# ENHANCED GRANULATION IN UPFLOW ANAEROBIC SLUDGE-BED DIGESTERS (UASB) BY PROCESS INDUCTION AND MICROBIAL STIMULATION

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## EXECUTIVE SUMMARY



## BACKGROUND AND MOTIVATION

The 1990's were introduced as a decade of water conservation and environmental reclamation. Among the main challenges, especially in South Africa, few are more critical than the need to protect our water resources. As a result of the importance of environmental pollution, coupled to the expected future shortage of usable water, a renewed interest has arisen in the anaerobic digestion process. The direct treatment of waste waters was greatly stimulated by the development of the upflow anaerobic sludge blanket (UASB) process and its successful full-scale application.

In bioreactors of the UASB design, the biomass retention is promoted by bacterial self-aggregation into dense granules. This granulation enhances the performance since the good settling properties of granules minimizes biomass washout and the close cell packing in the granules optimizes the interspecies exchange of metabolites. However, one of the main problems still remaining in the application of the UASB process is the extensively long start-up periods needed for the development of granules. This may in part be related to the extended generation time of the acetogenic and methanogenic bacteria. It can take several months before a highly effective granular bed can be cultivated. Since the operational efficiency and performance of these systems are mainly dictated by the formation, amount and specific activity of the granules, the rather extended start-up times limit the potential use of the system.

## OBJECTIVES

The objective of this research programme was to enhance granulation in UASB systems and promote a more rapid start-up procedure. This was be done by:

1. Setting-up and operating a series of batch systems simulating UASB operating parameters. The granule growth was monitored in terms of yield and metabolic activity. Environmental "stress" conditions were applied to the batch systems and the influence of these as granule stimulants, evaluated. Three waste waters were used as treatment substrates and included synthetic effluents with either glucose, sucrose or lactate as carbon source, a canning industry effluent and a petrochemical effluent containing only volatile fatty acids. The best conditions for granulation were identified and optimized;

2. Setting-up and operating UASB laboratory-scale digesters using a synthetic and a carbohydrate rich canning industry wastewater as treatment substrates. The induction of specific `stress' conditions and their influence as granule stimulants, was evaluated. Changes in the environmental conditions included: organic and hydraulic overloading; changes in C:N:P ratios; as well as the addition of cysteine;

3. Furthermore, based on the data obtained from the batch- and laboratory-scale bioreactors, a UASB anaerobic biological model was constructed and evaluated on a 50 litre pilot-scale UASB reactor; and

4. Start-up procedures were established as part of the batch-, laboratory- and pilot-scale digestion systems.

## RESULTS AND CONCLUSIONS

The research in this study focused on the granulation process in batch-, laboratory-, and 50-l pilot-scale UASB systems, and in particular, the enhancement of the process by placing stress conditions on the microbial consortium. The following conclusions were made from the results obtained:

1. A hypothesis for enhanced granulation was developed and validated against experimental observations. The hypothesis was used to develop a biological model for the simulation of the process and to compare results from both batch- and laboratory-scale anaerobic systems. A good correlation was evident between the hypothesis and the experimental data examined. The results were used to demonstrate the usefulness of a granulation model in correlating process response and testing the hypothesis theories. From the biological model, the operational parameters that must be applied to enhance the granulation were identified and applied in the start-up and operation of the 50-l pilot-scale UASB bioreactor. It was found to be essential that controlled environmental stress be applied and maintained to sustain granulation;

2. It was found that the environmental conditions in batch cultures could be changed (carbon source and concentration, type and age of the sludge inoculum and culturing conditions) to give the propionic acid producers a competitive advantage, leading to an enhancement of the granulation process. The data obtained and the increase in granule formation clearly indicated that granules can be cultured in batch systems using glucose, lactate, canning fruit and petrochemical waste waters as carbon sources. The granulation process is facilitated by a rapid drop in pH at the start resulting from the sudden increase in propionic and acetic acids, followed by a subsequent increase and stabilization in pH, with a steady decrease in propionic and acetic acid concentrations until the formation stabilized. The data showed that metabolic activity and granule growth was dependent on the type and age of the anaerobic sludge used. An increase in granule formation (based on the increase in granule numbers) of 354%, 559%, 36% and 600% was found for the Glucose, Lactate, Petrochemical and Fruit batch units respectively. It was clear from the studies that a more reliable granule counting method must be developed and there has already been preliminary investigations into the use of image-analysis as an option. One advantage of this method is that the aggregation and disintegration of granules can be accurately measured and monitored;

3. The influence of multi-shock conditions, using standardized inoculum sizes, on the basic growth substrates showed that the characteristic pH, acetic and propionic acid profiles were obtained and a further enhancement of the granulation process (based on the increase in granule numbers) could be facilitated (400% - 1 000%). This confirmed the hypothesis that by changing environmental conditions on batch-scale, the propionic acid producers can be given a competitive advantage that leads to enhanced granulation. The results also showed that the response of the anaerobic consortium was strongly dependent on the specific carbon source and its concentration as well as a suitable nitrogen source and the necessary growth factors. A suitable environment was essential to prevent a too fast acidification situation. This was

also necessary to facilitate a suitable microbial recovery environment. One factor that was found to be extremely important was the condition (type, age and concentration) of the inoculum sludge.

4. An UASB bioreactor inoculated only with fresh anaerobic sludge results in a very extended start-up, stabilization time and biomass washout, when compared to the enhanced start-up of a bioreactor inoculated with batch grown granules. When used in the treatment of a canning industry effluent, this led to a COD removal of 89 - 93% at organic loadings of 9.8 - 10.95 $kgCOD.m^{-3}.d^{-1}$, at a HRT of 10 h and substrate pH of 5.5;

5. The data obtained also showed that when stress conditions are applied to an UASB bioreactor, an enhancement of granulation would occur. It was also found that the type and concentration of stress applied must be carefully chosen otherwise over-stressing could lead to an extended recovery period or even to total system failure; and

6. The use of batch cultivated granules as inoculum for the 50 l UASB pilot-plant led to the successful and efficient treatment of a full strength canning factory effluent. This was at a HRT of 10 h, with an average COD removal of 81 - 84% and removal rate of 7.5 $kgCOD.m.^{-3}.d^{-1}$ at an OLR of 9.2 $kgCOD.m^{-3}.d^{-1}$ and substrate pH of 5.0 with an effluent alkalinity level of 700 $mg.l^{-1}$ and an effluent pH of 6.5.

## RECOMMENDATIONS FOR FUTURE RESEARCH

In the study, several technical problems were also encountered that will have to be investigated:

1. The increase in granulation was determined by a direct counting of diluted samples. The method was not very efficient as the increase was seen as rather small and gritty granule-like particles, making a direct count very difficult. The development of a more practical method to determine the increase in terms of the numbers and size of the granules must be investigated;

2. A technique will also have to be developed to determine the increase in granular sludge in terms of a mass-increase without disturbing or disrupting the granule bed;

3. It was found that the characteristics of the anaerobic sludge used in the study differed from season to season and from digester to digester source. A suitable method of characterizing the sludge that will be used as inoculum has become essential; and

4. It is also important that a method for the determination of acidogenic and methanogenic granule activity be developed.

The successful cultivation of granules has important economic implications for the optimization of biological treatment processes in terms of improved sludge settlability, biomass retention and high loading rates. The promising results obtained in this investigation show that the separate culturing of granules hold a lot of promise for application of the UASB technology as a high-rate biological waste water treatment option. This study has also opened new avenues for investigation which include: the mass culturing of granules; analysis of the microbial consortium; use of the granulation model as a stable multi-level kinetic model; inclusion of selected microbes into the granules to enhance degradation; and the use of the granule consortia to produce value-added products from waste water.

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The Steering Committee responsible for this project consisted of the following persons:

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# CONTENTS

# GLOSSARY AND ABBREVIATIONS

## GLOSSARY

Acidogens – bacteria that depolymerize organic polymers, carbohydrates, proteins and lipids and ferment these to organic acids, alcohol's, hydrogen and carbon dioxide.

Aerobes – microorganisms whose growth requires the presence of air or free oxygen.

Anaerobes – microorganisms that grow in the absence of air or oxygen; organisms that do not use molecular oxygen in respiration.

Anaerobic digestion – a microbial fermentation of organic matter to methane and carbon dioxide that occurs in the near absence of air.

Anaerobic sludge - accumulated solids separated from waste water under conditions in which no free oxygen or nitrates are present.

Assimilation – the incorporation of nutrients into biomass of a microorganism.

Batch fermentation – a fermentation process that can run for a period, in which raw materials are supplied and products and microorganisms are removed in batch.

Biodegradable – a substance that can be broken down into smaller molecules by microorganisms or their enzymes.

Catabolic pathway – a degradative metabolic pathway in which molecules are broken down into smaller ones.

Chemical oxygen demand – the amount of oxygen required to completely oxidize the organic matter in an effluent sample.

Consortium – an interactive association between microorganisms that generally results in a combined metabolic activity.

Culture – to encourage the growth of particular microorganisms under controlled conditions; the growth of particular types of microbes on or within a medium as result of inoculation and incubation.

Domestic sewage – household liquid wastes.

Ecosystem – a functional self-supporting system that includes the organisms in a natural community and their environment.

Effluent – the liquid discharge from industrial sites or from digesters.

Fruit effluent – waste water obtained from the fruit canning industry.

Granules (flocs) – a mass of microbes cemented together in a slime or extracellular matrix produced by certain bacteria, usually found in waste treatment plants or specifically in upflow sludge blanket bioreactors.

Granule formation - in this study it refers only to the increase in countable granules and was not determined as an mass increase.

Growth rate – increase in the number of microorganisms per unit time.

Metabolite – product of a microbial biochemical activity, eg. propionic or acetic acids.

Metabolic productivity – refers to the production of volatile fatty acids by microbes after growth for a period in a specific carbon source.

Methanogens – methane-producing prokaryotes; a group of archaebacteria capable of reducing carbon dioxide or low-molecular-weight fatty acids to produce methane.

ABBREVIATIONS

| | |
|---|---|
| ADP | Adenosine diphosphate |
| ATP | Adenosine triphosphate |
| $CH_4$ | Methane |
| $CO_2$ | Carbon dioxide |
| COD | Chemical oxygen demand |
| GC | Gas chromatography |
| $H_2$ | Dimolecular hydrogen gas |
| HRT | Hydraulic retention time |
| TKN | Total Kjeldahl nitrogen |
| UASB | Upflow anaerobic sludge bed |
| VFA | Volatile fatty acids |

# CHAPTER 1

# INTRODUCTION

## 1.1 Water Situation

In South Africa the supply of water is at best limited and the average annual rainfall of 497 mm is well below the world average of 860 mm (Department of Water Affairs, 1986). The country's rainfall patterns account for long periods of drought, while the quality of the water sources is declining because of salinity, eutrophication and pollution (Republic of South Africa, 1991). Pollution is also a fundamental factor resulting in pressure on the country's water resources while the population has already exceeded 40 million (Weaver *et al.*, 1986). The expected rise in standard of living of a large part of the population and a higher demand for agricultural and industrial products will increase the demand for water. A 2 - 5% risk of undersupply for future demand was estimated from the year 1995 onwards (Department of Water Affairs, 1986).

To meet the increased demand for water, several courses of action will have to be taken (Bekker, 1982). Organic wastes from the manufacture of foods create serious problems because of their high organic load. Each step in the food industry system - food production, processing, transportation, storage, distribution and marketing has some impact on the environment, creating wastes of different quality and quantity, and thus having the potential to cause severe pollution problems (Kroyer, 1995). Anaerobic treatment, provides a biotechnological solution for industrial wastewaters (Iza *et al.*, 1991; Ross, 1989) and the process has been shown to be ideal for the treatment of a wide spectrum of organic effluents (Ditchfield, 1986). Anaerobic treatment involves the metabolic activity of a wide range of symbiotic microorganisms. Of these, the methanogens are the most significant group but are also the slowest growing population with generation times between 48 - 480 h (Ditchfield, 1986).

The direct treatment of waste waters was greatly stimulated by the development of the upflow anaerobic sludge blanket (UASB) process (Lettinga *et al.*, 1980; Weiland & Rozzi, 1991) and its successful full-scale application (Rinzema *et al.*, 1993). The UASB design containing granular sludge (Chynoweth, 1987), has permitted high space loading rates (30 $kgCOD.m^{-3}$) to be achieved at low hydraulic retention times (6 - 24 h) and this implies that smaller digester volumes are required (Iza *et al.*, 1991). It has now also been shown that even difficult degradable waste waters and those containing potentially toxic compounds, like pentachlorophenol, can be successfully treated with the UASB system (Hendriksen & Ahring, 1993). However, one of the main problems still remaining in the application of the UASB process, is the extensively long start-up periods (Goodwin *et al.*, 1992). With non-carbohydrate wastes, it can take a few months before a highly effective granular sludge blanket can be cultivated. Since the operational efficiency and performance of these systems are mainly dictated by the formation, amount and specific activity of the granules, the potential use of the UASB is limited by the extended start-up periods. The characteristics of the sludge, which develops in the blanket, are the

striking feature of the UASB system as the bacteria somehow bunch together to form the granules with a diameter of up to 5 mm.

## 1.2 Motivation

The mechanism of granule formation is still poorly understood (Slobodkin & Verstraete, 1993) and it would appear that granule sludges can only be formed with certain types of carbohydrate/protein containing waste waters generated in the agricultural and food processing industries. With other types of waste waters, granulation has not been possible (Sam-Soon *et al.*, 1991). In many cases, even after seeding the systems with granules it has been found that in time these have disintegrated or washed out (Sorensen *et al.*, 1991). This clearly restricts the general application, unless the granulation reaction can be induced. Full exploitation of this cell immobilization method can thus not be realized until the granule formation conditions are defined. The granulation process appears to be a unique type of bioflocculation, which is similar to an agglutination reaction as induced by polymers. Moosbrugger *et al.* (1992) concluded that the granules are formed by the generation of an extracellular polypeptide produced by a hydrogenotrophic methanogen of the genus *Methanobacterium*. In contrast, Vanderhaegen *et al.* (1992) found the granules rather to contain equal amounts of extracellular proteins and carbohydrates. According to Riedel & Britz (1993) and Slobodkin & Verstraete (1993), these compounds are produced by the propionate forming acidogens that are effective slime and aggregate formers.

During stable state, the anaerobic digestion process requires the concerted action of various microbial metabolic groups. Under these balanced operational conditions no lactate and very little propionate can be detected in the UASB digester. However, when 'stress' conditions are put on a digester treating carbohydrate rich waste waters, the first metabolite that appears is propionate (Myburg & Britz, 1993), while simultaneously, hydrogen can be detected in the gas phase and lactate starts to accumulate (Eng *et al.*, 1986). These metabolic changes result in a shift of the population dynamics of the anaerobic community. This was confirmed by Riedel & Britz (1993). Subsequently, slime producing and aggregate forming *Propionibacterium* strains can be isolated under these organic 'stress' conditions. We have found that under these unbalanced 'stress' conditions granule formation is stimulated. The increase in the lactate concentration, as a result of the unbalanced conditions, results in an orderly shift between the predominant lactate-utilizing bacteria, in response to the gradual decrease in the pH and increase in $H_2$ partial pressure. This possibly begins with the acid sensitive *Veillonella* and *Selenomonas* genera and then being superseded by the more acid tolerant *Propionibacterium*. These *Propionibacterium* strains gain a competitive advantage during the 'stress' condition, as they obtain a maximum of ATP per mol of lactate fermented. Once they have the advantage at the lower pH, they start producing extracellular compounds, with the subsequent formation of aggregates (Vanderhaegen *et al.*, 1993). The production of the extracellular polymers by the acidogenic bacteria (Slobodkin & Verstraete, 1993) could contribute directly to the formation of the highly settable granules found in efficient operating UASB digesters.

In order to shorten the start-up period of the UASB process, the need exists to stimulate the aggregation of microbes into granules. A faster UASB digester start-up period as well as the exploitation of the UASB technology in the treatment of difficult degradable waste waters will be of great value in conservation of our water resources. The results from this study will enable us to understand and optimize the process and therefore assist in managing the digestion process with maximum environmental pollution control.

## 1.3 Research Aims

The objective of this research programme will be to enhance granulation in UASB systems and promote a more rapid start-up procedure. This will be done:

Firstly, in a series of batch systems simulating UASB operating parameters, the granule growth will be monitored in terms of yield and metabolic activity. Environmental "stress" conditions will also be applied to the batch systems and the influence of these as granule stimulants will be evaluated. The best conditions for granulation will be identified and optimized;

Secondly, aim is to promote a faster start-up procedure in UASB laboratory-scale digesters using a synthetic and a food industry wastewater as treatment substrates. The induction of specific `stress' conditions and their influence as granule stimulants, will be evaluated. Changes in the environmental conditions will include: organic overloading; hydraulic overloading; changes in C:N:P ratios; as well as the addition of cysteine; and

Thirdly, based on the data obtained, a biological model will be constructed and evaluated on a 50 litre pilot-scale UASB bioreactor.

Chap2

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 BACKGROUND

Microbial methanogenesis is a natural process occurring in most anaerobic environments and is responsible for the overall degradation of organic compounds to biogas (Chynoweth, 1987). The biogas, produced as an end-product of the digestion process, consists of 50 - 70% methane ($CH_4$), 25 - 45% carbon dioxide ($CO_2$), small amounts of hydrogen, nitrogen, hydrogen sulphate and other minor compounds (Price, 1992). In 1881, the usefulness of the anaerobic process was recognized for the treatment of municipal waste waters. Since that time, the applications of anaerobic treatment have grown, as knowledge of the chemistry, biochemistry and microbiology of the process has increased. Interest in the methane by-product as a useful energy source is also growing (Chynoweth, 1987).

The first significant application of the process was in 1860 in France (Moigno, 1882), when M. Mouras liquefied organic waste water in an airtight chamber, called the "Mouras Automatic Scavenger". Other studies on liquefaction of waste water solids in the absence of air, were also conducted in England. Scott-Moncrieff, in 1890 and 1891 (Metcalf & Eddy, 1915), constructed a tank with an empty space below and a bed of stones above. It was the first application of an anaerobic filter and was probably done to retain the methanogenic organisms within the system.

Cameron constructed a tank similar to Mouras's tank for the treatment of 60 000 US gallons of waste water per day (McCarty, 1982). He called it a "septic tank". He developed the tank to a high degree and as a result the system was approved in 1897 for the treatment of the city of Exeter's waste water. The value of the methane gas was recognized by Cameron (McCarty, 1982) and was used for heating and lighting at the disposal works of Exeter, England. The effluents from these tanks were still black and offensive and aroused negative feelings towards the system. In 1899, Clark (McCarty, 1982) suggested that the sludge be fermented in a separate tank. In 1904, Travis (McCarty, 1982) put a two-stage process in operation at the Hampton Waste Water Disposal Plant. Problems with septic conditions in the effluent were however still experienced (McCarty, 1982).

In 1905 Imhoff (Metcalf & Eddy, 1915), the sewrage engineer of the Emscher Drainage District Board, modified the Travis tank (McCarty, 1982) by retaining the sludge in the digester chamber and then disposing it when it was considered to be "inoffensive". In 1927, the Rührverband (Imhoff, 1938) installed a clarification plant at Essen-Rellinghausen with sludge-heating in a separate tank. It proved to be very efficient and grew in popularity. In 1934, 600 000 Germans were served by septic tanks, 6 500 000 by Imhoff tanks and 5 600 000 by separate sludge digestion units (McCarty, 1982). The heating of the tanks was facilitated by re-using the biogas produced during the process.

The first reported observation on anaerobic degradation was made by Volta in 1776 (Barker, 1956), when it was observed that "combustible air" was formed in lakes, ponds and streams (McCarty, 1982). In 1856, Reiset (Buswell & Hatfield,

1938), identified this combustible air to be methane, while Bechamp (Barker, 1956) gave the first indication that this gas was formed during a microbiological process in 1868.

Omelianski in 1890 (Buswell & Hatfield, 1938) did the first so-called classical studies on the methane fermentation. He isolated the organisms producing hydrogen, acetic and butyric acids. He also reported the formation of methane from hydrogen and carbon dioxide and formulated the following reaction: $4H_2 + CO_2 = CH_4 + 2H_2O$ (McCarty, 1982). Shöngen (1910) confirmed Omelianski's findings and reported that the fermentation was via oxidation-reduction reactions and that methane is formed via a decarboxylation reaction from acetic acid. These findings were of significance in the control of the anaerobic digestion process. In 1916, Imhoff (Imhoff, 1938) was the first to use the terms of "acid digestion" and "methane digestion". From 1920 to 1930, Buswell (Buswell & Hatfield, 1938) showed the importance of volatile organic acids as intermediates in the process which led to a better understanding of process control. In 1948, Söhngen's original suggestion that methane is formed from $CO_2$ reduction and acetate decarboxylation, became the accepted theory (McCarty, 1982).

The isolation of the methane producing microbial group was found to be very difficult, as a result of their extreme sensitivity to oxygen and very slow replication rate. The breakthrough came when Barker (1940) isolated *Methanobacterium omelianski*, which oxidised ethanol to acetate and methane. In 1967, Bryant (Bryant *et al.*, 1967) reported that the methanogenic bacteria rather contained two species and not just one. They demonstrated clearly that one converted ethanol to acetate and hydrogen and the other $CO_2$ and $H_2$ to $CH_4$. Today it is generally accepted that four groups of bacteria are involved in the anaerobic digestion process (McCarty, 1982).

From 1950 to 1980, many process developments for the enhancement of the anaerobic treatment process were made. In 1950, mixing was introduced to the bioreactors and the anaerobic contact process was developed (McCarty, 1982). Before 1959, no mixing was used in the tanks and scum-layers developed which reduced the capacity of the digester. Mixing overcame the problem. Stander (1950), recognized the value of keeping large populations of bacteria in the methane-producing reactor. The solid retention time (SRT) of the waste water in the bioreactors varied from 20-30 days to just less than a year. Several processes were developed including the clarigester process, the contact process, two-phase concept, anaerobic filter, Upflow Anaerobic Sludge Blanket, expanded-bed and the fluidized-bed process (Ross, 1989).

## 2.2 THE UASB PROCESS

In 1972, the Upflow Anaerobic Sludge Blanket (UASB) process was developed. It was a move towards a more efficient and stable anaerobic process. Low-strength waste waters with 3 - 4 h retention times were treated (Lettinga *et al.*, 1980). From 1974 to 1977, three UASB pilot-plants with working volumes of 6, 30 and 200 $m^3$ were constructed in the Netherlands. These studies showed that the process was able to handle chemical oxygen demand (COD) loads of 15 - 40 $kg.d^{-1}$ at 3 - 8 h

retention times. In 1978, a full-scale UASB reactor with a working volume of 800 $m^3$ was constructed for the treatment of beet sugar waste water (Lettinga *et al.*, 1980). An 88% COD removal was achieved under organic loading of 16.25 $kg.m^{-3}$ per day. Since then, many full-scale UASB reactors have been installed around the world for the treatment of various types of wastes. Waste waters from sugar, potato processing, brewery, winery, alcohol distillery, slaughterhouse, meat packing, paper mill, soft drink, food starch and yeast industries have been treated successfully (Lin & Yan, 1991).

Up to 1988, 82 full-scale UASB reactors were constructed around the world (Ross, 1991). In Europe and Canada, the UASB system is generally known as the commercial BIOPAQ system. By October 1991, 106 full-scale BIOPAQ UASB plants were in operation throughout the world (Ross, 1991). In Holland, the leader in anaerobic digestion is PAQUES (Ross, 1991). Today 451 UASB plants are in operation, with 225 being manufactured by PAQUES (John West, Bio-Water Pty. Ltd. - personal communication, Ross 1991 ). Most full scale reactors can treat organic loading of 5-18 $kg.m^{-3}.d^{-1}$. Peak loadings of up to 30 - 40 $kg.m^{-3}.d^{-1}$ can also be achieved, especially in the sugar industry. The UASB process presents a stable and effective treatment method (Lin & Yan, 1991). High methane production rates and yields are achieved.

Favourable environmental factors are necessary for anaerobic digestion. Temperature, pH, volatile acids, alkalinity, grease, fibre and toxic substances all play important roles in digestion efficiency. Most digesters operate in the mesophilic range between 20° and 45°C (Price, 1992) and the optimum pH range for anaerobic digestion is between 6.5 and 8.0 (Fannin & Biljetina, 1987). Failure during the anaerobic digestion of industrial waste water is often ascribed by the sensitivity of the methanogenic bacteria to chemicals such as ammonia, fatty acids and heavy metals (Kroecker *et al.*, 1979). Inhibition of anaerobic digestion by heavy metals was found to decrease in the sequence Ni>Cu>Cd>Cr>Pb. Factors like high ammonia concentrations, finely dispersed solids, low pH and organic compounds like organic solvents, pesticides, alcohols and high concentrations of long chain fatty acids, can all be inhibitory to the anaerobic digestion process (Price, 1992). The process thus needs to be monitored daily.

The UASB design concept is based on the upward movement of soluble organic feeds through a blanket of bio-solids consisting primarily of microorganisms. The "sludge bed" in the UASB is a layer of biomass settled at the bottom of the bioreactor. This layer is responsible for 80 - 90% of the degradation of the waste water and occupies 30% of the reactor volume. The "sludge blanket", in contrast, is a suspension of sludge particles and microbial biomass mixed with gas produced in the process and occupies 70% of the reactor volume. Waste water enters the bottom of the reactor and is degraded in both the sludge bed and sludge blanket (Lin & Yan, 1991). Gas entrapped in the sludge granules often forces them to travel to the upper section of the reactor because of their reduced density (Annachhatre, 1996). This creates a need for efficient separation of the sludge granules, treated effluent and gas. This is achieved through the use of a gas-solid-separator in the top of the reactor. Granules are returned to the reactor, while the treated effluent is discharged.

The main reasons for the increasing popularity of this system are due to the ability of the UASB to handle high organic loading rates and to reduce the chemical

oxygen demand (COD) of waste waters efficiently and economically in a short time. In addition, biogas is produced, which can be used as an energy source (Quarmby & Forster, 1995). 1 kg COD can yield 355 litre $CH_4$, which is equal to 14 132 kJ energy (Britz, US, 1996, personal communication).

The UASB process differs from other anaerobic treatment systems in that no added compounds or carrier material are required for the biomass to adhere to. There are no support systems within the reactor for bacterial attachment and biofilm formation, only a gas-solids separator at the top of the reactor to separate the biogas from the sludge (Quarmby & Forster, 1995).

## 2.3 GRANULES

The successful operation of UASB bioreactors are thus dependant on the spontaneous formation of granular methanogenic sludge with high settling velocities and high methanogenic activity (Fukuzaki *et al.*, 1995). The aggregates produced, referred to as bacterial granules, are an essential feature of the UASB process and show excellent settling properties because of their large size (MacLeod *et al.*, 1995). The adhesion of the bacteria into settable granules causes them to stay in a nutritionally favourable environment, even though no inert carrier material is available. The upflow velocity in the UASB reactor causes the organisms to adhere to each other and these are then exposed to the continual supply of nutrients (MacLeod *et al.*, 1990). The granular sludge has the advantages of settling well under favourable environmental conditions, are extremely stable and can withstand high mixing forces making their use of significant importance (Lettinga *et al.*, 1980). Ideally, the active biomass must be able to operate at high organic loading rates and short hydraulic retention times (Quarmby & Forster, 1995). Furthermore, their compact nature ensures that these granules can withstand the high hydraulic shear caused by the upward flow of the waste water.

The stability of the granules is based upon the firm aggregation of the cells and the self-immobilisation of various suitable types of bacteria associated with the methanogenic fermentation (Fukuzaki *et al.*, 1995). The granular sludge can be described as a spherical biofilm consisting of a densely packed anaerobic microbial consortium (Forster & Quarmby, 1995). No space is lost for inert supports so that the granule provides a maximum microorganism-to-space ratio (Guiot *et al.*, 1992). The densities of the populations in the granules vary widely, depending on the chemical composition of the waste water and the operational conditions of the bioreactor (Forster & Quarmby, 1995). The actual microbial composition of the granulated activated sludge is a result of the strong competition for energy, carbon sources and nutrients (Wanner, 1994).

As previously stated, one of the major problems when operating an UASB system is the long start-up times as a result of the slow process of granulation. An understanding of the mechanisms which govern granule formation and stability during the operation of a UASB is thus of considerable significance (Forster & Quarmby, 1995). The anaerobic sludge, in time, obtains and maintains superior settling characteristics if environmental conditions favourable to sludge flocculation and maintenance are provided (Lettinga *et al.*, 1980). Many environmental factors

affect granule formation. Different concentrations of nutrients were found to affect granulation in different ways. Carbon, nitrogen and phosphorus are important ingredients for biomass growth (Annachhatre, 1996). A COD/N/P ratio of 300:5:1 is recommended. Morgan *et al.* (1991b) reported that calcium ion concentrations of up to 158 $mg.l^{-1}$ were found to enhance granulation. When calcium (80 $mg.l^{-1}$) and phosphate (192 $mg.l^{-1}$) were both added to the feed, more stable granules were produced. Of the trace elements manganese, cobalt and iron ($Fe^{+2}$) were also found to be growth stimulators. Nickel was the most stimulating. Different pH values result in changes of the physical characteristics of granules, including size, density and volatile solids content. A slightly acid to neutral pH (6.5 - 7.8) was found to favour optimal granule growth. In contrast, a number of factors are strongly detrimental to granular sludge growth. High levels of proteins, $NH4^{+}$ and ammonium concentrations between 1 500 and 3 000 $mg.l^{-1}$ at a pH higher than 7.4, inhibit granular growth. pH levels below 6.0 can become toxic to methanogenic bacteria. High levels of free suspended solids either interfere with the granular formation or lead to gradual filling up of the sludge-bed with inactive particulate (Vanderhaegen *et al.*, 1992). The nature of the substrate, seed sludge and start-up conditions have all been found to cause different types of sludges (Forster & Quarmby, 1995).

Granules can be as large as 5 mm in diameter, but are generally smaller (Quarmby & Forster, 1995). Substrate type and composition is important for granule colour. Granules are usually black but granules from papermill and wheat starch effluents are large and grey-white. Granules from molasses effluents are reported to be small and gritty, while coffee waste waters give less dense light brown granules.

Nutrient deficiency around densely packed cells at low loading rates also results in granule changes and white and grey types form. Kosaric *et al.* (1990) reported that sulphur and iron had an influence on the colour. High concentrations of iron resulted in small, black granules, while higher concentrations of sulphur resulted first in grey and later in whiter granules.

Kosaric *et al.* (1990) made an interesting observation on the colours of the granules in an UASB reactor. They found that granules can change composition at different organic loading rates. At low organic loading rates of 0.65 g COD, the granules became soft and grey or white. A hollow core develops which can entrap gas and cause the granule to float to the top of the reactor. At high organic loading rates of 1.60 g COD, the granules stayed black and seldom formed hollows. The hollow appears to develop when the granule reaches a certain size. The substrate concentration in the granule becomes too low to serve as energy source for all the bacteria and autolysis of the bacteria occurs towards the centres of these granules. Gas can be entrapped, the granule floats to the top and is washed out of the system, with a resulting loss of granules.

The methanogenic consortium in the granule are chemically comparable to bacteria in general. Inorganic mineral content (or ash) of the granule is typically 10 - 29%, but an ash content of up to 73% has been reported (Forster & Quarmby, 1995). The ash content depends on the waste water composition and process conditions (Schmidt & Ahring, 1996). Under mesophilic conditions, granules grown on complex waste water had a lower ash content than granules grown on simple substrates like acetate, propionate and butyrate. Thermophilic conditions resulted in higher ash contents. Fukuzaki *et al.* (1995) made a comparison between granules grown on different substrates. They found an ash content of 56-63% in granules grown on a

butyrate-propionate mixture. Granules grown on starch, sucrose and ethanol had ash contents between 24 and 40%. The major components of the ash were calcium (28 - 32%), phosphorus (18 - 21%), magnesium (3 - 4%), sodium (2 - 3%), potassium (0.5 - 1%) and trace elements such as Fe, Ni, Co (0.4 - 0.6%). Accumulation of inorganic salts is thought to be stimulated under alkaline conditions caused by the bioconversion of metabolic fatty acids to $CH_4$. Granules grown under slightly acidic conditions had a lower ash content. Protein content was found to be 35 - 60%. Total organic carbon was 41 - 47%, while Kjeldahl nitrogen was 10 - 15%.

Forster & Quarmby (1995) found that 30% of the ash contained FeS, which resulted in the typical black colour of granules. High levels of calcium have also been found in the core of the granules. Aluminium and sulphur silicon were found only at the surface of the granules. Precipitated or insoluble inorganic compounds like sulphides and silicates are part of the sludge structures.

Total number of bacteria by direct counting gave typical cell concentrations of $5 \times 10^{10}$ to $1.4 \times 10^{12}$ cells.ml$^{-1}$ of granules (Schmidt & Ahring, 1996). The number of bacteria was confirmed using staining techniques or transmission electron microscopy (TEM).

## 2.4 EXTRACELLULAR POLYMER PRESENCE, FUNCTION AND PRODUCTION IN GRANULES

Many microorganisms excrete high molecular weight polymers (molecular weight > 10 000), which may be retained as part of the cell structure or may be released into the environment. The extracellular polymers (ECP) can be rigid, flexible, tightly or loosely bound to the cell (Beech & Gaylarde, 1991). ECP is generally excreted by microbial cells under favourable conditions. Substrate plays an important role in ECP excretion but the amount of ECP excreted depends on the composition of the waste water. An increase in the C:N ratio of the substrate stimulates the production of ECP (Forster, 1994). Fukuzaki *et al.* (1995) found that starch and sucrose grown granules were large with abundant ECP. Ethanol and fatty acid grown granules produced only the minimum amount of ECP required for granulation.

Schmidt & Ahring (1994a) extracted and analysed ECP from different granules grown on substrates of acetate, volatile fatty acids, sugar, paper mill and fish meal waste water. The highest protein content of 5.1 mg.l$^{-1}$ was found in the ECP of granules grown on acetate and other volatile fatty acids. The granules from simple substrates like acetate, propionate and butyrate had a higher lipid ECP than granules grown on complex substrates such as sugar, papermill and fish meal waste waters.

The main components of ECP (Table 1) have been found to be made up of proteins and carbohydrates in a 1:3 ratio (Quarmby & Forster, 1995). The carbohydrates include glucose, galactose, mannose and rhamnose. The proteins can be made up of asparagine, glutamine and alanine. The remaining components include lipopolysaccharides, RNA, DNA and inorganic molecules.

The two dominating elements detected by EDAX (Morgan *et al.*, 1990) in ECP were found to be phosphorous and calcium, which constituted of up to 85% of all elements. Calcium is ascribed to be important in the linking of polymers. Na, Mg, Al,

Si, S, Cl, K and Fe were also present. The relative concentration of each metal is dependent on its concentration in the feed. Iron may play an important role in the formation of ECP by binding cysteine and causing the formation and excretion of extracellular polymers (Shen *et al.*, 1993). Mesophilic conditions resulted in a higher amount of ECP. Quarmby & Forster (1995) found that granules tended to become weaker as the organic load increased and that ECP from weak granules had a lower polysaccharide content, 1 - 4% of total solids in granular sludge consist of ECP.

The type of bacterial species has a strong influence on ECP excretion. The main ECP producers are found to be the acidogens, rather than the methanogens (Forster & Quarmby, 1995). Racine *et al.* (1991) studied the production of polysaccharide by *Propionibacterium* on a whey-based medium. They found that the polysaccharide production started after 20 - 40 h growth and reached a stationary phase after approximately 100 h. In some cases, a decrease in the amount of polysaccharide was observed at the end of the fermentation. If the growth conditions of the organism are stressed it may promote polysaccharide formation.

**Table 1.** Composition of extracellular polymers extracted from different types of granules.

| Author | Content |
|---|---|
| Grotenhuis *et al.* (1991) | 3.5 mg polysaccharide per gram<br>5.5 mg protein per gram |
| Bhatti *et al.* (1995) | 1.7 - 2.7% carbohydrate of TSS<br>2.5 - 0.1% nucleic acids of TSS<br>8.3 - 6.3% proteins of TSS |
| Schmidt & Ahring (1994) | 5.1 : 8.3 protein : polysaccharides ($g.vss^{-1}$) |
| Forster & Quarmby (1995) | 3-5 : 1 protein:carbohydrate<br>(100 gram ECP) |
| Morgan *et al.* (1990) | 1-2 : 1 protein : carbohydrate per g SS (amino acids) |
| Vanderhaegen *et al.* (1992) | Equal amounts of extracellular proteins and carbohydrates |

More polysaccharide was produced at 25°C - a temperature slightly lower than optimal growth temperature. Polysaccharide production also increased when the culture medium was low in nitrogen and rich in carbon. Optimal pH for maximum polysaccharide production was close to 6.0. Phosphate had an enhancing effect on polysaccharide production. Petrovic *et al.* (1989) did microscopical studies on activated sludge from municipal waste water. All the microorganisms present showed a high production of extracellular material. Extracellular material formed a dense, homogeneous covering or a cobweb-like matrix in some cases.

ECP has a number of functions depending on the specific microorganism. It protects the organisms against phages and traps soluble nutrients. However, the main function of ECP within the granule is thought to be linked with the formation of bacterial aggregates and as the mediator in cell to cell adhesion (Forster & Quarmby, 1995). The high molecular weight polymers bind electrostatically and physically to microbial cell surfaces and are able to bridge between different microbial surfaces to form a three-dimensional floc matrix. MacLeod *et al.* (1995) found ECP to form thick, dense structures around bacterial cells that often completely filled the intercellular spaces in the granule structure.

ECP is ideal for permanent adhesion. The fibrous nature ensures that all the fibres would not desorb at the same time (MacLeod *et al.*, 1995). A network is formed for microbes to settle in (Forster *et al.*, 1995). The amount of ECP is one of the factors which can influence the stability of granules. As the amount of ECP in the granules increase, flocs become more stable and resistant to break-up, although excessive granular growth with abundant ECP formation inhibits gas release and a fluffy appearance occurs (Fukuzaki *et al.*, 1995). Too much ECP can cause deterioration in floc formation as ECP has a negative charge and, therefore, repulsion can occur. Floc strength is also influenced by waste water composition and physical, biological and biochemical factors (Forster & Quarmby, 1995).

Many attempts to extract and quantify sludge biopolymers have been made because of their role in bioflocculation (Grotenhuis *et al.*, 1991; MacLeod *et al.*, 1995; Morgan *et al.*, 1990). These techniques include steaming, centrifugal stripping, alkali stripping and ethanolic extraction. A comparison of the efficiency of these techniques shows a considerable variation, so that no standard method exists to extract biopolymers. Comparative studies can be used if the technique is initially standardised.

## 2.5 GRANULE STRUCTURE

Anaerobic degradation in granules is a multi-step process (Fang *et al.*, 1995b). Complex organic substrates like proteins and carbohydrates are first hydrolysed by enzymes to form soluble amino acids and sugars. These are then degraded by acidogenic bacteria into volatile fatty acids, which are then further degraded by acetogenic bacteria to form acetate, formate, carbon dioxide and hydrogen. In the final step these intermediates are converted to methane.

The rate of each step depends on the concentration of the reactants, the bacterial species and factors like pH and temperature (Fang *et al.*, 1995b). In cases

where the initial step of substrate degradation is faster than the intermediate steps, most of the substrate will be consumed at the granule surface. Intermediate concentrations will thus build up, and cause them to diffuse towards the interior of the granule. A layered structure will thus develop where the outer layer is responsible for the rapid degradation of initial substrate, while the rest of the layers are responsible for the degradation of the intermediates. The thickness of the outer layer is dependent on the complexity of the carbohydrate. The more complex the substrate, the thicker the outer layer. With only one intermediate forming, as in the case of butyrate being degraded to only acetate, a granule with only two layers will develop. Biogranules treating complex carbohydrates like starch and brewery waste waters will develop well defined three-layered microstructures. When the initial degradation is slow in relation to the intermediates, a complex, uniform structure will develop. Simple substrates converted by a one-step process will lead to a simple, uniform structure (Fang *et al.*, 1995b).

The microstructure of biogranules can thus be either layered or uniform (Fang *et al.*, 1995b). The layered granules can be either two- or three-layered, while the uniform granules can be either simple or complex with intertwined bacteria.

MacLeod *et al.* (1990) examined granules obtained from a mesophilic reactor, which was fed over a 30 d period with a sucrose substrate. Their findings showed that these granules were irregular spheres exhibiting a three-layered structure and that each layer had a characteristic morphology. The external layer was approximately 10-20 µm thick and had a large diversity of bacterial morphotypes on the surface. The population included chain forming cocci (0.7 micrometer in diameter), large rods (1.0 by 2.0 micrometer), long thin filaments (0.27 micrometer in width) and small rods and cocci (smaller than 1.0 micrometer). Large numbers of clustering cocci, resembling the order *Methanococcales,* were also observed. A few chain-forming rods, similar to those of the *Methanothrix* species, were occasionally observed on the surface. Gram-positive and gram-negative cell wall structures were observed. ECP was found to form a structural matrix around the diverse variety of morphotypes present in the granule surface. Sections obtained from a depth of approximately 20 micrometer contained regions in which two or three species were juxtaposed. At a depth of 50 - 100 micrometers, the bacteria predominantly exhibited one shape. The cells were rod shaped, with a width of 0.4 to 0.5 micrometer and an average length of 2.5 micrometer. Short chains of two or three cells were sometimes observed. The cells had an angular packing. The cell structures were characteristic of the genus *Methanothrix*. The cell membranes were bordered by a thin inner wall and an amorphous layer separated the inner wall from the outer wall. Cells in the filaments were separated from each other by septa.

The interior of the granules consisted of a central core of cavities surrounded by several distinct layers of bacteria (MacLeod *et al.*, 1990). Gas spaces separated the surface layer from the underlying one. Thin filamentous organisms similar to *Methanospirillum* species, acted as bridging species in these spaces. The second layer consisted of a tightly packed bacterial structure embedded in extracellular polymer and was about 10-20 µm thick. The bacteria were predominantly rod-shaped. An electron-dense organism resembling a *Methanobrevibacter* species and a larger, shorter rod resembling *Syntrophobacter* species were among these organisms. The third layer consisted of large micro-colonies of almost exclusively angular-shaped rods with a structure similar to those of the *Methanothrix* spp. These

bacteria were surrounded by an extracellular polymer and formed the walls of the cavities in the core of the granules. These cavities are most probably the sites of vigorous gas production in the granules.

Fang *et al.* (1995d) also studied the characteristics of granules, but those grown on waste waters containing concentrated mixed volatile fatty acids. These were mostly acetate, propionate and butyrate in a 2:1:1 ratio. The granules that formed were 1 - 2 mm in size and settled well. In this case no patterned structure was found and the granules had a fluffy surface of interwoven filamentous *Methanothrix* type bacteria. *Methanothrix* is an acetoclastic bacterium, which can use acetate as sole substrate. It outcompetes other methanogenic bacteria, such as *Methanosarcina*, when the acetate concentration is low, as is possibly found in the granule interior. The *Methanothrix* group appeared to be a key structural element in all the granules. These bacteria are dominant in the granule core, with few colonies in the middle and outer layers. In granules with no layered structure, a *Methanothrix* network was found throughout the granule and it was thus concluded that *Methanothrix* filaments probably play an important role in sludge granulation (Fang *et al.*, 1994d).

Fang *et al.* (1995a) also studied the degradation of butyrate in an UASB reactor. In this case they found the granules to be 1 - 2 mm in size with a simple structure consisting of a densely packed outer layer and an interior of mainly *Methanothrix*-like bacteria. These granules settled well. Ruptures were present in the outer layers of a large number of the granules. This was very unlike UASB granules. Through the ruptures, the *Methanothrix* bacteria of the interior were exposed. These bacteria had a bamboo shape when observed by TEM and the filaments were entwined into rope-shaped aggregates.

The microstructure of granules from systems treating sucrose, glutamate and brewery waste waters were found to be dependent on the nature of the substrate (Fang *et al.*, 1994b). A typical sucrose-degrading granule was 1 - 2 mm in size and had a dense outer layer with a loosely packed interior. Methanogens were distributed throughout the granule. In the outer layer they were of diverse morphologies, including cocci, bacilli and some filaments, while those in the interior were filamentous *Methanothrix*.

Chui & Fang (1992) studied the microstructure of over a 100 granules from UASB reactors also treating sucrose wastewater. They reported the granules to have a dense surface layer with a 20 - 40 micrometer thickness and a loosely packed interior. The surface layer consisted of a variety of hydrogen consuming methanogenic cocci and bacilli, while the interior was mainly composed of *Methanothrix*-like bacteria. It was concluded that knowledge on the microstructure of UASB granules could be important for the basic understanding of the granulation mechanism and its effect on settleability (Chui & Fang, 1992).

Guiot *et al.* (1992) confirmed the multilayer structure model of granules by MacLeod *et al.* (1990) and found the syntrophic bacterial associations to be located between an external acidogenic layer and an acetoclastic core. Studies on granules fed on a sucrose medium clearly showed a three-layered structure with each layer possessing different distinguishing bacterial morphotypes. The external layer contained a variety of organisms including acidogen morphotypes, *Methanococcales* and *Methanospirillum*-like organisms. The middle layer consisted of a large number of cocci and rod-shaped bacteria, resembling *Methanobrevibacter* species which

were juxtaposed to *Syntrophobacter*-like organisms. The central layer or core had cavities surrounded by rod-shaped bacteria with flat ends. They resembled bacteria of the species *Methanosaeta* (*Methanothrix*).

Guiot *et al.* (1992) observed a change in the specific activities coupled with a change in the size of the granules. Acidogenic activity was found to be higher in suspended sludge, flocs and small particles, while acetoclastic activities were higher in larger granules. The outer layer of a small particle takes up a greater proportion of the whole volume than in large particles, so does the specific activity corresponding to the dominant organisms in that layer i.e. the acidogens. Granule development results from a dynamic equilibrium between biofilm being sloughed off and growth of organisms in the granule. Acetoclastic organisms are thus sheltered from detachment in the inner space of the granule. This leads to an increase of specific activity of acetoclastic organisms in larger granules.

Granules from brewery waste water were 2 - 4 mm in size and showed a complex layered structure (Fang *et al.*, 1995c). In this case methanogens were distributed in the three layers. The outer layer included cocci, bacilli and some colonies of *Methanothrix* and *Methanosarcina* but most of the bacteria in this layer were acidogens. The middle layer was dominated by syntrophic microcolonies with scattered *Methanothrix* colonies. The core was packed with rod-shaped *Methanothrix*. The typical glutamate degrading granules were 0.5 - 1.5 mm in size and showed no layered structure, but consisted of a network of *Methanothrix* with packets of non-methanogenic bacilli throughout. The presence of the layered structure in the granules was limited to granules treating soluble carbohydrate rich substrates, like sucrose, glucose and brewery waste waters. No layered structure was observed for granules treating non-carbohydrate substrates (Fang *et al.*, 1994b).

Propionate and butyrate are important intermediates in the degradation of carbohydrates (Fang *et al.*, 1994a). The degradation of propionate and butyrate are thermodynamically unfavourable, except if hydrogen and acetate concentrations are at low levels. The syntrophic acetogens thus have to be in the close vicinity of hydrogen and acetate consuming methanogens. This syntrophic relationship is enhanced if the bacteria are in juxtaposition, so that the diffusion distance for the metabolites is minimised. For the granules used for treating brewery wastewater, two juxtapositioned syntrophic microcolonies were observed (Fang *et al.*, 1995c). They consisted of hydrogen producing acetogens and hydrogen consuming methanogens.

In the cases where proteins or amino acids like glutamate are degraded, acidogenesis is the rate limiting step. Glutamate, because of its slow degradation and molecular diffusion, will be evenly distributed throughout the granule, as will the other metabolites like volatile fatty acids, acetate and hydrogen. Bacteria are then rather evenly distributed throughout the granule and no layered structure is found (Fang *et al.*, 1994a).

The *Methanothrix* bacteria are a key structural element in all the granules. These bacteria are the dominant bacteria in the granule core, with few colonies in the middle and outer layers. In granules with no layered structure, a *Methanothrix* network was found throughout the granule (Fang *et al.*, 1994b). Thus, it appears that *Methanothrix* filaments play an important role in sludge granulation. Granules that were grown on complex carbohydrate effluents (Fang *et al.*, 1994b) included a

wide variety of bacterial trophic groups like acidogenic fermenters, sulphate reducers, proton reducing acetogens, homoacetogens, hydrogenophilic and acetoclastic methanogens.

Arcand *et al.* (1994) stripped granules of their surface bacteria and found a significant downward gradient of acidogenic activity, with a clear predominance of fermentative bacteria in the external layer of the granules. They developed an intragranular model to predict substance and biomass concentrations at any depth within the granule. Predictions were made that acidogens constitute more than 40% of the total biomass of the surface layer while less than 5% of the surface biomass was made up of *Methanothrix* type bacteria. Immediately beneath this layer, 30% were found to be hydrogenotrophic microorganisms and 20% *Methanosarcina*-like organisms. At a depth of 1 mm and more, 85% of the population consisted of *Methanosaetea* bacteria, which were constant towards the centre of the granule.

The significant upward pH gradient could be the major factor for selecting a *Methanothrix* culture in the granule core. While a pH of over 7.5 is probably unfavourable for most of the bacterial species, *Methanothrix* species can still grow at pH 8.3. The unionized form of acetic acid and pH are interdependent and an upward pH gradient creates a downward unionised acetic acid gradient, which is the substrate for methanogens. Because of their higher affinity for acetic acid, *Methanothrix* species outcompete other methanogens at low acetic acid concentrations. A pH close to eight makes the granule core the most active zone for *Methanothrix* species (Arcand *et al.*, 1994).

Fang *et al.* (1994b) studied the characteristics of granules grown on hydrolysed proteins. They found granules between 1 - 2 mm in size, which settled satisfactorily. The granule surface had a coarse and loosely packed structure with multiple cracks. No layered structure or dominant bacteria were found in the granules and under the porous surface, the granules had a densely packed structure of intertwined bacteria with different morphologies. Cocci, filaments, bacilli, sarcina and spirochetes were found. The complex nature of protein degradation requires a large number of different microorganisms. *Spirochetes*, *Methanosarcina* and *Methanothrix* were distributed throughout the granule and *Syntrophobacter*-like bacteria and *Methanospirillum* species were found in juxtapositioned associations.

In 1994, Chui *et al.* studied the removal of formate from waste water with the UASB process. They found small granules (0.5 mm or less) of irregular shape that settled well. The dominant bacteria in this case were found to be the filamentous rod-shaped *Methanobacterium formicicum* that has the ability to convert formate to methane. Another formate degrading bacterium, *Methanococcus vannielli*, with an irregular shape, was also identified.

Quarmby & Forster (1995) studied a wide variety of waste waters and made a comparison between their surface and internal structures. The nature of the substrate has been found to yield different types of sludges. Potato, sugar beet and volatile fatty acid (VFA) feeds gave rod shaped bacteria. In pure VFA feeds, filamentous species dominated, while maize-starch gave uniform "spiky" granules with a 60% calcium carbonate content. Species of the methanogens *Methanobacterium, Methanospirillum* and *Methanosarcina* are frequently found in granules, but the filamentous methanogen *Methanothrix* is especially dominant. Most of the granular samples appeared to be compact with a few craterous pores, possibly for the release of gasses produced.

Coffee-fed samples had a rippled surface (Quarmby & Forster, 1995). The microstructure of the granules was found to be dependent on the substrate, but other factors like pH, temperature, organic loading rate and COD must also be taken into consideration. Granules fed on potato wastes displayed a three-layered structure. The surface layer was heterogeneous, with a second tightly packed layer of many types of rods followed by a central layer of *Methanothrix*-like organisms and cavities. The heterogeneous outer section became more homogeneous towards the centre and consisted of tightly packed bacteria.

Wheat-starch and papermill samples, as studied by Quarmby & Forster (1995), had no distinct three-layered structures, rather very thin bands which formed a honeycomb pattern. In this study sucrose-degrading granules were also found to have two layers, a dense outer layer of diverse morphologies surrounding a loosely packed inner centre which contained mostly *Methanothrix*. Granules from a brewery sample had three layers. The outer layer had many morphological types of bacteria including a few colonies of *Methanosarcina* and *Methanothrix*. The middle layer was dominated by synthrophic microcolonies with a few *Methanothrix* colonies, while the inner core was densely packed with *Methanothrix* rods. Glutamate-degrading granules did not exhibit any layered structure, instead a network of *Methanothrix* and non-methanogenic colonies were scattered throughout the granules. In granules fed on a pure VFA feed, the majority of organisms were filamentous, while with mixed VFA a high percentage of rods were found.

Most authors working on granule structure found that in the "typical" granule, more Gram-positive bacteria occupied the outer region, surrounding an increasing number of Gram-negative organisms towards the interior of the granule. In granules grown on wheat starch an interior of more than 90% Gram-negative bacteria were found (Quarmby & Forster, 1995). Protein and lipid was widespread throughout the granule. The amount of carbohydrate was seen to decrease slightly in the more central sections, whereas the areas which were positively stained for protein increased in size. Stronger granules were associated with a higher percentage of carbohydrate. Gram-positive organisms were found to be extremely rare (less than 5% of the total bacteria present) in granules (Quarmby & Forster, 1995). Most of the bacteria in granules, including the filamentous bundles, gave negative reactions when stained with the Gram-stain. Among the bacteria which give a positive response are the rod-shaped bacteria (Morgan *et al.*, 1991a). Daffonchio *et al.* (1995) did studies on the cell hydrophobicity of granular sludge and found the acidogens to be hydrophylic, while the acetogens and methanogens were hydrophobic.

Grotenhuis *et al.* (1991) studied the ultrastructure of mesophilic granular methanogenic sludge treating waste water from a sugar plant and of granules adapted to ethanol and propionate. They found that in the propionate-grown sludge, *Methanothrix* and *Methanothrix arboriphilus* clustered together with propionate-oxidising bacteria throughout the whole granule. The bacteriological structure of the propionate-grown granules did not confirm the layered structure predicted by MacLeod *et al.* (1990).

Granules grown on a synthetic phenol medium were spherical with numerous cavities (Chang *et al.*, 1995). The granules were small, ranging between 0.61 and 0.77 mm in diameter. Filamentous organisms resembling *Methanothrix* were found on the surface as well as in the centre of the granules.

Morgan *et al.* (1991a) examined granules grown on paper-mill and sugar waste waters and found the dominant bacteria to be *Methanothrix*. The organisms occurred as single cells and filaments of varying lengths, orientated randomly throughout the granules. Towards the centre of the granules, the degree of compaction increased. The minimal space between the cells resulted in a "honeycomb-like" appearance.

Scanning electron microscopy has mainly been used to examine the surface of granules (Schmidt & Ahring, 1996) and cavities and holes were usually observed. In contrast, with transmission electron microscopy the internal structure of the granules was studied where microcolonies were observed and identified on the basis of structure, shape and cell envelope structures. In a study on syntrophic and competitive interactions in complex ecosystems, such as granules, microscopic identification is not too helpful. New molecular methods based on 16S rRNA probes have been reported to be used to study community composition, structure and microbial growth of granules. Harmsen *et al.* (1996) and Raskin *et al.* (1995) reported on the use of 16S rRNA-targeted probes to study the orientation of organisms in the syntrophic consortia.

In summary, it can be stated that a typical granule structure can be found. The granule's bacterial profile or microstructure is dependent on the concentration of the substrate and metabolites inside the granule. These are dependent on degradation and diffusion rates of metabolites like volatile fatty acids, acetate and hydrogen. Acidogens appear to be concentrated in the outer layer, where carbohydrates are degraded. The volatile fatty acids produced by the acidogens appear to diffuse inward because of concentration gradients and become substrate for syntrophic acetogens. Acetate is then the key substrate for the bacteria, especially the methanogens, in the core. The granule structure typically consists of an extremely heterogeneous surface zone surrounding a homogeneous central core. The surface zone is generally composed of small clusters of Gram-negative bacteria within a matrix of Gram-positive organisms. The core in contrast, appears to be made up primarily of Gram-negative organisms. The outer surfaces contain more carbohydrates and less protein than the central core. *Methanothrix*-like bacteria have been reported to be the most common methanogen present in the granules.

## 2.6 GRANULATION

The granulation process appears to be a unique type of bioflocculation similar to an agglutination reaction induced by polymers. The precise nature of the mechanisms involved in the formation of granules and their continued stability is still not fully understood. Bacterial community composition and the presence of extracellular polymeric substances (ECP) are probably involved (Forster & Quarmby, 1995).

Moosbrugger *et al.* (1992) concluded from their research that granules are formed by the generation of an extra-cellular polypeptide produced by *Methanothrix*. Vanderhaegen *et al.* (1992) found the granules to contain equal amounts of extracellular proteins and carbohydrates. In contrast to the work of Moosbrugger *et al.* (1992), Riedel & Britz (1993) hypothesised that the ECP compounds are rather produced by propionate forming acidogens of the genus *Propionibacterium*. The

ECP production let them gain a metabolic advantage under environmental "stress" conditions (Riedel & Britz, 1993). Excessive amounts of extracellular polysaccharides are then produced, leading to clumping characteristics.

Chen & Lun (1993) also suggested a mechanism for granulation. According to their hypothesis, the first step is the formation of a nucleus. The bacteria involved in the formation of this nucleus are mainly *Methanosarcina* and *Methanothrix*. With a rise in organic loading rate, *Methanosarcina* grow into clumps through secreting ECP. Large clumps are then in time formed with a sufficient diameter so that they are not washed out of the system. In contrast, *Methanothrix* can easily grow and attach to different surfaces or insert into the clumps. When acetic acid concentrations are high, *Methanosarcina* dominates over *Methanothrix*, but once the acetic acid concentration decreases, *Methanothrix* will grow faster than the *Methanosarcina* because of a higher selection for substrate. *Methanosarcina* can then no longer play any role in granulation. *Methanothrix* can attach to an inert particle in the sludge and grow competitively with *Methanosarcina* to form a nucleus in which these bacteria dominate.

A number of investigators have described the prominence of *Methanothrix* spp. in anaerobic waste water systems. Morgan *et al.* (1991a) suggested that granules initially develop from a precursor which consists of a small aggregate of *Methanothrix* and other bacteria and in time the *Methanothrix* filaments grow, forming characteristic bundles, separated by the surrounding matrix where other methanogenic and non-methanogenic bacteria are found. As the bundles increase, the surrounding matrix becomes excluded, forming a region in the centre of the granule where only compact filaments of *Methanothrix* are found. The separate bundles can no longer be seen. In time, larger, older colonies of *Methanothrix* are found towards the centre of the granule and the growth of the granules may be largely as a consequence of the development of *Methanothrix*.

Similarly, MacLeod *et al.* (1990) suggested that the presence of *Methanothrix*-like cells in the central core of the granules may function as nucleus centres for the initial development of granules. A loose mat of *Methanothrix* filaments forms an excellent framework that could be colonised by a succession of other organisms. Some of the first colonising organisms, probably acidogens like *Propionibacterium*, would be those which produce acetate and propionate. These bacteria would provide the *Methanothrix* spp. with the required substrate. During this time other volatile fatty acids like propionate and butyrate are degraded to acetate by the $H_2$-producing acetogens. High concentrations of $H_2$ inhibit the degradation of propionate and butyrate by these bacteria and they thus require a syntrophic association with $H_2$-using bacteria for substrates like propionate and butyrate. The formation of a second layer around the *Methanothrix* mat would thus include $H_2$-producing acetogens and $H_2$-consuming organisms. Fermentative bacteria would then probably adhere to the mini-aggregate to form the external layer of the granule. The volatile fatty acids produced would then serve as substrates to the underlying acetogens and homo-acetogens. Hydrogen-using organisms (homo-acetogens and methanogens) in the second layer would be able to remove any remaining hydrogen produced by the acetogens so that a high level of metabolic activity by the acetogens are possible. Such a three-layered aggregate would be a complete and stable metabolic consortium that would create optimal environmental conditions for all its members. High levels of metabolic activity and biomass accumulation should permit

the aggregate to reach the size of granules, which are not easily washed out of the anaerobic system.

Bossier & Verstraete (1996) suggested that environmental factors like substrate gradient and slow growth can also trigger aggregation. They found in their studies that a higher substrate concentration could be found in the vicinity of a granule particle or a floc. It is generally accepted that up to 50% of the COD in waste water is found in a colloidal or particulate form and these can adsorb to a microbial floc. When these macromolecules are degraded by the extracellular enzymes, the concentration of metabolites can increase in the vicinity of the floc. Bacteria might then experience a substrate gradient in the direction of the floc. ECP can also trap nutrients from the liquid by loose binding, with the same effect. Aggregation by slow growth starts with a depletion of substrate. Faced with starvation, the bacteria are able to change cell surface characteristics and become more hydrophobic. This might facilitate adhesion (Bossier & Verstraete 1996).

Myburg & Britz (1993) found a shift in population dynamics of anaerobic communities under environmental "stress" conditions with an organic overload or a shortened hydraulic retention time. The accumulation of lactate in the substrate would favour the growth of *Propionibacterium* strains with an abundant production of extracellular polymers, contributing to the formation of granules. These ECP forming acidogens were subsequently isolated from stressed bioreactors (Riedel & Britz, 1993).

Schmidt & Ahring (1996) compared the granulation process, with the formation of a biofilm. According to them, the process consists of four steps: transport of the cells to the surface of an inert material; adsorption to the substrate; adhesion to the substrate; and multiplication of the cells. The initial adsorption can possibly be achieved through the collision of the microbial cell and the macromolecules of the substrate. The macromolecules can include bacterial aggregates, organic or inorganic material present in the feed or precipitations or particles like straw present in the sludge. Adsorption will be due to forces like ionic, dipolar or hydrogen bonds or hydrophobic interactions. Irreversible adhesion will be established by strong bonds of Van Der Waal forces and electrostatic binding with cations like $Ca^{2+}$ and $Mg^{2+}$. ECP is then produced by acidogens which forms a matrix for the cells to divide in and a macrocolony of identical cells is formed.

Sanin & Vesilind (1996) confirmed the above theories and suggested that the building blocks of a floc are colonies, which are formed when two or more microorganisms become attached to each other. Bacterial surfaces are covered with slime or an extracellular polysaccharide layer. These polymers react through hydrophobic or hydrogenic interactions, or the formation of metal/polymer complexes through cations like $Ca^{2+}$ or $Cu^{2+}$. Once the colonies are formed, they react with other colonies to form larger structures. Other microorganisms can also be entrapped by the extracellular polymer network. Aggregation of anaerobic microorganisms into granules optimises the co-operation between the partner organisms by reducing the diffusion distance for the transfer of metabolites and creating the close cell associations (Guiot *et al.*, 1992). Aggregation of bacteria of different metabolic groups is thus important for the energetics and kinetics of the overall conversion of substrate during anaerobic digestion.

## 2.7 CONCLUSIONS

Biodegradable high-strength industrial and food effluents can be successfully treated using anaerobic digestion systems and specifically the UASB process which was developed to present a stable, high rate and efficient treatment method. However, the successful operation of UASB bioreactors is dependent on the formation of highly settable methanogenic granules which are responsible for the degradation of the organic component of waste waters.

The major problems in operating the UASB system are the long start-up times as a result of the slow process of granulation, as well as the need for a speedy replacement of granules once they have been washed out of the system. These two limitations clearly restrict the general application of this system, unless the granulation reaction can be enhanced. Full exploitation of this rather unique biomass immobilisation technique can thus not be realised until the granule formation conditions are defined and optimised. The precise nature of the mechanisms involved in the formation of granules and the reason for their stability, is still not fully understood and many researchers have suggested theories for granulation, but community composition, structure and microbial growth of granules still needs to be investigated in more detail.

In this hypothesis it is postulated that, through the implementation of environmental "stress" conditions, a shift in the population dynamics of the anaerobic community can be obtained. This results in a concurrent increase in ECP formation that appears to enhance aggregate formation. The hypothesis on granulation presented by Myburg & Britz (1993) and Riedel & Britz (1993) is one that is worth further investigation and exploitation.

chap3

# CHAPTER 3

# INFLUENCE OF SUBSTRATE COMPOSITION ON THE METABOLIC ACTIVITY OF RAW ANAEROBIC SLUDGE AND GRANULES

## 3.1 Summary

Batch cultures with the different carbon sources and effluents were inoculated with either anaerobic sludge or granules and grown in shake waterbaths over a period of 14 - 20 d. Growth and metabolic activity were monitored in the form of pH, volatile fatty acids, production of granules and COD removal. For all the substrates a drop in pH within the first two days was experienced. In the glucose units the pH dropped to 6.0 and to below 5.5 for the Sucrose units where after in both cases a continual drop was experienced until day 14. With the Lactate units, the pH dropped to 6.5 by day 2, with a subsequent climb until the pH stabilized at around 7.7 - 7.9. With the Fruit effluent, the pH dropped to 6.0 by day 3, after which it remained stable. For the Petrochemical effluent, the pH increased from 7.0 to 7.8 and then remained stable.

The volatile fatty acid (VFA) profiles of the Glucose units showed a slow increase of acetic and propionic acids in conjunction with the decrease in pH and lower COD removal and indicated the start of instability and final acidification. In the case of the Sucrose units, a faster VFA formation took place, which led to total acidification and no clear granule formation. The Petrochemical profiles showed a steady increase in acetic and propionic acids up to day 7 and then a slow decrease in concentration before stabilization. With Lactate and the canning effluent, the profiles showed a sharp increase in acetic and propionic acids with the latter at the highest concentration and a good COD removal. Data showed that metabolic activity and granule growth was very dependent on the type and age of the anaerobic sludge used. An increase in granule formation was found for the Glucose (354%), Lactate (559%), Petrochemical (36%) and Fruit units (46 - 600%), probably because of a high production of propionic. This confirms the hypothesis that a sudden increase in a substrate leads to a shift in the population dynamics giving the propionic acid producers a competitive advantage leading to the formation of ECP as an alternative hydrogen sink medium. Enhanced granulation within these systems could thus be experienced.

## 3.2 Introduction

The Upflow Anaerobic Sludge Blanket (UASB) process is one of the most extensively applied anaerobic treatment systems in the world (Wieland & Rozzi, 1991; Lettinga *et al.*, 1997). This process depends on the upward movement of waste waters through a blanket of granules which are (Chynoweth, 1987) a characteristic feature of the UASB system. The mechanism of granule formation is, however, still poorly understood (Slobodkin & Verstraete, 1993) and it would appear that granular sludges can only be formed with certain types of carbohydrate/protein containing waste waters generated in the agricultural and food processing industries. With other waste waters, granule formation has not been possible (Sam-Soon *et al.*, 1991). In many of these cases, even

after seeding the systems with granules, it has been found that in time these have disintegrated and washed out (Sorensen *et al.*, 1991). This clearly restricts the general application unless the granulation reaction can be induced in other treatment systems. Since the operational efficiency and performance of these systems are mainly dictated by the amount and activity of the granules, the rather extended start-up times limit the potential use of the system (Wentzel *et al.*, 1994). Full exploitation of this cell immobilization method can thus not be realized until the granule formation conditions are defined.

Moosbrugger *et al.* (1992) reported that the granules are formed as a result of the generation of an extracellular polypeptide produced by a hydrogenotrophic methanogen of the genus *Methanobacterium.* In contrast, Vanderhaegen *et al.* (1992) found the granules rather to contain equal amounts of extracellular proteins and carbohydrates. According to Riedel & Britz (1993) as well as Slobodkin & Verstraete (1993), these can be formed by propionate-producing acidogens that are effective slime and aggregate formers. When "stress" conditions are put on a digester treating carbohydrate rich waste water, one of the first metabolites that appears is propionate and simultaneously hydrogen can be detected in the gas phase (Riedel & Britz, 1993). A sudden increase in the loading of readily degradable substrates also leads to the accumulation of lactate, with a resulting shift of the population dynamics in the anaerobic consortium. Organisms producing propionic acid from lactate can then gain a competitive advantage as they obtain a maximum of ATP per mol of lactate fermented (Myburg & Britz, 1993). These propionic acid producers can then also produce excessive amounts of extracellular polymers (ECP) as an alternative hydrogen sink mechanism, and then display clumping characteristics.

In this study the influence of different substrates on the metabolic activity of anaerobic sludge and granules, was investigated. The main purpose was to determine which substrate could be used to lead to the formation of propionic acid as major metabolite so as to give organisms producing propionic acid under "stress conditions," a competitive advantage.

## 3.3 Materials and methods

*Experimental setup*

Two linear-shake water baths (manufactured by Scientific Manufacturing, Paarden Eiland, Cape Town), each with a capacity for 10 x 500 ml bottles, were used to cultivate the granules and sludge in a batch system. The temperature of the waterbaths was maintained at 35°C, with a shake-speed of 150 r.p.m. Bottles containing 400 ml of each specific sterile growth medium were inoculated with either 50 ml sludge from an anaerobic tank from the local Kraaifontein Sewerage Works or with granular sludge from a SA Brewery Plant (supplied by Dr. A. Wood). Each day, for a period of 14 days, a 100 ml of the batch units volume was removed and replaced with sterile substrate so as to simulate UASB operational parameters under sudden increases in the loading rate.

*Substrate*

During the study five different substrates were used ($g.l^{-1}$):

* Lactate medium (YEL - Riedel & Britz, 1993) which consisted of lactate 20.0, yeast extract 5.0, peptone 2.0, Tween 80 1 $ml.l^{-1}$ and a trace element solution 10 $ml.l^{-1}$ (Nel *et al.*, 1985);
* Sucrose medium (Quarmby & Forster, 1995) which consisted of sucrose 9.0, yeast extract 0.5, urea 1.0, Tween 80 2 $ml.l^{-1}$ and a trace element solution 10 $ml.l^{-1}$ (Nel *et al.*, 1985);
* Glucose medium (Lens *et al.*, 1993) which consisted of glucose 9.0, yeast extract 0.5, urea 1.0 and a trace element solution 10 $ml.l^{-1}$ (Nel *et al.*, 1985);
* Fruit effluent was obtained from the Ashton Canning Company (Mr. J. Visser) and included as an example of a carbohydrate rich food effluent with a COD concentration of 4 000 which was diluted to 2 000 $mg.l^{-1}$ and the pH poised at 7.0; and
* The Petrochemical effluent was collected as waste water generated during the Fischer-Tropsch Sasol coal-to-oil process, Secunda (Mrs. R. Augustyn). It consisted mainly of mono-carboxylic acids (1.0 - 1.3% $M.vol^{-1}$), small amounts of emulsified oils, phenols, alcohols and ketones (0.01 - 0.03%). The batch of the petrochemical effluent used had a COD value of 11 660 $mg.l^{-1}$ and a volatile fatty acid (VFA) concentration of 7 500 $mg.l^{-1}$. The VFA's were mainly acetic (66%), propionic (16%), iso-butyric (3%), n-butyric (9%), iso-valeric (2%) and n-valeric acid (4%). The pH of the effluent was 3.5 and for the study it was poised at 7.0 using 1N NaOH. The COD of the solution used in the batch studies was 2 000 $mg.l^{-1}$, and a trace element solution 10 $ml.l^{-1}$ (Nel *et al.*, 1985) and enriched with 100 $mg.l^{-1}$ of urea (Sam-Soon *et al.*, 1991).

  The pH of all the media was poised at 7.0 using 1N NaOH and the media were steam sterilized at 121°C for 15 minutes.

*Granule counts*

The amount of granules were counted by directly and physically counting the amount of granules formed over time, by using a round glass container with a graded grid underneath. The graded field was divided into 52 fields each with a diameter of 2 x 2 cm. For each count, an amount of 10 ml was withdrawn every day and diluted 10 times before it was poured into the base of the glass container and counted. The amount of granules formed were calculated and recorded. The granules formed were very small, and in combination with a cloudy and very viscous solution, it was fairly difficult to always accurately detect the black nuclei.

*Analytical procedures*

The following parameters were monitored: pH; COD; Total Solids (TS); Total Non-Volatile Solids (TNVS) and Total Suspended Solids (TSS) (Standard Methods, 1985). The pH was determined using a Knick pH meter equipped with a standard Russell glass electrode. The amount of granules formed was determined by means of physical counts of diluted samples as well as a drying and weighing procedure.

Volatile fatty acids (VFA) were determined using a Varian Model 3700 gas chromatograph equipped with a flame ionization detector and a bonded phase 007FFAP (Quadrex Corp., New Haven) fused silica capillary column with a 30 m length and diameter of 0.32 mm. The column temperature was initially held at 105°C for 5 min,

then increased at a rate of 10°C per min. to 219°C and held for 5 min. The injector temperature was set at 250°C, while the detector was set at 260°C. Nitrogen was used as carrier gas at a flow rate of 2.5 ml.min$^{-1}$. A sample volume 0.5 microlitre was introduced via the injector port. A calibration standard was made up of acetic, propionic, i-butyric, n-butyric, i-valeric and n-valeric acids added to 0.5 ml 0.1N HCl with an internal standard of n-hexanol (24%). The internal standard was chosen because its polarity and boiling point allowed it to be closest to the fatty acid peaks.

## 3.4 Results and discussion

*pH profiles*

The pH was measured to indicate metabolic productivity (production of volatile fatty acids) at the start and during the study. It was found that the initial carbohydrate concentration of 9.0 g.l$^{-1}$ in the Glucose medium was too high as this resulted in a very sharp decrease in pH (as illustrated in Fig. 1) and finally system failure. The glucose concentration was thus subsequently reduced to 5.0 g.l$^{-1}$ which resulted in a more acceptable and stable pH profile (Fig. 1).

In the first experimental batch studies (Fig. 2), where the units had been inoculated with either anaerobic sludge (An) or granules (Gr) and incubated at 35°C over a period of 14 days, the pH dropped within the first 48 h from 7.0 to about 6.0 for the Glucose units (G) and from 7.0 to below 5.5 for the Sucrose units (S) (Fig. 2). This was expected due to the strong acid formation from the specific carbohydrates in spite of a high buffering capacity with 10.0 g.l$^{-1}$ $K_2HPO_4$. However, in both cases, from day 2 to day 4 the pH increased, whereafter a continual drop was experienced until day 14 of the experiment. When the Glucose and Sucrose units were incubated for a longer time (up to 20 days) the pH dropped to below 5.0 resulting in system failure.

In the case of the units with Lactate as carbon source (Y) (Fig. 2) inoculated with either sludge (An) or granules (Gr), the pH dropped to about 6.5 within the first 2 days, with a slight climb from there onwards until the pH stabilized at around 6.7 - 6.9. This stabilization persisted with an extended incubation of up to 20 days.

With the Fruit effluent (F), a large difference in the pH profile was found in the reaction of the units inoculated with either granules (Gr) or the anaerobic sludge (An). The sludge pH showed a drop to 6.0 by day 3, with a slight climb to day 4, after which it remained stable even up to day 20. The pH of the granule batches in contrast, showed a drop to pH 6.5 by day 3, and then increased to around 7.0 where it remained fairly stable until day 13 when it decreased again to around 6.5. This decrease continued to below 5.5 during an extended incubation of up to 20 days suggesting that the granules were not as stable as the anaerobic sludge when using Fruit effluent as a carbon source.

In the case of the Petrochemical effluent (P), the pH increased from 7.0 to 7.8. This increase was ascribed to the high rate of utilization of the volatile fatty acids by the microbes in the inoculum and a lack of carbohydrates in the effluent (Fig. 2). Similar results for the same petrochemical effluent were reported by Nel & Britz (1986). The pH in both the granule and anaerobic sludge units stabilized by days 6 and 11 respectively and remained so over a longer incubation period of up to 20 days.

fig8-1

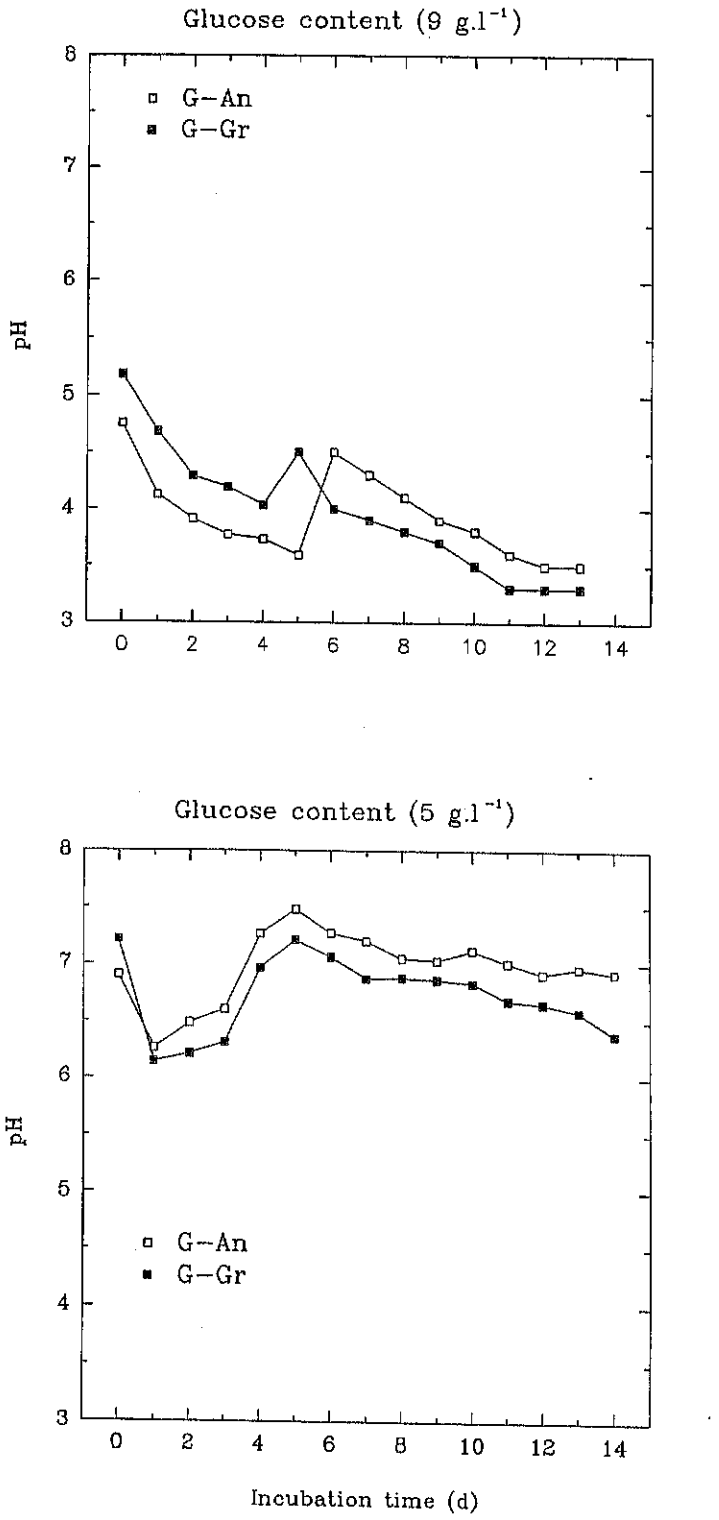


**Figure 1.** A comparison between pH values formed in batch cultures with a high and low glucose content of the batch systems operated over a period of 14 days at 35°C (Carbon source: glucose = G) (Inoculum type: Anaerobic sludge = An, Granules = Gr).

fig3-2

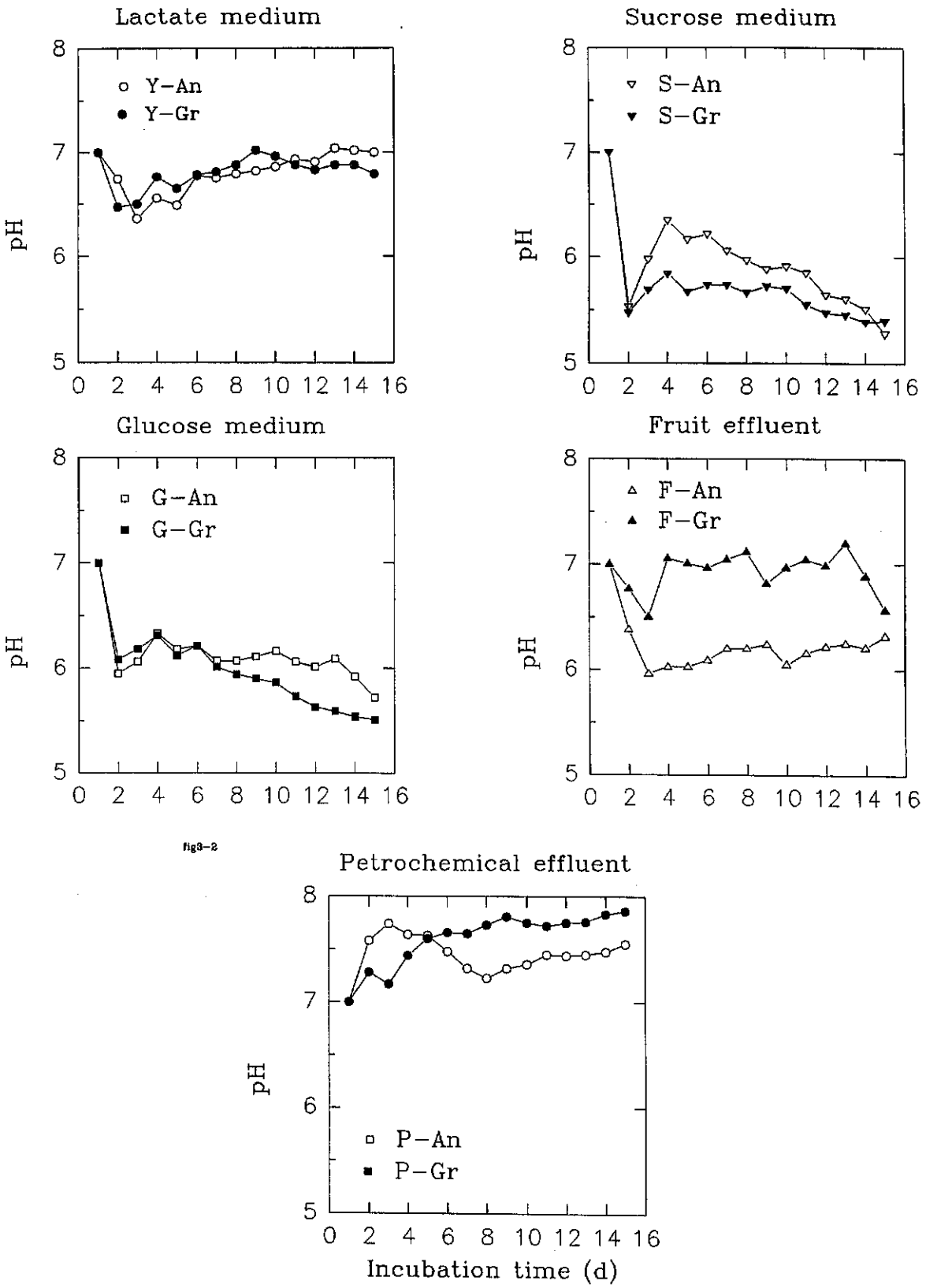


**Figure 2.** Influence of carbon sources and waste streams on the pH of the batch systems operated over a period of 14 days at 35°C (Carbon sources: Lactate = Y, Sucrose = S, Glucose = G, Fruit effluent = F, Petrochemical effluent = P) (Inoculum: Anaerobic sludge = An, Granules = Gr).

fig3-3

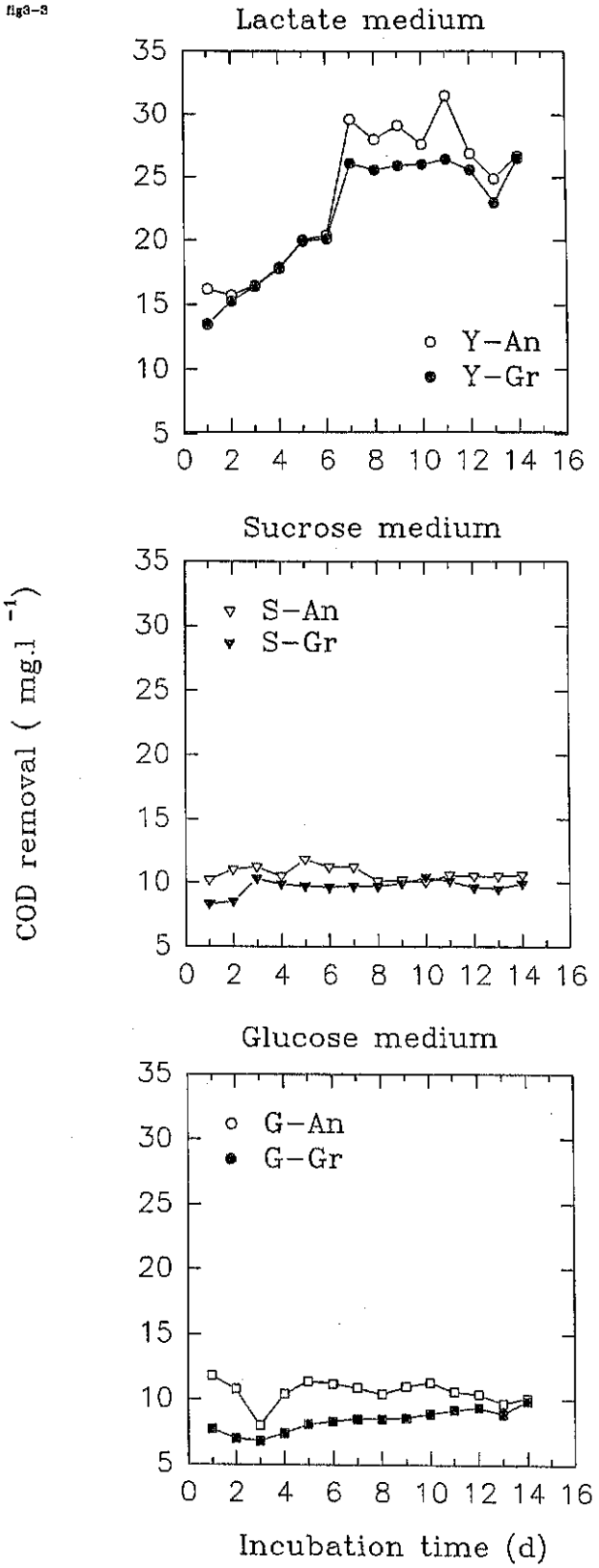


**Figure 3.** Influence of carbon sources on COD removal in batch systems operated over a period of 14 days at 35°C. (Carbon sources: Lactate = Y, Sucrose = S, Glucose = G) (Inoculum type: Anaerobic sludge = An, Granules = Gr).

*COD removal*

The COD removal efficiency for the Glucose (G), Sucrose (S) and Lactate (Y) batch units inoculated with either anaerobic sludge (An) or granules (Gr) over the experimental period of 14 days is illustrated in Fig. 3. In the case of the Lactate medium, a steady increase in the COD removal was experienced over the first 7 days of growth for both the sludge and granule units, indicating an active COD removal biological system. After day 7, the systems stabilized at a removal of between 25 - 30 $mg.l^{-1}$. This still indicated activity in both the systems. The stabilization in COD removal after day 7 corresponded with the pH stabilization as seen in Fig. 2. The highest COD removal in the Sucrose and Glucose media for both the sludge and granules was around 8 - 12 $mg.l^{-1}$. It can thus be concluded that the Glucose and Sucrose cultures were less active in terms of COD removal than the Lactate grown cultures. It is also possible that the low pH values (Fig. 2) of the batch cultures inhibited the growth and metabolic activity resulting in the low activity in terms of COD removal.

*Volatile fatty acid profiles*

The volatile fatty acid (VFA) profiles obtained during the study are shown in Fig. 4 and 5. For the VFA profiles of the Glucose units (Fig. 4) inoculated with anaerobic sludge (G-An) and the one with granules (G-Gr) at the lower glucose concentration (5.0 $g.l^{-1}$), both the acetic and propionic acid concentrations started to increase from the start of the experiment and, with the exception of the propionic acid in the sludge unit, increased during the course of the study. These steady increases in the acid concentrations in conjunction with the decrease in pH (Fig. 2) and low COD removal (Fig. 3) indicates the start of instability in the glucose units. After a longer incubation of up to 20 days, it was found that the Glucose units totally acidified with the pH dropping to below 5.0.

In the case of the Sucrose units (S-An and S-Gr) (Fig. 4), a similar but faster acidification took place with most of the VFA's showing increases which corresponded with the decrease in pH for both the sludge and granule units.

With the Lactate, the data (Fig. 4) showed that for both the granule (Y-Gr) and sludge (Y-An) inoculated units an initial, very sharp increase in acetic and propionic acids was found. During this time the propionic acid was produced at the highest concentration. However, in both cases after days 6 to 7 these acids decreased again and by day 14 in both granule and sludge units, had nearly stabilized and this was confirmed by the pH data (Fig. 2). The iso-butyric and butyric acids also showed increases in both units. It is interesting to note that the decrease in acetic and propionic acids corresponds to the stabilization of the COD removal (Fig. 3). An extended incubation of up to 20 days showed no further changes in the VFA profile nor a further change in the pH.

From the VFA profiles formed in the different batch units, the most active production system was clearly found in the Lactate units (Y-An and Y-Gr). This was confirmed by the COD removal data (Fig. 3) and the acetic and propionic profiles (Fig. 4 and 6). A large concentration of propionic and acetic acids were formed within the first 4 - 5 days of operation. The higher percentage of propionic to acetic acid formed during this time is typical of the growth and metabolic profiles of members of the genus *Propionibacterium* (Riedel & Britz, 1993).

fig3-4

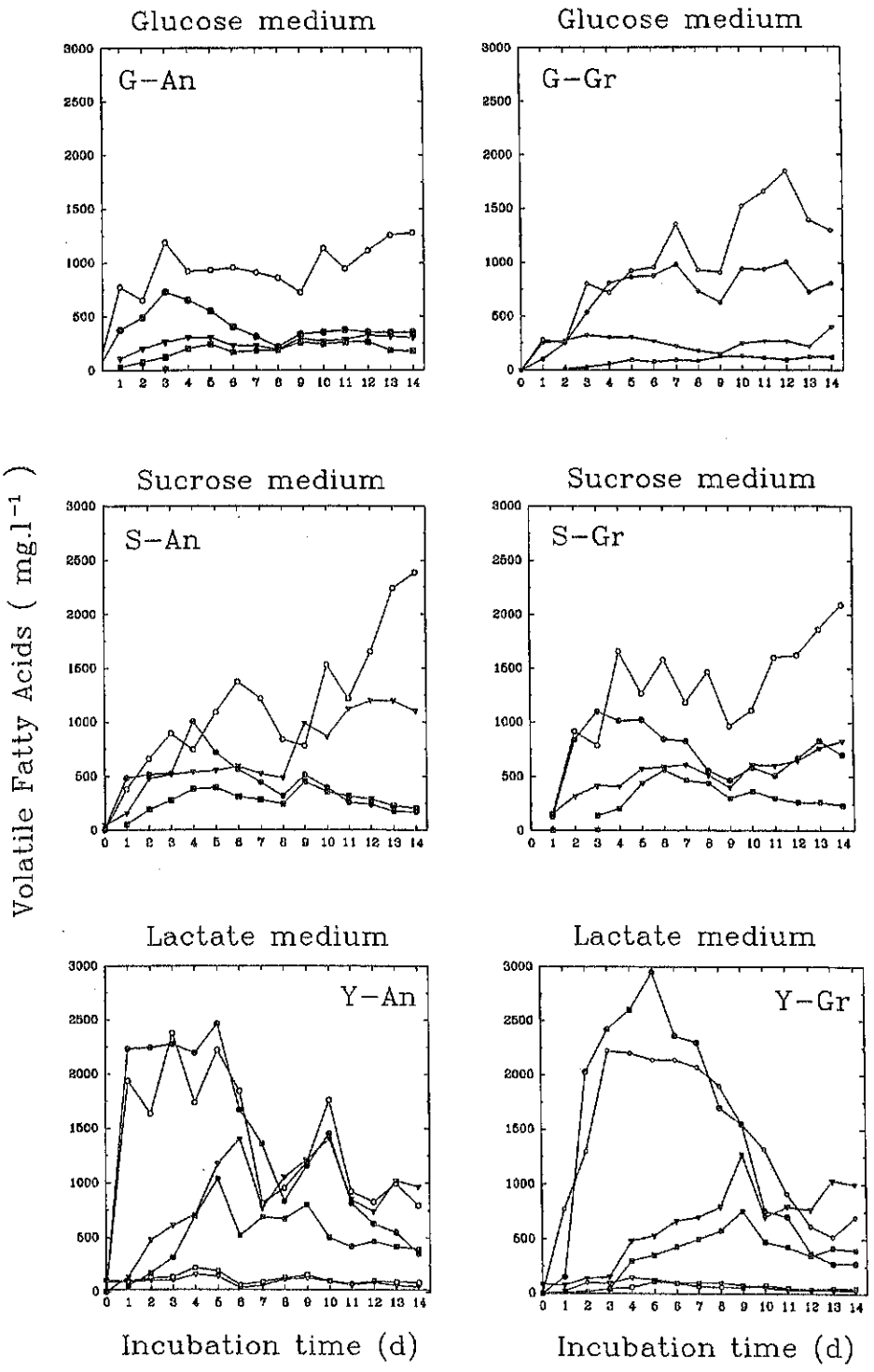


**Figure 4.** Influence of different carbon sources on the volatile fatty acid production of the batch systems operated over a period of 14 days at 35°C (Carbon sources: Lactate = Y, Sucrose = S, Glucose = G) (Acids: ● = Acetic, O = Propionic, ■ = *Iso*-Butyric, ▼ = Butyric, □ = *Iso*-Valeric, ∇ = Valeric) (Inoculum type: Anaerobic sludge = An, Granules = Gr).

fig3-5

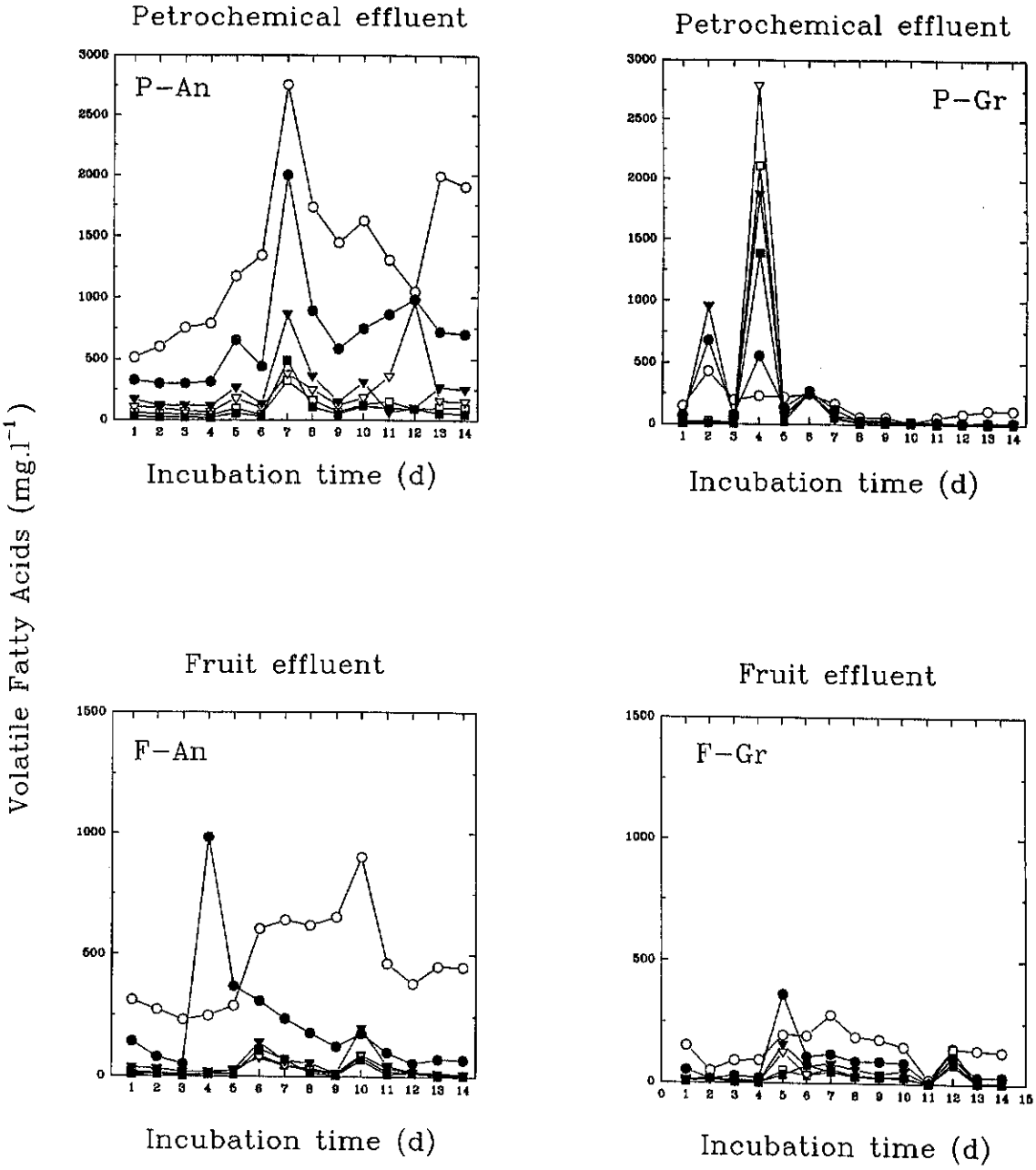


**Figure 5.** Influence of different waste streams on the volatile fatty acid production in batch systems operated over a period of 14 days at 35°C (Carbon sources: Fruit effluent = F, Petrochemical effluent = P) (Acids: ● = Acetic, O = Propionic, ■ = *Iso*-Butyric, ▼ = Butyric, □ = *Iso*-Valeric, ∇ = Valeric) (Inoculum type: Anaerobic sludge = An, Granules = Gr).

The growth conditions and the use of lactate as carbon source probably led to a more acceptable environment for the propionic acid producing bacteria. The data, with standard error bars, from 10 separate repetitions during a four month period, of the Lactate units inoculated with different batches of anaerobic sludge (Y-An) are illustrated in Fig. 6. From these it can be seen that even with different batches of anaerobic sludge, characteristic acetic and propionic acid and pH profiles were obtained suggesting that when using lactate as carbon, a repeatable metabolic pathway is obtained. This data confirms the hypothesis of Riedel & Britz (1993) that a sudden increase in a readily degradable substrate like lactate leads to a shift in the population dynamics, giving the propionic acid producers a competitive advantage, leading to the formation of ECP as an alternative hydrogen sink product.

The VFA data (Fig. 5) of the Petrochemical unit which was inoculated with anaerobic sludge (P-An) showed a steady increase in especially acetic and propionic acids up to day 7 and then a slow decrease in concentration. By day 14 this unit had not reached a stable state. It is extremely difficult to explain this data, especially the increase in VFA, as the substrate contained no other carbon sources. In the case of the Petrochemical unit inoculated with granules (P-Gr), a double increase was found before a stable state was reached, indicating that the granular biomass was utilizing the substrate with subsequent gas production (visual confirmation only).

With the Fruit unit (Fig. 5) inoculated with anaerobic sludge (F-An), a major propionic acid peak similar to that found with the Lactate sludge unit, was found at day 4 with a steady decrease in propionic acid until the formation stabilized. The highest concentration of acetic acid was found at day 10. For the Fruit effluent unit inoculated with granules (F-Gr), a very stable fatty acid profile was found for the whole period, with a slight increase in propionic acid at day 5. The acetic acid and the other fatty acids showed a relatively stable profile over the whole period, with a slight drop at day 11.

*Granule formation*

The influence of the different carbon sources and waste streams on granule formation is given in Fig. 7. The values are average values for four replicates. Data from the study showed that metabolic activity and granule growth was very dependent on the type of anaerobic sludge used. The sludge obtained from the Sewerage Works was found to differ from week to week. In the rainy season, it was found that a much more watery sludge was obtained and that it definitely had a negative effect on granule formation. During the dryer summer season, a more solid product was obtained and granulation was found to occur more rapidly. The age of the sludge also had an effect on granule formation. It was found that if a fresher sample was used, acid production and granulation occurred much more speedily, than when an older sample was used. Thus, a fresh sample during the summer season gave the best results. However, besides the age and constitution of the sludge it was extremely difficult to grade the sludge. An illustration of the variation in the granule producing quality of anaerobic sludge batches from the same source, is given in Fig. 7. Two units of Fruit effluent were inoculated with different batches of anaerobic sludge (F-An1 and F-An2) and incubated at 35°C for 18 - 26 d and an increase of between 46% (F-An1) and 600% (F-An2) depending on the batch of anaerobic sludge used, was found.

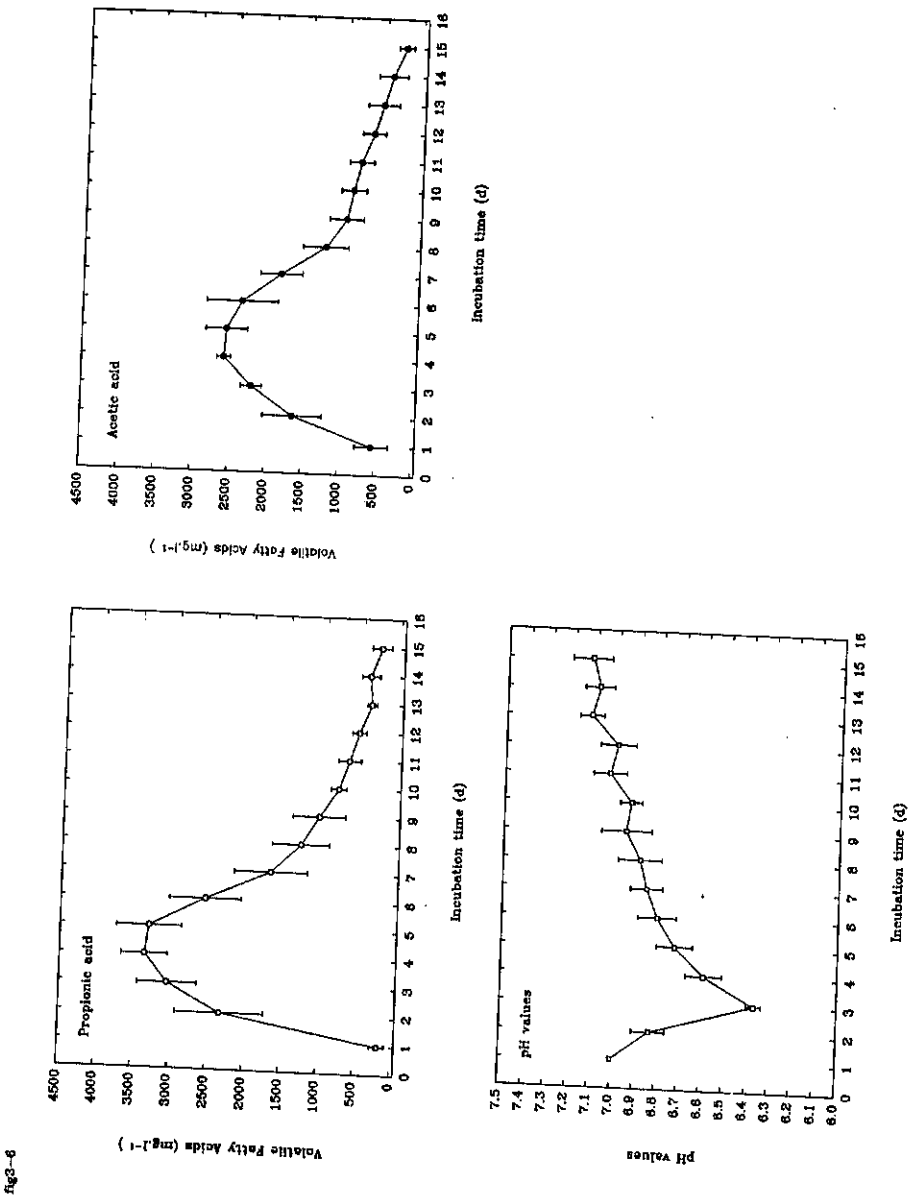


**Figure 6.** Variation in acetic and propionic acid production and pH in 10 separated lactate batch units inoculated with different batches of anaerobic sludge and incubated over a 15 day period at 35°C. The standard deviation was used as the error bar length.

In all the cases, after 6 days incubation on the shakers at 35°C (Fig 7), metabolic activity in the form of gas production was evident even during the phase where the pH had decreased to below 6.0. In the Glucose (G-An), Lactate (Y-An) and the Fruit (F-An) units that had been inoculated with anaerobic sludge, the formation of very small (fine) granules were found. The Lactate, Petrochemical and Fruit granules were dark (see Photo I) and an extra but clearly separated biomass layer, was evident at the end of the incubation period. For the Lactate medium (Y-An), a steady increase of 559% was found (Fig. 7). Fewer granules were formed in the Glucose units and these were in contrast to those from the Lactate units, a brownish colour and bigger (see Photo II). The granules grown on a Glucose medium (G-An) showed an increase of 354% over the first ten days, whereafter a decrease was found over the rest of the incubation period.

Visual granule production was found in both of the Petrochemical units (P-An and P-Gr) (an increase of 36%) but the granules were very small. Although the units for both the granules and the sludge were found to be active, especially within the first week, the high pH value probably had an effect on granule growth. Chang *et al.* (1993) in contrast reported that a higher pH of up to 8.4 leads to the stimulation of granules. The granule formation in the Petrochemical unit (P-An) as found in this study is in contradiction to the reports of Sam-Soon *et al.* (1991) who found that under the conditions used in their studies, granule formation directly in UASB systems using the same Petrochemical effluent as substrate did not take place.

In the Sucrose units (S-An and S-Gr) no clear granule formation was visible even though the viscosity of the solution increased as observed visibly. In contrast, Fukuzaki *et al.* (1995) reported that in sucrose waste waters, granular sludges developed well compared to those with fatty acid waste waters. They also demonstrated that the overall characteristics of the granules depended on the carbon source in the waste waters and the operational conditions. Based on their study they suggested that more energy was needed for granule formation and suggested a pH between 7.7 and 8.4 to be important in the formation of stable granules.

The sharp increase in the amount of granules formed within 10 to 15 days in all the systems can probably be ascribed to the high activity in the cultures and suitable growth conditions. This fact can be confirmed by the pH and fatty acid profiles of the batch cultures during the first 10 to 15 days. In all the cases a sudden sharp drop was found after this first very active period. Chang *et al.* (1995) found that granules could not be formed at an acidic pH of 6.0 and concluded that a pH of 7.0 may still be too low to simulate granule formation. In this study a characteristic drop in pH (Fig. 1 and 2) at the start of the process for all the carbon sources and waste waters seemed to stimulate granule formation. This agrees with the process of granule formation as hypothesized by Riedel & Britz (1993) and Myburg & Britz (1993). In response to the decrease in pH, this can result in an orderly shift of the population dynamics in the anaerobic consortium with the more acid-tolerant propionic acid producing bacteria gaining a competitive advantage (Mackie & Gilchrist, 1979; Riedel & Britz, 1993). Subsequently, an increase in more reduced metabolites like propionic acid is found (Bryant, 1979) and this can then also lead to the production of excessive amounts of extracellular polymers (ECP) as an alternative hydrogen sink mechanism, and the system then displays clumping characteristics.

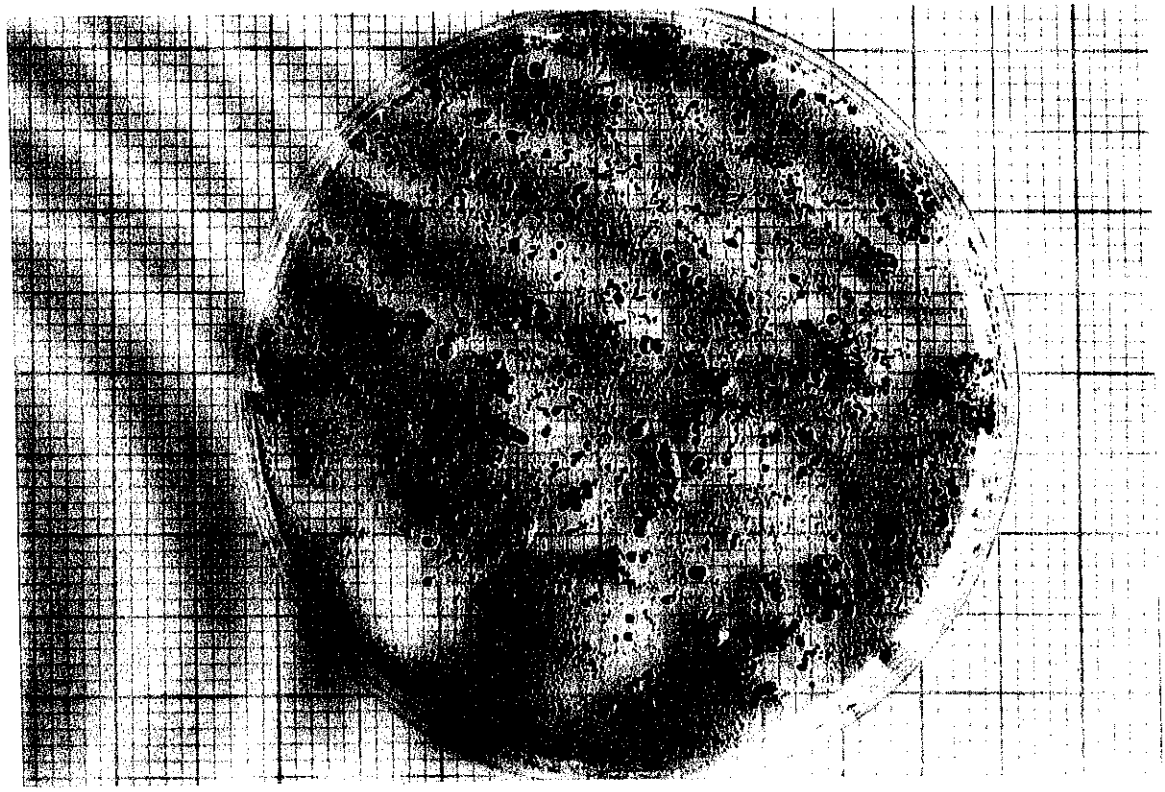

**Photo 1.** Quality of granules produced during the batch process induction and lactate as growth stimulants. Granule sizes vary from pin-point to 2 mm.

**Photo 2.** Quality of granules produced during the batch process induction and glucose as growth stimulants. Granule sizes vary from pin-point to 3 mm.

fig3-7

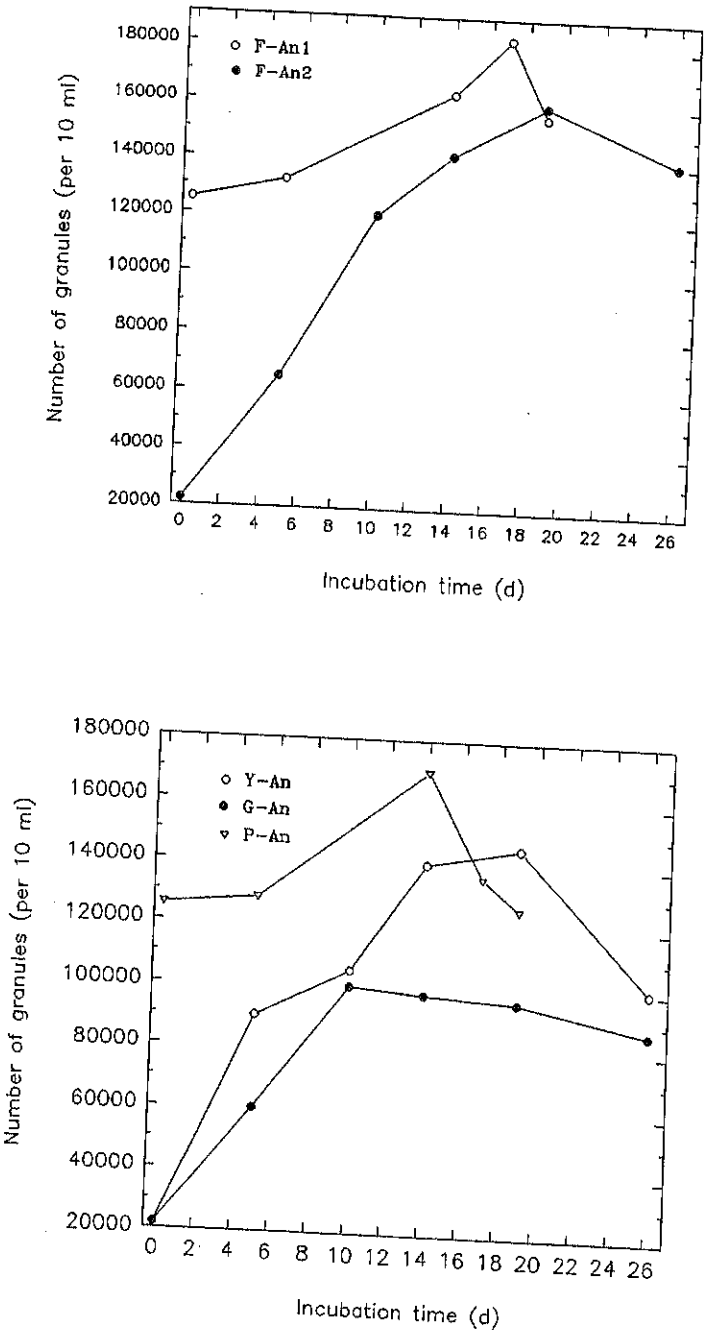


**Figure 7.** Influence of carbon sources and waste streams on the granule formation over a period of 14 - 26 days at 35°C (Carbon sources: Lactate = Y, Glucose = G, Fruit effluent = F, Petrochemical = P) (Inoculum type: Anaerobic sludge = An). F-An1 and F-An2 represents replicates of the Fruit effluent inoculated with different anaerobic sludges.

Vanderhaegen *et al.* (1992) in growth studies with granules in batch cultures, reported that the factor foremost in influencing the build-up in the reactor of granular sludge was the presence of high-energy sugars. They also found that shear forces were instrumental in providing cells growing in granules with a selective advantage over free suspended cells (Bossier & Verstraete, 1996). Thaveesri *et al.* (1994) reported that carbohydrate-rich substrates gave a good biomass growth yield, but that protein-rich substrates resulted in poor granular sludge growth. They also found that only when the medium was supplemented with a high energy substrate like sucrose, with a combination of a low surface tension in the environment, did the biomass start to granulate.

## 3.5 Conclusions

The aim of this study was to determine, in batch cultures, which substrate (Glucose, Sucrose, Lactate, a Petrochemical effluent and a Fruit canning factory effluent) could be used to lead to the formation of propionic acid as major metabolite so as to give organisms producing propionic acid under "stress conditions," a competitive advantage. This decision was based on the hypothesis that: propionic acid is one of the main intermediates in anaerobic digestion (Slobodkin & Verstraete, 1993); propionic acid is, after lactate and hydrogen, one of the first metabolites produced under "stress conditions" with a subsequent increase in granulation (Myburg & Britz, 1993; Riedel & Britz, 1993); propionate producers are effective slime and aggregate formers (Mulder *et al.*, 1989): and the formation of extra-cellular polysaccharides may serve as an alternative hydrogen sink reaction for the propionate producers (Quarmby & Forster, 1995; Vanderhaegen *et al.*, 1992).

From the fatty acid profiles formed in the different batch units, the most active VFA production system was clearly found in the Lactate unit (Y-An). This was confirmed by the COD removal data and the acetic and propionic profiles. High concentrations of propionic and acetic acids were formed within the first 4 - 5 days of operation. The higher percentage of propionic to acetic acid formed during this time was typical of the growth and metabolic profiles of members of the genus *Propionibacterium* (Riedel & Britz, 1993). The growth conditions and the use of lactate as carbon source probably led to a more acceptable environment for the propionic acid producing bacteria. The data showed that even with different batches of anaerobic sludge, characteristic acetic and propionic acid and pH profiles were obtained and that when using lactate as carbon source a repeatable metabolic pathway can be obtained. This confirms the hypothesis of Riedel & Britz (1993) that a sudden increase in a readily degradable substrate like lactate leads to a shift in the population dynamics giving the propionic acid producers a competitive advantage leading to the formation of ECP as an alternative hydrogen sink medium.

The results obtained and the increase in the formation of granules (even though only based on visual observations) clearly indicated that granules can be cultured in batch systems, and that a drop in pH at the start appears to facilitate the process. This supports the hypothesis of Riedel & Britz (1993) that granule formation is stimulated under 'stress' conditions like organic overloading and possibly higher hydrogen pressure at lower pH values.

# CHAPTER 4

# IMPACT OF PROCESS "STRESS" INDUCTION ON GRANULE GROWTH IN BATCH SYSTEMS

## 4.1 Summary

The influence of stress induction on lactate, glucose and fruit effluent containing batch systems at 35°C over a period of 14 to 30 days on granulation, was investigated. The anaerobic sludge inoculum used in all the studies was standardized to give an inoculum granule concentration of about 20 000 per 10 ml based on the visual counting method. For each experimental study a set of inoculated control units were included where no shock was applied to the systems. For each unit, a 100 ml was withdrawn and replaced with a 100 ml sterile medium. Growth and activity were measured in terms of pH and the volatile fatty acid profiles and visual increases in granule formation. The results showed the characteristic acetic, propionic and pH profiles with a clear enhancement in granule formation (400% - 1 000%) confirming the hypothesis of Riedel & Britz (1993) that by changing environmental conditions on batch scale, the propionic acid producers can be given a competitive advantage that leads to enhanced granulation. For all the systems the most active granulation period was found to be during the first 10 - 14 days of incubation. The best granule growth was in the Fruit effluent containing batches. The standardization of the inoculum size lead to reproducible results but only when the studies were done using inoculum from the same batch and same age. It was found that when repeating studies but using different inoculum batches, even from the same anaerobic digester, the increases in granule numbers could vary fairly widely. In most cases the granules were small and dense making it difficult to visually count. Another more accurate method will have to be found to enumerate the growth of granules. The data from the studies where a shock was applied (LG-shock-medium) on day 6 and once the fatty acid profiles had stabilized, showed that an enhancement (600% - 800%) of the granulation process could be facilitated. In the case of a double shock and a cysteine shock, a stimulation of only the Fruit effluent was found and with the *Propionibacterium* combination shocks, no clear enhancement was found.

## 4.2 Introduction

The characteristic granulation process in Upflow Anaerobic Sludge Blanket bioreactors (UASB) is an unique type of bioflocculation similar to the agglutination reaction induced by polymers. Moosbrugger *et al.* (1992) postulated that the granules are formed after the generation of extracellular polypeptides by a hydrogenotrophic methanogen of the genus *Methanobacterium.* In contrast, Vanderhaegen *et al.* (1992) found that the granules rather contained equal amounts of extracellular proteins and carbohydrates (Veiga *et al.*, 1997). According to Riedel & Britz (1993) as well as Slobodkin & Verstraete (1993), these can, under suitable environmental conditions, be formed by propionic acid producing acidogens that are effective slime and aggregate formers.

It has been reported (Marchaim & Krause, 1993) that when "stress" conditions are put on a digester, of the first metabolites that appear after lactate (Eng *et al.*, 1986), are propionic and acetic acids and hydrogen can (Hickey & Switzenbaum, 1991) simultaneously be detected in the gas phase (Myburg & Britz, 1993). The environmental "stress" can include differences in carbon sources and concentrations, organic overloading, increases in the operational hydrogen pressure, changes in the C:N:P ratio's, as well as the addition of cysteine (Moosbrugger *et al.*, 1992; Myburg & Britz, 1993).

The accumulation of lactate, especially in extreme cases, and a significant rise in hydrogen concentration, consequently results in a shift of the anaerobic consortium population dynamics (Riedel & Britz, 1993). At the higher hydrogen levels, the acidogens resort to utilization of the electrons of reduced pyridine nucleotides generated during fermentation in the increased catabolism of pyruvate to alternative more reduced hydrogen sink products (Bryant, 1979). In extreme cases, products such as lactate rather than acetate, $CO_2$, or $H_2$ may be formed. This represents a loss of potential growth-energy for most fermentative bacteria. However, bacteria utilizing the methyl-malonyl Coenzyme-A metabolic route for the production of propionic acid from lactate gain a competitive advantage during the unbalanced anaerobic conditions, as these bacteria obtain a maximum of ATP per mol of lactate fermented (Riedel & Britz, 1993). For these acidogens the formation of extracellular polymers may also serve as an alternative hydrogen sink reaction. Many propionic acid producers isolated from anaerobic digesters produce excessive amounts of extracellular polysaccharides and display clumping characteristics (Riedel & Britz, 1993). The production of extracellular polymers could thus contribute to the formation of granules. It appears therefore that the high partial hydrogen pressure and the subsequent production of lactate in response to organic loading "stress" or the shortening of the hydraulic retention time, all create an environment where strains which fulfill the above criteria could gain a competitive advantage, and subsequently, highly settable granules can be formed (Riedel & Britz, 1993). Balanced growth of acidogens, acetogens and methanogens is then necessary for further development of granular sludge.

In the previous chapter, the influence of different substrates on batch cultures was investigated so as to determine which would lead to the formation of propionic acid as major metabolite so that, under "stress" conditions, propionic acid producers could gain a competitive advantage. The substrates leading to the characteristic propionic profile and to an increase in granule formation were found to be either a Lactate, Fruit effluent or Glucose containing substrate. Based on the results it was decided to investigate the influence of multi-shock conditions on the basic growth substrates so as to stimulate granule formation.

## 4.3 Materials and methods

*Experimental setup*

Linear-shake waterbaths, each with a capacity of 10 x 500 ml bottles were used for the batch cultivation of the inoculum. The temperature of the water baths was set at 35°C, with a shaking speed of 150 r.p.m. Each specific sterile growth medium batch unit was inoculated with 50 ml anaerobic sludge from the Kraaifontein Sewerage Works. Each new batch of sludge used for each experimental study was diluted to give (as near

as possible) a visual countable granule concentration of 20 000 (variation between 18 000 and 22 000) granules per 10 ml. Each day for 14 days, a 100 ml of a batch culture was withdrawn and replaced with a 100 ml sterile medium so as to simulate conditions of sudden increases in the loading rate. To determine the influence of additional shocks, the batch units were incubated under the same growth conditions for 6 days, after which they were shocked with 200 ml of a specific 'stress medium'. For the rest of the growth period, the same culturing procedure was followed. Operational parameters were monitored on a daily basis.

*Substrate and Stress media*

Three different substrates were used as growth medium ($g.l^{-1}$):

* Lactate medium (Y-medium, Riedel & Britz, 1993) which consisted of lactate 20.0, yeast extract 5.0, peptone 2.0, Tween 80 1 $ml.l^{-1}$ and a trace element solution 10 $ml.l^{-1}$ (Nel *et al.*, 1985);
* Glucose medium (G-medium, Lens *et al.*, 1993) which consisted of glucose 9.0, yeast extract 0.5, urea 1.0, and a trace element solution 10 $ml.l^{-1}$ (Nel *et al.*, 1985); and
* Fruit effluent (F-medium) obtained from the Ashton Canning Company (Mr. J. Visser) and included as an example of a carbohydrate rich food effluent with a COD concentration of about 4 000 which was diluted to 2 000 $mg.l^{-1}$ and the pH poised at 7.0.

The following 'media and parameter changes' were used to create environmental stress conditions ($g.l^{-1}$):

* Lactate/Glucose shock medium (LG-shock-medium) which consisted of glucose 5.0, lactate 20.0, Tween 80 1 $ml.l^{-1}$, urea 1.0, peptone 2.0, yeast extract 5.0 and a trace element solution of 10 $ml.l^{-1}$ (Nel *et al.*, 1985);
* A cysteine shock medium (CYS-shock-medium, Moosbrugger *et al.*, 1992) which consisted of 12.5 $mg.l^{-1}$ cysteine in sterile, distilled water;
* A slime producing *Propionibacterium thoenii* culture originally isolated from an anaerobic digester (PAB-shock-medium), cultivated in 10 ml YEL-medium (Riedel & Britz, 1993) for 48 h and then transferred to 500 ml YEL-medium until a final cell concentration of $10^4$ $cfu.ml^{-1}$ was obtained; and
* A combined cysteine (12.5 $mg.l^{-1}$ cysteine) and *Propionibacterium thoenii* culture ($10^4$ $cfu.ml^{-1}$) (CYS-PAB-shock-medium).

The pH of the media was poised at 7.0 using a 1 N NaOH solution and the media were all steam sterilized at 121°C for 15 min.

*Experimental studies*

For each experimental study a set of inoculated control units were included where no additional shock was applied to the systems. For each control over the 14 d period, a 100 ml was withdrawn and replaced with a 100 ml sterile medium. Growth and activity were measured in terms of pH and volatile fatty acid profiles and increases in granule formation.

In the first Experimental Study (I), 400 ml of sterile Lactate (Y-medium) and Glucose (G-medium) media were each inoculated with 50 ml of sludge from the sewerage works and for each day up to day 6, a 100 ml was withdrawn and replaced

with a 100 ml of each sterile medium. On the sixth day, both the Y-medium and G-medium units were shocked with 200 ml of the LG-shock-medium. The rest of the growth period was continued with the normal growth medium and the 100 ml removal/replace procedure.

The second Experimental Study (II) was a repetition of the first study on the Y-medium and G-medium with the extra shock (200 ml of the LG-shock-medium) on day 6 and with an additional substrate in the form of the Fruit effluent (F-medium).

In the third Experimental Study (III), growth studies were conducted with Y-medium, G-medium and the F-medium units but over an extended period of 30 days with two shocks of 200 ml of the LG-shock-medium on days 6 and 18.

In the fourth Experimental Study (IV), a cysteine shock (200 ml of the CYS-shock-medium) was applied to each of the Y-medium, G-medium and the F-medium on day 6.

In the fifth Experimental Study (V), Y-medium, G-medium and the F-medium were each shocked with combinations of the slime producing *Propionibacterium* strain (PAB-shock-medium). This study included: the control units without the shock treatment; an extra Lactate unit inoculated with the *Propionibacterium* at the start of the study with no further shocks; a *Propionibacterium* shock on the Y-medium, G-medium and the F-medium on day 6; a combined *Propionibacterium* and cysteine shock treatment on the Y-medium, G-medium and the F-medium on day 6; and a combined *Propionibacterium* and LG-medium shock on a separate Fruit unit on day 6.

*Analytical procedures*

The amount of granules formed (per 10 ml of the contents of each batch unit) during each experimental study was determined by direct and physical counts of diluted samples in a round flat-bottomed glass container with a 2 x 2 cm graded grid underneath.

The operational parameters that were monitored according to Standard Methods (1985) included: pH; chemical oxygen demand COD; Total Solids (TS); Total Non-Volatile Solids (TNVS); and Total Suspended Solids (TSS).

Volatile fatty acids (VFA) were determined using a Varian Model 3700 gas chromatograph equipped with a flame ionization detector and a bonded phase 007FFAP (Quadrex Corp., New Haven) fused silica capillary column (30 m x 0.32 mm). The column temperature was initially held at 105°C, then increased at a rate of 10°C per min to 219°C and held for 5 min. The injector temperature was set at 250°C and the detector at 260°C. Nitrogen was used as carrier gas at a flow rate of 2.5 $ml.min^{-1}$. A sample volume of 0.5 microlitre was introduced via the injector port. A calibration standard was made up of acetic (Merck), propionic (Merck), *i*-butyric (Merck), butyric (Merck), *i*-valeric (Merck) and n-valeric acids added to 0.5 ml 0.1 N HCl with an internal standard of n-hexanol (24%). The internal standard was chosen because its polarity and boiling point allowed a suitable retention time close to the fatty acids.

## 4.4 Results and discussion

*Experimental Study I*

pH Profiles: The pH was measured to indicate metabolic activity during the growth period of the cultures. In the first Experimental Study (I), units with Glucose (G)

and Lactate (Y) as carbon sources were inoculated with anaerobic sludge (An) and one set was used as control. The duplicate control units for both the Y-medium and G-medium showed the characteristic drop in pH within the first 48 h (Fig. 1A) and recovery profiles as was found in Chapter 3. The pH of the Glucose units dropped from 7.0 to 6.4 within a day and then showed an increase to 6.6 - 6.7 whereafter it stabilized around 6.65 for the rest of the growth period. In the case of the Lactate units, the pH decreased from 7.0 to 6.45 by day 2 and then steadily increased to about 7.0 - 7.1 whereafter it remained stable until day 14.

In the second part of the study when the shock was applied to the units (Fig. 1B), the data showed similar profiles up to day 6 (Fig. 1A). On day 6, when the shock was applied to the system, the pH increased to 7.1 for the Lactate units, but remained fairly stable for the Glucose units. From days 6 to 8, a slight drop in pH was found with the Lactate units and for the Glucose units from 6.8 to 6.7. These decreases can be the result of the increase in the amount of volatile fatty acids produced, as a result of an increase in the metabolic activity in the systems due to the shocks applied. By day 10 the glucose units had stabilized at around 6.7 but the pH of the Lactate units appeared to be still increasing.

VFA Profiles: The VFA profiles for the Lactate and Glucose units inoculated with anaerobic sludge are shown in Fig. 2A and 2B. In the case of the Lactate unit (Fig. 2A), a sharp increase in the amount of acetic and propionic acids were found within the first three days. After day 4, the concentrations of these two acids started to decline and the shock treatment on day 6 did not show any change on the propionic acid profile. In the case of the acetic acid, an increase was found on day 6 just after the shock had been applied, suggesting a slight increase in the activity of the system. A decline was then found for the acetic acid, which continued for the rest of the experimental period. Both the butyric and valeric acids steadily increased until day 11 whereafter they were found to decrease and stabilize. Under conditions where no shock treatment was applied, the VFA profiles showed the same pattern as found in Chapter 3.

In the case of the Glucose units (Fig. 2B), no major changes in the values of acetic and propionic acids were found and the data corresponded well with that found in Chapter 3. After the shock treatment, the systems did not show any particular increase in the amount of acids produced, except for the butyric acid, which showed an increase from day 7 onwards.

Granule formation: In the first experimental study, the two growth media were shocked with the LG-shock-medium on day 6. Before the application of the shock treatment (Fig. 3A), the Lactate medium gave a granule increase of about 1 000% by day 14 (Fig. 3A and Table 1), while the Glucose units yielded a 750% increase by day 10. After the shock treatment (Fig. 3B), the Lactate units did not show any dramatic increase but a drop of about 100% by day 14 in the number of granules counted. In contrast, the Glucose units gave an increase of 1 400% by day 14 in granule numbers after the shock. The data obtained for the mass of the granules are given in (Fig. 4A and 4B). Before the shock treatment the granule mass of both the Lactate and Glucose units remained constant after day 2 for the rest of the study period (Fig. 4A). After the shock treatment (Fig. 4B), the granule mass yield showed a decrease for the Lactate units and an increase for the Glucose units. The data obtained (Fig. 3 and 4) shows that the most active time for granule formation appears to be within the first 10 days of growth.

fig4-1

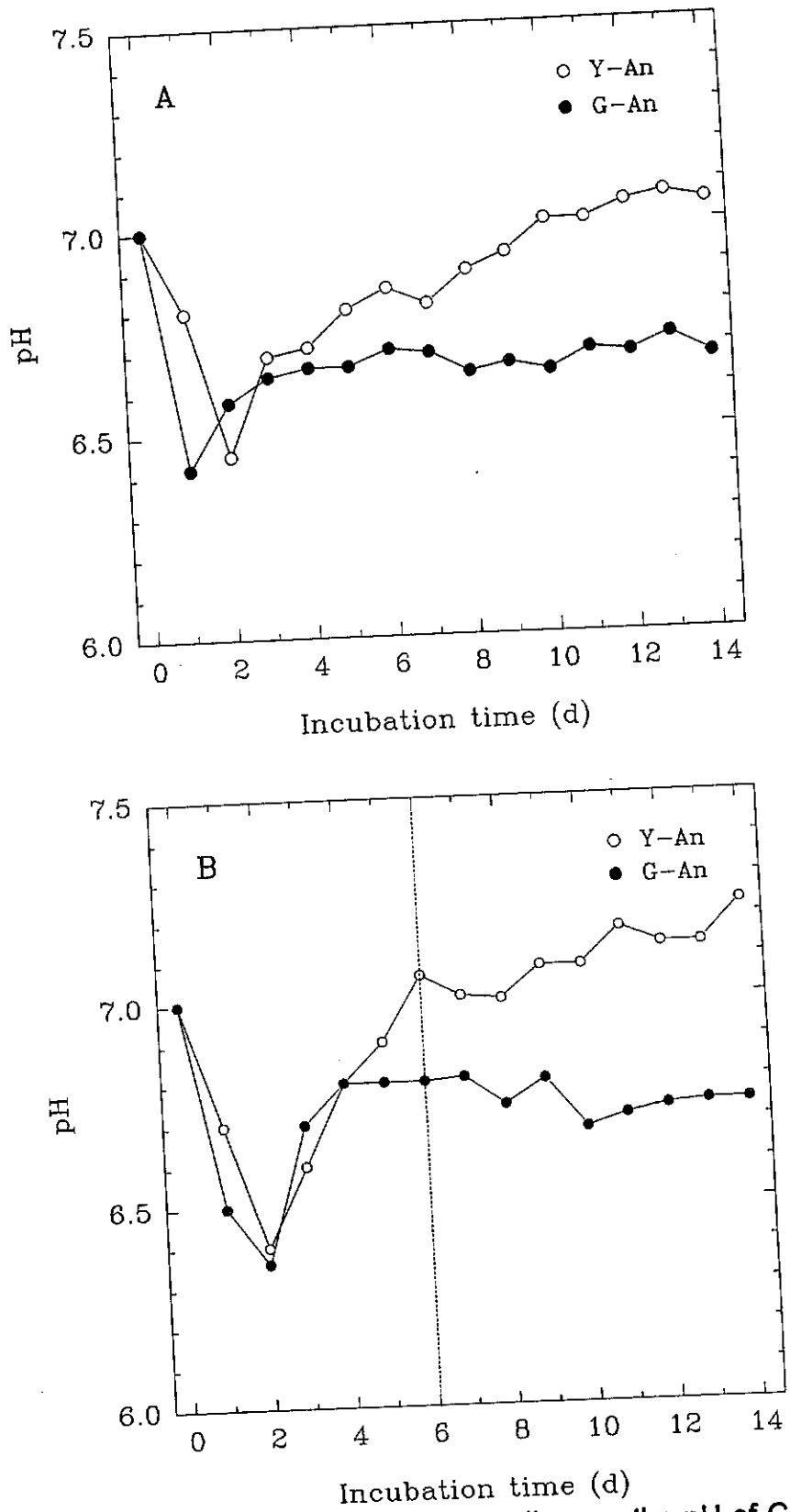


**Figure 1.** The influence of the LG-shock-medium on the pH of Glucose and Lactate batch units (A = without the shock treatment, B = after the shock treatment). The dotted line represents the application of the shock treatment. (Carbon sources: Lactate = Y; Glucose = G) (Inoculum type: Anaerobic sludge = An).

fig4-2

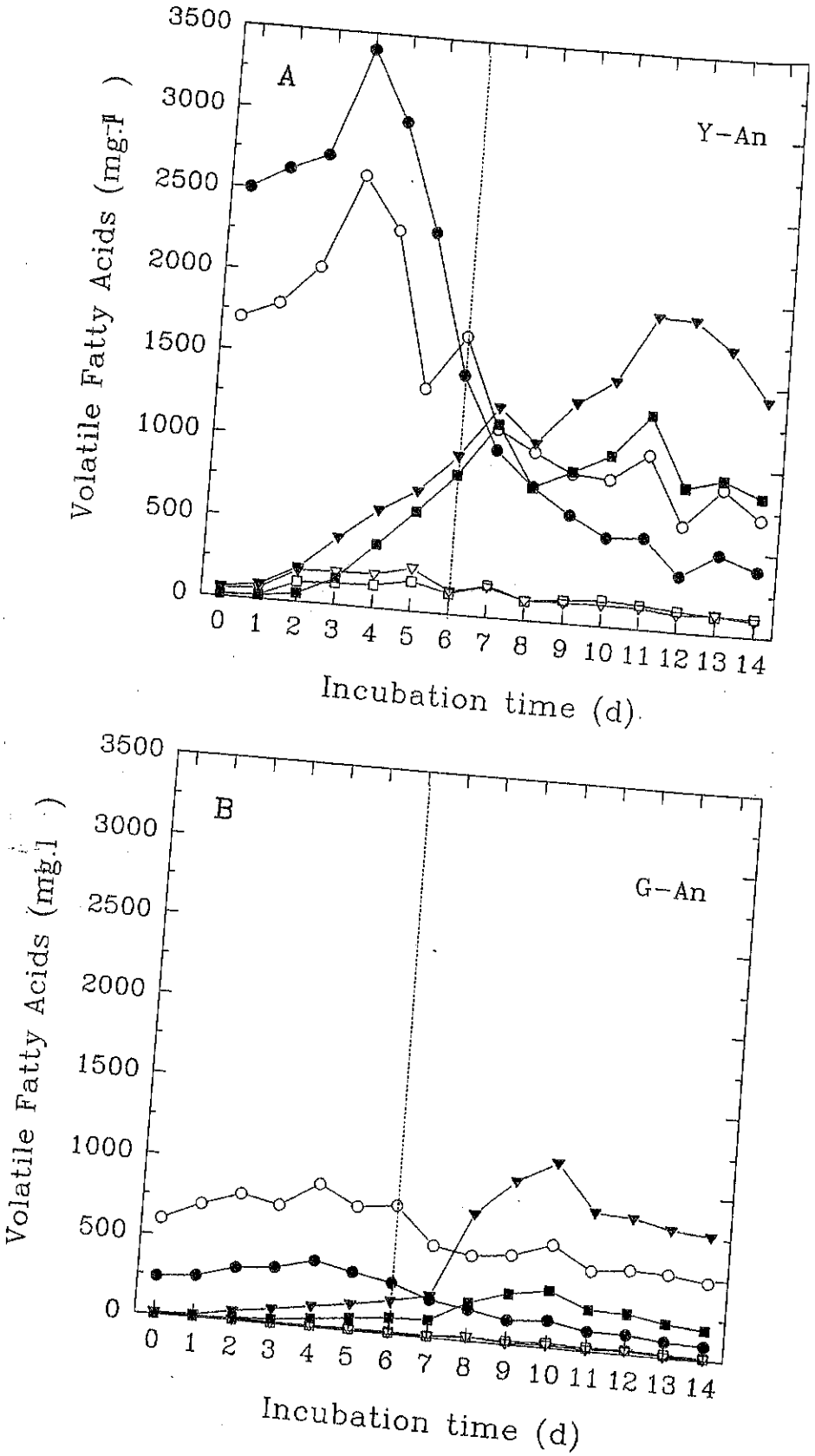


**Figure 2.** Influence of LG-shock on VFAs formed in the Glucose and Lactate units. The dotted line = application of the shock treatment on day 6. (Carbon sources: Lactate = Y; Glucose = G) (Inoculum type: Anaerobic sludge = An). (Acids: ○ = Acetic, ● = Propionic, ▼ = *Iso*-Butyric, ■ = Butyric, □ = *Iso*-Valeric, ▽ = Valeric) (Inoculum type: Anaerobic sludge = An).

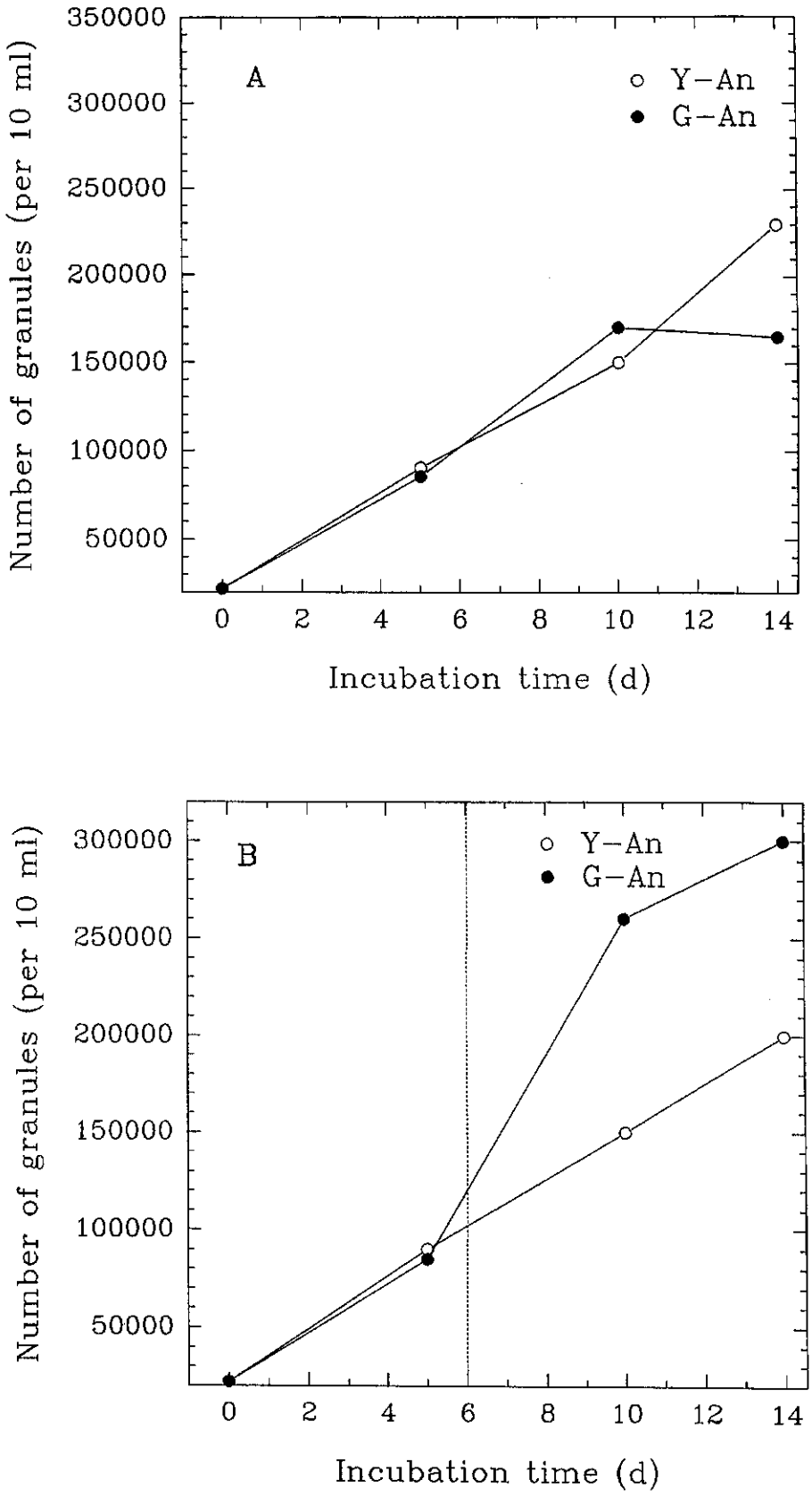


**Figure 3.** The influence of the LG-shock-medium on the granule formation in the Glucose and Lactate batch units (A = without the shock treatment; B = after the shock treatment). The dotted line represents the application of the shock treatment on day 6. (Carbon sources: Lactate = Y; Glucose = G) (Inoculum type: Anaerobic sludge = An).

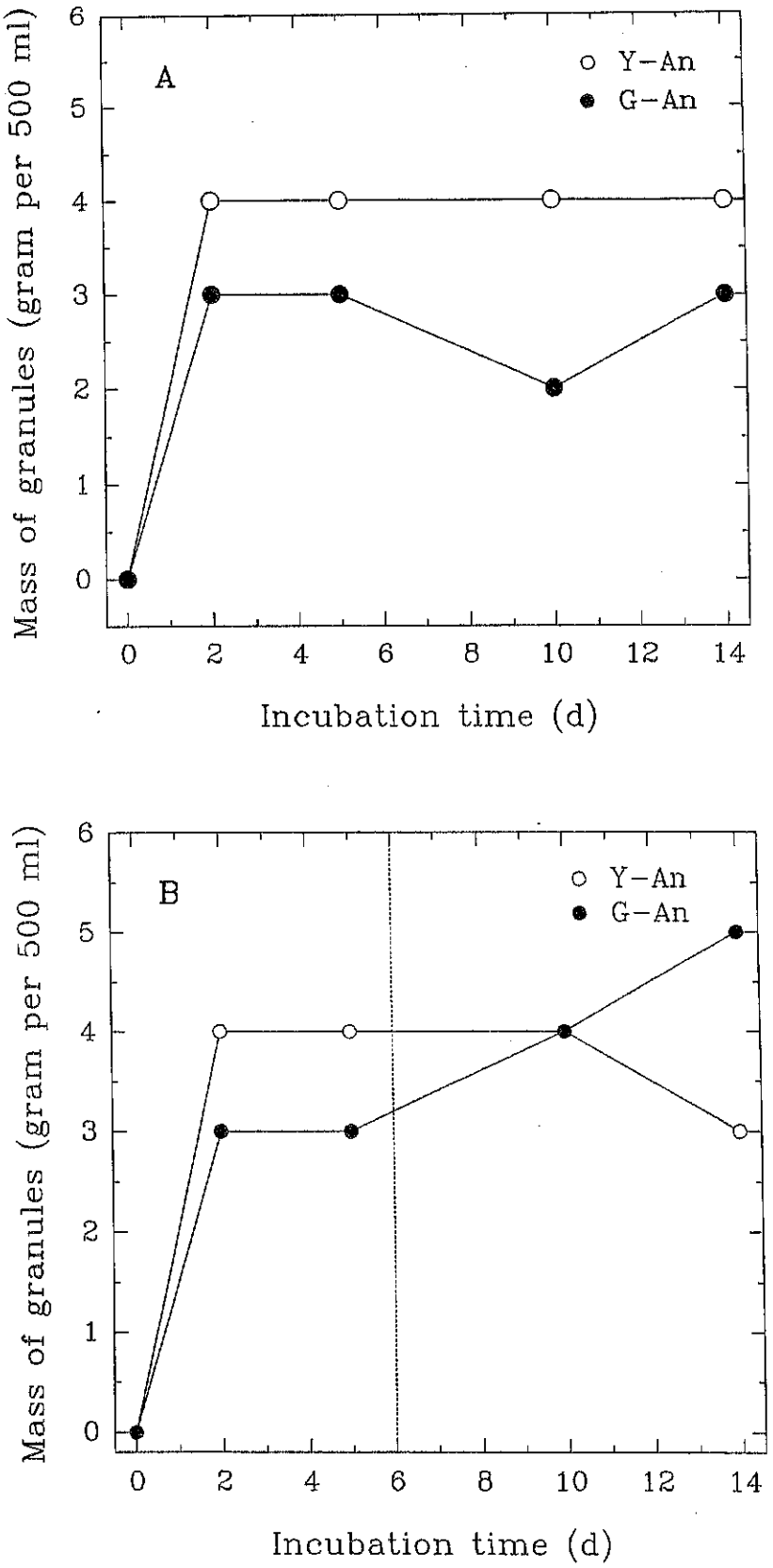


**Figure 4.** The influence of the LG-shock-medium on the granule mass yield in the Glucose and Lactate batch units (A = without the shock treatment; B = after the shock treatment). The dotted line represents the application of the shock treatment on day 6. (Carbon sources: Lactate = Y; Glucose = G) (Inoculum type: Anaerobic sludge = An).

*Experimental Study II*

pH Profiles: The second Experimental Study (II) was a repetition of the first on the Y-medium and G-medium and with the inclusion of an additional substrate (Fruit effluent). In this study, the pH again showed the characteristic drop within the first 48 h as found in Chapter 3. In the case of the Lactate (Y) units (Fig. 5A), the pH dropped from 7.0 to 6.46 within the first two days and for the Glucose and Fruit effluent units, from 7.0 to 6.4 and 7.0 to 5.8 respectively within 24 h. For the rest of the incubation period the pH of the Lactate unit steadily increased and stabilized around 7.1. For the Glucose unit, a slight climb to pH 6.75 was found by day 3 followed by a stabilization around 6.5. For the Fruit unit, the recovery was slower with a final stabilization evident only after days 13 -16. The much more drastic fall and slower recovery in the pH of the Fruit effluent unit is probably due to the higher concentration of readily degradable fruit sugars in this type of effluent.

In the second part of this study (Fig. 5B), the characteristic pH drop and recovery of the pH in the three units was again experienced. After the shock treatment was applied on day 6, the profile of the Lactate and Glucose units remained very similar to the ones where no shock had been applied. However, in the case of the Fruit units, the pH steadily decreased and reached 6.46 by day 16 (Fig. 5B). This appears to indicate that the shock was too drastic for the microbial populations to recover.

Granule formation: In this study the granule formation profiles for the Lactate and Glucose units (Fig. 6A) were similar to that found in Experimental Study I (Fig. 3A) but the percentage increases were smaller. The smaller increases can be attributed to different batches of anaerobic sludge being used. In the case of the Fruit effluent, a granule increase of 600% (Table 1) was found which was very similar to the profile obtained when using the Lactate as carbon source.

When the shock was applied on day 6 (Fig. 6B), a similar profile was obtained for the Glucose units in that an increase was found after day 10 in contrast to the stabilization found when no shock was applied (Fig. 6A). In the case of the Lactate and Fruit units there was again an increase after the shock but after day 10 there was a stabilization and even small decrease. In the case of the Lactate units, the granules rather appeared to aggregate into larger granules but this was difficult to determine visually and it is clear that another method will have to be used to confirm this fact. In the case of the fruit effluent units the granules appeared to be disintegrating rather than aggregating. The pH of this unit was also found to decrease (Fig. 5B) and the more unfavourable pH environment could possibly have played a role in the granule disintegrating situation.

*Experimental Study III*

pH Profiles: In the third Experimental Study (III), studies were again conducted in the Lactate, Glucose and the Fruit units but over an extended period of 30 days with shocks of 200 ml of the LG-shock-medium, on days 6 and 18. In this study (III), data similar to data from studies I and III, were obtained. Before the double shock treatments were applied to the batch units (Fig. 7A), the characteristic pH profiles were again found for all three the media. The extended operational period of up to 30 days showed that all three systems had reached a stable state and could probably be maintained indefinitely.

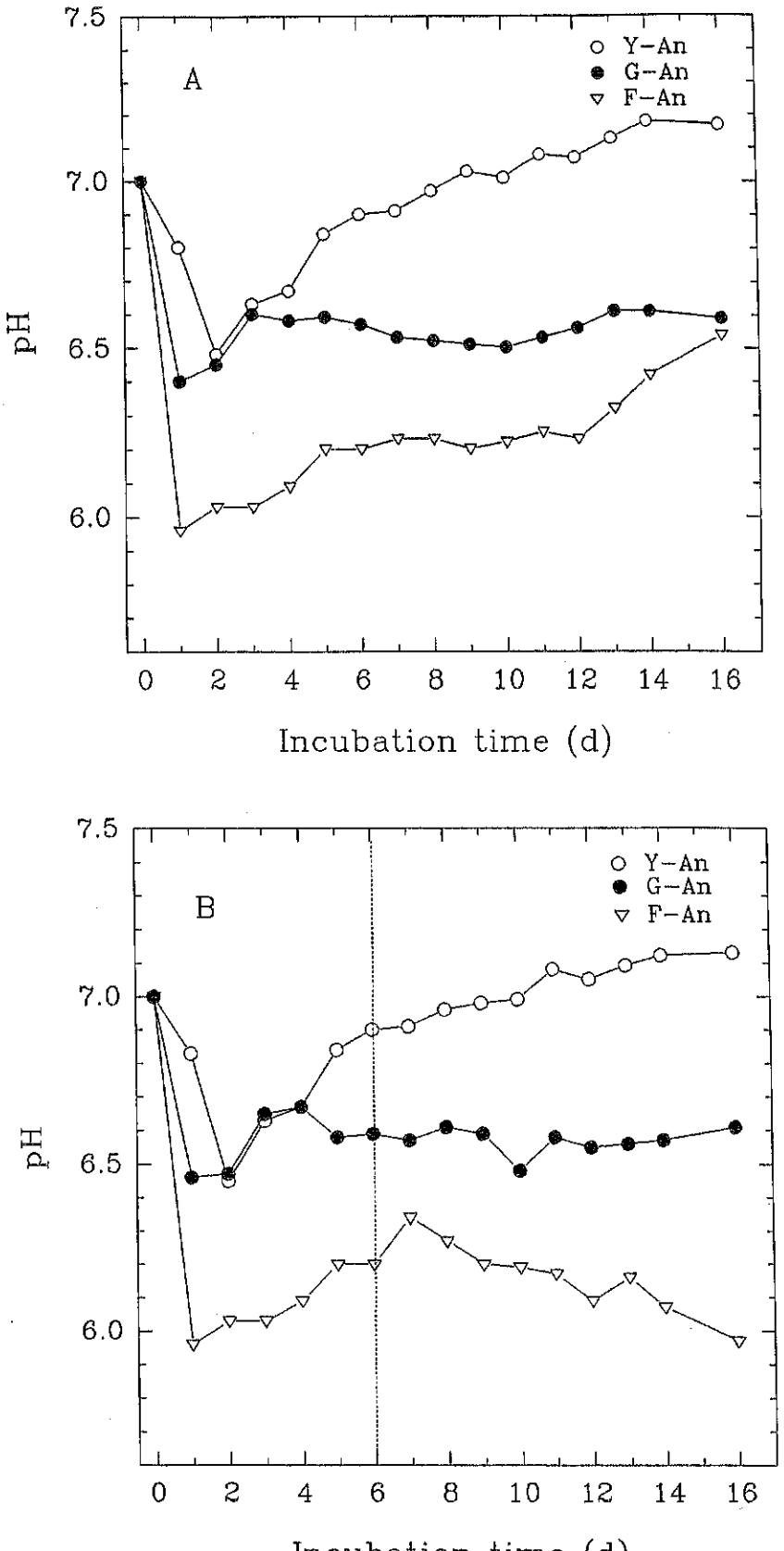


**Figure 5.** The influence of the LG-shock-medium on the pH of the Glucose, Lactate and Fruit effluent batch units (A = without the shock treatment; B = after the shock treatment). The dotted line represents the application of the shock treatment on day 6. (Carbon sources: Lactate = Y; Glucose = G; Fruit effluent = F) (Inoculum type: Anaerobic sludge = An).

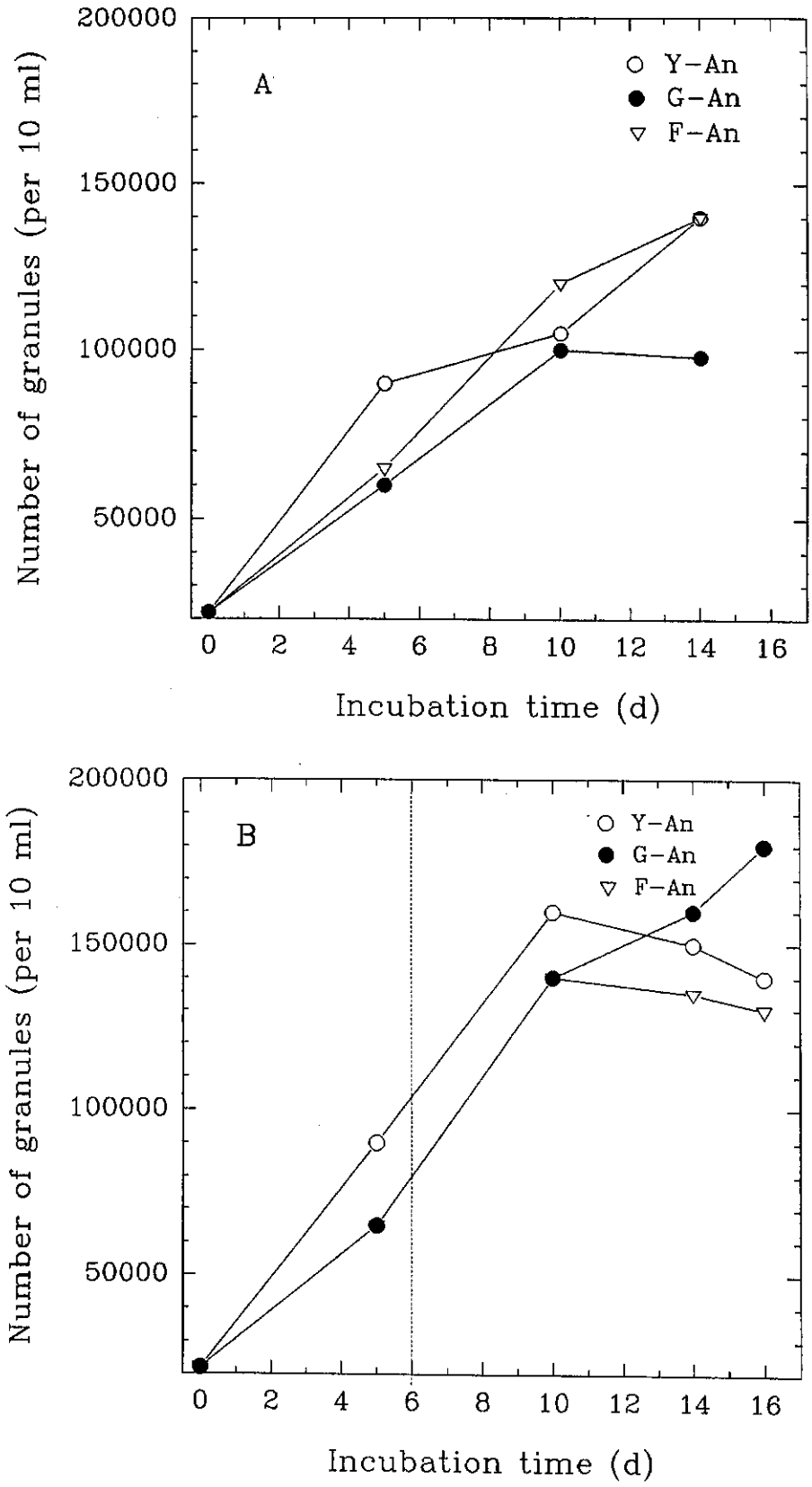


**Figure 6.** Influence of LG-shock-medium on the amount of granules formed in the Glucose, Lactate and Fruit units (A = without shock treatment; B = after shock treatment). The dotted line represents the application of the shock treatment on day 6. (Carbon sources: Lactate = Y; Glucose = G; Fruit effluent = F) (Inoculum type: Anaerobic sludge = An).

**Table 1.** Maximum percentage increase in granulation before and after the different shock conditions during the Experimental Studies. (The time of maximum granulation is given in parenthesis).

| Study | Before shock | After shock |
|---|---|---|
| Study I - Figure 3, LG-shock day 6 | | |
| Y-An | 1 050% (14 d) | 900% (14 d) |
| G-An | 750% (10 d) | 1 400% (14 d) |
| Study II - Figure 6, LG-shock day 6 | | |
| Y-An | 600% (14 d) | 700% (10 d) |
| G-An | 400% (10 d) | 800% (16 d) |
| F-An | 600% (14 d) | 600% (10 d) |
| Study III - Figure 8, LG-shock days 6 and 18 | | |
| Y-An | 600% (14 d) | 700% (14 d) |
| G-An | 400% (10 d) | 800% (16 d) |
| F-An | 700% (18 d) | 875% (29 d) |
| Study IV - Figure 10, CYS-shock day 6 | | |
| Y-An | 518% (14 d) | 436% (10 d) |
| G-An | 459% (10 d) | 590% (19 d) |
| F-An | 681% (19 d) | 1127% (10 d) |
| Study V - Figure 12, *different Propionibacterium* shocks | | |
| Y-An + PAB from start | 550% (5 d) | - |
| Y-An + PAB day 6 | 645% (10 d) | 460% (5 d) |
| G-An + PAB day 6 | 615% (5 d) | 615% (5 d) |
| F-An + PAB day 6 | 865% (14 d) | 800% (5 d) |
| Y-An + PAB + CYS day 6 | 645% (10 d) | 460% (5 d) |
| G-An + PAB + CYS day 6 | 615% (5 d) | 615% (5 d) |
| F-An + PAB + CYS day 6 | 865% (14 d) | 800% (5 d) |
| F-An + PAB + LG day 6 | 865% (14 d) | 800% (5 d) |

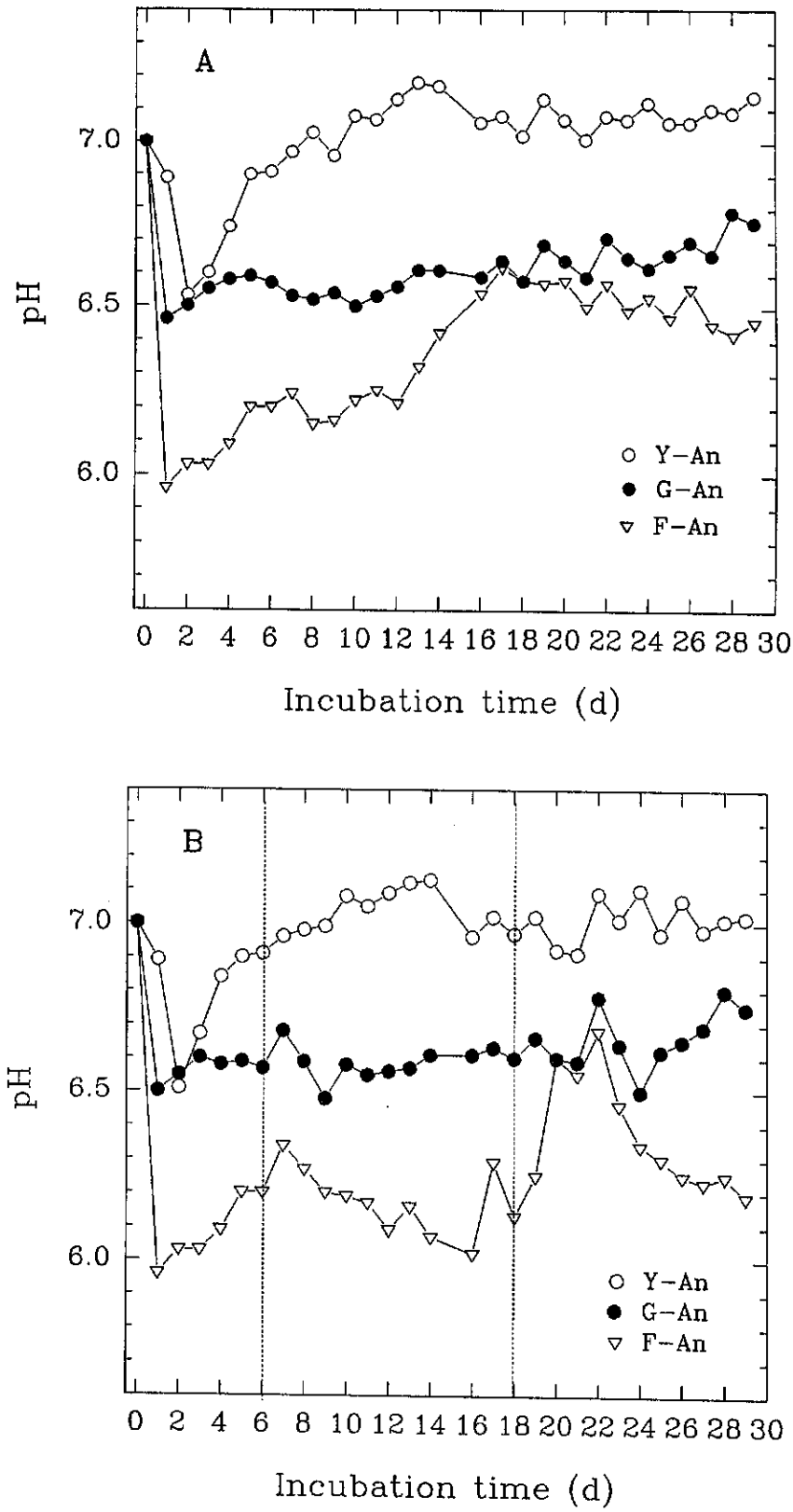


**Figure 7.** Influence of a double LG-shock on the pH of Lactate, Glucose and Fruit units over a period of 30 days (A = without the shock treatment; B = after the shock treatment). The dotted line represents the application of the shock treatments on days 6 and 18. (Carbon sources: Lactate = Y; Glucose = G; Fruit effluent = F) (Inoculum type: Anaerobic sludge = An).

When the double shock treatment was applied to the units (Fig. 7B) a repetition of data from the previous experiments (I and II) was again obtained. The Lactate and Glucose units did not show any major variations even after the second shock had been applied. The Fruit effluent unit showed the same decrease in pH as found previously and after the second shock, the pH increased up to day 22 whereafter the pH decreased and at the end of the study appeared to have stabilized at around 6.2.

Granule formation: In the first two studies (I and II), especially for the Lactate and Fruit units, it was found that there was a continuous increase in granulation up to day 14 and for the Glucose units, stabilization after day 10. The question then arose as to how long such an increase could be expected and how stable the granulation under the operational conditions would be.

The granule profiles obtained in this study (III) for all three batch systems were very similar to those from the previous studies (Fig. 8A) for the same period of 14 days with 400%, 600% and 700% increases (Table 1) respectively for the Glucose, Lactate and the Fruit systems. After this period it was found that all three systems stabilized (Glucose after 10 d, Lactate after 14 d and Fruit after 19 days) and remained so for the rest of the experimental period of up to 30 d.

With the first shock (Fig. 8B) on day 6, the Lactate and Glucose units were found to show an increase but started to decrease again after days 14 and 16 respectively. The Fruit unit was the first unit to show a decrease (after 10 days) but strongly increased after the second shock and reached an 875% increase at day 29. The other two units showed no positive response to the second shock, in contrast to the control systems (Fig, 8A) where the granulation stabilized, they were found to strongly decrease.

*Experimental Study IV*

pH Profiles: In this study (IV), a cysteine shock (200 ml of the CYS-shock-medium) was applied to each of the Lactate, Glucose and Fruit units on day 6. A Fruit effluent duplicate unit inoculated with anaerobic sludge from a separate municipal digester was also included to test the reproducibility. Before the application of the shock treatment (Fig. 9A), the same characteristic pH profiles for each of the three carbon sources were found. The application of the CYS-shock-medium on day 6 (Fig. 9B) did not lead to any major changes in the pH values of the three different systems examined. It was however found that the pH of all three systems stabilized much sooner and at lower values than had been found for the other experimental studies. The Lactate units stabilized around 6.7, the Glucose around 6.5 and the Fruit units around 6.2 (Fig. 9B).

Granule formation: The profiles of the changes in granule numbers found during this study before the cysteine shock was applied (Fig 10A) were similar to those from the first three studies (I, II and III). However, the increases were lower than found in the first study and this can probably be ascribed to differences in the batches of anaerobic sludge that was used as inoculum. The application of the CYS-shock-medium on day 6 (Fig. 10B) resulted in only small changes in the number of granules formed in the Lactate and Glucose units. In the case of the Fruit effluent, the cysteine shock led to a major increase (1 127%) in numbers by day 10 with a slow decrease up to day 19 (990%) (Table 1).

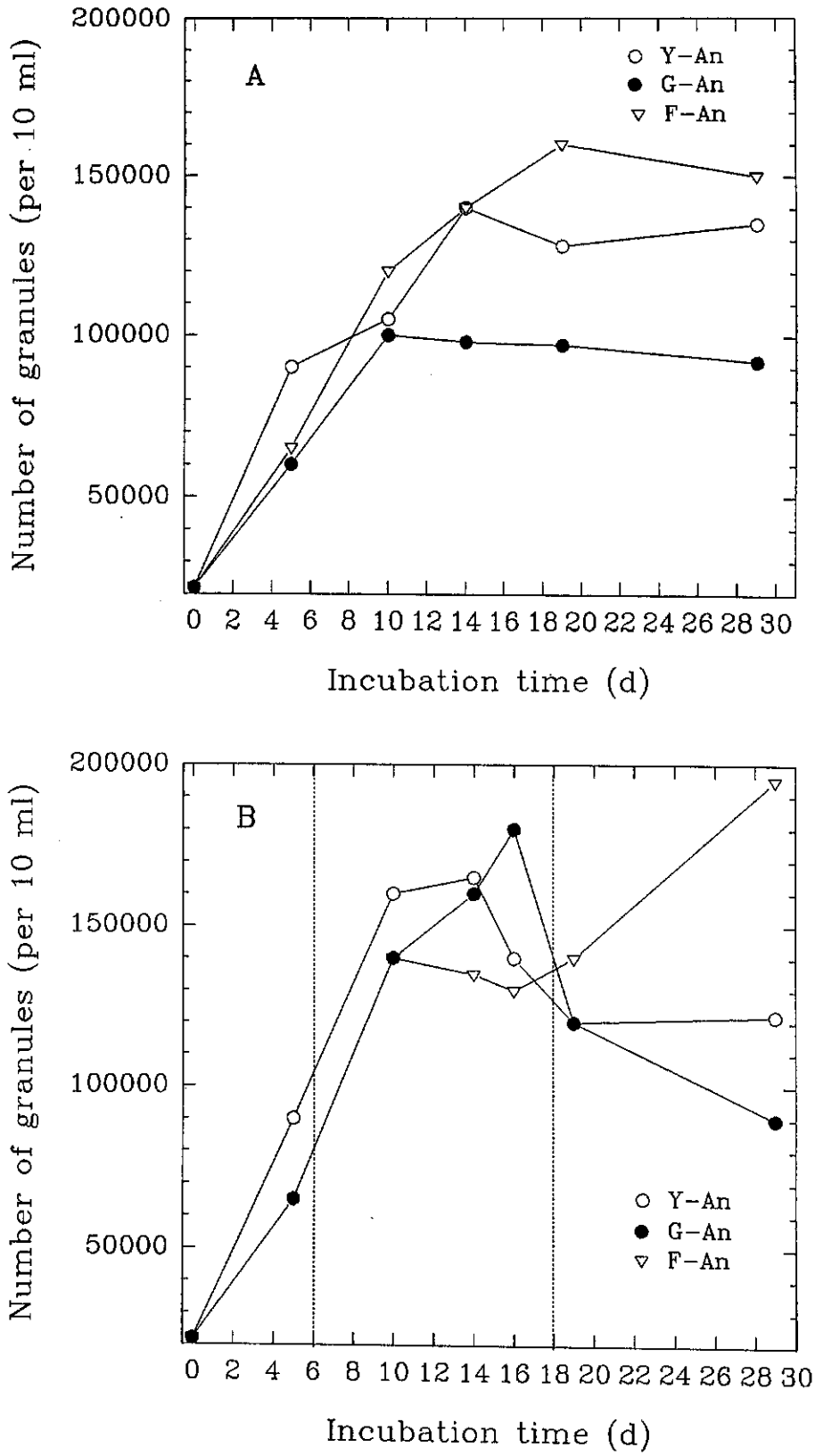


**Figure 8.** Influence of a double LG-shock on the amount of granules formed in the Lactate, Glucose and Fruit units over a period of 30 days (A = without shock treatment; B = after double shock treatment). The dotted lines represent the application of the shock treatments on days 6 and 18. (Carbon sources: Lactate = Y; Glucose = G; Fruit effluent = F) (Inoculum type: Anaerobic sludge = An).

fig4-9

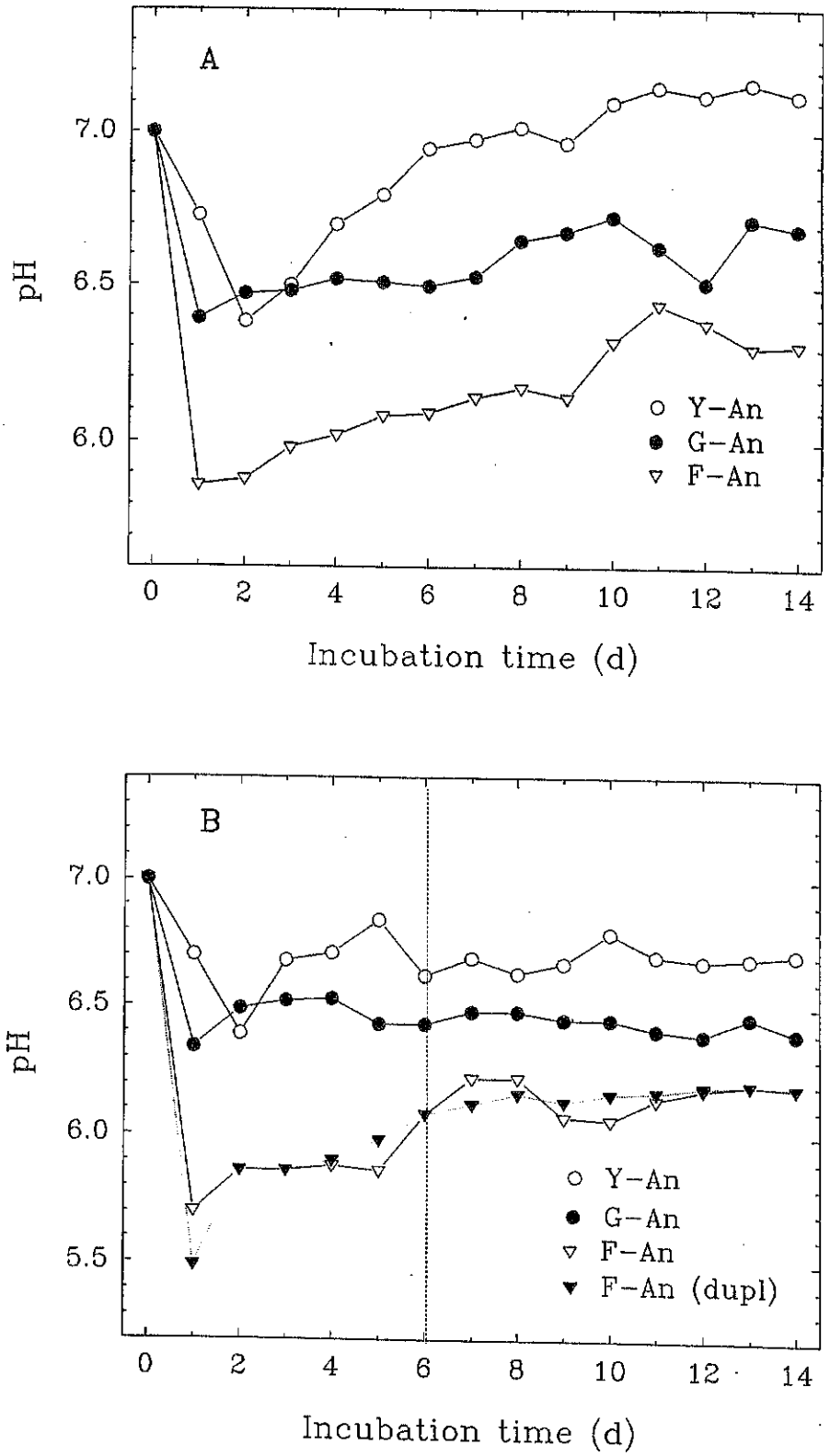


**Figure 9.** Influence of CYS-shock-medium (cysteine) on the pH of Lactate, Glucose and Fruit units over a period of 20 days. (A = without the shock treatment; B = after the shock treatment). The dotted line represents the application of the shock treatment on day 6. The Fruit effluent was done in duplicate (F-An (dupl)). (Carbon sources: Lactate = Y; Glucose = G; Fruit effluent = F) (Inoculum type: Anaerobic sludge = An).

fig4-10

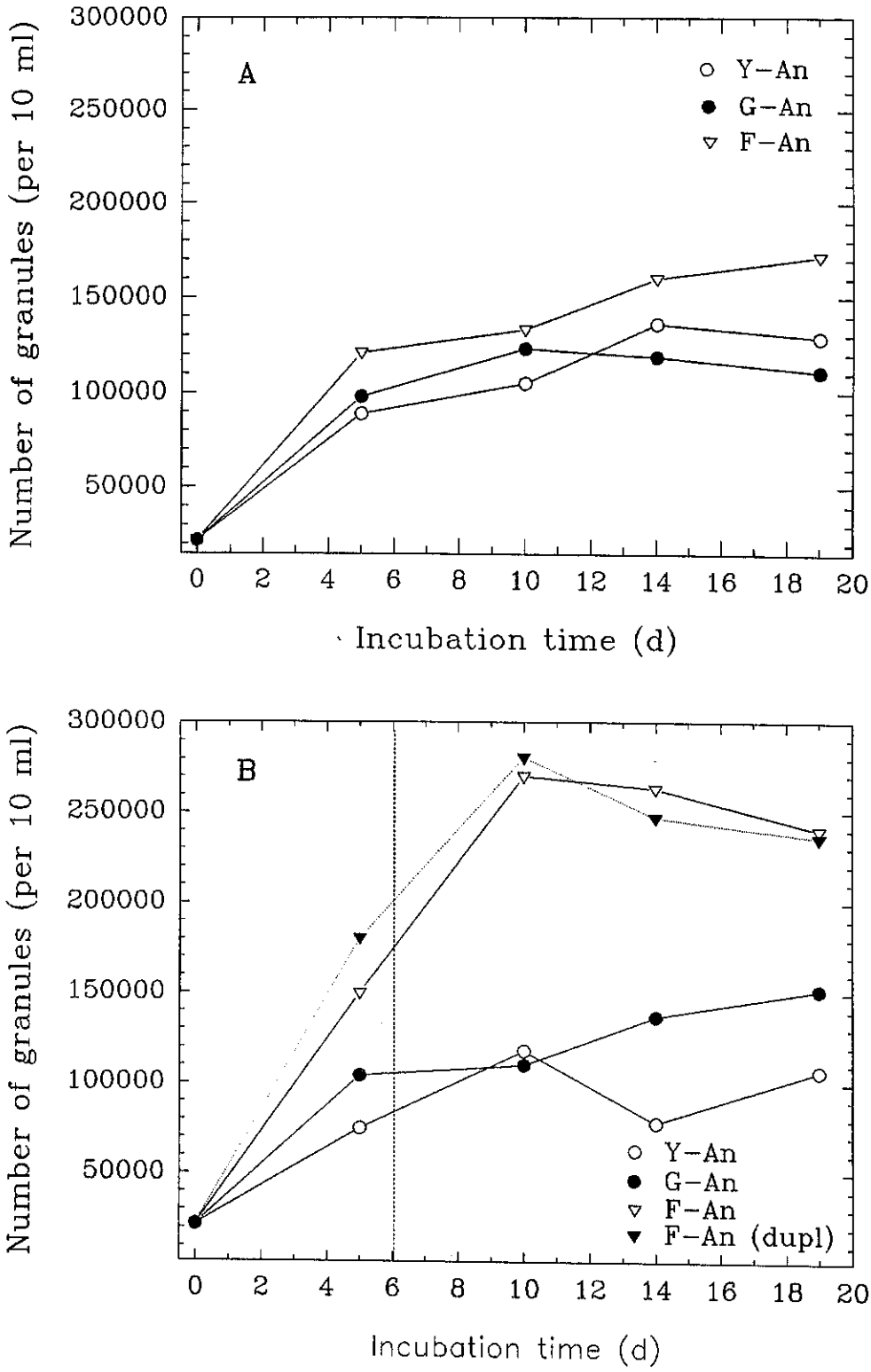


**Figure 10.** Influence of the CYS-shock-medium (cysteine) on the amount of granules formed in Lactate, Glucose and Fruit units over a period of 20 days. (A = without shock treatment; B = after shock treatment). The dotted line represents the application of a shock on day 6. The Fruit effluent was done in duplicate (F-An (dupl)). (Carbon sources: Lactate = Y; Glucose = G; Fruit effluent = F) (Inoculum type: Anaerobic sludge = An).

*Experimental Study V*

Since it has been postulated (Riedel & Britz, 1993) that propionic acid producing bacteria could possibly play a major role in the granule formation process, a set of experiments were included where a slime producing *Propionibacterium* strain isolated from an anaerobic digester, was included. In the fifth Experimental Study (V), each of the Lactate-medium, Glucose-medium and the Fruit-medium were shocked with combinations of the slime producing *Propionibacterium* strain (PAB-shock-medium). These included the control units; Fruit effluent with *Propionibacterium* added at the start; a *Propionibacterium* shock on day 6; a combined *Propionibacterium* and cysteine shock; and the Fruit effluent with a combined *Propionibacterium* and LG-shock-medium.

pH Profiles: Before the application of the different shock treatments (Fig. 11A), the characteristic pH profiles were again found. The Lactate unit inoculated with the slime producing *Propionibacterium* strain right at the start of the experiment gave the same profile as the Lactate control unit but the final pH stabilization was found to be lower than that of the controls. It was also found that this unit only reached the lowest pH value on the profile a day after that of the control.

In the cases where the units were given a *Propionibacterium* shock on day 6 (Fig. 11B), the profiles were again similar to the controls but it was found that they also stabilized at values of about 0.5 pH units lower than those from the controls. Similar results were found for both the units that received the combined *Propionibacterium*/cysteine shock treatment (Fig. 11C) and the one Fruit unit that was shocked with the *Propionibacterium*/Lactate combination (Fig. 11D).

Granule formation: The profiles of the changes in granule numbers found during this study before the cysteine shock was applied (Fig. 12A), were similar to those from the first three studies (I, II and III). The Lactate unit that was inoculated at the start with the *Propionibacterium* strain (Fig. 12A) showed a similar increase in granules up to day 5 (550% increase - Table 1) whereafter a decrease was found in contrast to the control Lactate unit where an increase of 645% was found up to day 10. In the case of all the shocks where *Propionibacterium* was included (Fig. 12B, 12C and 12D), a very characteristic increase similar to the controls was found up to day 5 whereafter a drastic decrease by day 10 was followed by a smaller increase on day 14. It was thus concluded that the direct addition of this propionic acid producing strain did not enhance the granulation process over the 14 day study period.

## 4.5 Conclusions

In the previous Chapter the influence of different substrates was investigated to determine which would lead to the formation of propionic acid as major metabolite. Based on the results found it was decided to investigate the influence of multi-shock conditions on the basic growth substrates so as to stimulate granule formation. The results from this study showed that for the control units the characteristic acetic, propionic, pH and granule formation profiles were again obtained, confirming the hypothesis of Riedel & Britz (1993) that by changing environmental conditions on batch scale (Bossier & Verstraete, 1996), the propionic acid producers can be given a competitive advantage that leads to enhanced granulation. The data from the studies where shocks were applied (LG-shock-medium) showed that a further enhancement of the granulation process could be facilitated. In the case of the double shock and the

cysteine shock, a stimulation of only the Fruit effluent was found and with the *Propionibacterium* combination shocks, no clear enhancement was found.

One of the problems experienced in the previous study (Chapter 3) was the quality of the anaerobic sludge that was used as inoculum. For this reason the inoculum used in all these experimental studies was standardized to give an inoculum granule concentration of about 20 000 per 10 ml based on the visual counting method. This standardization of the inoculum size did lead to reproducible results but only when the studies were done using inoculum from the same batch and same age. It was found that when repeating studies but using different inoculum batches, even from the same anaerobic digester, the increases in granule numbers could vary fairly widely. In most cases the granules were small and dense making it very difficult to visually count and another more accurate method will have to be found to enumerate the growth of granules.

fig4-11

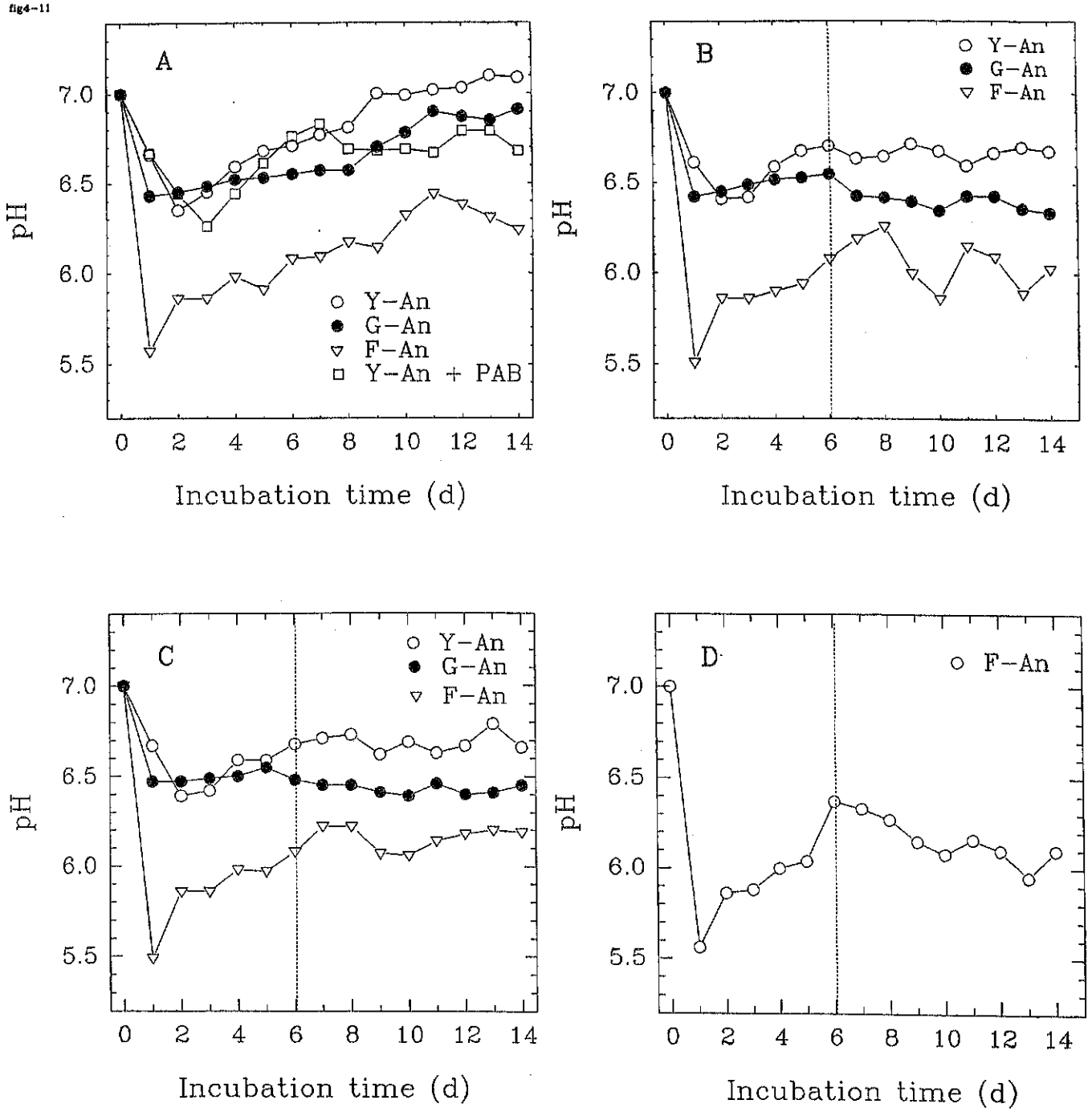


**Figure 11.** Influence of combinations of a slime producing *Propionibacterium* strain shock treatments on the pH of Lactate, Glucose and Fruit units (A = without a shock plus the fruit effluent with *Propionibacterium* added at the start of the study; B = after *Propionibacterium* shock on day 6; C = after combined *Propionibacterium* and cysteine shock; and D = after combined *Propionibacterium* and LG-shock). The dotted line represents the application of the shock treatments on day 6. (Carbon sources: Lactate = Y; Glucose = G; Fruit = F) (Inoculum type: Anaerobic sludge = An).

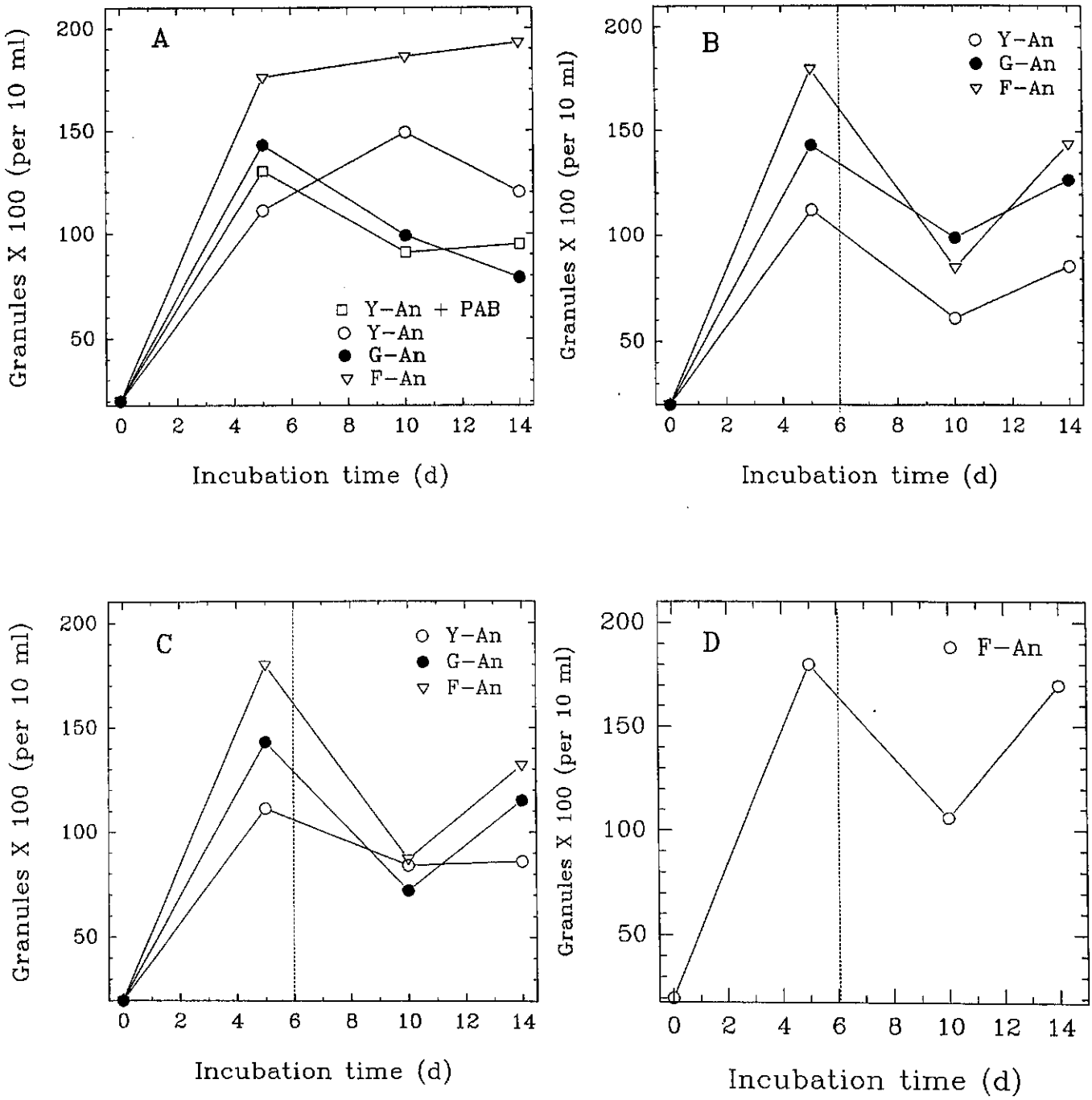


**Figure 12.** Influence of combinations of a slime producing *Propionibacterium* strain shock treatments on the amount of granules formed in Lactate, Glucose and Fruit units (A = without shock treatment plus the fruit effluent with the *Propionibacterium* added at the start of the study; B = after a *Propionibacterium* shock on day 6; C = after a combined *Propionibacterium* and cysteine shock treatment; and D = after a combined *Propionibacterium* and LG-shock). The dotted line represents the application of the shocks on day 6. (Carbon sources: Lactate = Y; Glucose = G; Fruit effluent = F) (Inoculum type: Anaerobic sludge = An).

# CHAPTER 5

# INFLUENCE OF HIGHER ORGANIC LOADING RATES AND SHORTER HYDRAULIC RETENTION TIMES ON THE EFFICIENCY OF AN UASB BIOREACTOR TREATING A CANNING FACTORY EFFLUENT

## 5.1 Summary

A mesophylic laboratory-scale upflow anaerobic sludge bed bioreactor design was evaluated for the treatment of a carbohydrate-rich effluent from the canning industry. The bioreactor was inoculated with 500 g of anaerobic granules and after the system had stabilized the HRT was set at 24 h and the substrate pH poised at 8.0 to prevent rapid acidification. In the first experimental study the chemical oxygen demand (COD) was increased stepwise from 2 300 to a full strength of 4 000 $mg.l^{-1}$. In the second study the organic loading rate was increased by shortening the HRT (24 to 8 h) to give an organic loading rate increase from 3.95 to 10.95 $kgCOD.m^{-3}.d^{-1}$ with an average COD removal of 90 - 93% and removal rate of 9.8 $kgCOD.m^{-3}.d^{-1}$. However, the recovery rate of the system at HRT values below 10 h was found to be very slow suggesting that the system had reached its minimum HRT. This was confirmed by the stabilization of the granule bed. An HRT of 10 h was thus taken as the optimum operational HRT. Since neutralization costs would influence economic aspects of the process, the influence of lower pH values was investigated in the third study where the pH of the canning effluent was lowered from 8.0 to 5.0. At the lower pH the COD removal dropped drastically, the biogas production decreased and the digester effluent pH dropped to 6.2. It was clear from the slow recovery of the digester and the low COD removal (66.1%) that the lower end of the operational pH had been reached and any further lowering of the substrate pH would lead to system failure. The economic implication of being able to operate at pH 5.5 means that canning effluent can be introduced to the digester without any neutralization is considerable.

## 5.2 Introduction

All industries are increasingly required to reduce their impact on the environment. Adequate treatment of food processing effluents is assuming increasing importance as this industry addresses the issue of responsible environmental management (Wayman, 1996). For the food industry this is frequently difficult as factors related to seasonal operation, changes in plant effluent characteristics due to the processing of different products, nutritional deficiencies and the location of food processing plants, heavily impact the treatment efficiency. Furthermore, the growing concern over the quality and quantity of fresh water has forced higher surcharges and fines in an attempt to reduce the pollution loading on treatment facilities and environmental pollution. Many local

authorities are now insisting that industries undertake some form of effluent treatment so as to protect the environment.

Considerable interest has been shown in the application of anaerobic digestion to waste waters from the food industry since the nature and strength of the waste waters often provide the ideal conditions for digester operation. The waste waters have a high organic content, have little or no toxic material present (Kroyer, 1995) and include the situation where waste waters are produced over a short period of the year such as in the canning industry. Anaerobic processes have been shown to be amenable to such variations and in particular where complete shutdown may take place.

Among the high-rate anaerobic reactors developed and successfully applied in recent years (Lettinga *et al.*, 1997), the upflow anaerobic sludge blanket (UASB) reactor has become one of the most popular designs for the biological treatment of effluents in particular those from the food processing industries (Lettinga *et al.*, 1997). Many UASB reactors are in operation throughout the world (Schmidt & Ahring, 1996). The advantage of the UASB design is the ability to retain high biomass concentrations despite the upflow velocity of the waste water and the production of biogas. Consequently, the reactor can operate at short hydraulic retention times since the sludge retention time is almost independent of the hydraulic retention time. In UASB reactors, the biomass is retained as granules, formed by the natural self-immobilization of the bacteria. These granules have good settling abilities and vary in size from 0.14 to 5 mm depending upon the waste water used and operational conditions. The granules vary widely in shape, but they usually have a spherical form. The development of granular sludge is the key factor for successful operation of UASB reactors. Today it is possible to develop granules on a variety of waste waters and defined media, but there have been several reports on lack of granulation on specific waste waters (Wentzel *et al.*, 1994). Furthermore, some researchers have reported sudden disintegration of granules without any obvious reason.

The objective of this study was to assess the effects of shortening the hydraulic retention times on the overall performance and stability of an UASB reactor, while treating a canning factory effluent.

## 5.3 Materials and methods

*Digester design*

A laboratory-scale upflow anaerobic sludge blanket bioreactor (UASB) was used. The digester had an operational volume of 2.3 litre (total height of 830 mm and internal diameter of 50 mm) and combined a UASB design with an open gas/solids separator at the top of the bioreactor (Fig. 1). The biogas exited through the top, while the substrate was introduced into the bioreactor at the base. The overflow of the bioreactor emptied through a U-shaped tube to prevent any atmospheric oxygen from entering the system. The upflow velocity within the reactor was set at 2 $m.h^{-1}$. The temperature of the insulated bioreactor was maintained at 35°C using a heating tape and an electronic control unit (Meyer *et al.*, 1985). The volume of the biogas was determined using a manometric unit equipped with an electronically controlled counter and a gas-tight valve and the volumes corrected to standard temperature and pressure. The substrate was fed semi-continuously to the bioreactor by means of a peristaltic pump (Watson-Marlow 101) controlled by an electronic timer.

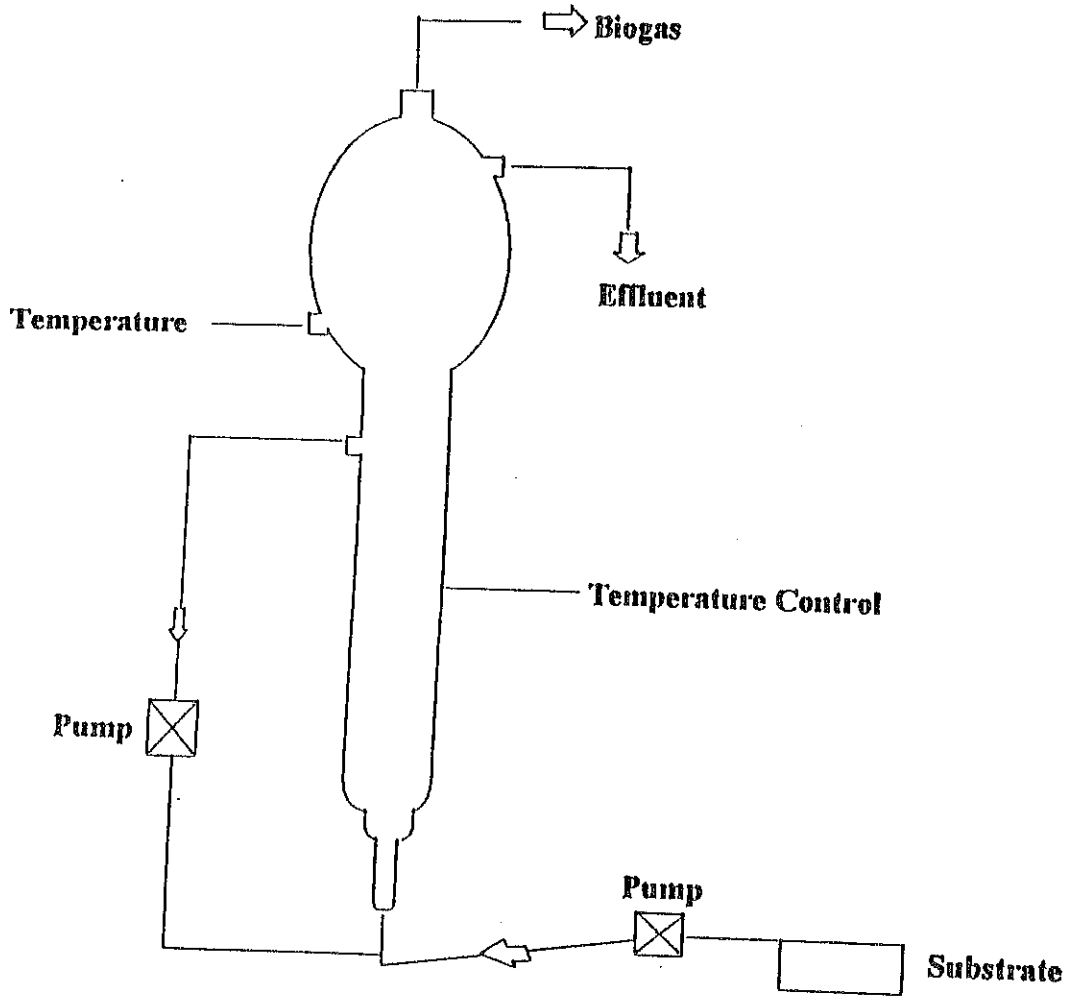


**Figure 1.** Laboratory-scale upflow anaerobic sludge blanket bioreactor.

*Bioreactor start-up*

The bioreactor was seeded with 500 g of water drained anaerobic granules obtained from batch reactors, to give a settled sludge-bed height of 300 mm. The bioreactor was then allowed to stabilize for 48 h in order to allow the bacterial community to acclimatize and fed with a diluted synthetic substrate (2 300 $mg.l^{-1}$ COD) and the HRT set at 24 h. After three weeks this was replaced with a fruit-canning factory effluent and the COD concentration was increased to 4 000 $mg.l^{-1}$.

*Substrate*

The composition of the synthetic substrate was ($mg.l^{-1}$): glucose 1 250; sodium lactate 5 000; acetic acid 10; urea, 500; and $K_2HPO_4$ 500. The substrate was also supplemented with 1.0 ml trace element solution (Nel *et al.*, 1985) and the pH poised at 8.5 with calcium hydroxide to optimize the environment for maximum granule growth. This substrate was then diluted to the required COD concentration.

The effluent from the fruit canning factory was sampled over the whole 1996/1997 canning season from the waste water stream before it reached the general stream that also contained the lye effluents (Mr. J. Visser, Ashton Canning Co, personal communication 1997).

*Analytical methods*

The following parameters were monitored according to the APHA (Standard Methods, 1985): pH; alkalinity; total solids (TS); total volatile solids (TVS); and total non-volatile solids (TNVS). COD, orthophosphate phosphorus and total Kjeldahl nitrogen were determined colorimetrically using a DR2000 spectrophotometer (Hach Co. Loveland, CO) and standardised procedures (Standard Methods, 1985). The general mineral analyses were done colorimetrically according to standard Hach procedures using a DR2000 spectrophotometer (Hach Co. Loveland, CO).

The total volatile fatty acids (TVFA) were determined using a Varian (Model 3700) gas chromatograph, equipped with a flame ionisation detector and a 30 m x 0.32 mm i.d. Fused Silica capillary column with 007 FFAP bonded phase (Quadrex Co. New Haven). The column temperature was initially held at 105°C for 5 min, then increased at a rate of 10°C $min^{-1}$ to 219°C. The detector and the inlet temperatures were set at 260°C and 250°C respectively and nitrogen gas was used as carrier gas at a flow rate of 2.5 $ml.min^{-1}$. The biogas composition was determined on a Fisons GC equipped with a thermal conductivity detector and 2.0 m x 3.0 mm i.d. column packed with Porapak Q (Waters Ass. Inc, Milford, MA), 80/100 mesh. The oven temperature was set at 55°C and helium was used as carrier gas at a flow rate of 40 $ml.min^{-1}$.

Carbohydrate composition was determined on a Dani GC equipped with a 2.0 m x 3.0 mm column packed with 1% OV-1 (Waters Ass. Inc, Milford, MA). The column temperature was initially held at 160°C, then increased at a rate of 5°C $min^{-1}$ to 193°C. The detector and the inlet temperatures were set at 250°C and 280°C respectively and nitrogen gas was used as carrier gas at a flow rate of 30 $ml.min^{-1}$.

*Experimental studies*

The study comprised three experimental studies (I to III). In the first study (I), the substrate COD concentration was increased stepwise from 2 300 to 4 000 $mg.l^{-1}$ in seven steps. In the second study (II), the COD concentration was kept constant at 4000 $mg.l^{-1}$, while the HRT was reduced stepwise from 24 to 8 h in 14 steps. In the third study (III), the HRT was reset at 10 h while the COD concentration was kept constant at 4 000 $mg.l^{-1}$. The substrate pH was then reduced stepwise from 8.5 to 5.0 in 7 steps. In all three studies, the bioreactor was allowed to reach stable-state conditions before each HRT or pH reduction. Stable-state is defined as a state, which can be maintained indefinitely without system failure (Cobb & Hill, 1990), during which the variation in bioreactor performance parameters is less than 10%. Thus, the length of each phase was based on the stability of the bioreactor effluent pH, alkalinity and COD removal.

## 5.4 Results and discussion

*Canning effluent composition*

The average composition of 15 different batches from a local fruit-canning factory is given in Table 1. The data clearly show that the composition of the effluent was fairly constant over the whole canning season. During the study period only the COD of this effluent was standardised.

*Study I - Increasing the organic loading rate*

The substrates used during these experimental studies were a dilution of the factory effluent given in Table 1. To prevent a shortage of nitrogen and phosphorus, a 100 $mg.l^{-1}$ of each of ureum and $K_2HPO_4$ and 1.0 $ml.l^{-1}$ of the trace element solution were added and the COD diluted to the required concentration. The pH was poised at 8.0 using a 1.0 N $Ca(OH)_2$ solution as initially at the start of the study the digester showed signs of pH instability with a tendency towards pH values below 6.5 units. A summary of the operational conditions and digester efficiency is given in Table 2. The hydraulic retention time was kept constant at 24 h and the OLR increased in seven steps from 2.28 to 3.95 $kgCOD.m^{-3}.d^{-1}$. The final value represented on average the full strength effluent as obtained from the factory and from these data it was concluded that it could be treated directly without dilution.

*Study II - Shortening the hydraulic retention time*

During this study the HRT was shortened from 24 h to 8 h over 14 steps (Table 3) with a subsequent increase in OLR from 3.95 to 10.95 $kgCOD.m^{-3}.d^{-1}$. Stable-state conditions plus 5 HRTs were used as criterion for increasing the OLR. During the different steps the pH of the digester effluent remained fairly constant (7.5 to 8.1) with the alkalinity in the range of 1 800 to 3 200 at the end of each step. However, directly on changing the OLR it was usually found that the pH dropped from 0.2 to 0.8 units but within 5 d the pH increased and stabilized. The pH stability can probably be ascribed to

the high alkalinity level (1 800 – 3 200 mg.l$^{-1}$). According to Duff & Kennedy (1982) and Lane (1984), alkalinity plays an important role in minimizing overloading effects.

During this study it was found that at HRT values of shorter than 10 d, the recovery rate in terms of pH and COD removal stabilization was slower than found with the longer HRTs with up to 14 h before the two parameters stabilized. The decrease in pH after increasing the OLR, as one of the indicators of impending digester failure, has been intensively studied (Hill & Bolte, 1989). According to Dohanyos *et al.* (1985), any change in operational parameters, such as organic loading, causes simultaneous increase in the concentration of all the volatile fatty acids resulting in a decrease in the pH.

**Table 1.** Average composition of the fruit canning factory effluent used as substrate for the UASB bioreactor (mg.l$^{-1}$).

| Parameter | Average | SD |
|---|---|---|
| pH | 5.45* | 1.4 |
| COD | 4432* | 297 |
| TS | 2114* | 625 |
| TVS | 1783* | 169 |
| TNVS | 331* | 152 |
| $PO_4$ | 5.32* | 3.9 |
| TKN | 21.4* | 12.4 |
| Alkalinity (as $CaCO_3$) | 25* | 6.5 |
| Glucose | 161.6+ | 25.3 |
| Fructose | 389.7+ | 34.6 |
| Sorbitol | 88.8+ | 9.7 |
| $Ca^{2+}$ | 21+ | nd |
| $Co^{2+}$ | <0.06+ | nd |
| Fe (total) | 7.9+ | nd |
| K | 84+ | nd |
| Mg | 15+ | nd |
| Na | 65+ | nd |
| $Ni^{2+}$ | <0.3+ | nd |
| $SO_4^{2-}$ | 12.8+ | nd |
| $S^{2-}$ | 0.03+ | nd |

* = Data are means of 15 batches
+ = Average of two determinations

Once the microbial biomass has recovered and stabilized the extra VFAs are normally metabolized and the pH stabilizes (Myburg & Britz, 1993). Based on the extended stabilization time at these HRTs, it was concluded that even though the COD removal after stable-state had been reached was still above 90%, the digester was reaching its maximum operational HRT and that any sudden changes in normal operating parameters would influence the efficiency negatively. An HRT of 10 h with an average COD removal of between 90 and 93% and removal rate of 9.8 kg COD was taken as the optimum operational conditions (Table 3).

*Study III - Lowering of the substrate pH*

Since neutralization costs would influence economic aspects of the process, the influence of lower pH values was investigated in the third experimental study. In this study, based on the data obtained during Study II, the HRT was kept constant at 10 h and the substrate pH lowered in seven steps over 60 d from 8.0 to 5.0. The data (Table 4) show that at substrate pH's of 6.0 and 5.5, the alkalinity drastically decreased to 1 150 $mg.l^{-1}$. However, the COD removal was still above 88% and the effluent pH above 6.8 which is still in the optimal recommended pH range (Nel & Britz, 1986). When the substrate pH was lowered to 5.0 the COD removal dropped drastically (Table 4) and the biogas production started to decrease. It was also found that just after the change to pH 5.0 the digester effluent pH dropped to 6.2 and once the system reached stable state slowly increased to 6.7. It was clear from the slow recovery of the digester and the low COD removal (66.1%) that the lower end of the operational pH had been reached and any further lowering of the substrate pH would lead to system failure. The substrate pH was then reset at 5.5 which is near the average pH of the canning effluent (Table 1) and the COD removal and digester effluent pH slowly recovered to about 90% COD removal and pH 6.8 - 7.0.

**Table 2.** Operating conditions and digester efficiency during experimental study I where the organic loading rate was increased.

| Parameter | Steps | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Substrate COD ($mg.l^{-1}$) | 2300 | 2500 | 3000 | 3200 | 3500 | 3700 | 4000 |
| COD removal (%) | 88 | 92 | 90 | 91 | 89 | 90 | 91 |
| HRT (h) | 24 | 24 | 24 | 24 | 24 | 24 | 24 |
| OLR ($kgCOD.m^{-3}.d^{-1}$) | 2.28 | 2.5 | 3.0 | 3.22 | 3.49 | 3.73 | 3.95 |
| Digester pH | 7.6 | 7.5 | 7.3 | 7.4 | 7.4 | 7.7 | 8.0 |
| Alkalinity ($mg.l^{-1}$ $CaCO_3$) | 1800 | 1850 | 1950 | 1125 | 1225 | 2010 | 2125 |
| Biogas ($l.d^{-1}$) | 1.1 | 1.37 | 2.36 | 1.84 | 2.27 | 2.35 | 2.52 |
| Methane (%) | 62 | 63 | 64 | 64 | 65 | 64 | 64 |

**Table 3.** Operating conditions and digester efficiency during Experimental Study II where the HRT was shortened.

| Parameter | Steps | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| Substrate COD ($mg.l^{-1}$) | 3947 | 4162 | 4011 | 3932 | 4119 | 4233 | 3912 | 3953 | 3967 | 4069 | 4125 | 3991 | 4099 | 4161 |
| COD removal (%) | 91 | 96 | 96 | 93 | 92 | 94 | 88 | 90 | 91 | 93 | 93 | 90 | 91 | 93 |
| HRT (h) | 24 | 22 | 20 | 18 | 17 | 16 | 15 | 14 | 13 | 12 | 11 | 10 | 9 | 8 |
| OLR ($kgCOD.m^{-3}.d^{-1}$) | 3.95 | 4.16 | 4.36 | 4.74 | 5.6 | 6.13 | 6.2 | 6.27 | 6.84 | 7.54 | 8.25 | 8.68 | 9.8 | 10.95 |
| Digester pH | 8.0 | 7.9 | 7.5 | 7.9 | 8.1 | 7.6 | 7.5 | 7.7 | 7.55 | 8.1 | 8.1 | 7.6 | 7.9 | 7.9 |
| Alkalinity ($mg.l^{-1}$ CaCO3) | 2125 | 2250 | 2100 | 1800 | 2250 | 2200 | 3200 | 3800 | 2600 | 2450 | 2625 | 2350 | 2700 | 3200 |
| Biogas ($l.d^{-1}$) | 2.52 | 2.29 | 2.78 | 4.4 | 4.7 | 4.72 | 4.5 | 5.33 | 5.58 | 6.14 | 6.39 | 5.72 | 6.68 | 8.7 |
| Methane (%) | 63 | 64 | 64 | 65 | 64 | 65 | 62 | 63 | 64 | 64 | 64 | 624 | 64 | 64 |

**Table 4.** Operating conditions and digester efficiency during experimental study III where the substrate pH was decreased.

| Parameter | Steps | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Substrate COD ($mg.l^{-1}$) | 3937 | 3980 | 4096 | 3834 | 4018 | 4104 | 4352 |
| COD removal (%) | 90.7 | 88.5 | 90.5 | 90.2 | 88.3 | 89.4 | 66.1 |
| HRT (h) | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| OLR ($kgCOD.m^{-3}.d^{-1}$) | 8.75 | 9.48 | 9.75 | 9.13 | 9.57 | 9.77 | 10.4 |
| Substrate pH | 8.0 | 7.5 | 7.3 | 6.5 | 6.0 | 5.5 | 5.0 |
| Digester pH | 8.0 | 7.6 | 7.89 | 7.95 | 6.82 | 6.91 | 6.7 |
| Alkalinity ($mg.l^{-1}$ $CaCO_3$) | 3475 | 2550 | 2775 | 2175 | 1150 | 1150 | 1050 |
| Biogas ($l.d^{-1}$) | 4.88 | 5.72 | 6.45 | 7.7 | 8.75 | 9.43 | 8.12 |
| Methane (%) | 64 | 63 | 63 | 64 | 65 | 65 | 63 |

The economic implication of being able to optimizing and operate the digester at a substrate pH of 5.5 means that canning effluent can be introduced to the digester without any neutralization, is considerable. It must also be remembered that the canning effluent is rich in carbohydrates and if the effluent is stored, fermentation will take place with a concurrent reduction in pH to values of about 3.0 - 3.5. Neutralization must then be applied before substrate introduction to the digester.

*UASB Bioreactor efficiency*

Since the efficiency of the UASB system is based on the formation and retention of granules, changes in the height of the UASB granule bed were monitored during the course of the study (Fig. 2). With a start bed height of 300 mm, growth at first was very slow but as soon as the HRT was lowered to below 24 h, a definite increase in the bed height was found, suggesting that the bioreactor had not reached its loading capacity. Around day 90, once an HRT of 10 h had been reached, the bed growth stabilized and remained stable for the rest of the study. At HRT values below 10 h the system was found to show an extended recovery time indicating maximum loading at the shorter HRTs. It is possible that if the COD concentration of the canning effluent had been higher, a further increase in granule bed height might have taken place but since 4 000 $mg.l^{-1}$ was the maximum concentration this was not followed up.

The UASB bioreactor efficiency in terms of the relationship between percentage COD removal and the COD removal rate (kg $COD.m^3.d^{-1}$) is plotted in Fig. 3 as a function of the OLR over all three the experimental studies. The data obtained at HRT values below 10 h (Table 3) and those at a pH value below 5.5 (Table 4) were not included as the slow bioreactor recovery clearly indicated that the lower end of the operational HRT and pH had been reached and any further changes in OLR would lead to system failure. The best removal rate (R-value) was found in the OLR region of about 11 $kgCOD.m^{-3}.d^{-1}$ (Fig. 3). Since the highest COD concentration (4 000 $mg.l^{-1}$) was already being loaded, any increase in OLR could only be obtained by further lowering of the HRT to below 10 h. However, changes to lower HRT values led to a slow recovery of the bioreactor suggesting that the system had reached its maximum organic loading rate and that system failure would be imminent even after small environmental changes (Verstraete & Vandevivere, 1997).

## 5.5 Conclusions

It has long been thought that anaerobic digestion is too slow and unreliable to be used by the canning industry as a treatment option (Borja & Banks, 1994; Wayman, 1996). From the results obtained during the three experimental studies it was clear that the UASB design is feasible for treatment of the carbohydrate-rich effluents produced in the canning industry. The most favourable COD removal of the canning-industry effluent was between 89 and 93% at organic loadings of 9.8 and 10.95 $kgCOD.m^{-3}.d^{-1}$, at an HRT of 10 h and substrate pH of 5.5. The UASB bioreactor in terms of HRTs, OLRs and substrate pH as operated in this study, was more efficient when compared to results reported by Austermann-Huan *et al.* (1997), where a UASB was used to treat a fruit-juice effluent.

fig6-2

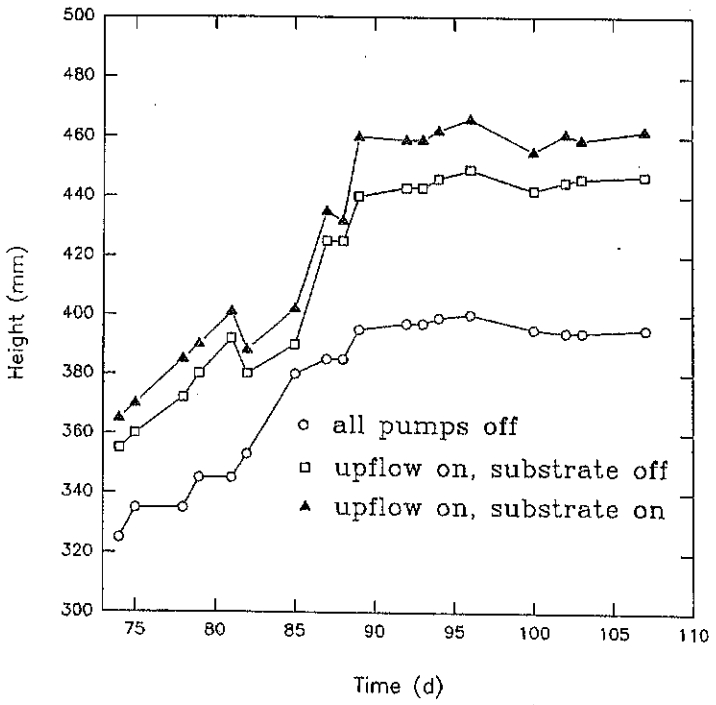


**Figure 2.** Changes in the height of the granule bed in the UASB.

Fig5-3

fig5-3

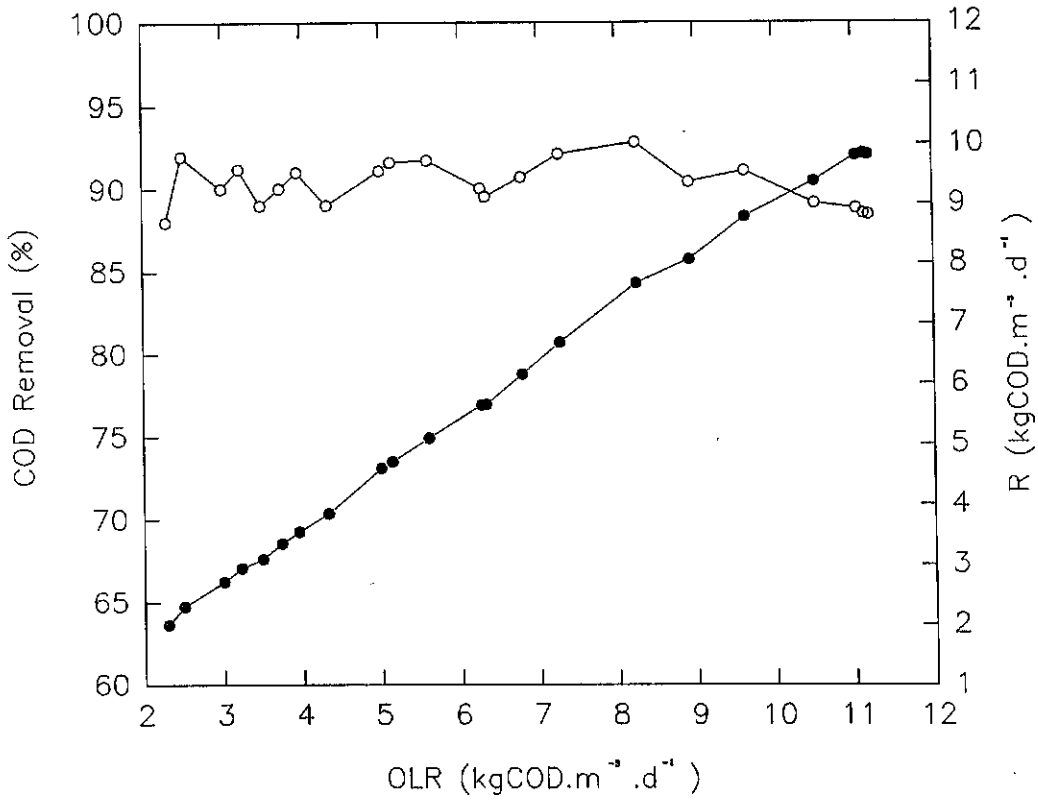


**Figure 3.** The effect of the increase in organic loading rate on the percentage COD removal ( □ ) and the COD removal rate ( ● ).

# CHAPTER 6

# IMPACT OF SHOCK LOADINGS ON THE GRANULE GROWTH AND PERFORMANCE EFFICIENCY OF AN UASB BIOREACTOR

## 6.1 Summary

The influence of environmental 'stress' conditions as microbial granule formation stimulants were evaluated using a mesophylic laboratory-scale upflow anaerobic sludge bed bioreactor design. An UASB bioreactor, just inoculated with anaerobic sludge from a local anaerobic digester, was used as control. To prevent system failure due to washout and lack of proper granulation, the control bioreactor had to be re-inoculated at regular periods. The continual operational setbacks showed the problem of long start-up times and the fundamental problem of biomass washout encountered with the UASB design when inoculating only with anaerobic sludge.

The application of a 40% organic shock loading (increase in OLR from 2.5 to 3.5 $kgCOD.m^{-3}.d^{-1}$) led to a prolonged recovery period even though an increase in granule bed was found. However, the bed height was found to decrease as the other efficiency parameters changed and this was probably due to the formation of smaller not so dense aggregates which washed out once the system started to stabilize. The volatile fatty acid and methane data profiles confirmed the increase in acidogen activity and lack of substantial methanogen growth. It was also found that acetic and propionic acids are excellent indicators of organic stress.

A 20% shock loading (increase in OLR from 2.5 to 3.0 $kgCOD.m^{-3}.d^{-1}$) (repeated three times) resulted in shorter recovery periods and again acetic acid showed the first change in stability of the system. The data also showed a stable increase of 14 - 19% in granule bed height. The cysteine shock resulted in an excellent increase (17%) in granule bed height.

The data obtained in this study shows that when stress conditions are applied to an UASB bioreactor an enhancement of granulation will occur but the type and concentration of stress applied must be carefully chosen otherwise over-stressing could lead to an extended recovery period or even to total system failure.

## 6.2 Introduction

The UASB bioreactor concept, where different bacterial types aggregate into firm, mechanical resistant granules, has been used to treat many industrial waste waters (Lettinga *et al.*, 1997). The mechanism of granule formation is still poorly understood (Sam-Soon *et al.*, 1987; Slobodkin & Verstraete, 1993) and it would appear that granules can only be formed with certain types of carbohydrate/protein containing wastes generated in the agricultural and food processing industries (Sam-Soon *et al.*, 1991; Sorensen *et al.*, 1991).

There are several hypotheses for explaining the granulation phenomenon, but actually no simple reasons seem to exist for it to occur, nor procedures to guarantee the formation of granules (Paula & Foresti, 1992). Since the operational efficiency

and performance of the UASB systems are mainly dictated by the formation, amount and specific activity of the granules, the potential use of the UASB bioreactor is limited by the extended start-up periods (Barber & Stuckey, 1997). The consequence of reactor failure following severe shock is usually re-seeding and/or total re-start. This is very expensive in terms of time and seed sludge and the possibility of litigation can also arise. Furthermore, the start-up period is influenced by the waste water composition and strength, as well as environmental parameters like carbon concentration, temperature and pH (Ghangrekar *et al.*, 1996). The digestion process has been found to be sensitive to changes in hydraulic and organic loading and only recovers slowly once it has been upset (Marchaim & Krause, 1993). During these changes, resulting in unbalanced operational conditions, propionate, hydrogen and lactate can be detected (Hickey & Switzenbaum, 1991; Myburg & Britz, 1993). A shift of the population dynamics of the anaerobic community possibly gives rise to the production of extracellular polymers by the acidogenic bacteria which could contribute directly to the formation of highly settable granules as found in efficient operating UASB bioreactors (Riedel & Britz, 1993). According to Hickey *et al.* (1991), the reduction of start-up time is one of the key parameters in increasing the efficiency of anaerobic reactors.

The main objective of this study was to determine the influence of 'stress' conditions (different organic shock loadings and cysteine addition) on an UASB bioreactor in order to evaluate these environmental effects as microbial granule formation stimulants.

## 6.3 Materials and methods

*Digester design*

Two laboratory-scale upflow anaerobic sludge blanket bioreactors (UASB) were used (UASB-Control and UASB-01). The bioreactors had an operational volume of 2.3 litre (total height of 830 mm and internal diameter of 50 mm) and combined an upflow anaerobic sludge blanket design with an open gas/solids separator at the top of the bioreactor. The biogas exited through the top, while the substrate was introduced into the bioreactor at the base. The overflow of the bioreactor emptied through a U-shaped tube to prevent any atmospheric oxygen from entering the system. The upflow velocity within the reactor was set at 1 $m.h^{-1}$. The temperature of the bioreactor was maintained at 35°C using a heating tape and an electronic control unit (Meyer *et al.*, 1985) and the bioreactor was insulated. The volume of the biogas ($CH_4$ and $CO_2$) was determined using a manometric unit equipped with an electronically controlled counter and a gas-tight valve and the volumes corrected to standard temperature and pressure. The substrate was fed semi-continuously to the bioreactors by means of a peristaltic pump (Watson Marlow 101) controlled by an electronic timer.

*Bioreactor start-up*

The control bioreactor (UASB-Control) was seeded with anaerobic sludge obtained from the sewerage works of Kraaifontein (Western Cape Province). In contrast, the experimental bioreactor (UASB-01) was seeded with an inoculum of

400 g water drained anaerobic granules produced in a batch reactor (P. Roos, 1997, US, personal communication), giving a settled sludge-bed height of 250 mm. The microbial community originated from anaerobic sludge from the same local municipal anaerobic digester (Kraaifontein, Western Cape Province), and was added to a 500 ml batch bioreactor and shaken at 150 r.p.m. for 14 days. After inoculation, the UASB bioreactor was allowed to stabilize for 72 h in order to allow the bacterial community to acclimatize. After the stabilization period, feeding was commenced with a dilute substrate (COD = 1 000 $mg.l^{-1}$ and pH = 8.5). During the start-up, the hydraulic retention time (HRT) was set at 4.5 d and after the first 20 days reset to an HRT of 1.0 d.

*Substrate*

A synthetic carbohydrate effluent was used for both the start-up and experimental phases and consisted of ($mg.l^{-1}$): glucose, 1 250; sodium lactate, 5 000; and acetic acid, 10. The substrate was supplemented with 500 $mg.l^{-1}$ urea and 500 $mg.l^{-1}$ $K_2HPO_4$ to prevent any nitrogen and phosphorus limitation during the start-up period. The substrate was also supplemented with 1.0 $ml.l^{-1}$ trace element solution, as described by Nel *et al.* (1985) and the pH poised at 8.5 with 1N calcium hydroxide to optimize the environment for maximum granule growth (Chang *et al.*, 1993). This substrate was diluted to the required COD concentration.

*Analytical methods*

The following parameters were monitored according to the APHA (Standard Methods, 1985): pH; alkalinity; total solids (TS); total volatile solids (TVS) and total non-volatile solids (TNVS). Chemical oxygen demand (COD), orthophosphate phosphorus and total Kjeldahl nitrogen were determined colorimetrically using a DR 2000 spectrophotometer (Hach Co. Loveland, CO) and standardised procedures (Standard Methods, 1985).

The volatile fatty acids (VFA) were determined using a Varian gas chromatograph (Model 3700), equipped with a flame ionisation detector and a 30 m x 0.32 mm i.d. Fused Silica capillary column with 007 FFAP bonded phase (Quadrex Co. New Haven). The column temperature was initially held at 105°C, then increased at a rate of 10°C per min to 219°C. The detector and the inlet temperatures were set at 260°C and 250°C respectively and nitrogen gas was used as carrier gas at a flow rate of 2.50 ml per min. The biogas composition was also determined on the Varian gas chromatograph equipped with a thermal conductivity detector and column (2.0 m x 3.0 mm i.d.) packed with Porapak Q (Waters Ass. Inc, Milford, MA), 80.100 mesh. The oven temperature was set at 55°C and helium was used as carrier gas at a flow rate of 40 ml per min.

*Experimental Studies*

Before the start of the experiment, the bioreactor (UASB-01) was successfully operated at a substrate COD concentration of 2 500 $mg.l^{-1}$. The HRT was maintained throughout the study at 1.0 d. During the first study (Experimental Study I), an organic overloading of 40% (3 500 $mg.l^{-1}$) over a period of 8 h, was applied to the experimental bioreactor at a background organic loading rate (OLR) of 2.5

kgCOD.m$^{-3}$.d$^{-1}$. Influent and effluent samples were taken every two hours for the first 30 h and then every 24 - 48 h. In the both the second and third Experimental Studies (II and III) respectively, three shock loadings of 20% (3 000 mg.l$^{-1}$) and one cysteine (12.5 mg.l$^{-1}$) shock were applied over a period of 8 h. Bioreactor influent and effluent samples were taken every two hours for the first 30 h and then every 24 - 48 h. Analyses were done to determine the effect of the shock loadings on the bioreactor and the recovery period needed to reach stable-state conditions. Stable-state is defined as a state which can be maintained indefinitely without system failure (Cobb & Hill, 1990), during which the variation in bioreactor performance parameters is less than 10%. The height of the granule bed was also measured to determine the effect of the shock loadings as a granule formation stimulant.

## 6.4 Results and discussion

*Control bioreactor*

The control bioreactor (UASB-Control) was seeded with anaerobic sludge obtained from the sewerage works of Kraaifontein (Western Cape Province). On several occasions (10, 20, 30, 40, 60, 70, 85 and again after 100 days) it was necessary to re-inoculate the control bioreactor to compensate for the loss of the microbial community due to biomass washout. This bioreactor was operated for four months with the HRT maintained at 1.0 d and the OLR at a COD of 2 500 mg.l$^{-1}$.d$^{-1}$. The COD removal varied between 45 and 65% (Fig. 1). The low COD removal was ascribed to the rather small and unstable granule content that formed in this bioreactor. A serious problem was also found with a continual drop in pH leading to acidification and thus this bioreactor was operated with the substrate pH set at 8.5. These continual operational setbacks clearly illustrates the problem of long start-up times and shows the fundamental problem of biomass washout encountered with the UASB bioreactor design when inoculating only with anaerobic sludge.

*Experimental Study I - 40% Shock loading*

The efficiency and operational parameters of the bioreactor (UASB-01) under normal operational conditions and that used during the 40% shock loading, are given in Table 1. In this Experimental Study, a continuous organic shock loading of 40% (based on the results obtained by Myburg & Britz, 1993) was applied at a background OLR of 2.5 kgCOD.m$^{-3}$.d$^{-1}$, for a period of 8 h. Samples were taken every 2 h for the first 30 h and then every 24 - 48 h. The results are illustrated in Fig. 2 to 6.

The pH of the bioreactor effluent (Fig. 2) was found to decrease immediately after the shock loading from around 6.9 to 6.74 within the first 8 h and then to the lowest value of 6.6 within 24 h. The pH only started to slowly recover after about 96 h and slowly returned to about pre-shock values after 360 h. Since the pH dropped to 6.5, which is just under the desired UASB operating range of between 7.4 and 6.6 (Wentzel *et al.*, 1994), it was concluded that the 40% COD shock was too drastic for the bioreactor. According to Elefsiniotis & Oldham (1994), pH affects the growth rate and thus changes in pH may cause dramatic shifts in the relative numbers of different species in a heterogeneous population such as is present during the anaerobic digestion process.

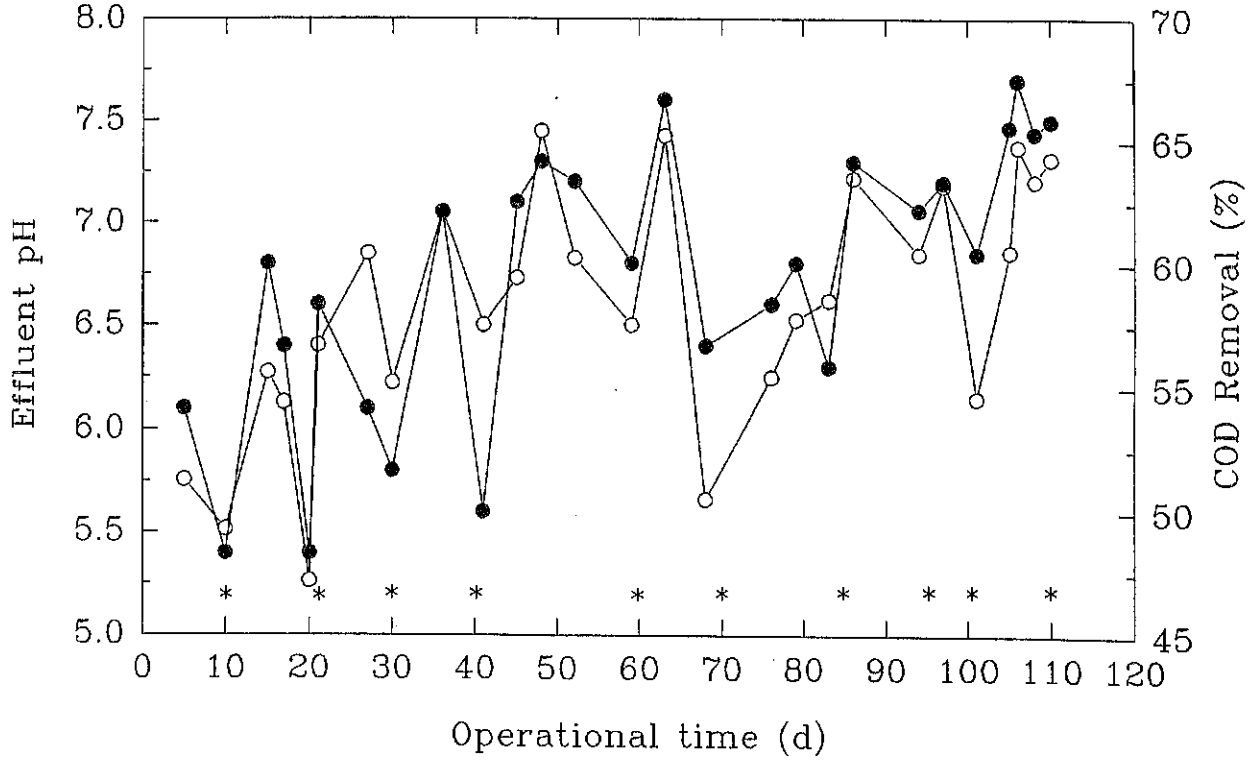


**Figure 1.** The influence of the start-up on the bioreactor effluent pH ( o ) and COD removal ( ● ) of the control bioreactor. (Each asterisk indicates the re-inoculation of the control bioreactor with sludge from a local anaerobic digester)

**Table 1.** Operational parameters and efficiency of the experimental bioreactor under normal operational conditions and the parameters used during the shock loading (40%) in Experimental Study I.

| Parameters | Normal loading(±SD)* | Shock loading (40%)** |
|---|---|---|
| Substrate COD ($mg.l^{-1}$) | 2 535 ±186 | 3 500 |
| COD removal (%) | 69 ±2.56 | - |
| HRT (h) | 24 | 24 |
| OLR ($kgCOD.m^{-3}.d^{-1}$) | 2.5 | 3.5 (for 8 h) |
| Substrate pH | 8.5 | 8.5 |
| Effluent pH | 6.9 ±0.2 | - |
| Alkalinity ($mg.l^{-1}$) | 980 ±68 | 575 |
| Biogas ($l.d^{-1}$) | 0.88 ±0.14 | - |

* = Data are means of 5 batches; ** = Data only represents the 8 h shock period

**Table 2.** Operational parameters and efficiency of the experimental bioreactor under normal operational conditions and the parameters used during the 20% shock loading in Experimental Study II.

| Parameters | Normal loading | Shock loading (20%) |
|---|---|---|
| Substrate COD ($mg.l^{-1}$) | 2 500 | 3 000 |
| HRT (h) | 24 | 24 |
| OLR ($kgCOD.m^{-3}.d^{-1}$) | 2.5 | 3.0 (for 8 hours) |
| Substrate pH | 8.5 | 8.5 |
| Alkalinity ($mg.l^{-1}$) | 300 | 425 |

The alkalinity of the effluent (Fig. 2) increased within the first 8 h to the highest value of 1 025 $mg.l^{-1}$ but was followed by a drastic decrease to the lowest value of 875 $mg.l^{-1}$ within 48 h after the shock loading. A sharp increase in the alkalinity value was followed by recovery to the pre-shock value of 925 $mg.l^{-1}$ whereafter a longer and slower decrease and increase took place. The sharp increase in the alkalinity straight after the shock loading showed the importance of alkalinity in minimizing overloading effects, which was also reported by Duff & Kennedy (1982) and Marsili-Libelli & Beni (1996). According to Moosbrugger *et al.* (1993), proteins, sulphates and/or short-chain fatty acids can generate internal buffering capacity, which may be the cause of the anaerobic process becoming partially or completely independent of buffer for pH control from an external source.

The COD removal (Fig. 3) was found to decrease immediately from 67% to 44% within the first 8 h. The lowest value of 43% was obtained 24 h after the shock loading. An increase to 47% was followed by another decrease to 45% at 72 h, whereafter it steadily increased and only stabilized after more than 300 h later to the pre-shock value. The highest COD concentration in the effluent (Fig. 3) was 1 436 $mg.l^{-1}$ at 26 h. The data clearly indicate that two dips occurred in terms of the COD removal and are probably partially due to the addition and removal of the higher COD during the shock period.

In Fig. 4, the volumes of $CH_4$ and $CO_2$ of the biogas are indicated. A sudden drop in the methane content (0.04 l) was found within the first 8 h, which was followed by a sharp increase (0.38 l) and another decrease (0.16 l), all within the first 24 h after the shock loading. The methane and carbon dioxide volumes then stabilized and returned to the pre-shock value within 50 h. From the data obtained in this study it appears as if the biogas production is the least sensitive parameter since it recovered and stabilized completely within 50 h and it was thus concluded that the biogas is not as good an indicator of stress as the other parameters. This could be due to the fact that the high alkalinity during the shock loading might possibly have buffered the system and stabilized the methane production.

In general, a low volatile fatty acid concentration indicates a 'healthy' bioreactor with an active methanogenic population (Hickey & Switzenbaum, 1991). As was originally suspected, the first parameter that would indicate 'stress' conditions in the bioreactor was the VFA's with an rapid increase in the acetic and propionic acids in the bioreactor effluent (Fig. 5). The valeric acid at the concentration produced and measured, appeared to be the least sensitive VFA indicator of applied stress. The occurrence of two major decrease/increase cycles in the VFA's just after the shock loading are very similar to those found for the pH and COD removal profiles (Fig. 2 and 3). In this study it appeared as if the response of the metabolites, acetic and propionic acids, to the shock loading are excellent indicators of organic stress. The highest values for both the acetic and propionic acids were obtained within the first 8 h after the shock load indicating a direct response in metabolic activity due to the increase in degradable compounds in the substrate. The increase in acetic and propionic acid also shows the response of the acidogens and lack of methanogens to remove those VFA's. Furthermore, the high values found 150 h after the shock loading are an indication that the bioreactor was still in a state of shock and is probably due to the inability of the acetate and propionate catabolizing populations to consume these VFA's as quickly as they are produced. Since these organisms are slow growing, with generation times of 3 to 12

fig6-2

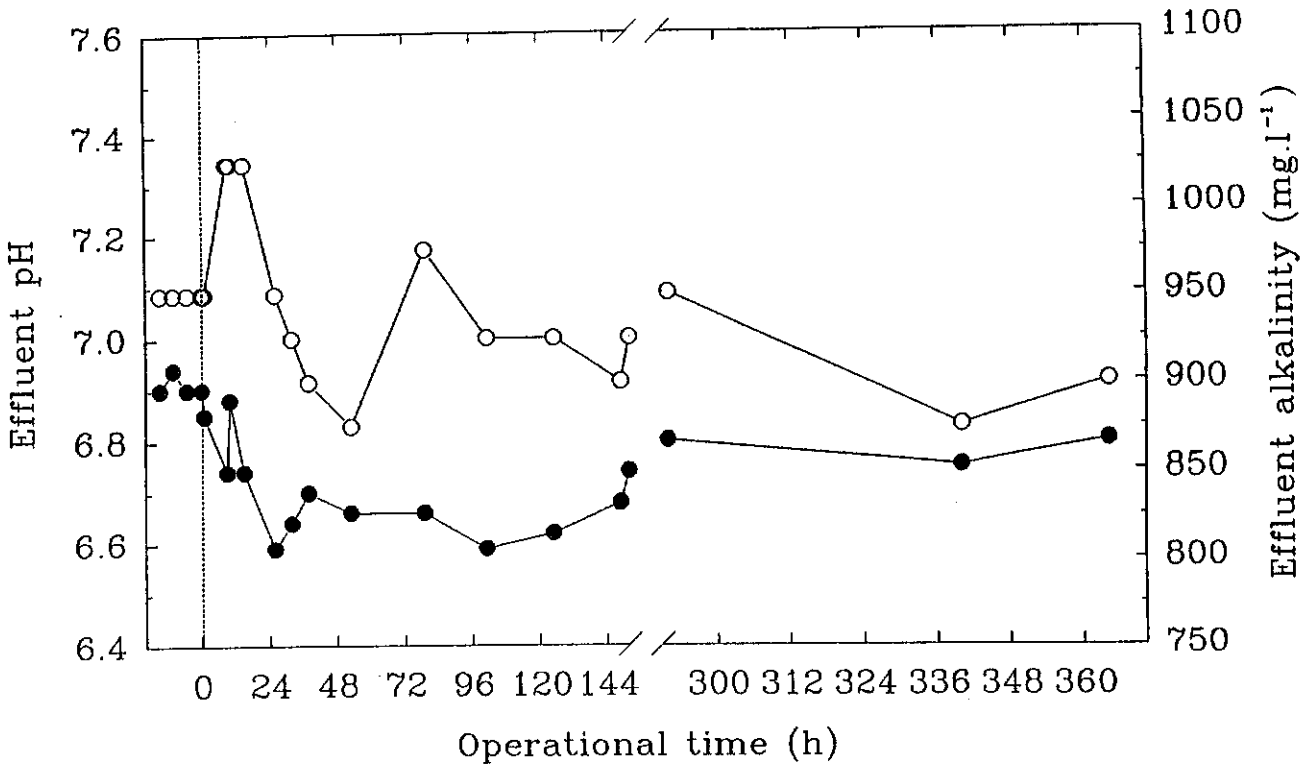


**Figure 2.** Influence of a 40% organic shock loading on the bioreactor effluent pH ( ● ) and effluent alkalinity ( O ). (The dotted line indicates the application of the shock loading).

fig6-3

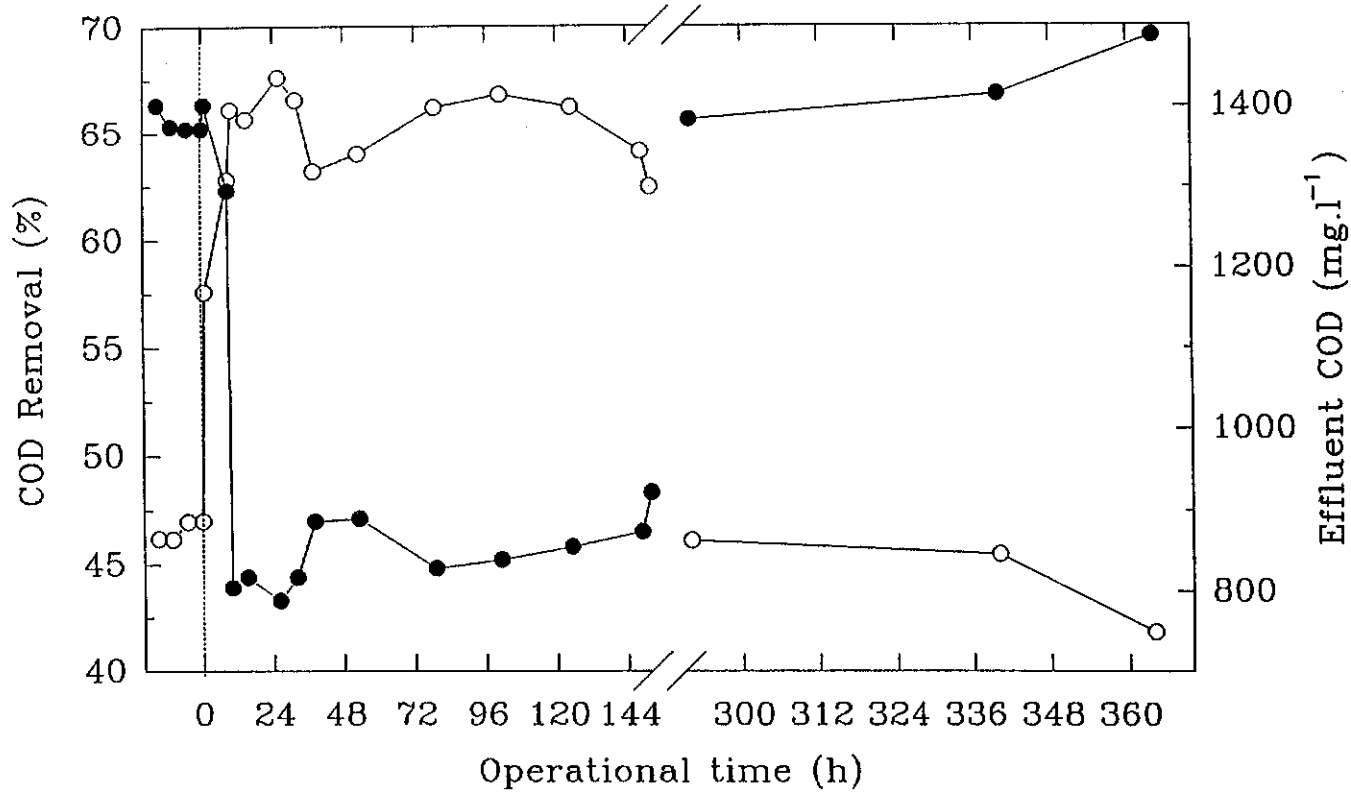


**Figure 3.** Influence of a 40% organic shock loading on the COD removal ( ● ) and bioreactor effluent COD ( O ). (The dotted line indicates the application of the shock loading).

fig6-4

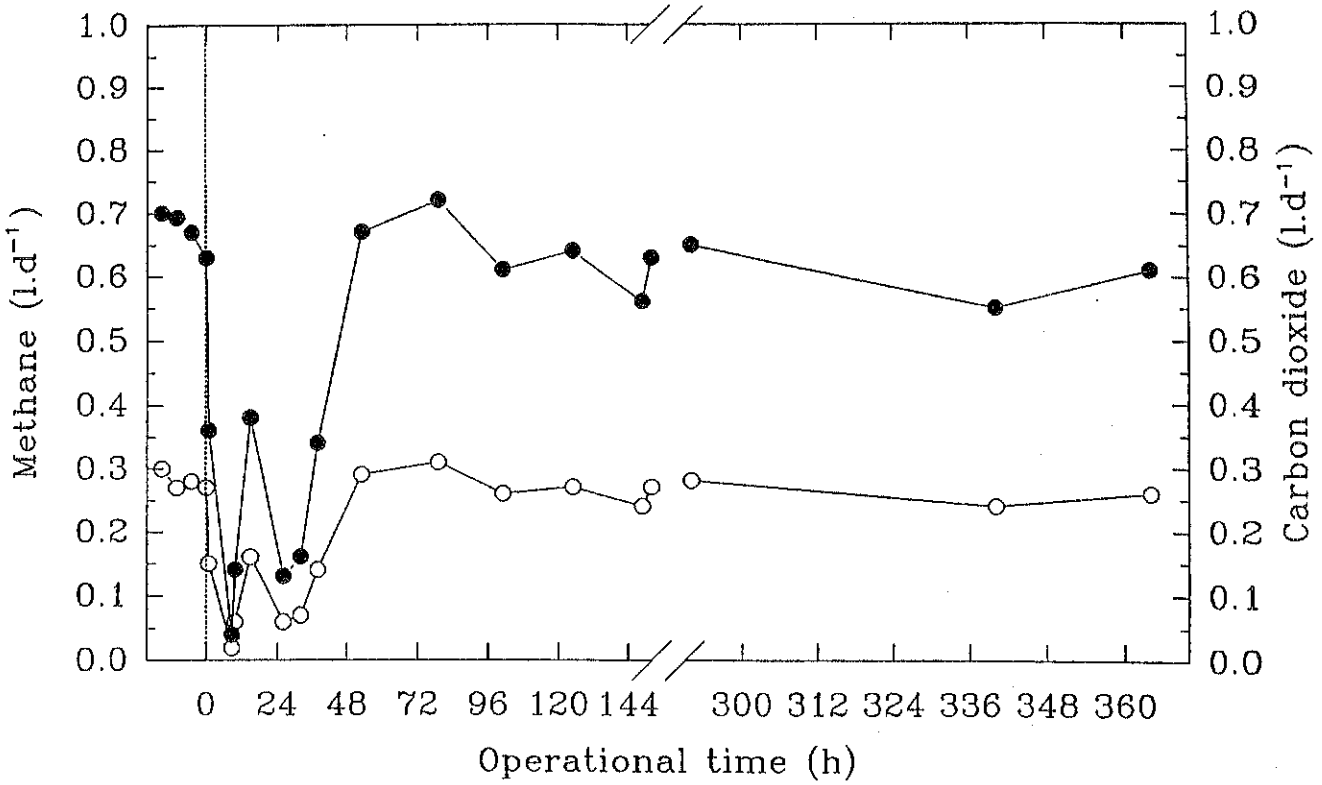


**Figure 4.** Influence of a 40% organic shock loading on the methane ( ● ) and the carbon dioxide ( O ) volumes. (The dotted line indicates the application of the shock loading)

fig6-5

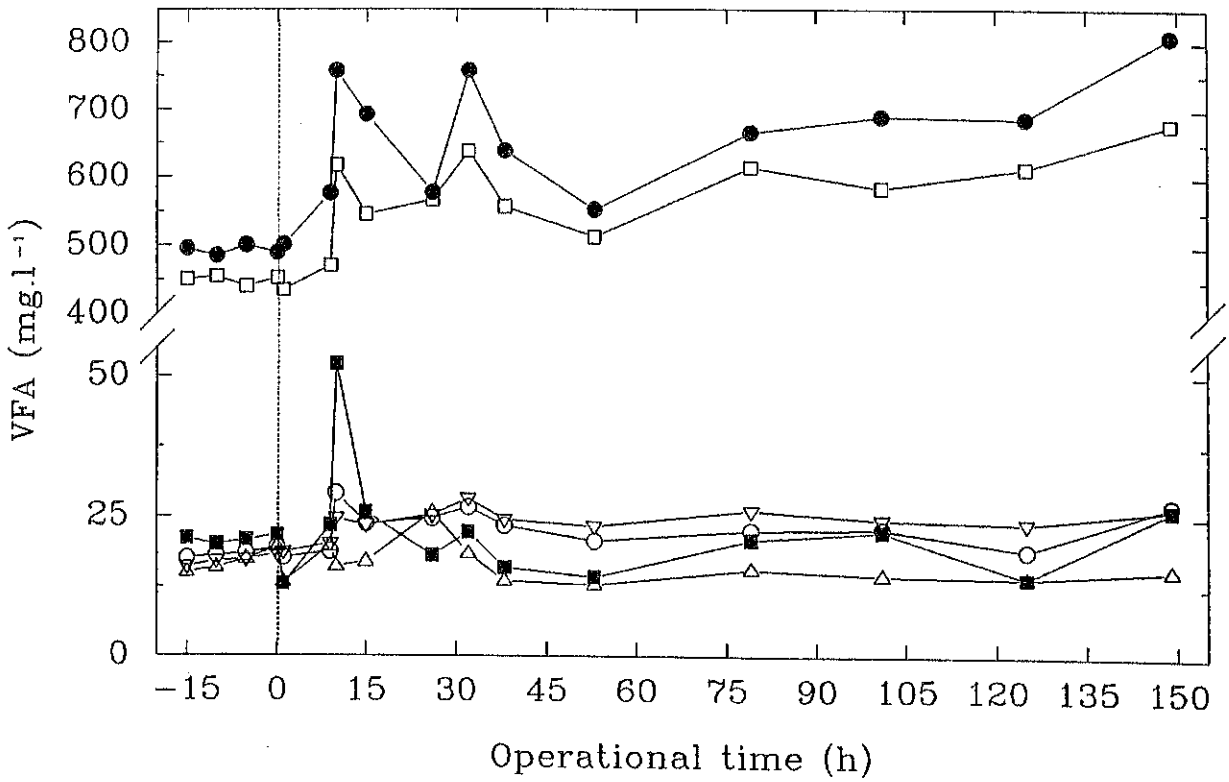


**Figure 5.** Influence of a 40% organic shock loading on the bioreactor effluent VFA content ( ● = AcAc; □ = PrAc; ■ = BuAc; O = ValAc; Δ = i-BuAc; ∇ = i-ValAc). (The dotted line indicates the application of the shock loading).

days (Hickey & Switzenbaum, 1991), enough additional biomass could not be produced fast enough to balance the situation.

The changes in the height of the granule bed are indicated in Fig. 6. Within the first 8 h there was an increase in the height of the granule bed with a maximum being reached 96 h after the shock loading. At this time it was noted that the bed contained a large portion of very small not so dense aggregates. The fast increase in the height of the granule bed after the shock induction appears to confirm that the induction of stress conditions may be the key to granule growth enhancement. As the other efficiency parameters (pH, $CH_4$ and $CO_2$ production volumes, VFA's and COD removal) started to stabilize, the granule bed height decreased. This was probably due to the fact that the 40% shock loading was fairly severe which subsequently resulted in the wash-out of biomass especially the smaller not so dense aggregates. These aggregates were probably made up of mostly acidogens since they have much shorter generation times than the methanogens and, with an increase in an easily degradable substrate like glucose, would be the first to respond in terms of metabolic activity and biomass increases. The data profiles confirm the increase in acidogen activity in that there was an increase in the production of acetate and propionate and no substantial increase in the volumes of methane, which would have increased if the methanogens had shown substantial growth. A similar situation was reported by Thaveesri *et al.* (1995) while treating a carbohydrate wastewater.

*Experimental Study II - 20% Shock loading*

From the results obtained in Experimental Study I, using an organic shock loading of 40%, it was clear that the recovery period was too prolonged as a result of a delayed second stage stress period. It was thus decided to decrease the shock loading to 20% for a period of 8 h. This study was only started when the efficiency of the bioreactor showed a variation of less than 10%.

This Experimental Study was done three times, with the second 20% shock being done three months after the first and the third 20% shock a further two months after the second shock. The 20% shock loading (3 000 $mg.l^{-1}$) was applied at a background loading of 2.5 $kgCOD.m^{-3}.d^{-1}$. The results of the second and third 20% shocks were very similar to the first and thus only the results for the first 20% organic COD shock loading are illustrated in Fig. 7 to 11. The operational parameters and composition of the bioreactor substrates used during the 20% shock loading are shown in Table 2.

The pH of the bioreactor effluent (Fig. 7) dropped from 6.95 to 6.76 within the first 8 h. This was followed by the characteristic decrease/increase effect over a period of 70 h, whereafter it increased and reached pre-shock values after 270 h and then slowly increased to 7.1. In this Experimental Study the decrease in the pH was not as drastic as in the 40% experiment but it still took an extended time for the reactor to stabilize. This was probably also as a result of the difference in the acidogen and methanogen cell growth and metabolic activity. The alkalinity (Fig. 7) again increased immediately after the shock loading but started to drop to just below pre-shock values within the first 30 h. A steep increase (1 300 $mg.l^{-1}$) was followed with a gradual decrease to pre-shock values only after 400 h. It is generally accepted (Caine *et al.*, 1991; Marchaim & Krause, 1993) that with sudden organic increases especially with carbohydrates, more VFA intermediates would be formed.

fig6-6

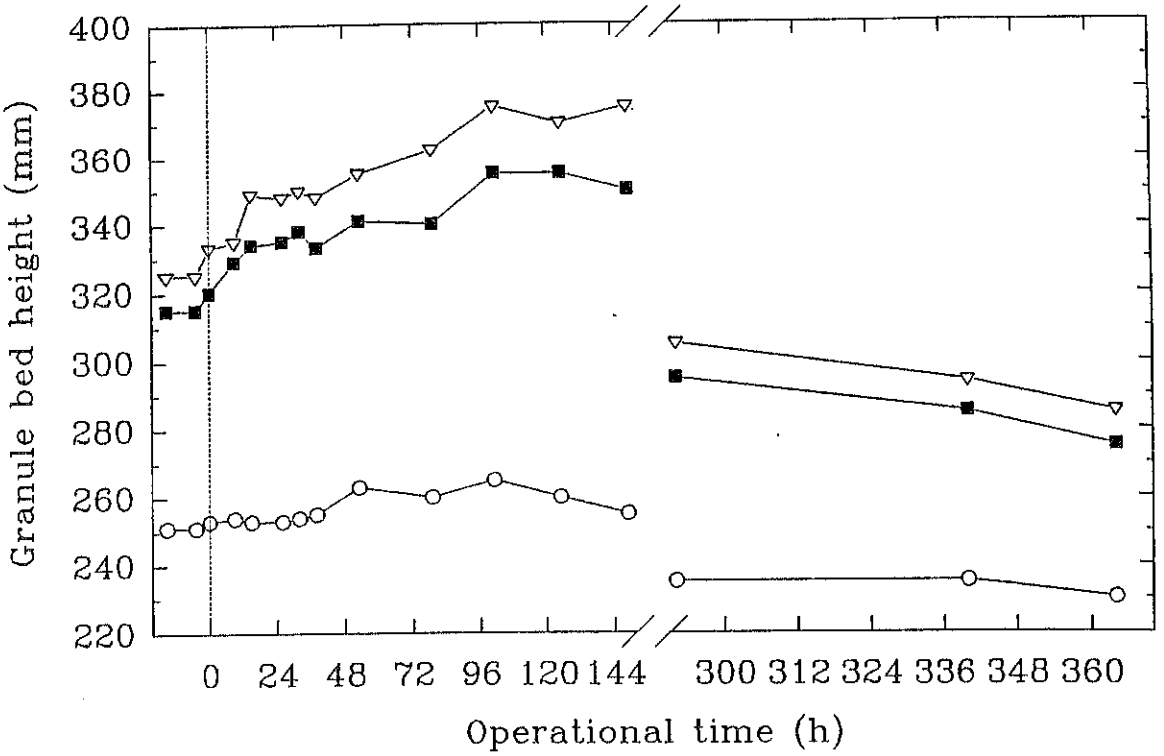


**Figure 6.** Influence of a 40% organic shock loading on the height of the bioreactor granule bed ( O = all pumps off; ■ = upflow on and substrate off; ▽ all pumps on). (The dotted line indicates the application of the shock loading).

fig6-7

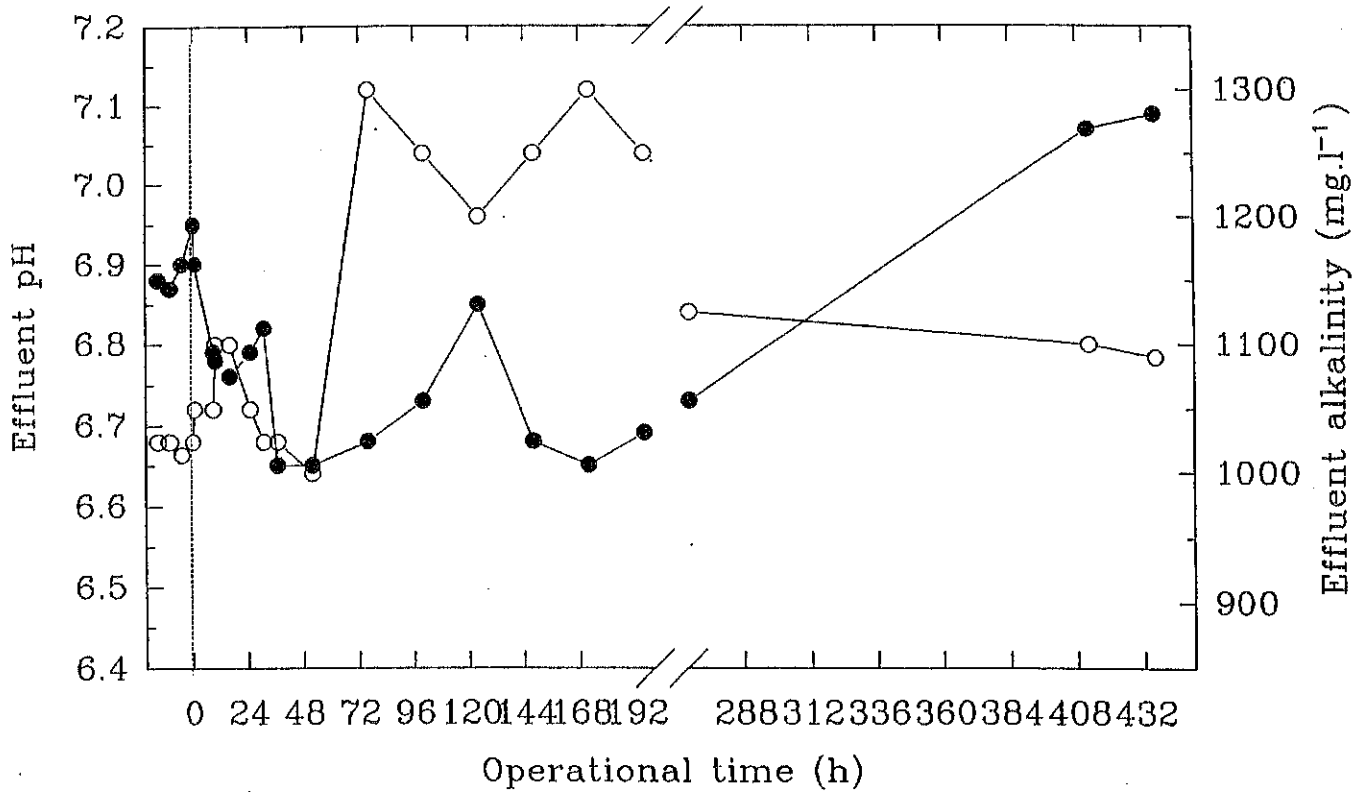


**Figure 7.** Influence of a 20% organic shock loading on the bioreactor effluent pH ( ● ) and effluent alkalinity ( O ). (The dotted line indicates the application of the shock loading).

Then the pH would be first to change. However, after the shock loading, there was an increase in the pH level, which could be the result of the increased buffering capacity in the bioreactor as suggested by Caine *et al.* (1991).

The COD removal (Fig. 8) showed several decrease/increase cycles during the first 48 h after the shock loading. The lowest COD removal (41%) was reached at 144 h, which was followed by a gradual increase to pre-shock values after about 450 h. The highest COD value in the bioreactor effluent was 1 470 $mg.l^{-1}$ and obtained during the shock loading period.

The methane and carbon dioxide (Fig. 9) volumes of the biogas showed that once again the biogas composition as well as the amount of biogas formed was less affected than the other parameters. There was a fast drop in biogas volumes after the shock loading, followed by two increase/decrease cycles and a fast recovery after 24 h to pre-shock values. The biogas composition for the 40% shock reached pre-shock values somewhat faster than the 20% shock loading, although the biogas composition was more stable after the 20% shock than the 40% shock.

The VFA values (Fig. 10) in this case showed a totally different profile to the ones from the 40% shock experiment (Fig. 5). An immediate drop with a very fast recovery to pre-shock values was found within the first 8 h after the shock loading. The sudden drop in the acetic, propionic and butyric acids is difficult to explain. Furthermore, in this study the acetic acid showed bigger variations to the stress conditions than the propionic acid. Both the propionic and *iso*-valeric acids showed the smallest variation and thus appeared to be least influenced by the 20% shock loading. According to the findings of Dohànyos *et al.* (1985) and Duarte & Anderson (1982), propionic acid is the slowest parameter to stabilize, but in this 20% study the shock loading had a much smaller influence on the bioreactor than was expected.

Hill *et al.* (1987) reported that in the digestion of cow or pig manure, acetic acid levels greater than 800 $mg.l^{-1}$ and propionic:acetic acid ratios greater than 1:1.4 indicated impending failure. Furthermore, Hill & Bolte (1989) reported that for full-scale plants the most reliable acids for the prediction of impending digester failure by as much as seven days in advance, were *iso*-butyric and *iso*-valeric acids. They recommended that the following guidelines be used with fixed media and conventional bioreactor types; no stress = *iso*-butyric and *iso*-valeric levels lower than 5 $mg.l^{-1}$; building stress and/or impending failure = levels between 5 and 15 $mg.l^{-1}$; and failure/damage having already occurred = levels exceeding 15 $mg.l^{-1}$. Based on these predictions, the data from these two Experimental Studies (40% and 20% shocks) appeared to indicate that the UASB bioreactor had already experienced stress conditions even before the shock loadings were started. For the 40% shock loading the values for the *iso*-butyric and *iso*-valeric levels were between 12 and 25 $mg.l^{-1}$ (Fig. 5), whereas the values for the 20% shock loading varied between 3 and 10 $mg.l^{-1}$ (Fig. 10). This anomaly can be explained by the fact that for all three Experimental Studies a relatively small granule bed height was used so as to determine if stress conditions do influence bed growth or not. Thus, it is possible that the granules used under the present operational conditions might have been under a sort of stress even before the 40% and 20% stress conditions were applied. This would also explain why the recovery rates took so long.

fig8-8

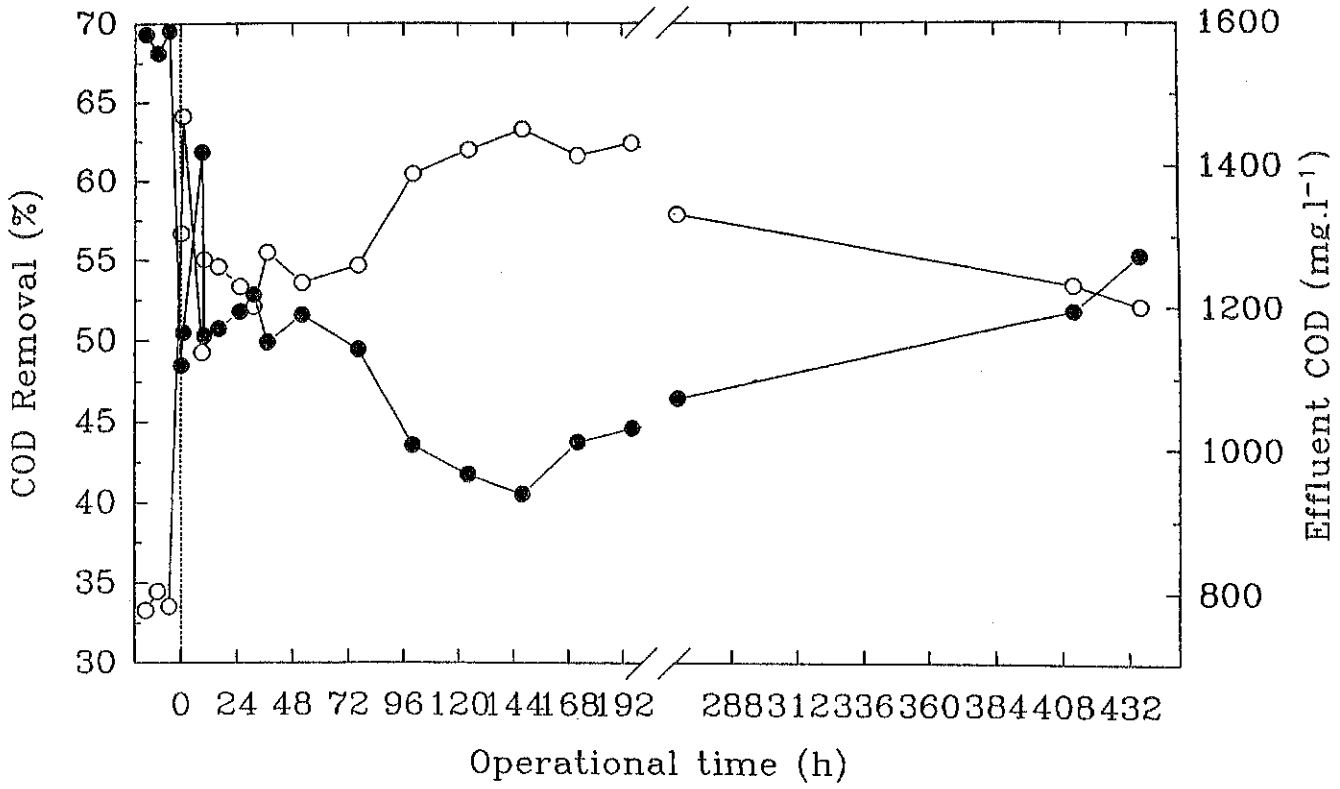


**Figure 8.** Influence of a 20% organic shock loading on the COD removal ( ● ) and the bioreactor effluent COD ( O ). (The dotted line indicates the application of the shock loading).

fig8-9

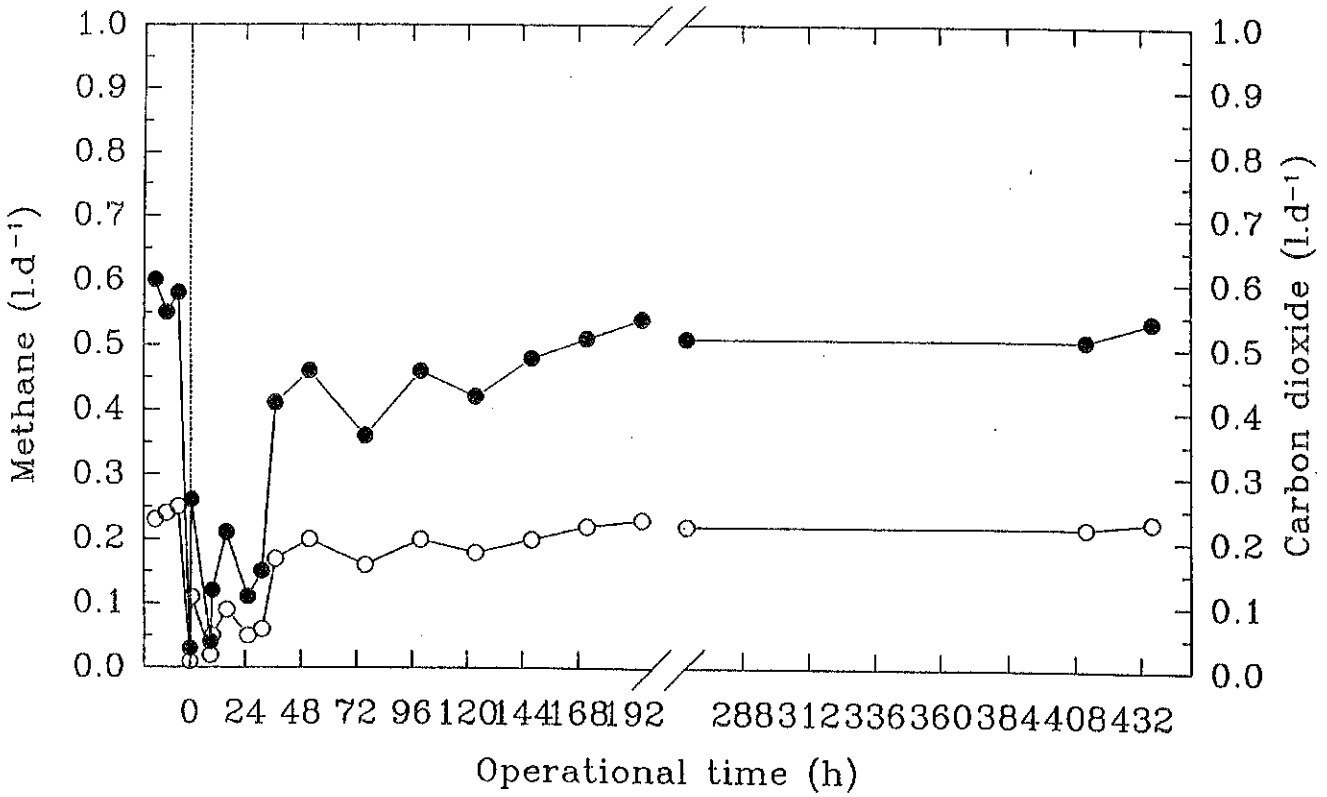


**Figure 9.** Influence of a 20% organic shock loading on the methane ( ● ) and carbon dioxide ( O ) volumes. (The dotted line indicates the application of the shock loading).

fig6-10

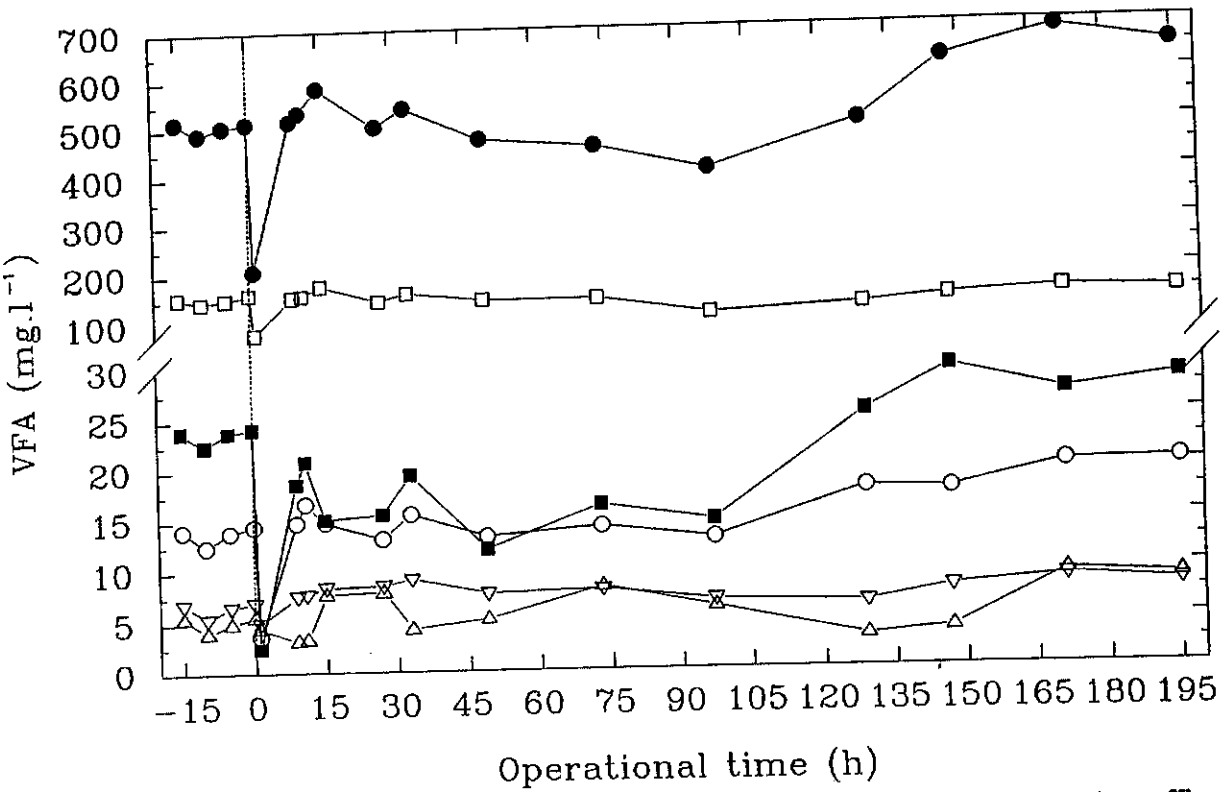


**Figure 10.** Influence of a 20% organic shock loading on the bioreactor effluent VFA content ( ● = AcAc; □ = PrAc; ■ = BuAc; ○ = ValAc; ▽ = i-ButAc; △ = i-ValAc). (The dotted line indicates the application of the shock loading)

fig6-11

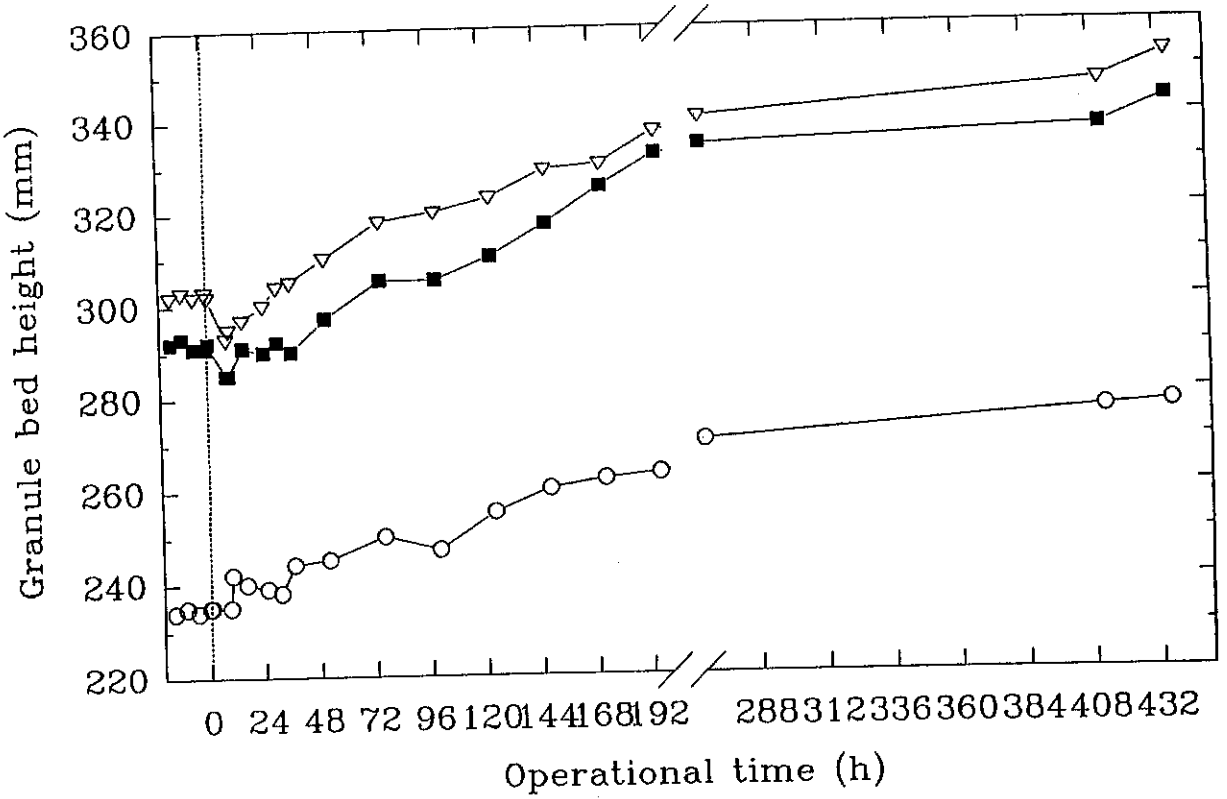


**Figure 11.** Influence of a 20% organic shock loading on the height of the bioreactor granule bed ( ○ = all pumps off; ■ = upflow on and substrate off; ▽ all pumps on). (The dotted line indicates the

The influence of the 20% shock loading on the granule bed height of the bioreactor is indicated in Fig. 11. After the shock loading was applied there was an increase of 10 mm (235 to 245 mm) in the height of the granule bed within the shock period. A slight decrease followed with a gradual increase to 278 mm, 430 h after the shock loading, giving an 18.3 % increase in bed height. In this case no decrease in granule bed was found over a longer operational period. To confirm the influence of the 20% shock loading as a granule stimulant, the study was repeated and similar results (14-19% increases in granule bed) were obtained.

*Experimental Study III - Addition of cysteine*

Moosbrugger and his co-workers (1992) postulated that granules are formed by the generation of an extracellular polypeptide produced by a hydrogenotrophic methanogen of the genus *Methanobacterium*. Furthermore, the species utilizes hydrogen as sole energy source and can produce all its amino acid requirements with the exception of the sulphur containing amino acid, cysteine. Thus, an external cysteine source is necessary for growth. According to Moosbrugger *et al.* (1992), in terms of the behavioral pattern of the specific *Methanobacterium*, if cysteine is supplied in increasing concentrations, polypeptide formation should correspondingly decrease but it is likely that even with a stoichiometrically adequate cysteine supply some polypeptide formation would persist. For this reason the influence of cysteine as granule stimulant or inhibitor was also investigated.

This Experimental Study (III) was started only after the bioreactor had recovered and stabilized from Experimental Study II. The composition of the bioreactor substrate used for the shock loading is given in Table 3. In this study a cysteine shock (12.5 $mg.l^{-1}$) was applied for 8 h at a background OLR of 2.5 $kgCOD.m^{-3}.d^{-1}$, with samples being taken every 2 h for the first 30 h and then every 24 - 48 h. The results are given in Fig. 12 to 16.

**Table 3.** Operational parameters applied during the cysteine shock loading in Experimental Study III

| Parameters | Value |
|---|---|
| Substrate COD ($mg.l^{-1}$) | 2 500 |
| HRT (h) | 24 |
| OLR ($kgCOD.m^{-3}.d^{-1}$) | 2.5 |
| pH | 8.0 |
| Alkalinity ($mg.l^{-1}$) | 200 |
| Cysteine shock loading ($mg.l^{-1}$) | 12.5 |

The pH of the effluent (Fig. 12) was found to decrease from the control level of 6.7 to 5.2 within the first 24 h after the shock loading. This was followed by a characteristic increase/decrease cycle and thereafter a sharp fairly pH increase to 5.7 which were similar to the ones found with the 40% and 20% shocks. The pH then started to slowly recover and to reach pre-shock values after more than 700 h. The alkalinity (Fig. 12) dropped immediately from 725 to 350 $mg.l^{-1}$ within the first 8 h of the shock loading. This was followed by two increase/decrease cycles reaching a maximum of 900 $mg.l^{-1}$ at 96 h, whereafter the alkalinity stabilized at a level of 575 $mg.l^{-1}$ which was slower than before the shock was applied. Once again the general alkalinity profile was similar to the ones found with the organic shock loadings.

The COD removal (Fig. 13) dropped immediately from 84% to 52% within the first 8 h. A further but slower drop was then found reaching a lowest value of 13% at 144 h. This was followed by a gradual increase to 45% and a very slow recovery to half of the pre-shock values by 672 h. The lowest and highest COD concentration in the bioreactor effluent respectively were 378 and 2 680 $mg.l^{-1}$.

The biogas ($CH_4$ and $CO_2$) production (Fig. 14) was once again the parameter that was the least influenced by the shock loading. There was a fast drop in production within the first 24 h with a recovery to pre-shock values after 96 h. After this a double decrease/increase cycle was found whereafter a steady increase in the volumes of both $CH_4$ and $CO_2$ were found. The biogas composition values are much lower than the values obtained with the 40% and 20% shock loadings.

The influence of the cysteine shock on the VFA formation is indicated in Fig. 15. The acetic and propionic acids appeared again to be the most sensitive indicators, with *iso*-butyric and *iso*-valeric acids showing smaller variations during the course of the study. The acetic acid dropped to the lowest value of 300 $mg.l^{-1}$ within the first 8 h followed by a sharp increase to a higher pre-shock value and fast recovery by 48 h after the shock loading. The occurrence of the two major dips during and after the shock loading for all the fatty acids, are very similar to those found for the COD removal (Fig. 13). The acetic, butyric and valeric acids showed a steady decrease in concentration from 72 h to 340 h with the propionic acid remaining more or less stable.

For the first 48 h after the application of the cysteine shock, the granule bed height remained constant at 249 mm (Fig. 16), followed by a slight decrease to 245 mm at 96 h, a steady increase (8%) to 269 mm by 242 h and a slower but gradual increase (17%) to 292 mm by 960 h. Moosbrugger *et al.* (1992) found that when cysteine was supplied in increasing concentrations polypeptide formation correspondingly decreased and led to a reduction of 50% in the specific granule yield. In contrast, in this study the data showed (Fig. 16), that there was a steady increase in the granule bed height after the bioreactor substrate was supplemented with 12.5 $mg.l^{-1}$ cysteine. The increase in bed height was still evident more than 650 hours after the shock loading, when most of the other efficiency parameters had stabilized.

fig9-12

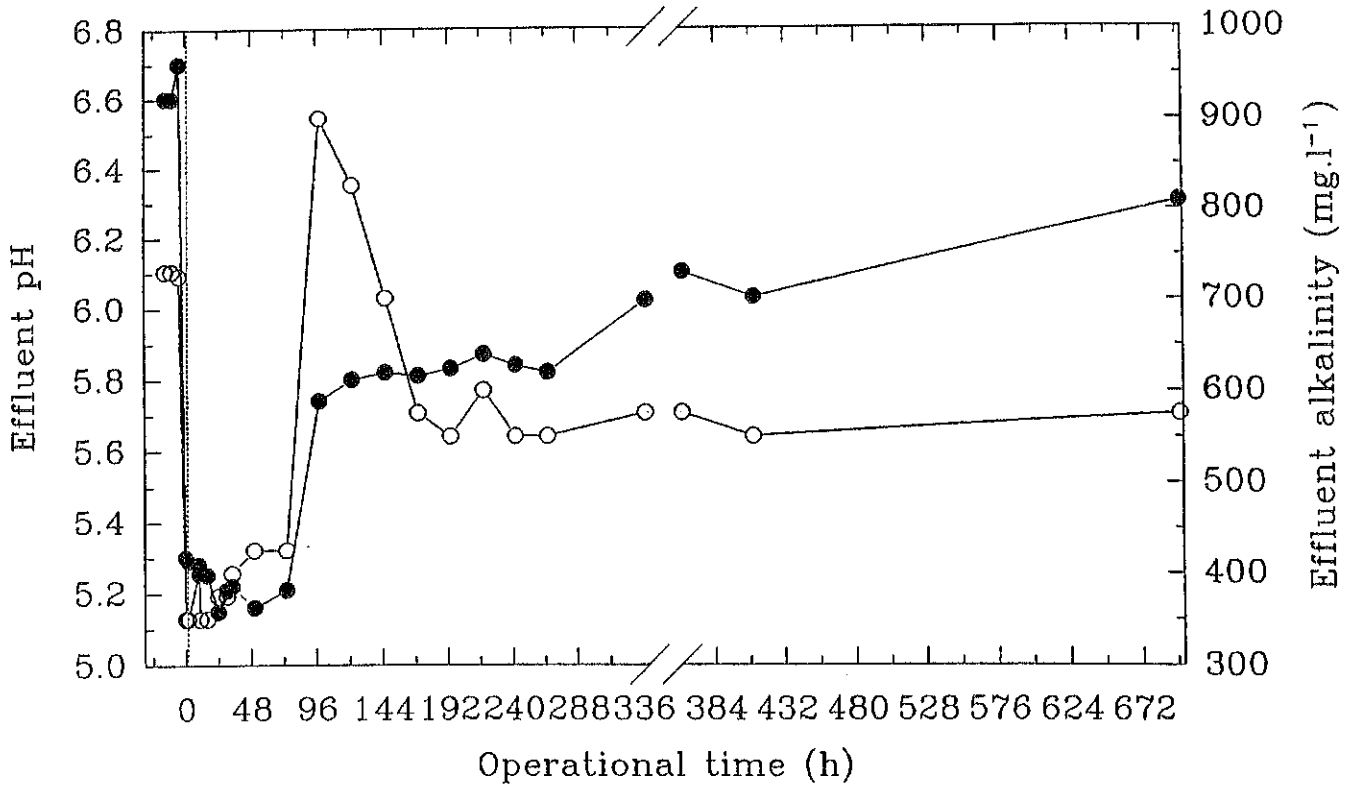


**Figure 12.** Influence of a cysteine shock loading on the bioreactor effluent pH ( ● ) and the effluent alkalinity ( O ). (The dotted line indicates the application of the shock loading).

fig9-13

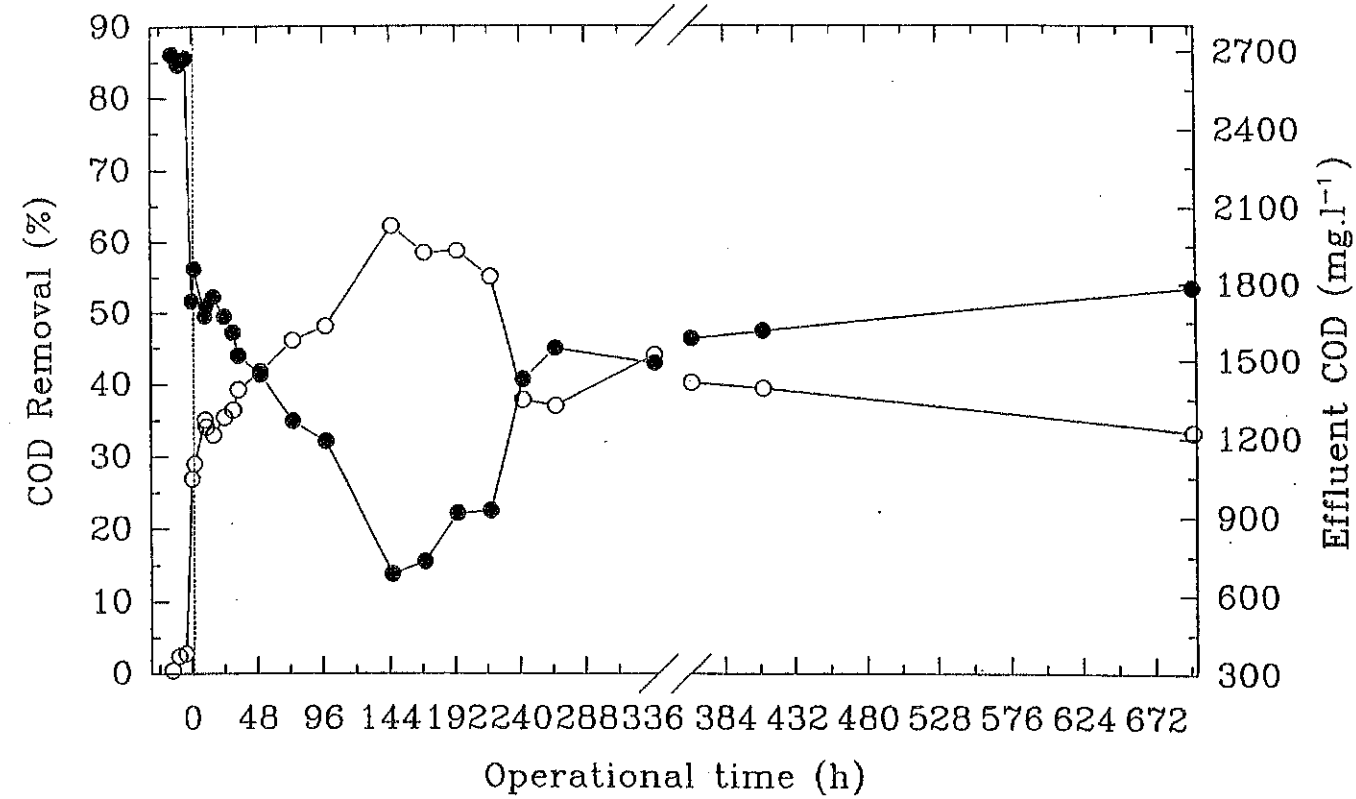


**Figure 13.** Influence of a cysteine shock loading on the COD removal ( ● ) and the bioreactor effluent COD ( O ). (The dotted line indicates the application of the shock loading).

fig6-14

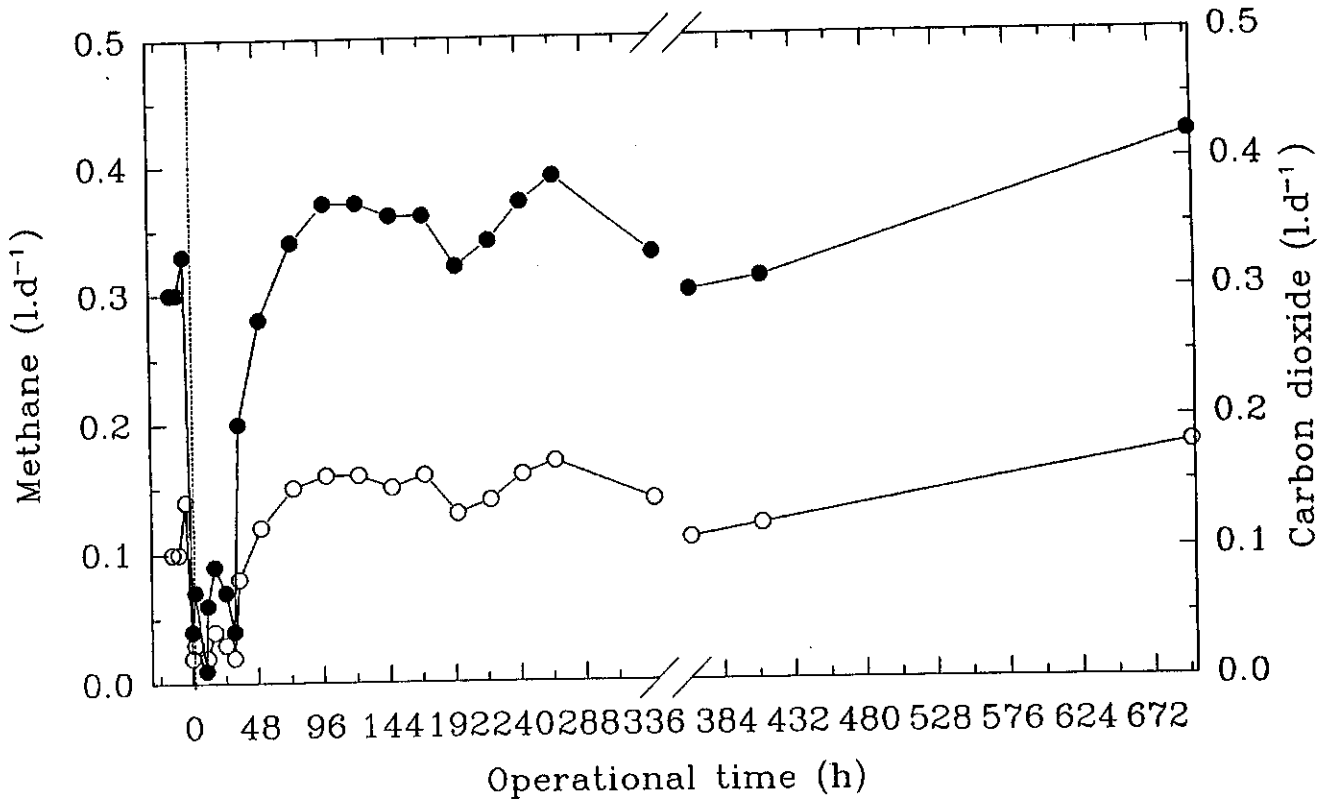


**Figure 14.** Influence of a cysteine shock loading on the methane ( ● ) and carbon dioxide ( O ) volumes. (The dotted line indicates the application of the shock loading).

fig6-15

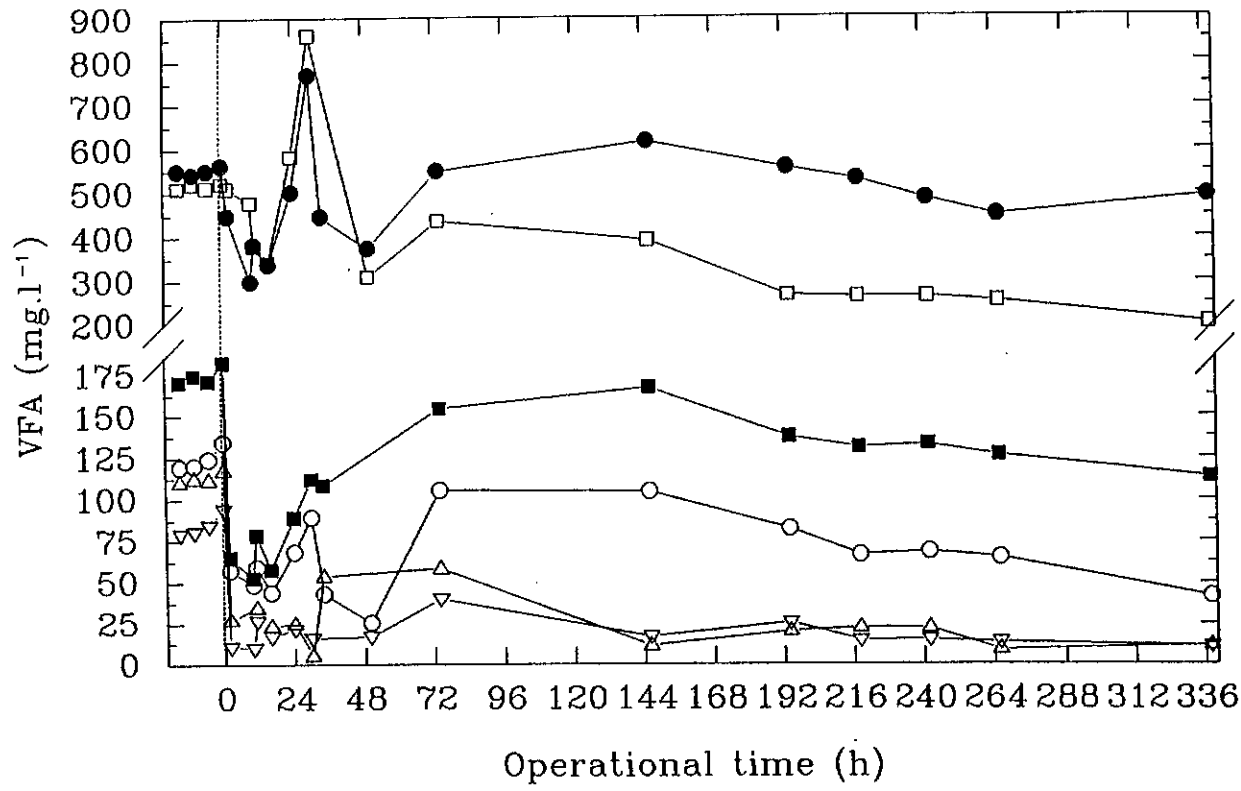


**Figure 15.** Influence of a cysteine shock loading on the bioreactor effluent VFA content ( ● = AcAc; □ = PrAc; ■ = BuAc; O = ValAc; Δ = i-BuAc; ∇ = i-ValAc). (The dotted line indicates the application of the shock loading).

fig6-16

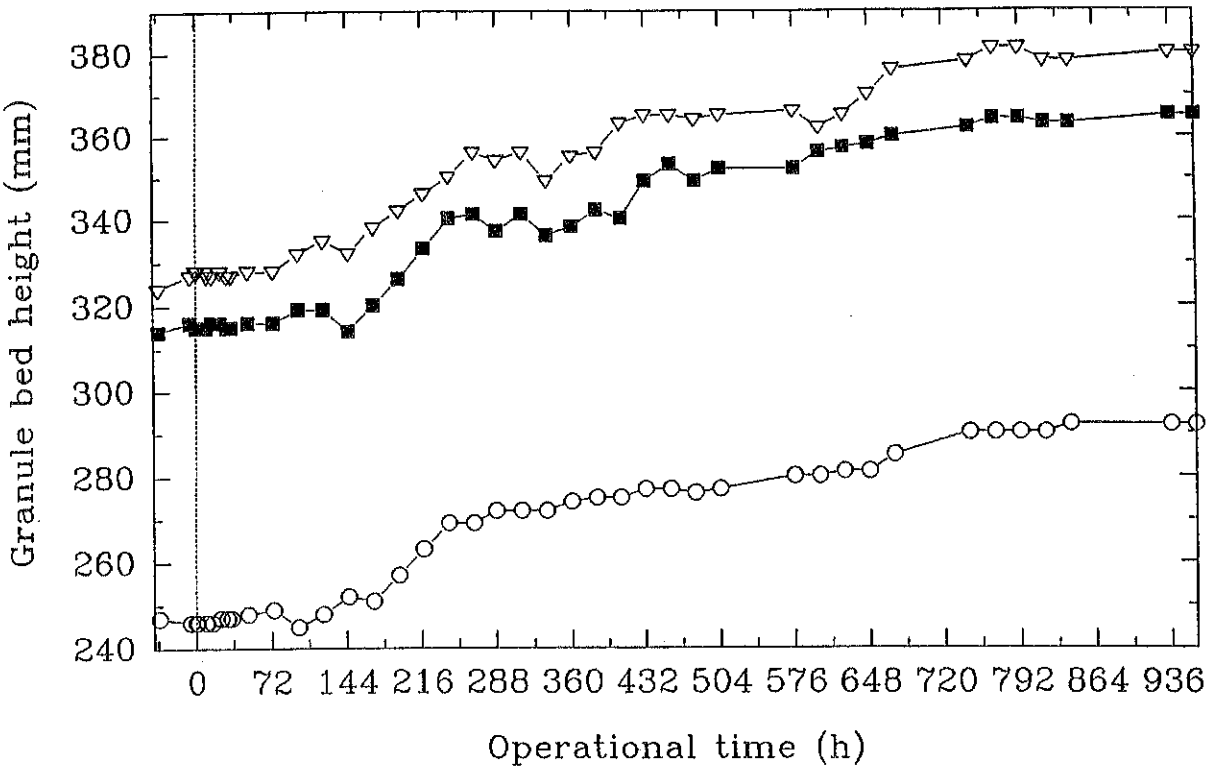


**Figure 16.** Influence of a cysteine shock loading on the height of the bioreactor granule bed ( O = all pumps off; ■ = upflow on and substrate off; ▽ = all pumps on). (The dotted line indicates the application of the shock loading).

## 6.5 Conclusions

Granules are a characteristic feature of the UASB system but the mechanism of their formation is still poorly understood and full exploitation of this cell immobilization method can thus not be realized until the granule formation conditions are defined. It has been reported that when 'stress' is placed on the microbial community in anaerobic bioreactors, an environment is created where specific strains gain a competitive advantage, which then leads to an increase in granules. The aim of this study was thus to evaluate the influence of 'stress` conditions as microbial granule formation stimulants.

In the first part of the study a control UASB bioreactor was used to simulate start-up and operation of a system just inoculated with anaerobic sludge from a local anaerobic digester. The continual operational setbacks showed the problem of long start-up times and the fundamental problem of biomass washout encountered with the UASB design when inoculating just with anaerobic sludge.

The results obtained from the first experimental study, where a 40% shock loading was applied to the UASB bioreactor, showed that this concentration was too high and led to a prolonged recovery period even though a small increase in granule bed was found. However, the bed height was found to decrease as the other efficiency parameters changed and this was probably due to the formation of smaller not so dense aggregates which washed out once the system started to stabilize. It was also found that acetic and propionic acids are excellent indicators of organic stress. The VFA and methane data profiles confirm the increase in acidogen activity and lack of substantial methanogen growth.

The 20% shock loading (repeated three times) resulted in shorter recovery periods and once again certain of the VFA's showed the first changes. The data also showed a stable increase of 14 - 19% in granule bed height. The cysteine shock resulted in an excellent and stable increase (17%) in granule bed height.

The increases in the granule bed obtained in this study showed that when stress conditions are applied to an UASB bioreactor an enhancement of granulation would occur. This supports the hypothesis that granule formation is stimulated under 'stress' conditions. However, the results clearly indicate that the type and concentration of stress applied must be carefully chosen otherwise over-stressing could lead to an extended recovery period or even to total system failure. This has far reaching implications for industries using the UASB bioreactor concept to treat industrial waste waters.

# CHAPTER 7

# THE UTILIZATION OF A 50 l UASB BIOREACTOR PILOT-PLANT TO TREAT A CANNING FACTORY EFFLUENT

## 7.1 Summary

A mesophylic pilot-scale upflow anaerobic sludge bed bioreactor (UASB) design was evaluated for the treatment of a carbohydrate rich effluent from the canning-industry. The UASB bioreactor was inoculated with 1 670 g anaerobic granules to give a granule bed height of 141 mm. After the system had stabilized, the HRT was set at 24 h and the substrate pH poised at 7.5 to prevent rapid acidification. In the first experimental study (I) the chemical oxygen demand (COD) was increased stepwise from 2 300 to a full strength of 4 000 $mg.l^{-1}$. In the second study (II) the organic loading rate (OLR) was increased by shortening the hydraulic retention time (HRT) from 24 to 10 h to give an OLR increase from 4.0 to 9.2 $kgCOD.m^{-3}.d^{-1}$ with an average COD removal of 81 - 84% and removal rate of 7.5 $kgCOD.m^{-3}.d^{-1}$. An HRT of 10 h was taken as the optimum operational HRT, as the recovery rate of the system at HRTs lower than 10 h were found to be longer than at longer HRTs. However, the data clearly showed that the bioreactor had not reached its optimal loading capacity, which was further confirmed by the constant increase in the granule bed height. Since neutralization costs would influence the economic aspects of the process, the influence of lower pH values was investigated in the second study and the pH of the canning effluent was lowered from 7.5 to 5.0. At the lower pH, the COD removal remained fairly constant between 79 - 82%. Stabilization occurred at a substrate pH of 5.0 with an alkalinity level of 700 $mg.l^{-1}$ and an effluent pH value of 6.5. It was clear from the low bioreactor effluent pH that the lower end of the operational pH had been reached and any further lowering of the substrate pH would lead to system failure. An increase of 144% in the granule bed height was found over the whole study period and the size of the granules obtained varied from smaller than 1.0 mm to about 4.0 mm.

## 7.2 Introduction

Concentrated organic industrial wastes such as distillery effluents and wastes resulting from the manufacture of various foods generally create serious treatment or disposal problems for the industry or local authority concerned because of their high organic load. These wastes are normally soluble or colloidal and have COD values varying from 2 000 to 200 000 $mg.l^{-1}$ as compared to domestic sewage with a COD of about 400 $mg.l^{-1}$ (Verstraete & Vandevivere, 1997). Effluents of this type are thus reluctantly received into communal sewers by the controlling authority and the manufacturer is faced with heavy trade-effluent charges. Investigations have shown that these effluents can seldom be treated satisfactorily by aerobic methods as they require excessive dilution to render them amenable to aerobic breakdown (Ross, 1989). Moreover, rising energy costs make aerobic treatment increasingly expensive.

Waste water from fruit processing plants consists mainly of process wash waters from various factory operations, including pre-washing of the fruit, cleaning of process vessels, pipelines and associated equipment. In the treatment of this type of waste it is normally necessary to include a flow-balancing tank or to use a completely mixed reactor system with an HRT in excess of 24 h (Borja & Banks, 1994).

The anaerobic digestion treatment process of industrial effluents has been extensively researched. It has been reported (Fang *et al.*, 1994) that there are over 300 full-scale operational anaerobic digesters world-wide with most of them designed for treating waste waters from the food and beverage industries. According to Monroy *et al.* (1994), the UASB design is the most common system with 30% of all installed plants of this design.

The key to the successful operation of UASB bioreactors is the development of granular sludge in which highly active granulated biomass with good settleability is formed (Pong & Verstraete, 1996, Gent University, personal communication). Alphenaar *et al.* (1994) noted that one of the most important disadvantages of anaerobic treatment is the extended start-up period. This has been overcome by the direct utilization of granular sludge as inoculum so as to accelerate the start of new bioreactors. However, the mechanical stability of granules decreases, resulting in disintegration or flotation and consequently loss of activity from the system.

Results obtained by Trnovec & Britz (1997) (Chapter 5) showed that carbohydrate rich effluents produced in the canning-industry can be successfully treated in a laboratory-scale UASB bioreactor. Prior to the implementation of the UASB bioreactor in the industry, extensive experimentation is required in order to assure similar results and consequently, a 50 litre UASB pilot-scale bioreactor was constructed. The objective of this study was to assess the efficiency of shorter HRT and lower pH values on the overall performance and stability of the 50 litre UASB bioreactor pilot-plant using canning factory effluent as substrate.

## 7.3 Materials and methods

*Digester design*

The pilot-plant UASB bioreactor had an operational volume of 50 litre (total height of 2 300 mm and internal diameter of 160 mm) and combined UASB design with gas/solids separator at the top. The biogas exited through the top, while the substrate was introduced into the bioreactor at the base. The overflow of the bioreactor emptied through a U-shaped tube to prevent any atmospheric oxygen from entering the system. The upflow velocity in the reactor was set at 2 $m.h^{-1}$ and the temperature maintained at 35°C using a heating tape and an electronic control unit (Meyer *et al.*, 1985) and the bioreactor was insulated. The volume of the biogas ($CH_4$ and $CO_2$) was determined using a manometric unit equipped with an electronically controlled counter and a gas-tight valve and the volumes corrected to standard temperature and pressure. The substrate was fed semi-continuously to the bioreactor by means of a peristaltic pump (Watson Marlow 101) controlled by an electronic timer.

*Bioreactor start-up*

The bioreactor was seeded with 1 670 g of liquid drained anaerobic granules obtained from a batch reactor to give a granule bed height of 141 mm. The bioreactor was then allowed to stabilize for 48 h in order to allow the bacterial community to acclimatize. After the stabilization period, feeding was commenced with a diluted canning factory effluent (COD = 2 500 $mg.l^{-1}$ ). Once the bioreactor had stabilized the COD concentration was gradually increased to 4 000 $mg.l^{-1}$ and the HRT set at 1.0 d. The canning factory effluent was supplemented with 500 $mg.l^{-1}$ urea and 500 $mg.l^{-1}$ $K_2HPO_4$ to prevent any nitrogen and phosphorus limitation during the start-up period. The substrate was also supplemented with 1.0 ml trace element solution, as described by Nel *et al.* (1985), and the pH poised at 7.5 with 1 N calcium hydroxide to optimize the environment for maximum granule growth.

The canning factory effluent used in this study was from the stream before it reached the general stream that also contained the lye effluents. The effluent was sampled over the whole 1996/1997 canning season (Mr. J. Visser, 1997, Ashton Canning Co, personal communication).

*Analytical methods*

The following parameters were monitored according to the APHA (Standard Methods, 1992): pH; alkalinity; total solids (TS); total volatile solids (TVS) and total non-volatile solids (TNVS). Chemical oxygen demand (COD), orthophosphate phosphorus and total Kjeldahl nitrogen were determined colorimetrically using a DR 2000 spectrophotometer (Hach Co. Loveland, CO) and standardized procedures (Standard Methods, 1992).

*Experimental studies*

The study comprised two experimental studies (I and II). In the first Experimental Study (I), the substrate COD concentration was kept constant at 4 000 $mg.l^{-1}$, while the HRT was reduced stepwise from 24 to 10 h in 12 steps. In the second Experimental Study (II) both the HRT and substrate COD concentration were kept constant respectively at 10 h and 4 000 $mg.l^{-1}$. The substrate pH was then reduced stepwise from 7.5 to 5.0 in six steps. In both studies, the bioreactor was allowed to reach stable-state conditions before each HRT or pH reduction. Stable-state is defined as a state, which can be maintained indefinitely without system failure (Cobb & Hill, 1990), during which the variation in bioreactor performance parameters is less than 10%. The length of each phase was based therefore on the stability of the bioreactor effluent pH, alkalinity and COD removal. The height of the granule bed was also measured to determine the effect of the shorter HRT and lower pH on the growth of the microbial community of the bioreactor.

## 7.4 Results and discussion

*Canning effluent composition*

The canning effluent was collected from the drain trough under a mechanical fruit cocktail canning line and stored at 4°C until required. The substrate for both experimental studies was a dilution of the substrate given in Table 1 of Chapter 5 of this thesis and pH poised at 7.5 with $Ca(OH)_2$.

**Table 1.** Operational parameters and bioreactor performance during Experimental Study I where the HRT was shortened. (Data taken after stable-state had been reached)

| Parameters | Phase | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| Substrate COD ($mg.l^{-1}$) | 3975 | 3802 | 3807 | 3885 | 3981 | 3951 | 4063 | 4050 | 3818 | 3926 | 4180 | 3866 |
| COD removal (%) | 83.1 | 83.5 | 82.8 | 83.8 | 83.8 | 83.7 | 82.5 | 82.5 | 81.9 | 82.2 | 81.9 | 81.7 |
| HRT (h) | 24 | 22 | 20 | 18 | 17 | 16 | 15 | 14 | 13 | 12 | 11 | 10 |
| OLR ($kgCOD.m^{-3}.d^{-1}$) | 4.0 | 4.1 | 4.6 | 5.2 | 5.6 | 5.9 | 6.5 | 7.0 | 7.1 | 7.9 | 9.1 | 9.2 |
| Effluent pH | 7.5 | 7.4 | 7.7 | 7.5 | 7.8 | 7.7 | 7.8 | 7.7 | 7.7 | 7.4 | 7.5 | 7.3 |
| Effluent alkalinity ($mg.l^{-1}$ $CaCO_3$) | 1350 | 1575 | 1600 | 1675 | 2200 | 2000 | 2125 | 2175 | 2125 | 2000 | 1900 | 1925 |
| Biogas ($l.d^{-1}$) | 1.13 | 1.19 | 1.30 | 1.49 | 1.61 | 1.70 | 1.84 | 1.95 | 1.98 | 2.22 | 2.55 | 2.59 |
| Methane content (%) | 63 | 63 | 65 | 64 | 63 | 64 | 65 | 63 | 65 | 65 | 64 | 63 |
| Methane yield ($m^3.d^{-1}.kg^{-1}$ $COD_{removed}$) | 0.216 | 0.217 | 0.222 | 0.220 | 0.216 | 0.220 | 0.225 | 0.213 | 0.222 | 0.224 | 0.219 | 0.217 |

*Experimental Study I - Shortening of the hydraulic retention time*

During this study the HRT was shortened from 24 to 10 h over 12 steps (Table 1) with a subsequent increase in organic loading rate (OLR) from 4.0 to 9.2 $kgCOD.m^{-3}.d^{-1}$. Both the substrate COD concentration and pH were kept constant at 4 000 $mg.l^{-1}$ and 7.5 respectively. It was decided not to lower the HRT to less than 10 h, since the recovery rate in terms of pH and COD removal stabilization was slower than at the longer HRTs. During the shortening of the HRT, the bioreactor effluent pH varied between 7.3 and 7.8 with the alkalinity from 1 350 to 2 200 $mg.l^{-1}$ at the end of each step. As the HRT decreased, an increase in the biogas production, from 1.13 to 2.59 $l.d^{-1}$, was found, with average methane yield of 0.219 $m^3.d^{-1}.kg^{-1}$ $COD_{removed}$. At the same time the percentage COD removal varied between 81% and 84% with a maximum removal rate of 7.5 $kgCOD.m^{-3}.d^{-1}$ at an HRT of 10 h (Table 1). This was found to be lower than when compared to the results obtained in the laboratory-scale bioreactor at the same HRT in Chapter 5 of this thesis. This could be due to the fact that the pilot-scale bioreactor had a larger component of more difficult degradable organic material (e.g. fiber) which accumulated within the bioreactor.

*Experimental Study II - Lowering of the substrate pH*

Since the pH of the carbohydrate rich stream from the canning factory is below the optimum range for granular growth, the influence of lower pH values, combined with a shorter HRT, was investigated. In this study, based on the results obtained during Study I, the HRT was kept constant at 10 h and the substrate pH lowered in six steps from 7.5 to 5.0 (Table 2). The data showed that at a substrate pH of 7.0, the alkalinity decreased from 1 925 to 1 450 $mg.l^{-1}$ (steps 1 and 2) with a second drastic drop in alkalinity from 1 375 to 500 $mg.l^{-1}$ (steps 4 and 5) as less alkalinity was produced in the bioreactor. This can possibly be explained by the regulation mechanism of the carbonate/bicarbonate/$CO_2$ buffering system of the process (Moosbrugger *et al.*, 1992; Nel & Britz, 1986). At the lower pH, the $CO_2$ is less soluble and the partial pressure of $CO_2$ is higher, thus an increase in the biogas production is also observed. As the substrate pH was lowered, the alkalinity level decreased with a corresponding decrease in the bioreactor effluent pH. Stabilization occurred at a substrate pH of 5.0 with an alkalinity level of 700 $mg.l^{-1}$ and a bioreactor effluent pH value of 6.5, which was at the lower end of the optimal recommended pH-range (Wentzel *et al.*, 1994). At the end of the study, the pH in the bioreactor effluent was higher than the substrate pH, due to the loss of $CO_2$ in the aqueous phase. When the substrate pH was lowered, the COD removal remained fairly constant between 79% and 82% with stabilization in the biogas production at around 2.78 $l.d^{-1}$. The economic implication of being able to optimize and operate the bioreactor at a substrate pH of 5.0 means that canning effluent can be introduced straight to the bioreactor without any neutralization. This is considerable, since the wastewater of the food, drinks and fermentation industries are often acidic (pH 4 to 5), due to the production of short chain organic acids in the holding or balancing tanks. This low substrate pH does however, not cause low pH conditions in the anaerobic bioreactor since, under efficient methanogenises the organic acids are converted rapidly to $CH_4$ and $CO_2$ and pH buffering in the form of bicarbonate alkalinity.

**Table 2.** Operational parameters and bioreactor performance during Experimental Study II where the substrate pH was decreased. (Data taken after stable-state had been reached)

| Parameters | Phase | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Substrate COD ($mg.l^{-1}$) | 3866 | 3933 | 4111 | 4144 | 4205 | 4238 |
| COD removal (%) | 81.7 | 81.6 | 80.4 | 80.5 | 80.3 | 79.6 |
| HRT (h) | 10 | 10 | 10 | 10 | 10 | 10 |
| OLR ($kgCOD.m^{-3}.d^{-1}$) | 9.2 | 9.4 | 9.8 | 9.9 | 10.01 | 10.09 |
| Substrate pH | 7.5 | 7.0 | 6.5 | 6.0 | 5.5 | 5.0 |
| Effluent pH | 7.3 | 7.1 | 7.0 | 6.8 | 6.5 | 6.5 |
| Effluent alkalinity ($mg.l^{-1}$ $CaCO_3$) | 1925 | 1450 | 1900 | 1375 | 500 | 700 |
| Biogas ($l.d^{-1}$) | 2.59 | 2.64 | 2.71 | 2.74 | 2.78 | 2.78 |
| Methane content (%) | 63 | 65 | 64 | 64 | 63 | 65 |
| Methane yield ($m^3.d^{-1}.kg^{-1}$ $COD_{removed}$) | 0.217 | 0.225 | 0.220 | 0.221 | 0.218 | 0.225 |

The pilot-scale bioreactor in terms of COD removal as operated in this study, was not as stable when compared to the laboratory-scale bioreactor operated in Chapter 5 of this report. This could possibly be due to an accumulation of more non-biodegradable material within the pilot-scale bioreactor. However, in this bioreactor bigger granules were observed, and according to Guiot *et al.* (1992), gas is produced inside the granules especially where the methanogens are located, which in terms limit the substrate diffusion into the granule centre. This leads to the formation of 'hollow' granules that fill with $CO_2$ and $CH_4$ and cause the granules to float which in turn leads to washout and lower COD removal values.

*UASB Bioreactor granulation efficiency*

The efficiency of granulation in the bioreactor was determined by monitoring the changes in the height of the granule bed (Fig. 1). With an initial bed height of 141 mm, growth at first was very slow but as soon as the HRT was lowered to below 20 h, a definite increase in the bed height was found. This sudden increase in granule formation can be ascribed to the sudden changes in OLR, which was equivalent to a 13% shock loading. Even after an HRT of 10 h had been reached (Study I), the granule bed still showed growth. This increase in bed height persisted during the second study (II) until the substrate pH was lowered to 5.5 when the bed growth stabilized at a height of 345 mm (increase of 144%).

fig7-1

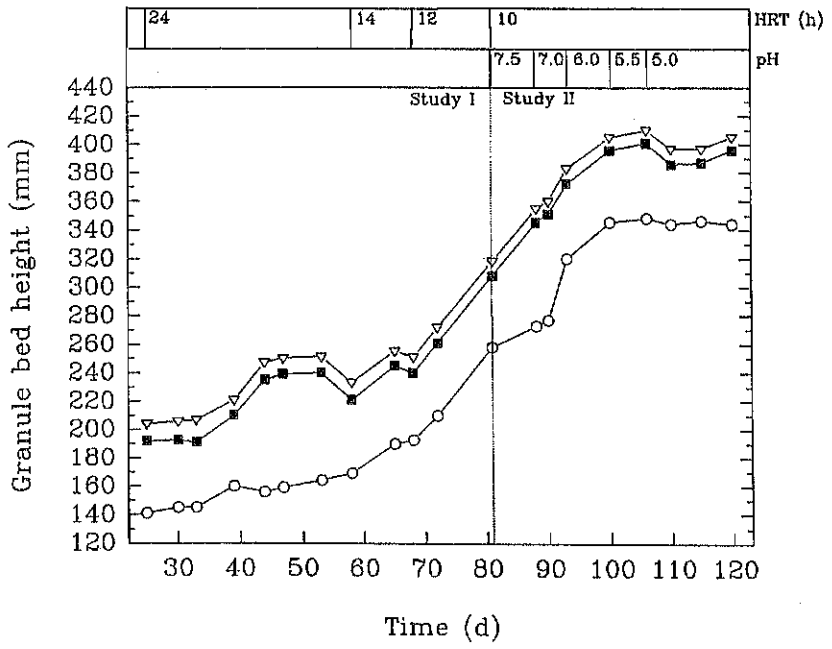


**Figure 1.** The effect of shorter HRTs and lower substrate pH values on the height of the digester granule bed ( O = all pumps off; ■ = upflow on, substrate off; ∇ = all pumps on).

It is possible that if the COD concentration of the canning effluent itself was higher, a further increase in the granule bed height might have taken place, since the granule to bioreactor volume ratio for the pilot-scale bioreactor (33.4 $g.l^{-1}_{reactor\ volume}$) is much higher than that of the laboratory-bioreactor (0.217 $g.l^{-1}_{reactor\ volume}$) ratio, thus creating a more favourable environment for the formation of granules should the COD concentration of the substrate be increased. Furthermore, an increase in the COD concentration would lead to further 'stress' conditions, thus resulting in a shift of the population dynamics of the anaerobic consortium and should, according to Riedel & Britz (1993), result in the formation of highly settable granules.

*Granule characteristics*

During start-up under ideal conditions, the biomass in an UASB bioreactor should aggregate to form stable and compact granules which may be up to 5 mm in diameter (Ross, 1989). The development of a suitable granulated sludge can be a lengthy process, nevertheless, it is considered vital to the success of the UASB operation. The granules obtained from the pilot-scale bioreactor used in this study varied in size from large granules (1 to 4 mm) to very fine particles (smaller than 0.5 mm). The larger granules from this bioreactor had clear spherical shapes as illustrated in Fig. 2. In comparison, the granules obtained from the 2.3 litre laboratory-scale UASB bioreactor (Chapter 5) were generally smaller, not so clearly spherical, and did not have such a wide size range (pinpoint to 2 mm) as are illustrated in Fig. 3. The periphery of the granule surface in both instances was well defined and no filaments extended beyond the surface, as is the case with most sludge flocs (Ross, 1989).

The granules collected from the 50-litre bioreactor showed different colours with black granules apparently being stronger than the grey granules. While the black granules had a smooth and shiny surface, the grey granules were more irregular in shape. A transparent film layer was found to cover the grey granules, which could possibly be ascribed to the extra-cellular polymers excreted by cells in the grey granules (Pong & Verstraete, 1996, Gent University, personal communication). Furthermore, it has been reported that black granules are microbiologically very rich in methanogens whereas the acidogens are most likely responsible for the grey colour and transparent layer on the grey granules. In this study, it was also found that the larger black granules are more susceptible to flotation and washout than the grey granules (Pong & Verstraete, 1996, Gent, personal communication). Fang *et al.* (1994) reported that the microbial distribution in an UASB granule is naturally dependent upon the degradation nature and type of the substrate. For all types of granules the aggregation reaction does not appear to be reversible as the granules can retain their shape and settling properties even after one year of storage without substrate, making them more accessible for further and future use as pre-granulated seed has often been used for rapid start-up in UASB bioreactors (Goodwin *et al.*, 1992; Wu *et al.*, 1995).

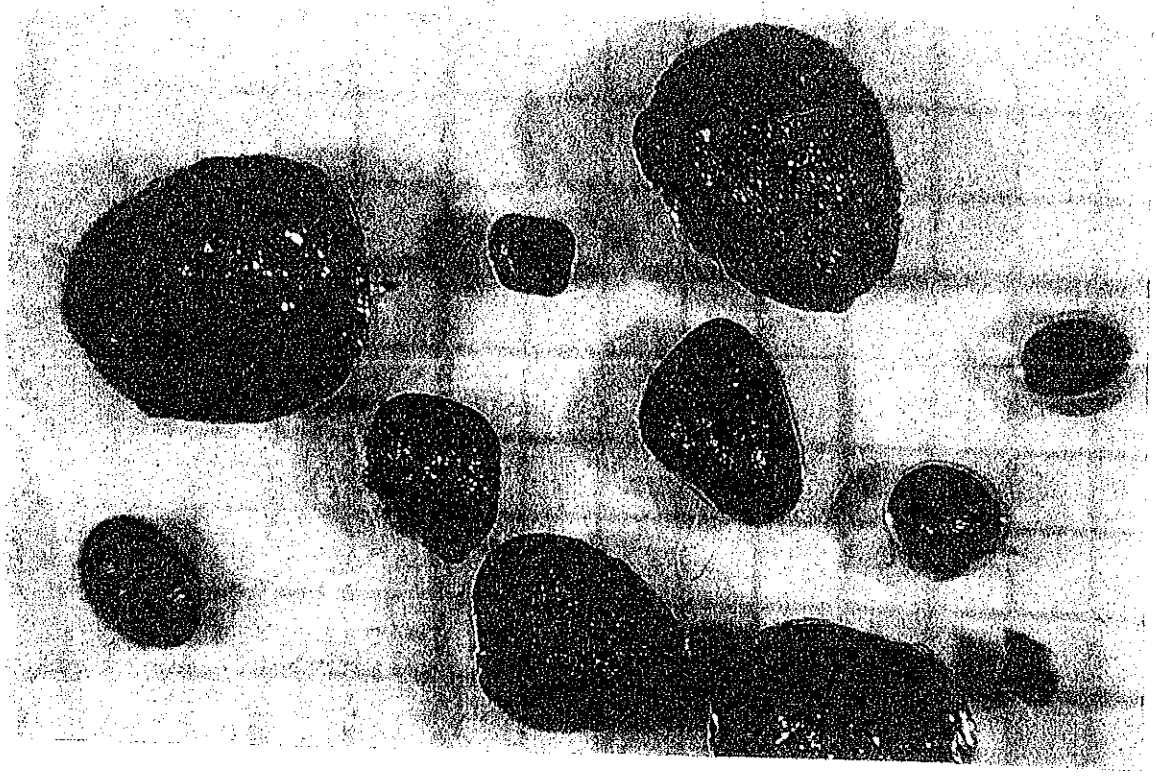

**Figure 2.** Granules from the 50 litre pilot-plant UASB bioreactor (grid size 1 $mm^2$).

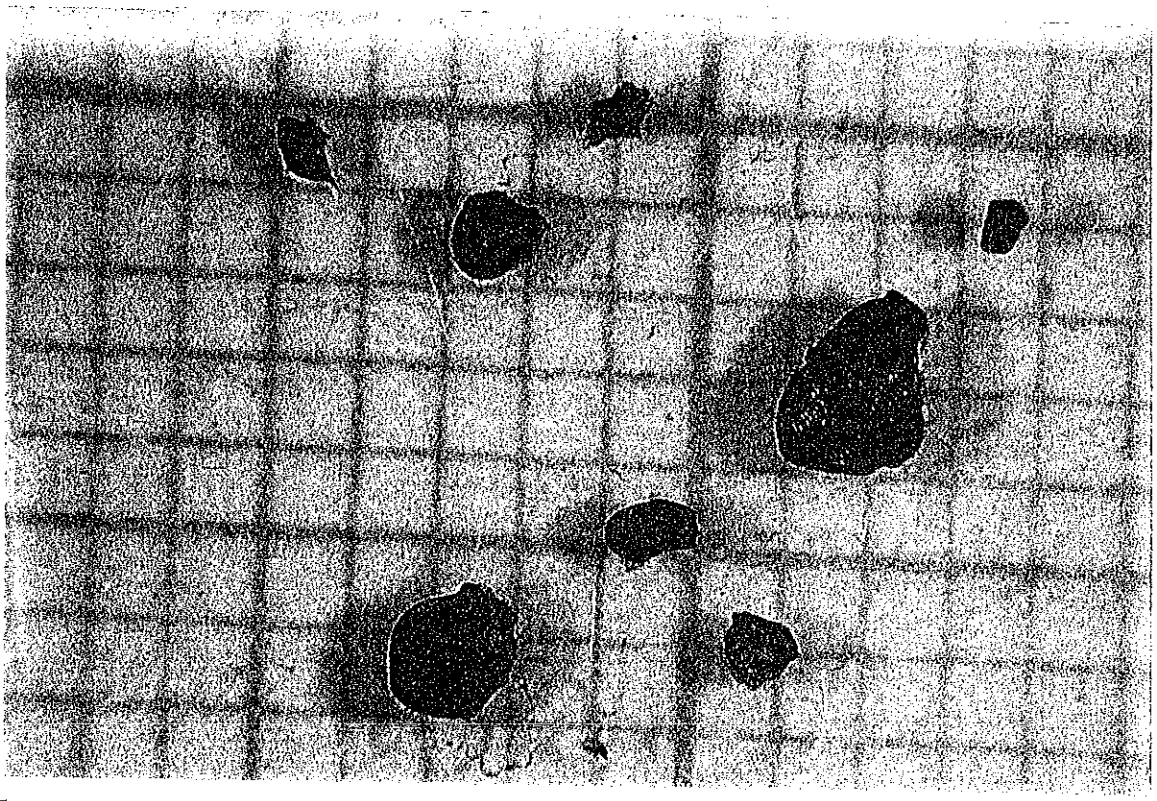

**Figure 3.** Granules from the 2.3 litre laboratory-scale UASB bioreactor (grid size 1 $mm^2$).

## 7.5 Conclusions

The successful cultivation of granules has important economic implications for the optimization of biological treatment processes in terms of improved sludge settleability, biomass retention and high loading rates. It has often been reported that methane-producing bacteria are sensitive to sudden changes of reactor conditions, such as pH fluctuation, loading variations, presence of toxicants, etc. According to Fang (1997), this is one of the major reasons which has hindered the broader acceptance of anaerobic biotechnology for waste water treatment. However, in granules, only a limited number of bacteria near the surface, are directly exposed to the mixed liquor and thus any adverse affects caused by the sudden changes of mixed conditions would be limited (Fang, 1997). Furthermore, the more vulnerable acetogens and methanogens, if they are as many believe, mostly situated in the interior, are thus shielded by the hydrolytic/fermentative acidogens of the surface layer. This implies that the methanogens present in biogranules should be less vulnerable to the sudden changes in environmental conditions.

From the results obtained during the two experimental studies (I and II) it was found that the UASB pilot-plant could be efficiently used for the treatment of canning factory effluent. There was an excellent increase (144%) in the height of the granule bed with an average COD removal of 82% over the course of the study (120 d). Raw canning factory effluent could be treated at a substrate pH of 5.0 without any neutralization, while the effluent pH never dropped below the optimum recommended pH range. This has far-reaching economic implications for the canning-industry, to be able to optimize and operate a bioreactor at a substrate pH of 5.0 without any neutralization. Furthermore, by being able to operate the bioreactor at a HRT of 10 h means that more factory effluent can be treated in a shorter time, resulting in smaller holding and balancing tanks and thus ensuring that less short chain organic acids will form, thus preventing a drop in substrate pH. The increase in granule bed height under these conditions will result in a continuous supply of granules, thus ensuring a 'healthy' and operational bioreactor under most circumstances.

Chap8

# CHAPTER 8

# DEVELOPMENT OF A MODEL FOR THE SIMULATION OF THE ENHANCEMENT OF THE GRANULATION PROCESS DURING ANAEROBIC DIGESTION

## 8.1 Summary

The hypothesis for enhanced granulation was used to develop a model for the simulation of the process and to compare results from both batch and laboratory-scale anaerobic systems. A good correlation was evident between the hypothesis and the experimental data examined. The results were used to demonstrate the usefulness of a granulation model in correlating process response and testing the hypothesis theories. It was found to be essential that controlled environmental stress be applied and maintained to sustain granulation.

## 8.2 Background

The 1990's were introduced as a decade of water conservation and environmental reclamation. Among the main challenges, especially in South Africa, few are more critical than the need to protect our water resources. As a result of the importance of environmental pollution, coupled to the expected future shortage of usable water, a renewed interest has arisen in the anaerobic digestion process.

In bioreactors of the upflow anaerobic sludge bed design, the biomass retention is promoted by bacterial self-aggregation into dense granules (El-Mamouni *et al.*, 1997). This granulation enhances the performance since the good settling properties of granules minimizes biomass washout and the close cell packing in the granules optimizes the interspecies exchange of metabolites. However, one of the main problems still remaining in the application of the UASB process is the extensively long start-up periods needed for the development of granules. This may in part be related to the extended generation time of the acetogenic and methanogenic bacteria. It can take several months before a highly effective granular bed can be cultivated. Since the operational efficiency and performance of these systems are mainly dictated by the formation, amount and specific activity of the granules, the rather extended start-up times limit the potential use of the system. In this chapter, the impact of changes in environmental conditions are examined and their direct influence on the enhancement of the granulation process are discussed.

## 8.3 Granulation hypothesis

*Hypothesis*

When sudden 'stress' conditions are applied to batch and UASB laboratory-scale UASB systems containing fresh anaerobic sludge under controlled conditions, an enhancement of the granulation process will take place.

*Theoretical aspects*

When sudden changes in operational parameters (stress conditions) are applied to a UASB bioreactor, it has been reported (Eng *et al.*, 1986) that lactate starts to accumulate and one of the first detectable metabolites that appear is propionic acid (Riedel & Britz, 1993). The accumulation of lactate, and significant rise in hydrogen (Hickey & Switzenbaum, 1991), consequently indicates a shift in microbial consortium dynamics. The acidogens now resort to utilization of the electrons of reduced pyridine nucleotides (NADH) generated during fermentation in the increased catabolism of pyruvate to alternative more reduced hydrogen sink products. These include metabolites such as lactic, propionic and even butyric acids rather than acetate, $CO_2$ or $H_2$. This unbalanced situation will also lead to the rapid decrease in the bioreactor pH as a result of the accumulation of organic acids like lactate. The increase in the lactate concentration subsequently results in an orderly shift between the normal acidogenic population and a more predominant lactate-utilising population (Riedel & Britz, 1993) in response to the gradual decrease in the pH. Once lactate utilizing microbes gain a competitive advantage, since they obtain a maximum of ATP per mol of lactate fermented (Thauer *et al.*, 1977), the propionic acid concentration will decrease and the pH will gradually increase and stabilize. The formation of extracellular polysaccharides may also serve as an alternative hydrogen-sink reaction (Vanderhaegen *et al.*, 1991; Wu *et al.*, 1996). This production of extracellular polymers by the lactate-utilizing population (Slobodkin & Verstraete, 1993) could contribute to the formation of highly settable granules.

## 8.4 Operational states

*Normal operational state*

Under normal efficiently balanced operational conditions hydrogen plays a central role in controlling the proportions of the metabolites produced by the fermentative bacteria and $H_2$-utilizing methanogenic bacteria. This control rests with NAD-linked $H_2$ formation:

$$NADH + H^+ \text{ <---> } H_2 + NAD^+ + 2e^- \qquad \text{.....1}$$
$$oG'_o = +4.3 \text{ kcal per reaction}$$

The oxidation of NADH with $H_2$ production is essential for the degradation of sugars to proceed but the equilibrium of the above reaction is strongly in the direction of NADH unless the hydrogen partial pressure is maintained at a very low level (Bryant, 1979). Under normal operating conditions, and the presence of efficiently metabolizing $H_2$-utilizing methanogenic bacteria, the hydrogen partial pressure is maintained at a very low level (Bryant 1979). Under these conditions, most of the carbohydrate is fermented via acetate, $CO_2$ and $H_2$, without major production of other volatile fatty acids, to the final products $CH_4$ and $CO_2$ or $H_2O$.

Normal loading:

C-source --> 2H + pyruvate --> $CO_2$ + acetyl-CoA --> acetate
and/or (Weber *et al.*, 1984)
--> $H_2$ + $CO_2$ --> $CH_4$ + $H_2O$
--> acetate + $2H_2O$ --> $CH_4$ + $CO_2$ + $2H_2O$ .....**2**

System response:

i) pH = 6.8 - 7.2;
ii) low concentration VFA's;
iii) granule bed - very little increase;
iv) biogas formation stabilized;
v) biogas composition stabilized.

*Data correlation to hypothesis under normal operating conditions*

The data obtained for a laboratory-scale UASB bioreactor under controlled conditions is presented in Chapter 5. Once the optimum OLR (9.7 - 10.95 $kgCOD.m^{-3}.d^{-1}$), shortest HRT (10 h), and lowest substrate feed pH (5.5 - 5.0) had been reached, the system stabilized. After stabilization, the reactor responses were in agreement with the predicted five system responses as noted above. This is also in agreement with general reports in the literature (Bossier & Verstraete, 1996; Schmidt & Ahring, 1996; El-Mamouni *et al.*, 1997). It was also found that at the start of the studies on the UASB bioreactor in Chapter 5 (Fig. 2), very little granule bed growth was found until environmental changes were made in the operational parameters (substrate pH, OLR and HRT).

*Stressed operational conditions*

When stress is applied to the anaerobic bioreactor by suddenly increasing the degradable carbon concentration or by changing the pH of the feed, there is an increase in hydrogen concentration. This results in an increasing tendency of the electrons of NADH generated during the fermentation step (2H) to be utilized in increased catabolism of pyruvate to the reduced products through lactate, especially major amounts of propionic rather than acetic acid, $CO_2$ and $H_2$. The concentrations of butyric, iso-butyric, valeric and iso-valeric acids may also increase. These VFA's are all considered to be indicators of stress or overloading when present in the anaerobic bioreactor effluent (Hill & Bolte, 1989).

Stress/Overloading:

C-source --> 2H + pyruvate --> 2H + lactate
--> acrylyl-CoA/oxaloacetate
--> propionate + acetate (Bryant, 1979) .....**3**

System response:

i) pH = fast decrease to below pH 6.5;
ii) increased concentration of VFA's;
iii) propionic acid as major VFA;
iv) decrease in biogas production;
v) decrease in $CH_4$ concentration;
vi) increase in effluent viscosity - (alternative H-sink reaction);
vii) enhanced granulation.

*Data correlation to hypothesis under stressed operating conditions*

To test the hypothesis that the application of stress conditions to the anaerobic digestion microbial community can enhance the granulation process, investigations were implemented on both batch and lab-scale UASB bioreactors.

Batch studies: As the start-up of an anaerobic bioreactor is a lengthy and complicated process, it was decided to also make use of batch systems to simulate UASB type conditions on small scale. As carbon sources, glucose (Lens *et al.*, 1993) and sucrose (Quarmby & Forster, 1995) were selected as representative carbohydrates that have been reported as granulation enhancers and can also be seen as the starter carbohydrates for the metabolic pathway given in equation **2**. The volatile fatty acid rich petrochemical effluent (Sam-Soon *et al.*, 1991) was included as an effluent that does not support granulation (equation **3**). As the accumulation of lactate (equation **3**) is seen as the 'source carbon' for the production of propionic acid (Riedel & Britz, 1993), its presence can give a competitive advantage to the propionic acid producers that are also active slime producers. The fruit effluent was included in the study as a representative of a true carbohydrate rich industrial wastewater.

In the simulated UASB batch studies, with only a daily 'draw and fill' environmental stress (Chapter 3), it was found that the reactions were in most cases in agreement with at least four of the predicted seven system responses as noted above. A rapid drop in pH (Chapter 3), followed by an increase in VFA's, especially propionic acid (equation **3**) (Chapter 3, Fig. 6), led to an increase in the number of countable granules. It was also clear that once the system had stabilized, the results were in agreement with most of the system responses predicted under normal operating conditions. The results thus do support the hypothesis that an enhancement of granulation will take place after controlled environmental stress is applied to an anaerobic system. Similar results and conclusions can be drawn for the data obtained on the application of the multi-shocks conditions to the batch systems (Chapter 4).

Laboratory-scale UASB bioreactors: In the case where a laboratory-scale UASB bioreactor (Chapter 5) and a 50-l UASB bioreactor (Chapter 7), were both inoculated with suitable granules, was submitted to small and controlled environmental changes (HRT, OLR and pH), the data once again obtained was in agreement with the predicted system responses and the hypothesis for enhanced granulation for both stable and stressed conditions. The application of the study

hypothesis (equations **2** and **3**) and granulation protocol lead to a fast start-up and efficiently operating high rate UASB digester.

In Chapter 6, where larger environmental stress conditions were applied (40%, 20% and cysteine shocks), similar results to the first bioreactor study (Chapter 5) were found. It was clear from this study (Chapter 6) that if the stress conditions were too extensive, the recovery time would be too long for the operation of an efficient anaerobic system and could lead to the danger of total system failure.

## 8.5 Conclusions

The hypothesis that when sudden 'stress' conditions are applied to anaerobic systems, an enhancement of the granulation process can take place, was able to provide a good comparison with the experimental data examined. The results obtained were used to demonstrate the usefulness of the granulation model in correlating process response and testing theories. It was however, clear from the studies that controlled environmental stress must be applied and maintained to sustain granulation. The response of the anaerobic consortium was strongly dependent on the specific carbon source and its concentration as well as a suitable nitrogen source and the necessary growth factors. One factor that was found to be extremely important was the condition (type, age and concentration) of the inoculum sludge. It was also clear from the studies that a more reliable granule counting method must be developed and there has already been preliminary investigations into the use of image-analysis as an option. One advantage of this method is that the aggregation and disintegration of granules can be accurately measured and monitored.

# CHAPTER 9

# CONCLUSIONS AND RECOMMENDATIONS

The objective of this research programme was to enhance granulation in UASB systems and promote a more rapid start-up procedure. This was be done by:

1. Setting-up and operating a series of suitable batch systems simulating UASB operating parameters. The granule growth was monitored in terms of yield and metabolic activity. Environmental "stress" conditions were applied to the batch systems and the influence of these as granule stimulants, evaluated. Three waste waters types were used as treatment substrates and included synthetic effluents with either glucose, sucrose or lactate as carbon source, a canning industry effluent, and a petrochemical effluent containing only volatile fatty acids. The best conditions for granulation were identified and optimized;

2. Setting-up and operating UASB laboratory-scale digesters using a synthetic and a carbohydrate rich canning industry wastewater as treatment substrates. The induction of specific `stress' conditions and their influence as granule stimulants, was evaluated. Changes in the environmental conditions included: organic and hydraulic overloading; changes in C:N:P ratios; as well as the addition of cysteine;

3. Furthermore, based on the data obtained from the batch- and laboratory-scale bioreactors, a UASB anaerobic biological model was constructed and evaluated on a 50 litre pilot-scale UASB reactor; and

4. Start-up procedures were established as part of the batch-, laboratory- and pilot-scale digestion systems.

The research in this study focused on the granulation process in batch-, laboratory-, and 50 l pilot-scale UASB systems, and in particular, the enhancement of the process by placing stress conditions on the microbial consortium. The following conclusions and recommendations can be made from data obtained:

1. A hypothesis for the enhancement of the granulation process was developed and validated against experimental observations. From the hypothesis, the operational parameters that must be applied to enhance the granulation were identified and applied in the start-up and operation of the 50 l pilot-scale UASB bioreactor;

2. It was found that the environmental conditions in batch cultures could be changed (carbon source and concentration, type and age of the sludge inoculum and culturing conditions) to give the propionic acid producers a competitive advantage, leading to an enhancement of the granulation

process. The data obtained and the increase in granule formation clearly indicated that granules can be cultured in batch systems using glucose, lactate, canning fruit and petrochemical waste waters as carbon sources. The granulation process is facilitated by a rapid drop in pH at the start resulting from the sudden increase in propionic and acetic acids, followed by a subsequent increase and stabilization in pH, with a steady decrease in propionic and acetic acid concentrations until the formation stabilized. The data showed that metabolic activity and granule growth was dependent on the type and age of the anaerobic sludge used. In the Sucrose units no clear granule formation was found. An increase in granule formation (increase in granule numbers) of 354%, 559%, 36% and 600% was found for the Glucose, Lactate, Petrochemical and Fruit batch units respectively. It was clear from the studies that a more reliable granule counting method must be developed and there have already been preliminary investigations into the use of image-analysis as an option. One advantage of this method is that the aggregation and disintegration of granules can be accurately measured and monitored;

3. The influence of multi-shock conditions, using standardized inoculum sizes, on the basic growth substrates showed that the characteristic pH, acetic and propionic profiles were obtained and a further enhancement of the granulation process (increase in granule numbers) could be facilitated (400% - 1 000%). This confirmed the hypothesis that by changing environmental conditions on batch-scale, the propionic acid producers can be given a competitive advantage that leads to enhanced granulation. In view of the fact that carbon may not be the only limiting macro-nutrient, more biochemical studies will be needed;

4. An UASB bioreactor inoculated only with anaerobic sludge results in a very extended start-up, stabilization time and biomass washout, when compared to the enhanced start-up of a bioreactor inoculated with batch grown granules. When used in the treatment of a canning industry effluent, this led to a COD removal of 89 - 93% at organic loadings of 9.8 - 10.95 $kgCOD.m^{-3}.d^{-1}$, at a HRT of 10 h and substrate pH of 5.5;

5. The data obtained also showed that when stress conditions are applied to an UASB bioreactor, an enhancement of granulation would occur. It was also found that the type and concentration of stress applied must be carefully chosen otherwise over-stressing could lead to an extended recovery period or even to total system failure; and

6. The use of batch cultivated granules as inoculum for the 50 l UASB pilot-plant led to the successful and efficient treatment of a full strength canning factory effluent. This was at a HRT of 10 h, with an average COD removal of 81 - 84% and removal rate of 7.5 $kgCOD.m.^{-3}.d^{-1}$ at an OLR of 9.2 $kgCOD.m^{-3}.d^{-1}$ and substrate pH of 5.0 with an effluent alkalinity level of 700 $mg.l^{-1}$ and an effluent pH of 6.5.

In the study, several technical problems were also encountered that will have to be investigated:

1. The increase in granulation was determined by a direct counting of diluted samples. The method was not very efficient as the increase was seen as rather small and gritty granule-like particles, making a direct count very difficult. The development of a more practical method to determine the increase in terms of the numbers and size of the granules must be investigated;

2. A technique will also have to be developed to determine the increase in granular sludge in terms of a mass-increase without disturbing or disrupting the granule bed;

3. It was found that the characteristics of the anaerobic sludge used in the study differed from season to season and from digester to digester source. A suitable method of characterizing the sludge that will be used as inoculum has become essential; and

4. It is also important that a method for the determination of acidogenic and methanogenic granule activity be developed.

The research work summarized in this report has addressed and met all the objectives set out at the start of the study with the exception of the studies on the aggregate forming members of the granule consortium. This aspect was not addressed as at the start (in conjunction with the Steering Committee) it was decided that the research effort must be focussed on the enhancement and formation of the granules and that propionic acid producing slime formers that had been isolated in a previous study, would be included in the batch system studies. This was done without any enhanced granulation being found.

The successful cultivation of granules has important economic implications for the optimization of biological treatment processes in terms of improved sludge settleability, biomass retention and high loading rates. The promising results obtained in this investigation show that the separate culturing of granules hold a lot of promise for application of the UASB technology as a high-rate biological waste water treatment option. This study has also opened new avenues for investigation which include: the mass culturing of granules; analysis of the microbial consortium; use of the granulation model as a stable multi-level kinetic model; inclusion of selected microbes into the granules to enhance degradation; and the use of the granule consortia to produce value-added products from waste water.

# CHAPTER 10

## REFERENCES


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