria

To degrade complex organic substrates to methane by anaerobic digestion, other organisms than the methanogenic bacteria are necessary because of the limited number of substrates catabolised by the methanogens (Klass, 1984; Pohland & Kim, 2000). The acidogenic population is thus by far the largest of the trophic groups of bacteria (Forday & Greenfield, 1983). Many of these bacteria have large substrate ranges, but their substrate preferences and utilisation patterns are different (McInerney & Bryant, 1981). The hydrolytic (acidogenic) bacteria depolymerise the complex organic polymers and these products are then fermented to primary (volatile fatty) acids, such as acetate, propionic and butyrate, with smaller quantities of formic, valeric, *iso*-valeric and caproic acids which can also be produced (Fang, 1997; Okamoto *et al.*, 2000). Ammonia, hydrogen gas (H_2), CO_2, *iso*-butyrate, *iso*-valerate, n-valerate, 2-methylbutyrate and certain aromatic compounds are produced from amino acids which are of the smaller sub-units formed after depolimerisation of the complex organic polymers (Bryant, 1976; Salminen *et al.*, 2000). Many aromatic compounds are also converted by bacteria to benzoate (Knoll & Winter, 1989).

The concentration of H_2 in the overall anaerobic digestion process plays a central role as regulator in controlling the proportions of the products produced by the bacteria (Bryant, 1979). In the presence of hydrogen-consuming bacteria (methanogenic, homo-acetogenic and sulphate-reducing bacteria), many bacteria will produce hydrogen for the disposal of excess electrons generated during the energy yielding oxidations of organic materials (Forday & Greenfield, 1983). When accumulation of hydrogen, due to the inhibition of the hydrogen-consuming methanogens occurs, unfavourable conditions for electron disposal will result (Forday & Greenfield, 1983). Under

stressed conditions, alternative electron sink products e.g. propionate, butyrate, succinate and ethanol are formed, which can be an indication of digester failure. Excessive activity of the acidogenic population can also result in digester failure (Forday & Greenfield, 1983). Acidogenic bacteria are tolerant to unionised organic acids, hydrogen ions and hydrogen, while these compounds are inhibitory to the other trophic groups of bacteria (Forday & Greenfield, 1983). The formation of acetate, CO_2 and H_2 are an indication of efficiently operating metabolic interactions in the anaerobic digestion process. The partial pressure of hydrogen must, however, be maintained at very low levels, since the pressure may affect the metabolic pathway of the acidogenic bacteria (Bryant, 1979; Okamoto *et al.*, 2000).

Most of the acidogenic bacteria are obligate anaerobic, however some are facultative and can also metabolise organic material via the oxidative pathway. This is important in anaerobic sewage treatment, as dissolved oxygen might become toxic to the obligate anaerobic organisms such as the methanogens (Ueki *et al.*, 1978; Van Haandel & Lettinga, 1994). Depending on the original complex organic substrate, the acidogens include members of the genera *Bacillus*, *Clostridium*, *Streptococcus*, *Micrococcus*, *Staphylococcus*, *Pseudomonas*, *Lactobacillus*, *Eubacterium*, *Klebsiella*, *Escherichia*, *Chryseomonas*, *Xanthomonas*, *Aerobacter*, *Acinetobacter*, *Synergistis*, *Bacteroides*, *Butyrivibrio*, *Butyribacterium*, *Propionibacterium*, *Acidaminobacter*, *Bifidobacterium* and other Enterobacteriaceae. The presence of *Peptococcus*, *Peptostreptococcus*, *Ruminococcus*, *Caprococcus*, *Acetivibrio* and *Sarcina* have also been reported (Siebert & Toerien, 1969; Scharer & Moo-Young, 1979; McInerney & Bryant, 1981; Klass, 1984; Hakulinen *et al.*, 1985; De Haast & Britz, 1986; Heijthuijsen & Hansen, 1987; Joubert & Britz, 1987; McSweeney *et al.*, 1993; Van Der Merwe, 1994; Okamoto *et al.*, 2000).

Acetogenic Bacteria

Further degradation of acidogenic substrates are anaerobically oxidised to acetate, formate, H_2 and CO_2 as main metabolic end-products (Bryant, 1979; Mountford & Bryant, 1982; Klass, 1984; Henson & Smith,

1985; Hanaki *et al.*, 1981; Fang, 1997; Salminen *et al.*, 2000). Different from the hydrogen-forming acidogenic bacteria, these bacteria do have an obligate requirement for the disposal of electrons such as hydrogen (Forday & Greenfield, 1983). The conversions are extremely sensitive to H_2 concentration and the growth of these bacteria can only occur at partial hydrogen pressures of less than 10^{-5} atmospheres (Forday & Greenfield, 1983). High hydrogen concentrations can inhibit acetogenesis which then results in the formation of fermentation products other than acetate (Zinder, 1984; Labib *et al.*, 1992). The conversion of these products (higher volatile fatty acids, caproate, butyrate and propionate) to methanogenic substrates (acetate, H_2 and CO_2) is an important step, as the unionised forms of these acids are toxic to the methanogenic group. Inhibition of the acetogens will result in a decrease in digester pH, which creates an unfavourable environment for methanogenic bacteria (Van Haandel & Lettinga, 1994; Broughton *et al.*, 1998). Since the acetogenic bacteria are the slowest growing of the trophic groups and hence represent a further rate-limiting step in the degradation process, which can lead to subsequent digester failure. The metabolic conversion of the acetogens are only possible if a low hydrogen partial pressure is maintained (5.8×10^{-5} to 1.6×10^{-6} atmospheres) by the methanogens and sulphate-reducers (Boone & Bryant, 1980; Boone & Mah, 1989).

Due to their hydrogen sensitivity and strict anaerobic growth requirements, the acetogens have generally not been well identified or physiologically characterised. On the basis of experimental data collected using co-cultures, the presence of these bacteria was first discovered in 1967 by Bryant and co-workers. It was found that the acetogens can only be cultured in the presence of a methanogenic hydrogen-utilising bacterium (Zeikus, 1980). A classic example of a hydrogen-producing acetogenic bacterium is the "*S*" organism, which was isolated from a *Methanobacillus omelianskii* mixed culture (Bryant *et al.*, 1967). After the isolation of the "*S*" organism, other strains of acetogens were also characterised. These bacteria are mostly syntrophic and are also called "obligate proton-reducing bacteria". These include members of the species of *Syntrophomonas*, *Clostridium*,

Syntrophococcus, *Syntrophospora*, *Syntrophobacter*, *Syntrophus* and *Acetomaculum* (Boone & Bryant, 1980; Heyes & Hall, 1983; Diekert *et al.*, 1984; Kellum & Drake, 1984; Mountford *et al.*, 1984; Stieb & Schink, 1985; Krumholtz & Bryant, 1986; Roy *et al.*, 1986; Iannotti *et al.*, 1987; Lux *et al.*, 1990; Zhao *et al.*, 1990; Guangsheng *et al.*, 1992; Wu *et al.*, 1992; Guiot *et al.*, 1997; Harper & Pohland, 1997; Zu *et al.*, 1997).

Homo-acetogenic Bacteria

The exact role of the homo-acetogenic bacteria in the anaerobic digestion process is still not fully understood. The homo-acetogenic bacteria display a mixotrophic metabolism and can utilise hydrogen and carbon dioxide, as well as other one-carbon compounds, with acetate as a major metabolic end-product (Britz, 1990). The homo-acetogenic bacteria can also catabolise complex carbohydrates (Forday & Greenfield, 1983), aromatic compounds, alcohol and fatty acids (Bache & Pfennig, 1981; Kellum & Drake, 1984; Schink, 1984). Other metabolites, such as acetate, CO_2, ethanol, propionate, butyrate and valerate may also be formed, depending on the oxidation status of the available substrates (Eichler & Schink, 1984; Dehning *et al.*, 1989; Scherer *et al.*, 2000).

The presence of homo-acetogens in digesters are very important as they maintain the low hydrogen partial pressure required in an efficiently operating anaerobic digester (Sam-Soon *et al.*, 1991). Homo-acetogens, however, compete with the methanogens for substrates (Conrad & Wetter, 1990). They can also donate hydrogen to the methanogenic bacteria by the "interspecies hydrogen transfer" phenomenon (Zeikus, 1980; Forday & Greenfield, 1983). An advantage of the homo-acetogens over the other bacteria, is their ability to use the bicarbonate (HCO_3^-) ion as electron acceptor (Balch *et al.*, 1977). The reduction to acetate occurs through CO_2 as an intermediate (Bryant, 1979; Diekert & Ritter, 1983; Diekert *et al.*, 1984).

There are very few easily recognised genera of this strict anaerobic, hydrogen-oxidising homo-acetogenic trophic group. These include *Acetobacterium*, *Acetoanaerobium*, *Acetogenium*, *Selenomonas*, *Clostridium*, *Eubacterium*, *Butyribacterium*, *Pelobacter* and *Sporomusa* (Zeikus, 1980;

Bache & Pfennig, 1981; Diekert & Ritter, 1983; Forday & Greenfield, 1983; Eichler & Schink, 1984; Zehnder *et al.*, 1982; Stieb & Schink, 1985; Dehning *et al.*, 1989; Schuppert & Schink, 1990; Zhao *et al.*, 1990; Buschhorn *et al.*, 1992; Guangsheng *et al.*, 1992; Van Der Merwe, 1994; Okamoto *et al.*, 2000).

Methanogenic Bacteria

The methanogens (MB) convert acetate, H_2 and CO_2 to methane and CO_2 as metabolic end-products (Ahring & Schmidt, 1992). These archaebacteria are a more diverse group of bacteria than the other three trophic groups of the consortium associated with anaerobic digestion. The methane-forming bacteria show considerable intra-species variations in cell dimensions (Zeikus, 1980; Forday & Greenfield, 1983; Guiot *et al.*, 1992) and are responsible for the terminal reactions in the anaerobic digestion process. They can thus be seen as "key" organisms in the process.

All the methanogens are obligate anaerobes, requiring an oxidation-reduction potential of less than -300 mV for growth (Bryant, 1976, Forday & Greenfield, 1983). Inhibition of methanogenesis can also be caused by organic overload when the rates of acetogenesis will exceed methanogenesis. Unionised volatile fatty acids will then start accumulating in the bioreactor (Tracey *et al.*, 1989; Nedwell & Reynolds, 1996; Salminen *et al.*, 2000) and thus, cause a decrease in pH as a result of the accumulation of mainly acetate (Nedwell & Reynolds, 1996). Wide deviations in pH can thus reduce the anaerobic microbial populations. The sensitivity of anaerobic digestion to extreme pH is mainly due to the sensitivity of the methanogenic populations to pH values above 7.8 and below 6.0 (Forday & Greenfield, 1983; Fang, 1997; Lens *et al.*, 1998). pH control at a neutral value facilitates more rapid establishment of an active methanogenic population and subsequent biogas production (Senior, 1995). If a neutral pH is not maintained, this may lead to the souring of the digester (Forday & Greenfield, 1983; Marchaim & Krause, 1993; Nedwell & Reynolds, 1996). The methanogens are very sensitive organisms, even to the toxicity caused by organic inhibitors such as toluene or chloroform and by accumulation of metals present in the wastewater being treated. All this can contribute to the

loss of methanogenic activity of the sludge present inside the anaerobic digester (Fang, 1997; Ruiz *et al.*, 1998).

This unique group of anaerobic organisms oxidise H_2, gaining energy by reducing CO_2 and acetate, as well as one of the methyl groups of either methanol, methylamines, trimethylamines or formate to produce methane as major end-product (Zehnder *et al.*, 1980; Zeikus, 1980; Miller & Wolin, 1985; Reeve *et al.*, 1997). The methanogens can grow either autotrophically, heterotrophically and some even show an amixotrophic pattern (Zeikus, 1980), but they are only able to utilise a limited range of substrates (Zehnder *et al.*, 1982). Methanogens are thus dependent on the metabolic activities of the other trophic groups in the anaerobic digestion process, as they do not ferment carbohydrates directly.

Acetate is the main substrate for methanogenic bacteria, but is a product of the acidogenic and acetogenic groups (Lens *et al.*, 1998). It is one of the most important substrates in the anaerobic degradation process leading to the generation of methane (Weber *et al.*, 1984). As much as 65 to 96% of the total methane produced can originate from acetate (Weber *et al.*, 1984). There is, however, another thermodynamically more favourable conversion than the direct conversion of acetate, namely the reduction of carbon dioxide to methane (Klass, 1984).

Methanogens include members of the genera *Methanobacterium*, *Methanobrevibacterium*, *Methanobacillus*, *Methanothermus*, *Methanococcus*, *Methanosarcina*, *Methanothrix*, *Methanolobus*, *Methanococoides*, *Methanomicrobium*, *Methanogenium*, *Methanospirillum*, *Methanoplanus*, *Methanocorpusculum*, *Methanoculleus*, *Methanosaeta* and *Methanosphaera* (McInerney & Bryant, 1981; Henson & Smith, 1985; Krumholtz & Bryant, 1986; Holland *et al.*, 1987; Zellner & Winter, 1987; Conrad & Wetter, 1990; Pauss *et al.*, 1990; Winter & Zellner, 1990; Ahring & Schmidt, 1992; Guiot *et al.*, 1992; Heppner *et al.*, 1992; Kitaura *et al.*, 1992; Reeve, 1992; Wu *et al.*, 1992; Shin *et al.*, 1996; Harper & Pohland, 1997; Reeve *et al.*, 1997; Zu *et al.*, 1997).

Sulphate-Reducing Bacteria

Just as the methanogenic bacteria (MB) are known to be able to couple the oxidation of molecular hydrogen (H_2) with the reduction of CO_2 to yield CH_4 as the electron sink product, the sulphate-reducing bacteria (SRB) are known to couple the oxidation of H_2 with the reduction of sulphate to yield hydrogen sulphide (H_2S) as the electron sink product (Tursman & Cork, 1989). Thus, the SRB behave similarly to the hydrogenotrophic MB. The electron donor in each case may be a dissolved gas from an exogenous source, or through a syntrophic association with an obligate proton/hydrogen-reducing acetogen. This interspecies hydrogen transfer mechanism can be seen as a type of intimate association or mutualism.

SRB compete with MB for substrates as well as electron donors, such as H_2 and acetate and normally outcompete the MB when sufficient sulphate is present in the environment (Fang, 1997; Lens *et al.*, 1998). MB can only utilise a restricted range of substrates (H_2 and acetate), while SRB can utilise a wider range of substrates (formate, acetate, methanol, pyruvate and H_2) (Fang, 1997) (Fig. 3). For this reason, the interactions between the SRB and the rest of the microbial community cannot be ignored. The SRB also convert sulphate into sulphide, which forms insoluble metal sulphides precipitating on the surface of bacterial cells (Fang & Liu, 1995). SRB can therefore be seen as more resilient to challenge by novel organic molecules than the MB, and digestion based upon sulphate reduction can thus be more resilient than methanogenic digestion (Nedwell & Reynolds, 1996).

In anaerobic digestion, sulphate reduction has generally been regarded as undesirable due to the production of hydrogen sulphide. Reis *et al.* (1992) reported that H_2S may have a direct and reversible toxic effect on the sulphate-reducing bacterial population. In terms of sulphide production, cytotoxicity, reaction rate kinetics and thermodynamic analysis, sulphate-reduction also result in the inhibition of acetoclastic methanogenesis, due to the distribution of sulphide precipitates on biogranule formation (Tursman & Cork, 1989; Reis *et al.*, 1992; Fang, 1997; Lens *et al.*, 1998).

The production of sulphide during anaerobic digestion also has beneficial effects through the precipitation of heavy metals as their insoluble

sulphides thus, preventing biotoxicity by removing the metals from the microbial environment in the digester (Dvorak *et al.*, 1992). The operation of the anaerobic digester at the maximum allowable pH will minimise the toxicological influence of sulphide:

$$H_2S \leftrightarrow \underset{\text{low pH}}{H^+} + HS^- \leftrightarrow \underset{\text{high pH}}{2H^+} + S^{-2}$$

The digester mixed liquid pH is therefore a primary effector in this distribution (Isa *et al.*, 1986). Results of batch activity experiments showed that the methanogenic activity dropped sharply at pH values above 7.7 and below 6, causing the predominance of sulphate-reducing bacteria at pH values above 8 (Visser *et al.*, 1992; Lens *et al.*, 1998).

Desulfovibrio is one of the most commonly studied genera in the sulphate-reducing group of bacteria. Other genera of sulphate-reducers found in anaerobic digesters include members of the genera *Desulfomonas*, *Desulfotomaculum*, *Desulfonema*, *Desulfobacterium*, *Desulfobacter*, *Thermodesulfobacterium*, *Desulfosarcina*, *Desulfobotulus*, *Desulfoarculus*, *Desulfomicrobium* and *Desulfococcus* (McInerney & Bryant, 1981; Holland *et al.*, 1987; Pfennig, 1989; Vainshtein *et al.*, 1992; Drzyzga *et al.*, 1993; Tasaki *et al.*, 1993; Van Houten *et al.*, 1996; Harper & Pohland, 1997; Lens *et al.*, 1998).

D. Conclusion

Conventional wastewater remediation methods employing physical and chemical treatment processes have been associated with a number of intrinsic advantages and disadvantages. The advantages of applying physical and chemical methods can be summarised as follows: start-up periods are short; flexible; simple equipment can be used; processes are generally insensitive to temperature; and many of the methods lend themselves to automation. The disadvantages include the production of more toxic intermediates, large amounts of sludge generated by the addition of chemicals, high investment and operating costs, low levels of efficiency, and applicability to limited effluent concentration ranges (Lema *et al.*, 1988;

Edwards *et al.*, 1998). These physical and chemical methods may become exceedingly expensive when low effluent pollutant concentrations need to be achieved (Sun *et al.*, 1992). Physical and chemical processes alone are inadequate for the complete treatment of a high-strength, noxious industrial effluent such as produced by the gelatin-manufacturing industry.

Great potential exists for the combination of various treatment options for the gelatin-manufacturing effluent, but the most efficient approach is considered to be biological wastewater treatment (Dall-Bauman *et al.*, 1990). Anaerobic digestion is a "clean and green technology" with promising possibilities for the removal of selective components in wastewater. Anaerobic digestion is furthermore cost effective over a long term and offers a viable option for the treatment of high concentration effluents, such as gelatin-manufacturing effluents.

Future research on anaerobic digestion for specific industrial effluents holds tremendous challenges in understanding the fundamental and interdependent microbiology and biochemistry of the digestion process. Furthermore, the need still exists to design the perfect anaerobic digester, since all the present digester configurations show some limitations for a specific industrial effluent. Whatever the means are to improve wastewater treatment technology, the aspiration remains to engineer the perfect anaerobic digester, inhabited by the perfect competitive microbial community.

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CHAPTER 3

TREATMENT OF GELATIN-MANUFACTURING EFFLUENT USING SINGLE-PHASE ANAEROBIC HYBRID DIGESTERS

Summary

Two different anaerobic hybrid laboratory-scale digester designs were used to evaluate the biological treatment efficiency of a high-strength, highly variable raw gelatin-manufacturing effluent (AD-1, UASB - polyethylene), as well as the raw effluent supplemented with anaerobic supernatant (AD-2, UASB - polyurethane), under mesophilic conditions. No chemical oxygen demand (COD) standardisation was done on the effluent, as varying batch and substrate composition were considered important in the simulation of the actual field conditions. Successful inoculation and start-up of the digesters resulted in good handling of the highly variable organic loading rates (OLR) and decreasing hydraulic retention time (HRT), with immediate sulphate (SO_4) (91 - 92%) and COD (22 - 30%) removal. During steady state, COD removal efficiencies of up to 90% (AD-1) and 83% (AD-2) at OLR of 9.56 (AD-1) and 4.62 kg $COD.m^{-3}.d^{-1}$ (AD-2), respectively, were achieved. High SO_4 removal efficiencies of up to 96% and 98% were obtained with the AD-1 and AD-2 bioreactors, respectively. The methane content of the biogas varied between 70 and 88% for AD-1 and between 69 and 89% for AD-2. The total methane yield per COD removed (Y_{CH4}) ranged between 0.41 and 7.16 $m^3.kg\ COD_{removed}.d^{-1}$ for AD-1 at OLR's of 1.89 to 9.56 kg $COD.m^{-3}.d^{-1}$, and for AD-2 the methane yield per COD removed varied between 0.45 and 6.75 $m^3.kg\ COD_{removed}.d^{-1}$ at OLR's of 1.48 to 9.30 kg $COD.m^{-3}.d^{-1}$. The digesters were pre-inoculated with a selected microbial population. This specific treatment process can be characterised as one having a short start-up period, good handling of extremely variable loading conditions, with no need for any additional pre-treatment of the gelatin-manufacturing effluent.

Introduction

South African industries are faced with critical pollution control issues and need to adopt changing attitudes towards pollution matters. Many of the problems that effluent producing industries face are attributed to the variable quality and noxious nature of the process effluents. Local authorities are increasingly and selectively reluctant to receive these effluents into communal sewers and industries are often faced with high trade-effluent charges. Under the new South African water and environmental laws, industries need to reconsider their water quality management strategies and treatment options (DEAT & DWAF, 1997) in order to accommodate the stricter water and pollution control regulations.

The gelatin-manufacturing industry is one of the industries that receives much attention (DEAT & DWAF, 1997) in terms of its influence on the treatment processes of the local wastewater purification works and hence, their compliance to water regulations (Van Der Merwe-Botha, 1998, Personal communication). During the gelatin-manufacturing process, reject hides which are not suitable for tanning purposes are conditioned, treated and processed. This involves a process of protein hydrolysis and denaturation, where insoluble hide collagen is converted into water soluble gelatin (Maree *et al.*, 1990). Effluents from the gelatin-manufacturing process have typically high organic as well as inorganic loads, with chemical oxygen demand (COD) values ranging between 500 - 77 000 $mg.l^{-1}$. Problems with high suspended solids, sulphate, fats, lipid emulsions, proteins and salt concentrations also contribute negatively to the effluent problems. The product process produces substantial effluent volumes of varying quality, of which the disposal to biological nutrient removal plants results in costly upsets of the treatment process (Van Der Merwe-Botha, 1998, Personal communication).

The treatment of gelatin-manufacturing effluent has not received much attention, hence little treatment data are available in this field. However, numerous studies have been done on tanning effluent and other high salinity wastewater types. These effluents are usually treated by means of expensive physical or chemical pre-treatment methods, such as either electrochemical methods, ion exchange, carbon adsorption, coagulation or flocculation

(Talinli, 1994; Garrote *et al.*, 1995; Petruzzelli *et al.*, 1995; Dalmacija *et al.*, 1996; Rajalo & Petrovskaya, 1996). Biological or anaerobic treatment of gelatin-manufacturing effluents have also been attempted with varying degrees of success (Maree *et al.*, 1990; Du Plessis *et al.*, 1993; Petruy *et al.*, 1999; Tommaso *et al.*, 1999).

Water quality managers are reconsidering biological processes with renewed interest as an alternative treatment option, so as to comply to the stricter pollution control regulations and satisfy the search for greater efficiency, economy and the use of natural energy sources. One of the biological processes involves anaerobic digestion which are well established for the treatment of high-strength industrial wastewaters. Since McCarty (1964) introduced the Upflow Anaerobic Filter (UAF) systems in the sixties, considerable progress has been made on the field of anaerobic reactor technology for wastewater treatment. This has led to the development of high-rate anaerobic bioreactor designs, with increased biomass retention and tolerance to toxic and shock loads, for the treatment of recalcitrant industrial wastewaters (Stronach *et al.*, 1987; Lettinga *et al.*, 1997). One of the newer designs is the anaerobic hybrid process instigated by Guiot & Van Den Berg (1985) and subsequently upgraded by Joubert & Britz (1987), Britz & Van Der Merwe (1993) and Guiot *et al.* (1997). Among these high-rate anaerobic designs developed and successfully applied in recent years, the Upflow Anaerobic Sludge Blanket (UASB) bioreactor has become one of the most extensively used designs for biological treatment of wastewaters (Lettinga *et al.*, 1997).

Against this background, the aim of this study was to evaluate the performance of single-phase anaerobic hybrid digesters for the treatment of raw and nutrient-enriched gelatin-manufacturing effluent.

Materials and methods

Digester design

Two upflow anaerobic hybrid laboratory-scale digesters (AD-1 and AD-2), each with a working volume of five litres, and an operating temperature of 35°C, were used to treat raw gelatin-manufacturing effluent. The temperature was regulated by means of a heating tape and temperature sensitive controls (Meyer *et al.*, 1985). The hybrid digesters combined a fixed-film and an upflow sludge blanket system. The inert porous polyethylene foam (Fig. 1a) and the polyurethane (Fig. 1b) material were fitted to the upper two thirds of the inner digester wall. The polyurethane material (Van Rompu *et al.*, 1990) had channels of 1.3 x 3.3 cm, edges of 1.3 x 3.0 cm and a back area 1.3 cm thick. The density of the polyethylene and polyurethane materials were estimated at 0.77 and 25.7 $kg.m^{-3}$, respectively (Van Der Merwe, 1994).

The substrate in each case was introduced semi-continuously via a horizontal inlet at the bottom of each digester by means of a peristaltic pump (Watson-Marlow 302S) controlled by an electronic timer. The overflow of the reactor emptied through an U-shaped tube to prevent any atmospheric oxygen from entering the system. The biogas exited at the top of the digester via a gas-solid separator and biogas production was determined by means of a brine displacement system (6N HCl, pH 2.0). The biogas volumes were corrected to standard temperature and pressure (STP) conditions.

Digester start-up

The digesters were originally seeded using a mixture of anaerobic sludge (Table 1) and biosolids obtained from other operational digesters, as well as acclimatised anaerobic and activated sludge from the local wastewater purification works treating the gelatin-manufacturing effluent. Initially, a mixture of gelatin-manufacturing effluent and raw sewage were fed to the digesters. The initial substrate flow rates were set at a hydraulic retention time (HRT) of 3.0 d and maintained until stable conditions persisted. Stable state conditions were assumed when, after five volume turnovers,

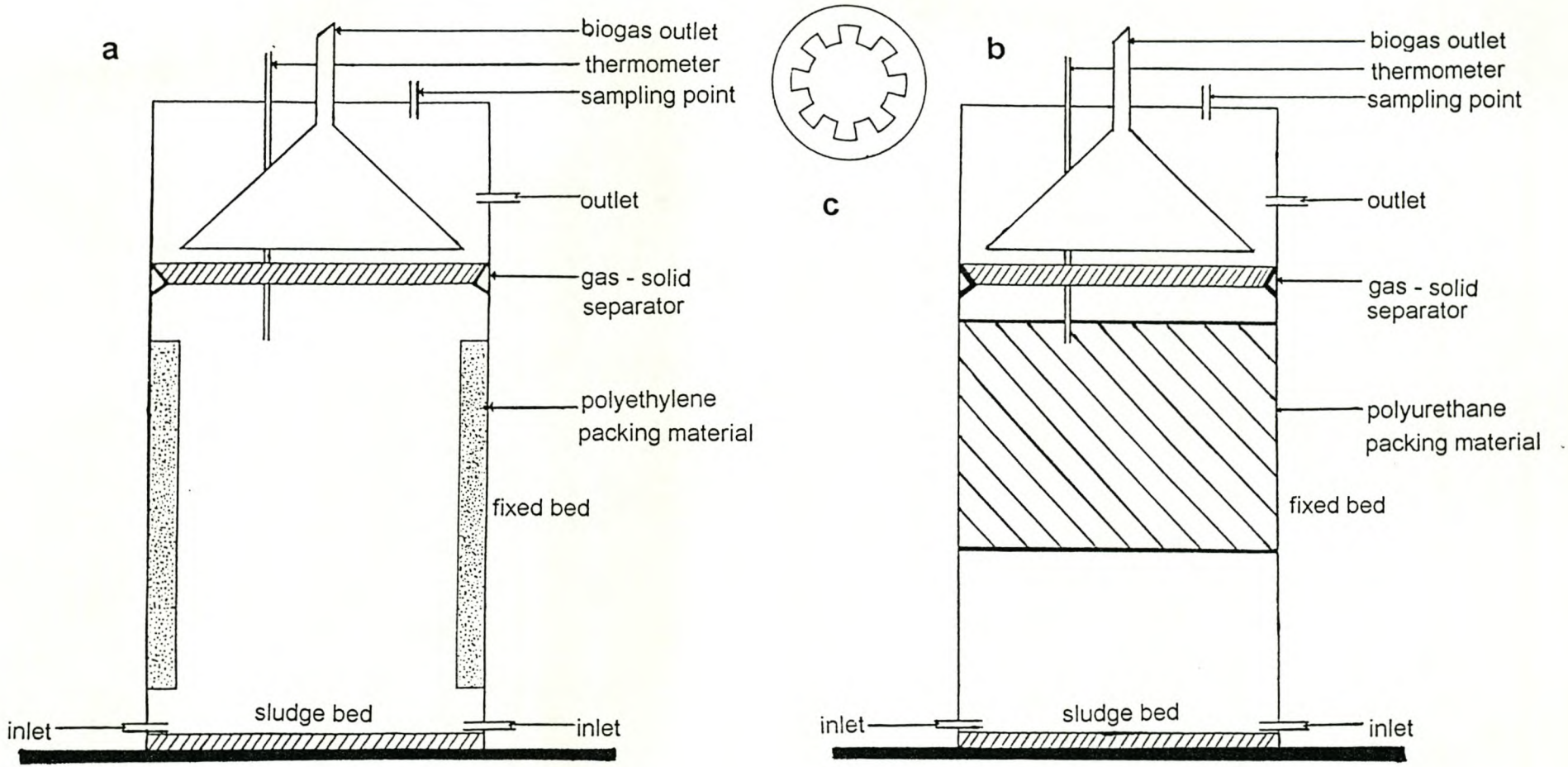

Figure 1. Schematic representation of the (a) polyethylene (AD-1) and (b) polyurethane (AD-2) laboratory-scale anaerobic hybrid digesters. The cross-section illustrates the fixed bed arrangement (c) of the polyurethane digester.

Table 1. Average composition of the anaerobic sludge used for the start-up process of the laboratoty-scale digesters.

Parameters	Minimum	Maximum	Average
Temperature (°C)	14.0	24.7	17.8
pH	6.9	7.7	7.4
TAlk (mg.l^{-1} as $CaCO_3$)	1 587	6 280	3 246
TS (%)	0.8	10.7	3.0
TVS (%)	0.6	3.5	1.7
TVS / TS (%)	26.6	91.3	64.5
PO_4-P (mg.l^{-1} as P)	18.5	112.2	53.2
TKN (mg.l^{-1})	969	3 024	1 607
COD (mg.l^{-1})	10 110	50 240	24 635
VFA (mg.l^{-1})	197	1 387	551
VFA:TAlk	0.05	0.37	0.17
NH_3-N (mg.l^{-1})	357	814	534
Cu (mg.l^{-1})	0.0	0.0	0.0
Fe (mg.l^{-1})	0.0	0.2	0.1
Co (mg.l^{-1})	0.0	0.0	0.0
Mn (mg.l^{-1})	0.3	1.0	0.6
Cr (mg.l^{-1})	0.0	0.0	0.0
Pb (mg.l^{-1})	0.0	0.3	0.1
Ni (mg.l^{-1})	0.1	0.3	0.2
Cd (mg.l^{-1})	0.0	0.1	0.0
Zn (mg.l^{-1})	0.0	0.1	0.1
Na (mg.l^{-1})	69	95	84
Mg (mg.l^{-1})	34	45	42
Ca (mg.l^{-1})	51	115	84

operational parameters showed a variation of less than 10%. Subsequently, the HRT was lowered to 2.0 d and then to 1.0 d for the rest of the study.

Gelatin-manufacturing effluent

The gelatin effluent was obtained from the local gelatin-manufacturing industry (Leiner Davis Gelatin South Africa (Pty) Ltd), in batches of 75 l and stored at room temperature until required. No COD standardisation was done on the effluent in an attempt to simulate the actual field conditions. However, the pH of the effluent used as digester substrate was adjusted to 6.5 using a standard 6N HCl solution. By this means, the direct effect of varying COD concentrations (organic loading rates) on the anaerobic treatment efficiency could be determined. The gelatin-manufacturing effluent used as digester substrate was initially supplemented with 100 $mg.l^{-1}$ urea, 100 $mg.l^{-1}$ K_2HPO_4, 10 ml acetic acid (CH_3COOH) and a sterile trace element solution (Nel *et al.*, 1985) during the start-up process, so as to stimulate the growth of the specific microbial consortium and to prevent any nutrient limitations.

Analytical methods

The following parameters were monitored on the digester substrate and effluent, according to Standard Methods (APHA, AWWA & WEF, 1995): pH; total alkalinity (TAlk); chemical oxygen demand (COD); total Kjeldahl nitrogen (TKN); volatile fatty acids (VFA); sulphate (SO_4^{2-}); total solids (TS); volatile solids (VS); total non-volatile solids (TNVS) and ortho-phosphate (PO_4-P).

Total metals which include most of the heavy metals (Cu, Fe, Co, Mn, Zn, Ni, Pb, Cr, Cd), as well as calcium and sodium concentrations, were determined using an Atomic Absorbance Spectrophotometer (Varian Model 250 Plus), equipped with hollow cathode lamps for the different metals, photoelectric detector with associated electronic amplifying and measuring equipment. Air/acetylene and nitrous oxide/acetylene burners were used with air as oxidant and acetylene (or nitrous oxide) as fuel. Pressure reducing regulators were used for the supply of the fuel and oxidant at appropriate levels. Control standards of known metal concentrations were prepared with a matrix similar to the samples, for the construction of a calibration curve.

Additional standard solutions were analysed between samples to confirm test control, as well as a blank to confirm the baseline stability. The sample concentrations were determined by reference to the calibration curve. The filtered samples (at room temperature) were aspirated into the air/acetylene or nitrous oxide/acetylene flame and atomised.

Volatile fatty acids (as acetate) were determined according to the titration method of Moosbrugger *et al.* (1992). The biogas composition (methane and carbon dioxide) was determined volumetrically according to the quantitative biogas carbon dioxide content method of Ross *et al.* (1992). The biogas volumes were corrected to STP conditions.

The accuracy of all tested chemical parameters were confirmed by participation in an inter-laboratory water testing program (SABS Water Check Proficiency Program).

Experimental phases

The study consisted of two experimental phases, the first during which the polyethylene hybrid digester (AD-1) was fed with full-strength raw gelatin-manufacturing effluent and the second where the polyurethane hybrid digester (AD-2) was fed with a mixture of 80% gelatin-manufacturing effluent and 20% anaerobic digester supernatant (as nutrient supplement). Standardisation was applied only to the extent of preparing the substrates from the same effluent batch, to make direct comparisons possible. The different batches were, however, not standardised in terms of COD concentration, in an attempt to simulate the actual field conditions using bench-scale systems.

Results and discussion

Gelatin-manufacturing effluent composition

The average composition of the different batches of gelatin-manufacturing effluent, obtained from Leiner Davis Gelatin (Pty.) Ltd, is given in Table 2. The average composition of the substrate fed to the hybrid

Table 2. General composition of gelatin-manufacturing effluent obtained over a 22 month period.

Parameters	Minimum	Maximum	Average	± SD
pH	1.8	13.4	9.8	8.0
EC ($mS.m^{-1}$)	73.9	3 870.0	530.7	456.8
TDS ($mg.l^{-1}$)	673	17 166	5 100	4 427
TAlk ($mg.l^{-1}$ as $CaCO_3$)	0	16 185	1 188	1 188
SS ($mg.l^{-1}$)	24	82 660	2 465	2 440
Cl ($mg.l^{-1}$)	49.3	6 145.7	716.7	667.4
PO_4-P ($mg.l^{-1}$ as P)	0.0	23.5	2.1	2.1
T-PO_4 ($mg.l^{-1}$ as P)	0.6	14.1	4.1	3.5
COD ($mg.l^{-1}$)	505	77 336	6 323	5 818
TKN ($mg.l^{-1}$)	61	4 362	588	527
SO_4^{2-} ($mg.l^{-1}$)	19	2 250	746	727
NH_3-N ($mg.l^{-1}$)	3	920	87	83
Cu ($mg.l^{-1}$)	0.0	0.7	0.0	0.0
Fe ($mg.l^{-1}$)	0.0	57.5	1.0	1.0
Co ($mg.l^{-1}$)	0.0	1.6	0.0	0.0
Mn ($mg.l^{-1}$)	0.0	5.3	0.2	0.2
Cr ($mg.l^{-1}$)	0.0	34.6	0.5	0.5
Pb ($mg.l^{-1}$)	0.0	3.9	0.2	0.2
Ni ($mg.l^{-1}$)	0.0	6.5	0.3	0.3
Cd ($mg.l^{-1}$)	0.0	0.9	0.0	0.0
Zn ($mg.l^{-1}$)	0.0	3.9	0.3	0.3
Total metals ($mg.l^{-1}$)	0.2	99.8	2.6	2.4
Na ($mg.l^{-1}$)	10	19 768	890	880
Ca ($mg.l^{-1}$)	10	376	160	150
Fats and Oils ($mg.l^{-1}$)	2.0	2 134.0	270.6	268.6

SD = Standard deviation

digesters during the experimental phases, is given in Table 3. The OLR ranging from 1.89 - 9.56 kg $COD.m^{-3}.d^{-1}$ for digester AD-1 and from 1.48 - 9.30 kg $COD.m^{-3}.d^{-1}$ for digester AD-2 clearly show the wide variation of OLR which a purification works can expect from the gelatin-manufacturing industry. Since the direct influence of varying batch and substrate composition was considered important, no standardisation was done on the effluent. The substrate pH was, however, adjusted to a value of 6.5.

Start-up period

A rapid start-up phase was observed for both digesters, with immediate SO_4 (91 - 92%) and COD removal (22 - 30%) at an OLR of 2.21 kg $COD.m^{-3}.d^{-1}$. This excellent start-up performance confirmed the successful selection of an active microbial community which were used as inoculum for the digesters. VFA production was observed in both digesters during the first 23 days, followed by VFA removal of 50% at an OLR of 2.04 kg $COD.m^{-3}.d^{-1}$ (AD-1) and 38% at an OLR of 1.76 kg $COD.m^{-3}.d^{-1}$ (AD-2). The VFA production then increased again in both digesters up to day 92, and this was followed by a steady increase in the removal efficiency of the VFA's. At this stage, VFA removal efficiencies of 26% (AD-1) at an OLR of 4.73 kg $COD.m^{-3}.d^{-1}$ and 61% (AD-2) at an OLR of 4.08 kg $COD.m^{-3}.d^{-1}$, were obtained, respectively. The effluent pH and alkalinity increased during the start-up period, also confirming the establishment of an active, balanced microbial community.

Digester Efficiency (AD-1)

The composition of the substrates used for the AD-1 digester, the digester effluent and the performance efficiency during the operational period, are summarised in Tables 4a, b and c. The data in Table 4 are arranged according to the numerical increases in OLR. The prominent variation in OLR over the 29 week period can clearly be seen (Fig. 2), with the higher OLR operational conditions found during weeks 9 to 11 and 23 to 25. The data in Fig. 2 reflects the variation in organic effluent composition which is found under typical field conditions where balancing facilities are not available.

Table 3. Average composition of the substrate used during the two experimental phases, obtained over a 29 week period, from an average of 34 samples.

Parameters	Digester AD-1 Substrate				Digester AD-2 Substrate			
	Minimum	Maximum	Average	± SD	Minimum	Maximum	Average	± SD
HRT (d)	1.0				1.0			
pH	6.3	7.0	6.7	0.7	6.9	8.3	7.2	0.7
COD ($mg.l^{-1}$)	1 891	9 560	3 975	1 935	1 477	9 300	3 652	2 535
OLR ($kg\ COD.m^{-3}.d^{-1}$)	1.89	9.56	3.97	1.93	1.48	9.30	3.65	2.53
VFA ($mg.l^{-1}$ as acetic acid)	68	3 387	722	671	128	2 263	816	901
TS ($mg.l^{-1}$)	1 400	10 800	5 485	4 100	2 100	10 100	5 191	3 000
VS ($mg.l^{-1}$)	600	4 500	2 321	1 800	1 000	4 400	2 276	1 200
TNVS ($mg.l^{-1}$)	600	7 000	3 164	2 500	900	6 200	2 915	2 000
SO_4 ($mg.l^{-1}$)	169	2 300	732	648	58	3 000	659	584
PO_4-P ($mg.l^{-1}$ as P)	0.1	26.8	5.1	3.6	3.3	26.8	11.0	6.2
TKN ($mg.l^{-1}$)	153	723	425	242	309	680	465	144
TAlk ($mg.l^{-1}$ as $CaCO_3$)	42	1 909	552	468	42	1 909	830	828
Na ($mg.l^{-1}$)	206	2 557	819	619	201	1 944	800	576
Ca ($mg.l^{-1}$)	183	376	240	60	81	512	253	165
Total metals ($mg.l^{-1}$)	0.6	7.9	2.8	2.2	0.4	6.3	2.3	1.9

SD = Standard deviation

Table 4a Composition of the substrate used during the single-phase operating conditions of digester AD-1, as a function of organic loading rate (OLR)*.

Digester Substrate													
OLR ($kg\ COD.m^{-3}.d^{-1}$)	COD ($mg.l^{-1}$)	TKN ($mg.l^{-1}$)	VFA ($mg.l^{-1}$)	SO_4^{2-} ($mg.l^{-1}$)	pH	TAlk ($mg.l^{-1}$ as $CaCO_3$)	TS ($mg.l^{-1}$)	VS ($mg.l^{-1}$)	TNVS ($mg.l^{-1}$)	PO_4-P ($mg.l^{-1}$)	Tmetals ($mg.l^{-1}$)	Na ($mg.l^{-1}$)	Ca ($mg.l^{-1}$)
1.89	1891	414	1004	N/D	6.5	284	10600	4500	6100	3.6	2.30	1982	N/D
1.98	1976	226	306	390	6.9	186	3300	1400	1900	2.1	3.80	633	N/D
2.02	2021	320	868	N/D	6.6	654	3400	800	2600	2.1	2.80	439	205
2.04	2040	410	272	376	6.6	769	4900	1600	3300	8.3	1.31	206	N/D
2.04	2040	350	1089	169	6.6	699	1400	600	800	1.8	1.24	338	N/D
2.13	2133	153	170	1120	6.5	140	5500	1400	4100	2.1	1.43	391	202
2.17	2172	580	323	307	6.8	42	3900	1600	2300	6.6	1.69	740	N/D
2.21	2212	580	375	365	6.6	416	3900	1600	2300	10.3	1.33	756	N/D
2.37	2367	260	536	750	6.7	160	4600	1700	2900	0.2	2.06	843	N/D
2.45	2445	512	383	278	6.8	749	4000	2100	1900	3.3	1.55	332	N/D
2.46	2459	486	357	485	6.7	836	4200	1900	2300	11.5	1.91	735	N/D
2.53	2534	580	204	597	6.8	874	4700	2000	2700	16.6	2.02	955	N/D
2.54	2539	380	68	186	6.9	724	1900	1300	600	0.8	0.56	460	N/D
2.60	2604	N/D	1208	415	6.9	223	3600	2400	1200	1.1	0.91	455	N/D
2.67	2665	269	740	400	6.7	529	5400	1400	4000	7.3	1.41	256	N/D
3.18	3183	243	468	330	6.6	128	4300	2300	2000	5.5	N/D	N/D	N/D
4.09	4094	214	257	364	6.9	368	4300	2500	1800	0.1	1.23	743	N/D
4.39	4393	356	357	630	6.6	235	7800	2600	5200	3.6	3.77	681	183
4.44	4443	293	272	1200	6.7	154	8100	2300	5800	2.7	3.71	2556	316
4.48	4476	417	596	N/D	6.3	195	5900	2600	3300	5.8	2.34	1032	N/D
4.48	4476	505	834	2300	6.6	546	5900	1600	4300	6.0	5.13	439	211
4.62	4619	511	1770	1300	6.9	910	5200	2600	2600	2.2	3.19	657	242
4.71	4709	512	1226	2200	6.6	943	6700	2400	4300	3.0	3.77	953	205
4.73	4729	338	587	290	6.7	716	3400	2000	1400	1.1	3.57	420	N/D
4.89	4890	385	604	850	6.6	258	9700	3400	6300	5.0	5.98	2037	N/D
4.90	4896	523	570	430	6.8	387	7400	3700	3700	2.1	3.34	1096	N/D
5.32	5321	482	1166	N/D	6.6	364	10400	3400	7000	4.7	3.17	1043	376
5.33	5333	642	536	1730	6.5	1356	4600	1600	3000	6.0	4.35	2035	200
5.46	5464	700	3387	460	7.0	903	4400	2400	2000	2.7	4.81	361	317
5.63	5626	431	800	480	6.6	326	5700	3600	2100	1.5	1.88	694	N/D
6.22	6215	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
6.28	6277	478	766	N/D	6.7	1910	6300	3500	2800	26.8	2.46	1195	N/D
8.34	8343	723	1208	1830	6.7	990	10800	4300	6500	10.7	7.88	300	185
9.56	9560	335	502	260	6.5	238	4800	3500	1300	0.1	2.45	460	N/D
Minimum 1.89	1891	153	68	169	6.3	42	1400	600	600	0.1	0.56	206	183
Maximum 9.56	9560	723	3387	2300	7.0	1910	10800	4500	7000	26.8	7.88	2556	376
Average 3.97	3975	425	722	732	6.7	552	5485	2321	3164	5.1	2.79	819	240

Above data have been arranged according to numerical increases in **OLR, while the numerical structure of **time** has been disregarded for this efficiency evaluation (Fig. 2).*

N/D = Not Determined

Table 4b Composition of the digester AD-1 effluent during the single-phase operating conditions at variable organic loading rates (OLR)*.

Digester Effluent														
OLR (kg COD.m^{-3}.d^{-1})		COD (mg.l^{-1})	TKN (mg.l^{-1})	VFA (mg.l^{-1})	SO_4^{2-} (mg.l^{-1})	pH	TAlk (mg.l^{-1} as $CaCO_3$)	TS (mg.l^{-1})	VS (mg.l^{-1})	TNVS (mg.l^{-1})	PO_4-P (mg.l^{-1})	Tmetals (mg.l^{-1})	Na (mg.l^{-1})	Ca (mg.l^{-1})
1.89		1215	366	664	N/D	7.6	1667	4600	700	3900	9.9	1.15	1514	N/D
1.98		618	207	230	23	8.0	1141	2100	400	1700	7.2	0.96	676	N/D
2.02		959	327	247	N/D	8.0	2097	3000	500	2500	5.8	0.72	462	133
2.04		2970	410	1328	343	7.9	1927	4100	1500	2600	43.7	1.58	446	N/D
2.04		944	331	545	14	8.0	1920	3000	800	2200	35.6	0.96	1094	N/D
2.13		587	161	213	110	7.6	1146	4600	800	3800	3.8	1.31	866	363
2.17		2352	615	936	N/D	8.1	102	4100	1300	2800	44.3	4.12	1089	N/D
2.21		1548	717	851	33	8.1	102	4100	1300	2800	42.4	1.43	962	N/D
2.37		779	287	1268	101	8.1	1301	3900	600	3300	3.8	1.12	1213	N/D
2.45		1327	528	538	28	8.0	2091	3900	1200	2700	37.1	1.50	143	N/D
2.46		3507	615	1430	42	8.0	2316	4900	2000	2900	54.5	1.91	1198	N/D
2.53		1307	615	545	35	8.0	1891	3600	1100	2500	41.0	0.85	818	N/D
2.54		1242	349	340	60	7.7	1596	2000	1100	900	15.0	1.59	362	N/D
2.60		865	N/D	1387	20	8.0	1106	2400	1000	1400	17.4	0.92	614	N/D
2.67		1170	272	528	150	8.4	1967	4100	700	3400	6.5	1.08	193	N/D
3.18		639	201	323	25	7.5	968	2600	400	2200	9.0	N/D	N/D	N/D
4.09		1357	280	647	20	7.9	1592	2600	900	1700	19.7	1.35	477	N/D
4.39		1843	302	323	83	7.9	1640	6300	800	5500	6.4	1.98	1243	83
4.44		2819	331	323	62	7.9	1565	6400	500	5900	6.3	2.26	1463	118
4.48		1112	319	494	N/D	7.5	1264	2800	500	2300	9.7	0.50	650	N/D
4.48		1631	484	970	140	8.4	2009	4300	800	3500	6.7	1.40	413	130
4.62		3557	474	323	175	8.2	2339	3700	1100	2600	4.2	1.62	897	90
4.71		1088	431	409	214	8.4	1879	4200	900	3300	3.5	0.72	998	54
4.73		2935	370	434	20	7.9	1635	2500	1100	1400	14.3	10.44	617	N/D
4.89		2188	353	417	174	8.0	1724	6000	900	5100	7.7	2.23	1607	N/D
4.90		1376	486	374	19	8.0	2088	4100	700	3400	8.9	1.13	993	N/D
5.32		3233	520	1345	N/D	8.2	2730	8600	800	7800	7.7	1.55	1449	216
5.33		1560	688	409	160	8.3	2618	2400	900	1500	7.0	1.48	1594	191
5.46		2424	587	1872	70	8.3	3342	6800	1300	5500	10.4	6.50	340	119
5.63		1444	440	655	43	7.7	1919	3300	1000	2300	9.9	2.70	720	N/D
6.22		2357	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
6.28		3447	341	1208	N/D	8.0	2325	5100	2100	3000	58.3	2.69	1135	N/D
8.34		3214	675	817	140	7.8	3318	7300	1500	5800	9.6	3.77	301	133
9.56		957	378	340	20	7.6	1252	1700	400	1300	9.7	1.16	973	N/D
Minimum	1.89	587	161	213	14	7.5	102	1700	400	900	3.5	0.50	143	54
Maximum	9.56	3557	717	1872	343	8.4	3342	8600	2100	7800	58.3	10.44	1607	363
Average	3.97	1782	421	689	86	8.0	1775	4094	958	3136	17.5	2.02	860	148

*Above data have been arranged according to numerical increases in **OLR**, while the numerical structure of **time** has been disregarded for this efficiency evaluation (Fig. 2).
N/D = Not Determined

Table 4c The influence of variable organic loading rates (OLR) on digester AD-1 performance.

Digester Efficiency																		
OLR ($kg\ COD.m^{-3}.d^{-1}$)		COD removal (%)	COD removal rate ($kg\ COD.m^{-3}.d^{-1}$)	SO_4^{2-} removal (%)	Biogas production (l/d)	Biogas yield ($m^3.m^{-3}$)	CH_4 content (%)	CH_4 yield ($m^3.kg\ COD_{rem}.d^{-1}$)	CH_4 yield ($m^3.kg\ COD_{load}.d^{-1}$)	VFA removal (%)	TS removal (%)	VS removal (%)	TNVS removal (%)	TKN removal (%)	PO_4-P removal (%)	Tmetal removal (%)	Na removal (%)	Ca removal (%)
1.89		35.7	0.68	N/D	5.60	1.12	86.4	7.16	2.56	33.9	56.6	84.4	36.1	11.6	0.0	50.0	23.6	N/D
1.98		68.7	1.36	94.1	5.60	1.12	83.2	3.43	2.36	25.0	36.4	71.4	10.5	8.7	0.0	74.7	0.0	N/D
2.02		52.5	1.06	N/D	2.28	0.46	N/D	N/D	N/D	71.6	11.8	37.5	3.8	0.0	0.0	74.3	0.0	35.1
2.04		0.0	-0.93	8.8	N/D	N/D	N/D	N/D	N/D	0.0	16.3	6.3	21.2	0.0	0.0	0.0	0.0	N/D
2.04		53.7	1.10	91.7	N/D	N/D	N/D	N/D	N/D	50.0	0.0	0.0	0.0	5.5	0.0	22.6	0.0	N/D
2.13		72.5	1.55	90.2	1.70	0.34	N/D	N/D	N/D	0.0	16.4	42.9	7.3	0.0	0.0	8.4	0.0	0.0
2.17		0.0	-0.18	N/D	N/D	N/D	N/D	N/D	N/D	0.0	0.0	18.8	0.0	0.0	0.0	0.0	0.0	N/D
2.21		30.0	0.66	91.0	N/D	N/D	N/D	N/D	N/D	0.0	0.0	18.8	0.0	0.0	0.0	0.0	0.0	N/D
2.37		67.1	1.59	86.5	6.10	1.22	84.1	3.23	2.17	0.0	15.2	64.7	0.0	0.0	0.0	45.6	0.0	N/D
2.45		45.7	1.12	89.9	N/D	N/D	N/D	N/D	N/D	0.0	2.5	42.9	0.0	0.0	0.0	3.2	56.9	N/D
2.46		0.0	-1.05	91.3	N/D	N/D	N/D	N/D	N/D	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	N/D
2.53		48.4	1.23	94.1	N/D	N/D	N/D	N/D	N/D	0.0	23.4	45.0	7.4	0.0	0.0	57.9	14.3	N/D
2.54		51.1	1.30	67.7	2.30	0.46	76.2	1.35	0.69	0.0	0.0	15.4	0.0	8.0	0.0	0.0	21.3	N/D
2.60		66.8	1.74	95.2	1.50	0.30	73.1	0.63	0.42	0.0	33.3	58.3	0.0	N/D	0.0	0.0	0.0	N/D
2.67		56.1	1.50	62.5	3.07	0.61	N/D	N/D	N/D	28.7	24.1	50.0	15.0	0.0	10.6	23.4	24.6	N/D
3.18		79.9	2.54	92.4	8.20	1.64	80.4	2.59	2.07	30.9	39.5	82.6	0.0	17.3	0.0	N/D	N/D	N/D
4.09		66.9	2.74	94.5	1.60	0.32	69.7	0.41	0.27	0.0	39.5	64.0	5.6	0.0	0.0	0.0	35.8	N/D
4.39		58.0	2.55	86.8	6.02	1.20	76.9	1.82	1.05	9.5	19.2	69.2	0.0	15.1	0.0	47.5	0.0	54.9
4.44		36.6	1.62	94.8	6.08	1.22	84.4	3.16	1.16	0.0	21.0	78.3	0.0	0.0	0.0	39.1	42.8	62.5
4.48		75.2	3.36	N/D	6.90	1.38	87.6	1.80	1.35	17.1	52.5	80.8	30.3	23.4	0.0	78.6	37.0	N/D
4.48		63.6	2.85	93.9	4.12	0.82	N/D	N/D	N/D	0.0	27.1	50.0	18.6	4.2	0.0	72.7	6.0	38.3
4.62		23.0	1.06	86.5	3.89	0.78	N/D	N/D	N/D	81.7	28.8	57.7	0.0	7.3	0.0	49.2	0.0	62.8
4.71		76.9	3.62	90.3	5.59	1.12	N/D	N/D	N/D	66.7	37.3	62.5	23.3	15.8	0.0	80.9	0.0	73.7
4.73		37.9	1.79	93.1	3.54	0.71	N/D	N/D	N/D	26.1	26.5	45.0	0.0	0.0	0.0	0.0	0.0	N/D
4.89		55.3	2.70	79.5	6.40	1.28	73.1	1.73	0.96	31.0	38.1	73.5	19.0	8.4	0.0	62.7	21.1	N/D
4.90		71.9	3.52	95.6	5.70	1.14	85.1	1.38	0.99	34.3	44.6	81.1	8.1	7.2	0.0	66.2	9.5	N/D
5.32		39.2	2.09	N/D	6.44	1.29	N/D	N/D	N/D	0.0	17.3	76.5	0.0	0.0	0.0	51.1	0.0	42.4
5.33		70.7	3.77	90.8	3.85	0.77	N/D	N/D	N/D	23.8	47.8	43.8	50.0	0.0	0.0	66.0	21.7	4.5
5.46		55.6	3.04	84.8	11.04	2.21	N/D	N/D	N/D	44.7	0.0	45.8	0.0	16.2	0.0	0.0	5.7	62.4
5.63		74.3	4.18	91.0	5.80	1.16	79.1	1.10	0.82	18.1	42.1	72.2	0.0	0.0	0.0	0.0	0.0	N/D
6.22		62.1	3.86	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
6.28		45.1	2.83	N/D	N/D	N/D	N/D	N/D	N/D	0.0	19.0	40.0	0.0	28.6	0.0	0.0	5.0	N/D
8.34		61.5	5.13	92.3	3.26	0.65	N/D	N/D	N/D	32.4	32.4	65.1	10.8	6.6	10.7	52.2	0.0	27.9
9.56		90.0	8.60	92.3	8.70	1.74	83.2	0.84	0.76	32.2	64.6	88.6	0.0	0.0	0.0	52.7	0.0	N/D
Minimum	1.89	0.0	-1.05	8.8	1.50	0.30	69.7	0.41	0.27	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Maximum	9.56	90.0	8.60	95.6	11.04	2.21	87.6	7.16	2.56	81.7	64.6	88.6	50.0	28.6	10.7	80.9	56.9	73.7
Average	3.97	52.7	2.19	86.0	5.01	1.00	80.2	2.19	1.26	19.9	25.3	52.5	8.1	5.7	0.6	33.7	10.2	42.2

N/D = Not Determined

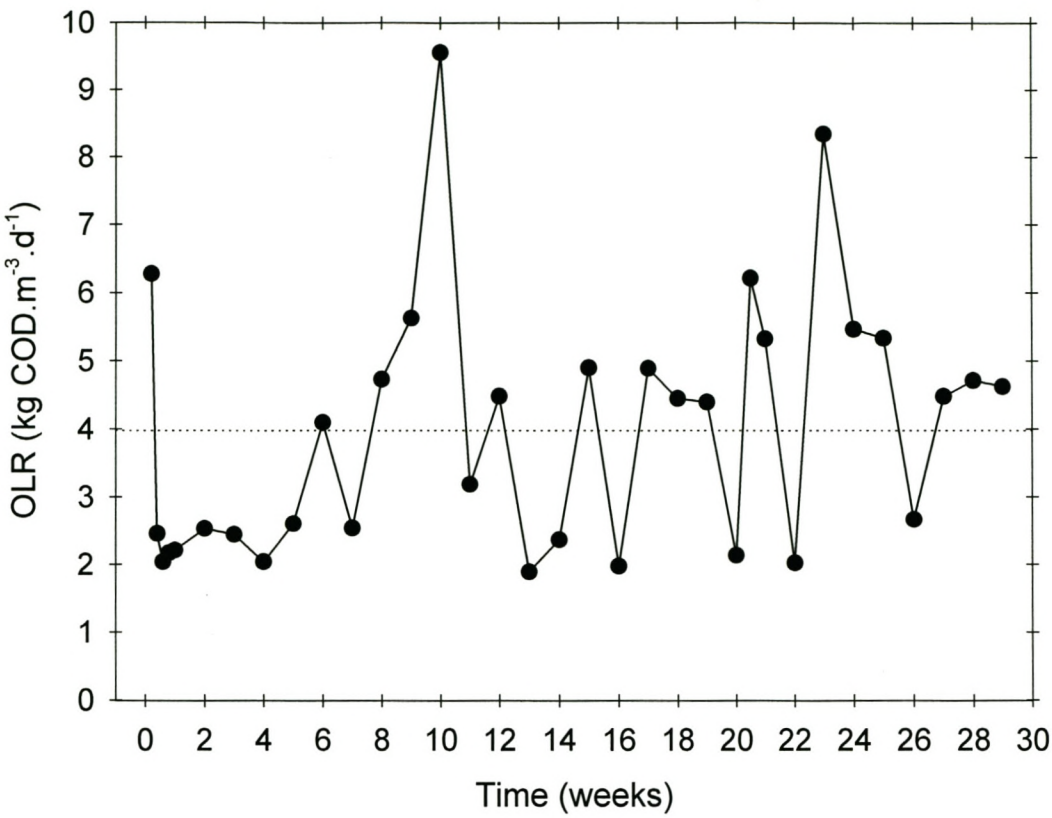

Figure 2. The variation in substrate OLR during the study on digester AD-1 over a 29 week period. The dotted line represents the average OLR.

In Fig. 3, the COD removal and COD removal rates (R-value) are plotted as a function of the OLR. The highest COD removal (90%), as well as the highest R-value (8.60 kg $COD.m^{-3}.d^{-1}$), were found at the higher OLR of 9.56 kg $COD.m^{-3}.d^{-1}$. The COD removal steadily increased from 40% at an OLR of 2 - 3 kg $COD.m^{-3}.d^{-1}$ to 80 % at an OLR of 4.5 - 6.0 kg $COD.m^{-3}.d^{-1}$ and finally to 88% at an OLR of 6.5 - 10 kg $COD.m^{-3}.d^{-1}$. Similarly, the R-value increased as the OLR increased from 2 kg $COD.m^{-3}.d^{-1}$ at an OLR of 2 - 3 kg $COD.m^{-3}.d^{-1}$ to 8.6 kg $COD.m^{-3}.d^{-1}$ at an OLR of 8.5 - 10 kg $COD.m^{-3}.d^{-1}$. The data indicates that the polyethylene hybrid digester design (AD-1) clearly had the potential to maintain a high biomass retention, resulting in an increased substrate utilisation at higher OLR's (Fig. 3).

The biogas yield of digester AD-1 was, however, found to increase slowly, but in contrast, the methane yield (both removed and loaded) decreased as the OLR increased (Fig. 4). The calculation of the methane yield (loaded) was based on the OLR, whilst the calculation of the methane yield (removed) was based on the R-values which explains why the CH_4 yield values are decreasing as the OLR and R-values increases. The total methane yields per COD removed varied between 0.41 and 7.16 $m^3.kg\ COD_{removed}.d^{-1}$ for AD-1 at OLR's of 1.89 to 9.56 kg $COD.m^{-3}.d^{-1}$. The highest biogas yield (2.21 $m^3.m^{-3}$) was observed at an OLR of 5.46 kg $COD.m^{-3}.d^{-1}$, as opposed to the highest methane yield (removed and loaded) at a lower OLR of 1.89 kg $COD.m^{-3}.d^{-1}$.

The average percentage methane produced in digester AD-1 was 80% (Table 4c), with the highest production at an OLR of 4.48 kg $COD.m^{-3}.d^{-1}$ and the lowest at 4.09 kg $COD.m^{-3}.d^{-1}$. The data indicates the presence of an active methanogenic population, which were able to compete with the sulphate-reducing bacteria for the available substrates (Lens *et al.*, 1998).

It was apparent from Fig. 5 that the sulphate removal was fairly stable throughout the study with removal efficiencies up to 96%, showing the presence of an active SRB community working in balance with the methanogens. Maree *et al.* (1990) found that the increase in sulphide was the one environmental factor that was mostly responsible for digester failure while treating gelatin-manufacturing effluent. The high sulphide concentrations led to the inhibition of the methanogenic bacteria, thus acetate

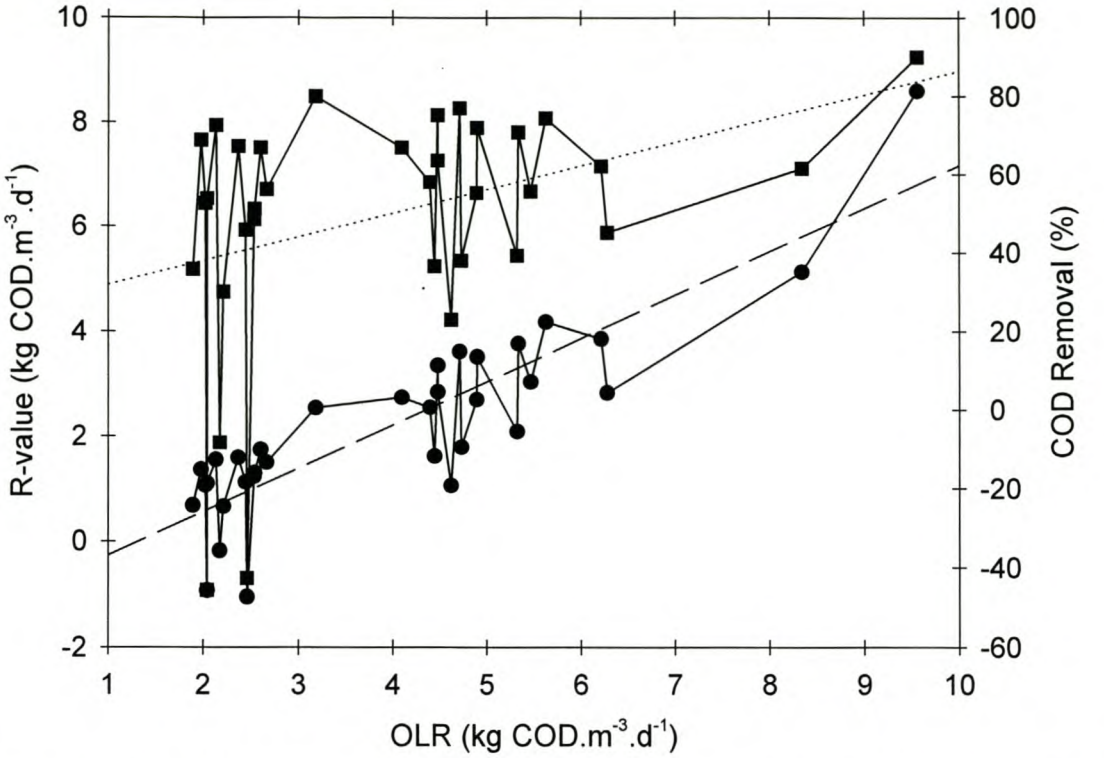

Figure 3. The COD removal rate (R-value) (● ; ----) and COD removal (■ ; .....) as a function of OLR for digester AD-1. The solid lines represent the actual data and the dotted and dashed lines represent the regression calculations.

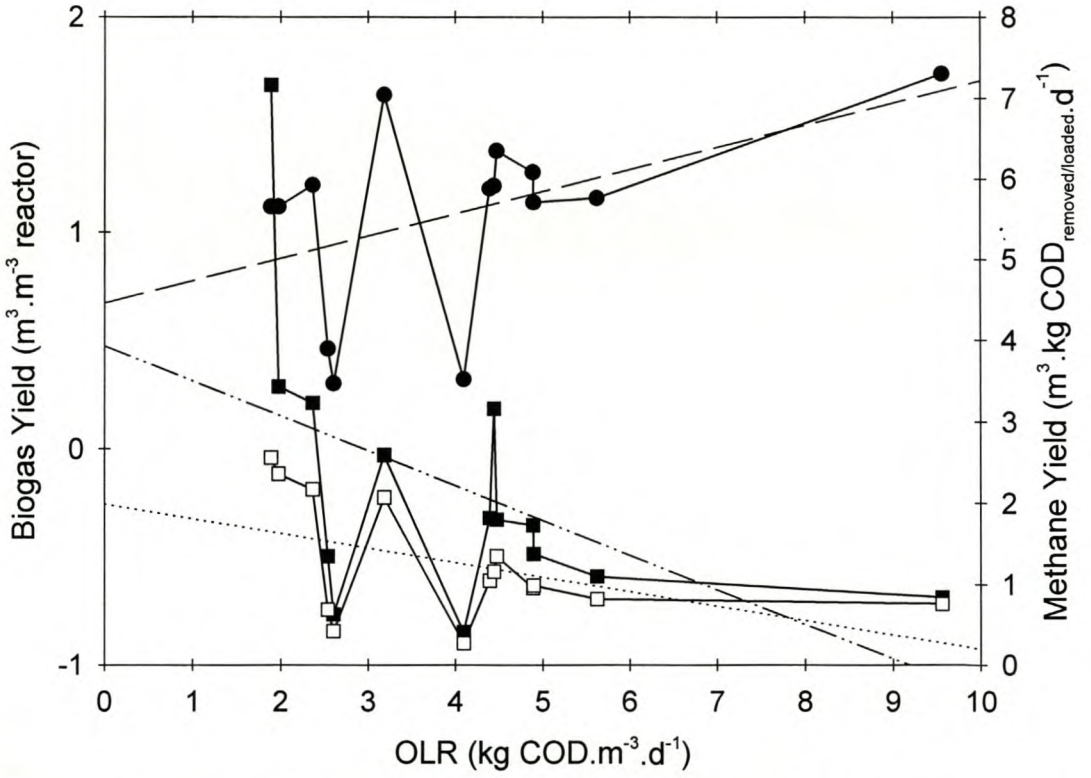

Figure 4. The influence of varying OLR's on biogas yield (● ; -----), methane yield per $COD_{removed}$ (■ ; —·—·—) and methane yield per COD_{loaded} (□ ; .....) in AD-1. The solid lines represent the actual data and the dotted and dashed lines represent the regression calculations.

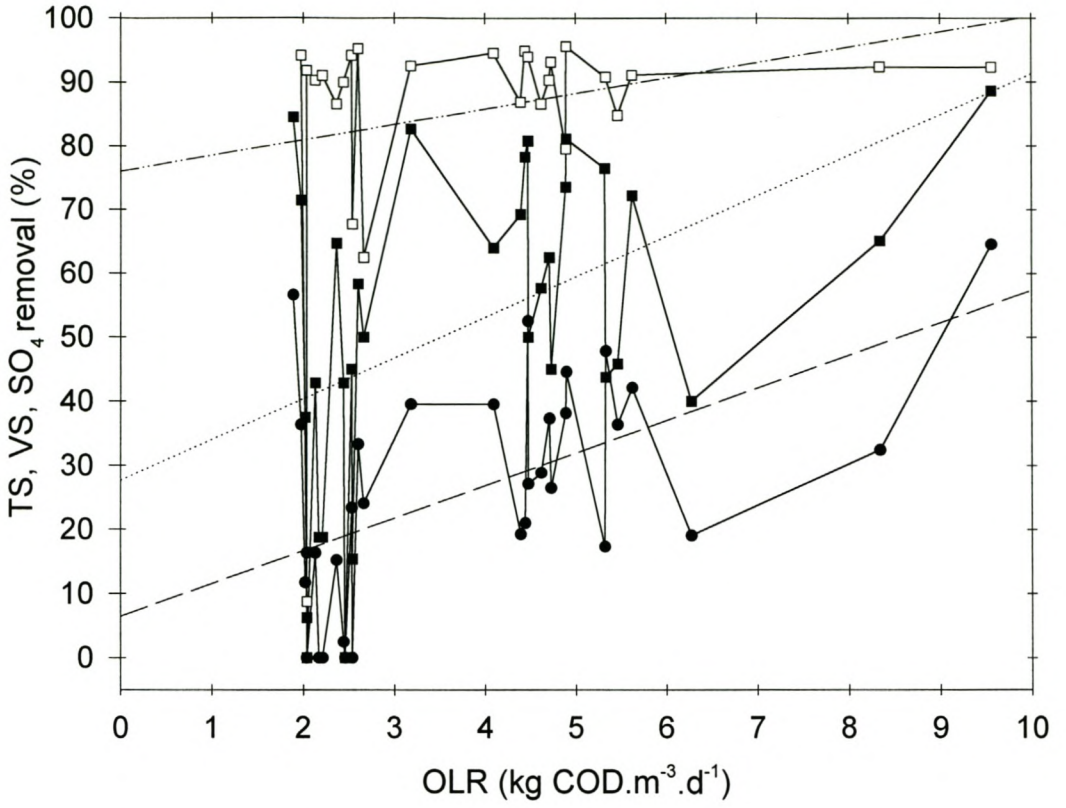

Figure 5. The TS (● ; -----), VS (■ ; …) and SO_4 (□ ; —··—··—) removal, as a function of OLR in digester AD-1. The solid lines represent the actual data and the dotted and dashed lines represent the regression calculations.

was not utilised effectively and led to the souring of the digester (Lens *et al.*, 1998).

The TS and VS removal efficiencies (Fig. 5) generally increased with an increase in OLR. The highest TS and VS removal efficiencies were again, as for the COD removal, found at an OLR of 9.56 kg $COD.m^{-3}.d^{-1}$ (65 and 89%, respectively).

During this study no consistency in TKN, total metals and TNVS removals were observed (Table 4c). The remaining nitrogen amounts indicated insufficient protein or amino acid degradation which involves the production of VFA and ammonia. This also explains why no significant accumulation of VFA were found which may led to digester failure (Okamoto *et al.*, 2000). This was contradictory to the findings of Maree *et al.* (1990), who reported a gradual accumulation of VFA, which subsequently became toxic to the microbial population. However, it was also clear from this study that significant amounts of VFA, phosphate and nitrogen were still present and unutilised, in the effluent (Table 4b), suggesting additional alternative experimentation at shorter HRT's or by using a multi-phase digester configuration.

The alkalinity of the digester was found to vary between 1 000 and 3 300 $mg.l^{-1}$, indicating a good buffering capacity. This exceeded the recommended limit of 1 500 $mg.l^{-1}$ (Hawkes *et al.*, 1992). The data in Fig. 6 indicates a steady increase in alkalinity, as a function of increasing OLR's up to OLR of 8.34 kg $COD.m^{-3}.d^{-1}$. The alkalinity was then found to decrease drastically at higher OLR values, suggesting that the threshold buffering capacity of the system had been reached. In Fig. 7, it can be seen that the effluent pH remained (to some extent) a function of the VFA concentration in the effluent, but did not necessarily reflect the increase in OLR (Table 4b).

Digester Efficiency (AD-2)

The composition of the digester substrate, the effluent and the efficiency during the operational period of 29 weeks, are given in Tables 5a, b and c. The data in Fig. 8 again illustrates the large variation in OLR's. When comparing the data of Fig. 2 with the data of Fig. 8, it is clear that the OLR's for digester AD-1 was generally higher than the OLR's for digester AD-2 (Fig.

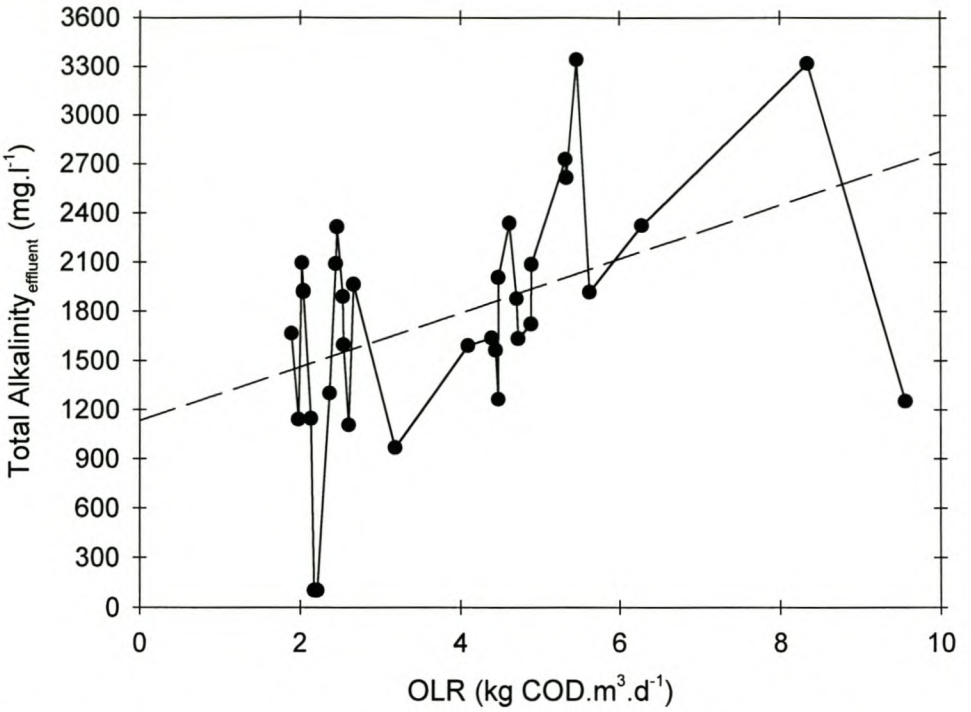

Figure 6. The variation of the total alkalinity (● ; -----) of the effluent, as a function of OLR for digester AD-1. The solid line represents the actual data and the dashed line represents the regression calculation.

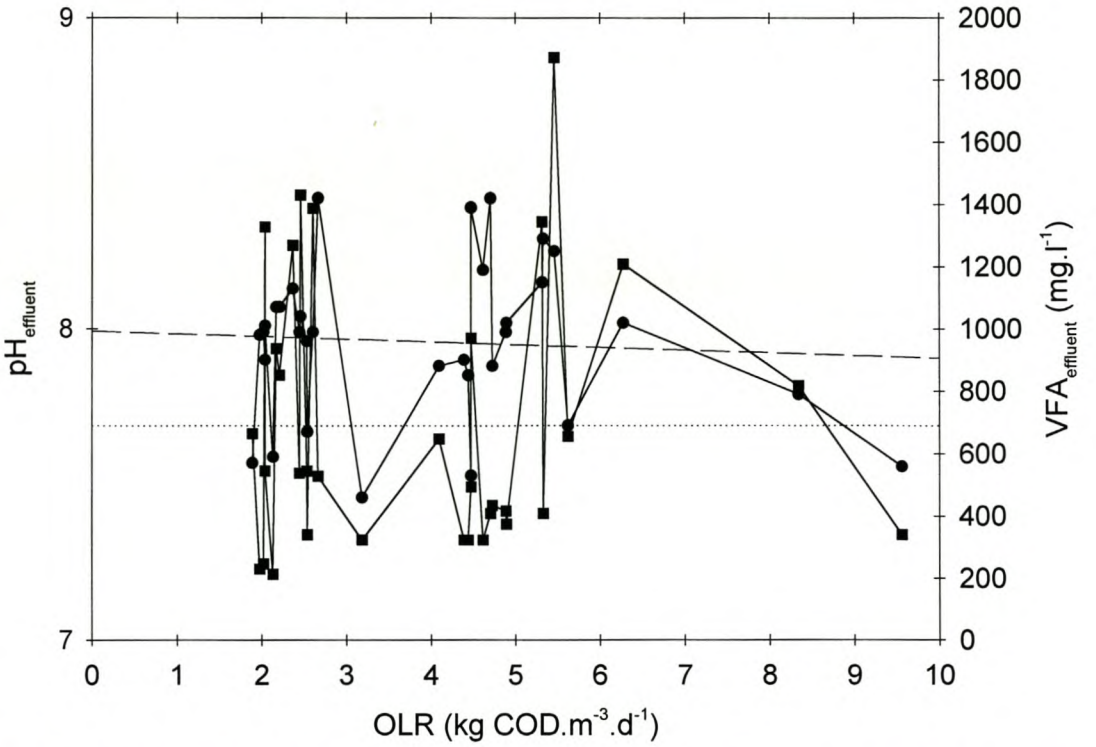

Figure 7. The variation of the reactor effluent pH (● ; -----) and the VFA content (■ ; .....) of the effluent, as a function of OLR for digester AD-1. The solid lines represent the actual data and the dotted and dashed lines represent the regression calculations.

Table 5a Composition of the substrate used during the single-phase operating conditions of digester AD-2, as a function of organic loading rate (OLR)*.

Digester Substrate														
OLR (kg COD.m^{-3}.d^{-1})		COD (mg.l^{-1})	TKN (mg.l^{-1})	VFA (mg.l^{-1})	SO_4^{2-} (mg.l^{-1})	pH	TAlk (mg.l^{-1} as $CaCO_3$)	TS (mg.l^{-1})	VS (mg.l^{-1})	TNVS (mg.l^{-1})	PO_4-P (mg.l^{-1})	Tmetals (mg.l^{-1})	Na (mg.l^{-1})	Ca (mg.l^{-1})
1.48		1477	413	953	N/D	7.2	771	4700	1700	3000	12.6	1.35	1130	N/D
1.76		1756	446	800	58	7.0	699	2100	1000	1100	16.9	0.91	350	N/D
1.87		1868	309	391	410	7.1	541	3100	1300	1800	10.5	3.03	600	N/D
2.04		2040	410	272	376	7.2	769	4900	1600	3300	8.3	1.31	206	N/D
2.11		2111	347	323	550	7.0	583	4000	1400	2600	4.5	0.94	948	N/D
2.17		2172	580	323	307	7.8	42	3900	1600	2300	6.6	1.69	740	N/D
2.21		2212	580	375	365	7.8	416	3900	1600	2300	10.3	1.33	756	N/D
2.45		2445	512	383	278	7.2	749	4000	2100	1900	3.3	1.55	332	N/D
2.46		2459	486	357	485	7.2	836	4200	1900	2300	11.5	1.91	735	N/D
2.53		2534	580	204	597	7.6	874	4700	2000	2700	16.6	2.02	955	N/D
2.81		2806	N/D	1753	257	7.3	774	3800	2400	1400	5.3	0.88	392	N/D
2.82		2824	512	477	70	6.9	1242	2100	1200	900	4.6	0.41	485	N/D
2.94		2944	533	1634	2150	7.0	1117	4600	2300	2300	7.6	3.19	516	202
3.09		3086	355	366	580	6.9	381	6200	2100	4100	15.2	2.04	1441	81
3.16		3156	521	834	1700	8.3	1036	5400	1600	3800	4.6	2.58	842	154
3.16		3157	347	128	790	7.0	461	6500	1800	4700	14.7	2.93	1642	323
3.19		3186	321	179	1090	6.9	604	6600	2600	4000	11.0	1.20	789	512
3.59		3593	347	1668	200	7.2	1044	4100	2300	1800	3.9	1.31	311	N/D
3.62		3620	365	689	200	7.1	721	4800	2600	2200	14.8	N/D	N/D	N/D
3.73		3726	360	936	320	7.2	852	5600	2000	3600	15.7	1.37	201	N/D
3.82		3818	448	1208	N/D	7.0	645	5100	2500	2600	14.7	1.74	770	N/D
3.93		3926	511	528	330	7.1	670	6200	2900	3300	4.8	1.84	1286	N/D
4.08		4080	366	1030	260	7.2	1032	3700	2200	1500	12.7	3.96	525	N/D
4.32		4321	440	826	650	7.2	631	7800	2700	5100	10.5	3.77	1936	N/D
4.38		4376	545	596	3000	7.3	894	6300	3200	3100	10.8	3.95	610	196
4.48		4481	676	1064	310	7.1	1165	4300	2300	2000	9.1	2.90	390	325
4.62		4623	370	587	300	6.9	693	4700	3300	1400	14.2	1.67	999	N/D
4.65		4646	537	1447	N/D	7.2	772	9000	2800	6200	9.2	2.34	1157	340
4.77		4775	482	877	360	6.9	733	5300	3200	2100	13.3	2.83	488	N/D
5.00		4997	412	1864	N/D	7.1	1310	6900	2600	4300	4.5	2.58	616	143
5.64		5639	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
5.74		5740	607	834	1180	7.6	1299	6400	2400	4000	17.6	5.52	1944	366
6.28		6277	478	766	N/D	7.2	1910	6300	3500	2800	26.8	2.46	1195	N/D
9.30		9300	680	2264	1280	7.2	1108	10100	4400	5700	16.4	6.25	320	144
Minimum	1.48	1477	309	128	58	6.9	42	2100	1000	900	3.3	0.41	201	81
Maximum	9.30	9300	680	2264	3000	8.3	1910	10100	4400	6200	26.8	6.25	1944	512
Average	3.65	3652	465	816	659	7.2	829	5191	2276	2915	11.0	2.31	800	253

Above data have been arranged according to numerical increases in **OLR, while the numerical structure of **time** has been disregarded for this efficiency evaluation (Fig. 8).*
N/D = Not Determined

Table 5b Composition of the digester AD-2 effluent during the single-phase operating conditions at variable organic loading rates (OLR)*.

Digester Effluent														
OLR (kg COD.m^{-3}.d^{-1})		COD (mg.l^{-1})	TKN (mg.l^{-1})	VFA (mg.l^{-1})	SO_4^{2-} (mg.l^{-1})	pH	TAlk (mg.l^{-1} as $CaCO_3$)	TS (mg.l^{-1})	VS (mg.l^{-1})	TNVS (mg.l^{-1})	PO_4-P (mg.l^{-1})	Tmetals (mg.l^{-1})	Na (mg.l^{-1})	Ca (mg.l^{-1})
1.48		777	375	681	N/D	8.1	1731	3300	500	2800	17.7	0.72	1031	N/D
1.76		880	461	494	19	8.0	1921	2800	800	2000	38.4	0.86	698	N/D
1.87		547	301	238	12	8.0	1330	1900	300	1600	11.5	0.97	636	N/D
2.04		970	546	562	N/D	7.9	1856	3800	1100	2700	38.3	1.17	449	N/D
2.11		662	370	417	79	7.7	1477	3200	400	2800	7.5	1.18	987	N/D
2.17		1950	615	460	N/D	8.1	95	3600	900	2700	34.1	1.27	699	N/D
2.21		1729	785	579	29	8.1	95	3600	900	2700	30.2	0.92	894	N/D
2.45		1267	589	519	35	8.1	2123	4300	1100	3200	48.3	1.04	130	N/D
2.46		2511	683	1379	48	8.1	2432	5000	2000	3000	59.0	1.64	455	N/D
2.53		1367	990	528	187	8.0	1749	3800	1000	2800	41.4	0.96	151	N/D
2.81		952	N/D	1617	29	8.0	1449	2300	1000	1300	14.1	0.68	471	N/D
2.82		1569	482	664	140	7.5	761	2200	1200	1000	10.9	0.82	405	N/D
2.94		1358	506	562	130	8.0	2392	3200	800	2400	8.0	1.78	768	63
3.09		1257	373	340	94	7.9	1702	5100	600	4500	12.6	1.34	821	193
3.16		763	454	391	202	8.3	2019	3900	700	3200	7.5	0.55	915	62
3.16		1026	363	221	21	8.0	1728	5300	500	4800	11.7	1.78	1692	184
3.19		586	251	281	190	7.7	1196	4000	800	3200	5.1	0.97	989	255
3.59		1279	243	2111	102	7.8	1805	2600	900	1700	13.9	1.46	694	N/D
3.62		695	278	519	20	7.6	1249	2600	500	2100	16.4	N/D	N/D	N/D
3.73		1107	404	468	90	8.5	2564	3900	900	3000	9.4	0.63	132	N/D
3.82		842	373	596	N/D	7.8	1492	2200	500	1700	19.4	0.46	564	N/D
3.93		891	500	264	9	8.0	2114	3800	500	3300	10.3	0.83	820	N/D
4.08		1481	404	400	20	7.8	1650	2400	1100	1300	25.9	13.94	430	N/D
4.32		1279	403	451	173	8.0	1897	4900	600	4300	12.2	1.65	1461	N/D
4.38		2009	469	1089	90	8.5	2674	3400	700	2700	10.1	1.08	454	171
4.48		2368	592	953	120	8.2	3030	6000	1400	4600	13.3	2.62	361	186
4.62		787	394	494	6	7.6	1527	1800	400	1400	20.7	1.67	1010	N/D
4.65		2499	547	536	N/D	8.2	2625	7800	900	6900	8.8	1.22	2001	331
4.77		1421	483	596	8	7.7	2064	3200	1000	2200	21.2	2.47	469	N/D
5.00		941	363	391	N/D	8.1	2907	4500	800	3700	5.7	0.56	592	39
5.64		1615	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
5.74		1546	591	417	150	8.2	3008	2800	900	1900	1.5	1.13	1362	235
6.28		1873	341	1591	N/D	7.9	2141	4700	1900	2800	45.0	1.56	1200	N/D
9.30		2628	535	443	140	7.8	2955	6500	1500	5000	15.0	2.45	340	119
Minimum	1.48	547	243	221	6	7.5	95	1800	300	1000	1.5	0.46	130	39
Maximum	9.30	2628	990	2111	202	8.5	3030	7800	2000	6900	59.0	13.94	2001	331
Average	3.65	1336	471	644	82	8.0	1871	3770	882	2888	19.5	1.64	752	167

Above data have been arranged according to numerical increases in **OLR, while the numerical structure of **time** has been disregarded for this efficiency evaluation (Fig. 8).*

N/D = Not Determined

Table 5c The influence of variable organic loading rates (OLR) on digester AD-2 performance.

Digester Efficiency																		
OLR ($kg\ COD.m^{-3}.d^{-1}$)	COD removal (%)	COD removal rate ($kg\ COD.m^{-3}.d^{-1}$)	SO_4^{2-} removal (%)	Biogas production (l/d)	Biogas yield ($m^3.m^{-3}$)	CH_4 content (%)	CH_4 yield ($m^3.kg\ COD_{rem}.d^{-1}$)	CH_4 yield ($m^3.kg\ COD_{load}.d^{-1}$)	VFA removal (%)	TS removal (%)	VS removal (%)	TNVS removal (%)	TKN removal (%)	PO_4-P removal (%)	Tmetal removal (%)	Na removal (%)	Ca removal (%)
1.48	47.4	0.70	N/D	5.70	1.14	82.8	6.75	3.20	28.6	29.8	70.6	6.7	9.2	0.0	46.7	8.7	N/D
1.76	49.9	0.88	67.2	N/D	N/D	N/D	N/D	N/D	38.3	0.0	20.0	0.0	0.0	0.0	5.5	0.0	N/D
1.87	70.7	1.32	97.1	3.90	0.78	79.1	2.34	1.65	39.1	38.7	76.9	11.1	2.4	0.0	68.0	0.0	N/D
2.04	52.4	1.07	N/D	N/D	N/D	N/D	N/D	N/D	0.0	22.4	31.3	18.2	0.0	0.0	10.7	0.0	N/D
2.11	68.6	1.45	85.6	5.90	1.18	79.7	3.25	2.23	0.0	20.0	71.4	0.0	0.0	0.0	0.0	0.0	N/D
2.17	10.2	0.22	N/D	N/D	N/D	N/D	N/D	N/D	0.0	7.7	43.8	0.0	0.0	0.0	24.9	5.5	N/D
2.21	21.8	0.48	92.1	N/D	N/D	N/D	N/D	N/D	0.0	7.7	43.8	0.0	0.0	0.0	30.8	0.0	N/D
2.45	48.2	1.18	87.4	N/D	N/D	N/D	N/D	N/D	0.0	0.0	47.6	0.0	0.0	0.0	32.9	60.8	N/D
2.46	0.0	-0.05	90.1	N/D	N/D	N/D	N/D	N/D	0.0	0.0	0.0	0.0	0.0	0.0	14.1	38.1	N/D
2.53	46.0	1.17	68.7	N/D	N/D	N/D	N/D	N/D	0.0	19.1	50.0	0.0	0.0	0.0	52.5	84.2	N/D
2.81	66.1	1.85	88.7	1.20	0.24	69.7	0.45	0.30	7.8	39.5	58.3	7.1	N/D	0.0	22.7	0.0	N/D
2.82	44.5	1.26	0.0	1.90	0.38	73.8	1.12	0.50	0.0	0.0	0.0	0.0	5.9	0.0	0.0	16.5	N/D
2.94	53.9	1.59	94.0	2.68	0.54	N/D	N/D	N/D	65.6	30.4	65.2	0.0	5.0	0.0	44.2	0.0	68.7
3.09	59.3	1.83	83.8	3.10	0.62	87.6	1.49	0.88	7.0	17.7	71.4	0.0	0.0	17.5	34.3	43.1	0.0
3.16	75.8	2.39	88.1	2.67	0.53	N/D	N/D	N/D	53.1	27.8	56.3	15.8	12.8	0.0	78.7	0.0	59.7
3.16	67.5	2.13	97.3	5.72	1.14	88.9	2.39	1.61	0.0	18.5	72.2	0.0	0.0	20.2	39.2	0.0	43.2
3.19	81.6	2.60	82.6	2.00	0.40	N/D	N/D	N/D	0.0	39.4	69.2	20.0	22.0	53.5	19.2	0.0	50.2
3.59	64.4	2.31	49.0	2.10	0.42	68.9	0.63	0.40	0.0	36.6	60.9	5.6	30.0	0.0	0.0	0.0	N/D
3.62	80.8	2.93	90.0	5.40	1.08	81.6	1.51	1.22	24.7	45.8	80.8	4.5	23.8	0.0	N/D	N/D	N/D
3.73	70.3	2.62	71.9	3.43	0.69	N/D	N/D	N/D	50.0	30.4	55.0	16.7	0.0	40.5	54.0	34.2	N/D
3.82	77.9	2.98	N/D	6.80	1.36	83.4	1.91	1.49	50.7	56.9	80.0	34.6	16.8	0.0	73.6	26.8	N/D
3.93	77.3	3.04	97.3	4.10	0.82	80.4	1.09	0.84	50.0	38.7	82.8	0.0	2.1	0.0	54.9	36.2	N/D
4.08	63.7	2.60	92.3	1.29	0.26	N/D	N/D	N/D	61.2	35.1	50.0	13.3	0.0	0.0	0.0	18.1	N/D
4.32	70.4	3.04	73.4	5.94	1.19	71.2	1.39	0.98	45.4	37.2	77.8	15.7	8.4	0.0	56.2	24.5	N/D
4.38	54.1	2.37	97.0	3.86	0.77	N/D	N/D	N/D	0.0	46.0	78.1	12.9	14.0	7.0	72.7	25.6	12.6
4.48	47.2	2.11	61.3	2.71	0.54	N/D	N/D	N/D	10.4	0.0	39.1	0.0	12.4	0.0	9.7	7.6	42.8
4.62	83.0	3.84	98.0	4.10	0.82	82.5	0.88	0.73	15.9	61.7	87.9	0.0	0.0	0.0	0.0	0.0	N/D
4.65	46.2	2.15	N/D	3.83	0.77	N/D	N/D	N/D	62.9	13.3	67.9	0.0	0.0	4.8	47.9	0.0	2.5
4.77	70.2	3.35	97.8	3.80	0.76	78.9	0.89	0.63	32.0	39.6	68.8	0.0	0.0	0.0	12.7	3.9	N/D
5.00	81.2	4.06	N/D	2.46	0.49	N/D	N/D	N/D	79.0	34.8	69.2	14.0	11.8	0.0	78.3	3.9	72.7
5.64	71.4	4.02	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
5.74	73.1	4.19	87.3	6.87	1.37	N/D	N/D	N/D	50.0	56.3	62.5	52.5	2.6	91.4	79.5	30.0	35.9
6.28	70.2	4.40	N/D	N/D	N/D	N/D	N/D	N/D	0.0	25.4	45.7	0.0	28.6	0.0	36.6	0.0	N/D
9.30	71.7	6.67	89.1	2.84	0.57	N/D	N/D	N/D	80.5	35.6	65.9	12.3	21.3	8.2	60.8	0.0	17.1
Minimum 1.48	0.0	-0.05	0.0	1.20	0.24	68.9	0.45	0.30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Maximum 9.30	83.0	6.67	98.0	6.87	1.37	88.9	6.75	3.20	80.5	61.7	87.9	52.5	30.0	91.4	79.5	84.2	72.7
Average 3.65	59.9	2.32	81.8	3.77	0.75	79.2	1.86	1.19	25.8	27.6	58.2	7.9	7.2	7.4	36.3	14.6	36.9

N/D = Not Determined

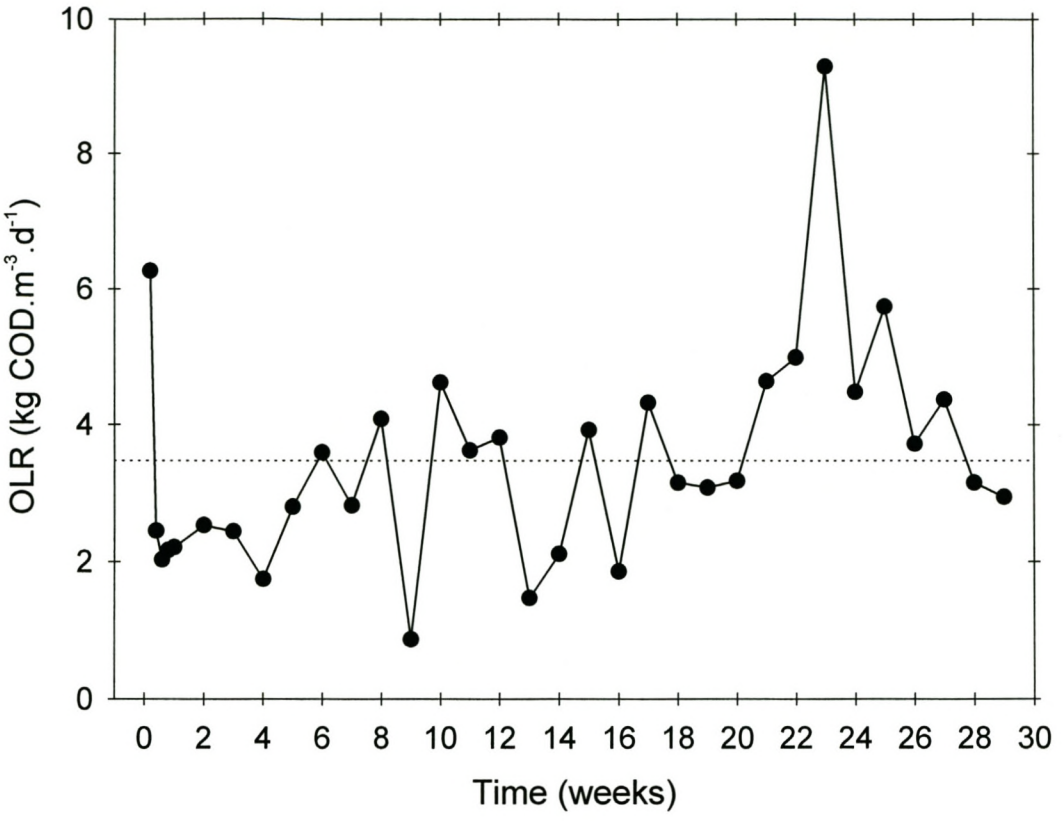

Figure 8. The variation in substrate OLR during the study on digester AD-2 over a 29 week period. The dotted line represents the average OLR.

8). Subsequently, AD-1 functioned at a higher average OLR (3.97 kg COD.m^{-3}.d^{-1}) operating conditions than AD-2 (3.65 kg COD.m^{-3}.d^{-1}), with the highest OLR found during weeks 22 to 24 (9.30 kg COD.m^{-3}.d^{-1}) for digester AD-2.

The COD removal data (Fig. 9) indicated that digester AD-2 gave the highest COD removal (83%) at an OLR of 4.62 kg COD.m^{-3}.d^{-1}, while the best R-values (6.67 kg COD.m^{-3}.d^{-1}) were found at the highest OLR of 9.30 kg COD.m^{-3}.d^{-1}, clearly indicating that the best R-values were obtained at higher OLR's. The R-value can be considered as the amount of COD (in kg) which is effectively removed from the substrate COD per reactor volume (m^3) in one day. The R-value increased as the OLR increased (Fig. 9) from 1.0 kg COD.m^{-3}.d^{-1} at an OLR of 1.5 - 2.5 kg COD.m^{-3}.d^{-1} to 6.67 kg COD.m^{-3}.d^{-1} at an OLR of 6.0 - 9.3 kg COD.m^{-3}.d^{-1}. The COD removal was found to steadily increase from below 60% at an OLR of 1.5 - 2.5 kg COD.m^{-3}.d^{-1} to 80% at an OLR of 6.0 - 9.3 kg COD.m^{-3}.d^{-1}. During the study, it was found that higher COD removal and R-values were obtained for digester AD-1 (90% COD removal at a R-value of 8.60 kg COD.m^{-3}.d^{-1}), when compared to digester AD-2 (83% COD removal and a R-value of 6.67 kg COD.m^{-3}.d^{-1}). It can be concluded that a higher feedload resulted in better digester performance and treatment efficiencies, thus explaining the better performance of digester AD-1. The difference in OLR (substrate strength) can be explained by the dilution effect of the lower-strength supernatant in the digester AD-2 substrate.

The major trends in biogas and methane yields were fairly similar for both digesters (Fig. 4 and 10), with the highest biogas yield of 1.37 m^3.m^{-3} found at an OLR of 5.74 kg COD.m^{-3}.d^{-1} (Table 5c). The highest methane yields (removed and loaded) were observed at a lower OLR (1.48 kg COD.m^{-3}.d^{-1}). The average percentage methane produced was 79% (Table 5c), again indicating the presence of an active methanogenic population in the digester.

In Fig. 11, the SO_4 removal efficiency, with one major exception, was found to be stable, with the highest removal of up to 98%. This indicates an active SRB population present in the digester. The highest TS and VS removals (Fig. 11) of 62 and 88%, respectively were not found at the highest OLR, but at an OLR of 4.62 kg COD.m^{-3}.d^{-1}. This could possibly be as a result of a threshold or saturation value for TS and VS in the system at which

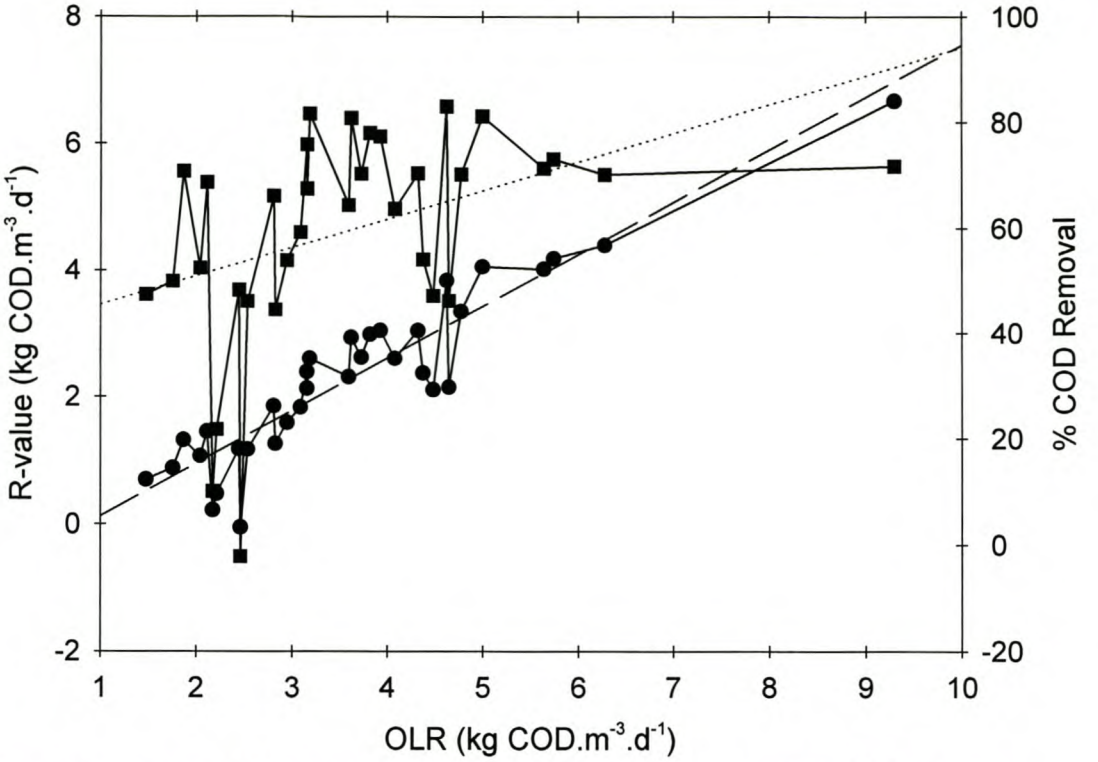

Figure 9. The COD removal rate (R-value) (● ; -----) and COD removal (■ ; .....) as a function of OLR for digester AD-2. The solid lines represent the actual data and the dotted and dashed lines represent the regression calculations.

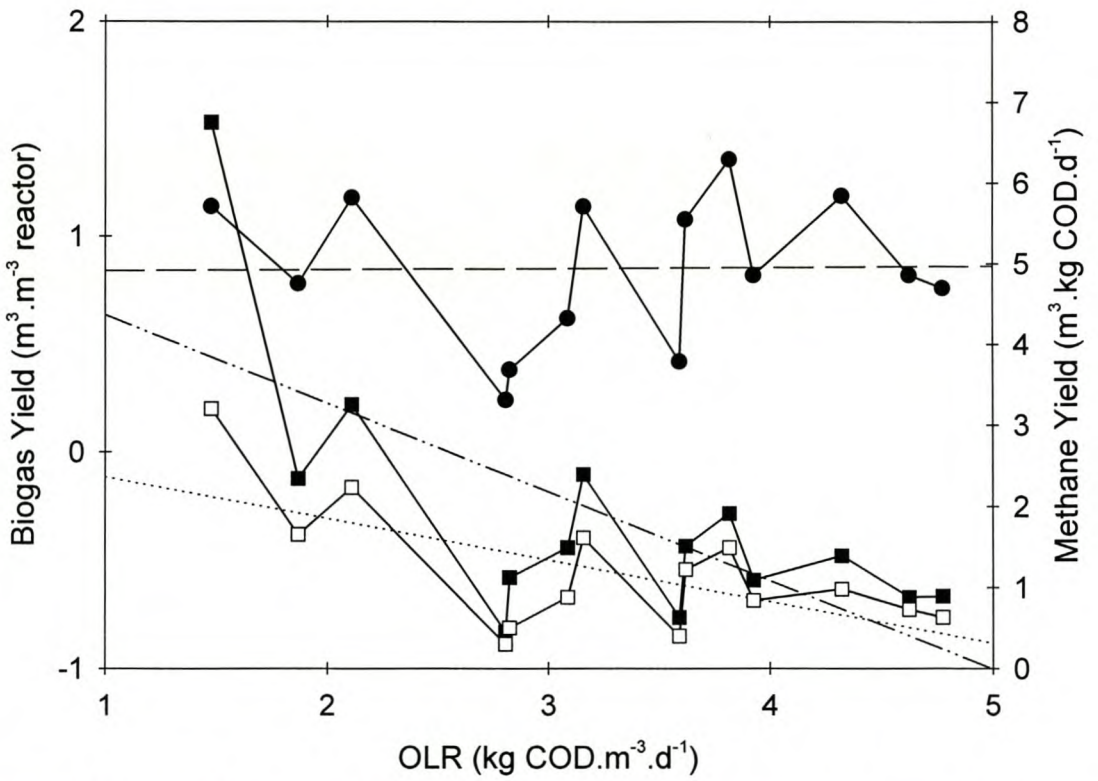

Figure 10. The influence of varying OLR's on biogas yield (● ; -----), methane yield per $COD_{removed}$ (■ ; —··—··—) and methane yield per COD_{loaded} (□ ; ....) for digester AD-2. The solid lines represent the actual data and the dotted and dashed lines represent the regression calculations.

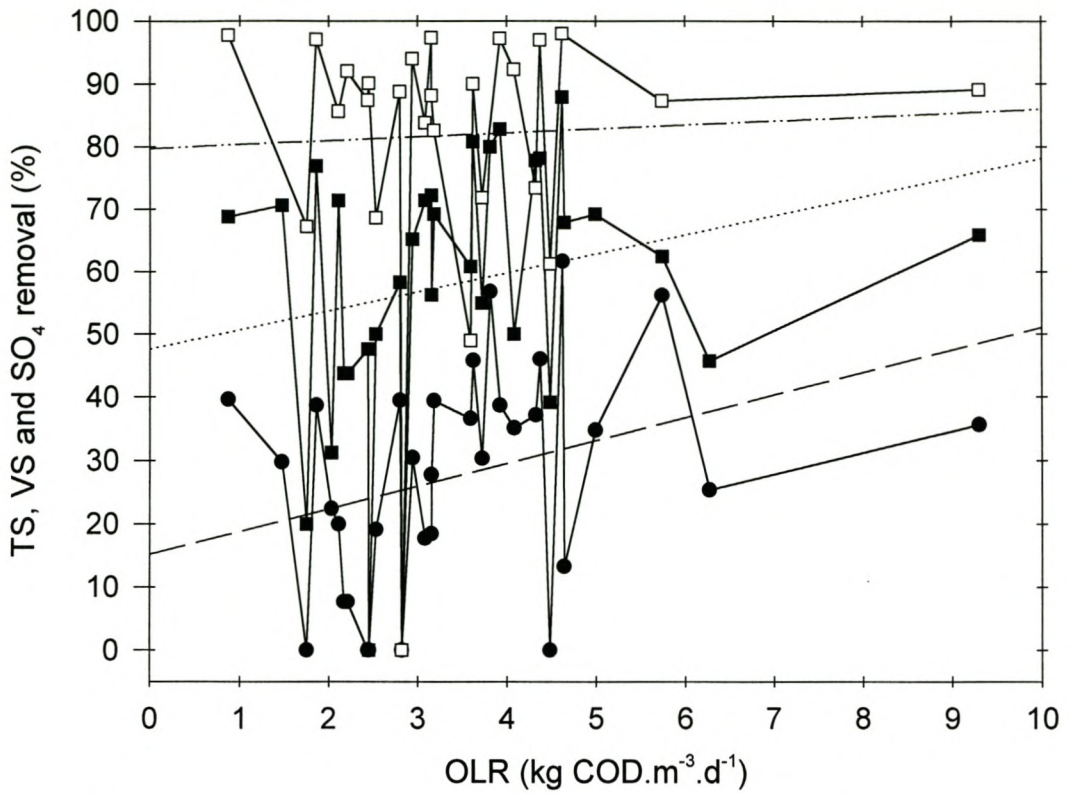

Figure 11. The TS (● ; -----), VS (■ ; .....) and SO_4 (□ ; —··—··—), as a function of OLR for digester AD-2. The solid lines represent the actual data and the dotted and dashed lines represent the regression calculations.

value the TS and VS release will occur. A steady increase in the trend of TS and VS removals were observed with increasing OLR's.

Again no consistency in TKN, total metals and TNVS removal efficiencies were observed (Table 5c). However, the data indicated an improvement in the removal of heavy metals towards the end of the study at an OLR of 5.74 kg $COD.m^{-3}.d^{-1}$. Similarly, no significant VFA accumulation, which are known to be toxic to the microbial community, was observed. There was, however, still VFA present in the digester effluent, which could probably indicate that the concentration of active methanogenic biomass is still to low for the maximum conversion of volatile acids to methane gas (Malina & Pohland, 1992).

The performance tendencies in terms of digester effluent pH and alkalinity are illustrated in Fig. 12 and 13. The digester alkalinity varied considerably between 100 and 3 000 $mg.l^{-1}$ (Table 5b), but generally increased with increasing OLR. The VFA content of the effluent varied between 221 and 2 111 $mg.l^{-1}$ (Table 5b). The effluent pH also varied between 7.53 - 8.51 over the spectrum of OLR (Fig. 13).

Microbial Community

It was reported that the type of support material is important in determining the size and activity of the microbial community colonising the support material (Britz & Van Der Merwe, 1993; Van Der Merwe & Britz, 1993). The ability of the community bacteria, especially the methanogens, when operated under upflow stream conditions, to aggregate into dense particles (granules) is important in the treatment of various industrial wastewaters (Kim *et al.*, 2000). These granules are known to occasionally disintegrate in industrial reactors and result in loss of activity and washout of biomass from the systems (Kosaric *et al.*, 1990). During the studies on digester AD-1, dark coloured and fluffy granules were observed. The average granule diameter was approximately 0.5 - 1.5 mm. Dark granules are typical of digesters treating effluent with high organic loading rates (Kosaric *et al.*, 1990). In contrast, only flocs and no granules were observed in digester AD-2. Although very little is known about the development of sludge granules under continuous upflow conditions, several researchers have reported the

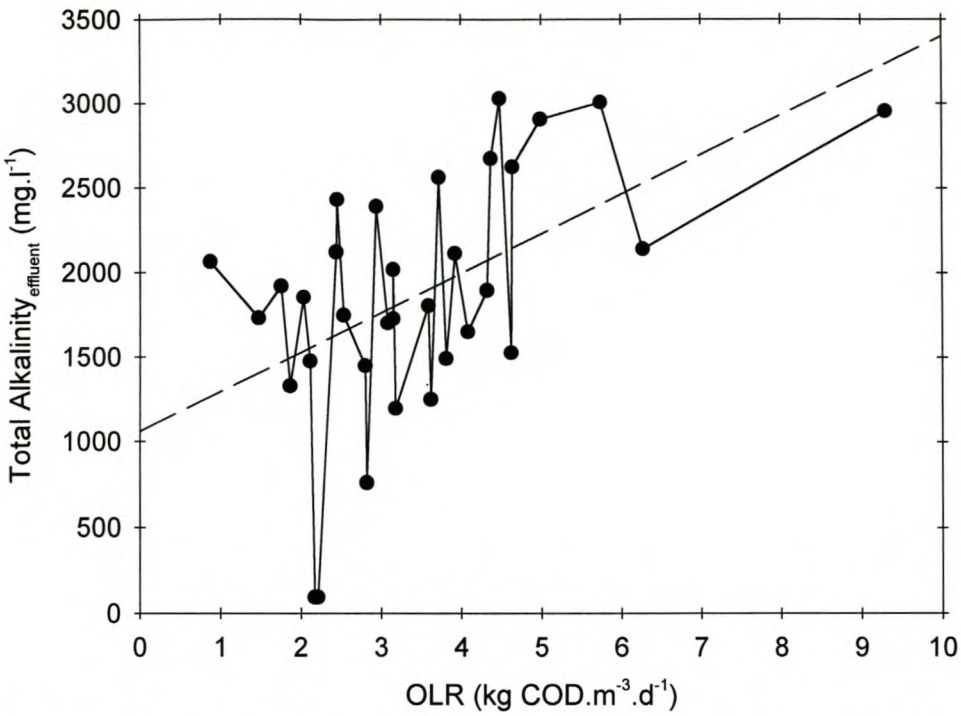

Figure 12. The variation of the total alkalinity (● ; -----) in the digester effluent, as a function of OLR for digester AD-2. The solid line represents the actual data and the dashed lines represent the regression calculation.

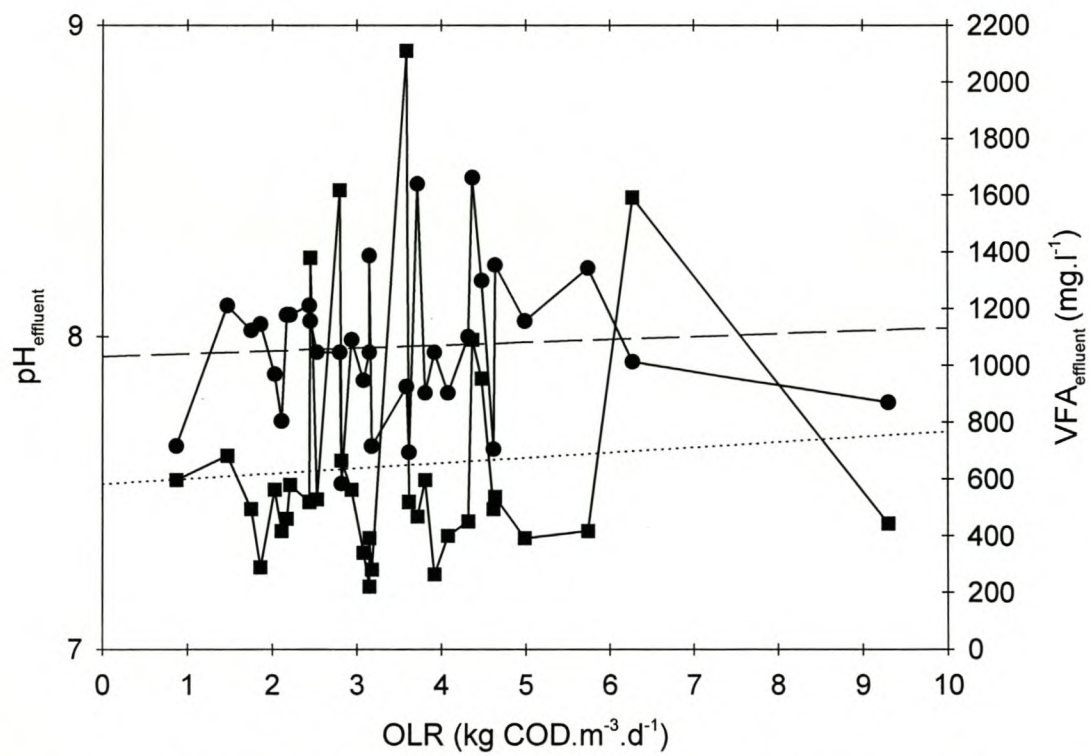

Figure 13. The variation of the pH (● ; -----) and the VFA content (■ ; .....) in the digester effluent, as a function of OLR for digester AD-2. The solid lines represent the actual data and the dotted and dashed lines represent the regression calculations.

presence of bacterial granules in their hybrid digesters (Alibhai & Forster, 1986; Kosaric *et al.*, 1990; Moosbrugger *et al.*, 1993). After a continuous operation of 22 months no signs of clogging in the digesters were observed.

Conclusions

Anaerobic digestion is one of the most promising possibilities for the treatment of recalcitrant waste (Lettinga *et al.*, 1997). Therefore, the disposal strategies must always be based on knowledge of the interactions between organic/inorganic chemicals present in the effluent, wastewater variations over time, digester designs and the condition of the microbial community present in the specific digester. The data from this study demonstrate the potential of the anaerobic hybrid digesters with an increased fixed-bed surface area, to treat a highly variable high-strength, complex and problematic wastewater. The extent of large variations in most of the operational parameters for the two digesters are an indication and reflection of the type of wastewater variation produced by the industry and thus in the substrate fed to the digesters.

The results obtained in this study show that the use of numerical OLR increases resulted in a better representation and indication of the performance efficiency compared to a time function. Higher OLR's generally resulted in higher COD removal rates and biogas yields. For both digesters it were found that extreme variations occurred in the COD removal efficiencies and other parameters, although an increase in biogas yield, alkalinity, TS, VS and SO_4 removal, occurred. This could be an indication of the selection of a suitable and specific microbial community as part of the stabilisation in the digester and subsequent adaptation and enhancement of the microbial degradation properties. Since the study was conducted over an extended period of 29 weeks, it can be assumed that an optimally adapted consortium of microbes were present. A longer operational period may even have resulted in more specific species selection.

The data from this study clearly indicates that anaerobic digestion with upflow anaerobic sludge digester designs can be considered a successful

pre-treatment method for the generally noxious full-strength gelatin-manufacturing effluent, without the need for nutrient supplementation. Implementation of these results on commercial scale, would directly benefit the industry - by reducing the COD and SS concentration in their effluent and thereby also reducing the trade effluent charge. The local wastewater purification works will also benefit by receiving a reduced organic load which has been anaerobically pre-balanced and treated before disposing off to the plant.

The results from this study do present the opportunity for further research. Average concentrations of VFA (689 $mg.l^{-1}$ as acetic acid), phosphate (18 $mg.l^{-1}$ as P) and nitrogen (421 $mg.l^{-1}$) were still present and unutilised in the effluent, suggesting alternative experimentation at shorter HRT's or using a multi-phase digester configuration. With cases of such complex effluents with rate-limiting steps during the treatment process, it will thus be worthwhile to evaluate multi-phase systems in order to facilitate more prominent species differentiation with enhanced degrading abilities.

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CHAPTER 4

TREATMENT OF GELATIN-MANUFACTURING EFFLUENT USING MULTI-PHASE ANAEROBIC HYBRID DIGESTERS

Summary

Multi-phase anaerobic digestion has often been reported to be beneficial for the treatment of complex and high-strength wastewater (Battistoni *et al.*, 2000). It was found by researchers that phase-separation improves process stability due to better working conditions for the acidogenic and methanogenic bacteria (Anderson *et al.*, 1994; Fongsatitkul *et al.*, 1995; Battistoni *et al.*, 2000). This study was therefore conducted as an extension of the single-phase digesters to determine the effect of phase-separation on the performance of the two digesters operated in series, while treating gelatin-manufacturing effluent. Two anaerobic mesophilic hybrid digesters AD-1 (UASB, polyethylene) and AD-2 (UASB, polyurethane), operated in series, were evaluated for the treatment of a highly variable raw gelatin-manufacturing effluent. This was done by coupling the digesters so that digester AD-2 received the effluent from digester AD-1 which in turn received the full-strength raw gelatin-manufacturing effluent. No chemical oxygen demand (COD) standardisation was done on the raw gelatin-manufacturing effluent so as to simulate actual field conditions. The pH of the raw effluent from the gelatin-manufacturing industry, was adjusted to 6.5 and the hydraulic retention time (HRT) set at 1.0 d. In the first section of this study the two reactors were evaluated separately. The two reactors achieved COD removal efficiencies of up to 93% (AD-1) and 80% (AD-2) at OLR's of 8.32 (AD-1) and 3.00 kg $COD.m^{-3}.d^{-1}$ (AD-2). High sulphate (SO_4) removal efficiencies were also obtained in both separate digesters (97% and 84%, respectively). The methane content varied between 70 and 84% for digester AD-1 and 39 and 95% for digester AD-2. A methane yield per COD removed of 0.96 m^3.kg $COD_{removed}.d^{-1}$ at an OLR of 7.56 kg $COD.m^{-3}.d^{-1}$ for digester AD-1 and a

methane yield per COD removed of 0.56 m^3.kg $COD_{removed}$.d^{-1} at an OLR of 5.53 kg COD.m^{-3}.d^{-1} for digester AD-2, were obtained.

In the second section of this study the total removal efficiency of the multi-phase system was evaluated. The substrate of digester AD-1 and the effluent of digester AD-2 were used for the calculation of the total removal efficiencies in this section. A total COD removal efficiency of 97% at an OLR of 8.32 kg COD.m^{-3}.d^{-1}, was achieved. Total SO_4 removal of 96% was obtained at OLR's of 4.33 kg COD.m^{-3}.d^{-1}. Higher total COD and sulphate removal efficiencies were obtained with the combined digesters than with the single-phase digestion, as well as with the phase separated digesters.

Introduction

The general performance of the anaerobic digestion process and the wide diversity of waste that can be treated, has increased steadily over the last few years, due to an array of breakthroughs related to reactor design, operating conditions and shock loadings (Austermann-Haun *et al.*, 1997). This treatment option has also been evaluated extensively by other scientists (Guiot & Van Den Berg, 1985; Stronach *et al.*, 1987; Van Der Merwe & Britz, 1993; Guiot *et al.*, 1997; Lettinga *et al.*, 1997; Verstraete & Vandevivere, 1997; Battistoni *et al.*, 2000).

Substantial proof exists that phasing/staging of the anaerobic process can improve the overall performance of the biological process (Speece *et al.*, 1997; Battistoni *et al.*, 2000). Separate phasing/staging seems to be the most effective approach to optimise environmental conditions for each phase, while using single-phase processes, both classes of organisms are forced to operate in a common environment (Speece *et al.*, 1997; Ghosh *et al.*, 2000). Potential advantages of phase separation include: improved process stability due to optimised environmental conditions for both acidogenic and methanogenic bacteria; altered intermediate product formation; enhanced volatile solid (VS) reduction efficiency; minimised H_2 concentration and maximised free energy for propionate conversion; minimised inhibitory

acetate concentrations in the presence of propionate degrading bacteria and *visa versa*; increased potential for a better organic loading rate (OLR); reducing risk of digester overloading; minimised residual volatile fatty acids (VFA) in the effluents as well as altered energy yields to various classes of microbial populations (Ghosh & Klass, 1978; Massey & Pohland, 1978; Shin *et al.*, 1992; Fongsatitkul *et al.*, 1995; Battistoni *et al.*, 2000; Ghosh *et al.*, 2000). Anderson *et al.* (1994) studied the changes in microbial populations in multi-phase digestion systems and confirmed that these designs have advantages over single-phase systems, especially in terms of the selection and enrichment of different bacteria in each phase, increased process stability and that the methanogenic phase is buffered by the first acid phase (Ghosh *et al.*, 2000).

The process used by gelatin-manufacturing industries to convert insoluble hide collagen into water soluble gelatin produces a large volume of effluent, high in COD, suspended solids (SS), SO_4 and salts. This result costly upsets to the treatment processes of the local wastewater purification process (Van Der Merwe-Botha, 1998, Personal communication). Aiming at the reduction of the load of the discharged wastewater, it was decided to evaluate anaerobic digestion as a pre-treatment option for this gelatin-manufacturing effluent before it is discharged into the municipal wastewater purification works.

This study was done as an extension to the previous laboratory-scale single-phase studies (Chapter 3 of this thesis) in an attempt to separate the dominant microbial populations and establish their interactions in two separately phased digesters, thereby optimising the nutrient utilising and metabolite formation dynamics by these specific phase-dominating populations. The aim of this study was thus to evaluate the performance efficiencies of both individually operated digesters, as well as the overall performance of the same two digesters operated in series, while treating raw gelatin-manufacturing effluent.

Materials and methods

Digester design

The two digesters were operated in series, each with a working volume of five litres, and operated at 35°C. The temperature was regulated by means of a heating tape and temperature sensitive controls (Meyer *et al.*, 1985). The hybrid digesters combined a fixed-film and an upflow sludge blanket design. The inert porous polyethylene (AD-1) foam and the polyurethane (AD-2) materials were fitted to the upper two thirds of each of the inner digester walls. The polyurethane material (Van Rompu *et al.*, 1990) had channels of 1.3 x 3.3 cm, edges of 1.3 x 3.0 cm and a back area 1.3 cm thick. The density of the polyethylene and polyurethane materials were estimated at 0.77 and 25.7 $kg.m^{-3}$, respectively (Van Der Merwe, 1994).

The substrate was introduced semi-continuously via a horizontal inlet at the bottom of each digester by means of a peristaltic pump (Watson-Marlow 302S) controlled by an electronic timer. The final overflow of the reactor system emptied through an U-shaped tube to prevent any atmospheric oxygen from entering the system. The biogas exited at the top of the digester via a gas-solid separator and biogas production was determined by means of a brine displacement system (6N HCl, pH 2.0). The biogas volumes were corrected to standard temperature and pressure (STP) conditions. Stable state conditions were assumed when, after five volume turnovers, operational parameters showed a variation of less than 10%. The hydraulic retention time (HRT) was set at 1.0 d for the entire duration of the experimental study for both digesters.

There was no need for a start-up process since the digesters were already activated using the same substrate used for the single-phase digestion reported in Chapter 3 of this thesis.

Gelatin-manufacturing effluent

The gelatin effluent was obtained from a local gelatin-manufacturing industry in batches of 75 l and were stored at room temperature until required. In an attempt to simulate the actual field conditions, no standardisation was

done on the effluent, except for the pH of the substrate which had to be adjusted to 6.5. The direct influence of varying COD concentrations (organic loading rates) on the anaerobic treatment efficiency can thus be studied. The gelatin-manufacturing effluent used as digester substrate was initially supplemented with 100 $mg.l^{-1}$ urea, 100 $mg.l^{-1}$ K_2HPO_4, 10 ml acetic acid (CH_3COOH) and a sterile trace element solution (Nel *et al.*, 1985), to stimulate the growth of the specific microbial consortium and to prevent any nutrient limitations.

Analytical methods

The following parameters were determined on the digester substrate and effluent during the experimental study according to Standard Methods (APHA, AWWA & WEF, 1995): pH; total alkalinity (TAlk); chemical oxygen demand (COD); total Kjeldahl nitrogen (TKN); chlorides (Cl); ammonia (NH_3-N); volatile fatty acids (VFA); sulphate (SO_4^{2-}); total solids (TS); volatile solids (VS); total non-volatile solids (TNVS) and ortho-phosphate (PO_4-P).

Total heavy metals (Tmetals) comprising of Cu, Fe, Co, Mn, Cr, Pb, Ni, Zn, Cd, as well as calcium and sodium cocentrations, were determined using an Atomic Absorbance Spectrophotometer (Varian Model 250 Plus), equipped with hollow cathode lamps for the different metals, photoelectric detector with associated electronic amplifying and measuring equipment. Air/acetylene and nitrous oxide/acetylene burners were used with air as oxidant and acetylene or nitrous oxide as fuel. Pressure reducing regulators were used for the supply of the fuel and oxidant at appropriate and prescribed levels. Control standards of known metal concentrations were prepared with a matrix similar to the samples, for the construction of a calibration curve. Additional standard solutions were analysed between samples to confirm test control, as well as a blank to confirm the baseline stability. The sample concentrations were determined by reference to the calibration curve. The filtered samples (at room temperature) were aspirated into the air/acetylene or nitrous oxide/acetylene flame and atomised.

Volatile fatty acids (as acetate) were determined according to the titration method of Moosbrugger *et al.* (1992). The biogas composition

(methane and carbon dioxide) was determined volumetrically according to the quantitative biogas carbon dioxide content method of Ross *et al.* (1992). The biogas volumes were corrected to standard temperature and pressure (STP) conditions.

The accuracy of all tested parameters were confirmed by participation in an inter-laboratory water testing program (SABS Water Check Proficiency Program).

Experimental phases

During the multi-phase experimental study, the two hybrid digesters were operated in series, with digester AD-1 (UASB, polyethylene) receiving the raw gelatin-manufacturing effluent as digester substrate (pH correction to 6.5 with 6N HCl), and digester AD-2 (UASB, polyurethane) receiving the treated effluent from AD-1, as substrate (pH 6.5). In the first section of the study the two reactors were evaluated separately to determine if phase-separation was possible. Thereafter, in the second section of the study the total removal efficiency of the multi-phase system treating gelatin-manufacturing, was evaluated.

Results and discussion

Digester substrate

The average composition of the substrate fed to the hybrid digesters during the multi-phase digestion, is given in Table 1. Since the direct influence of varying batch and thus substrate composition was considered as important field and bench parameters, no standardisation was done on the effluent, with the exception of the pH.

Digester efficiency (AD-1)

The composition of the digester substrate, effluent and digester efficiency, are given in Tables 2a, b and c. The first phase substrate (AD-1) was used as baseline in all efficiency calculations. The data in Fig. 1 shows

Table 1. Average composition of the digester substrates used during the multi-phase experimental studies, over a 48 week period, based on 100 batches of effluent.

Parameters	AD-1		AD-2	
	Average	± SD	Average	± SD
HRT (d)	1		1	
OLR (kg COD.m^{-3}.d^{-1})	4.05	3.34	1.42	1.04
COD (mg.l^{-1})	4 172	3 341	1 421	1 036
SO_4 (mg.l^{-1})	564	500	144	131
VFA (mg.l^{-1})	854	719	474	343
TKN (mg.l^{-1})	490	445	499	358
pH	6.3	0.7	6.6	0.7
TAlk (mg.l^{-1} as $CaCO_3$)	558	448	995	770
TS (mg.l^{-1})	4 458	3 200	3 194	2 000
VS (mg.l^{-1})	1 919	1 400	677	500
TNVS (mg.l^{-1})	2 540	1 800	2 517	1 700
Cl (mg.l^{-1})	388.5	298.1	916.4	502.8
NH_3 (mg.l^{-1})	60	56	199	141
PO_4-P (mg.l^{-1} as P)	2.6	2.5	5.0	4.5
Na (mg.l^{-1})	614	506	741	617
Ca (mg.l^{-1})	78	66	68	56
Tmetals (mg.l^{-1})	1.6	1.3	1.3	1.0

SD = Standard deviation

Table 2a. Composition of the substrate used during multi-phase operating conditions of AD-1, as a function of organic loading rate (OLR)*.

Digester Substrate																
OLR ($kg\ COD.m^{-3}.d^{-1}$)		COD ($mg.l^{-1}$)	TKN ($mg.l^{-1}$)	VFA ($mg.l^{-1}$)	SO_4^{2-} ($mg.l^{-1}$)	pH	TAlk ($mg.l^{-1}$ as $CaCO_3$)	Cl ($mg.l^{-1}$)	NH_3-N ($mg.l^{-1}$)	TS ($mg.l^{-1}$)	VS ($mg.l^{-1}$)	TNVS ($mg.l^{-1}$)	PO_4-P ($mg.l^{-1}$)	Tmetals ($mg.l^{-1}$)	Na ($mg.l^{-1}$)	Ca ($mg.l^{-1}$)
0.78		781	197	1226	190	7.1	335	N/D	15.1	2500	1200	1300	0.8	1.28	312	12
1.81		1813	55	255	380	7.4	327	266.1	45.4	2100	1100	1000	0.3	0.44	357	30
1.92		1917	102	698	120	7.3	273	440.3	69.6	2300	1100	1200	0.3	0.52	298	38
2.03		2027	246	136	84	6.8	277	155.9	76.8	2800	1500	1300	3.7	2.04	139	134
2.03		2030	19	784	630	6.4	88	N/D	2.3	1800	700	1100	2.7	1.22	763	117
2.07		2070	43	232	120	7.0	135	N/D	11.3	3000	1100	1900	1.6	0.38	578	19
2.07		2070	198	179	130	7.2	202	521.0	60.2	2600	1200	1400	0.9	0.45	558	58
2.16		2159	113	213	350	6.9	198	345.0	27.5	1700	500	1200	0.5	0.81	234	53
2.38		2377	166	340	590	6.4	152	N/D	7.1	3800	1300	2500	0.4	0.47	794	15
2.52		2521	278	485	340	6.8	506	251.5	120.0	2000	1100	900	0.5	0.72	340	40
2.55		2548	279	315	470	7.0	348	837.7	41.5	3400	1400	2000	0.4	1.30	690	81
2.63		2630	267	417	150	7.3	266	91.7	32.0	2600	1600	1000	0.2	1.09	263	10
2.66		2655	258	672	151	7.1	756	N/D	N/D	2000	900	1100	2.9	1.11	575	78
2.73		2734	340	281	380	7.5	268	N/D	6.2	3800	1500	2300	0.6	0.73	1044	23
2.76		2759	304	808	680	7.2	602	N/D	N/D	3000	600	2400	1.7	2.02	952	120
2.80		2802	4459	2365	550	6.8	597	N/D	N/D	3700	1300	2400	2.4	1.67	98	32
2.89		2887	264	706	290	7.1	667	N/D	N/D	2500	1100	1400	1.0	1.72	608	31
2.90		2901	317	340	270	7.0	214	N/D	N/D	3700	1500	2200	1.9	3.18	460	130
3.00		2996	330	179	510	7.1	152	378.8	13.1	3100	2000	1100	0.2	0.46	362	38
3.05		3051	289	2128	340	7.0	787	N/D	N/D	3600	1200	2400	3.9	2.08	724	N/D
3.17		3170	346	230	480	6.3	358	502.7	11.8	3200	1700	1500	0.5	0.49	444	42
3.29		3294	314	323	790	6.6	189	201.8	29.7	3700	2100	1600	4.9	2.23	287	236
3.39		3387	625	221	740	6.8	248	N/D	43.4	10000	1900	8100	0.9	1.60	794	38
3.42		3418	438	894	300	6.9	808	N/D	246.0	4300	1700	2600	2.6	0.72	1023	18
3.42		3418	619	1208	700	6.8	393	N/D	8.4	2400	1000	1400	0.5	2.63	582	55
3.51		3513	228	281	160	7.5	310	N/D	8.7	3300	2000	1300	0.0	2.04	592	20
3.54		3544	324	494	610	7.2	242	N/D	10.9	4100	2300	1800	1.0	1.86	729	63
3.60		3602	309	1368	590	7.5	378	N/D	13.8	1300	500	800	0.3	0.43	18	27
3.67		3670	249	374	360	6.9	355	N/D	53.2	4400	2000	2400	2.6	1.73	636	47
3.87		3865	583	885	590	6.4	596	N/D	226.0	3000	1100	1900	2.4	0.72	282	51
3.90		3899	458	451	210	7.1	477	669.3	68.2	3200	1700	1500	0.3	2.68	392	14
4.15		4152	417	385	130	7.5	349	N/D	7.5	3900	2400	1500	0.3	1.21	510	15
4.33		4333	473	183	1000	7.3	611	N/D	N/D	5200	1900	3300	14.3	2.91	1000	N/D
4.46		4456	665	2391	520	7.0	1022	N/D	283.0	2600	1000	1600	0.3	1.29	341	31
5.26		5257	830	2340	900	6.5	2274	N/D	N/D	6400	1900	4500	2.1	1.55	1448	212
5.34		5340	606	1847	1860	7.0	651	N/D	N/D	7800	2500	5300	5.9	4.59	181	31
5.90		5899	654	1140	410	7.1	963	N/D	177.3	3400	2400	1000	0.2	0.63	280	58
6.25		6245	707	1489	1590	7.2	1716	N/D	N/D	6900	2300	4600	5.2	2.89	874	220
6.33		6332	819	1709	1910	7.0	1350	N/D	N/D	8500	2700	5800	4.0	4.89	119	72
6.74		6740	460	1804	270	7.0	1314	N/D	N/D	5500	1900	3600	5.6	1.72	2404	346
6.81		6812	512	494	207	7.0	430	N/D	N/D	8000	3800	4200	7.4	2.08	838	184
7.08		7079	637	783	1610	7.0	1143	N/D	N/D	7800	3000	4800	4.6	2.91	1523	245
7.53		7525	606	230	320	7.6	482	N/D	70.4	5100	3900	1200	0.8	1.06	47	64
7.56		7562	524	936	140	6.9	721	N/D	N/D	6800	2800	4000	5.8	2.33	1179	82
8.32		8323	567	204	390	7.3	233	N/D	14.1	3800	2800	1000	0.2	0.65	386	76
8.56		8559	700	2196	2310	6.6	527	N/D	N/D	11700	4300	7400	6.0	3.42	178	41
9.26		9260	491	834	230	7.0	920	N/D	N/D	8900	4100	4800	8.6	2.50	1632	111
9.87		9874	846	2536	1000	6.8	747	N/D	N/D	12800	6500	6300	12.4	3.95	1230	137
Minimum	0.78	781	19	136	84	6.3	88	91.7	2.3	1300	500	800	0.0	0.38	18	10
Maximum	9.87	9874	4459	2536	2310	7.6	2274	837.7	283.0	12800	6500	8100	14.3	4.89	2404	346
Average	4.17	4172	490	854	564	7.0	562	388.5	60.0	4458	1919	2540	2.6	1.70	627	78

Above data have been arranged according to numerical increases in **OLR, while the numerical structure of **time** have been disregarded for this study's purposes (Fig. 1).*

N/D = Not Determined

Table 2b. Composition of the digester AD-1 effluent during multi-phase operating conditions at variable organic loading rates (OLR)*.

Digester Effluent																
OLR (kg COD.m^{-3}.d^{-1})		COD (mg.l^{-1})	TKN (mg.l^{-1})	VFA (mg.l^{-1})	SO_4^{2-} (mg.l^{-1})	pH	TAlk (mg.l^{-1} as $CaCO_3$)	Cl (mg.l^{-1})	NH_3-N (mg.l^{-1})	TS (mg.l^{-1})	VS (mg.l^{-1})	TNVS (mg.l^{-1})	PO_4-P (mg.l^{-1})	Tmetals (mg.l^{-1})	Na (mg.l^{-1})	Ca (mg.l^{-1})
0.78		451	189	579	70	7.4	845	N/D	184.6	3100	600	2500	6.1	0.89	851	143
1.81		309	142	128	110	7.3	324	483.9	120.0	1100	200	900	1.3	0.31	191	14
1.92		455	157	323	110	7.6	491	662.2	80.3	1600	300	1300	0.8	0.49	399	44
2.03		1064	280	357	250	7.1	830	576.2	214.7	1800	500	1300	5.6	0.96	241	192
2.03		871	100	432	158	7.5	776	N/D	52.0	1700	200	1500	4.5	1.46	595	111
2.07		918	129	355	40	7.1	335	N/D	80.2	2900	500	2400	2.4	0.43	717	23
2.07		459	123	128	160	7.5	525	517.5	64.2	1500	200	1300	3.0	0.33	440	53
2.16		984	142	281	240	7.1	405	622.7	61.2	1600	200	1400	2.4	0.74	149	33
2.38		523	131	162	86	6.6	286	N/D	87.5	1800	300	1500	2.1	0.24	467	15
2.52		612	255	255	110	7.3	649	619.3	199.0	1500	500	1000	1.2	0.60	363	40
2.55		971	349	204	170	7.7	736	1831.3	165.0	3000	500	2500	2.2	1.14	788	71
2.63		1018	444	272	90	7.3	503	1415.9	250.0	1900	500	1400	0.3	0.95	404	15
2.66		394	264	196	119	7.2	885	N/D	N/D	2000	400	1600	7.9	0.87	530	56
2.73		1481	199	272	250	7.4	503	N/D	102.0	2800	300	2500	2.5	0.55	790	12
2.76		850	304	196	23	7.5	871	N/D	N/D	2900	600	2300	4.6	1.51	1012	108
2.80		815	5633	1387	170	7.9	813	N/D	N/D	3100	500	2600	5.5	1.05	94	36
2.89		1382	338	366	110	7.0	1214	N/D	N/D	3400	700	2700	9.7	1.48	1609	156
2.90		948	373	306	14	7.1	1047	N/D	N/D	2500	700	1800	7.5	1.15	360	83
3.00		746	345	204	210	7.3	1284	593.4	292.0	3000	1600	1400	1.2	0.33	412	36
3.05		306	431	417	210	8.3	878	N/D	N/D	3800	400	3400	6.6	1.77	1091	N/D
3.17		693	315	162	310	7.2	1020	978.1	207.0	1500	300	1200	0.9	0.31	358	33
3.29		764	303	196	190	7.1	736	1235.1	148.9	2300	500	1800	2.5	0.69	425	82
3.39		813	566	136	142	7.8	858	N/D	172.0	1800	300	1500	2.1	1.54	735	43
3.42		1635	324	298	110	7.1	857	N/D	246.8	3400	900	2500	5.0	0.51	846	26
3.42		1375	607	638	150	7.5	1218	N/D	194.0	2300	400	1900	3.3	1.60	602	57
3.51		538	167	162	90	7.4	203	N/D	106.0	1900	400	1500	1.7	0.85	643	22
3.54		3028	313	460	121	7.2	866	N/D	176.0	2000	400	1600	3.0	1.60	846	71
3.60		3000	303	536	120	7.7	738	N/D	164.0	1800	300	1500	3.6	0.43	458	35
3.67		2415	238	196	190	6.9	709	N/D	179.6	3000	700	2300	7.5	0.52	716	46
3.87		1554	502	357	90	6.9	1147	N/D	324.0	2900	500	2400	4.2	0.78	407	63
3.90		2501	447	426	92	7.5	869	1461.0	244.3	2300	400	1900	0.5	0.86	738	29
4.15		816	426	230	110	7.7	668	N/D	247.0	2200	400	1800	0.5	0.75	647	17
4.33		1982	642	681	52	7.5	2060	N/D	N/D	6100	900	5200	11.9	3.11	1660	N/D
4.46		1080	656	1030	60	7.2	1572	N/D	332.0	1900	1200	700	2.5	0.67	353	32
5.26		1914	758	417	290	7.4	2027	N/D	N/D	6000	1500	4500	7.0	1.01	1493	48
5.34		2959	726	1260	260	7.1	1712	N/D	N/D	11200	1500	9700	12.2	3.80	763	80
5.90		918	618	672	60	7.3	1550	N/D	391.0	2000	800	1200	3.0	0.53	345	47
6.25		2118	643	604	370	7.1	2246	N/D	N/D	5400	1000	4400	8.4	2.20	866	164
6.33		1920	624	1716	176	7.0	1512	N/D	N/D	5400	1000	4400	8.4	2.69	115	60
6.74		1670	469	400	90	7.3	1271	N/D	N/D	5200	800	4400	10.2	1.49	3242	288
6.81		1480	311	247	185	7.7	863	N/D	N/D	2500	600	1900	5.8	1.25	706	64
7.08		3034	569	374	220	7.3	1438	N/D	N/D	5500	1100	4400	8.8	2.40	987	85
7.53		1877	553	417	60	8.0	1494	N/D	760.0	1700	700	1000	0.9	0.82	367	64
7.56		2139	460	306	202	7.3	1277	N/D	N/D	5300	1000	4300	8.6	2.42	1220	77
8.32		582	176	179	110	7.3	545	N/D	138.0	1400	400	1000	4.2	0.36	277	57
8.56		609	321	1506	169	7.2	681	N/D	N/D	3900	900	3000	4.0	2.09	1018	90
9.26		3693	788	970	90	7.3	1744	N/D	N/D	7600	1800	5800	16.4	3.74	2000	99
9.87		5533	779	1336	110	7.0	1657	N/D	N/D	7800	2100	5700	17.2	4.01	1231	118
Minimum	0.78	306	100	128	14	6.6	203	483.9	52.0	1100	200	700	0.3	0.24	94	12
Maximum	9.87	5533	5633	1716	370	8.3	2246	1831.3	760.0	11200	2100	9700	17.2	4.01	3242	288
Average	4.17	1421	505	471	146	7.3	998	916.4	200.0	3196	679	2517	5.0	1.26	739	67

Above data have been arranged according to numerical increases in **OLR, while the numerical structure of **time** have been disregarded for this study's purposes (Fig. 1).*

N/D = Not Determined

Table 2c. The influence of variable organic loading rates (OLR) on digester AD-1 performance during multi-phase digestion.

Digester Efficiency																				
OLR ($kg\ COD.m^{-3}.d^{-1}$)	COD removal (%)	COD removal rate ($kg\ COD.m^{-3}.d^{-1}$)	SO_4^{2-} removal (%)	Biogas production (l/d)	Biogas yield ($m^3.m^{-3}$)	CH_4 content (%)	CH_4 yield ($m^3.kg\ COD_{rem}.d^{-1}$)	CH_4 yield ($m^3.kg\ COD_{load}.d^{-1}$)	VFA removal (%)	Cl removal (%)	NH_3-N removal (%)	TS removal (%)	VS removal (%)	TNVS removal (%)	TKN removal (%)	PO_4-P removal (%)	Tmetal removal (%)	Na removal (%)	Ca removal (%)
0.78	42.3	0.33	63.2	2.39	0.48	N/D	N/D	N/D	52.8	N/D	0.0	0.0	50.0	0.0	3.7	0.0	30.5	0.0	0.0
1.81	83.0	1.50	71.1	2.45	0.49	N/D	N/D	N/D	50.0	0.0	0.0	47.6	81.8	10.0	0.0	0.0	29.5	46.5	53.6
1.92	76.3	1.46	8.3	N/D	N/D	N/D	N/D	N/D	53.7	0.0	0.0	30.4	72.7	0.0	0.0	0.0	5.8	0.0	0.0
2.03	47.5	0.96	0.0	N/D	N/D	N/D	N/D	N/D	0.0	0.0	0.0	35.7	66.7	0.0	0.0	0.0	52.9	0.0	0.0
2.03	57.1	1.16	74.9	0.46	0.09	N/D	N/D	N/D	44.8	N/D	0.0	5.6	71.4	0.0	0.0	0.0	0.0	22.1	4.8
2.07	55.7	1.15	66.7	2.60	0.52	N/D	N/D	N/D	0.0	N/D	0.0	3.3	54.5	0.0	0.0	0.0	0.0	0.0	0.0
2.07	77.8	1.61	0.0	N/D	N/D	N/D	N/D	N/D	28.6	0.7	0.0	42.3	83.3	7.1	38.1	0.0	26.7	21.2	8.6
2.16	54.4	1.18	31.4	N/D	N/D	N/D	N/D	N/D	0.0	0.0	0.0	5.9	60.0	0.0	0.0	0.0	8.6	36.4	37.5
2.38	78.0	1.85	85.4	N/D	N/D	N/D	N/D	N/D	52.5	N/D	0.0	52.6	76.9	40.0	20.9	0.0	48.9	41.1	0.0
2.52	75.7	1.91	67.6	3.16	0.63	N/D	N/D	N/D	47.4	0.0	0.0	25.0	54.5	0.0	8.5	0.0	16.7	0.0	0.0
2.55	61.9	1.58	63.8	5.48	1.10	80.8	2.81	1.74	35.1	0.0	0.0	11.8	64.3	0.0	0.0	0.0	12.3	0.0	12.3
2.63	61.3	1.61	40.0	N/D	N/D	N/D	N/D	N/D	34.7	0.0	0.0	26.9	68.8	0.0	0.0	0.0	12.8	0.0	0.0
2.66	85.2	2.26	21.2	3.53	0.71	N/D	N/D	N/D	70.9	N/D	N/D	0.0	55.6	0.0	0.0	0.0	21.6	7.8	28.2
2.73	45.8	1.25	34.2	N/D	N/D	N/D	N/D	N/D	3.0	N/D	0.0	26.3	80.0	0.0	41.4	0.0	24.7	24.3	47.8
2.76	69.2	1.91	96.6	5.25	1.05	N/D	N/D	N/D	75.7	N/D	N/D	3.3	0.0	4.2	0.0	0.0	25.2	0.0	10.3
2.80	70.9	1.99	69.1	3.37	0.67	N/D	N/D	N/D	41.4	N/D	N/D	16.2	61.5	0.0	0.0	0.0	37.1	3.6	0.0
2.89	52.1	1.61	62.1	6.53	1.31	84.0	3.42	1.90	48.2	N/D	N/D	0.0	36.4	0.0	0.0	0.0	14.0	0.0	0.0
2.90	67.3	1.95	94.8	3.10	0.62	N/D	N/D	N/D	10.0	N/D	N/D	32.4	53.3	18.2	0.0	0.0	63.8	21.7	36.0
3.00	75.1	2.25	58.8	N/D	N/D	N/D	N/D	N/D	0.0	0.0	0.0	3.2	20.0	0.0	0.0	0.0	28.3	0.0	5.0
3.05	90.0	2.75	38.2	3.33	0.67	N/D	N/D	N/D	80.4	N/D	N/D	0.0	66.7	0.0	0.0	0.0	14.9	0.0	N/D
3.17	78.1	2.48	35.4	N/D	N/D	N/D	N/D	N/D	29.6	0.0	0.0	53.1	82.4	20.0	8.8	0.0	36.7	19.4	20.9
3.29	76.8	2.53	75.9	N/D	N/D	N/D	N/D	N/D	39.5	0.0	0.0	37.8	76.2	0.0	3.6	49.0	69.1	0.0	65.3
3.39	76.0	2.57	80.8	1.44	0.29	N/D	N/D	N/D	38.5	N/D	0.0	82.0	84.2	81.5	9.5	0.0	3.8	7.4	0.0
3.42	52.2	1.78	63.3	4.48	0.90	80.0	2.01	1.05	66.7	N/D	0.0	20.9	47.1	3.8	26.1	0.0	29.2	17.3	0.0
3.42	59.8	2.04	78.6	3.68	0.74	N/D	N/D	N/D	47.2	N/D	0.0	4.2	60.0	0.0	1.9	0.0	39.2	0.0	0.0
3.51	84.7	2.98	43.8	N/D	N/D	N/D	N/D	N/D	42.4	N/D	0.0	42.4	80.0	0.0	26.7	0.0	58.3	0.0	0.0
3.54	14.6	0.52	80.2	2.79	0.56	N/D	N/D	N/D	6.9	N/D	0.0	51.2	82.6	11.1	3.3	0.0	14.0	0.0	0.0
3.60	16.7	0.60	79.7	1.93	0.39	N/D	N/D	N/D	60.8	N/D	0.0	0.0	40.0	0.0	1.8	0.0	0.0	0.0	0.0
3.67	34.2	1.26	47.2	3.70	0.74	N/D	N/D	N/D	47.7	N/D	0.0	31.8	65.0	4.2	4.3	0.0	69.9	0.0	2.0
3.87	59.8	2.31	84.7	3.32	0.66	N/D	N/D	N/D	59.6	N/D	0.0	3.3	54.5	0.0	13.9	0.0	0.0	0.0	0.0
3.90	35.9	1.40	56.2	N/D	N/D	N/D	N/D	N/D	5.7	0.0	0.0	28.1	76.5	0.0	2.2	0.0	67.9	0.0	0.0
4.15	80.3	3.34	15.4	N/D	N/D	N/D	N/D	N/D	40.3	N/D	0.0	43.6	83.3	0.0	0.0	0.0	38.0	0.0	0.0
4.33	54.3	2.35	94.8	8.07	1.61	N/D	N/D	N/D	0.0	N/D	N/D	0.0	52.6	0.0	0.0	16.3	0.0	0.0	N/D
4.46	75.8	3.38	88.5	5.29	1.06	N/D	N/D	N/D	56.9	N/D	0.0	26.9	0.0	56.3	1.3	0.0	48.1	0.0	0.0
5.26	63.6	3.34	67.8	9.81	1.96	N/D	N/D	N/D	82.2	N/D	N/D	6.3	21.1	0.0	8.6	0.0	34.8	0.0	77.3
5.34	44.6	2.38	86.0	9.56	1.91	N/D	N/D	N/D	31.8	N/D	N/D	0.0	40.0	0.0	0.0	0.0	17.2	0.0	0.0
5.90	84.4	4.98	85.4	7.55	1.51	N/D	N/D	N/D	41.0	N/D	0.0	41.2	66.7	0.0	5.4	0.0	15.9	0.0	19.6
6.25	66.1	4.13	76.7	7.06	1.41	70.7	1.21	0.80	59.4	N/D	N/D	21.7	56.5	4.3	9.1	0.0	23.9	0.9	25.5
6.33	69.7	4.41	90.8	4.27	0.85	N/D	N/D	N/D	0.0	N/D	N/D	36.5	63.0	24.1	23.8	0.0	45.0	3.4	16.5
6.74	75.2	5.07	66.7	9.71	1.94	80.8	1.55	1.16	77.8	N/D	N/D	5.5	57.9	0.0	0.0	0.0	13.4	0.0	16.7
6.81	78.3	5.33	10.6	10.20	2.04	78.6	1.50	1.18	50.0	N/D	N/D	68.8	84.2	54.8	39.2	21.1	39.9	15.8	65.3
7.08	57.1	4.05	86.3	12.81	2.56	81.7	2.59	1.48	52.2	N/D	N/D	29.5	63.3	8.3	10.7	0.0	17.5	35.2	65.2
7.53	75.1	5.65	81.3	2.88	0.58	N/D	N/D	N/D	0.0	N/D	0.0	66.7	82.1	16.7	8.8	0.0	22.6	0.0	0.0
7.56	71.7	5.42	0.0	7.13	1.43	73.1	0.96	0.69	67.3	N/D	N/D	22.1	64.3	0.0	12.2	0.0	0.0	0.0	5.8
8.32	93.0	7.74	71.8	2.67	0.53	N/D	N/D	N/D	12.5	N/D	0.0	63.2	85.7	0.0	69.0	0.0	44.6	28.1	24.3
8.56	92.9	7.95	92.7	2.59	0.52	N/D	N/D	N/D	31.4	N/D	N/D	66.7	79.1	59.5	54.1	33.9	38.9	0.0	0.0
9.26	60.1	5.57	60.9	10.02	2.00	70.8	1.27	0.77	0.0	N/D	N/D	14.6	56.1	0.0	0.0	0.0	0.0	0.0	10.0
9.87	44.0	4.34	89.0	8.27	1.65	69.9	1.33	0.59	47.3	N/D	N/D	39.1	67.7	9.5	7.9	0.0	0.0	0.0	14.2
Minimum 0.78	14.6	0.33	0.0	0.46	0.09	69.9	0.96	0.59	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Maximum 9.87	93.0	7.95	96.6	12.81	2.56	84.0	3.42	1.90	82.2	0.7	0.0	82.0	85.7	81.5	69.0	49.0	69.9	46.5	77.3
Average 4.17	64.6	2.75	61.2	5.17	1.03	77.0	1.86	1.13	38.0	0.1	0.0	26.6	61.5	9.0	9.7	2.5	26.3	7.3	14.6

N/D = Not Determined

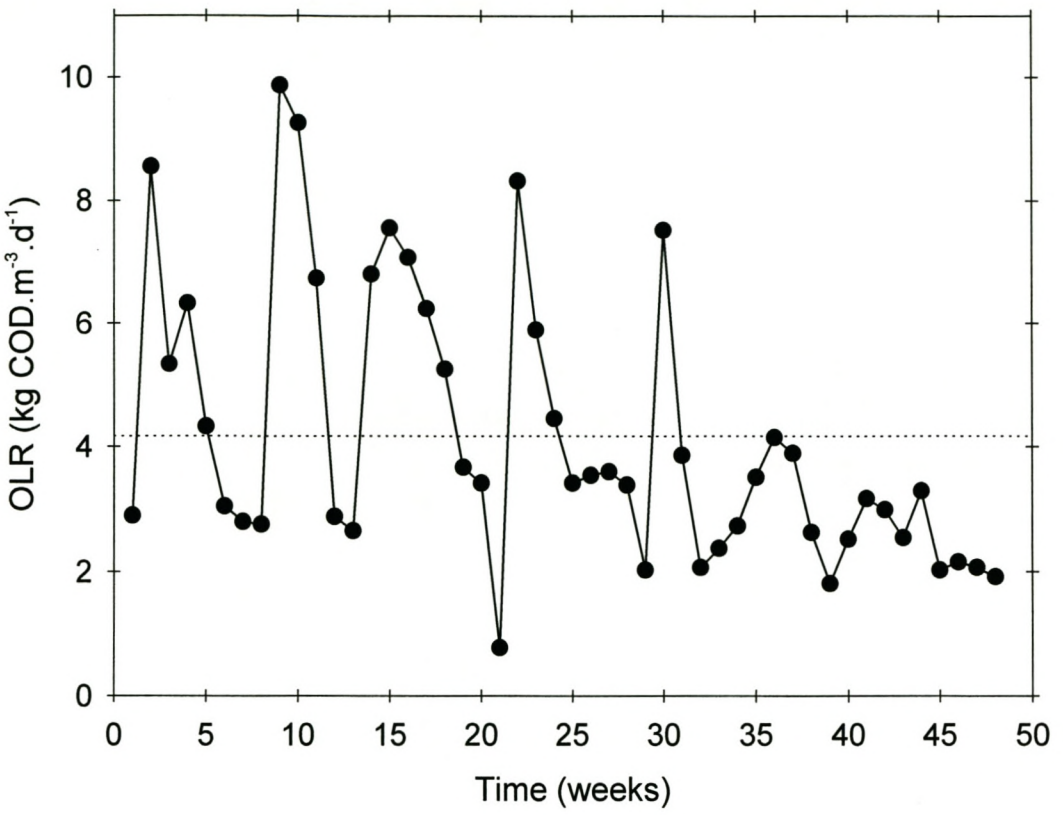

Figure 1. Variation in the substrate OLR during the 48 week study of digester AD-1 during multi-phase digestion. The dotted line represents the average OLR.

the time frame of the study and the extreme variations in the substrate OLR for digester AD-1, over the 48 week study period. Higher OLR operational conditions (> 7.0 kg $COD.m^{-3}.d^{-1}$) were found during weeks 2 to 4, 9 to 11, 14 to 17, 22 to 23 and 29 to 31. All data are discussed as a function of OLR due to the relative insignificance of time during this study. The influence of the variations in OLR (Fig. 1) on anaerobic treatment efficiency in terms of COD removal, R-value, biogas and methane yield, pH, total alkalinity and volatile fatty acid content of the effluent are discussed below.

The highest COD removal (93%) and COD removal rate (R-value) (7.95 kg $COD.m^{-3}.d^{-1}$) were found at the higher OLR of 8.32 and 8.56 kg $COD.m^{-3}.d^{-1}$, respectively. The R-value increased significantly with the increasing OLR (Fig. 2). The COD removal efficiency ranged from 15 to 93% with an average of 65% (Table 2c).

The increase in the substrate OLR was similarly reflected by a subsequent increase in the biogas yield (Fig. 3). This increased biogas yield possibly indicates that the microbial community prefer higher OLR's where more energy was available. The highest biogas yield of 2.56 $m^3.m^{-3}$ reactor was observed at an OLR of 7.08 kg $COD.m^{-3}.d^{-1}$, with an irregular production tendency in the yield at higher OLR's. The maximum methane yields per COD removed or COD loaded (3.42 and 1.90 $m^3.kg\ COD.d^{-1}$, respectively) were obtained at a lower OLR of 2.89 kg $COD.m^{-3}.d^{-1}$. Thus, as the OLR increased, the organic loading surpassed the metabolic capacity of the methanogenic population and, therefore, the methane yield decreased. The average percentage methane produced was 84% (Table 2c), indicating an active methanogenic population in the digester, which is able to compete with the sulphate reducing bacteria (SRB) bacteria. The highest SO_4 removal efficiency was 97% at an OLR of 2.76 kg $COD.m^{-3}.d^{-1}$ (Table 2c).

The TS removal also generally increased throughout the study with the highest TS removal efficiency of 82%, at an OLR of 3.39 kg $COD.m^{-3}.d^{-1}$. The highest VS removal efficiency (86%) was obtained at an OLR of 8.32 kg $COD.m^{-3}.d^{-1}$. After the maximum removal of TS and VS had been reached, further increasing of the OLR's resulted in decreasing TS and VS removals (Table 2c).

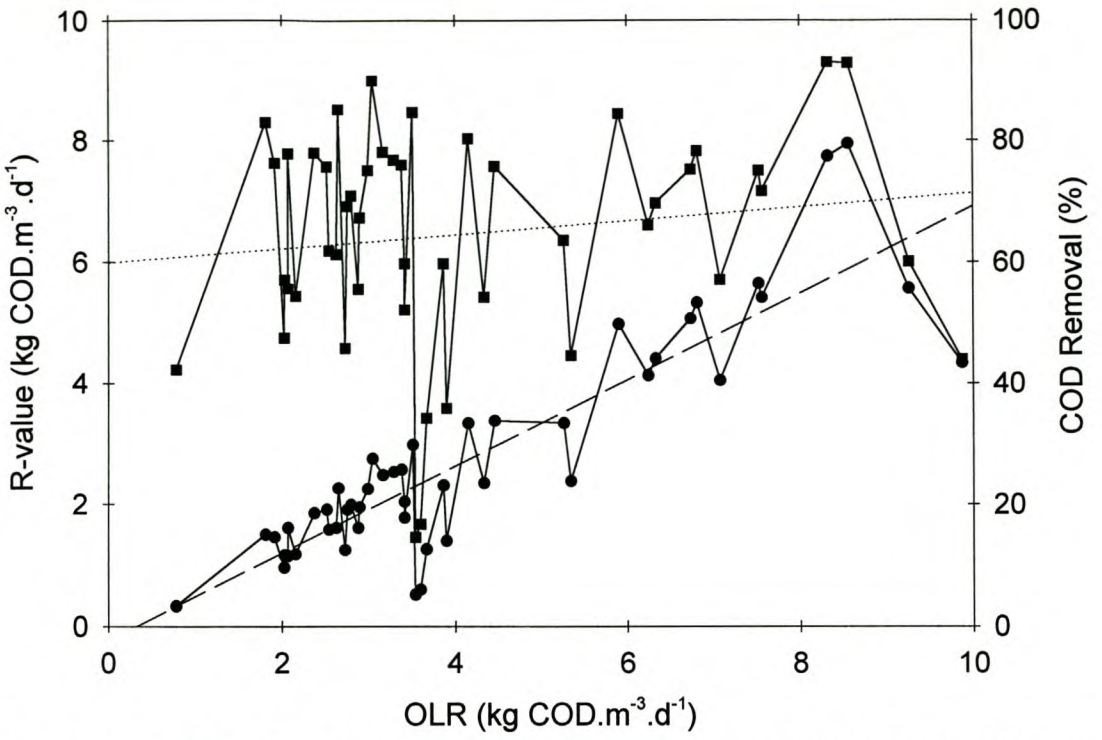

Figure 2. The COD removal rate (R-value) (● ; -----) and COD removal (■ ; .....), as a function of OLR for digester AD-1. The solid lines represent the actual data and the dotted and dashed lines represent the regression calculations.

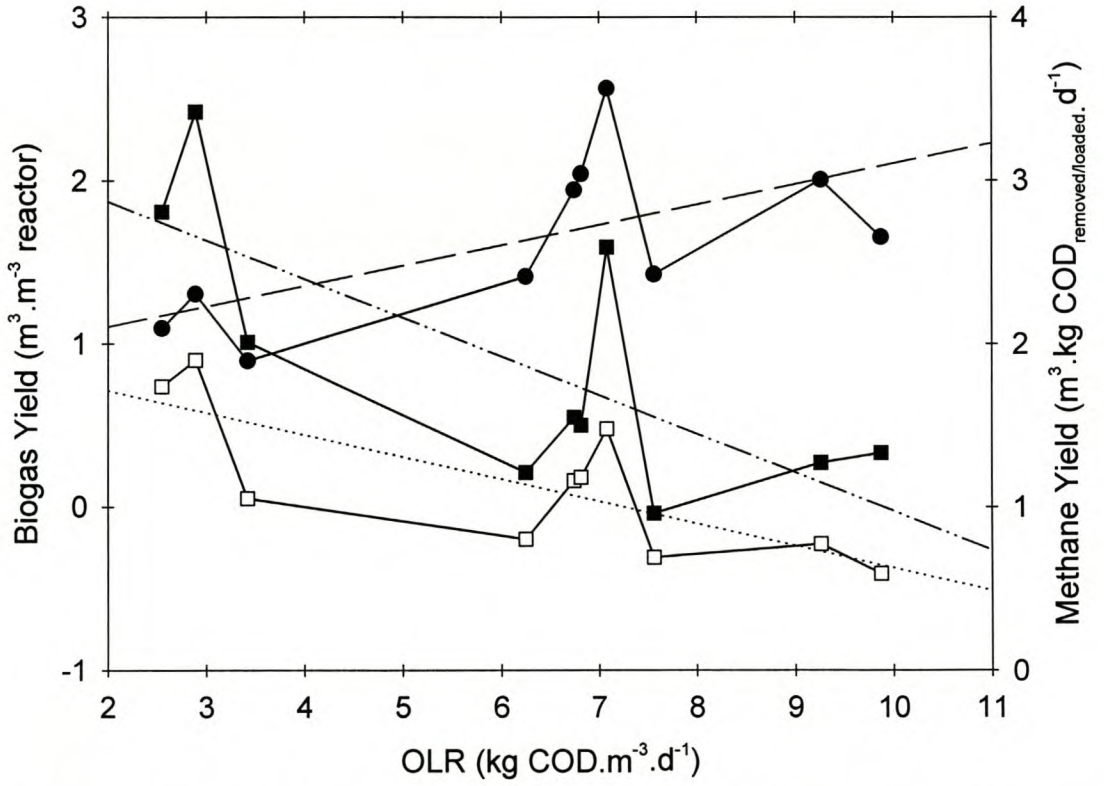

Figure 3. The influence of increasing OLRs on biogas yield (● ; -----), methane yield per $COD_{removed}$ (■ ; —··—··—) and methane yield per COD_{loaded} (□ ; .....) for digester AD-1 during multi-phase digestion. The solid lines represent the actual data and the dotted and dashed lines represent the regression calculations.

The pH was not strongly influenced by the increase in OLR's (Fig. 4). An average effluent pH of 7.3 (Table 2b) was obtained during the study. The effluent alkalinity also showed a strong variation but a general increase was found with increases in OLR (Fig. 5). The effluent alkalinity varied between 500 - 2 250 $mg.l^{-1}$. The effluent alkalinity decreased drastically at OLR's of > 6.25 kg $COD.m^{-3}.d^{-1}$ (Table 2b), indicating a depletion of the threshold buffering capacity of the system. The VFA content of the effluent varied between 128 and 1 716 $mg.l^{-1}$ (Table 2b).

Digester efficiency (AD-2)

The composition of the substrate fed to digester AD-2 and effluent composition, as well as the digester efficiency are summarised in Tables 3a, b and c. For this study all efficiency calculations were based on the values of the effluent of digester AD-1, which was used as substrate for digester AD-2. The data is arranged according to the numerical increases in OLR's. The data in Fig. 6 again illustrates the large variations in OLR found during the 48 week study period. Digester AD-2 received a substrate with a lower average OLR (1.42 kg $COD.m^{-3}.d^{-1}$) than digester AD-1 which received effluent with an average OLR of 4.17 kg $COD.m^{-3}.d^{-1}$ (Fig. 1 and 6). The lower OLR could be explained due to the fact that digester AD-1 already converted most of the organic material into more readily compounds and methane. The highest OLR was found during weeks 8 to 10 (OLR > 3.00 kg $COD.m^{-3}.d^{-1}$).

The highest COD removal (80%) achieved by digester AD-2 was found at an OLR of 3.00 kg $COD.m^{-3}.d^{-1}$, whilst the best COD removal rate (R-value) of 3.53 kg $COD.m^{-3}.d^{-1}$ was found at an OLR of 5.53 kg $COD.m^{-3}.d^{-1}$. Both the COD removal and R-values generally increased with increasing OLR (Fig. 7). Negative COD removal rates (Table 3c and Fig. 7) were experienced at times, especially at the beginning just after digester inoculation. Problems were also at times experienced with blockages in the multi-phase system, which led to negative COD removals. This resulted the intake of oxygen when cleaning the system, thus inhibiting the methanogenic performance efficiency of the digester.

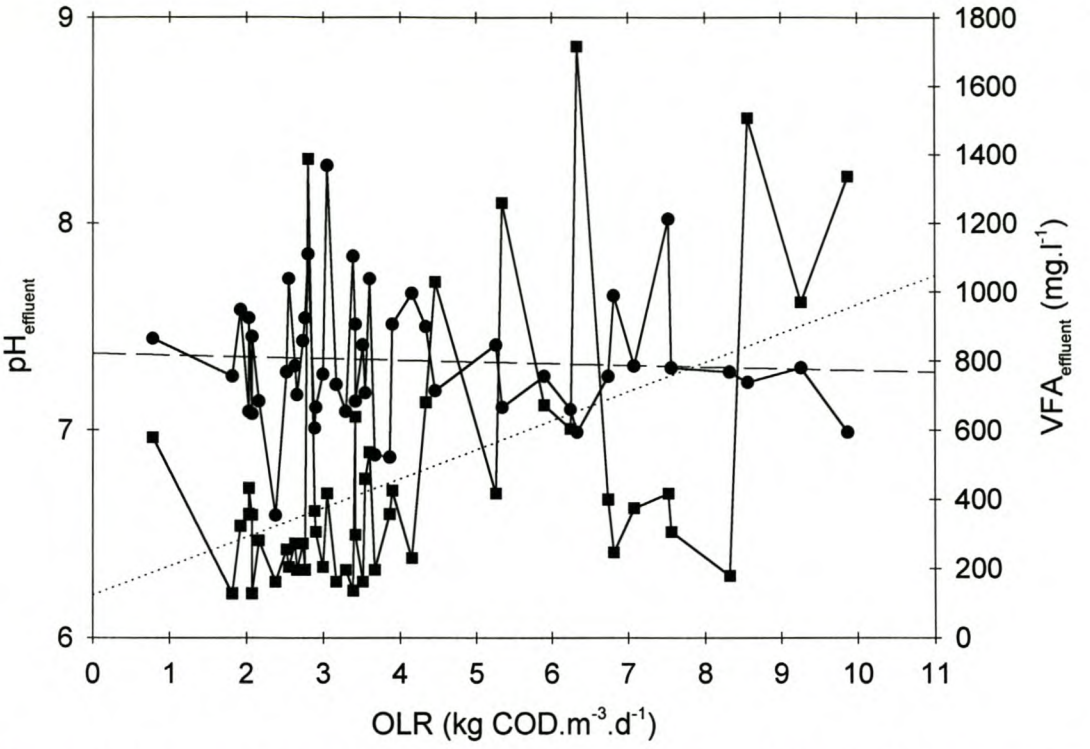

Figure 4. The variation in effluent pH (● ; -----) and the VFA content (■ ; .....) of the effluent, as a function of OLR for digester AD-1 during multi-phase digestion. The solid lines represent the actual data lines and the dotted and dashed lines represent the regression lines.

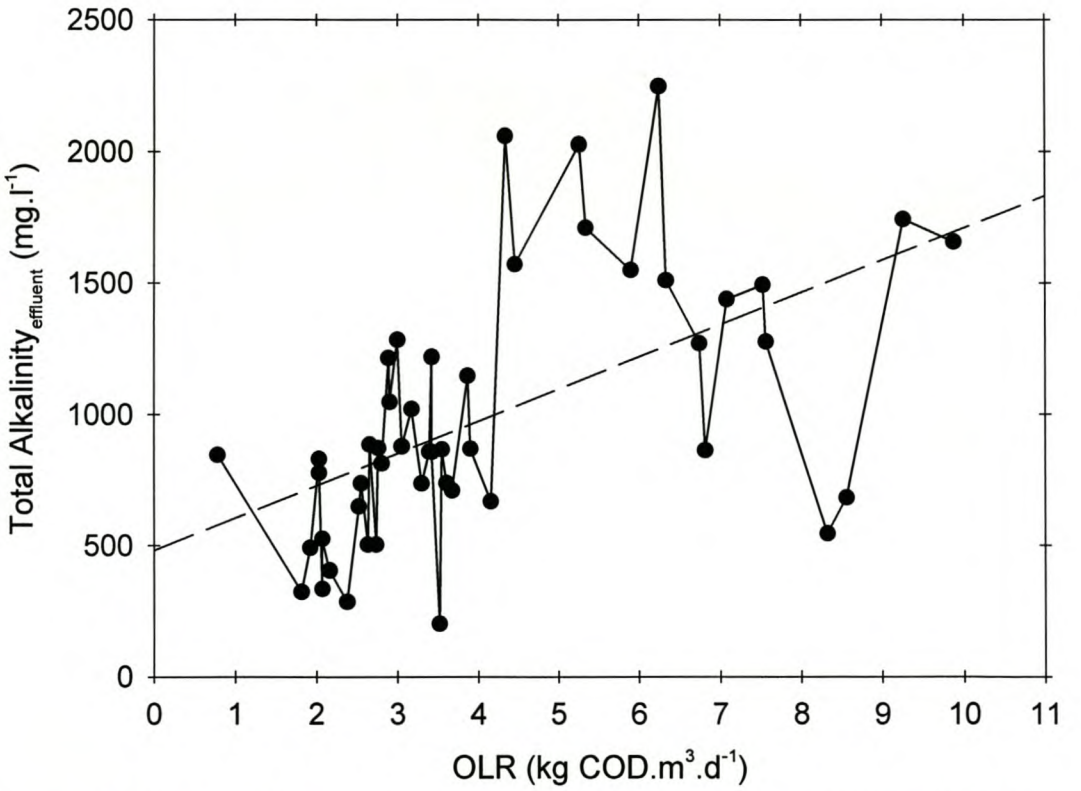

Figure 5. The variation of the total alkalinity (● ; -----) of the effluent, as a function of OLR for digester AD-1 during multi-phase digestion. The solid line represents the actual data and the dashed lines represent the regression calculations.

Table 3a. Composition of the substrate used for digester AD-2 during multi-phase operating conditions, as a function of organic loading rate (OLR)*.

Digester Substrate																
OLR ($kg\ COD.m^{-3}.d^{-1}$)		COD ($mg.l^{-1}$)	TKN ($mg.l^{-1}$)	VFA ($mg.l^{-1}$)	SO_4^{2-} ($mg.l^{-1}$)	pH	TAlk ($mg.l^{-1}$ as $CaCO_3$)	Cl ($mg.l^{-1}$)	NH_3-N ($mg.l^{-1}$)	TS ($mg.l^{-1}$)	VS ($mg.l^{-1}$)	TNVS ($mg.l^{-1}$)	PO_4-P ($mg.l^{-1}$)	Tmetals ($mg.l^{-1}$)	Na ($mg.l^{-1}$)	Ca ($mg.l^{-1}$)
0.31		306	431	417	210	8.3	878	N/D	N/D	3800	400	3400	6.6	1.77	1091	N/D
0.31		309	142	128	110	7.3	324	483.9	120.0	1100	200	900	1.3	0.31	191	14
0.39		394	264	196	119	7.2	885	N/D	N/D	2000	400	1600	7.9	0.87	530	56
0.45		451	189	579	70	7.4	845	N/D	184.6	3100	600	2500	6.1	0.89	851	143
0.46		455	157	323	110	7.6	491	662.2	80.3	1600	300	1300	0.8	0.49	399	44
0.46		459	123	128	160	7.5	525	517.5	64.2	1500	200	1300	3.0	0.33	440	53
0.52		523	131	162	86	6.6	286	N/D	87.5	1800	300	1500	2.1	0.24	467	15
0.54		538	167	162	90	7.4	203	N/D	106.0	1900	400	1500	1.7	0.85	643	22
0.58		582	176	179	110	7.3	545	N/D	138.0	1400	400	1000	4.2	0.36	277	57
0.61		609	321	1506	169	7.2	681	N/D	N/D	3900	900	3000	4.0	2.09	1018	90
0.61		612	255	255	110	7.3	649	619.3	199.0	1500	500	1000	1.2	0.60	363	40
0.69		693	315	162	310	7.2	1020	978.1	207.0	1500	300	1200	0.9	0.31	358	33
0.75		746	345	204	210	7.3	1284	593.4	292.0	3000	1600	1400	1.2	0.33	412	36
0.76		764	303	196	190	7.1	736	1235.1	148.9	2300	500	1800	2.5	0.69	425	82
0.81		813	566	136	142	7.8	858	N/D	172.0	1800	300	1500	2.1	1.54	735	43
0.82		815	5633	1387	170	7.9	813	N/D	N/D	3100	500	2600	5.5	1.05	94	36
0.82		816	426	230	110	7.7	668	N/D	247.0	2200	400	1800	0.5	0.75	647	17
0.85		850	304	196	23	7.5	871	N/D	N/D	2900	600	2300	4.6	1.51	1012	108
0.87		871	100	432	158	7.5	776	N/D	52.0	1700	200	1500	4.5	1.46	595	111
0.92		918	618	672	60	7.3	1550	N/D	391.0	2000	800	1200	3.0	0.53	345	47
0.92		918	129	355	40	7.1	335	N/D	80.2	2900	500	2400	2.4	0.43	717	23
0.95		948	373	306	14	7.1	1047	N/D	N/D	2500	700	1800	7.5	1.15	360	83
0.97		971	349	204	170	7.7	736	1831.3	165.0	3000	500	2500	2.2	1.14	788	71
0.98		984	142	281	240	7.1	405	622.7	61.2	1600	200	1400	2.4	0.74	149	33
1.02		1018	444	272	90	7.3	503	1415.9	250.0	1900	500	1400	0.3	0.95	404	15
1.06		1064	280	357	250	7.1	830	576.2	214.7	1800	500	1300	5.6	0.96	241	192
1.08		1080	656	1030	60	7.2	1572	N/D	332.0	1900	1200	700	2.5	0.67	353	32
1.38		1375	607	638	150	7.5	1218	N/D	194.0	2300	400	1900	3.3	1.60	602	57
1.38		1382	338	366	110	7.0	1214	N/D	N/D	3400	700	2700	9.7	1.48	1609	156
1.48		1480	311	247	185	7.7	863	N/D	N/D	2500	600	1900	5.8	1.25	706	64
1.48		1481	199	272	250	7.4	503	N/D	102.0	2800	300	2500	2.5	0.55	790	12
1.55		1554	502	357	90	6.9	1147	N/D	324.0	2900	500	2400	4.2	0.78	407	63
1.64		1635	324	298	110	7.1	857	N/D	246.8	3400	900	2500	5.0	0.51	846	26
1.67		1670	469	400	90	7.3	1271	N/D	N/D	5200	800	4400	10.2	1.49	3242	288
1.88		1877	553	417	60	8.0	1494	N/D	760.0	1700	700	1000	0.9	0.82	367	64
1.91		1914	758	417	290	7.4	2027	N/D	N/D	6000	1500	4500	7.0	1.01	1493	48
1.92		1920	624	1716	176	7.0	1512	N/D	N/D	5400	1000	4400	8.4	2.69	115	60
1.98		1982	642	681	52	7.5	2060	N/D	N/D	6100	900	5200	11.9	3.11	1660	N/D
2.12		2118	643	604	370	7.1	2246	N/D	N/D	5400	1000	4400	8.4	2.20	866	164
2.14		2139	460	306	202	7.3	1277	N/D	N/D	5300	1000	4300	8.6	2.42	1220	77
2.42		2415	238	196	190	6.9	709	N/D	179.6	3000	700	2300	7.5	0.52	716	46
2.50		2501	447	426	92	7.5	869	1461.0	244.3	2300	400	1900	0.5	0.86	738	29
2.96		2959	726	1260	260	7.1	1712	N/D	N/D	11200	1500	9700	12.2	3.80	763	80
3.00		3000	303	536	120	7.7	738	N/D	164.0	1800	300	1500	3.6	0.43	458	35
3.03		3028	313	460	121	7.2	866	N/D	176.0	2000	400	1600	3.0	1.60	846	71
3.03		3034	569	374	220	7.3	1438	N/D	N/D	5500	1100	4400	8.8	2.40	987	85
3.69		3693	788	970	90	7.3	1744	N/D	N/D	7600	1800	5800	16.4	3.74	2000	99
5.53		5533	779	1336	110	7.0	1657	N/D	N/D	7800	2100	5700	17.2	4.01	1231	118
Minimum	0.31	306	100	128	14	6.6	203	483.9	52.0	1100	200	700	0.3	0.24	94	12
Maximum	5.53	5533	5633	1716	370	8.3	2246	1831.3	760.0	11200	2100	9700	17.2	4.01	3242	288
Average	1.42	1421	499	474	144	7.3	995	916.4	199.4	3194	677	2517	5.0	1.26	741	68

** Above data have been arranged according to numerical increases in **OLR**, while the numerical structure of **time** have been disregarded for this study's purposes (Fig. 6).*

N/D = Not Determined

Table 3b. Composition of the digester AD-2 effluent during multi-phase operating conditions at variable organic loading rates (OLR)*.

Digester Effluent																
OLR (kg COD.m^{-3}.d^{-1})		COD (mg.l^{-1})	TKN (mg.l^{-1})	VFA (mg.l^{-1})	SO_4^{2-} (mg.l^{-1})	pH	TAlk (mg.l^{-1} as $CaCO_3$)	Cl (mg.l^{-1})	NH_3-N (mg.l^{-1})	TS (mg.l^{-1})	VS (mg.l^{-1})	TNVS (mg.l^{-1})	PO_4-P (mg.l^{-1})	Tmetals (mg.l^{-1})	Na (mg.l^{-1})	Ca (mg.l^{-1})
0.31		254	342	306	196	8.3	1531	N/D	N/D	4600	300	4300	4.2	2.24	816	N/D
0.31		205	143	119	110	7.7	520	472.0	141.0	1100	200	900	3.5	0.27	331	21
0.39		605	263	255	108	7.8	1155	N/D	N/D	2100	300	1800	13.8	0.98	684	80
0.45		504	179	502	70	7.7	1064	N/D	220.1	3200	600	2600	10.2	1.73	886	89
0.46		888	166	391	90	6.8	235	658.8	80.6	1800	300	1500	3.3	0.49	382	43
0.46		343	123	136	160	7.9	551	502.0	65.8	1500	200	1300	3.6	0.77	527	55
0.52		344	130	128	44	7.3	383	N/D	91.1	1900	300	1600	5.0	0.40	435	16
0.54		275	142	145	120	7.9	499	N/D	131.0	1600	200	1400	4.2	0.79	581	22
0.58		235	171	170	70	7.7	708	N/D	147.0	1400	200	1200	8.0	0.46	325	52
0.61		427	300	2945	123	7.7	880	N/D	N/D	3400	600	2800	7.3	1.42	795	70
0.61		265	253	153	200	7.3	647	662.4	189.0	1500	400	1100	3.3	0.63	412	55
0.69		330	262	145	290	7.7	882	664.1	185.0	1300	200	1100	3.4	0.30	492	25
0.75		618	314	221	180	7.5	1154	714.2	284.0	1800	300	1500	3.1	0.41	467	35
0.76		559	351	162	190	7.8	866	1638.8	184.2	2500	400	2100	4.8	0.78	609	84
0.81		439	490	136	57	8.2	951	N/D	155.0	1800	200	1600	5.3	1.38	661	70
0.82		387	4817	817	114	8.0	1192	N/D	N/D	3500	200	3300	10.4	0.83	89	20
0.82		260	376	187	70	8.0	1039	N/D	234.0	2200	300	1900	5.6	0.78	732	20
0.85		408	296	238	54	7.7	1043	N/D	N/D	2700	300	2400	7.6	1.27	914	96
0.87		827	170	162	204	7.3	343	N/D	76.0	1900	200	1700	7.6	1.39	622	44
0.92		847	605	409	110	7.7	1602	N/D	385.0	2300	900	1400	7.6	0.62	398	45
0.92		634	365	328	32	7.7	768	N/D	162.8	2700	600	2100	6.3	0.53	904	32
0.95		565	420	323	17	7.8	1953	N/D	N/D	2500	400	2100	13.9	0.90	575	85
0.97		762	436	187	90	7.7	1154	1990.7	224.0	3200	700	2500	6.1	1.26	384	84
0.98		528	180	179	340	7.7	534	552.0	91.5	1400	200	1200	3.4	0.60	179	75
1.02		341	469	187	45	7.6	857	1502.4	266.5	2200	400	1800	5.1	1.00	464	25
1.06		575	340	187	39	7.8	909	664.1	217.1	1800	400	1400	4.8	0.79	202	103
1.08		641	630	562	70	7.7	1812	N/D	316.0	1900	700	1200	6.4	0.77	356	29
1.38		827	602	502	90	7.8	1523	N/D	284.0	1400	500	900	6.1	1.41	483	52
1.38		1072	374	298	70	7.6	1554	N/D	N/D	4200	400	3800	14.1	1.65	2579	218
1.48		660	259	170	102	7.7	871	N/D	N/D	1900	300	1600	11.5	1.00	537	48
1.48		670	184	238	145	7.6	703	N/D	106.0	2500	100	2400	5.7	0.54	770	15
1.55		843	403	213	60	7.2	1187	N/D	362.0	2700	600	2100	7.3	0.83	231	81
1.64		851	304	204	150	7.9	865	N/D	243.2	3200	500	2700	8.4	0.62	960	40
1.67		1560	471	289	80	7.7	1471	N/D	N/D	5400	600	4800	14.2	1.32	2808	327
1.88		1549	493	340	60	7.9	1513	N/D	569.0	2200	600	1600	7.9	1.07	495	86
1.91		1051	708	289	130	7.8	2518	N/D	N/D	5500	1100	4400	10.3	1.11	1538	19
1.92		1960	572	140	180	7.7	1716	N/D	N/D	5700	500	5200	12.5	2.02	113	78
1.98		1116	694	400	44	8.1	2081	N/D	N/D	5400	500	4900	3.0	2.37	1060	N/D
2.12		1240	601	230	160	7.9	2520	N/D	N/D	5000	700	4300	11.0	1.54	847	140
2.14		847	375	340	107	8.3	1361	N/D	N/D	4700	700	4000	13.4	1.61	1195	73
2.42		523	228	145	90	7.6	1243	N/D	270.0	3500	1000	2500	9.8	0.89	749	63
2.50		1082	444	315	90	8.1	1151	1393.7	261.5	2400	400	2000	4.0	0.98	795	28
2.96		1306	657	1915	310	7.8	2126	N/D	N/D	7500	700	6800	13.4	2.86	695	70
3.00		600	296	477	110	8.0	1168	N/D	179.0	2600	300	2300	6.7	0.63	731	44
3.03		622	286	409	53	7.8	1233	N/D	185.0	2300	300	2000	6.3	1.35	3462	296
3.03		2046	515	494	210	7.6	1691	N/D	N/D	5300	800	4500	12.1	1.87	985	66
3.69		2598	819	349	90	7.9	2171	N/D	N/D	7700	1600	6100	17.3	3.88	2657	99
5.53		2001	671	545	60	7.7	1684	N/D	N/D	6600	1600	5000	12.9	2.40	1138	136
Minimum	0.31	205	123	119	17	6.8	235	472.0	65.8	1100	100	900	3.0	0.27	89	15
Maximum	5.53	2598	4817	2945	340	8.3	2520	1990.7	569.0	7700	1600	6800	17.3	3.88	3462	327
Average	1.42	794	473	375	116	7.7	1200	951.2	210.2	3073	496	2577	7.8	1.16	814	73

** Above data have been arranged according to numerical increases in **OLR**, while the numerical structure of **time** have been disregarded for this study's purposes (Fig. 6).*

N/D = Not Determined

Table 3c. The influence of variable organic loading rates (OLR) on digester AD-2 digester performance during multi-phase digestion.

Digester Efficiency																				
OLR ($kg\ COD.m^{-3}.d^{-1}$)	COD removal (%)	COD removal rate ($kg\ COD.m^{-3}.d^{-1}$)	SO_4^{2-} removal (%)	Biogas production (l/d)	Biogas yield ($m^3.m^{-3}$)	CH_4 content (%)	CH_4 yield ($m^3.kg\ COD_{rem}.d^{-1}$)	CH_4 yield ($m^3.kg\ COD_{load}.d^{-1}$)	VFA removal (%)	Cl removal (%)	NH_3-N removal (%)	TS removal (%)	VS removal (%)	TNVS removal (%)	TKN removal (%)	PO_4-P removal (%)	Tmetal removal (%)	Na removal (%)	Ca removal (%)
0.31	17.0	0.05	6.7	0.62	0.12	N/D	N/D	N/D	26.5	N/D	N/D	0.0	25.0	0.0	20.8	36.7	0.0	25.2	N/D
0.31	33.7	0.10	0.0	0.06	0.01	N/D	N/D	N/D	6.7	2.5	0.0	0.0	0.0	0.0	0.0	0.0	12.9	0.0	0.0
0.39	0.0	-0.21	9.2	0.37	0.07	N/D	N/D	N/D	0.0	N/D	N/D	0.0	25.0	0.0	0.2	0.0	0.0	0.0	0.0
0.45	0.0	-0.05	0.0	0.57	0.11	N/D	N/D	N/D	13.2	N/D	0.0	0.0	0.0	0.0	5.3	0.0	0.0	0.0	37.7
0.46	0.0	-0.43	18.2	N/D	N/D	N/D	N/D	N/D	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.2	2.3
0.46	25.3	0.12	0.0	N/D	N/D	N/D	N/D	N/D	0.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.52	34.2	0.18	48.8	N/D	N/D	N/D	N/D	N/D	21.1	N/D	0.0	0.0	0.0	0.0	0.9	0.0	0.0	6.9	0.0
0.54	48.9	0.26	0.0	N/D	N/D	N/D	N/D	N/D	10.5	N/D	0.0	15.8	50.0	6.7	14.8	0.0	7.1	9.6	0.0
0.58	59.6	0.35	36.4	0.25	0.05	N/D	N/D	N/D	4.8	N/D	0.0	0.0	50.0	0.0	2.9	0.0	0.0	0.0	8.9
0.61	29.9	0.18	27.2	0.06	0.01	N/D	N/D	N/D	0.0	N/D	N/D	12.8	33.3	6.7	6.6	0.0	32.1	21.9	22.8
0.61	56.7	0.35	0.0	0.46	0.09	N/D	N/D	N/D	40.0	0.0	5.0	0.0	20.0	0.0	0.7	0.0	0.0	0.0	0.0
0.69	52.4	0.36	6.5	N/D	N/D	N/D	N/D	N/D	10.5	32.1	10.6	13.3	33.3	8.3	16.9	0.0	3.2	0.0	26.5
0.75	17.2	0.13	14.3	N/D	N/D	N/D	N/D	N/D	0.0	0.0	2.7	40.0	81.3	0.0	9.1	0.0	0.0	0.0	2.6
0.76	26.8	0.21	0.0	N/D	N/D	N/D	N/D	N/D	17.4	0.0	0.0	0.0	20.0	0.0	0.0	0.0	0.0	0.0	0.0
0.81	46.0	0.37	59.9	0.29	0.06	N/D	N/D	N/D	0.0	N/D	9.9	0.0	33.3	0.0	13.4	0.0	10.4	10.0	0.0
0.82	52.5	0.43	32.9	0.06	0.01	N/D	N/D	N/D	41.1	N/D	N/D	0.0	60.0	0.0	14.5	0.0	21.0	6.1	45.4
0.82	68.1	0.56	36.4	N/D	N/D	N/D	N/D	N/D	18.5	N/D	5.3	0.0	25.0	0.0	11.7	0.0	0.0	0.0	0.0
0.85	52.0	0.44	0.0	0.16	0.03	N/D	N/D	N/D	0.0	N/D	N/D	6.9	50.0	0.0	2.6	0.0	15.9	9.6	10.6
0.87	5.1	0.04	0.0	0.06	0.01	N/D	N/D	N/D	62.5	N/D	0.0	0.0	0.0	0.0	0.0	0.0	4.8	0.0	60.0
0.92	7.7	0.07	0.0	1.14	0.23	N/D	N/D	N/D	39.2	N/D	1.5	0.0	0.0	0.0	2.2	0.0	0.0	0.0	4.4
0.92	30.9	0.28	20.0	0.59	0.12	N/D	N/D	N/D	7.7	N/D	0.0	6.9	0.0	12.5	0.0	0.0	0.0	0.0	0.0
0.95	40.4	0.38	0.0	0.97	0.19	N/D	N/D	N/D	0.0	N/D	N/D	0.0	42.9	0.0	0.0	0.0	21.7	0.0	0.0
0.97	21.5	0.21	47.1	0.58	0.12	75.0	2.08	0.45	8.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	51.3	0.0
0.98	46.3	0.46	0.0	N/D	N/D	N/D	N/D	N/D	36.4	11.4	0.0	12.5	0.0	14.3	0.0	0.0	18.9	0.0	0.0
1.02	66.5	0.68	50.0	N/D	N/D	N/D	N/D	N/D	31.2	0.0	0.0	0.0	20.0	0.0	0.0	0.0	0.0	0.0	0.0
1.06	46.0	0.49	84.4	N/D	N/D	N/D	N/D	N/D	47.6	0.0	0.0	0.0	20.0	0.0	0.0	13.8	17.7	16.1	46.4
1.08	40.6	0.44	0.0	1.69	0.34	N/D	N/D	N/D	45.5	N/D	4.8	0.0	41.7	0.0	3.9	0.0	0.0	0.0	8.6
1.38	39.9	0.55	40.0	0.34	0.07	N/D	N/D	N/D	21.3	N/D	0.0	39.1	0.0	52.6	0.8	0.0	11.9	19.7	8.0
1.38	22.4	0.31	36.4	0.85	0.17	88.5	2.43	0.54	18.6	N/D	N/D	0.0	42.9	0.0	0.0	0.0	0.0	0.0	0.0
1.48	55.4	0.82	44.9	0.81	0.16	94.2	0.93	0.52	31.0	N/D	N/D	24.0	50.0	15.8	16.7	0.0	20.0	23.9	24.2
1.48	54.8	0.81	42.0	N/D	N/D	N/D	N/D	N/D	12.5	N/D	0.0	10.7	66.7	4.0	7.6	0.0	1.8	2.5	0.0
1.55	45.8	0.71	33.3	1.51	0.30	N/D	N/D	N/D	40.5	N/D	0.0	6.9	0.0	12.5	19.8	0.0	0.0	43.2	0.0
1.64	48.0	0.78	0.0	1.16	0.23	76.5	1.13	0.54	31.4	N/D	1.5	5.9	44.4	0.0	6.2	0.0	0.0	0.0	0.0
1.67	6.6	0.11	11.1	1.46	0.29	84.1	11.16	0.74	27.7	N/D	N/D	0.0	25.0	0.0	0.0	0.0	11.4	13.4	0.0
1.88	17.5	0.33	0.0	0.66	0.13	N/D	N/D	N/D	18.4	N/D	25.1	0.0	14.3	0.0	10.7	0.0	0.0	0.0	0.0
1.91	45.1	0.86	55.2	1.46	0.29	N/D	N/D	N/D	30.6	N/D	N/D	8.3	26.7	2.2	6.6	0.0	0.0	0.0	60.0
1.92	0.0	-0.04	0.0	1.80	0.36	N/D	N/D	N/D	91.9	N/D	N/D	0.0	50.0	0.0	8.3	0.0	24.9	1.9	0.0
1.98	43.7	0.87	15.4	1.77	0.35	N/D	N/D	N/D	41.2	N/D	N/D	11.5	44.4	5.8	0.0	74.5	23.8	36.1	N/D
2.12	41.5	0.88	56.8	1.28	0.26	39.1	0.57	0.24	62.0	N/D	N/D	7.4	30.0	2.3	6.4	0.0	30.0	2.1	14.6
2.14	60.4	1.27	47.0	1.31	0.26	90.9	0.94	0.56	0.0	N/D	N/D	11.3	30.0	7.0	18.5	0.0	33.5	2.0	4.9
2.42	78.3	1.89	52.6	0.48	0.10	N/D	N/D	N/D	26.1	N/D	0.0	0.0	0.0	0.0	4.0	0.0	0.0	0.0	0.0
2.50	56.7	1.42	2.2	N/D	N/D	N/D	N/D	N/D	26.0	4.6	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0	3.1
2.96	55.9	1.65	0.0	2.05	0.41	N/D	N/D	N/D	0.0	N/D	N/D	33.0	53.3	29.9	9.4	0.0	24.7	8.9	12.6
3.00	80.0	2.40	8.3	0.12	0.02	N/D	N/D	N/D	11.1	N/D	0.0	0.0	0.0	0.0	2.2	0.0	0.0	0.0	0.0
3.03	79.5	2.41	56.2	0.74	0.15	N/D	N/D	N/D	11.1	N/D	0.0	0.0	25.0	0.0	8.8	0.0	15.6	0.0	0.0
3.03	32.6	0.99	4.5	1.66	0.33	95.2	1.60	0.52	0.0	N/D	N/D	3.6	27.3	0.0	9.4	0.0	22.1	0.2	23.0
3.69	29.7	1.10	0.0	2.85	0.57	90.5	2.36	0.70	64.0	N/D	N/D	0.0	11.1	0.0	0.0	0.0	0.0	0.0	0.0
5.53	63.8	3.53	45.5	2.44	0.49	80.9	0.56	0.36	59.2	N/D	N/D	15.4	23.8	12.3	13.9	25.1	40.1	7.6	0.0
Minimum 0.31	0.0	-0.43	0.0	0.06	0.01	39.1	0.56	0.24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Maximum 5.53	80.0	3.53	84.4	2.85	0.57	95.2	11.16	0.74	91.9	32.1	25.1	40.0	81.3	52.6	20.8	74.5	40.1	51.3	60.0
Average 1.42	39.2	0.63	21.9	0.93	0.19	81.5	2.38	0.52	23.2	4.5	2.2	5.9	24.9	4.0	5.8	3.1	8.9	6.7	9.3

N/D = Not Determined

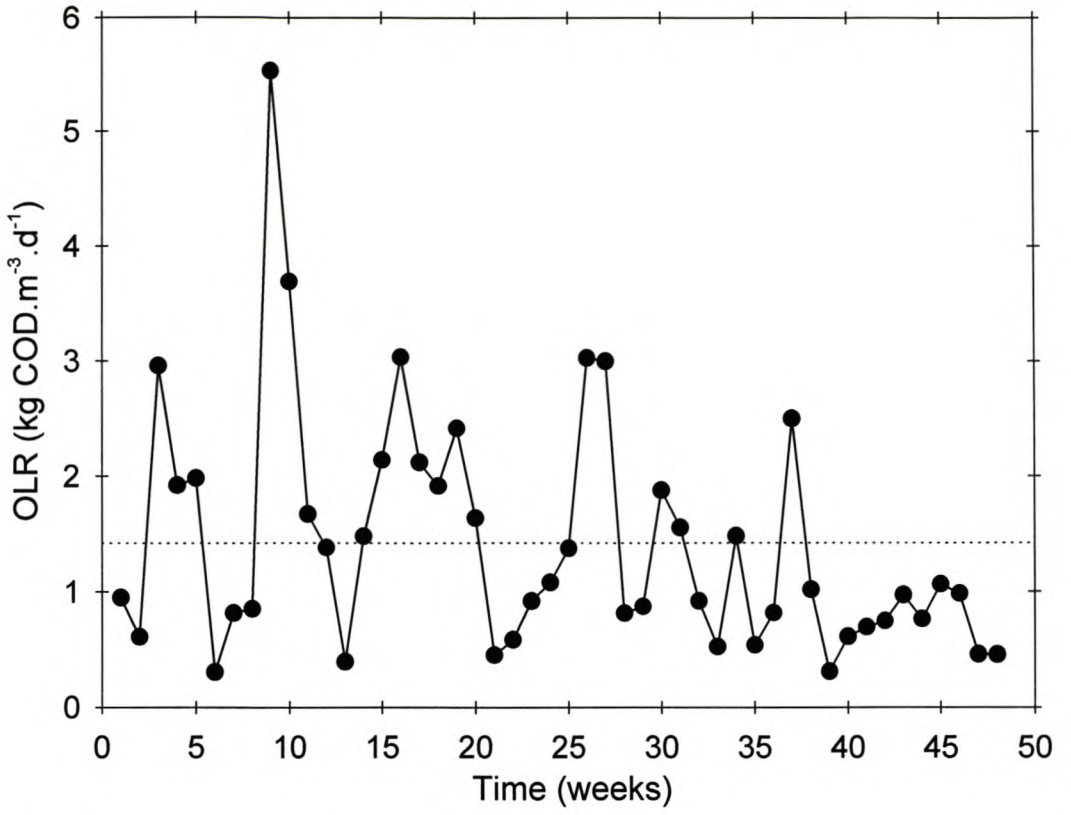

Figure 6. The variation in substrate OLR during the study on digester AD-2 over the 48 week period. The dotted line indicates the average OLR.

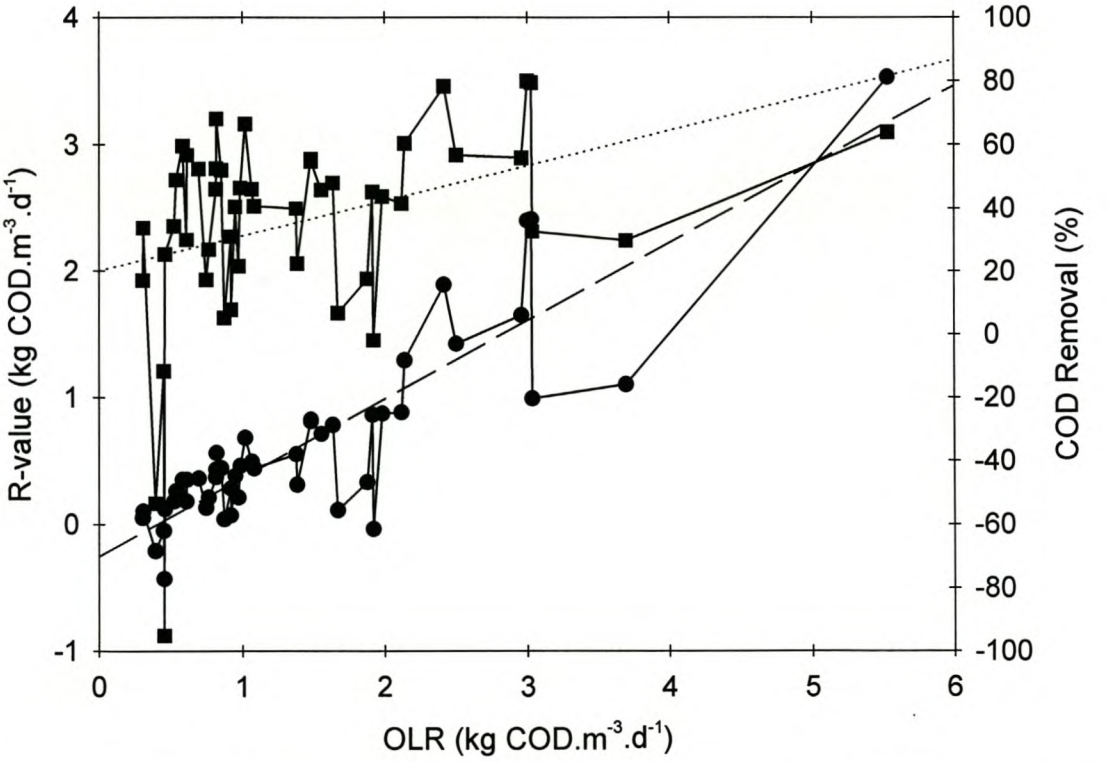

Figure 7. The COD removal rate (R-value) (● ; -----) and COD removal (■ ; .....), as a function of OLR for digester AD-2. The solid lines represent the actual data and the dotted and dashed lines represent the regression calculations.

The best biogas yields (0.57 $m^3.m^{-3}$ reactor) were found at an OLR of 3.69 kg $COD.m^{-3}.d^{-1}$ (Table 3c). The average biogas yield data of digester AD-2 (0.19 $m^3.m^{-3}$) was also much lower than the biogas yield recorded for digester AD-1 (1.03 $m^3.m^{-3}$ reactor). This suggests that the first digester (AD-1) possibly utilised most of the easily degradable compounds and probably depleted other nutrients before its effluent was fed as substrate to digester AD-2. The biogas yield increased as the OLR increased while the methane yield per COD removed decreased with increasing OLR (Fig. 8). Both the methane yield per COD removed and per COD loaded were the highest (11.16 and 0.74 $m^3.kg\ COD.d^{-1}$; respectively) at an OLR of 1.67 kg $COD.m^{-3}.d^{-1}$. The methane yield per COD loaded was found to be fairly stable throughout the study, indicating the selective but consistent use and conversion of acetate to CH_4. This was supported by the low VFA concentration present in the final effluent. The average methane content of digester AD-2 was 95% (Table 3c), suggesting that an active and acclimatised methanogenic population was present in this eco-system.

A SO_4 removal efficiency of 84% was found at an OLR of 1.06 kg $COD.m^{-3}.d^{-1}$ (Table 3c), indicating the presence of an active sulphate reducing bacterial population. The highest TS and VS removal (40 and 81%, respectively) were found at an OLR of 0.75 kg $COD.m^{-3}.d^{-1}$. The effluent pH and VFA profiles as a function of the OLR, are illustrated in Fig. 9. The effluent pH (average pH of 7.7) generally remained fairly constant with increasing OLR. The effluent alkalinity was found to increase significantly with increased OLR (Table 3b) and ranged between 600 - 2 500 $mg.l^{-1}$, indicating a fairly good buffering capacity (Fig. 10) (Hawkes *et al.*, 1992). These results suggest that the AD-2 system functioned fairly well under continuously changing conditions and several extreme organic shock loads.

Total digester efficiency (AD-1 + AD-2)

The total digester performance (Table 4b) was calculated using the substrate values of digester AD-1 (Table 2a) and the effluent values of digester AD-2 (Table 4a). This data represent the total treatment efficiency of the two digesters (AD-1 + AD-2) applied in series. When the data for the total

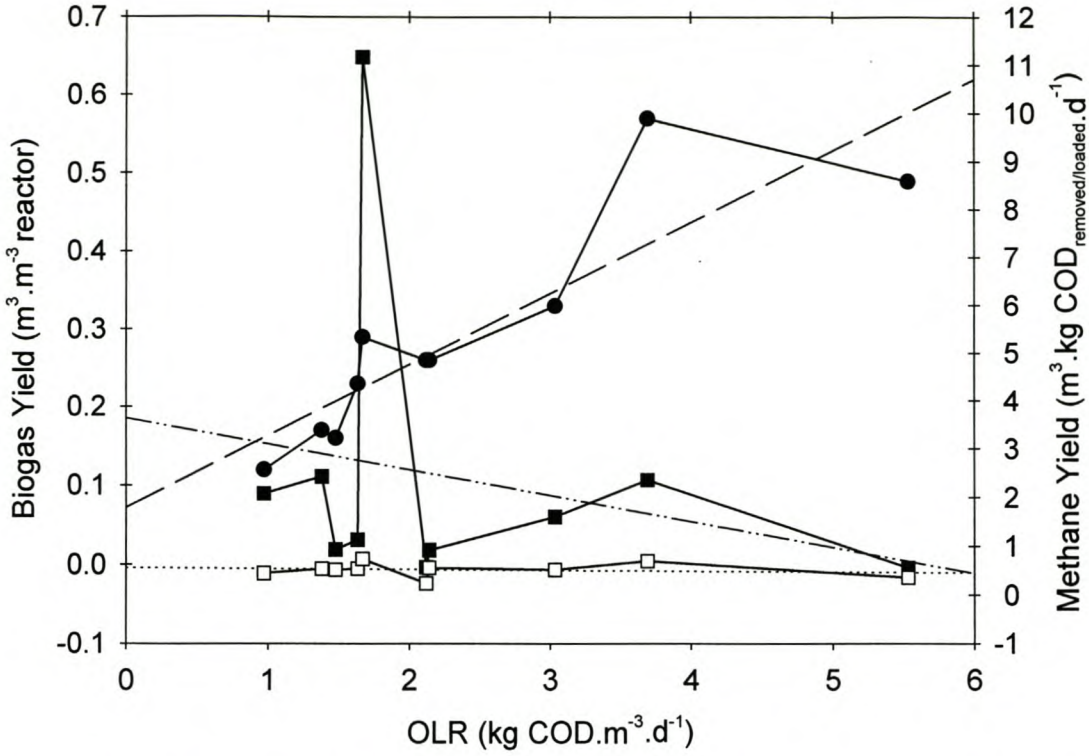

Figure 8. The influence of varying OLR's on biogas yield (● ; -----), methane yield per $COD_{removed}$ (■ ; —··—··—) and methane yield per COD_{loaded} (□ ; .....) for digester AD-2. The solid lines represent the actual data and the dotted and dashed lines represent the regression calculations.

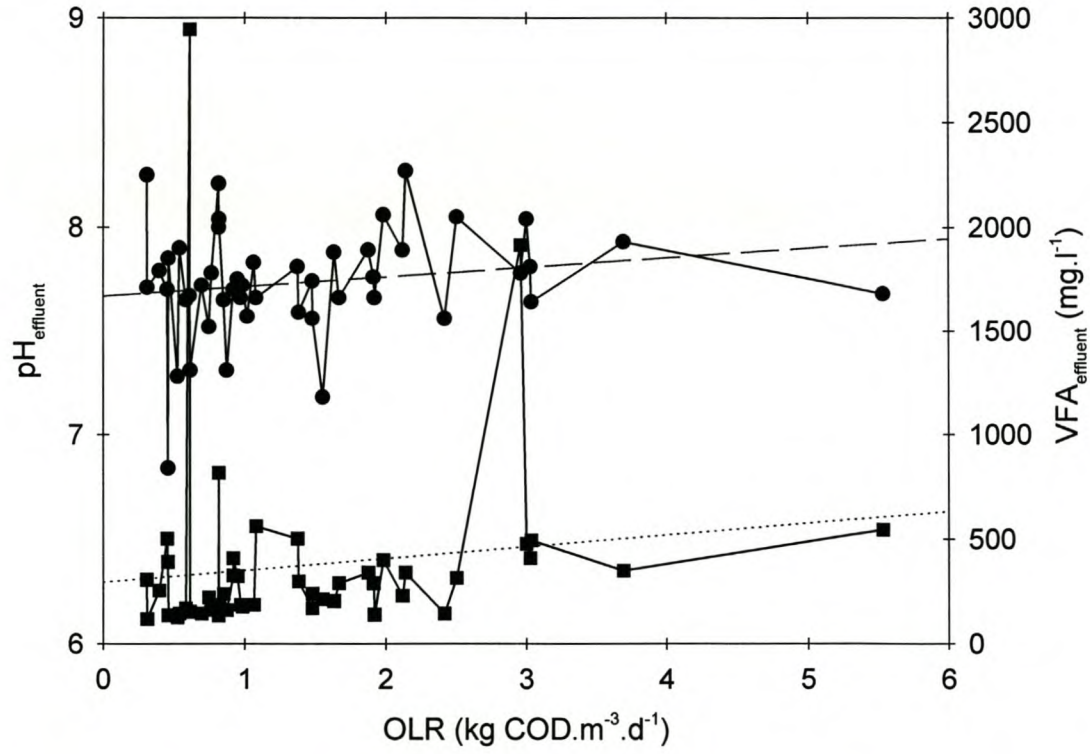

Figure 9. The variation of pH (● ; -----) and VFA content (■ ; .....) in the effluent, as a function of OLR for digester AD-2. The solid lines represent the actual data and the dotted and dashed lines represent the regression calculations.

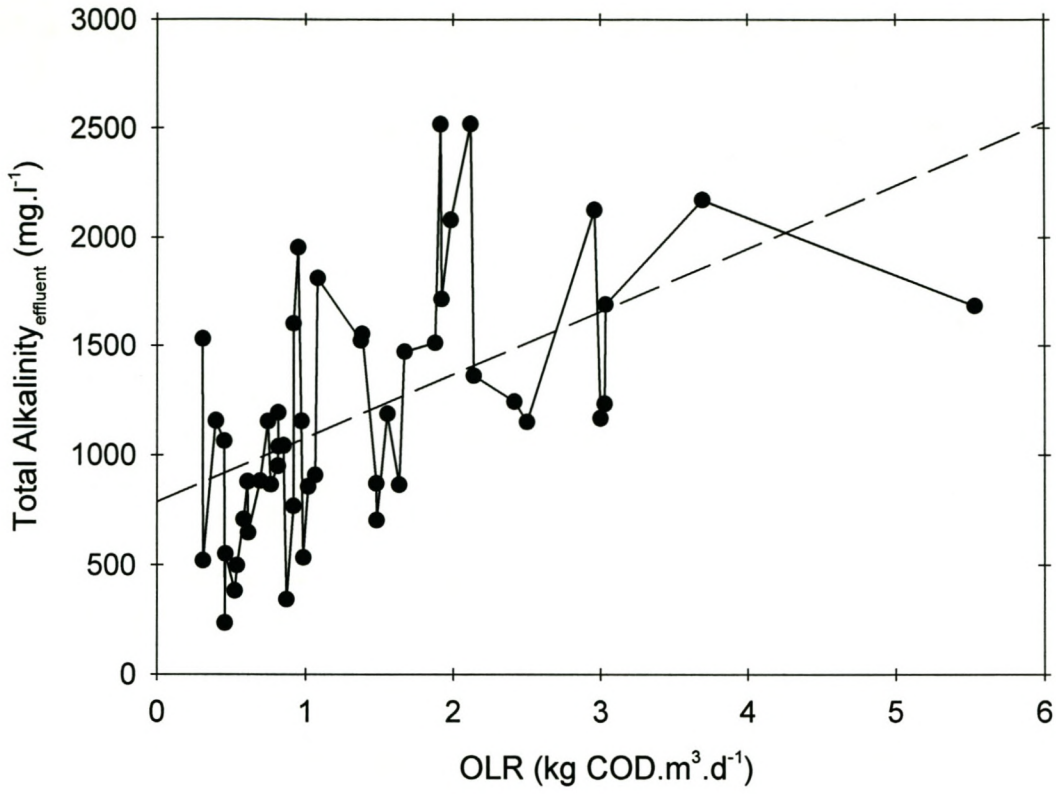

Figure 10. The variation of the total alkalinity (● ; -----) of the effluent, as a function of OLR for digester AD-2. The solid line represents the actual data and the dashed line represents the regression calculations.

Table 4a. Composition of the AD-2 effluent based on the total multi-phase operating conditions using the raw gelatin-manufacturing effluent (AD-1) as basis for the efficiency calculations*.

Digester Effluent																
OLR (kg COD.m^{-3}.d^{-1})		COD (mg.l^{-1})	TKN (mg.l^{-1})	VFA (mg.l^{-1})	SO_4^{2-} (mg.l^{-1})	pH	TAlk (mg.l^{-1} as $CaCO_3$)	Cl (mg.l^{-1})	NH_3-N (mg.l^{-1})	TS (mg.l^{-1})	VS (mg.l^{-1})	TNVS (mg.l^{-1})	PO_4-P (mg.l^{-1})	Tmetals (mg.l^{-1})	Na (mg.l^{-1})	Ca (mg.l^{-1})
0.78		504	179	502	70	7.7	1064	N/D	220.1	3200	600	2600	10.2	1.73	886	89
1.81		205	143	119	110	7.7	520	472.0	141.0	1100	200	900	3.5	0.27	331	21
1.92		888	166	391	90	6.8	235	658.8	80.6	1800	300	1500	3.3	0.49	382	43
2.03		575	340	187	39	7.8	909	664.1	217.1	1800	400	1400	4.8	0.79	202	103
2.03		827	170	162	204	7.3	343	N/D	76.0	1900	200	1700	7.6	1.39	622	44
2.07		634	365	328	32	7.7	768	N/D	162.8	2700	600	2100	6.3	0.53	904	32
2.07		343	123	136	160	7.9	551	502.0	65.8	1500	200	1300	3.6	0.77	527	55
2.16		528	180	179	340	7.7	534	552.0	91.5	1400	200	1200	3.4	0.60	179	75
2.38		344	130	128	44	7.3	383	N/D	91.1	1900	300	1600	5.0	0.40	435	16
2.52		265	253	153	200	7.3	647	662.4	189.0	1500	400	1100	3.3	0.63	412	55
2.55		762	436	187	90	7.7	1154	1990.7	224.0	3200	700	2500	6.1	1.26	384	84
2.63		341	469	187	45	7.6	857	1502.4	266.5	2200	400	1800	5.1	1.00	464	25
2.66		605	263	255	108	7.8	1155	N/D	N/D	2100	300	1800	13.8	0.98	684	80
2.73		670	184	238	145	7.6	703	N/D	106.0	2500	100	2400	5.7	0.54	770	15
2.76		408	296	238	54	7.7	1043	N/D	N/D	2700	300	2400	7.6	1.27	914	96
2.80		387	4817	817	114	8.0	1192	N/D	N/D	3500	200	3300	10.4	0.83	89	20
2.89		1072	374	298	70	7.6	1554	N/D	N/D	4200	400	3800	14.1	1.65	2579	218
2.90		565	420	323	17	7.8	1953	N/D	N/D	2500	400	2100	13.9	0.90	575	85
3.00		618	314	221	180	7.5	1154	714.2	284.0	1800	300	1500	3.1	0.41	467	35
3.05		254	342	306	196	8.3	1531	N/D	N/D	4600	300	4300	4.2	2.24	816	N/D
3.17		330	262	145	290	7.7	882	664.1	185.0	1300	200	1100	3.4	0.30	492	25
3.29		559	351	162	190	7.8	866	1638.8	184.2	2500	400	2100	4.8	0.78	609	84
3.39		439	490	136	57	8.2	951	N/D	155.0	1800	200	1600	5.3	1.38	661	70
3.42		851	304	204	150	7.9	865	N/D	243.2	3200	500	2700	8.4	0.62	960	40
3.42		827	602	502	90	7.8	1523	N/D	284.0	1400	500	900	6.1	1.41	483	52
3.51		275	142	145	120	7.9	499	N/D	131.0	1600	200	1400	4.2	0.79	581	22
3.54		622	286	409	53	7.8	1233	N/D	185.0	2300	300	2000	6.3	1.35	3462	296
3.60		600	296	477	110	8.0	1168	N/D	179.0	2600	300	2300	6.7	0.63	731	44
3.67		523	228	145	90	7.6	1243	N/D	270.0	3500	1000	2500	9.8	0.89	749	63
3.87		843	403	213	60	7.2	1187	N/D	362.0	2700	600	2100	7.3	0.83	231	81
3.90		1082	444	315	90	8.1	1151	1393.7	261.5	2400	400	2000	4.0	0.98	795	28
4.15		260	376	187	70	8.0	1039	N/D	234.0	2200	300	1900	5.6	0.78	732	20
4.33		1116	694	400	44	8.1	2081	N/D	N/D	5400	500	4900	3.0	2.37	1060	N/D
4.46		641	630	562	70	7.7	1812	N/D	316.0	1900	700	1200	6.4	0.77	356	29
5.26		1051	708	289	130	7.8	2518	N/D	N/D	5500	1100	4400	10.3	1.11	1538	19
5.34		1306	657	1915	310	7.8	2126	N/D	N/D	7500	700	6800	13.4	2.86	695	70
5.90		847	605	409	110	7.7	1602	N/D	385.0	2300	900	1400	7.6	0.62	398	45
6.25		1240	601	230	160	7.9	2520	N/D	N/D	5000	700	4300	11.0	1.54	847	140
6.33		1960	572	140	180	7.7	1716	N/D	N/D	5700	500	5200	12.5	2.02	113	78
6.74		1560	471	289	80	7.7	1471	N/D	N/D	5400	600	4800	14.2	1.32	2808	327
6.81		660	259	170	102	7.7	871	N/D	N/D	1900	300	1600	11.5	1.00	537	48
7.08		2046	515	494	210	7.6	1691	N/D	N/D	5300	800	4500	12.1	1.87	985	66
7.53		1549	493	340	60	7.9	1513	N/D	569.0	2200	600	1600	7.9	1.07	495	86
7.56		847	375	340	107	8.3	1361	N/D	N/D	4700	700	4000	13.4	1.61	1195	73
8.32		235	171	170	70	7.7	708	N/D	147.0	1400	200	1200	8.0	0.46	325	52
8.56		427	300	2945	123	7.7	880	N/D	N/D	3400	600	2800	7.3	1.42	795	70
9.26		2598	819	349	90	7.9	2171	N/D	N/D	7700	1600	6100	17.3	3.88	2657	99
9.87		2001	671	545	60	7.7	1684	N/D	N/D	6600	1600	5000	12.9	2.40	1138	136
Minimum	0.78	205	123	119	17	6.8	235	472.0	65.8	1100	100	900	3.0	0.27	89	15
Maximum	9.87	2598	4817	2945	340	8.3	2520	1990.7	569.0	7700	1600	6800	17.3	3.88	3462	327
Average	4.17	794	473	375	116	7.7	1200	951.2	210.2	3073	496	2577	7.8	1.16	814	73

** Above data have been arranged according to numerical increase in **OLR**, while the numerical structure of **time** have been disregarded for this study's purposes (Fig. 1).*

N/D = Not Determined

Table 4b. The influence of variable organic loading rates (OLR) on the total digestion performance (AD-1 + AD-2) during multi-phase digestion.

Total Digester Efficiency																				
OLR (kg COD.m^{-3}.d^{-1})	COD removal (%)	COD removal rate (kg COD.m^{-3}.d^{-1})	SO_4^{2-} removal (%)	Biogas production (l/d)	Biogas yield (m^3.m^{-3})	CH_4 content (%)	CH_4 yield (m^3.kg COD_{rem}.d^{-1})	CH_4 yield (m^3.kg COD_{load}.d^{-1})	VFA removal (%)	Cl removal (%)	NH_3-N removal (%)	TS removal (%)	VS removal (%)	TNVS removal (%)	TKN removal (%)	PO_4-P removal (%)	Tmetal removal (%)	Na removal (%)	Ca removal (%)
0.78	35.5	0.28	63.2	2.96	0.59	N/D	N/D	N/D	59.0	N/D	0.0	0.0	50.0	0.0	8.8	0.0	0.0	0.0	0.0
1.81	88.7	1.61	71.1	2.51	0.50	N/D	N/D	N/D	53.3	0.0	0.0	47.6	81.8	10.0	0.0	0.0	38.6	7.1	28.6
1.92	89.3	1.03	85.8	N/D	N/D	N/D	N/D	N/D	82.9	0.0	0.0	52.2	90.9	0.0	0.0	0.0	48.1	0.0	0.0
2.03	71.6	1.45	53.6	N/D	N/D	N/D	N/D	N/D	0.0	0.0	0.0	35.7	73.3	0.0	0.0	0.0	61.3	0.0	23.1
2.03	59.3	1.20	67.6	0.52	0.10	N/D	N/D	N/D	79.3	N/D	0.0	0.0	71.4	0.0	0.0	0.0	0.0	18.6	61.9
2.07	69.4	1.44	73.3	3.19	0.64	N/D	N/D	N/D	0.0	N/D	0.0	10.0	45.5	0.0	0.0	0.0	0.0	0.0	0.0
2.07	83.4	1.73	0.0	N/D	N/D	N/D	N/D	N/D	23.8	3.6	0.0	42.3	83.3	7.1	37.9	0.0	0.0	5.6	5.2
2.16	75.5	1.63	2.9	N/D	N/D	N/D	N/D	N/D	16.0	0.0	0.0	17.6	60.0	0.0	0.0	0.0	25.9	23.6	0.0
2.38	85.5	2.03	92.5	N/D	N/D	N/D	N/D	N/D	62.5	N/D	0.0	50.0	76.9	36.0	21.6	0.0	14.9	45.2	0.0
2.52	89.5	2.26	41.2	3.62	0.72	N/D	N/D	N/D	68.4	0.0	0.0	25.0	63.6	0.0	9.1	0.0	12.5	0.0	0.0
2.55	70.1	1.79	80.9	6.06	1.21	77.9	4.89	2.19	40.5	0.0	0.0	5.9	50.0	0.0	0.0	0.0	3.1	44.3	0.0
2.63	87.0	2.29	70.0	N/D	N/D	N/D	N/D	N/D	55.1	0.0	0.0	15.4	75.0	0.0	0.0	0.0	8.3	0.0	0.0
2.66	77.2	2.05	28.5	3.90	0.78	N/D	N/D	N/D	62.0	N/D	N/D	0.0	66.7	0.0	0.0	0.0	11.7	0.0	0.0
2.73	75.5	2.06	61.8	N/D	N/D	N/D	N/D	N/D	15.2	N/D	0.0	34.2	93.3	0.0	45.9	0.0	26.0	26.2	34.8
2.76	85.2	2.35	92.1	5.41	1.08	N/D	N/D	N/D	70.5	N/D	N/D	10.0	50.0	0.0	2.6	0.0	37.1	4.0	19.8
2.80	86.2	2.42	79.3	3.43	0.69	N/D	N/D	N/D	65.5	N/D	N/D	5.4	84.6	0.0	0.0	0.0	50.3	9.4	37.9
2.89	62.9	1.81	75.9	7.38	1.48	86.2	5.85	2.44	57.8	N/D	N/D	0.0	63.6	0.0	0.0	0.0	4.1	0.0	0.0
2.90	80.5	2.34	93.7	4.07	0.81	N/D	N/D	N/D	5.0	N/D	N/D	32.4	73.3	4.5	0.0	0.0	71.7	0.0	34.7
3.00	79.4	2.38	64.7	N/D	N/D	N/D	N/D	N/D	0.0	0.0	0.0	41.9	85.0	0.0	5.1	0.0	10.9	0.0	7.5
3.05	91.7	2.80	42.4	3.95	0.79	N/D	N/D	N/D	85.6	N/D	N/D	0.0	75.0	0.0	0.0	0.0	0.0	0.0	N/D
3.17	89.6	2.84	39.6	N/D	N/D	N/D	N/D	N/D	37.0	0.0	0.0	59.4	88.2	26.7	24.1	0.0	38.8	0.0	41.9
3.29	83.0	2.74	75.9	N/D	N/D	N/D	N/D	N/D	50.0	0.0	0.0	32.4	81.0	0.0	0.0	2.4	65.0	0.0	64.4
3.39	87.0	2.95	92.3	1.73	0.35	N/D	N/D	N/D	38.5	N/D	0.0	82.0	89.5	80.2	21.6	0.0	13.8	16.7	0.0
3.42	75.1	2.57	50.0	5.64	1.13	78.2	3.14	1.59	77.1	N/D	1.1	25.6	70.6	0.0	30.7	0.0	13.9	6.2	0.0
3.42	75.8	2.59	87.1	4.02	0.80	N/D	N/D	N/D	58.5	N/D	0.0	41.7	50.0	35.7	2.7	0.0	46.4	17.0	4.9
3.51	92.2	3.24	25.0	N/D	N/D	N/D	N/D	N/D	48.5	N/D	0.0	51.5	90.0	0.0	37.5	0.0	61.3	1.8	0.0
3.54	82.4	2.92	91.3	3.53	0.71	N/D	N/D	N/D	17.2	N/D	0.0	43.9	87.0	0.0	11.8	0.0	27.4	0.0	0.0
3.60	83.3	3.00	81.4	2.05	0.41	N/D	N/D	N/D	65.2	N/D	0.0	0.0	40.0	0.0	4.0	0.0	0.0	0.0	0.0
3.67	85.7	3.15	75.0	4.18	0.84	N/D	N/D	N/D	61.4	N/D	0.0	20.5	50.0	0.0	8.1	0.0	48.6	0.0	0.0
3.87	78.2	3.02	89.8	4.83	0.97	N/D	N/D	N/D	76.0	N/D	0.0	10.0	45.5	0.0	30.9	0.0	0.0	18.0	0.0
3.90	72.2	2.82	57.1	N/D	N/D	N/D	N/D	N/D	30.2	0.0	0.0	25.0	76.5	0.0	3.1	0.0	63.4	0.0	0.0
4.15	93.7	3.89	46.2	N/D	N/D	N/D	N/D	N/D	51.4	N/D	0.0	43.6	87.5	0.0	9.8	0.0	35.5	0.0	0.0
4.33	74.2	3.22	95.6	9.84	1.97	N/D	N/D	N/D	0.0	N/D	N/D	0.0	73.7	0.0	0.0	78.7	18.6	0.0	N/D
4.46	85.6	3.82	86.5	6.98	1.40	N/D	N/D	N/D	76.5	N/D	0.0	26.9	30.0	25.0	5.2	0.0	40.3	0.0	5.9
5.26	80.0	4.21	85.6	11.27	2.25	N/D	N/D	N/D	87.6	N/D	N/D	14.1	42.1	2.2	14.7	0.0	28.4	0.0	90.9
5.34	75.5	4.03	83.3	11.61	2.32	N/D	N/D	N/D	0.0	N/D	N/D	3.8	72.0	0.0	0.0	0.0	37.7	0.0	0.0
5.90	85.6	5.05	73.2	8.69	1.74	N/D	N/D	N/D	64.2	N/D	0.0	32.4	62.5	0.0	7.5	0.0	1.6	0.0	23.2
6.25	80.1	5.01	89.9	8.34	1.67	54.9	1.78	1.04	84.6	N/D	N/D	27.5	69.6	6.5	15.0	0.0	46.7	3.0	36.4
6.33	69.0	4.37	90.6	6.07	1.21	N/D	N/D	N/D	91.8	N/D	N/D	32.9	81.5	10.3	30.1	0.0	58.7	5.3	0.0
6.74	76.9	5.18	70.4	11.17	2.23	82.4	12.71	1.90	84.0	N/D	N/D	1.8	68.4	0.0	0.0	0.0	23.3	0.0	5.6
6.81	90.3	6.15	50.7	11.01	2.20	86.4	2.43	1.70	65.5	N/D	N/D	76.3	92.1	61.9	49.3	0.0	51.9	35.9	73.7
7.08	71.1	5.03	87.0	14.47	2.89	88.5	4.19	2.00	37.0	N/D	N/D	32.1	73.3	6.3	19.2	0.0	35.7	35.3	73.2
7.53	79.4	5.98	81.3	3.54	0.71	N/D	N/D	N/D	0.0	N/D	0.0	56.9	84.6	0.0	18.6	0.0	0.0	0.0	0.0
7.56	88.8	6.69	23.6	8.44	1.69	82.0	1.88	1.25	63.6	N/D	N/D	30.9	75.0	0.0	28.4	0.0	30.9	0.0	10.5
8.32	97.2	8.09	82.1	2.92	0.58	N/D	N/D	N/D	16.7	N/D	0.0	63.2	92.9	0.0	69.9	0.0	29.2	15.6	31.1
8.56	95.0	8.13	94.7	2.65	0.53	N/D	N/D	N/D	0.0	N/D	N/D	70.9	86.0	62.2	57.1	0.0	58.5	0.0	0.0
9.26	71.9	7.26	60.9	12.87	2.57	80.7	3.63	1.47	58.2	N/D	N/D	13.5	61.0	0.0	0.0	0.0	0.0	0.0	10.0
9.87	79.7	7.27	94.0	10.71	2.14	75.4	1.89	0.95	78.5	N/D	N/D	48.4	75.4	20.6	20.7	0.0	40.2	7.4	0.8
Minimum 0.78	35.5	0.28	0.0	0.52	0.10	54.9	1.78	0.95	0.0	0.0	0.0	0.0	30.0	0.0	0.0	0.0	0.0	0.0	0.0
Maximum 9.87	97.2	8.13	95.6	14.47	2.89	88.5	12.71	2.44	91.8	3.6	1.1	82.0	93.3	80.2	69.9	78.7	71.7	45.2	90.9
Average 4.17	80.1	3.38	68.8	6.10	1.22	79.3	4.24	1.65	48.4	0.3	0.0	29.0	71.0	8.2	13.6	1.7	27.9	7.2	15.8

N/D = Not Determined

system is taken into consideration, it was found that the COD removals, R-values, SO_4 removals, TS removals, biogas yields, effluent alkalinity's and effluent VFA removals generally increased with increasing OLR. The multi-phase digestion showed optimal removal efficiencies at OLR > 4.00 kg $COD.m^{-3}.d^{-1}$ (Table 4b). In this study no NH_3-N removal was found (Table 4b) which could possible be ascribed to the breakdown of proteins in the digestion process present in the gelatin-manufacturing effluent. This aspect of increased NH_3-N ($mg.l^{-1}$) concentrations must be taken into consideration in any scale-up possibilities and disposal strategies of the final effluent. Another factor to consider with multi-phase digestion is the fact that the ammonia released from the degradation of proteins leads to the selection of the growth of acidifying consortiums in the first phase, which has a significant influence on the down stream process (Tommaso *et al.*, 1999).

A total COD removal efficiency of 97% was found at an OLR of 8.32 kg $COD.m^{-3}.d^{-1}$ (Fig. 11) and SO_4 removal of 96% at 4.33 kg $COD.m^{-3}.d^{-1}$ (Fig. 13). The methane content varied between 55% and 89% (Table 4b). The highest methane yields (12.71 $m^3.kg\ COD_{removed}.d^{-1}$ and 2.44 $m^3.kg\ COD_{loaded}.d^{-1}$ were observed at OLR's of 6.74 and 2.89 kg $COD.m^{-3}.d^{-1}$, respectively (Fig. 12). A VFA removal of up to 92% was observed throughout the study. No VFA accumulation was observed (Table 4b). The effluent pH (Fig. 14) and the total alkalinity (Fig. 15) increased throughout the digestion process as a function of OLR, and an average pH of 7.7 and average total alkalinity of 1 200 $mg.l^{-1}$ was recorded.

Conclusions

The use of multi-phase anaerobic systems represents an innovative process in which each digestion stage harbours more or less the separate metabolic groups of the fermentative and methanogenic bacteria (Ramjeawon *et al.*, 1997) and is considered beneficial for the treatment of high-strength wastewater.

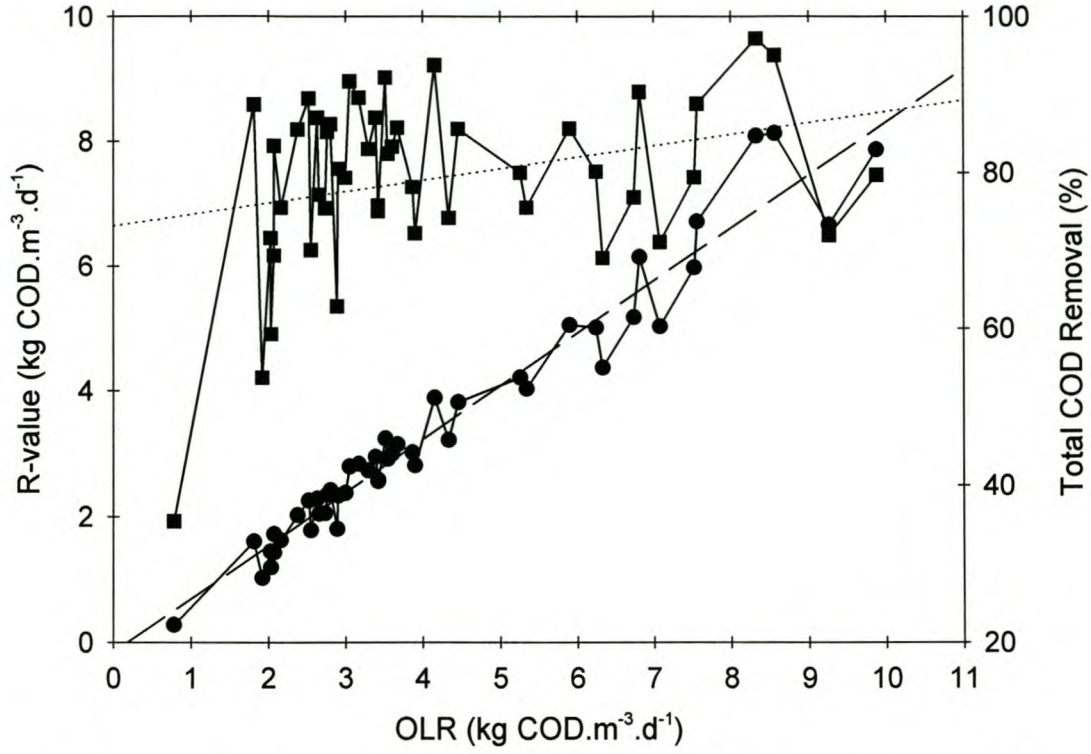

Figure 11. The total digester performance (AD-1+AD-2) of the COD removal rate (R-value) (● ; -----) and COD removal (■ ; .....) as a function of OLR. The solid lines represent the actual data and the dotted and dashed lines represent the regression calculations.

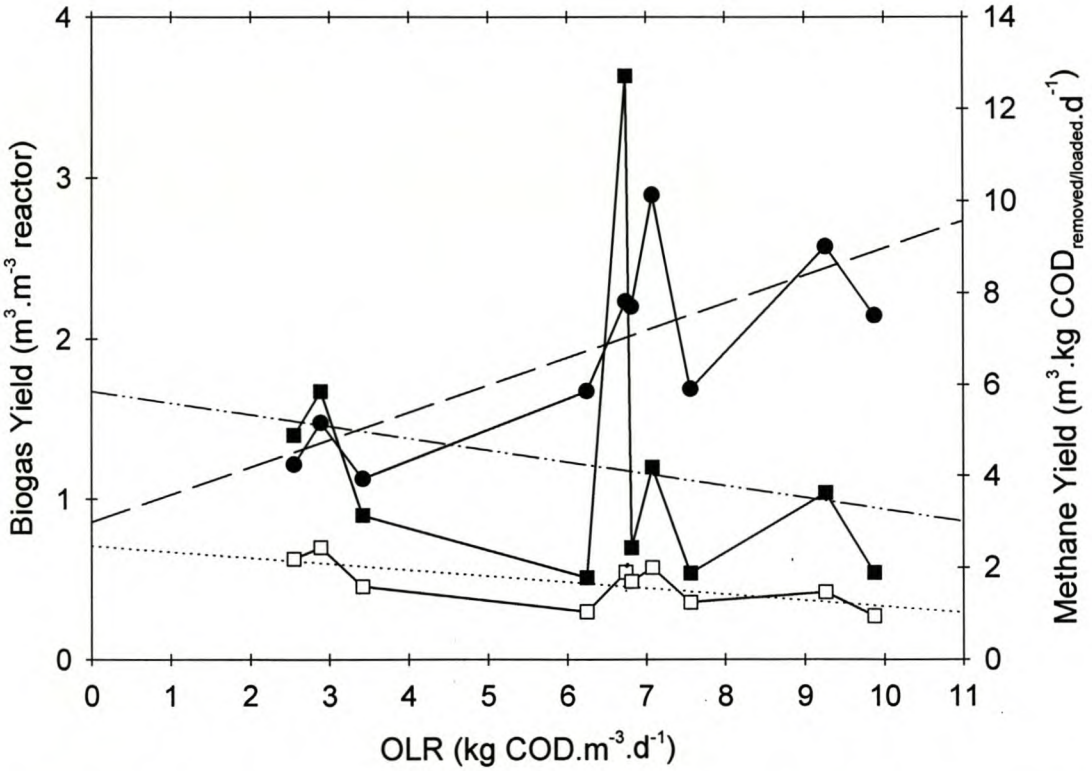

Figure 12. The influence of varying OLR's on biogas yield (● ; -----), methane yield per $COD_{removed}$ (■ ; —··—··—) and methane yield per COD_{loaded} (□ ; .....) for the total digester performance. The solid lines represent the actual data and the dotted and dashed lines represent the regression calculations.

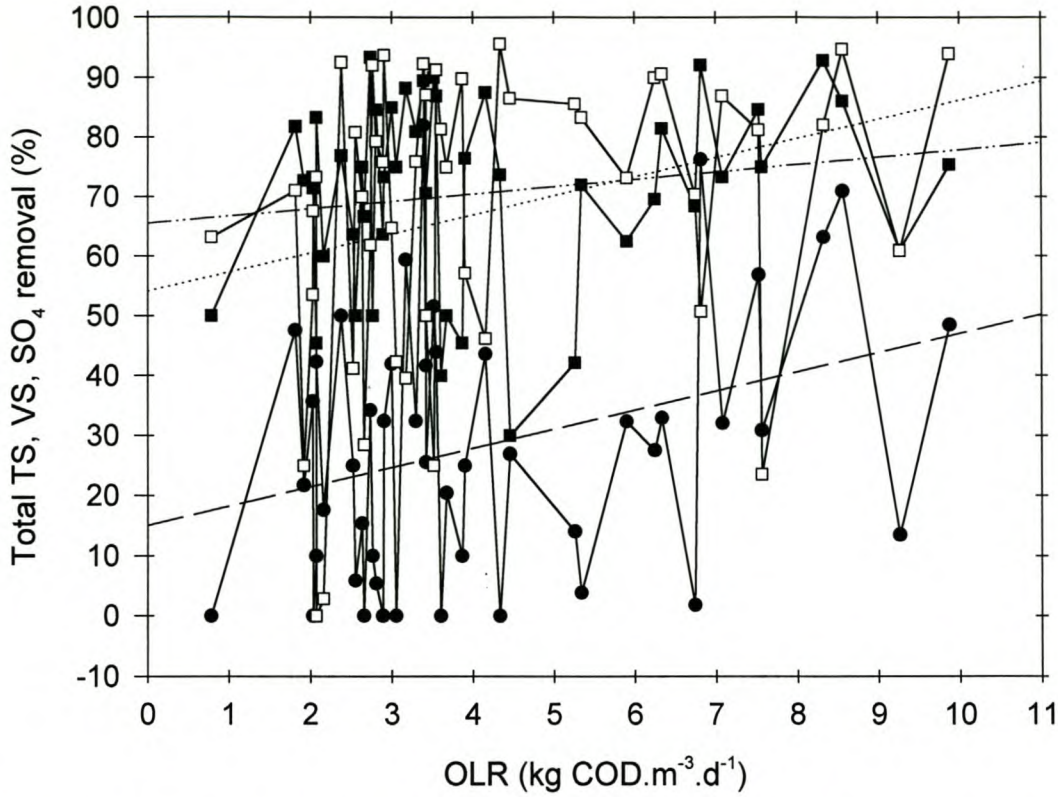

Figure 13. The TS (● ; -----), VS (■ ; —··—··—) and SO_4 (□ ; .....) removals, as a function of OLR for the total digester performance. The solid lines represent the actual data and the dotted and dashed lines represent the regression calculations.

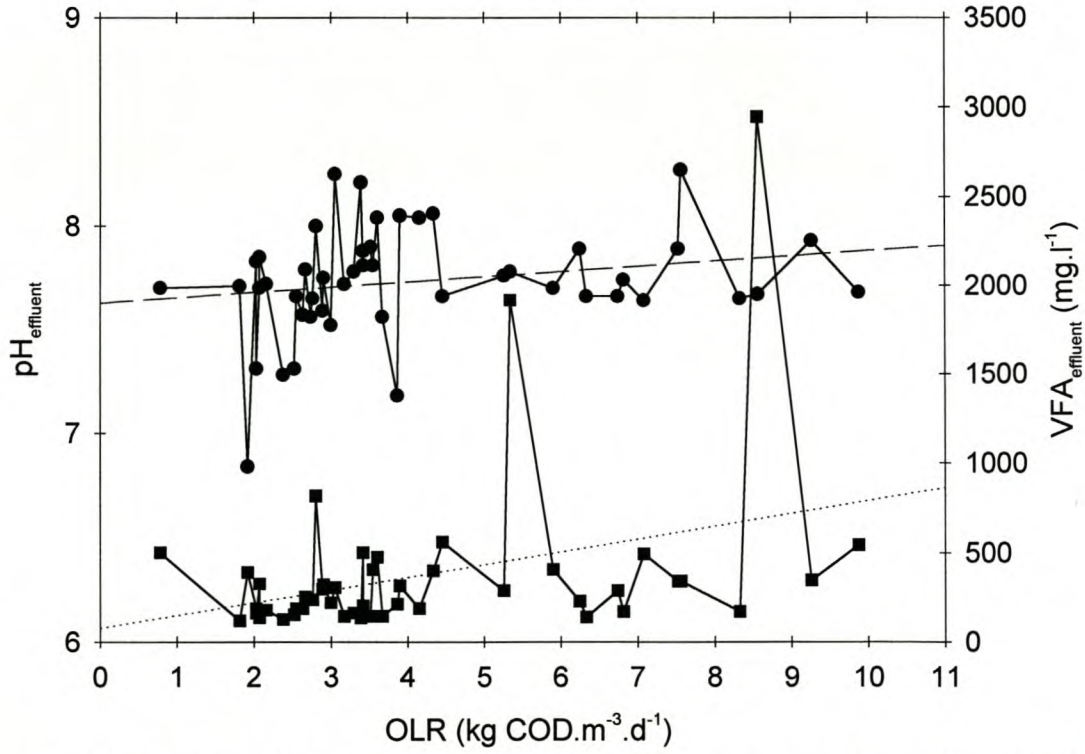

Figure 14. The variation in the effluent pH (● ; -----) and the effluent VFA content (■ ; .....), as a function of OLR for the total digester performance. The solid lines represent the actual data and the dotted and dashed lines represent the regression calculations.

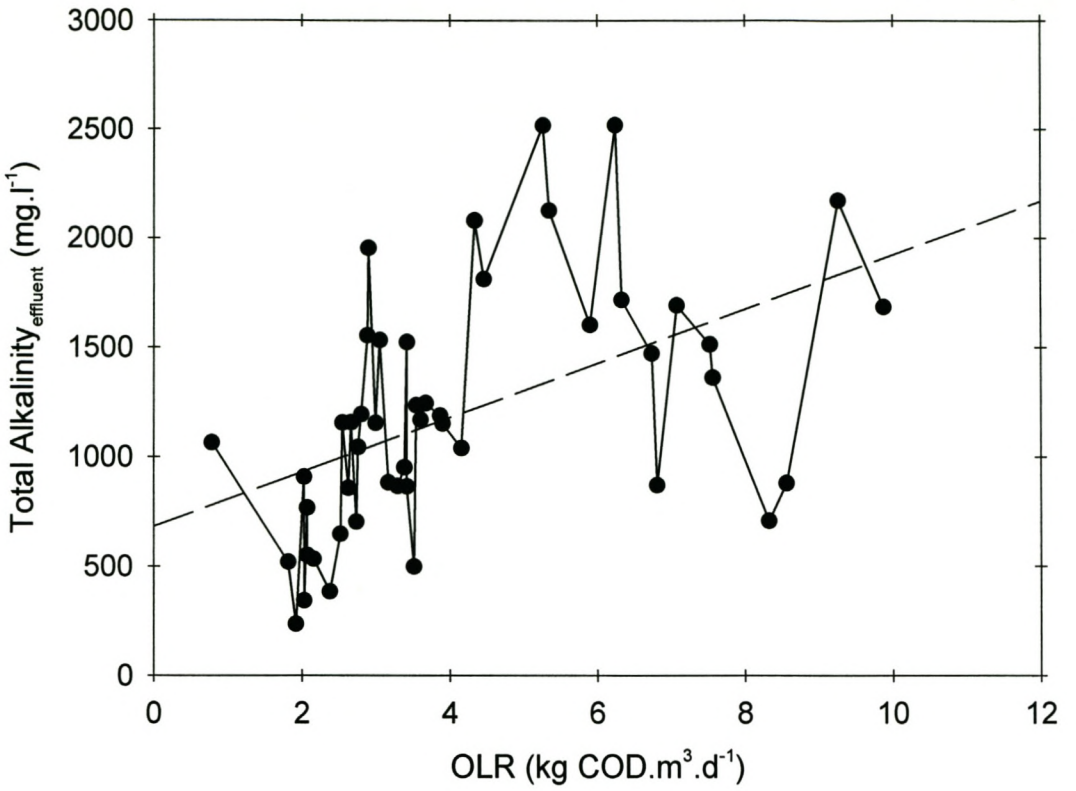

Figure 15. The variation of the effluent total alkalinity (● ; -----), as a function of OLR in the total digester performance. The solid line represents the actual data and the dashed line represents the regression calculation.

In this study, large variations were found in most of the operational performance parameters, which in turn were indicative of the variation in substrate and the organic load applied to the digesters. For both digesters (AD-1 and AD-2) therefore, significant and inconsistent variations occurred in the COD removal efficiencies and other parameters, although an overall increase in biogas yield, alkalinity, TS, VS, as well as SO_4 removal, occurred. This could also be an indication of the natural process selection of a specific microbial community as part of the stabilisation in the digester.

From the results obtained in this study and data from Chapter 3, the options and performances of single vs multi-phase anaerobic digestion of gelatin-manufacturing effluent can be compared (Table 5). Digester AD-1 and digester AD-2 of the single-phase digestion and the total multi-phase digesters (AD-1 + AD-2) operated at OLR's as high as 9.56, 9.30 and 9.87 kg $COD.m^{-3}.d^{-1}$, respectively. Although very good results were obtained with single-phase digestion, the best digester performance efficiencies were found during the multi-phase treatment of the gelatin-manufacturing effluent: COD (97%); biogas production (14.47 $l.d^{-1}$); biogas yield (2.89 $m^3.m^{-3}$); methane yield per COD removed (12.71 m^3.kg $COD.d^{-1}$); VFA (92%); TS (82%); VS (93%) and TNVS (80%). From the data it is clear that the multi-phase system can be successfully incorporated when a need arises to optimise the fermentation steps, by separating certain stages in separate digesters. Hence, the results of the overall process efficiency are better than those of conventional single-phase processes (Ghosh *et al.*, 2000).

One disadvantage of the use of this technology is, however, the high capital costs required to implement this type of treatment on commercial basis. The high capital costs involved in upscaling of a multi-phase reactor system must be seriously considered especially when comparing and evaluating the option of achieving good final effluent qualities with a lower cost single-phase digester with satisfactory final effluent qualities. Although these bench-scale studies clearly indicated the greater advantage of phase separation, pilot and full-scale studies will have to be done to address the economic viability of such treatment systems.

Table 5. Comparison of the maximum operational efficiency between the single- and multi-phase anaerobic digestion systems.

Parameters	Single phase		Multi phase
	AD-1	AD-2	Total (AD-1 + AD-2)
Organic Loading Rate (kg COD.m^{-3}.d^{-1})	9.56	9.30	9.87
COD removal (%)	90.0	83.0	97.2
COD removal rate (kg COD.m^{-3}.d^{-1})	8.60	6.67	8.13
SO_4 removal (%)	95.6	98.0	95.6
Biogas production (l.d^{-1})	11.04	6.87	14.47
Biogas yield (m^3.m^{-3} reactor)	2.21	1.37	2.89
CH_4 content (%)	87.6	88.9	88.5
CH_4 yield (m^3.kg $COD_{removed}$.d^{-1})	7.16	6.75	12.71
CH_4 yield (m^3.kg COD_{loaded}.d^{-1})	2.56	3.20	2.44
VFA removal (%)	81.7	80.5	91.8
TS removal (%)	64.6	61.7	82.0
VS removal (%)	88.6	87.9	93.3
TNVS removal (%)	50.0	52.5	80.2
Total metal removal (%)	80.9	79.5	71.7

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CHAPTER 5

TREATMENT OF GELATIN-MANUFACTURING EFFLUENT USING AN UPFLOW ANAEROBIC SLUDGE BLANKET (UASB) BIOREACTOR

Summary

The gelatin-manufacturing industry produces high volumes of high-strength effluent with distinctive peak and low periods in terms of organic, hydraulic and toxic loads. These must then be accommodated by the local wastewater purification works. As a result of the large volumes of noxious effluent received, the purification works has in the past experienced many operational problems. In order to secure the position of the industry and minimise the toxic load disposed to the wastewater purification works an efficient and cost-effective pre-treatment technology has to be developed. This will also lead to the reduction of high municipal trade-effluent charges of the industry and enable compliance to the legal effluent disposal requirements. Anaerobic digestion appears to be a feasible and economic option for the treatment of this effluent, as excellent treatment results were obtained in previous studies using hybrid reactor designs. The importance of upscaling the digester design has necessitated further evaluation of other anaerobic process configurations to treat the gelatin-manufacturing effluent.

During this study, a mesophilic laboratory-scale upflow anaerobic sludge bed (UASB) bioreactor design with recirculation was evaluated for its suitability in treating high-strength, highly variable raw gelatin-manufacturing effluent. Successful inoculation and start-up of the digester resulted in good handling and led to increases in removal efficiencies. The digester was operated at a pH of 6.5. Immediate sulphate (SO_4) (66 - 79%) and chemical oxygen demand (COD) (45 - 72%) removals were achieved during the start-up period at a hydraulic retention time (HRT) of 3.0 d. Chemical oxygen demand (COD), SO_4 and total solid (TS) removal efficiencies of up to 96, 86 and 79%, respectively, were achieved at the 3.0 d HRT. At an HRT of 1.0 d,

COD, SO_4 and TS removal efficiencies of up to 95, 97 and 67%, respectively, were observed. The biogas production, however, was found to be very low throughout the study (0.05 - 0.63 $l.d^{-1}$).

Introduction

Different wastewater types are produced daily which must be accommodated by local wastewater purification works. This often presents a problem to the local authorities, as the disposal of certain noxious industrial effluents can inhibit the biological treatment processes, with significant adverse cost and effluent quality implications. Gelatin-manufacturing effluent specifically, can be considered as one of the most difficult effluents to treat and poses a potential environmental pollution hazard due to its highly variable and complex nature. It is, therefore, imperative that this effluent be treated in an environmentally responsible manner.

Considerable interest has been shown in the application of anaerobic digestion, due to it being seen as a "clean technology", its cost-effectiveness and adaptable nature. The general performance of the anaerobic digestion process and the wide diversity of wastewaters that can be treated has increased steadily over the past few years. This is mainly as a result of an array of breakthroughs related to digester designs, operating conditions and shock loads, for the treatment of recalcitrant industrial wastewater (Austermann-Haun *et al.*, 1997; Guiot *et al.*, 1997).

Among the different high-rate anaerobic reactors developed and successfully applied in recent years, the upflow anaerobic sludge blanket (UASB) system represents a popular design for the biological treatment of effluents (Lettinga *et al.*, 1997). The ability of bacterial cells when grown in an upflow stream, to aggregate into dense particles (granules) is one of the reasons why the UASB configuration is one of the more suitable designs (Britz *et al.*, 1999). The microbial "blanket" is retained by its own mass and by baffles or screens forming the settler unit in the upper portion of the reaction vessel, whilst gas and liquid escape from the top of the tank. This mode of

bacterial growth can increase the performances of high-rate digesters, since the favourable settling properties of granules can minimise biomass wash-out and the close cell packing improves the metabolite interspecies transfer and the overall granular activity (Schmidt & Ahring, 1996). However, the development of sludge granulation and augmentation under continuous upflow conditions is not yet fully understood as it is a complex process with many factors involved in this development (Wu *et al.*, 1985; Van Lier *et al.*, 1996; Verstraete *et al.*, 1996). Granules have been reported to vary in size from 1 - 3 mm (Fang, 1997) and is known that granules occasionally disintegrate for unknown reasons and thus result in a washout of biomass (Kosaric *et al.*, 1990; Lettinga *et al.*, 1997).

The advantages of an UASB contact bioreactor design includes: less dead digester volume (Visser *et al.*, 1993); higher loading rates; lower HRT's; the ability to retain high biomass concentrations despite the upflow velocity of the wastewater; no support material is required for the retention of a high density of anaerobic activated sludge (Fukuzaki *et al.*, 1991); and the production of biogas. The disadvantages of this digester configuration are the slow start-up time and the dependence on the formation of a well-settleable granular sludge. This particular design can therefore be seen as an attractive alternative option to comply with the stricter pollution control regulations currently enforced on industries and authorities by the National Water Act (1998) and the National Environmental Management Act (1998).

The aim of this study was to evaluate the operational performance of an UASB anaerobic bioreactor design for the treatment of full-strength gelatin-manufacturing effluent. This digester option is the third design (AD-3) in a series of design tests and comparisons to provide a database for future full-scale application of the anaerobic biological technology for the treatment of gelatin-manufacturing effluent.

Materials and methods

Digester design

A laboratory-scale upflow anaerobic sludge blanket bioreactor (UASB), with an operational volume of 2.3 litres, was used. The total height of the digester was 830 mm with an internal diameter of 50 mm. The digester (AD-3) combined an upflow anaerobic sludge blanket design with a gas/solids separator at the top of the bioreactor (Fig. 1). The biogas exited at the top, while the substrate was introduced into the bioreactor at the base. The overflow of the bioreactor emptied through an U-shaped tube to prevent any atmospheric oxygen from entering the system. The temperature of the bioreactor was maintained at 35°C, using a heating tape and an electronic control unit (Meyer *et al.*, 1985). The substrate was introduced semi-continuously to the bioreactor by means of a peristaltic pump (Watson-Marlow 302S) controlled by an electronic timer. The upflow velocity within the reactor was set at 2 $m.h^{-1}$ by means of recirculation (Trnovec & Britz, 1998). The biogas production was measured automatically by means of an electronic counter with a gas-tight valve. The biogas volumes were corrected to standard temperature and pressure (STP) conditions.

Digester start-up

The bioreactor was seeded using a mixture of acclimatised and activated anaerobic sludge, as well as gelatin-manufacturing effluent from the local wastewater purification works. The biomass in the bioreactor was then allowed to stabilise for 72 h to allow the bacterial community to acclimatise. The substrate flow rate was initially set at an hydraulic retention time (HRT) of 3.0 d and was maintained until stable state conditions were observed. After the start-up phase, the HRT was decreased to 2.0 d and later to 1.0 d where it was kept constant for the rest of the study.

Digester substrate

Full-strength gelatin-manufacturing effluent obtained from a local gelatin-manufacturing industry, was used as substrate for the bioreactor. The

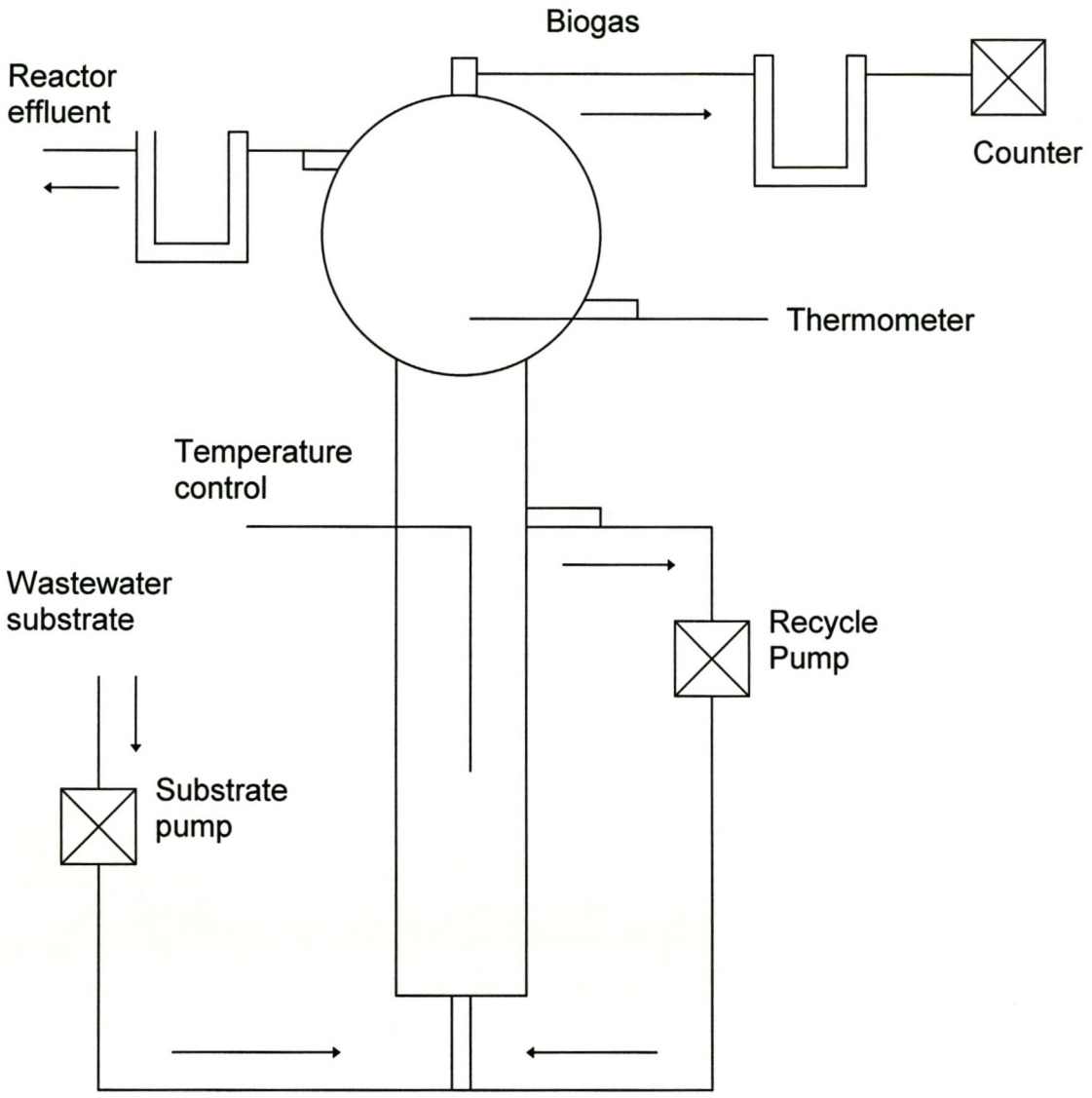

Figure 1. Laboratory-scale upflow anaerobic sludge blanket bioreactor (Trnovec & Britz, 1998).

effluent was collected in batches of 75 l and stored at room temperature until required. No standardisation was done on the effluent, except for the adjustment of the pH to 6.5 with standard 6 N HCl solution. The composition of this substrate is given in "Table 2" of Chapter 3 of this thesis. The effluent was supplemented with 100 $mg.l^{-1}$ urea, 100 $mg.l^{-1}$ K_2HPO_4, 10 ml acetic acid (CH_3COOH) and a sterile trace element solution (Nel *et al.*, 1985) during the start-up phase.

Analytical methods

During the experimental study, the following parameters were monitored on the digester substrate and effluent according to Standard Methods (APHA, AWWA & WEF, 1995): pH; total alkalinity (TAlk); total solids (TS); volatile solids (VS); total non-volatile solids (TNVS), chemical oxygen demand (COD); total Kjeldahl nitrogen (TKN); volatile fatty acids (VFA); sulphate (SO_4^{2-}); chlorides (Cl); ammonia (NH_3-N) and ortho-phosphate (PO_4-P). Heavy (total) metals (Cu, Fe, Co, Cr, Mn, Ni, Pb, Cd, Zn), as well as sodium, calcium and magnesium, were measured with an Atomic Absorbance Spectrophotometer (Varian Model 250 Plus).

Volatile fatty acids (as acetate) were determined according to the titration method of Moosbrugger *et al.* (1992). The biogas composition (CH_4 and CO_2) was determined volumetrically with a brine displacement system, according to the quantitative biogas carbon dioxide content method described by Ross *et al.* (1992).

Experimental phase

During this single-phase experimental study, the UASB anaerobic reactor (AD-3) received full-strength gelatin-manufacturing effluent as substrate. No standardisation was done on the substrate fed to the digester, so as to simulate the actual field conditions. The pH of the substrate was adjusted to 6.5. The bioreactor was operated at an HRT of 3.0 d for 30 weeks, and the HRT was then reduced to 1.0 d for the rest of the study.

Results and discussion

Digester substrate

The compositions of the digester substrate and effluent as well as the performance of the anaerobic digester (AD-3) at HRT's of both 3.0 and 1.0 d, are summarised in Tables 1 and 2.

UASB bioreactor efficiency

The reactor was started at an initial organic loading rate (OLR) of 1.69 kg COD.m^3.d^{-1} at an HRT of 3.0 d (Fig. 2). The data in Fig. 2 show the variation of the OLR ranging from 0.16 to 12.94 kg COD.m^3.d^{-1}, with an average OLR of 2.08 kg COD.m^3.d^{-1}, during the total experimental study of 75 weeks.

In Fig. 3, the reactor efficiency is plotted as a function of OLR in terms of the relationship between COD removal (%) and the COD removal rate (R-value) at a HRT of 1.0 d. The highest COD removal (96%) and best R-value (2.78 kg COD.m^3.d^{-1}) were both found at the OLR of 2.89 kg COD.m^3.d^{-1} at a HRT of 3.0 d (data not shown). When the reactor was operated at a HRT of 1.0 d, a maximum COD removal of 95% and a R-value of 7.18 kg COD.m^3.d^{-1} were achieved at OLR's of 2.61 and 8.00 kg COD.m^3.d^{-1}, respectively. The average COD removals during the 3.0 d HRT and 1.0 d HRT operations were very similar (51% and 54%) (Tables 1 and 2). In the case of the HRT of 1.0 d, the R-value showed a good increase as a function of increasing OLR (Fig. 3), whereas the COD removal showed a more gradual increase as a function of the OLR conditions.

Sulphate (SO_4) removals of up to 86% and 97% were observed at HRT's of 3.0 d and 1.0 d, respectively with an average removal of 51%. The data in Fig. 4 shows the very large variation in SO_4 removal as a function of increasing OLR. A more stable removal was found for the period of OLR of 4 - 7 kg COD.m^3.d^{-1}, ranging between 70% to 80% SO_4 removal. The drastic decrease in SO_4 removal for OLR period of 7 - 10 kg COD.m^3.d^{-1} can not be ascribed to OLR increases, but could possibly be due to the adverse

Table 1. Composition of the digester substrate and effluent, and the digester efficiency obtained during the 30 week operational period at a HRT of 3.0 d.

Parameters	Substrate			Effluent			Digester Efficiency (%)		
	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average
OLR (kg COD.m^{-3}.d^{-1})	0.26	3.82	1.28	-	-	-	-	-	-
COD (mg.l^{-1})	772	11 450	3 832	89	6 958	1 672	0	96	51
TKN (mg.l^{-1})	60	906	287	64	697	254	0	82	16
VFA (mg.l^{-1})	240	6 252	1 991	120	7 416	1 819	0	95	24
SO_4 (mg.l^{-1})	66	2 290	484	30	1 820	280	0	86	42
pH	6.2	8.2	7.2	6.9	8.7	8.2	-	-	-
TAlk (mg.l^{-1})	79	3 225	887	269	5 045	1 734	-	-	-
Cl (mg.l^{-1})	124	1 482	593	186	6 151	814	0	79	7
NH_3-N (mg.l^{-1})	5	1 110	194	28	1 077	242	0	53	5
TS (mg.l^{-1})	1 000	12 900	4 364	1 200	16 700	3 715	0	79	21
VS (mg.l^{-1})	200	6 000	1 870	100	2 800	789	0	96	51
TNVS (mg.l^{-1})	600	10 800	2 494	200	15 300	2 926	0	24	5
P-PO_4 (mg.l^{-1})	0.0	53.3	5.6	0.5	63.8	9.9	0	83	6
Total metals (mg.l^{-1})	0.6	4.6	1.9	0.3	6.7	1.7	0	83	19
Na (mg.l^{-1})	66	2 708	774	327	3 362	1 061	0	34	4
Ca (mg.l^{-1})	8	914	179	8	691	142	0	80	22
R-value (kg COD.m^{-3}.d^{-1})	-	-	-	-	-	-	-0.72	2.78	0.72
Biogas production ($l.d^{-1}$)	-	-	-	-	-	-	0.05	0.63	0.30
Biogas Yield (m^3.m^{-3} reactor)	-	-	-	-	-	-	0.00	0.13	0.01
CH_4 content	-	-	-	-	-	-	30	89	70
CH_4 yield per $COD_{removed}$	-	-	-	-	-	-	0.00	1.93	0.08
CH_4 yield per COD_{loaded}	-	-	-	-	-	-	0.00	0.54	0.04

Table 2. Composition of the digester substrate and effluent, and the digester efficiency obtained during the 45 week operational period at a HRT of 1.0 d.

Parameters	Substrate			Effluent			Digester Efficiency (%)		
	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average
OLR (kg COD.m^{-3}.d^{-1})	0.16	12.94	2.60	-	-	-	-	-	-
COD (mg.l^{-1})	158	12 940	2 598	141	10 930	1 197	0	95	54
TKN (mg.l^{-1})	66	968	244	65	928	233	0	72	9
VFA (mg.l^{-1})	120	7 318	756	96	7 054	735	0	87	21
SO_4 (mg.l^{-1})	90	1 550	500	17	975	215	0	97	59
pH	6.4	8.4	7.1	6.6	8.7	8.0	-	-	-
TAlk (mg.l^{-1})	43	1 231	317	64	2 145	933	-	-	-
Cl (mg.l^{-1})	27	3 390	660	6	2 185	452	0	97	40
NH_3-N (mg.l^{-1})	0	355	76	0	684	176	0	1	0
TS (mg.l^{-1})	900	12 200	3 574	700	9 900	2 736	0	67	26
VS (mg.l^{-1})	200	7 400	1 329	100	7 600	650	0	89	57
TNVS (mg.l^{-1})	700	6 900	2 245	600	6 400	2 086	0	50	17
P-PO_4 (mg.l^{-1})	0.0	7.9	1.2	0.0	10.9	1.9	0	72	7
Total metals (mg.l^{-1})	0.2	10.8	2.1	0.1	7.3	1.2	0	96	38
Na (mg.l^{-1})	60	2 002	510	72	1 620	500	0	79	10
Mg (mg.l^{-1})	2	58	18	2	57	17	0	69	11
Ca (mg.l^{-1})	6	2 000	217	4	1 084	176	0	81	22
R-value (kg COD.m^{-3}.d^{-1})	-	-	-	-	-	-	-0.03	7.18	1.40
Biogas production ($l.d^{-1}$)	-	-	-	-	-	-	0.05	0.52	0.29
Biogas Yield (m^3.m^{-3} reactor)	-	-	-	-	-	-	0.00	0.10	0.01
CH_4 content	-	-	-	-	-	-	45	90	71
CH_4 yield per $COD_{removed}$	-	-	-	-	-	-	0.00	0.19	0.01
CH_4 yield per COD_{loaded}	-	-	-	-	-	-	0.00	0.17	0.01

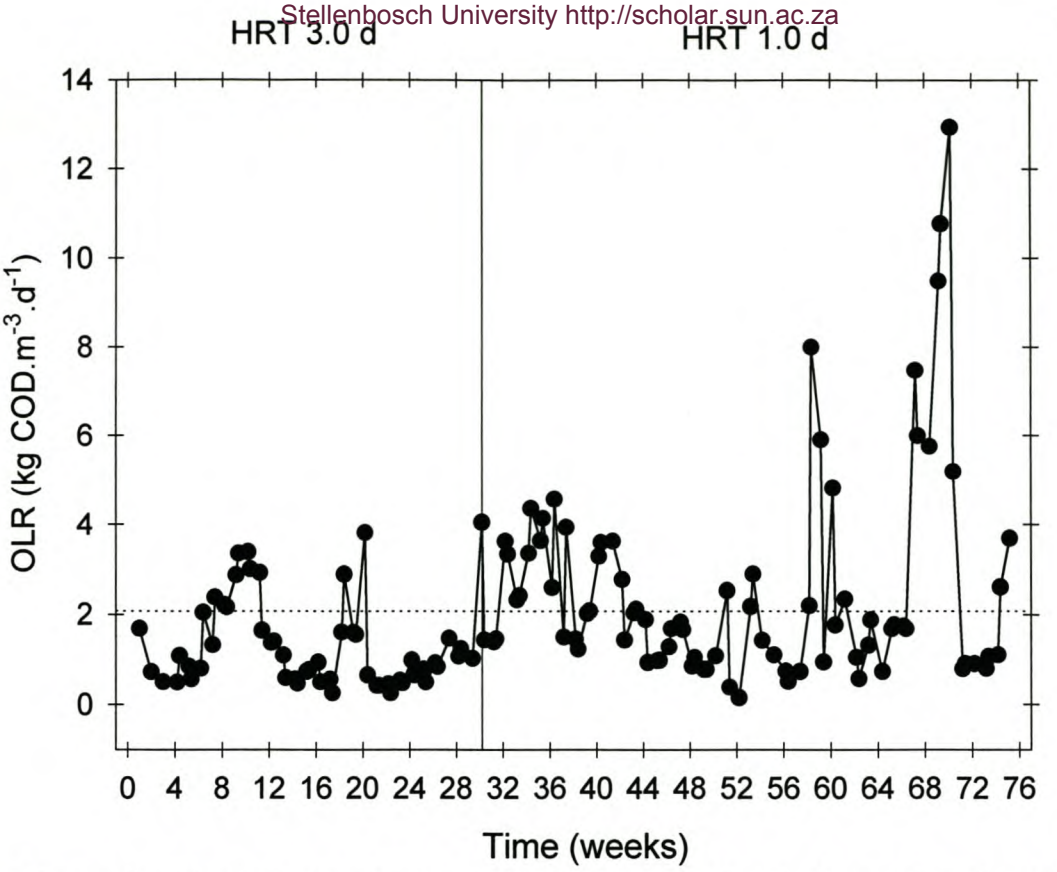

Figure 2. The variation in substrate OLR during the study on digester AD-3 over a 75 week period. The dotted line represents the average OLR. The vertical solid line separates the data of the 3.0 d and 1.0 d HRT studies.

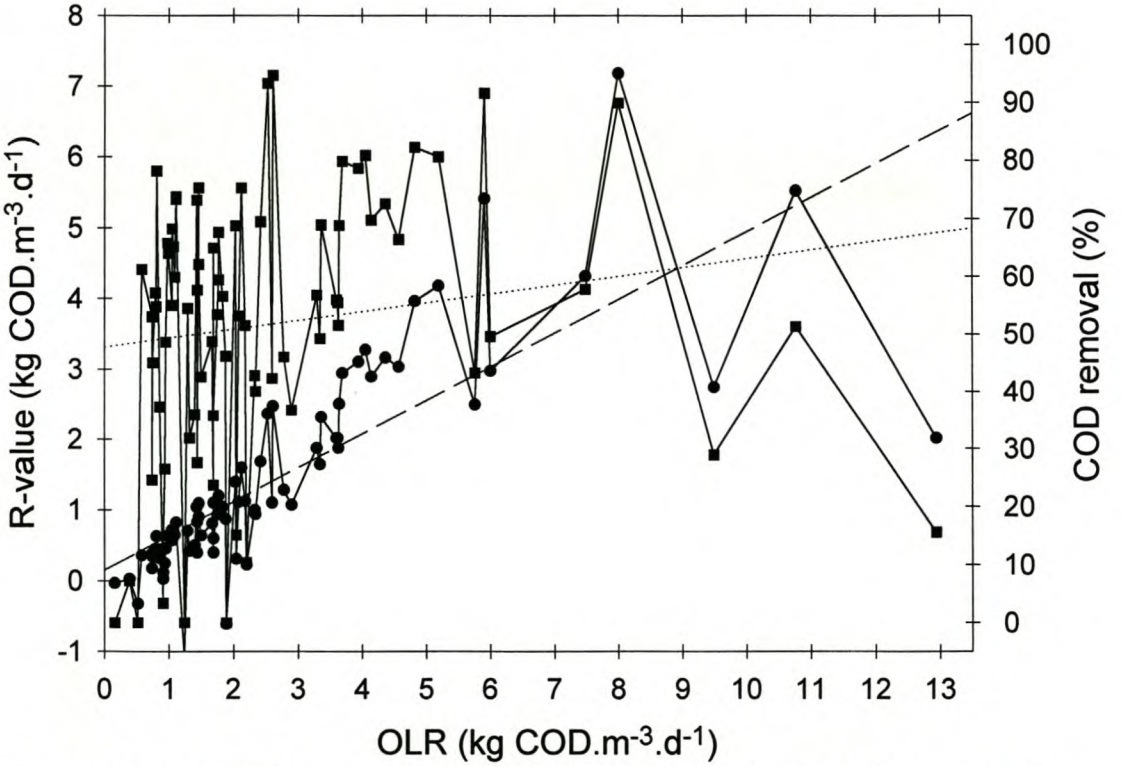

Figure 3. The COD removal rate (R-value) (● ; -----) and COD removal (■ ; ....) as a function of OLR in AD-3 at a HRT of 1.0 d. The solid lines represent the actual data and the dotted and dashed lines are the regression calculations.

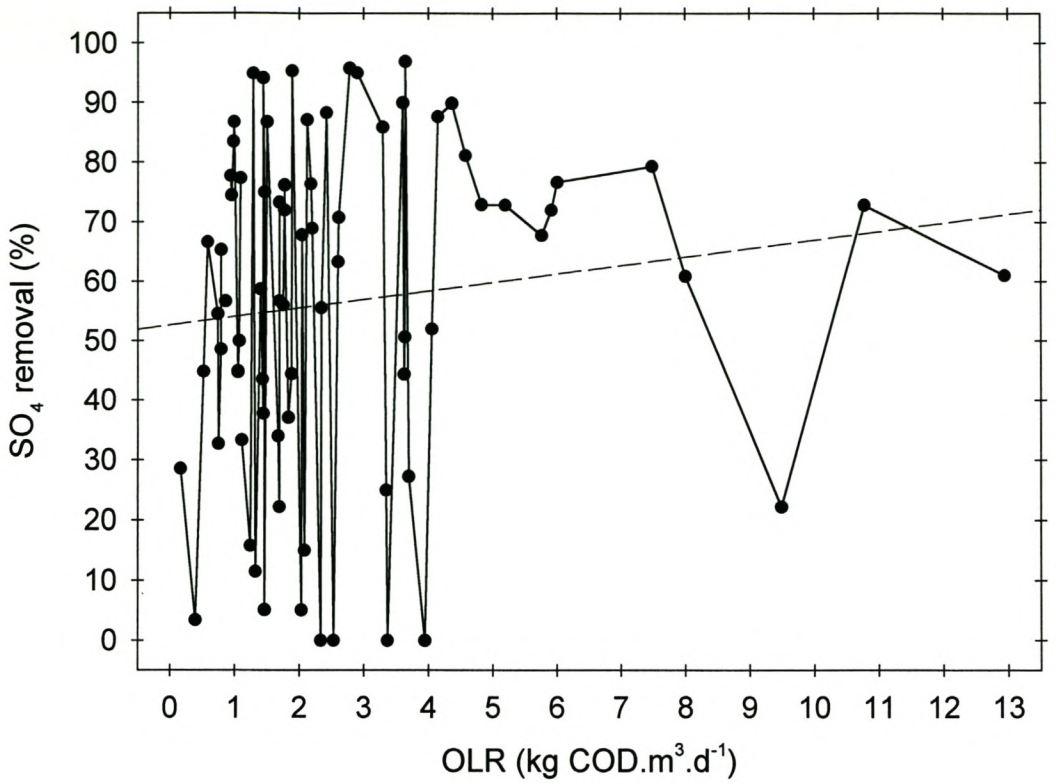

Figure 4. The SO_4 removal (● ; -----) as a function of OLR in AD-3 at a HRT of 1.0 d. The solid line represents the actual data and the dashed line represents the regression calculation.

influence of the introduction of other chemicals at the gelatin-manufacturing industry.

The variations in the UASB bioreactor effluent pH and the total effluent alkalinity during the total experimental study, are shown in Fig. 5. During the study a very high average effluent pH of 8.71 and a fairly good average total alkalinity of 1 334 $mg.l^{-1}$, were obtained. In a balanced anaerobic digestion system, the volatile fatty acids are proportionally converted to methane and carbon dioxide and an optimum operational pH of 6.8 to 7.4, should be maintained. When the pH in an anaerobic system decreases, the alkalinity produced in the anaerobic system should provide an efficient buffering capacity (Malina & Pohland, 1992). If the pH is lower than 6.3 or higher than 7.8, the rate of methanogenesis is known to decrease leading to a lower operational efficiency (Van Haandel & Lettinga, 1994). During this study, very little biogas production was observed, which probably relates to the non-optimal operational pH of the system. This would also influence the methane production negatively. It was also seen that gas bubbles adhered to the bacterial flocs, which in turn resulted in poor settling properties of the anaerobic biomass. The biomass and bubbles thereby were lost from the system via the digester overflow to the open effluent collection flask. This phenomenon was also reported by other researchers when treating protein and lipid containing gelatin effluent (Tommaso *et al.*, 1999) and ascribed to the absence of the formation of suitable granules in the bioreactor.

Conclusions

Anaerobic wastewater treatment processes can provide several advantages over other aerobic biological, chemical and/or physical applications if a suitable digester is properly selected, designed and operated for the treatment of a specific effluent.

The UASB configuration can be utilised to overcome some of the disadvantages of the other digesters by recycling the biomass of the digester to obtain an optimal food-biomass contact situation. With this recirculation

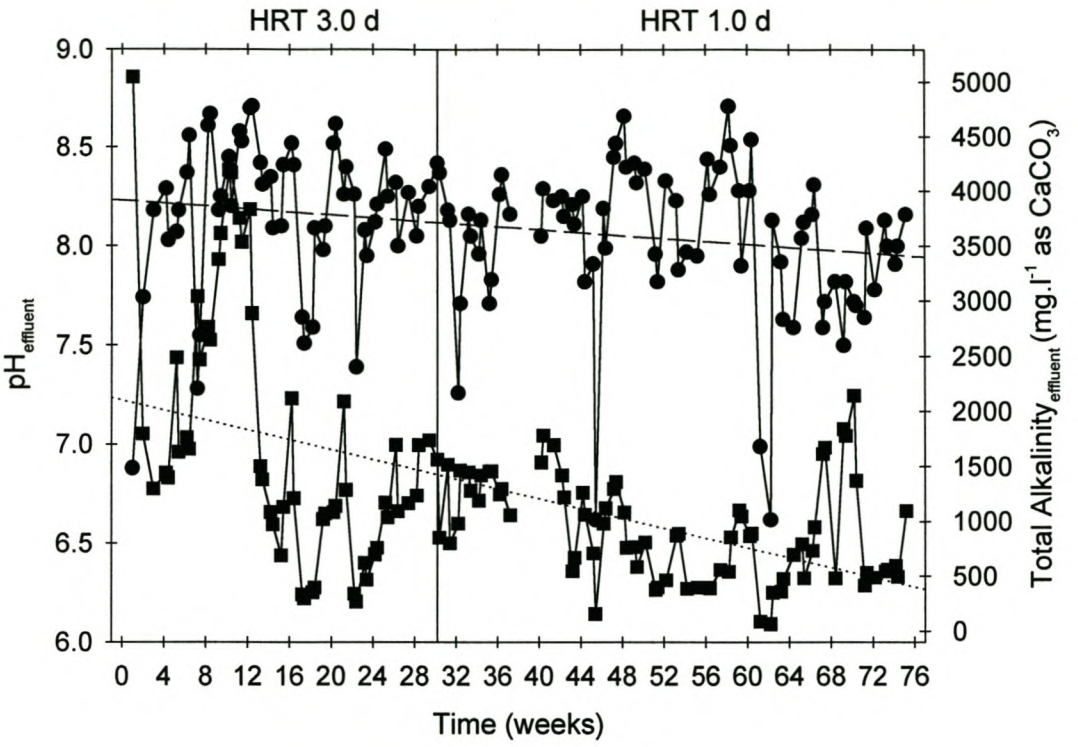

Figure 5. The variation in the bioreactor effluent pH (● ; -----) and effluent total alkalinity (■ ; .....) obtained over a period of 75 weeks in AD-3. The solid lines represent the actual data and the dotted and dashed lines represent the regression calculations. The vertical solid line separates the data of the 3.0 d and 1.0 d HRT studies.

option it will be possible to retain both active microorganisms and undigested substrate suspended solids, which can promote more extensive biodegradation of the wastewater particles. The UASB bioreactor retains most of the advantages of a conventional digester with the extra benefits of shorter sludge retention times (SRT), smaller reactor volumes, better alkalinity control, as well as optimal food-biomass contact.

However, one disadvantage of the UASB digester found during this study, was the poor settleability and retention of the granular biomass within the digester. Adequate biomass settleability is crucial for successful operation of this digester configuration. Tommaso *et al.* (1999) also reported the washout of biomass when treating a complex protein and lipid containing gelatin effluent. Usually pre-treatment of the sludge in the bioreactor is needed before it is recirculated back into the digester, to produce a more settleable floc. A number of approaches have been developed to enhance sludge settleability, such as gas stripping, stirred or vacuum degasification, as well as the addition of coagulants and flocculants to promote floc formation in the digester (Malina & Pohland, 1992). Petruy *et al.* (1999) reported that the inflow of low lipid concentrations (100 $mg.l^{-1}$) in the form of milk-fat emulsion did not affect the COD removal of their expanded granular sludge bed (EGSB) system. Even a concentration of 100 000 $mg.l^{-1}$ was not toxic, however, a lipid/fat removal mechanism of adsorption into the sludge is activated under such conditions, which again affects biogas and methane yields. These reports correspond well with the data from this study which reflect the relatively high COD removal at a short HRT of 1.0 d, but in contrast, only low methane yields were obtained .

During this study it was found that the UASB bioreactor had a stable operation at an HRT of 1.0 d. A good COD removal was also observed during a 1.0 d HRT operation with an average of 54 % and maximum at 94%.

There are several advantages of this system that must be kept in mind for upscaling possibilities. These include the fact that the system operation was stable and a fairly good COD removal was obtained at a HRT of 1.0 d. Thus, future studies must be done to incorporate and evaluate some of these approaches to deliver a better granular biomass quality. Further

experimentation with shorter hydraulic retention times may also improve the digester efficiencies (Trnovec & Britz, 1998). The use of tailored made granules, as described by Britz *et al.* (1999), especially to treat this type of effluent, can also be evaluated.

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CHAPTER 6

PILOT-SCALE ANAEROBIC PRE-TREATMENT OF UNDILUTED GELATIN-MANUFACTURING EFFLUENT USING AN UASB AND A CONTACT CONFIGURATION

Summary

Based on the laboratory-scale studies, a pilot-scale digester was evaluated under actual field conditions, while treating full-strength gelatin-manufacturing effluent. The pilot-scale anaerobic digester had an operational volume of 300 litre with an alternate UASB- and contact process configuration. In the first phase, the anaerobic treatment of the gelatin-manufacturing effluent using an UASB configuration resulted in excellent chemical oxygen demand (COD) removal efficiencies of up to 96% (average 58%) at a hydraulic retention time (HRT) of 1.0 d. The organic loading rates (OLR) ranged from 1.42 to 63.09 kg $COD.m^{-3}.d^{-1}$. The highly variable OLR's applied resulted in extreme performance variations with total solids (TS), volatile fatty acids (VFA) and sulphate (SO_4) removals of up to 93, 80 and 96%, respectively. Biogas production volumes of up to 176 $l.d^{-1}$ (average 49 $l.d^{-1}$) were observed, with an average methane content of 85%. From the data obtained it was clear that the microbial community which developed in the UASB system could handle the highly variable and unfavourable feed composition and shockloads efficiently and thus this reactor configuration was considered as an acceptable option for the pre-treatment of the gelatin-manufacturing effluent.

In the second phase, the contact process was used and resulted in fairly low COD, SO_4, VFA and TS removal efficiencies of 41, 62, 64 and 39%, respectively, at a HRT of 1.0 d. Due to the varying quality of the gelatin-manufacturing effluent and high solids content (2 900 to 53 800 $mg.l^{-1}$) during this study, several serious and limiting operational problems were experienced, one being the constant blocking/obstruction (calcium carbonate precipitation, grit, hair and fat) in the feed and recirculation lines. The data

from this study, where the contact configuration was used, indicated that this configuration was not entirely suitable as a treatment option.

Based on the data obtained using the 300 litre anaerobic pilot plant and two reactor configurations, it was concluded that gelatin-manufacturing effluent can be successfully treated under actual field conditions, with highly variable pH, OLR, TS values and temperature shockloads, using an UASB digester configuration, while the contact design appeared to be less suitable for this application.

Introduction

Gelatin-manufacturing wastewater is characterised by a high organic matter load originating mainly from the hide processing and the chemical addition during the gelatin-manufacturing process and is thus very difficult to treat. In addition to a high COD concentration, the wastewater also has high concentrations of suspended solids (SS), fats, SO_4 and salts, which may inhibit biological processes. Currently, biological wastewater treatment systems offer a number of significant advantages and fewer serious drawbacks compared to other physical and chemical methods of wastewater treatment.

The complex nature of the gelatin-manufacturing effluent prescribes the vital importance of implementing a wastewater pre-treatment step prior to discharge to the conventional municipal plant. This pre-treatment step must combine acceptable treatment efficiencies with low maintenance costs and operational problems. For this reason, anaerobic treatment, with the benefit of a low energy consumption and low sludge generation system, was selected to reduce the high organic loads during the pre-treatment of gelatin-manufacturing effluent.

To determine effective removal efficiencies during wastewater treatment, several factors have to be considered, namely: the nature of the organic matter to be removed; the suitability of environmental factors for anaerobic digestion; the retained amount of viable biomass; the design of the

anaerobic bioreactor system and the retention time of the sludge in the anaerobic digester (Van Haandel & Lettinga, 1994).

Taking all these requirements into consideration, it was decided to evaluate an UASB and a contact configuration to pre-treat the gelatin-manufacturing effluent. Data from previous studies (Chapters 3, 4 and 5) showed good removal efficiencies using laboratory-scale digesters treating the effluent. As a follow-up study, a 300 litre pilot-scale digester was constructed and operated at actual field conditions.

The aim of this study was to evaluate the stability and overall performance of a 300 litre pilot-scale reactor treating gelatin-manufacturing effluent at actual field conditions and to use this useful database for future full-scale considerations and applications of this technology.

Materials and methods

Digester design

The pilot-scale anaerobic digester had an operational volume of 300 litres and could either be operated as an UASB or as a contact process configuration by switching the mixing device in the digester on or off (Fig. 1). The substrate was introduced continuously at the bottom of the reactor by means of a peristaltic pump (Watson-Marlow 504S) and the biogas exited at the top of the digester. The biogas production was determined by means of a brine displacement system (6N HCl, pH 2.0) and the biogas volumes were corrected to standard temperature and pressure (STP) conditions.

The overflow of the reactor emptied through a U-tube to prevent atmospheric oxygen from entering the system. For the UASB configuration, the overflow emptied into a container for sampling purposes only, from there the overflow went to the inletworks of the purification plant, to be treated with the rest of the incoming wastewater.

For the contact configuration, the overflow of the digester emptied into a settling cone (clarifier). The settled sludge (biosolids) from the bottom of the clarifier was then recirculated back to the digester with the substrate. The

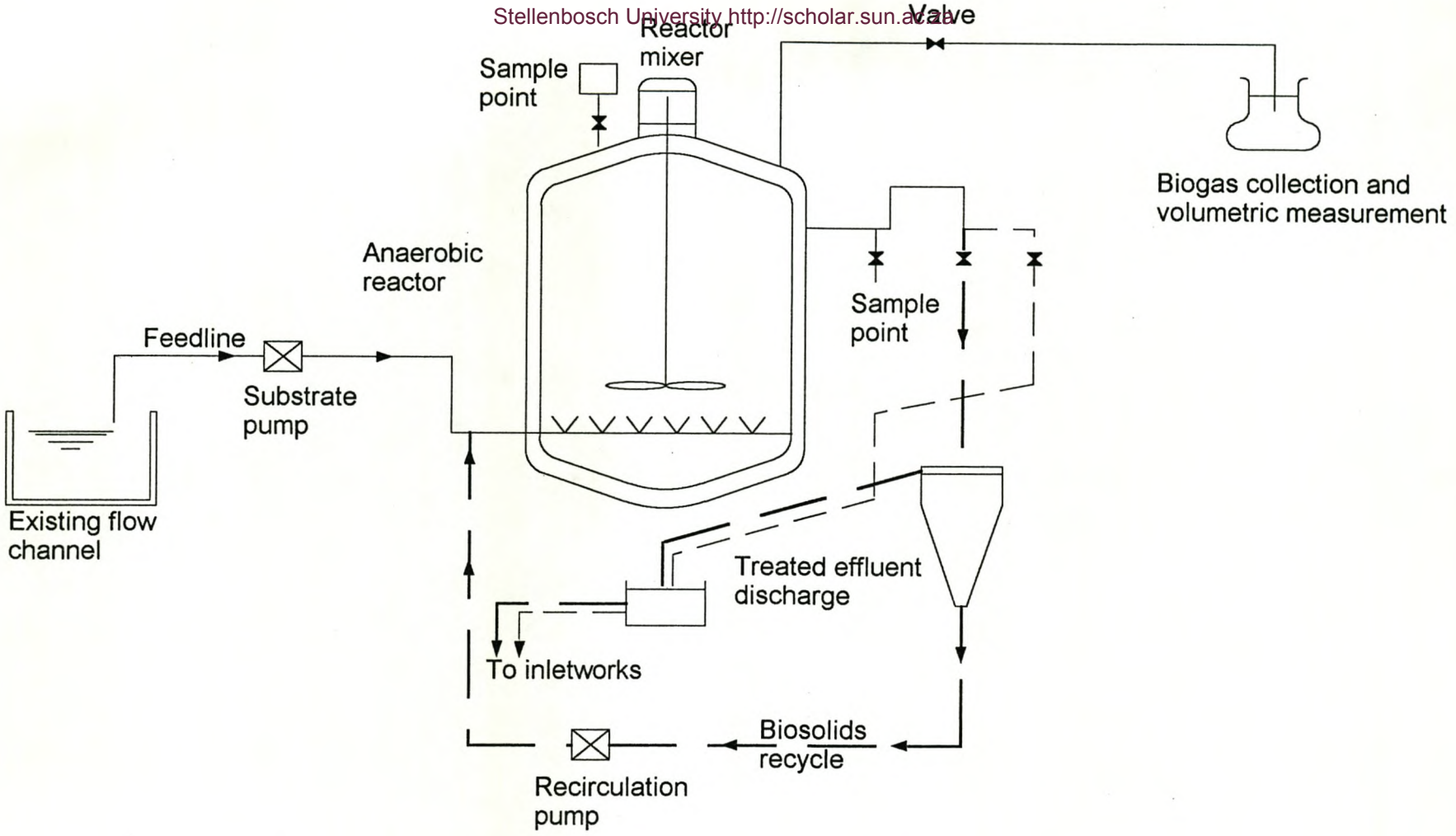

Figure 1. The 300 litre pilot-scale plant combining an UASB and contact configurations. The solid line (⟶) and the dashed (- - - -) lines represent the pipelines used for the UASB configuration. The solid line (⟶) and the thick dashed (▬ ▬) lines represent the pipelines used for the contact configuration.

overflow of the clarifier also went to the inletworks of the purification plant, to be treated with the rest of the wastewater. The digester consisted of a mixing device to distribute the biomass evenly throughout the contents of the reactor so as to maximise the food-biomass contact. Good internal mixing can minimise dead volume accumulation and flow channeling. Thus, no consideration was needed for settleability characteristics of anaerobic microorganisms.

Digester start-up

The pilot-scale digester was seeded with a mixture of anaerobic sludge and activated sludge obtained from the existing wastewater treatment works treating the gelatin-manufacturing effluent. Full-strength gelatin-manufacturing effluent was also added to the digester with a small booster volume of acetic acid (500 ml). The biomass within the digester was allowed 72 h to for acclimatise. It is important that an appropriate and stable microbial community must develop to ensure an efficient and reliable biological treatment performance (Silvey *et al.*, 2000). The substrate flow rate was set to 300 $l.d^{-1}$, thus at an HRT of 1.0 d. After acclimatisation, the digester was operated firstly in an UASB and then as a contact configuration.

Analytical methods

The following parameters on the digester substrate and effluent were monitored: temperature; pH; total alkalinity (TAlk); chemical oxygen demand (COD); total Kjeldahl nitrogen (TKN); volatile fatty acids (VFA as acetate); sulphate ($SO4^{2-}$); chlorides (Cl); ammonia (NH_3-N); total solids (TS); volatile solids (VS); total non-volatile solids (TNVS); ortho-phosphate (PO_4-P); total metals (Tmetals); sodium (Na); magnesium (Mg) and calcium (Ca). All analyses were performed according to Standard Methods (APHA, AWWA & WEF, 1995).

The total metals (Cu; Fe; Co; Mn; Cr; Pb; Ni; Cd and Zn) were determined, as well as sodium, magnesium and calcium concentrations with an Atomic Adsorption Spectrophotometer (Varian Model 250 Plus), equipped with hollow cathode lamps for the different metals, a photoelectric detector

with associated electronic amplifying and measuring equipment. Air/acetylene and nitrous oxide/acetylene burners were used with air as oxidant and acetylene/nitrous oxide as fuel. Standards of known metal concentrations were used to calibrate the instrument and also to verify the accuracy of the data obtained.

Volatile fatty acids (as acetate) were determined according to the titration method of Moosbrugger *et al.* (1992). The biogas production and composition was measured volumetrically with a brine displacement system, according to the quantitative biogas carbon dioxide content method of Ross *et al.* (1992). The accuracy of all tested chemical parameters were confirmed by the participation in an inter-laboratory water testing program (SABS Water Check Proficiency Program).

Experimental studies

The study consisted of two experimental studies (I and II). In the first study (I), the pilot-scale digester was operated as an UASB process configuration for 25 weeks. In the second study (II), the digester was operated as a contact process configuration for 14 weeks. Since the digester was started during winter and was made of stainless steel, the need arises to isolate the digester to maintain a constant temperature at all times. No other external heat was applied to maintain the digester at a specific temperature. The performance efficiencies of both the UASB and contact configurations were evaluated under field conditions at an HRT of 1.0 d.

Results and discussion

Digester substrate

The average composition (Table 2) and large compositional variations of the gelatin-manufacturing effluent was discussed in detail in Chapter 3 of this thesis. The pilot-scale digester was fed directly from the gelatin-manufacturing effluent discharge channel. No standardisation was done on

the incoming effluent, except for the removal of the harshest non-biodegradable grit by means of a filter.

Flow patterns

The general flow patterns of the gelatin-manufacturing effluent were found to be highly variable due to the nature of the gelatin production batch process. The associated organic load was therefore, as shown in Fig. 2, found to fluctuate considerably. Process upsets and spills were also found under field conditions, which contributed to unexpected flow and pollution load patterns compared to a normal effluent flow pattern. Experience gained from this study showed that it was important to regulate the volume of the effluent to ensure sufficient substrate availability for submerged digester feed at all times and to prevent atmospheric oxygen from entering the anaerobic system.

Considering the batch nature of the gelatin production process, upstream flow load equalisation of the biological treatment processes, is essential to protect the anaerobic digester against possible major organic shock and toxic overloads (Van Niekerk & Bohmer, 1998).

Experimental study I - UASB process configuration as pilot-scale

In this study the digester was operated at a HRT of 1.0 d. The composition of the substrate, effluent and the performance efficiency of the UASB pilot-scale digester are summarised in Table 1.

In Fig. 3, the variation of the operational OLR applied during the study period ranged from 1.42 to 63.09 kg $COD.m^{-3}.d^{-1}$. An average OLR of 9.94 kg $COD.m^{-3}.d^{-1}$ was fed to the digester over the 25 week operational period. From the data it can be seen that the digester received several severe "shocks" in terms of OLR, temperature and pH variations (Fig. 3, 4 and 5).

The data summarised in Fig. 4 shows the variation in the temperature of the discharged gelatin-manufacturing effluent as well as the temperature of the digester effluent. Gelatin extraction takes place at temperatures elevated above and below ambient atmospheric temperature, as reflected by the typical range of effluent temperatures during the manufacturing process:

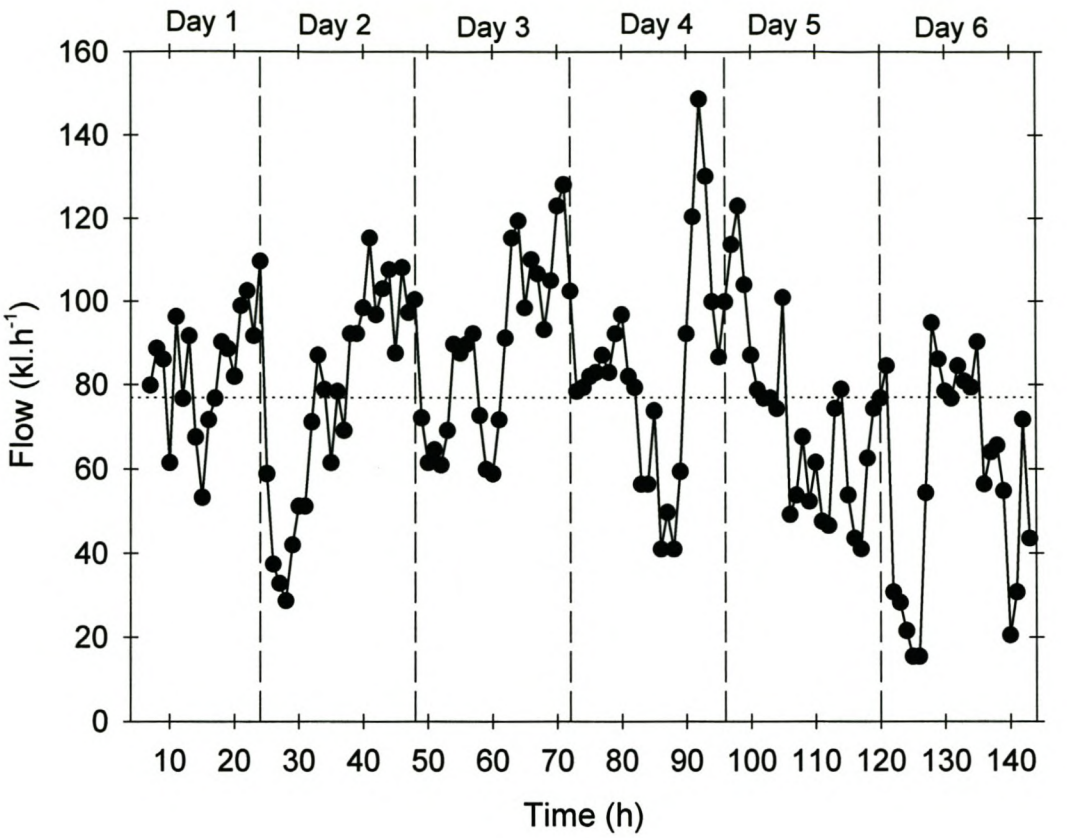

Figure 2. The fluctuations in flow received from the gelatin-manufacturing industry during a typical 6 day week. The dotted line represents the average flow.

Table 1. Composition of the substrate, effluent and digester efficiency during the UASB pilot-scale operating conditions.

Parameters	Substrate			Effluent			Digester Efficiency (%)		
	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average
OLR (kg COD.m^{-3}.d^{-1})	1.42	63.09	9.94						
COD (mg.l^{-1})	1 418	63 090	9 944	445	7 130	3 388	0	96	58
TKN (mg.l^{-1})	151	1 520	538	56	691	326	0	91	42
VFA (mg.l^{-1})	216	9 530	1 815	636	6 000	2 042	0	80	49
SO_4 (mg.l^{-1})	130	2 300	554	23	2 100	447	0	96	44
Temp (°C)	15.5	25.5	21.1	10.0	19.0	14.3	-	-	-
pH	7.2	12.3	9.7	7.5	10.5	8.3	-	-	-
TAlk (mg.l^{-1} as $CaCO_3$)	109	21 833	2 663	475	5 934	1 373	-	-	-
Cl (mg.l^{-1})	57	724	342	32	1 188	534	0	90	47
NH_3 (mg.l^{-1})	4	903	117	10	994	218	0	64	27
TS (mg.l^{-1})	2 900	53 800	13 208	1 700	8 200	3 725	0	93	63
VS (mg.l^{-1})	1 300	40 600	8 436	700	4 000	1 608	0	95	72
TNVS (mg.l^{-1})	900	29 500	4 772	900	4 200	2 116	0	92	48
P-PO_4 (mg.l^{-1})	0.0	7.4	1.6	0.4	8.4	2.4	0	74	6
Total metals (mg.l^{-1})	0.3	5.0	1.8	0.4	4.8	1.8	0	52	22
Na (mg.l^{-1})	58	1 501	535	105	1 230	653	0	81	25
Mg (mg.l^{-1})	0	751	80	1	465	63	0	98	60
Ca (mg.l^{-1})	8	545	128	7	489	147	0	94	43
R-value (kg COD.m^{-3}.d^{-1})	-	-	-	-	-	-	-2.03	59.32	6.56
Biogas production ($l.d^{-1}$)	-	-	-	-	-	-	3.32	175.54	48.88
Biogas Yield (m^3.m^{-3} reactor)	-	-	-	-	-	-	0.66	35.11	9.78
CH_4 content	-	-	-	-	-	-	70	95	85
CH_4 yield (m^3.kg $COD_{removed}$. d^{-1})	-	-	-	-	-	-	1.53	42.57	1.91
CH_4 yield (m^3.kg COD_{loaded}. d^{-1})	-	-	-	-	-	-	0.24	55.21	5.72

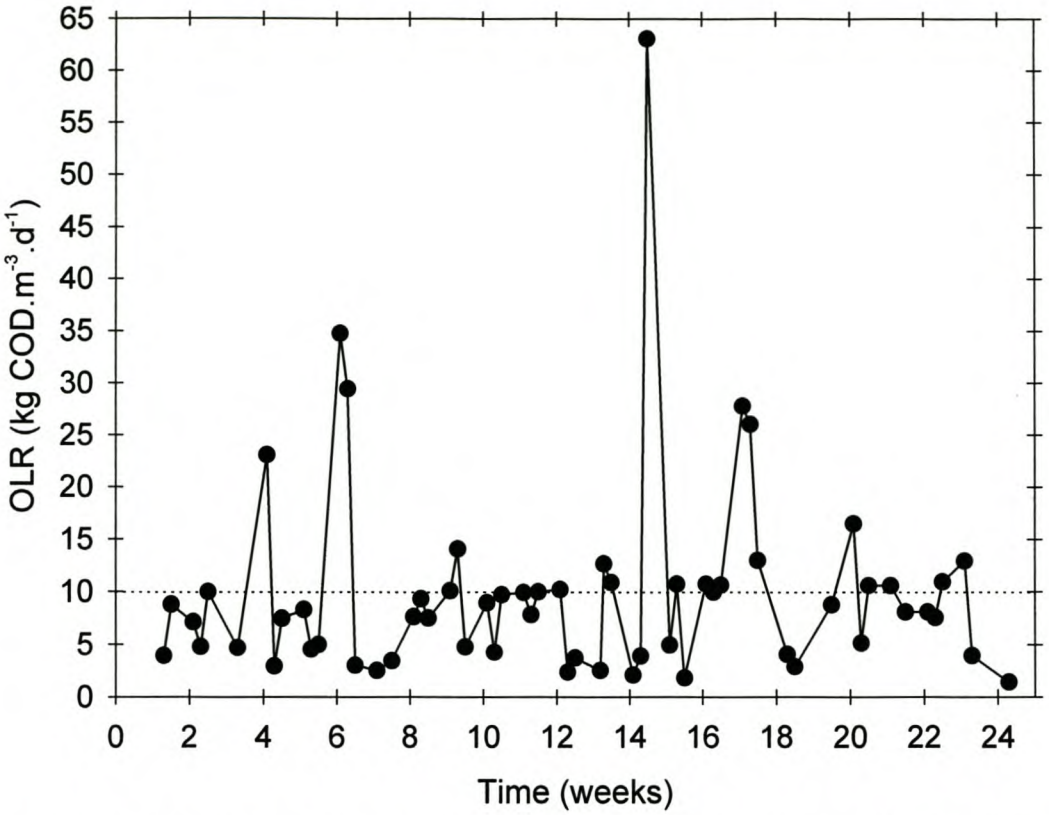

Figure 3. Variation of the substrate organic load for the pilot-scale digester over a 25 week period. The dotted line represents the average OLR.

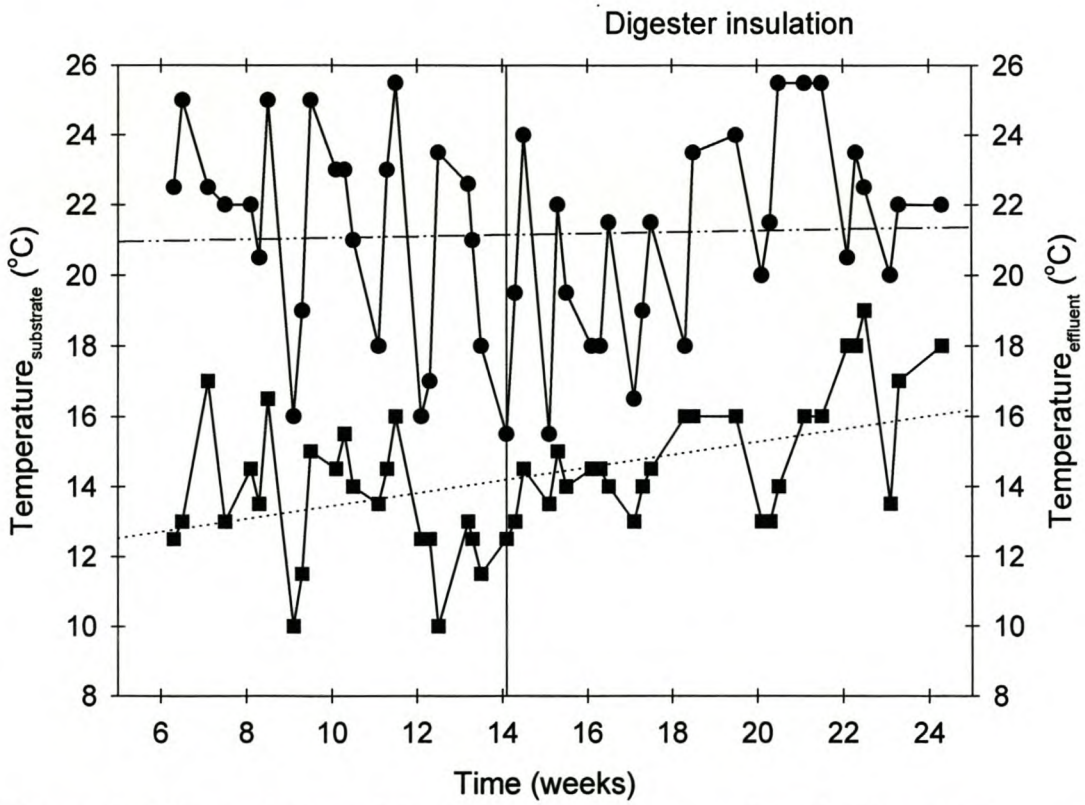

Figure 4. The variation of the substrate (● ; —··—··—) and effluent (■ ; .....) temperature. The solid lines represent the actual data and the dotted and dashed lines represent the regression calculations.

extraction (44° - 80°C); primary evaporation (50°C); sterilisation (140°C); chill and extrusion (-10°C) and drying of gelatin (28° - 60°C) (Cole, 1997, Personal communication). The environmental temperature also influences the temperature of the gelatin-manufacturing effluent. Sharp and frequent fluctuations in environmental temperature will affect the performance of the methane-producing bacteria to a greater extent than variations in operating temperatures within the mesophilic or thermophilic ranges (Malina & Pohland, 1992). It was, therefore, decided to insulate the digester (Fig. 4) after the 14th week of operation. However, the results indicate that the reactor insulation did not have a marked effect on the biodegradation performance and temperature variations (Fig. 6).

Substrate pH is also known to be an important regulatory parameter for the cellular metabolism of anaerobic digestion systems (Pretorius, 1994). In a balanced anaerobic digestion system, where volatile fatty acids are converted to methane and carbon dioxide, a pH of between 6.5 and 7.5 should be maintained for optimum methane production (Pretorius, 1994). It has, however, been reported that the best pH for the direct conversion to methane can also be within a range from 5.5 to 6.0, and if the correct species are present, even as low as a pH of 3.5 (Matsunaga & Sekine, 1997). The extreme variations of the substrate pH can be seen in Fig. 5. An average substrate pH of 9.7 was fed to the digester (Table 1). The effluent pH of the UASB digester ranged from 7.5 and 10.5 with an average of 8.3 (Table 1). According to the data in Fig. 7, the methane composition of the biogas production was more affected at the higher pH values. The methanogens, however, appeared to have recovered from extreme shock loads within a few days, where after the methane composition of the biogas increased again (Fig. 7).

In a system where the methanogens are inhibited, the acidogenic population usually prevail, since they are significantly less sensitive to low or high pH environments and this will lead to the acidification of the reactor systems. No acidification of the reactor were, however, reported during this study. In another gelatin effluent study Maree *et al.* (1990) reported the gradual increase in volatile fatty acids and sulphide concentrations which

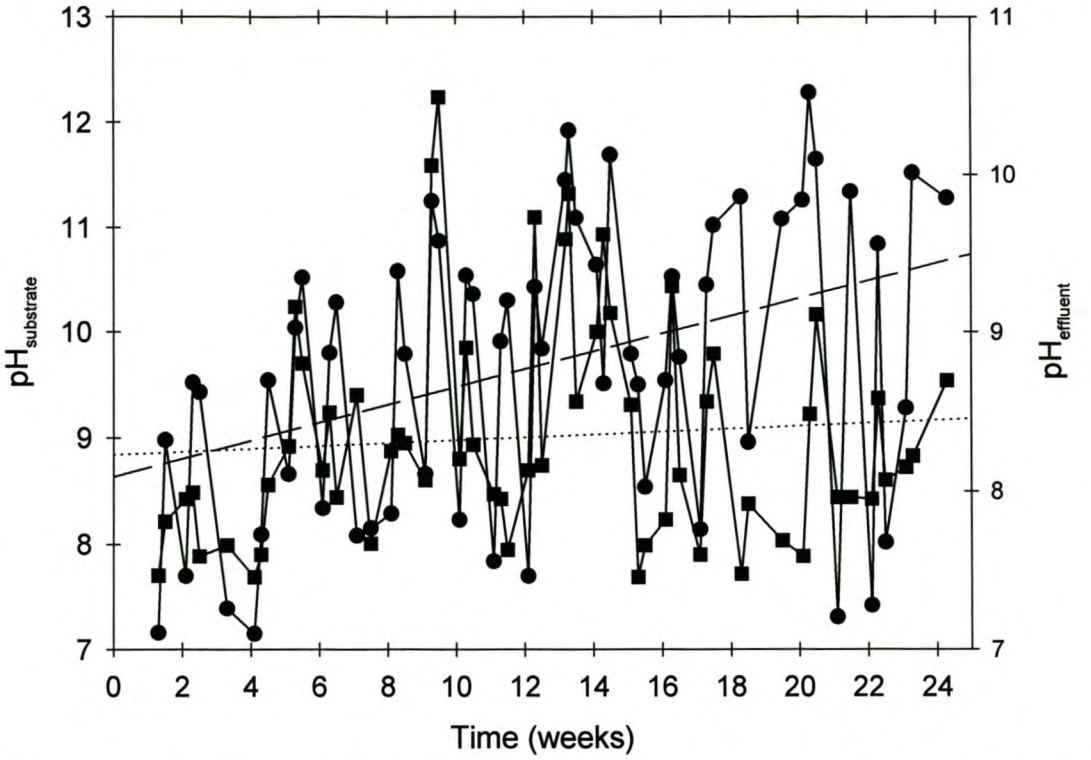

Figure 5. The variation of the substrate pH (● ; -----) and digester effluent pH (■ ; .....) as a function of time. The solid lines represent the actual data and the dotted and dashed lines represent the regression calculations.

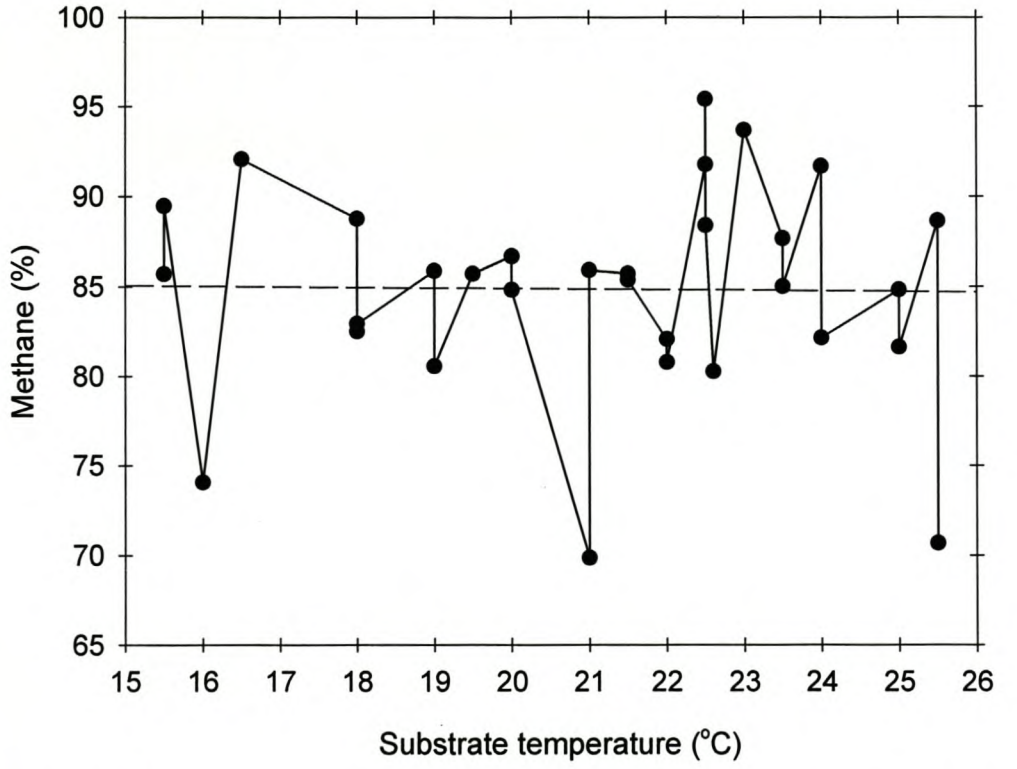

Figure 6. Methane as a function of substrate temperatures. The dashed line represents the regression calculation.

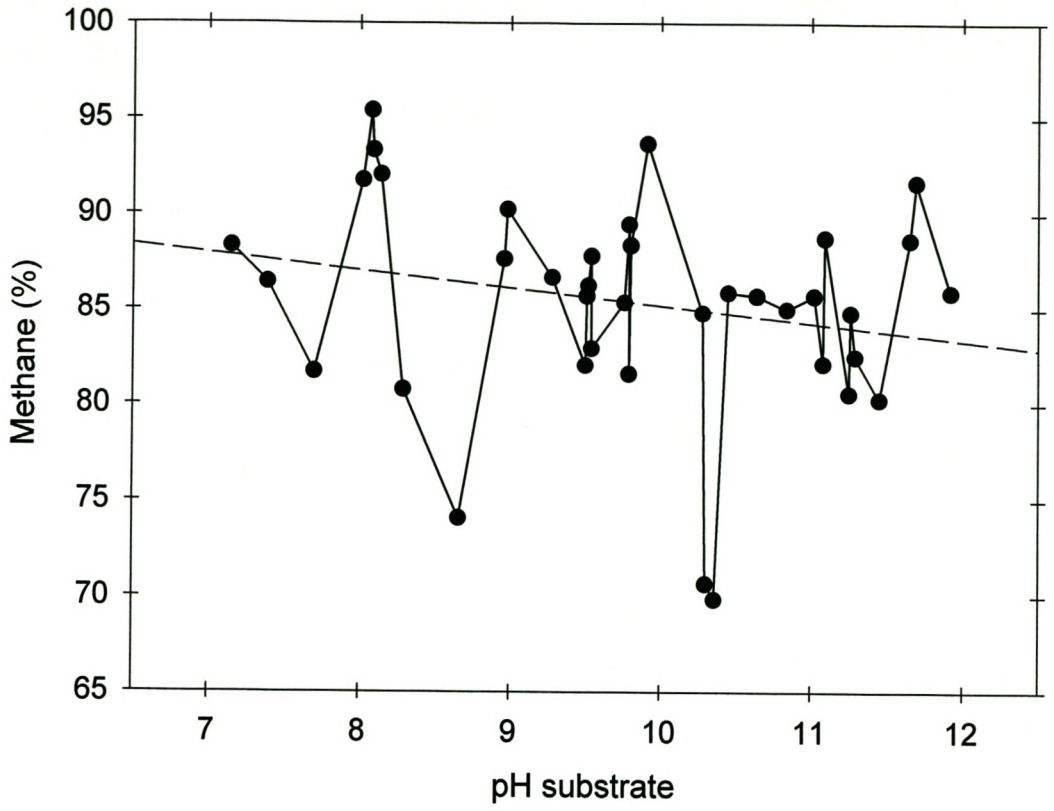

Figure 7. Methane as a function of substrate pH. The dashed line represents the regression calculation.

accumulated to such an extent that the anaerobic system eventually collapsed. This did not happen during this study.

The UASB pilot-scale reactor as operated in this study performed relatively well throughout the test period under the highly variable OLR and other shock conditions. An excellent increase was found in the COD removal and R-value at the higher OLR's (Fig. 8). COD removals of up to 96% (an average of 58%) were achieved at an OLR of 10.72 kg $COD.m^{-3}.d^{-1}$. An average R-value of 6.56 kg $COD.m^{-3}.d^{-1}$ was obtained under the same OLR's. The maximum R-value of 59.32 kg $COD.m^{-3}.d^{-1}$ was obtained at an OLR of 63.09 kg $COD.m^{-3}.d^{-1}$.

The biogas yield was found to increase over the experimental period (Fig. 9). Similar trends were found for increases in OLR (Fig. 10). Week 1 to 7 were part of the start-up phase of the digester (Fig. 9 and 10). The increase in biogas yield after week 7 indicated the presence of an active digester population which rapidly adapted the specific substrate. The highest biogas yields (35.1 and 34.6 $m^3.m^{-3}$ reactor) were observed at an OLR of 10.88 and 26.03 kg $COD.m^{-3}.d^{-1}$, respectively. The high methane content of the biogas (85%) suggested the presence of an active methanogenic population which was able to compete with the sulphate reducing bacteria (SRB) for the available substrate. SO_4 removals of up to 96% were obtained during the study (Table 1), also indicating an active SRB community.

The TS removal was also found to increase with increasing OLR. The highest TS removal efficiency of 93% was found at an OLR of 34.78 kg $COD.m^{-3}.d^{-1}$ (Fig. 11).

The alkalinity of the digester effluent was found to vary between 470 to 5 930 $mg.l^{-1}$ indicating a good buffering capacity (Hawkes *et al.*, 1992). An average total alkalinity of 1 370 $mg.l^{-1}$ was obtained during the UASB study (Fig. 12).

Experimental study II - Contact process configuration as pilot-scale

The anaerobic contact configuration was originally designed to overcome some of the limitations/disadvantages of conventional digester processes, especially in terms of separating and recirculating the effluent

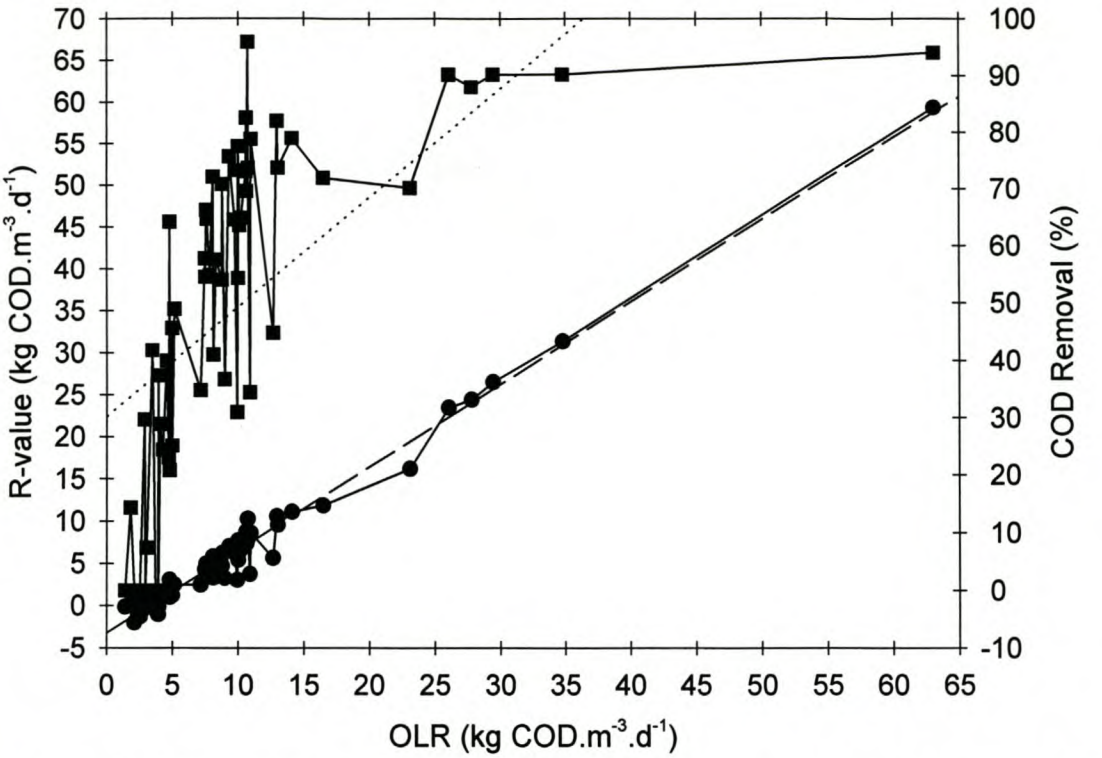

Figure 8. The COD removal rate (R-value) (● ; -----) and COD removal (■ ; .....) as a function of the OLR. The solid lines represent the actual data and the dotted and dashed lines represent the regression calculations.

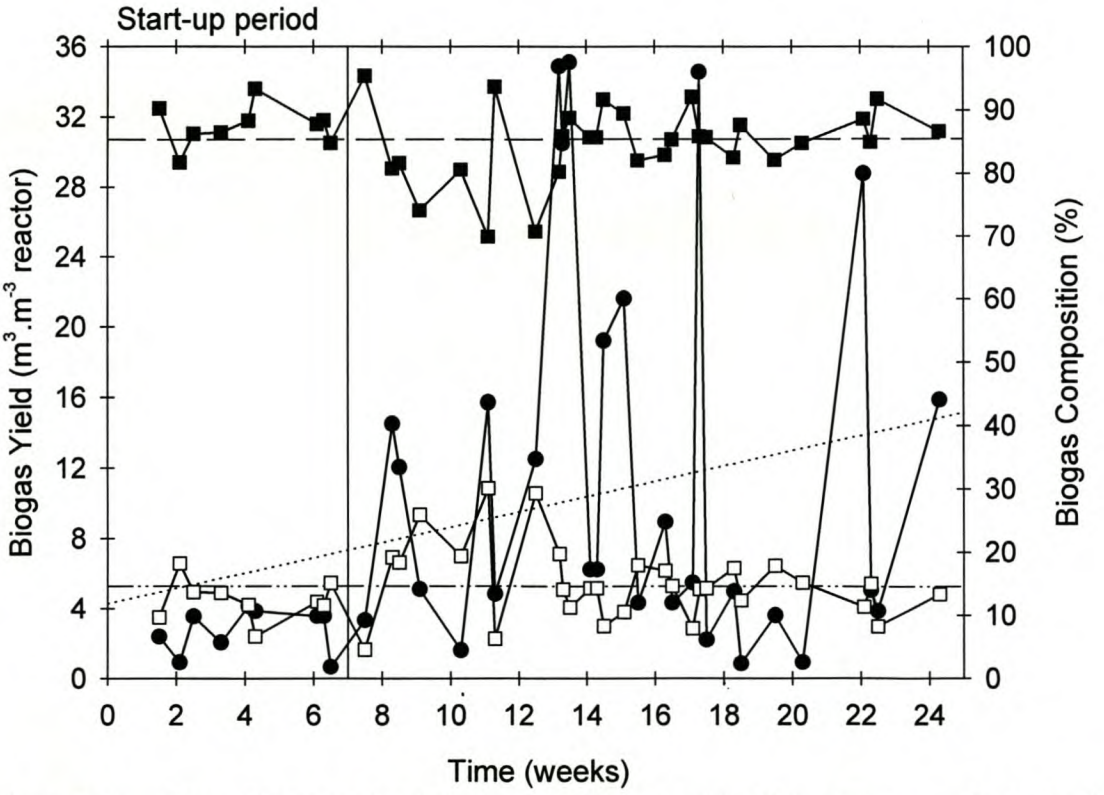

Figure 9. The biogas yield (● ; .....) and theCH_4 (■ ; -----) and CO_2 composition of the biogas (□ ; —··—··—) during the experimental study. Up to week 7 was part of the start-up period. The dashed and dotted lines represent the regression calculations.

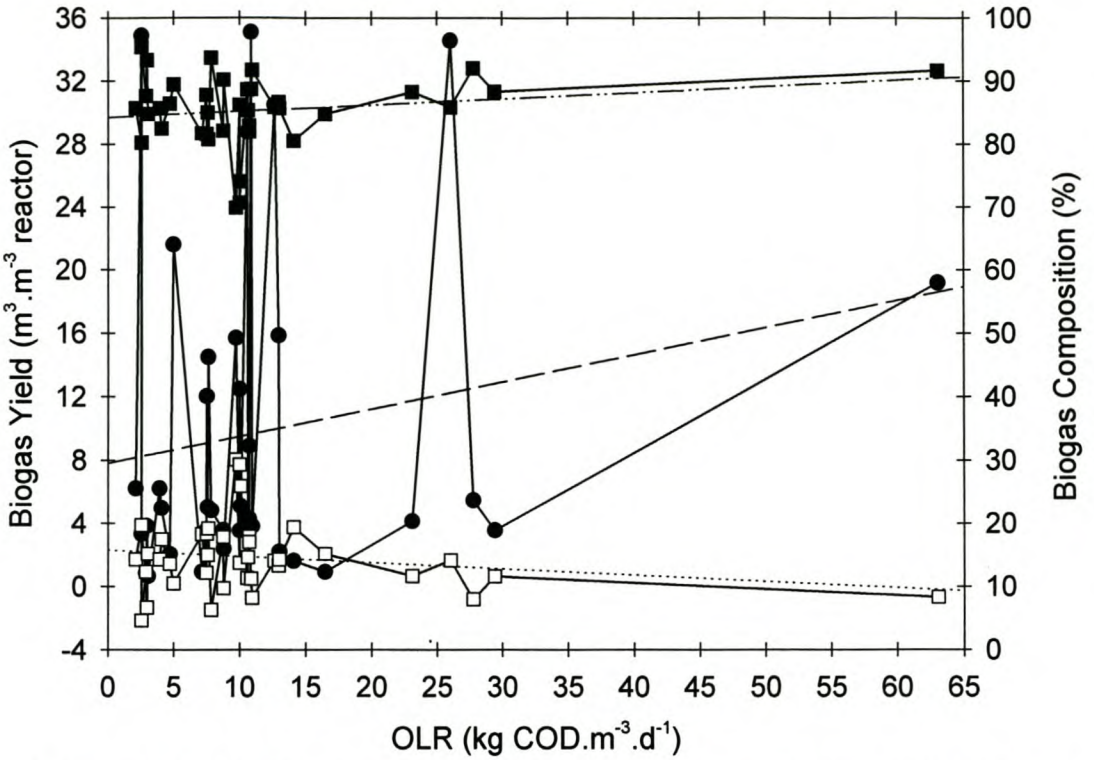

Figure 10. The influence of increasing OLR's on biogas yield (● ; -----) and the CH_4 (■ ; —··—··—) and CO_2 content (□ ; .....) during the pilot-scale digestion. The solid lines represent the actual data. The dotted and dashed lines represent the regression calculations.

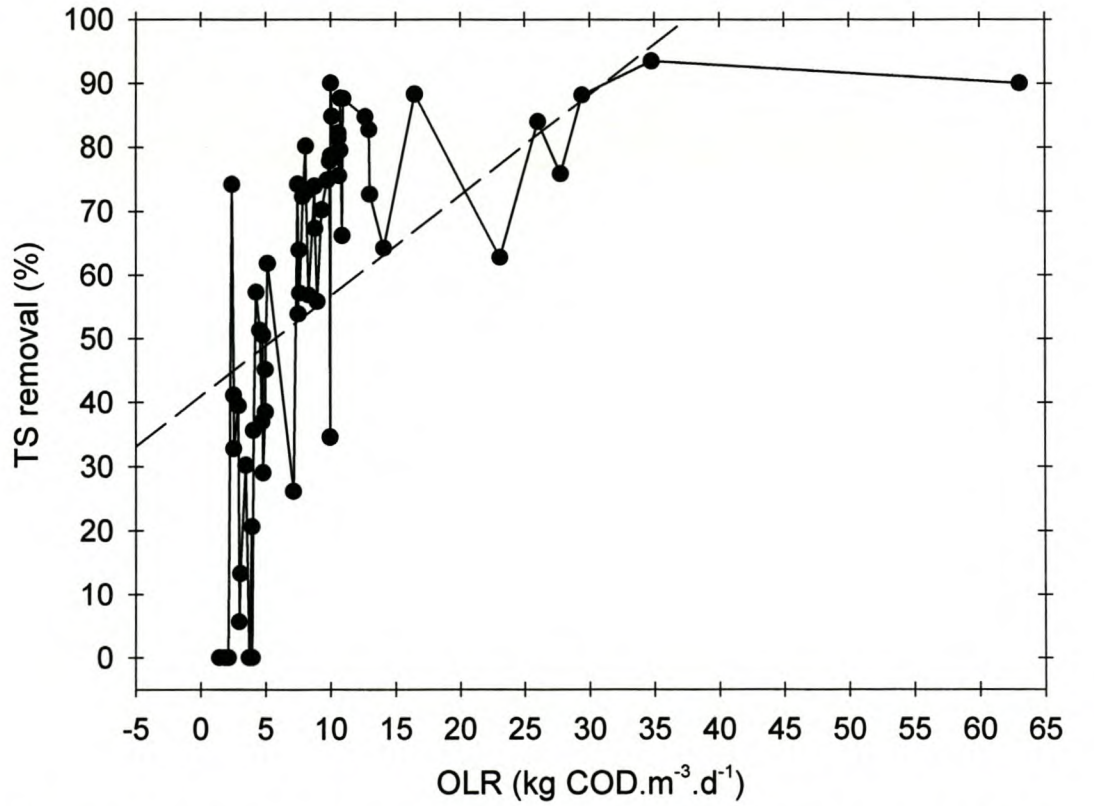

Figure 11. The total solid removal as a function of OLR. The dashed line represents the regression calculation.

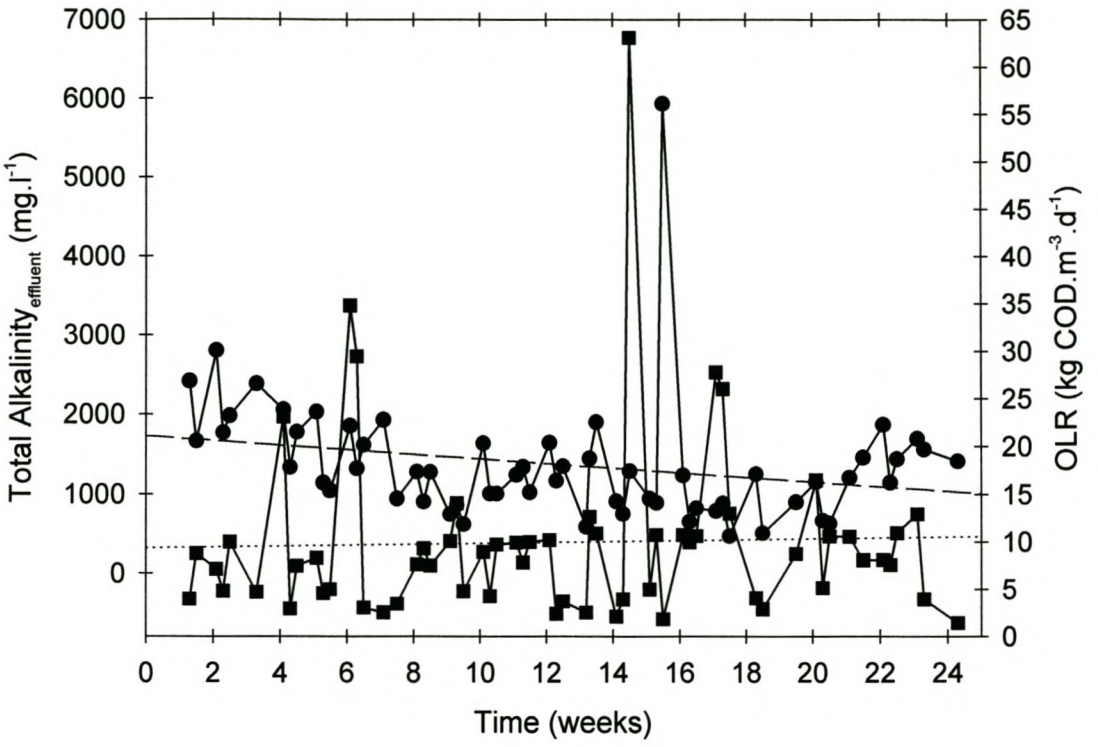

Figure 12. The variation of the digester effluent total alkalinity (● ; -----) and OLR (■ ; .....) as a function of time. The solid lines represent the actual data and the dotted and dashed lines represent the regression calculations.

suspended solids back to the anaerobic reactor (Malina & Pohland, 1992). The separation and the recirculation of the biomass using this configuration has the advantage of retaining both the active microorganisms as well as the slowly biodegradable suspended solids, thus promoting a more complete biodegradation of wastewater particles. A possible problem that can be expected while using anaerobic contact systems is that the configuration is heavily dependent on the settling properties of the anaerobic sludge (Malina & Pohland, 1992). Active anaerobic sludge flocs are usually associated with biogas, thus solids settleability may be problematic.

The contact process configuration as used in this study (Fig. 1), suffered from constant blocking (grit, fat, salts and calcium carbonate) in the feed and recirculation lines. Relatively poor performances were achieved in COD, SO_4, VFA, TS and VS removals, (a maximum of 41, 62, 64, 39 and 60%, respectively) at a HRT of 1.0 d (Table 2). A relatively low digester total alkalinity (1 257 $mg.l^{-1}$) was apparent throughout the study of 14 weeks, indicating a poor system buffering capacity. The high reactor effluent pH (over 8.1) (Table 2) did influence the complete conversion of fatty acids to methane by inhibiting the rate of methanogenesis, when considering the optimal pH for methanogenesis is between 6.5 and 7.5 (Pretorius, 1994).

The volume of biogas produced was low, but when biogas production could be measured an average of 86% methane content was present in the biogas. This indicated that, although constant blocking was experienced, the contact configuration could be effective if the current problems could be eliminated. The high methane production indicated that an active methanogenic population were present. Furthermore, solid washout occurred from time to time. Various researchers have previously reported problems with foaming and scum formation while treating a gelatin effluent containing high protein and lipid contents (Tommaso *et al.*, 1999). This again induced sludge flotation and led to solids and biomass washout.

Table 2. Composition of the substrate, effluent and digester efficiency during the pilot-scale operating conditions using the contact configuration.

Parameters	Substrate			Effluent			Digester Efficiency (%)		
	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average
OLR (kg COD.m^{-3}.d^{-1})	1.19	7.60	3.93						
COD (mg.l^{-1})	1 192	7 604	3 934	2 351	4 503	3 479	0	41	27
TKN (mg.l^{-1})	136	671	341	159	310	261	0	57	23
VFA (mg.l^{-1})	600	6 910	2 918	938	3 828	2 066	0	64	27
SO_4 (mg.l^{-1})	145	345	262	55	425	237	0	62	41
Temp (°C)	23.0	23.5	23.2	18.0	18.8	18.4	-	-	-
pH	7.7	9.6	8.2	7.6	8.1	8.0	-	-	-
TAlk (mg.l^{-1} as $CaCO_3$)	158	3 026	1 094	554	1 642	1 257	-	-	-
Cl (mg.l^{-1})	162	508	319	225	698	450	0	17	3
NH_3 (mg.l^{-1})	18	635	187	139	220	174	0	65	13
TS (mg.l^{-1})	1 600	5 200	3 520	2 300	3 200	2 920	0	38	21
VS (mg.l^{-1})	600	3 000	1 780	800	1 300	1 140	0	60	42
TNVS (mg.l^{-1})	1 000	2 500	1 740	1 700	2 200	1 940	0	12	11
P-PO_4 (mg.l^{-1})	0.0	3.9	1.4	0.3	3.3	1.8	0	29	9
Total metals (mg.l^{-1})	0.6	2.0	1.0	0.5	1.9	1.0	0	52	22
Na (mg.l^{-1})	120	348	256	172	683	428	0	0	0
Mg (mg.l^{-1})	23	292	87	37	85	59	0	73	15
Ca (mg.l^{-1})	76	411	233	172	484	331	0	0	0
R-value (kg COD.m^{-3}.d^{-1})	-	-	-	-	-	-	-1.40	3.10	0.46
Biogas production ($l.d^{-1}$)	-	-	-	-	-	-	0.67	1.43	1.05
Biogas Yield ($m^3.m^{-3}$ reactor)	-	-	-	-	-	-	0.00	0.29	0.11
CH_4 content	-	-	-	-	-	-	82	90	86
CH_4 yield (m^3.kg $COD_{removed}$. d^{-1})	-	-	-	-	-	-	-0.41	0.42	0.00
CH_4 yield (m^3.kg COD_{loaded}. d^{-1})	-	-	-	-	-	-	0.00	0.23	0.13

Operational Problems

Several major problems were experienced during the studies while using the UASB and contact process configurations and included the following:

1. The nature of the effluent plays an important role in the determination of the removal efficiency of the organic matter while being treated anaerobically. As used in this study, the effluent contained non-biodegradable material such as hair, grit, fats, reject hide pieces and large sludge volumes, which are part of the gelatin-manufacturing process. Due to the nature of the effluent, a filter had to be inserted at the beginning of the feedline to prevent hair and large grit/solids particles from entering the system. However, this resulted in clogging of the filter and the filter had to be cleaned regularly. The result of the filter being clogged was that no substrate could enter the feedline, thereby causing a vacuum in the feedline which affected peristaltic pumping negatively;
2. The gelatin-manufacturing industry utilises a batch process and therefore discharge variable volumes of effluent. To eliminate this problem and to ensure a submerged system at all times, a sluice was incorporated to regulate and ensure a regular flow of the effluent, since the digester system was fed continuously. The sluice channel had to be cleaned daily to prevent the build-up of solids/sludge/grit in the channel;
3. Problems were also experienced with the blocking of the feedline as the main channel was situated ± 20 m from the digester. With time, fats and grit accumulated in the feedline and led to blockages that had to be removed regularly;
4. As previously stated more problems were experienced during the operation of the contact process. The solids present in the digester were forced into the outlet of the digester, which resulted in further down-stream blockages preventing the effluent to exit the system. This was followed by an increased pressure in the digester which forced the contents of the digester in the opposite direction back to the main channel through the feedline of the digester; and

5. Another problem experienced during the contact process was at the cone shaped clarifier. The effluent of the digester exited via the top into the clarifier. The biosolids were then extracted at the bottom of the clarifier to be recirculated with a peristaltic pump, back into the digester. These settled solids often blocked the bottom of the clarifier. Therefore, no effluent could be recirculated and a vacuum was created in the recirculation lines, which affected the recirculation pump negatively. It was vital to clean the clarifier regularly by forcing air or substrate into the pipelines to remove all blockages.

All these problems emphasised the need for the continuous attention and monitoring of the pilot-scale digester, especially the contact process, to prevent failure. The above mentioned problems are typical operational problems that must be taken into consideration when planning a full-scale reactor.

Conclusion

Gelatin-manufacturing effluents contain relatively high concentrations of nominal or slowly biodegradable organic compounds. It was taken that, due to the highly variable nature and the complex composition of the effluent, biological breakdown would be a slow process. Hence, it was decided to evaluate different high-rate anaerobic digester configurations to determine the ideal design and conditions for optimal biological breakdown. In previous studies (Chapters 3, 4 and 5) the laboratory-scale UASB and contact digesters proved that successful breakdown of the compounds can be obtained.

As a continuance to the results of the laboratory-scale digesters, a 300 litre pilot-scale digester was subsequently evaluated in this study under actual field conditions. From the results obtained during the two experimental studies (I and II) it was found that the UASB pilot plant (Study I) can be efficiently used as a pre-treatment option for this effluent. There was no need for chemical pre-treatment of the effluent, such as neutralisation of pH, before

anaerobic digestion. Maximum performance efficiencies were achieved with COD, TS, VFA and SO_4 of up to 96, 93, 80 and 96%, respectively for the UASB configuration. Relatively few operational problems were experienced with this configuration.

The contact process (Study II) only gave maximum removals of up to 41, 39, 64 and 62% of COD, TS, VFA and SO_4, respectively. Continuous operational problems were, however, experienced during the contact process due to the constant blocking of the reactor system, and specifically, sludge wash-out was experienced during the different stages while using this configuration. Tommaso *et al.* (1999) also reported the wash-out of biomass while treating a complex protein and lipid containing gelatin effluent using a similar reactor configuration.

One possible reason for the successful start-up of the anaerobic digesters can be the use of well-acclimatised sludge, which may already have a highly selective and adapted microbial consortium for the gelatin-manufacturing effluent, another good advantage for the construction of a possible full-scale digester. The problems experienced during this study, especially with the contact configuration should be addressed appropriately.

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CHAPTER 7

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

Background

Adequate long term conservation and management of South Africa's water resources is of vital importance for sustained economic growth and development. The country is situated in a semi-arid region where rainfall and waterbodies are unevenly distributed. The rapidly expanding demand arising from positive population growth and demographic changes has resulted in water becoming increasingly scarce in many parts of South Africa. Greater pollution loads and reduced flows in the country's rivers, due to the expanding demand, will in future place additional pressure on the already limited water resources. For these reasons water must be treated, whether to produce water for general consumption, or for specific industrial uses, or to limit the discharge of pollution into the environment. Nationally, local authorities are thus forced to implement higher trade-effluent charges, especially to wastewater producing industries, in an attempt to reduce the pollution load on conventional purification facilities and to minimise downstream water and environmental pollution. The purification facilities often experience difficulties with the treatment of discharged wastewaters, due to the noxious nature of the effluents. These effluents often inhibit biological treatment processes, consequently resulting in higher chemical dosing demands, operational problems and inefficient treatment of the sewage.

One such industrial effluent which has recently received much attention, is that of the gelatin-manufacturing industry. This is the only industry in South Africa that produces edible and technical gelatin and is situated in Krugersdorp, Gauteng. During this process, reject hides received from abattoirs and tanneries are conditioned, treated and processed. Insoluble hide collagen is converted into water soluble gelatin through a process of alkali conditioning, protein hydrolysis and denaturation (Maree *et al.*, 1990). The gelatin-manufacturing industry use approximately 57 000 Ml

of water per month, based on a 6 working day operation. The effluent which the industry produce varies considerably in volume as a result of the large volume of water consumption. The organic strength also varies from batch to batch, and typically has an alkaline nature with high concentrations of COD, SS, fats, lipid emulsions, protein and salts.

The composition of the effluent has a negative influence on the biological treatment processes of the local wastewater purification works. The main problems that have been experienced include a loss of nitrification and denitrification ability, reduced ortho-phosphate ($P\text{-}PO_4$) and chemical oxygen demand (COD) removal, as well as biosolid carry-over during secondary clarification. Approximately 12% (v/v) of the total flow received at this specific purification works and 65% of the organic load into the purification works, are contributed by the gelatin-manufacturing industry.

Considering the impact of these high and irregular organic discharges to the purification works and the subsequent high trade-effluent charges paid by the industry, it was decided to find a mutually suitable pre-treatment method to deal with the effluent before discharging to the local purification works.

Pre-treatment options

Considering the various options available (physical vs chemical vs biological) for the treatment of industrial effluents, anaerobic biological treatment with concomitant production of methane has distinct advantages over other processes. Recognition of these benefits have resulted in the wide international application of anaerobic processes, mainly because it compares favourably with the costly physical and chemical technologies.

Very little application data are unfortunately available on the biological and anaerobic treatment of gelatin-manufacturing effluent (Maree *et al.*, 1990; Du Plessis *et al.*, 1993; Petruy *et al.*, 1999; Tommaso *et al.*, 1999). Numerous studies have, however, been done on the biological treatment of

similar tannery and high salinity wastewaters (Shipin *et al.*, 1994; Petruzelli *et al.*, 1995; Dalmacija *et al.*, 1996; Rajalo & Petrovskaya, 1996).

The successful improvements of the basic digester design has over the last decade led to a remarkable improvement in the removal efficiencies of compounds present in effluents, compared to the conventional anaerobic systems. Among these designs, UASB digesters have become one of the most extensively used designs for treatment of high-strength wastewaters (Lettinga *et al.*, 1997).

Against this background, this study was conducted to evaluate the performance of different laboratory and pilot-scale anaerobic digester configurations for the pre-treatment of the gelatin-manufacturing effluent. This was also done to obtain a suitable database for future reference in reactor upscaling and application of anaerobic effluent treatment.

Laboratory-scale investigation

Different experimental studies were conducted on laboratory-scale anaerobic digesters to determine the optimal operational conditions and digester design. The studies included three single-phase UASB anaerobic digesters (AD-1;polyethylene, AD-2; polyurethane, AD-3; contact) and a multi-phase unit (AD-1 + AD-2). For the laboratory-scale studies, with the exception of pH adjustment to 6.5 and implementation of mesophilic temperatures, no standardisation was done on the effluent in an attempt to simulate actual field conditions. The laboratory-scale digesters were started at a hydraulic retention time (HRT) of 3.0 d and the HRT was subsequently decreased to 1.0 d after stable state conditions had been reached. The average operational efficiencies of the four laboratory-scale systems treating gelatin-manufacturing effluent, are summarised in Table 1.

Single-phase systems

In the first study (AD-1), an upflow hybrid digester, fitted with polyethylene foam as fixed-film basis, was evaluated while treating the full-

Table 1. Comparison of the average digester removal efficiencies of the single, multi-phase and pilot-scale anaerobic digesters treating the gelatin-manufacturing effluent at an HRT of 1.0 d.

Parameters	Single-phase			Multi-phase	Pilot-scale	
	AD-1	AD-2	AD-3		UASB	Contact
HRT	1	1	1	1	1	1
OLR (kg COD.m^{-3}.d^{-1})	3.97	3.65	2.60	4.17	9.94	3.93
COD (%)	53	60	54	80	58	27
COD removal rate (kg COD.m^{-3}.d^{-1})	2.19	2.32	1.40	3.38	6.56	0.46
SO_4 (%)	86	82	59	69	44	41
Cl (%)	N/D	N/D	40	0	47	3
NH_3-N (%)	N/D	N/D	0	0	27	13
TKN (%)	6	7	9	14	42	23
PO_4-P (%)	1	7	7	2	6	9
Biogas production ($l.d^{-1}$)	5.01	3.77	0.29	6.10	48.88	1.05
Biogas yield ($m^3.m^{-3}$)	1.00	0.75	0.01	1.22	9.78	0.11
CH_4 content (%)	80	79	71	79	85	86
CH_4 yield (m^3.kg $COD_{rem}.d^{-1}$)	2.19	1.86	0.01	4.24	1.91	0.00
CH_4 yield (m^3.kg $COD_{load}.d^{-1}$)	1.26	1.19	0.01	1.65	5.72	0.13
VFA removal (%)	20	26	21	48	49	27
TS removal (%)	25	28	26	29	63	21
VS removal (%)	53	58	57	71	72	42
TNVS removal (%)	8	8	17	8	48	11
Na removal (%)	10	15	10	7	25	0
Mg removal (%)	N/D	N/D	11	N/D	60	15
Ca removal (%)	42	37	22	16	43	0
Total metal removal (%)	34	36	38	28	22	22

strength gelatin-manufacturing effluent. The highest COD removal (90%) and organic removal rate (R-value) (8.6 kg $COD.m^{-3}.d^{-1}$) was observed at an OLR of 9.56 kg $COD.m^{-3}.d^{-1}$ (Chapter 3). However, other process parameters were found to vary considerately, both as a function of time and of OLR. These variations were indicative of the adverse influence of the unstable and inconsistent substrate composition on the performance of the succeeding microbial populations. Good results were obtained with SO_4 removals (up to 96%), indicating the presence of an active SRB community working in balance with the system methanogenic population. The methane content varied between 70 and 88%, with an average methane yield per COD removed of 2.2 $m^3.kg\ COD_{removed}.d^{-1}$. The alkalinity of digester AD-1 varied between 100 to 3 300 $mg.l^{-1}$ showing good buffering capacities to neutralise possible toxic and / or loads. These alkalinity levels exceeded the recommended limit of 1 500 $mg.l^{-1}$ for a properly balanced and stable system (Hawkes *et al.*, 1992). Furthermore, no VFA accumulation was observed during the study, which is contradictory to other studies where a gradual accumulation of VFA was reported, which eventually became toxic to the microbial population. No consistent trends in TKN, total metals and TNVS removals were observed during the study. These results are contradictory to the results reported by Maree *et al.* (1990), who found, while using a different digester design, failure as a result of increasing sulphide concentrations.

The aim of the second phase of the study was to determine if substrate nutrient supplementation would contribute to better digester performance and to prevent digester failure. An upflow hybrid digester (AD-2), fitted with polyurethane foam received 80% gelatin-manufacturing effluent enriched with 20% supernatant from an existing full-scale sewage sludge digester. The highest COD removal (83%) and R-value (6.7 kg $COD.m^{-3}.d^{-1}$) was observed at OLR of 4.62 and 9.30 kg $COD.m^{-3}.d^{-1}$, respectively (Chapter 3). The performance tendencies of digester AD-2 were similar to digester AD-1, but digester AD-2 only functioned at a relatively lower OLR. The only distinctive difference between digester AD-1 and digester AD-2 was the granule formation which was observed in digester AD-1. The granules observed were fluffy and dark coloured with an average diameter of 0.5 to 1.5 mm. Thus, it

was concluded that the raw substrate used in this study lent itself to granule production under the specific conditions of anaerobic dynamics. Even though the type granules could be classified as a stable high quality granule, it was clearly consistent in its production cycle and immediately reflected reactor upset by washout of the granules along with the biomass particles. The results obtained from these studies on digester AD-1 and digester AD-2, indicated the feasibility of UASB systems while treating high-strength gelatin-manufacturing effluent. It was also clear from this study that nutrient supplementation, using high VFA supernatant with the raw substrate, did not benefit the total reactor performance and no reason could be argued to pursue the supplementation project.

It was decided to determine the influence of a higher biomass contact area in an upflow contact digester configuration during a third study (AD-3) while treating the same effluent. At an HRT of 1.0 d, the highest COD removal (95%) and R-value (7.2 kg $COD.m^{-3}.d^{-1}$) was observed at an OLR of 2.61 and 8.00 kg $COD.m^{-3}.d^{-1}$, respectively (Chapter 5). The average COD removals at HRT's set at 3.0 d and 1.0 d, were 51 and 54%, respectively. The R-value was found to increase rapidly as a function of increasing OLR, whereas the COD removal showed a more gradual increase. Again the efficiency tendencies for all the process parameters were fairly similar to those found for digester AD-1 and digester AD-2. Sulphate removals of between 86 and 97%, were observed and a very high average pH (8.71) and very low total alkalinity (933 $mg.l^{-1}$) was found in this study and it was concluded that the alkalinity was not high enough to buffer the system effectively. During the study very little biogas production was observed, which probably could be the result of the non-optimal operational pH of the digester. Furthermore, gas bubbles were found to adhere to the bacterial flocs, which in turn resulted in biomass carry over and poor settling of the anaerobic biomass in the reactor. These results corresponds with the data reported by Petruy *et al.* (1999) who found that the inflow of low lipid concentrations (100 $mg.l^{-1}$) in the form of milk-fat emulsion did not affect the COD removal of a EGSB system. Even a concentration of 100 000 $mg.l^{-1}$ was not toxic, but a

lipid/fat removal of adsorption to the sludge is activated under anaerobic conditions, which again affects the biogas and methane yields.

It was also found in this study that the contact digester required a shorter period to stabilise after the start-up period. The digester also recovered more rapidly from toxic and shock loads, probably due to the recirculation in the reactor. Cost considerations for upscaling an anaerobic contact reactor would include: higher capital costs; more intensive operation and maintenance of the process; higher energy consumption for recirculation practices and additional sludge settling infrastructures.

Multi-phase system

The same two laboratory-scale digesters used during the single-phase digestion (AD-1 and AD-2) were connected in series during this study. Digester AD-1 received the full-strength gelatin-manufacturing effluent as substrate and digester AD-2 received the effluent from digester AD-1 as substrate for further digestion. This research was done in an attempt to try and partially separate the dominant microbial populations and to establish their interactions in two separate digesters, as well as to demonstrate the feasibility of a multi-phase system while treating gelatin-manufacturing effluent. It was originally argued that an increased bacterial activity and higher density biomass would occur as a result of creating optimum digester operating conditions for the different microbial populations. This phase separation could also be seen to represent a specific nutrient separation for the phase-dominating populations as well as a metabolite production system for the subsequent dependent populations. Other researchers have already confirmed the feasibility of using multi-phase separations in UASB biosystems (Shin *et al.*, 1992; Fongastitkul *et al.*, 1995; Speece *et al.*, 1997).

This multi-phase system showed optimal COD (97%) and SO_4 (96%) removal efficiencies at OLR's of 8.3 and 4.3 kg $COD.m^{-3}.d^{-1}$, respectively. The methane content varied between 55 and 89%. A significant difference between the single and multi-phase systems was noticed, namely the production and removal of VFA's, which occurred in the multi-phase system, but which was not so clear or incomplete in the single-phase systems. Up to

92% of the VFA's were removed during this study with the multi-phase system. Petruy *et al.* (1999) also found that ammonia released from the degradation of proteins leads to the selection of an acidifying consortium during the first phase, which had a significant influence on the downstream process. The high ammonia gelatin-manufacturing effluent also appeared to enhance the activity of the acidogenic population in the first digester, with subsequent high VFA removal in the total process.

From the data generated during the multi-phase study it can be concluded that the multi-phase system is highly effective for the treatment of high-strength gelatin-manufacturing effluent. Higher removal efficiencies were achieved during multi-phase digestion than with the tested single-phase systems, mainly as a result of the second "finishing" digestion process during multi-phase digestion. In commercial practise, however, this enhanced performance would not motivate the additional mechanical and civil costs associated with the construction of two digesters.

Pilot-scale investigation

The successful application of the different types of hybrid and contact configurations on laboratory-scale led to the evaluation of the stability and overall performances of a 300 litre pilot-scale reactor at actual field conditions. This was also done so as to obtain a useful database for possible future full-scale application. Again, no standardisation was done on the effluent, with the exception of the removal of the harshest non-biodegradable solids.

The 300 litre pilot-scale reactor performed relatively well throughout the study (Chapter 6), under highly variable shock and organic loads. The COD removals and R-values increased strongly during OLR's increments. COD removals of up to 96%, with an average of 58%, were obtained at OLR's of up to 10.72 kg $COD.m^{-3}.d^{-1}$. The average R-values (6.56 kg $COD.m^{-3}.d^{-1}$) were achieved under similar loading rates. Sulphate and TS removals of up to 96

and 93%, respectively, were achieved. An average methane content of 85% was also recorded, suggesting that rate-limiting factors were minimal.

The data generated was of great value for the future planning and design of a full-scale digester. Many operational problems were experienced and valuable experience in operating larger digesters gained. These problems were mainly associated with the clogging and blocking of the system, pumps, nozzles and recirculation lines, as well as sludge washout.

Concluding remarks

The study on the laboratory-scale investigations proved that the hybrid design, especially the configuration used in digester AD-1, had the potential to retain a high portion of the active biomass, and subsequently resulted in good substrate utilisation by the adapted microbial consortium. The results also clearly showed that no nutrient supplementation was needed, as it did not contribute significantly to enhance the digester performance. The data also showed that at higher organic loads, better digester performance efficiencies were obtained, thereby also explaining the better performance of digester AD-1 over digester AD-2.

With the anaerobic contact configuration (AD-3) some of the disadvantages of this type of design could be overcome by returning the biomass back into the digester. It would then be possible to retain both the active microorganisms and undigested suspended solids, which would lead to a better solid retention time and further biodegradation of the solids. The major disadvantage of this configuration is the dependence on good biomass settleability for successful operation and the option of recirculation in the digester will also increase operational costs of the digester. This configuration is more suitable for wastewaters with low or intermediate levels of suspended solids, lipids and proteins. Relatively short HRT's can result in reduced equalisation capacities when receiving shock loads. It must be taken into consideration that the digester will serve a multiple purpose on larger

scale, namely the equalisation (balancing) of peak organic concentrations in the incoming substrate and the subsequent treatment thereof.

The average results of the three laboratory-scale digesters also indicated that the conventional UASB anaerobic hybrid digesters with support material achieved more reliable average treatment results than the UASB bioreactor. However, according to the effect of the increasing OLR's on the efficiencies of the different anaerobic digester designs, it is clear that the digesters reacted in a similar manner.

During the multi-phase digestion good removal efficiencies and phase separation were achieved. High capital costs will, however, be required to implement the use of this technology on commercial scale, although the bench-scale study clearly indicate the greater advantage of phase separation. Careful consideration should thus be given to the economic viability of pilot to full-scale systems. The promising results obtained from the different studies, thus motivated further experimentation to implement a pilot-scale digester.

The pilot-scale digester also revealed valuable information on the application of shock loads to a single-phase UASB and a contact configuration at actual field conditions. The pilot-scale studies indicated the need for mechanical removal of solids in excess of 5 to 10 mm in size, fat and grit removal of particles bigger than 0.3 mm, equalisation and solid and fats separation. The application of a contact configuration may also prove to be successful, if the problems experienced during the field set-up can be clearly identified and addressed appropriately for full-scale application. This implies that, in addition to the construction of a large-scale digester, a fully equipped inletworks with adequate screening and grit removal will have to precede the digester.

Recommendations

It could be concluded that the UASB design, both on laboratory and pilot-scale, was feasible for the treatment of the complex high-strength gelatin-manufacturing effluent. It was shown in this study that highly variable

gelatin-manufacturing effluent can successfully be treated at actual field conditions, with fluctuating pH, organic and solid concentrations.

Based on the data obtained in this study, a mutual decision was made by the management of the gelatin-manufacturing industry and the local municipal authority, that a full-scale treatment can be applied successfully (Van Niekerk & Der Merwe-Botha, 1999, Personal communication). The pilot-scale UASB functioned satisfactorily under field conditions and experienced relatively few operational problems. Although an average COD removal of 58% was obtained, it was decided to use a 50% COD removal target for full-scale considerations. This is an acceptable engineering approach which reduces the risk to the client and the authority and allows for unexpected negative influences on digester performance and full-scale digester design inefficiencies. These figures will result in a treated COD of 1 500 to 2 000 $mg.l^{-1}$, which is acceptable both from a downstream treatment point of view and economic viewpoint. As mentioned, screening to remove solids, grit removal and equalisation should be seriously considered to prevent shockloads from entering the anaerobic system.

This proposed plant upgrading will extend the life of the existing plant and will allow the local wastewater purification works to produce an acceptable effluent quality. This full-scale application will also be a good investment for the gelatin-manufacturing industry, since it will have a positive impact on the trade-effluent tariffs. The cost structure associated with the gelatin-wastewater collection and treatment will incorporate capital redemption, interest on capital loans, operational and maintenance costs. The use of this technology has been well received by the industry (Van Der Merwe-Botha, 1999, Personal communication), who view this pre-treatment plant as an alternative approach to comply with the stricter pollution control legislations. This approach will also have positive implications for the industry regarding lower trade-effluent tariffs, the enhancement of the industry's environmental policy and continuance of their core business in a suitable and economically viable manner.

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