ANAEROBIC DIGESTION OF DAIRY FACTORY EFFLUENTS

J P Strydom[1], J F Mostert[1] and T J Britz[2]

Report to the Water Research Commission

by the

[1]ARC-Animal Nutrition and Animal Products Institute, Irene
[2]Department of Food Science, University of Stellenbosch

WRC Report No 455/1/01
ISBN No 1 86845 724 9

April 2001

EXECUTIVE SUMMARY

BACKGROUND AND MOTIVATION

Some years ago, it was estimated that the dairy industry consumes approximately 4.5 million m^3 water per annum in over 150 dairies in South Africa (Water Research Commission, 1989). Between 75% and 95% of the water intake volume is discharged as effluent. South African dairies and dairy factories received and processed approximately 1.86×10^6 kl milk during the 1989/1990 year (Dairy Board, 1990). Milk and milk products have exceptionally high chemical oxygen demand (COD) values (milk: 218 000 $mg.l^{-1}$, skimmed milk: 100 000 $mg.l^{-1}$, whey: 80 000 $mg.l^{-1}$) and the inevitable wastage of milk and milk products contributes greatly to pollution loads discharged by dairies. The average COD of dairy effluents is approximately 3 800 $mg.l^{-1}$.

While most larger dairy factories dispose of their effluent into municipal sewers, cases of effluent disposal into the sea and disposal by means of land irrigation do occur. In contrast to this, most smaller dairy factories dispose of their effluent by irrigation onto lands or pastures. Surface and ground water pollution is therefore a potential threat posed by these practices.

The aim of this study was to firstly determine the extent of effluent related problems experienced by the dairy industry in South Africa. Secondly, the feasibility of using the anaerobic hybrid digester for the treatment of dairy factory waste water had to be determined. Finally, the aim was to assess the hybrid digester as a treatment option for effluents emanating from three dairy factories producing different types of dairy products.

OBJECTIVES

The objectives of this research programme were as follows:

1. To survey the South African dairy industry to determine the present situation, requirements and need for effluent treatment;
2. To investigate the use of anaerobic digestion of dairy waste water;
3. To investigate the use of the anaerobic digestion-ultrafiltration (ADUF) system for the treatment of dairy waste water
4. To investigate the possible development of the ADUF-system into an efficient process for the treatment of waste water produced by dairy factories in South Africa.

RESULTS AND CONCLUSIONS

Effluent production and disposal in the South African dairy industry: A postal survey

In South Africa, where water has been identified as the country's most important natural resource, the dairy industry is significant, both from a water intake and discharge point of view. The requirements of the dairy industry in relation to on-site effluent treatment were thus determined by means of a postal survey. Of the 247 questionnaires sent out, 81 were returned. The

data obtained indicated that the respondents from the survey receive and process 70% of the total milk production in South Africa. A diverse range of effluents and effluent problems were described by the respondents. The survey results indicated that larger factories generally dispose their effluents into municipal sewer treatment works resulting in high disposal costs. The majority of smaller factories and dairies dispose of their effluents by means of irrigation onto lands and pastures. A possible side-effect of this practice is ground-water pollution. Most of the respondents expressed a need for more information on the subject and a proposed project for the development of a biological effluent treatment procedure was supported by 49% of the respondents. These respondents represent 40% of the total milk volume processed in the country. The supportive respondents were also responsible for 84% of the reported municipal levies.

Anaerobic treatment of a synthetic dairy effluent using a hybrid digester

A mesophylic laboratory-scale hybrid anaerobic digester, combining an upflow sludge blanket and a fixed-bed design, was evaluated for the anaerobic treatment of a synthetic dairy effluent. In the first experimental study, the chemical oxygen demand of the dairy effluent was increased stepwise from 3 700 to 10 300 $mg.l^{-1}$. In the second experimental study the COD of the synthetic dairy effluent was kept constant at 10 000 $mg.l^{-1}$ and the hydraulic retention time was shortened stepwise from 4.1 to 1.7 d. A COD removal of between 90 and 97% was achieved at organic loading rates of between 0.82 and 6.11 kg $COD.m^{-3}.d^{-1}$. At an HRT of 1.7 d, the digester achieved a methane yield of 0.354 m^3 CH_4 per kg $COD_{removed}$. The best results in terms of methane yield were achieved at an HRT of 1.9 d. The data also showed that the maximum operational potential of the digester had been reached, as indicated by the drop in methane yield observed at the end of the second experimental study. The results clearly show that this particular type of digester would be suitable for the anaerobic treatment of dairy effluents. An important consequence of the data from this study is that a two-phase set-up will be required to protect the methanogens in the digester from inhibitively low pH values and high concentrations of volatile fatty acids (VFAs) produced during the acidogenic phase. A two-phase system will allow pH control in the acidogenic phase, should it be needed at a full-scale or pilot-scale treatment plant.

Two-phase anaerobic digestion of three different dairy effluents using a hybrid digester

A mesophilic hybrid digester was used in conjunction with a pre-fermentation step to treat effluents from three different dairy factories which included a cheese, a fresh milk and a milk powder/butter factory. The effluents from these factories were analyzed and the chemical oxygen demand, pH and effluent volumes were found to be highly variable over short time intervals. The pH was found to vary between 2.2 and 11.8 units, and the COD values ranged from 800 to 15 000 $mg.l^{-1}$ over a period of two hours. Significant differences were also found in the composition of the effluents from the three factories. The average COD of effluents emerging from the three factories

varied between 1 908 and 5 340 mg.l^{-1}. During the anaerobic treatment of these effluents using the hybrid digester, the COD of the effluents was reduced by between 91 and 97%. The methane yield (per kg of $COD_{removed}$) varied between 73 and 91% of the theoretical maximum yield. The data clearly indicated that anaerobic treatment of the different dairy effluents was successful. However, it was also clear that balancing tanks will be essential in full-scale treatment plants due to the high variations in effluent quality that were found over very short intervals. It was also found that the pre-fermentation step may be unnecessary when using highly diluted effluents as a digester substrate.

Optimization of acidogenesis of dairy effluents using a selected *Peptostreptococcus productus* strain

The optimization of acidogenesis of a synthetic dairy effluent was investigated in this study, using a continually operated mixed-culture acidogenic bioreactor. A population study was conducted and 47 isolates were obtained. These isolates were characterized and found to be very similar in terms of biochemical characteristics. By using both the Jaccard and the Sokal and Michener coefficient, a percentage similarity of above 80% was obtained for 93% of the isolates. The isolate with the highest volumetric lactic acid productivity (Q_p(max)), was subsequently identified. Three isolates had Q_p(max) values below 10.0 g.l^{-1}.d^{-1}, while two isolates had Q_p(max) values above 30.0 g.l^{-1}.d^{-1}. The dominant isolate, both in terms of Q_p and total counts in the mixed culture, was *Peptostreptococcus productus*. The strain (F06) with the highest Q_p(max) was subjected to a factorial design experiment, to determine the optimal levels of the factors temperature, COD and pH. With the exception of pH level, the optimal operational parameters as found in the factorial design, closely correlated with the levels as used in the acidogenic bioreactor. This underlines the highly selective pressure exerted by a chemostat on a bacterial population. In this study, an optimization procedure was developed which can possibly be used for the microbial and initial operational optimization of acidogenesis, even in existing full-scale acidogenic bioreactors. The optimal values of the various operational parameters must be verified and possibly adapted, when the pure cultures obtained in this study, are used for pilot or full-scale acidogenesis. However, the results obtained during the process optimization in this study will facilitate start-up and initial operation of such a full-scale acidogenic bioreactor. The use of a known isolate has yielded the unexpected bonus of odour control. Similar advantages, such as the elimination of unknown and possibly pathogenic bacteria are also implied.

DISCUSSION

Postal survey

The South African dairy industry was shown in this study, to be in a highly favourable position to contribute to water conservation in South Africa. Specific actions that the dairy industry can take to contribute to water conservation in South Africa, involve the very obvious "prevention is better

than cure" approach. An environmental audit can be used to identify problem areas and it is recommended that this be the first step, to ensuring environmental compatibility of any individual factory. The logical way to deal with dairy effluents, as with any other waste, is to include the following steps in an effluent action programme:

i) Waste prevention;
ii) Waste minimization;
iii) Waste recycling; and,
iv) Waste treatment.

These measures can then be taken to the point where they complement each other in the most favourable manner, both environmentally and economically. From the data obtained in this study it is clear that, due to the differences between individual factories in terms of their products and effluents, no two detailed effluent action programmes will be similar.

The impression gained from the postal survey, was that at least the first three of the above steps are applied at various levels of sophistication by the South African dairy industry. While there is certainly scope for improvement in many areas, such as specific water consumption figures, it is also believed that the current levels of technology available in South Africa for the prevention, minimization and recycling of wastes in the dairy industry, have virtually been taken to its limits in terms of cost effectiveness and practicality.

From a microbiological viewpoint, the fourth step, namely wastewater treatment, is the one outstanding area where the current state of the biotechnology can be improved considerably, without necessarily adding to the overall complexity and cost of existing processes. Bearing in mind that previously, wastewater treatment received very little specific attention from the dairy industry in South Africa, it was believed from the outset, that this study would serve a dual purpose. In the first instance, a contribution to the existing body of scientific knowledge could be made and in the second instance, the availability and practicality of existing biotechnological options for the treatment of dairy wastewaters, could be brought under the attention of the South African dairy industry.

It may be argued that dairy factories, who pay for the privilege to dispose effluent to municipal sewage treatment works, are indeed applying the fourth step. However, this can be very expensive and thus, it is unlikely to be the most economical option. More important, however, occasional concentrated effluent discharges from a large dairy factory can cause a negative environmental impact, especially if the receiving municipal treatment plant is running at or near its full capacity. This phenomenon has been observed in South Africa and Europe, the latter being described in the literature. Finally, the technical requirements for the treatment of domestic sewage sludge differ markedly from those for the treatment of dairy factory effluents. Moreover, dairy factory effluents in itself show vast differences, in terms of composition and overall character, between the different types of factories. An on-site effluent treatment plant can be tailored to the specific demands of that particular effluent and thus offer improved cost-effectiveness to the factory as well as reliable organic overload protection to the municipal treatment plant.

Laboratory-scale anaerobic digestion

The hybrid anaerobic digester used in this study was deliberately chosen as subject for the experimental investigation of the anaerobic digestion of dairy factory effluents, as it has previously yielded excellent results during the treatment of "difficult" wastes such as bakers' yeast factory effluent and landfill leachate. In addition, it was also clear from the literature review that the hybrid digester had never before been used for the treatment of dairy factory effluents.

The very positive results from the anaerobic treatment of the three different types of effluents, underlines the suitability of the synthetic effluent to serve as a laboratory replacement substrate in effluent treatment trial runs.

Optimization of acidogenesis

Pre-acidification was found to be a pre-requisite for the successful high rate anaerobic treatment of dairy factory effluents. Prior to this study, the continuous pre-acidification of dairy factory effluents has never been studied with microbial strains, selected specifically to optimize acidogenesis during two-phase anaerobic treatment. The optimization of the pre-fermentation process in general, was considered essential in order to ensure the successful practical application of the process.

It is believed that, based on the data obtained in this study, acidogenesis of virtually any dairy effluent can be microbially optimized by studying the naturally occurring bacterial population in an operational acidogenic bioreactor. Furthermore, this process can be replicated at any scale.

By using the capabilities of naturally occurring but specially selected micro-organisms, it is believed that any waste treatment biotechnology can be developed in an elegant and cost-effective manner. In this study, the most suitable bacterium was employed under the most favourable set of operational parameters, primarily for the production of organic acids. An unexpected bonus was odour control. Other benefits include the control of pathogens and possibly also bacteriophage. It is believed that the factorial design experiment, as used in this study, provides a starting point for the optimization of all types of dairy factory effluents.

RECOMMENDATIONS FOR FUTURE RESEARCH

This study is not the final and complete answer to the effluent problems of the South African dairy industry. It does, however, provide a starting point from which the dairy industry can proceed to make an informed decision, based on viable effluent treatment options. When anaerobic digestion is considered as an option for the on-site treatment of dairy effluents, the results of this study will hopefully remove many fundamental uncertainties.

However, several measures should still be taken to ensure that the application of on-site anaerobic treatment for the treatment of dairy effluents is as successful and efficient as possible.

1. The application of the results from this study, in an on-site pilot-scale setup, will provide practical information that will help to ensure the successful design and operation of a full-scale treatment plant. Thus, as far as future research is concerned, a pilot-scale study will have the greatest practical impact of immediate consequence.

2. The single aspect where the results from this study compares unfavourably to the results reported in the literature, namely the rather long hydraulic retention time, should also receive further attention. This problem can be addressed in a variety of ways, both in the laboratory and on pilot-scale. The most practical option is to establish the functional and optimal volume of the digester, which was used in the laboratory.

   It is likely that channeling may have occurred, as the digester was continually operated for more than two years. A tracer test and a residence time distribution (RTD) analysis will describe the mixing characteristics of the digester and thus identify so-called dead spaces. This will have important consequences and is, therefore, considered as a prerequisite for successful pilot-scale work.

3. Since the quality of the final effluent, after anaerobic digestion, does not allow its direct disposal to rivers and waterways, further research will have to include an investigation of secondary, "polishing" steps. This may include either a physical treatment such as ultrafiltration, a chemical treatment step or preferably a secondary biological treatment step, such as aerobic algal ponds or maturation ponds. The use of spray irrigation of anaerobically digested dairy effluents and the effects on soil condition and soil fertility would also provide interesting information of practical value.

   Research of a more fundamental nature is also justified by the results obtained during this study. From the literature it was seen that the kinetic constants of acidogenesis in synthetic dairy effluents are well established. Furthermore, in this study, a very important contribution to the understanding of acidogenic bacterial populations, was made. Methanogenesis, the key aspect of the anaerobic digestion process, remains open for further investigation. Due to the unique configuration of the hybrid digester and the two-phase set-up which was used in this study, the biochemical kinetics and the microbial dynamics of the methanogenic bacterial population in the hybrid digester, may prove an important study field.

   The current state of waste-treatment biotechnology will allow successful on-site treatment of dairy factory effluents. However, further research of both applied and fundamental nature, should not be neglected since it can only enhance the efficiency and practicality of the process, thereby rendering it more attractive to the dairy industry and thus increasing the probability that the results of this study will eventually find a practical application.

Evaluation of contract objectives

The first contractual objective was to survey the South African dairy industry and the successful results of this part of the study were published in Water SA in 1993. As a result of this survey, many members of the dairy industry in South Africa were made aware of the potential of anaerobic digestion, and many enquiries were received.

The investigation into the use of anaerobic digestion as an effluent treatment option for the dairy industry, was carried out in two phases. The first dealt with a synthetic effluent, and the basic operational parameters were established. The second part had a more practical approach in the sense that actual effluents were used as digester substrate. The results of these two studies, are considered to meet the second objective as listed above, and the results from both phases have been published in Water SA.

The ADUF part of this laboratory scale project was not successfully carried out, due to unsurmountable technical difficulties. The minimum reactor size required for successful ADUF work is around 250 litres, and the biggest digester available at the Institute had a capacity of only 97 litres. Initial trials using this digester proved unsuccessful and results are not reported.

Thus, instead of reiterating previous small-scale ADUF failures, it was decided to reschedule the ADUF work and to include it in the follow-up pilot-scale project where it could be carried out on a more practical scale. A project proposal ("The complete treatment of dairy factory effluents by means of primary anaerobic digestion and secondary algal production") was subsequently approved by the WRC and work will start in January 1996. This constitutes the only deviation from the original aims as set out in the contract.

ACKNOWLEDGEMENTS

The research in this report emanated from a project funded by the Water Research Commission entitled: "Anaerobic digestion of dairy factory effluents".

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

Dr O O Hart	-	Water Research Commission
Prof T J Britz	-	University of Stellenbosch
Mr J C Kruger	-	Dairy Belle
Mr H McVicker	-	Nestlé
Dr S A Mitchell	-	Water Research Commission
Dr J F Mostert	-	ARC-Animal Nutrition and Animal Products Institute
Mr J P Nel	-	NCD
Mr J P Strydom	-	ARC-Animal Nutrition and Animal Products Institute
Mr P W Weideman	-	Water Research Commission

The financing of the project by the Water Research Commission and the contribution of the members of the Steering Committee is gratefully acknowledged.

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.

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

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

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

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

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.

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
ADUF	Anaerobic digestion – ultrafiltration
COD	Chemical oxygen demand
HRT	Hydraulic retention time
$Q_{p(max)}$	Maximum volumetric lactic acid productivity
VFAs	Volatile fatty acids
K_2HPO_4	Dipotassium hydrogen phosphate
TKN	Total Kjeldahl nitrogen
TS	Total solids
TNVS	Total non-volatile solids
VS	Volatile solids
OLR	Organic loading rate
PO_4-P	Orthophosphate phosphorus
TOC	Total organic carbon
UASB	Up-flow sludge blanket
CH_4	Methane
Ks	Monod half-saturation constant
μ_{max}	Maximum specific growth rate

CSTR	Completely stirred tank reactors
SRT	Solids retention time
STP	Standard temperature and pressure

CHAPTER 1

INTRODUCTION

1.1 Water Situation

South Africa is a semi-arid country with limited water supplies. Pollution of rivers, waterways and other water sources is prohibited by the Water Act, Act 54 of 1956 (Department of Water Affairs, 1986). The Department of Water Affairs (1986) also reports that water pollution is rapidly increasing in South Africa. Moreover, the anticipated demand for water will outstrip the available supplies by the year 2020 and intercatchment transfers of water will, only to a limited extent, improve the regional matching of supply and demand (Bekker, 1982). Among the recommendations made in a report by the President's Council on a National Environmental Management System (Republic of South Africa, 1991), is a proposal to re-use factory effluent. This demands serious consideration if industrial growth is to be sustained. The prevention of pollution and the development of technology to facilitate the re-use of effluents, are both of equal importance to industrial water users.

Ten years ago, it was estimated that the dairy industry consumes approximately 4.5 million m^3 water per annum in over 150 dairies in South Africa (Water Research Commission, 1989). Between 75% and 95% of the water intake volume is discharged as effluent. South African dairies and dairy factories received and processed approximately 1.86×10^6 kl milk during the 1989/1990 year (Dairy Board, 1990). Milk and milk products have exceptionally high chemical oxygen demand (COD) values (milk: 218 000 $mg.l^{-1}$, skimmed milk: 100 000 $mg.l^{-1}$, whey: 80 000 $mg.l^{-1}$) and the inevitable wastage of milk and milk products contributes greatly to pollution loads discharged by dairies. The average COD of dairy effluents is approximately 3 800 $mg.l^{-1}$ (Jones, 1974).

1.2 Motivation

Dairy Factory Effluent

While most larger dairy factories dispose of their effluent into municipal sewers, cases of effluent disposal into the sea and disposal by means of land irrigation do occur. In contrast to this, most smaller dairy factories dispose of their effluent by irrigation onto lands or pastures. Surface and ground water pollution is therefore a potential threat posed by these practises.

Since the dairy industry in South Africa is a major water user, it is a potential candidate for effluent re-use. Purified effluent can possibly be utilized in boilers and cooling systems. The bacteriological and chemical quality of foodstuffs produced is unlikely to suffer from such measures. Even if the purified effluent is initially not re-used, the dairy industry will still benefit from in-house effluent treatment in a direct way, since levies charged by municipalities for effluent reception will be significantly reduced. In the United Kingdom, 70% of the total savings that have been achieved with anaerobic digestion are due to reduced discharge costs (Senior, 1986). The industry will also benefit where effluents are currently used for irrigation of pastures, albeit in a more indirect way. All these facts underline the need for dairy effluent treatment.

Biological Waste Water Treatment Systems

Biological treatment systems are preferable treatment options since they do not have certain health and environmental hazards sometimes associated with chemical systems, especially systems where chlorine is involved. Chlorination of any water may result in the formation of chlorite and chlorate. These compounds have been shown to cause haemolytic anemia in laboratory animals (Daniel *et al.*, 1990, as cited by Dietrich *et al.*, 1992) and these substances are being considered for regulation by the American Environmental Protection Agency (Dietrich *et al.*, 1992). The potential health hazards associated with chlorine suggest that biological treatment systems are probably safer than chemical systems. Furthermore, in chemical systems the actual chemicals have to be added, in contrast to biological systems where the organisms used during treatment, are added only once during start-up. This suggests that biological systems are also much less demanding in terms of operational costs.

Anaerobic Digestion

Several different biological waste water treatment systems are available at present. Anaerobic digestion, traditionally used for the stabilization of municipal sludges, has recently received new impetus through the development of new digester designs. The development of high rate anaerobic systems have made anaerobic digestion a viable option for the treatment of industrial waste waters. All the various known configurations have been applied successfully at full-scale (Iza *et al.*, 1991); of the UASB type more than 200 full-scale digesters have been constructed in the USA and Western Europe (Lettinga and Hulshoff-Pol, 1991). The anaerobic digestion of dairy waste water has received much attention over the years and in 1990, at least six full-scale anaerobic treatment plants were known to be used by dairy factories world-wide (Anon., 1990). Compared to other biological treatment systems, such as aerobic systems, anaerobic digestion offers greater reduction in chemical oxygen demand, lower sludge yield, the formation of biogas, low nutrient requirement and low running costs (Anon., 1990). Considering these benefits of anaerobic digestion, it is the most promising biological treatment system and deserves further study.

A high rate anaerobic digester is preferable for the treatment of large volumes of industrial waste waters. A high rate anaerobic digester is defined as a digester where the solids retention time (SRT) and the hydraulic retention time (HRT) are effectively separated (Hickey *et al.*, 1991). A short HRT translates into a smaller digester volume and less initial capital expenditure. To achieve this however, solely through the cultivation of granules in an up-flow sludge blanket (UASB) digester, is time consuming and this, as well as the slow growth rates of the acetogenic and methanogenic microbial populations, account for the long start-up times associated with anaerobic digesters. Cultivation of granules is also difficult, if a source of granules is not available (Weiland and Rozzi, 1991).

A new high-rate digester system is the hybrid digester system, which is a combination of an UASB and fixed-bed digester types (Guiot and Van Den Berg, 1984). In the hybrid system, the SRT and HRT are separated, through the double action of sludge formation and biofilm development. The application of the hybrid system for the treatment of dairy waste waters has never before been investigated. It is a promising design and its suitability for the treatment of dairy waste waters should be investigated.

1.3 Research Aims

The aim of this study was:

Firstly, to determine the extent of effluent related problems experienced by the dairy industry in South Africa;

Secondly, the feasibility of using the anaerobic hybrid digester for the treatment of dairy factory waste water had to be determined; and

Thirdly, the aim was to assess the hybrid digester as a treatment option for effluents emanating from three dairy factories producing different types of dairy products.

CHAPTER 2

LITERATURE REVIEW

2.1 Background

Anaerobic digestion has become such a specialized field of study that it is impossible to discuss it meaningfully without first defining it. The majority of the general public has never even heard of anaerobic digestion, in spite of the many exciting possibilities offered and the wide application it already found in the treatment of industrial and domestic wastewaters. In spite of this, almost each and every municipal sewage treatment works has one or more operational anaerobic digesters.

Anaerobic digestion is a biological process performed by active microbes in the absence of exogenous electron acceptors. Methane and carbon dioxide are the most important by-products of the anaerobic digestion process. The most important asset of the process is that considerable purification of wastewater is achieved without the formation of excessive biomass. Up to 95% of the organic load in a waste stream can be converted to biogas (methane and carbon dioxide) and the remainder is utilized for cell growth and maintenance (Anon., 1990; Weber *et al.*, 1984). Biochemical energy, locked-up in the waste stream, is thus converted and conserved by the anaerobic digestion process. In this regard, anaerobic digestion is in a class of its own when compared to aerobic wastewater purification systems. In the case of aerobic systems, up to 50% of the organic load is converted to biomass. This has to be disposed of, while the remainder is lost through heat and carbon dioxide production.

Many ancient civilizations unwittingly used anaerobic digestion for the stabilization of their wastes. The citizens of Knossos, Crete, used circular walled containers as a type of solid state bioreactor as long ago as 1 900 B.C. (Senior, 1990). The stabilization of household wastewater by means of anaerobic digestion was first described in 1881. In 1914, the process came into extensive use at municipal sewage treatment works (McCarty, 1981). This trend continued while the process was improved to such an extent that it has become indispensable at modern sewage treatment works.

Anaerobic digestion has only much more recently been applied on the industrial level. During the 1950's, the process was understood well enough to allow the development of full-scale anaerobic digesters treating industrial effluents (Ross *et al.*, 1989). Anaerobic digestion has since successfully been tried and tested on many types of industrial effluents, both in the laboratory and on full-scale.

The treatment of dairy factory effluents have also come under scrutiny as a potential application for anaerobic digestion. Yet, in spite of the many papers that have been published on the subject of digestion of dairy effluents (Pico, 1987), only six of the 205 full-scale up-flow anaerobic sludge blanket (UASB) digesters operating world-wide in 1990, were built at dairy factories (Lettinga and Hulshoff-Pol, 1991). The reasons for this are not clear, eventhough it may be that either dairy effluents present more of a challenge than many other food industry effluents, or the dairy industry is experiencing less environmental problems than other types of food industries.

2.2 Application of anaerobic digestion in the dairy industry

Anaerobic digestion of dairy effluents has been studied on both laboratory and pilot-scale (Anon., 1990; Pico, 1987; Van Den Berg, 1984). During the last decade several full-scale digesters for the treatment of dairy effluents have been taken into operation (Anon, 1990; Iza *et al.*, 1991; Lettinga and Hulshoff-Pol, 1991). Despite all the available information on the anaerobic digestion of dairy factory effluents, very few dairies treat their effluents in this manner. Effluents are either disposed of untreated or treated by means of aerobic processes such as activated sludge or biofilter systems.

Treatment processes currently used by the dairy industry in South Africa are described as either capital intensive, unreliable or very basic pre-treatment processes (Water Research Commission, 1989). Considering all these facts, anaerobic digestion presents a real alternative. This is especially true for dairies which are situated in areas where adequate sewer connections, to correctly designed and operated sewage works, are not available. However, even dairies situated in areas where sewage works are available, may benefit from wastewater treatment. In 1990, Odegaard and Rusten reported on sewage plants in Norway that receive total organic loads in the range of 200 to 300 kg biological oxygen demand (BOD) per day. The municipal areas contributed up to 80% of the hydraulic load, but only 25 to 35% of the total organic load. The organic overloading of these sewage works was mostly due to dairies discharging untreated wastewater directly to the treatment plants. This example illustrates the impact that industrial effluents can have on a water treatment works which was originally designed to cater only for domestic sewage.

Dairy effluents, as produced in South Africa, are also suitable for anaerobic digestion treatment. Table 2.1, taken from the Water Research Commission's report (1989) on "Water and Waste-Water Management in the Dairy Industry", shows that even butter and cheese factory effluents have chemical oxygen demand (COD) values well inside the operational potential of the latest high rate anaerobic digesters.

From Table 2.1, it is estimated that the average COD of South African dairy effluents is about 2 500 $mg.l^{-1}$. Jones (1974) reported that the average COD of effluents produced by dairies in the USA is 3 800 $mg.l^{-1}$. The difference may be due to different water management practices. However, it should be borne in mind that the average COD, as calculated from Table 2.1, is largely of academic interest. Specific factories, such as butter and cheese factories (Table 2.1), produce effluents with COD values ranging from 3 000 to 4 000 $mg.l^{-1}$. In the event of such a factory deciding to treat its effluent by means of anaerobic digestion, the average COD of all the other dairy effluents will, of course, be irrelevant.

2.3 Digesters used for dairy effluent treatment

Despite the relatively limited application of anaerobic digestion in the dairy industry, enough examples are available in the literature to show that anaerobic digestion is indeed a viable treatment option. It is difficult, however, to assess and compare the advantages and disadvantages of different anaerobic digester types. Weiland and Rozzi (1991) advised caution in the selection of any type of digester based solely on results reported in the literature. Researchers sometimes compare only the advantages of their own system with the disadvantages of certain other digester systems. Thus, published data is not always objective in terms of performance efficiency when different types of digesters are compared. However, a review of the

relevant literature does provide a useful understanding of the digester types that have been used in dairy effluent treatment.

Since the definitions of digester types are sometimes rather vague and overlapping, it is difficult to accurately categorize each digester design. However, in terms of their biomass retention mechanism, all digesters can be broadly categorized into one of only four types. These are attached biofilm systems, systems where gravitational sludge settling takes place, systems where solids and liquids are separated by some mechanical means such as membranes and finally completely mixed systems with no mechanism for biomass retention. In this review, however, the digester designs, as described in the literature, are divided into ten categories, namely:

Anaerobic lagoon

The anaerobic lagoon, also called an anaerobic pond, is the simplest type of anaerobic digester. It consists of a pond which is covered, to exclude air and thus oxygen, and to prevent methane flux to the atmosphere. Lagoons are far easier to construct than vertical digester types, but the biggest drawback is the large surface area required.

In New Zealand (Anon., 1990), a lagoon covered with butyl rubber was used to treat dairy effluent. This system, however, was also described as a contact digester. The lagoon had a volume of 26 000 m^3, and the total load amounted to 40 000 kg COD per day, at a rather low organic loading rate (OLR) of 1.5 kg $COD.m^{-3}.d^{-1}$. The temperature was controlled in an unspecified manner at 35 °C. The pH was held between 6.8 and 7.2 units, although no mention was made of the actual method used to control the pH of a 26 000 m^3 lagoon. The digester effluent was clarified and the settled biomass recycled through the digester feed. The clarified digester effluent was then treated in a 18 000 m^3 aerated lagoon, presumably for the removal of phosphorus and nitrogen. The effluent from the aerated lagoon was held in a sedimentation basin before discharge. The system was claimed to achieve a 99% reduction in COD. From the data supplied, it was calculated that the anaerobic lagoon was operated at a HRT of approximately 1.0 day, and the aerobic lagoon at a HRT of approximately 0.68 day.

Contact digester

The contact digester was defined by Dunican *et al.* (1986) as essentially similar to the aerobic activated sludge process. Some form of biomass recycling was required since it was claimed that anaerobic sludge does not settle. On the other hand, should the definition of Van Den Berg (1984) be used, the contact digester can be regarded as similar to the up-flow anaerobic sludge blanket (UASB) digester. The basic principle of the contact digester was described by Van Den Berg (1984) as the settling of biological flocs and other suspended solids which form a sludge. This sludge should be in close contact with the raw waste. Complete mixing, without disturbing the sludge settling capability, is therefore claimed to be essential. These unacceptably ambiguous descriptions are due to the incomplete definitions of the contact digester as described above.

Table 2.1 Typical total effluent loads for South African dairies*

Factory type	Effluent volume ($kl.month^{-1}$)	COD ($mg.l^{-1}$)	Total dissolved solids ($mg.l^{-1}$)
Pasteurized milk	2 200	2 600	1 800
Fruit Juice and milk	4 700	1 500	1 100
Milk and miscellaneous	12 000	2 750	1 500
Butter	1 000	4 000	2 000
Cheese	800	3 000	3 000

*(Water Research Commission, 1989)

The contact digester was apparently first used in 1955 (Van Den Berg, 1984) and was developed further by Schroepfer *et al.* (1955). In this case, the anaerobic sludge was recycled after being collected in an external, conically shaped sludge settling tank. This external, secondary settling stage, distinguishes the contact digester from other, more recent designs. Due to modern developments, designs presently described as contact digesters can no longer be seen as the original contact digester. According to the confusing definitions of the contact digester, as found in the above cited literature, virtually any modern digester can be described as a contact digester. The contact digester, properly defined as a design with complete mixing and external gravitational sludge settling, is considered to be obsolete and only of historical interest.

Up-flow anaerobic sludge blanket

The up-flow anaerobic sludge blanket (UASB) digester was originally developed by Professor Lettinga in the Netherlands (Lettinga *et al.*, 1980; Lettinga and Hulshoff-Pol, 1991). In this system, the wastewater passes upwards through a sludge blanket consisting of microbial aggregates or granules with good settling properties. These granules are retained in the digester by means of a barrier which also serves to separate the biogas from the liquid phase. The growth and development of granules is important for the success of the UASB digester.

It must be noted that the presence of granules in the UASB system ultimately serves to separate the hydraulic retention time (HRT) from the solids retention time (SRT). Thus, good granulation is essential to achieve a short HRT without inducing biomass washout. The superiority of the UASB design over the completely stirred tank reactor (CSTR) is obvious when a HRT of 18.4 hours (Goodwin *et al.*, 1990) is compared to the minimum retention time of at least 9.0 days for the CSTR (Lebrato *et al.*, 1990). The rather long retention time of that particular CSTR probably was due to the slow growth rate and washout of methanogens.

Goodwin *et al.* (1990) treated a synthetic ice-cream wastewater using the UASB process. They also tested several different substrate supplements such as sucrose, calcium and cobalt salts. Of these, only calcium seemed to have a positive effect on the performance of the UASB. Granulation was observed after 60 to 70 days and a total organic carbon (TOC) removal of up to 86% was achieved. These workers

achieved a very short hydraulic retention time (HRT) of 18.4 hours. The maximum organic loading rate (OLR) was 3.06 kg $TOC.m^{-3}.d^{-1}$.

Cheese effluent has also been treated in the UASB digester (De Man and De Bekker, 1986). A pilot-scale wastewater treatment plant was set up at a cheese factory in Wisconsin, USA. The main part of the system consisted of an UASB digester which was operated at a HRT of 16.0 hours, albeit with recycling. The untreated effluent had a COD of 33 000 $mg.l^{-1}$ and a COD removal of 86% was achieved. From the above, it was calculated that the UASB part of the system was operated at an OLR of 49.5 kg $COD.m^{-3}.d^{-1}$. This calculation discounts the volume of the mixing tank. It should be mentioned that the maximum OLR for high rate digesters is considered to be around 16.0 kg $COD.m^{-3}.d^{-1}$ (Hickey *et al.*, 1991). Though the authors make no mention of pH, alkalinity requirements or methane yields, the success of this pilot-plant led to the construction of a full-scale plant on the same site. The UASB digester is, however, only a part of the complete full-scale treatment plant. The digester effluent from the UASB digester is recycled to a mixing tank which also receives the incoming effluent. Thus, the HRT in the actual UASB section of the design could be kept low. Although the system is described as an UASB system, it could also pass as a separated or two phase system, since some degree of pre-acidification is presumably attained in the mixing tank. Furthermore, the pH in the mixing tank was controlled by means of lime dosing when necessary. This was, however, required only under abnormal circumstances. The effluent emerging from the mixing tank was treated in an aerobic system, serving as a final polishing step, to provide an overall COD removal of 99%.

One of the six full-scale treatment plants described in 1990 (Anon., 1990) was in Finland at the Mikkeli Cooperative Dairy which produces Edam type cheese, butter, pasteurized and sterilized milk. The effluent volume was 165 million litres per year and the digester volume was 650 m^3, which included a balancing tank of 300 m^3 (Carballo-Caabeira, 1990; Ikonen *et al.*, 1985). This treatment plant had an operating temperature of between 20 and 25 °C. The digester initially contained a carrier material which became plugged, and after the carrier material was removed, the digester resembled an up-flow anaerobic sludge blanket digester (Anon., 1990). The final digester effluent was released to the town effluent treatment plant. The COD value was reduced by between 70 and 90%. The 400 m^3 biogas produced daily had a methane content of around 70% and it was used to heat process water in the factory.

One of the most successful full-scale treatment plants described in the literature was in the United Kingdom at South Caernarvon Creameries. This effluent treatment plant was used to treat whey and other effluent from the creamery with extraordinary success (Anon., 1984). The whey alone reached volumes of up to 110 kl per day. This treatment plant combined anaerobic digestion, in the form of an up-flow anaerobic sludge blanket digester with recirculating sludge, and an aerobic denitrification system. The anaerobic digester had a working volume of 2000 m^3. Biogas, with a calorific value of 21 $MJ.m^{-3}$, was produced in sufficient quantities to meet the total energy need of the whole factory. The HRT was about 20.0 days, and the COD was reportedly reduced by 95%. The effluent passed from the digester into a sedimentation tank which removed suspended matter and from there it flowed to aerobic tanks where the BOD was reduced to 20.0 $mg.l^{-1}$ and the NH_3-nitrogen reduced to 10.0 $mg.l^{-1}$. The effluent was finally disposed of into a nearby river. The whey disposal costs, which originally amounted up to £30 000 per year, was reduced to zero and the biogas also replaced heavy fuel oil, i.e. diesel oil. On full output, this biogas had a value of up to £109 000 per year as an oil replacement and a value of about £60 000 as an electricity replacement. These values were, however, calculated in terms of the oil and electricity

prices of 1984. This case-study, nevertheless illustrates the economic potential of the anaerobic digestion process.

Completely stirred tank reactors

Completely stirred tank reactors (CSTR), which are also defined as constant volume stirred tank reactors (Feilden, 1983), are, next to lagoons, the simplest type of anaerobic digester. In this case there is no biomass retention. Consequently the HRT and SRT are not separated, necessitating long retention times, which are dependent on the growth rate of the slowest-growing bacteria involved in the digestion process.

This type of digester was used by Lebrato *et al.* (1990) to assess the suitability of treating cheese factory wastewater using an anaerobic digester. While 90% COD removal was achieved, the digester could only be operated at a minimum HRT of 9.0 days, most probably due to biomass washout. The wastewater, consisting of 80% washing water and 20% whey, had a COD of 17 120 $mg.l^{-1}$. This study makes the important assumption that success with the CSTR on lab-scale probably means that other, biomass retaining digester types will also achieve success.

It seems that, while the CSTR is very useful for laboratory studies and kinetic studies, it is hardly a practical option for full-scale effluent treatment due to the HRT limitation. An efficient UASB system, operated at a HRT of 1.0 day, will need one-ninth the working volume of a CSTR operated at 9.0 days HRT, to treat the same effluent. This has obvious implications when the economics of the two systems are compared.

Fixed-film digester

The fixed-film digester contains solid baffles or porous carrier materials which are permanently fixed to the walls of the digester. Fixed-bed digesters also fit this description, and the two terms are therefore treated as synonymous. By means of extracellular polysaccharides, bacteria can attach to the surface of the packing material and still remain in close contact with the passing wastewater. Usually digester designers strive to achieve an optimum surface-to-volume ratio for the material. This ratio obviously differs for different types of carrier materials. A large variety of packing materials have been tested in fixed-film digesters. Particular trophic bacterial groups are claimed to favour different materials, each according to its own specific hydrophobicity (Yu and Pinder, 1992). According to these authors, support surfaces can have selective action which determines the relative quantities of acetogens and methanogens that are immobilized in a symbiotic biofilm.

In the fixed-film digester, wastewater is added either at the bottom or at the top of the digester to create up-flow or down-flow configurations. A down-flow fixed-film digester was used by Cánovas-Diaz and Howell (1987a) to treat deproteinized cheese whey with an average COD of 59 000 $mg.l^{-1}$. The stability of this down-flow digester was investigated using different digester volumes. When operated in partially submerged or half-filled mode, the digester achieved a COD reduction of 90 to 95% at a HRT ranging between 2.0 and 2.5 days. This efficiency was achieved at an OLR of 12.5 kg $COD.m^{-3}.d^{-1}$. The authors also very clearly stated that no pH control was ever needed. The deproteinized cheese whey had an average pH of 2.9, while the digester pH was consistently above pH 7.0 (Cánovas-Diaz and Howell, 1987b). Although gas composition was measured, no indication of methane yield was given. It was also not possible to calculate the methane yield from the data supplied. This study (Cánovas-Diaz and Howell, 1987a) also provides a good example of the confusing nomenclature

currently applied to anaerobic digester types. The digester is alternately referred to as a fixed-film digester and, incorrectly when considering the above definition, as an anaerobic filter. Judging from the description it should be classified as a fixed-film.

A laboratory-scale fixed-bed digester was also used by De Haast *et al.* (1986), to treat cheese whey. The fixed-bed consisted of an inert polyethylene bacterial carrier. The whey was diluted, prefermented and neutralized before being used as digester substrate. These workers found a severe pH drop when the diluted, untreated whey was directly used as digester substrate. As a result, the whey was prefermented, creating in effect, a type of two-phase system. The best results were obtained at a HRT of 3.5 days, where between 85 and 87% of the substrate COD was removed. The OLR was 3.8 kg $COD.m^{-3}.d^{-1}$ and biogas yield amounted to 0.42 $m^3.kg^{-1}COD_{added}.d^{-1}$. The biogas had a methane content of between 55 and 60%, and 63.7% of the calorific value of the substrate was conserved in the methane.

Anaerobic filters

The up-flow anaerobic filter was developed in 1967 (Van Den Berg, 1984) by the researchers Young and McCarthy. An anaerobic filter consists of a container filled or partially filled with loose material (Bonastre and Paris, 1989) such as small rocks, clay fragments, glass fragments, shells, sand and other similar materials. In spite of the packing material, most of the bacteria are in suspension (Van Den Berg, 1984) and tend to settle at the bottom. The packing material serves to separate the gas, providing quiescent areas for bacterial growth. However, due to plugging, channeling and other difficulties, this digester type was claimed to have had limited applications (Van Den Berg, 1984).

In a comprehensive review on anaerobic filter installations, Bonastre and Paris (1989) listed 51 pilot and industrial-scale anaerobic filter applications. Also listed were 63 laboratory-scale applications of the anaerobic filter, of which five were used for dairy effluent treatment. These digesters treating dairy effluent were operated at HRTs between 12.0 and 48.0 hours, while COD removal ranged between 60 and 98%. The OLR of these digesters varied between 1.7 and 20.0 kg $COD.m^{-3}.d^{-1}$. Under industrial-scale applications of the anaerobic filter, Bonastre and Paris (1989) listed three full-scale applications treating whey or dairy wastes. However, no mention is made of HRTs, while the OLR varied between 10.4 and 16.0 kg $COD.m^{-3}.d^{-1}$ and COD removals between 80 and 90%.

Expanded bed digester

The expanded bed, or as they are sometimes called, fluidized bed digesters, have a carrier medium similar to those found in anaerobic filters. In this case however, the carrier medium is constantly kept in suspension by powerful recirculation of the liquid phase. The carrier media include plastic granules with a large surface area, sand particles, glass beads, clay particles and activated charcoal fragments. This design actually offers a combination of two types of biomass retention: the active microbes are attached, by means of a biofilm, to the carrier medium, while the latter is retained in the digester through gravitational settling.

Several advantages are claimed for this digester configuration (Toldrá *et al.*, 1987; Iza *et al.*, 1991). These include the high specific surface area available to micro-organisms; uniform flow distribution and thus minimal problems associated with channeling, plugging and gas hold-up; the control of biofilm thickness due to the

particle movement and liquid up-flow and consequently minimal mass transfer resistance; and finally, the small size bioreactor required.

Toldrá *et al.* (1987) used the expanded bed process to treat three effluents (hog slaughterhouse, dairy and brewery wastewaters) and studied the effects of retention time and varying temperatures on the process. The dairy wastewater was treated at a retention time of 8.0 hours while achieving a soluble organic removal efficiency of 80%. This dairy effluent, however, had a COD of only 200 - 500 $mg.l^{-1}$, compared to a COD of between 1 500 and 4 000 $mg.l^{-1}$ for South African dairies. Moreover, the pH of this particular effluent was 9.05, and had to be adjusted to pH 7.4 by the addition of hydrochloric acid. The brewery effluent that was also included in their study, had a total COD of between 670 and 2 500 $mg.l^{-1}$, and of the three digesters, only the one treating brewery effluent could produce appreciable amounts of biogas. Thus, no methane yield figures were available for the dairy effluent digester. Bearing in mind the very wide variations found between different dairy effluents, it can be deduced that this particular dairy effluent is at the bottom end of the scale in terms of its COD concentration and organic load. The dairy effluent probably was produced by a dairy with very good product-loss control and rather high water use.

Hybrid digesters

The hybrid anaerobic digester is essentially a combination of different digester types in a single design. The fixed-film/sludge blanket combination has been used successfully for wastewater treatment, combining the advantages of the two different digester types. In this particular combination, the two zones are interdependent. The sludge blanket section, located beneath the fixed-film section, maintains high biomass concentrations, while the fixed-film section achieves high organic removal rates without the accumulation of excess solids or sludge (Joubert and Britz, 1987).

The hybrid digester, combining the UASB and fixed-film types, was first described in 1984 by Guiot and Van Den Berg. Several references to the use of hybrid digesters for effluent treatment are found in the literature. Britz *et al.* (1989) and Myburg and Britz (1993), for example, used the hybrid digester as described above for the treatment of a high-strength and complex landfill leachate, while it was used by Van Der Merwe and Britz (1993) for the treatment of bakers yeast factory effluent. However, no reference to the use of hybrid digesters for dairy wastewater treatment could be found in the available literature.

Membrane anaerobic digesters

A membrane anaerobic digester is any digester type where the digester effluent is filtrated by means of a filtration membrane. The use of membranes enhances biomass retention and immediately separates the HRT from the SRT.

Li and Corrado (1985) described a membrane anaerobic reactor system, abbreviated MARS, which made use of a completely mixed digester to which a micro-filtration membrane system was attached. The digester effluent was filtrated through the membrane and the permeate discharged, while the retentate, containing biomass and suspended solids was returned to the digester. Two MARS systems are described. Two pilot-scale units, both with an operating volume of 186 litres and a demonstration plant, with an operational volume of 37 850 litres were used. The substrate consisted of whey permeate with a COD of up to 62 000 $mg.l^{-1}$. The large demonstration plant performed exceptionally well, removing 99.5% of the COD. The HRT was set at 7.49

days, and the influent COD was 59 790 mg.l^{-1}. However, the system had a drawback in the form of pH control. Caustic was added to maintain pH between 6.80 and 7.00 units, and ammonium chloride was added to balance the carbon:nitrogen ratio. The ammonium addition will probably be unnecessary when whole whey, instead of whey permeate, is used as digester substrate. The most important conclusion these authors made was that the process control parameters, obtained in the small pilot plants could effectively be applied to their full-scale demonstration plant.

A similar membrane anaerobic digester system, the anaerobic digestion-ultrafiltration system (ADUF) has successfully been used on bench and pilot-scale studies, treating wine distillery effluent. The ADUF has also been evaluated for several other effluents (Ross *et al.*, 1989). The ADUF system does not use micro-filtration but rather an ultrafiltration membrane, and therefore, far greater biomass retention efficiency is possible. The ADUF system is a unique South African development, and uses locally manufactured polyethersulfone membranes. It has, however, not yet been used for the treatment of dairy factory effluents.

Separated phase digesters

Separated phase digesters are digesters where the acid-forming bacteria and the acid-consuming bacteria are spatially separated. The operation of a single phase digester requires bacterial populations whose metabolic rates are in perfect equilibrium. The use of two-phase digesters protects the methanogens from volatile fatty acid (VFA) inhibition during high organic loading rates. In a single phase digester an excessively high organic loading rate may lead to digester failure because of VFA inhibition of the methanogens. Separated phase digesters are especially useful for the treatment of wastes either with unbalanced carbon to nitrogen (C:N) ratio's, such as wastes with high protein levels, or wastes that acidify quickly (Anon., 1990). The use of separate phases has been refined by many workers. Burgess (1985) described a two-phase system which was basically the separation of the acidogenic and methanogenic phases, with each phase being operated at its own specific optimal conditions. High OLRs and short HRTs are claimed to be the major advantages of the separated phase digester.

Burgess (1985) also described two cases where dairy effluents were treated using a separated phase full-scale process. The one dairy had an effluent with a COD of 50 000 mg.l^{-1} and a pH of 4.5. Both digester phases were operated at 35 °C, while the acidogenic reactor was operated at a HRT of 24.0 hours and the methanogenic reactor at a HRT of 3.3 days. In the acidification tank, 50% of the COD was converted to organic acids while only 12% of the COD was removed. The OLR for the acidification reactor was 50.0 kg COD.m^{-3}.d^{-1}, and for the methane reactor, 9.0 kg COD.m^{-3}.d^{-1}. An overall COD reduction of 72% was achieved. The biogas had a methane content of 62%, and from the data supplied, it was calculated that a methane yield ($Y_{CH4}/COD_{removed}$) of 0.327 $m^{-3}kg^{-1}$CODremoved was obtained.

Lo and Liao (1986; 1988) also used separated phase digesters to treat cheese whey. The digesters were described as anaerobic rotating biological contact reactors (AnRBC) but can be described as tubular fixed-film digesters orientated horizontally, with internally rotating baffles. In the methane reactor, these baffles were made from cedar wood as it has, according to these authors, been proved that the desired bacterial biofilms develop very quickly on wood. The acidogenic reactor was mixed by means of the recirculation of the biogas. However, it achieved a COD reduction of only 4%. More important, the total volatile fatty acids concentration was increased from 168

$mg.l^{-1}$ to 1 892 $mg.l^{-1}$. This was then used as substrate for the second phase where a COD reduction of up to 87% was achieved. The original COD of the whey was 6 720 $mg.l^{-1}$, which indicates that the whey was diluted approximately ten-fold.

Many other examples of two phase digesters are found in the literature. It was the opinion of Kisaalita *et al.* (1987) that two phase processes may be more successful in the treatment of lactose containing wastes. These workers studied the acidogenic fermentation of lactose, determined the kinetics of the process (Kissalita *et al.*, 1989) and also found that the presence of whey protein had little influence on the kinetics of lactose acidogenesis (Kisaalita *et al.*, 1990). Venkataraman *et al.* (1992) also used a two phase packed bed (i.e., an anaerobic filter) system to treat dairy wastewater. Their main aims were to determine the kinetic constants for biomass and biogas production rates and substrate utilization rates in this configuration.

While only the above references are included to show the successful application of two-phase systems, many more references on the subject of two-phase systems are available still, the discussion of which falls beyond the scope of this review.

2.4 Anaerobic digestion: microbiological and biochemical considerations

Anaerobic digestion is a complex biological process, carried out by a wide range of bacteria living in a close symbiotic association. The bacteria present in an anaerobic digester can be divided into four distinct bacterial populations, but from the literature the impression is gained that the four groups are practically inseparable, due to their symbiotic association. The process and kinetics of anaerobic digestion has been extensively modelled using Monod kinetics and other mathematical models (McCarty and Mosey, 1991).

Originally only two trophic groups were recognized, namely the acid forming or acidogenic bacteria and the methane forming or methanogenic bacteria. Later a third intermediate group, the obligate hydrogen forming or acetogenic bacteria, was discovered and eventually a fourth group, namely the homoacetogenic bacteria was recognized. These last two groups are capable of producing acetic acid directly from hydrogen and carbon dioxide. These four groups exist in close symbiotic association. Nutrients supplied to an anaerobic digester are utilized through complex feed chains carried out by the four trophic groups (Ryder, 1985).

The end result of anaerobic digestion is the breakdown of organic matter into carbon dioxide and methane, i.e. biogas. Lactose, for example will be broken down into biogas in the following nett stoichiometric manner (Gottschalk, 1986):

$C_{12}H_{22}O_{11}.H_2O \rightarrow 6\ CH_4 + 6\ CO_2$

The above equation is valid only if no biomass is formed from the lactose supplied (Product to substrate yield (Yp/s) = 0, where p represents biomass and s lactose). In practice the $Y_{p/s} \approx 0.05$. The transformation is also by no means a direct one. It is the end result of the interrelated metabolic activities of the four bacterial populations, as is shown in Fig. 2.1, adapted from Giraldo *et al.* (1990). The interactions as shown in Fig. 2.1., are by no means complete and much more complex schematic representations are to be found in the literature.

All bacteria in the anaerobic food chain are chemotrophic, in other words, chemical compounds are used as the source of energy. This includes both chemo-organotrophs using organic compounds for electron donors during ATP synthesis, and chemolithotrophs which use inorganic compounds for the same purpose (Gottschalk, 1986). A detailed account of the specific microbes involved in each of the four groups is beyond the scope of this review.

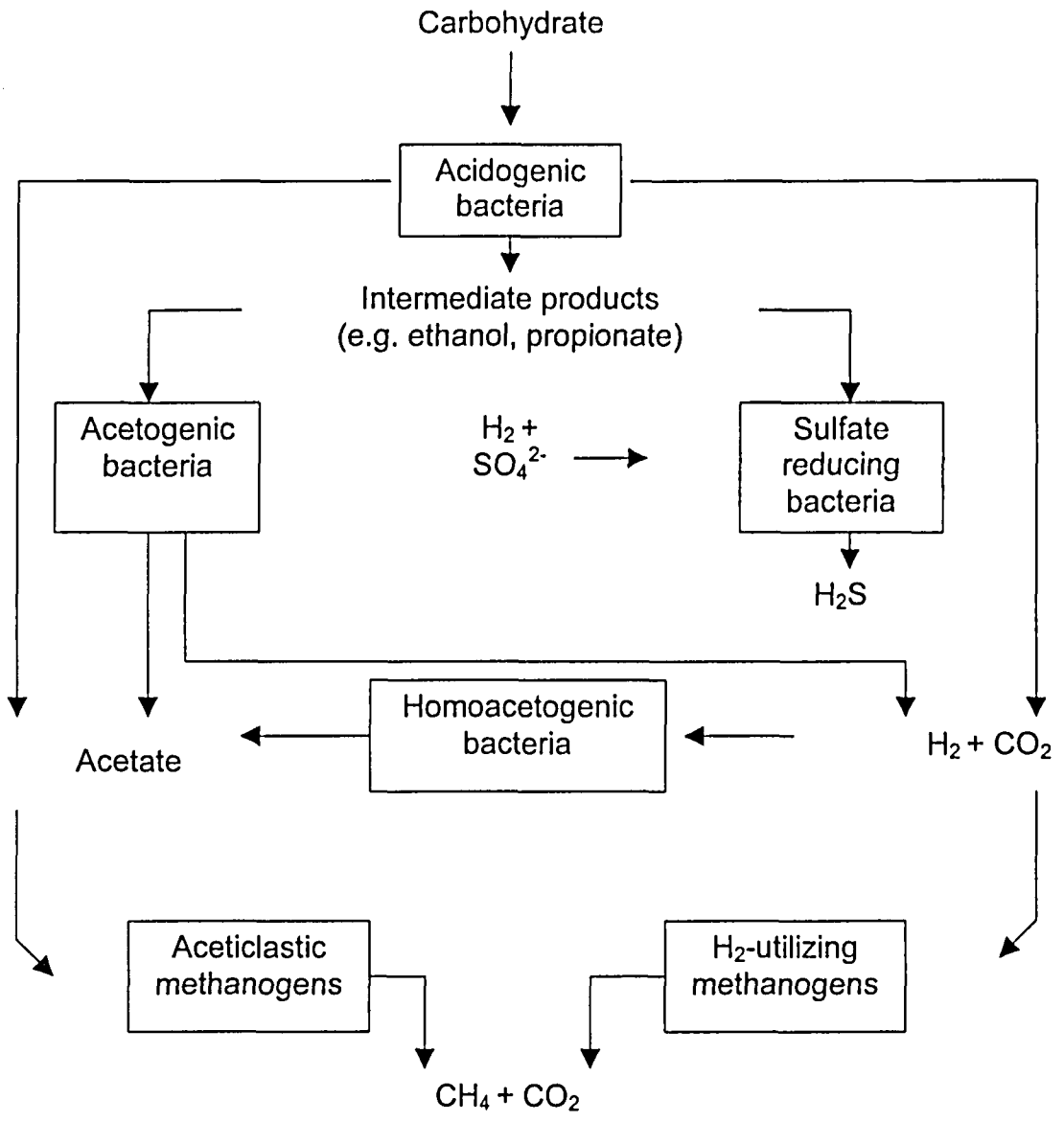

Figure 2.1 A simplified scheme of the inter-related metabolic pathways as found in an anaerobic digester, adapted from Giraldo *et al.* (1990)

2.5 Operational parameters

Operational parameters are those physical variables which can be used to control the anaerobic digestion process or assess its performance. Included in this review are hydraulic retention time, organic loading rate, temperature, alkalinity, pH, gas production and measurement, digester configuration and nutritional requirements.

Hydraulic Retention Time

Hydraulic retention time (HRT) is an indication of the time needed for the treatment of a certain volume of effluent, and is therefore also an indication of the digester size needed if a full-scale treatment facility is being considered. A smaller digester volume translates into less expenditure for the set-up of a treatment facility, and therefore a HRT as short as possible is usually aimed at.

Digestion efficiency is directly affected by the HRT (Toldrá *et al.*, 1987). The anaerobic digestion of hog slaughterhouse, brewery and dairy wastewaters at retention

times of 8, 6, 4, and 2 hours indicated that any reduction in HRT would severely affect the soluble COD removal. Although maximum removal efficiency was not the aim of their study, it was clear that even eight hours of HRT was not sufficient for maximal removal of soluble COD, when treating dairy wastewater. Only the brewery effluent could be treated at shorter HRTs without significant reductions in the soluble COD removal efficiency. It is obvious to note that a reduction in the efficiency of the anaerobic process is undesirable and that different effluents will each have a unique response to changes in the hydraulic retention time.

Bearing in mind the fact that high rate anaerobic digesters are capable of organic loading rates of up to 16.0 kg $COD.m^{-3}.d^{-1}$ (Hickey *et al.*, 1991) a high rate digester should theoretically be capable of treating an effluent with an average COD of 3 800 $mg.l^{-1}$, using an HRT of 0.24 d (3.8 kg $COD.m^{-3}$ - 16 kg $COD.m^{-3}.d^{-1}$). This is equivalent to 5.76 hours, which is much less than the eight hour retention time which was found to be unsuitable by Toldrá *et al.* (1987). It is therefore very difficult to draw a conclusion on the HRT that can be used in practice. This probably depends on the actual effluent and the optimum HRT should therefore be determined experimentally.

Organic Loading Rate

The loading rate of an anaerobic digester is usually expressed as the amount of COD in kg, per m^3 of digester volume, that is fed to an anaerobic digester during one day. The organic loading rate can be as high as 16 $kg.m^{-3}.d^{-1}$ for certain high rate anaerobic systems (Hickey *et al.*, 1991). In the event of the organic loading exceeding the design limit of an anaerobic digester, the acidogenic bacteria, which are the fastest growing of the four trophic groups, will become dominant. The end result is an accumulation of volatile fatty acids, a drop in pH and ultimately, digester failure.

Temperature

Temperature is an environmental factor which, according to some (Toldrá *et al.*, 1987), has a profound effect on the performance of an anaerobic digester and according to others (Kelly and Switzenbaum, 1984), has relatively little impact on the performance of an anaerobic digester. Again, the differences between individual digesters and effluents are probably the cause of this differing opinions.

Kelly and Switzenbaum (1984) studied temperature and nutrient effects on the anaerobic expanded bed process treating reconstituted whey. At constant HRT, the COD removal was not severely affected by lower temperatures. This is however, true only of the nutrient supplemented effluent. Treatment of the un-supplemented effluent was markedly affected by temperature differences. However, even at its optimum temperature, the digester treating the nutrient limited effluent could only achieve 60% COD removal. The nutrient supplemented digester had very similar COD removals at 35 °C and 30 °C, respectively 70% and 72%, but at 25 °C the removal efficiency fell to 54%.

Toldrá *et al.* (1987) also considered the effects of temperature on the COD removal from three different effluents. No detrimental effect was observed when treating slaughterhouse effluent but temperature did affect the treatment efficiency of digesters fed with dairy and brewery effluents. The COD removal from the dairy effluent dropped from ± 80% to less than 40% with a drop in operating temperature from 35 to 20 °C. This drop in removal efficiency was observed after the digester was allowed to stabilize for ten days at the lower temperature. Joubert and Britz (1987)

conducted a factorial design study, and established that the optimum operational temperature of their hybrid digester, treating a volatile fatty acid containing substrate, was 37 °C. From these results, it can be concluded that temperature is indeed an important operational parameter, and 35 to 37 °C is widely considered as the optimum temperature for mesophilic effluent treatment.

Alkalinity and pH

Alkalinity is a measure of the buffering capacity of an anaerobic digester. It is the reverse of titratable acidity, and is usually expressed as mg calcium carbonate equivalents per liter. Since the main buffering system of an anaerobic digester is the carbonate-bicarbonate system (Schröder and De Haast, 1988; Moosbrugger *et al.*, 1992), the bicarbonate alkalinity is a useful parameter to evaluate the buffering capacity of an anaerobic digester and also its possible resistance to pH changes during shock loading conditions. While the bicarbonate acid/base system is the main buffer against pH change, dissociation of the volatile fatty acids is the main cause for a decrease in pH in anaerobic digesters. The optimum pH range for anaerobic digesters is from 6.6 to 7.4 pH units (Moosbrugger *et al.*, 1992).

Gas formation and measurement

Gas measurement is important to monitor the success of the anaerobic digestion process. The maximum theoretical methane yield is 0.350 m^3 CH4 per kg of COD removed.

Petrozzi and Dunn (1991) reviewed various methods, which are used for the measurement of gas production, claiming that the rate of gas production, especially methane, is the most important indicator of operational performance. This is in contrast to Giraldo *et al.* (1990), who utilized hydrogen and carbon monoxide as early warning indicators of digester instability. Petrozzi and Dunn (1991) also state that instantaneous gas flow rates are more useful than gas volume accumulation over long time periods, since accumulated volumes indicate events that have already taken place.

Gas production measurements certainly are important to evaluate the efficiency of the digestion process. However, bearing in mind the schematic representation of the microbial interactions between the four trophic groups (Fig. 2.1.), hydrogen and carbon monoxide will indeed provide a more useful indication of the operational stability than the methane production rate. The methane production rate will show an increase with an increase in substrate concentration. However, the methanogenic population is likely to have a finite capacity for methanogenesis, which implies that a certain maximum rate of methane generation will not be exceeded. Thus, the formation of carbon monoxide and hydrogen, as well as an increase in the level of volatile fatty acids, probably gives a better indication on the operational stability of the digester when compared to methane production rate.

However, the methane yield, in terms of $m^3.kg^{-1}$ COD removed (Colin *et al.*, 1983), is the most important indicator of the nett energy recovery achieved by the anaerobic process. Using this parameter, it is also possible to assess the digester in terms of the theoretical values that should be obtained under ideal conditions. Therefore, biogas composition and biogas production rate are important parameters for assessing the performance of an anaerobic digester, although care must be exercised to ensure correct interpretation of the results.

Digester configuration

Not all effluents are suitable for treatment in any type of anaerobic digester. De Man and De Bekker (1986) used an up-flow anaerobic sludge blanket (UASB) type digester for the treatment of effluent from a multiple product dairy in the Netherlands. This digester configuration caused precipitation problems and severe scaling, but it performed very well treating a cheese factory effluent from a factory in Wisconsin. The first dairy's needs were catered for with a combination of anaerobic stabilization and bio-aeration with continuous external inoculation. The success of the trial experiments on the Wisconsin cheese effluent lead to the construction of a full-scale up-flow anaerobic sludge blanket treatment facility, the results of which were not yet reported.

Nutritional requirements

The extent to which the nutrient requirements of the bacterial population are satisfied or supplemented may, among other factors, have a profound effect on the synthesis of macroscopic structures in which bacterial agglomerates will reside. In many types of digesters, the most important and desirable macroscopic structure is granules, since granulation helps prevent biomass washout, thereby enhancing efficiency.

Methanol is an organic nutrient supplement that apparently aids the start-up process (Cayless *et al.*, 1990). During the start-up of two up-flow anaerobic sludge blanket digesters, fed with effluent from an ice-cream manufacturing plant, the substrate fed to one of the digesters was supplemented with methanol. In this supplemented digester, granule formation was observed three weeks after start-up, and complete granulation was observed at four weeks. This quick granulation was attributed to the addition of methanol. The digester fed with the methanol supplement also displayed a greater degree of COD removal (62%) than the control digester (45% COD removal). Washout was severe, however, and the residual biomass was sensitive to methanol withdrawal, which indicate the methanol supplement would have to be sustained in order to maintain these quickly formed granules. The sensitivity towards methanol withdrawal might be attributed to the fact that methanol is a direct pre-cursor of methane. Methanol can be utilized as substrate by Methanosarcina, Methanolobus and Methanococcoides species. (Gottschalk, 1986). However, Cayless *et al.* (1990) gives no indication as to the possible mechanism through which methanol aids granulation.

Inorganic trace-nutrient supplements will also affect digester performance. Goodwin *et al.* (1990) compared the effects of two trace nutrients, namely cobalt and calcium and one organic supplement, sucrose, to results obtained with un-supplemented effluent. This was done in five duplicate up-flow anaerobic sludge blanket treatment systems, using the effluent from an ice-cream manufacturer. The process efficiency was measured mainly in terms of biogas production rates, total organic carbon (TOC) removal rates and effluent acetic acid concentrations against the control pair. The formation of granules were studied with a scanning electron microscope. Only the added calcium had any significant effect on the performance of the anaerobic digestion process, when measured against the control in terms of the three parameters mentioned above.

In terms of granulation, the calcium supplemented sludge formed granules at day 47, about nine days earlier than any of the control experiments. Granulation not only occurred earlier but also was significantly improved overall against the control, and the digester could also withstand much more rapid increases in organic loading.

The authors (Goodwin *et al.*, 1990) postulate an enhanced efficiency in the utilization of the volatile fatty acids due to the high calcium concentration, but also admit that the calcium is responsible for the superior granulation observed. This alone might have been the reason for the greater efficiency since more of the slower growing methanogens would have been retained in the digester.

The effect of many other trace nutrients have been studied by other workers. Kelly and Switzenbaum (1984) found the trace nutrients nickel, iron and cobalt to significantly influence digester performance when an anaerobic film expanded bed was used to treat nitrogen and phosphorus supplemented whey. This whey was reconstituted using whey powder and water, and the authors also found that the mineral composition of the dilution water had an effect on digester performance. These authors rightly suggest that careful attention should be given to nutrient requirements for successful anaerobic waste treatment. Nel at al. (1985) used a fixed-film reactor to treat effluent containing mainly monocarboxylic acids. They assessed the effects of many trace nutrients, and found that the addition of the trace elements silicon, selenium, nickel and tungsten resulted in an improvement in digester performance. These authors concluded that the order of magnitude, by which certain trace elements stimulate the performance of an anaerobic digester, warrants the trace element supplementation of anaerobic digester feedstocks.

2.6 Kinetics of anaerobic digestion

In the past, growth kinetic studies have contributed greatly to the success of mono-culture fermentation and aerobic single cell protein production technologies. Similarly, an understanding of the kinetics of the anaerobic digestion process is essential if the latest treatment systems are to be developed to their full potential (Lin *et al.*, 1989). However, a complete understanding of the kinetics of the anaerobic digestion process is hampered by the complex nature of the microbial populations and their interactions. Furthermore, both the populations and their interactions can easily change as a result of changes in the substrate concentration or composition. For example, a change in substrate concentration may favour bacteria that were not previously competitive, and as a result, new kinetic constants may apply (Kissalita *et al.*, 1989). Changes in substrate composition will have similar effects, with additional effects on bacterial decay rates and maintenance energy requirements when toxic compounds, which may be present in the substrate, are co-metabolized in the digester (Criddle, 1993). Despite these limitations, much information on the kinetics of the anaerobic digestion process can still be found in the literature, albeit with some contradictions.

The term "kinetics" and the context in which it is used, applies to the time-related dynamics of the anaerobic digestion process. It mainly concerns the rate of substrate transformation or rates of metabolite and biomass production. In other words, physical variables are usually related to or expressed as units of time.

The Monod equation

Since the anaerobic digestion process is generally divided into four main phases, performed by four distinct but highly interactive microbial populations, the kinetics of the process may likewise be considered from different points of view. However, the starting point of any discussion on microbial kinetics in a continuous flow system, such as an anaerobic digester, is the Monod equation (Monod, 1949 as cited by Pavlostathis and Giraldo-Gomez, 1991):

$$\mu = \frac{s}{\mu_{max}\,(Ks + s)}$$

where μ = specific growth rate (h^{-1})
μ_{max} = maximum specific growth rate
s = concentration of limiting substrate ($g.l^{-1}$)
Ks = Monod half-saturation constant

This equation mathematically describes the relationship between the growth rate and substrate concentration by means of the maximum possible growth rate and the so-called half-saturation constant Ks. The term "specific" indicates the amount of new biomass formed per unit of existing biomass within a specified time interval, e.g. 10 mg cells formed from 100 mg of cells within an hour will indicate a specific growth rate, of 0.01 $mg.mg^{-1}.h^{-1}$, or simply $0.01h^{-1}$. The half-saturation constant Ks, is that substrate concentration where the growth rate is at exactly half its maximum. Ks should in theory, have the same value for identical systems utilizing identical substrates. However, each bacterial strain has its own maximum specific growth rate, μ_{max}. Furthermore, μ_{max} is influenced strongly by the operational conditions such as pH and temperature, and thus, different values for μ_{max} will be arrived at for every set of operational parameters.

The Monod equation, as described above, is valid only for completely mixed systems without any cell retention, which might be caused by biofilm or granule development. This poses several limitations for the application of the Monod equation to anaerobic digester systems. However, in systems where cells are immobilized, for example in granules or in biofilms, the equation can be used to describe the product formation rate or substrate utilization rate.

The literature which is available on the subject of the kinetics of anaerobic digestion, covers all the aspects of the successive substrate conversions found in the anaerobic digester. Some authors even modelled the anaerobic digestion process as a one-step chemical transformation (Pavlostathis and Giraldo-Gomez, 1991).

Kinetics of acidogenesis

The kinetics of acidogenesis has been studied by many workers on many types of substrates. In this study on the anaerobic treatment of dairy effluents, the primary concern is with the acidogenesis of lactose as substrate.

Kissalita *et al.* (1989) used the general Monod equation to describe the growth of a mixed, undefined anaerobic culture, converting lactose to organic acids in a fermenter system without any biomass retention mechanism. A continually mixed fermenter with an operating volume of 1.5 liters was used. After initial batch mode operation, the system was switched to chemostat mode operation with an initial dilution rate (D) of 0.05 h^{-1} (a HRT of 20 hours). These studies were conducted using a chemically defined, lactose containing substrate, with no milk protein. This was done in order to minimize the drawbacks of using a complex substrate in a chemostat. The acidogenesis of lactose was studied at pH values of 4.5 and 6.0 units. It was found that the predominant fermentation products were acetate, propionate, n-butyrate and lactate. The concentrations of these products was strongly dependant on the HRT. The maximum acetate concentration of approximately 1 300 $mg.l^{-1}$ was found at an HRT of 20.0 h. At shorter retention times (i.e, higher dilution rates), lactate became the

dominant product, with the highest concentration of approximately 1 400 $mg.l^{-1}$ observed at an HRT of 5.0 hours. The observed shift in product distribution coincided with a transient drop in biomass concentration. This drop in biomass concentration was presumably due to a shift in the microbial population. This phenomenon was observed during both experiments. These authors (Kissalita et al, 1989) concluded that the Monod equation, without provision for maintenance energy requirements, modelled the acidogenesis of lactose very well, at a pH value of 4.5 units. However, at a pH value of 6.0, abundant cell growth occurred at dilution rates near the critical dilution rate, which invalidated the Monod model.

Kissalita *et al.* (1989) commented that lactate was the preferred product for the operation of an acidogenic reactor in a two-phase process. This is in strong contrast to the well established fact that aceticlastic methanogenesis is the most important pathway of methane fermentation in an anaerobic digester. However, when compared to lactose, lactate is indeed preferable as a substrate for an anaerobic digester. This notion is supported by the findings of De Haast *et al.* (1983). These authors found that prefermented whey, in which 50% of the lactose was converted to lactate, was suitable as substrate for an anaerobic fixed-film digester. When these authors used untreated whey as substrate, severe acidification and eventual digester failure were observed.

Yu and Pinder (1993) studied the fermentation kinetics of lactose in an acidogenic biofilm. This study was conducted using a continuous flow fermenter. The pH was held at 4.6 units and the temperature at 35 °C. The mass transfer resistances were eliminated or minimized by using a thin biofilm and recycled medium. These authors found that the utilization rate of the lactose could be described as a function of the lactose concentration by means of the Michaelis-Menten equation, which in essence is a graphical transformation of the Monod equation. The production rates of acetate, butyrate and ethanol could also be described in this manner. A very significant observation that Yu and Pinder (1993) made, was that the concentration of acetate quickly reached a constant, while any additional lactose consumption resulted in additional butyrate production. The production of propionic acid was well suppressed at the low pH level.

In a review on anaerobic digestion kinetics, Pavlostathis and Giraldo-Gomez (1991) concluded that all the steps in the anaerobic digestion process can be modelled using the Monod equation, with the exception of the hydrolysis step. However, as hydrolysis of particulate substrates precedes the acidogenesis step, it will only influence the overall process if the substrate is of such recalcitrant nature that it will cause the hydrolysis step to become rate-limiting. These authors indeed found hydrolysis to be rate-limiting whenever the treatment of complex substrates was studied (Pavlostathis and Giraldo-Gomez, 1991). A very interesting summary of the kinetic data in the literature is also presented by these authors. Eventhough no mention is made of lactose containing substrates, the data on the kinetics of glucose acidogenesis reveals an interesting pattern. Here, three of the reported four μ_{max} values for glucose acidogenesis are 0.30 h^{-1} within a standard deviation of ± 0.01 h^{-1}. This corresponds to an HRT of 3.33 hours. The fourth reported value was 1.25 h^{-1}, corresponding to an HRT of 19.2 hours, which is almost the same retention time as used by Kisaalita *et al.* (1987).

Kinetics of methanogenesis

No literature could be found where the kinetics of methanogenesis were studied using dairy effluent as substrate. However, this is not a limitation since methanogenic bacteria use substrates such as methanol, formic acid, acetic acid, ethanol and gaseous products, i.e. hydrogen and carbon dioxide (Gottschalk, 1986). Therefore, the type of substrate in the untreated effluent will only influence the acidogenic and possibly the acetogenic processes, and not the methanogenic processes. This is true unless high levels of toxic compounds, which will influence the methanogenic bacteria, are present in the substrate. Thus, kinetic rates, which were obtained in systems where methanogenesis is rate-limiting, should also apply to similar systems treating lactose containing wastes. Mass-transfer limitations are one final consideration besides toxic compounds and substrate saturation. This becomes clear when it is considered that modern anaerobic digesters all use some type of biomass immobilization, in order to separate HRT from SRT (solids retention time). Therefore, the substrate utilization rate and gas production rate will be more important than specific growth rate. For this reason, mass-transfer resistance will influence the kinetic constants and maximum transformation rates (Pavlostathis and Giraldo-Gomez, 1991). Mass-transfer resistance, in its turn, can be influenced by the type of biomass immobilization. Factors such as the size of granules and the thickness and density of a biofilm can conceivably influence the rate of substrate and product transfer. Therefore, care should be exercised when kinetic data obtained from one system are applied to another, altogether different system, even if both are used to treat identical wastes.

Still, valuable data on the kinetics of the overall process and of methanogenesis are available in the literature. Table 2.2, adapted from Pavlostathis and Giraldo-Gomez (1991), is a summary of the values of kinetic constants obtained on various substrates.

Table 2.2 Summary of values of kinetic constants for various substrates utilized in mesophilic anaerobic digesters (Pavlostathis and Giraldo-Gomez, 1991)

Substrate	Process	Ks ($mg.l^{-1}$ COD)	$\mu_{max}(d^{-1})$	Corresponding HRT (d)
Carbohydrates	Acidogenesis	22.5 – 630	7.20 – 30	0.14 – 0.033
Long-chain fatty acids	Anaerobic oxidation	105 – 3180	0.085 – 0.55	11.8 - 1.8
Short-chain fatty acids*	Anaerobic oxidation	12 – 500	0.13 – 1.2	7.7 – 0.8
Acetate	Aceticlastic methanogenesis	11 – 421	0.08 – 0.7	12.5 – 1.4
Hydrogen/ Carbon dioxide	Methanogenesis	$4.8x10^{-5}$ – 0.60	0.05 – 4.07	20 – 0.25

* Excluding acetate

As can be seen from the values presented for the half-saturation constant, Ks, the average dairy effluent with a COD value of 2 500 to 3 800 $mg.l^{-1}$ (Water Research Commission, 1989; Jones, 1974) will easily exceed the saturation point of almost all the steps associated with the anaerobic digestion process. This implies that the bacteria involved in the process will grow at their maximum growth rate in batch processes, and that washout of bacteria will occur if no immobilization is used in high-rate continuous processes.

The data, as summarized by Pavlostathis and Giraldo-Gomez (1991) and presented in Table 2.2, clearly does not differentiate between processes where separation of SRT and HRT are achieved, and processes where no separation is achieved. This is evident from the reported maximum growth rates of aceticlastic methanogens. It is highly unlikely that an aceticlastic methanogen will be able to grow fast enough to prevent washout in a digester operated at an HRT of 1.4 days, without some type of biomass retention. Therefore, it is highly likely that the kinetic results presented in Table 2.2 were obtained using both growth rate and substrate utilization rate. Modern high-rate digester systems all achieve a measure of separation between SRT and HRT. Therefore, the substrate utilization rate will provide a more useful parameter for comparison than maximum growth rate. Bearing in mind the result of biomass retention, the minimum and maximum HRT values given in Table 2.2, might be considered as representative of systems with and without separation of SRT and HRT, respectively.

2.7 Start-up of anaerobic digesters

Usually, an anaerobic digester is seeded with active sludge from another digester and many other sources of suitable bacteria. This practise results in a heterogeneous mixture of different and unidentified bacteria being present in an anaerobic digester, which probably is responsible, among other factors, for the long start-up times associated with anaerobic digesters.

The reason for a delayed start-up is the slow growth rate of the acetogenic and methanogenic bacteria compared to the acidogenic bacteria (Cánovas-Diaz and Howell, 1987b). These authors succeeded in reducing the start-up time of a down-flow percolating fixed-film anaerobic digester to 35 days by growing acetogenic and methanogenic bacteria in a synthetic medium until a stable biogas production rate was achieved. The digester substrate was then changed from the synthetic medium to deproteinized cheese whey which allowed growth of the acidogenic bacteria. Thirty five days after start-up the digester was able to handle 8 kg $COD.kl^{-1}.d^{-1}$. The complete anaerobic "food chain" was established only four days after the change from synthetic medium to the deproteinized cheese whey was made.

The practise of inoculation with defined, pure cultures was taken to the extreme by Schug *et al.* (1987). They studied bi- and tricultures of several known bacterial strains and found a very efficient combination in *Lactobacillus plantarum*, *Acetobacterium woodii* and *Methanosarcina barkeri*. The combination of these bacteria in a chemostat culture lead to 90% lactose converted to methane and carbon dioxide at dilution rates of 0.27 to 0.37 per day. This translates to HRTs of 3.7 to 2.7 days, respectively. The use of these defined bacterial strains may aid in the reduction of long start-up times of both laboratory and full-scale anaerobic digesters.

A very important factor during start-up of up-flow anaerobic sludge blanket (UASB) digesters, is the formation of granules. These granules enhance the settling properties of the sludge and are essential for successful operation of an UASB

digester. Even though granules will eventually form in a digester seeded with a diffuse sludge (Hickey *et al.*, 1991), it is common practice to seed new digesters with pre-granulated sludge. To test the necessity of using pre-granulated seeds for new digesters, Goodwin *et al.* (1992) used two different sludges, one granular and one diffuse, to inoculate ten digesters using different mixtures of sludge. The digesters were fed a synthetic wastewater containing sucrose. The authors concluded that the use of pre-granulated sludge seeds allows greatly reduced start-up times. They also saw no evidence that diffuse sludge can be stimulated to form granules by adding small amounts of granular sludge. The digesters seeded only with granular sludge achieved high performance levels after only a few days, despite using granules that were stored for two months at 4 °C. The digesters receiving only diffuse sludge required start-up times longer than 60 days. In spite of the many references in the literature on granulation, the actual mechanism of granulation, and thus also specific procedures to ensure granule formation, remains uncertain.

In conclusion, anaerobic digesters are evidently best started by using sludge obtained from a similar digester system, which was used for the treatment of the same or a similar waste. Where temperature is concerned, the desired operational temperature should evidently be attained and also maintained from the outset. As far as initial loading rate is concerned, the levels of volatile fatty acids and biogas yield can provide an indicator of methanogenic activity. The loading rate initially should be well below the desired loading rate, while it is gradually raised until the desired loading rate is achieved.

2.8 Conclusions

Anaerobic digestion is widely accepted as treatment method for raw sewage sludge since the 1930's. The more recent applications of anaerobic digestion for industrial effluent treatment since the 1950's, have grown steadily in number and sophistication. However, anaerobic digestion remains a complex process and all the events that occur during the anaerobic digestion of complex substrates are not yet understood. Therefore, even now after almost a hundred years, the process still offers much scope for research and development. Many bacteria involved in anaerobic digestion have been identified, but many more remain to be identified. Despite the many unknown complexities and mysteries which still pervades the process, great strides have been made to understand and effectively utilize the process.

World-wide, several applications of anaerobic digestion for the treatment of dairy effluent are to be found. However, there is none in South Africa. Currently, inefficient or very basic effluent treatment procedures are applied to dairy effluents in South Africa, yet dairy effluents in general are eminently suitable as substrates for anaerobic treatment.

The various types of digesters developed over the years are described in the literature in a confusing array of nomenclature and acronyms. Regrettably, no standard convention exists for the classification and description of digester types. It is believed that such a standard convention will greatly assist in the interpretation and application of the masses of research results available in the literature. This certainly was the case with the convention proposed by Colin *et al.* (1983). This convention suggests a standard definition of parameters and analytical measurements applicable to the anaerobic digestion process. A similar set of standard definitions concerning digester types will indeed be very useful for comparison purposes. Therefore it is suggested that digesters be classified on the basis of the type of biomass retention.

Using this criterion, only four basic digester types and hybrid combinations thereof, can be distinguished. The current wide variety of digester names and types are most certainly due to each worker promoting his or her own results and ideas, and not due to any great variety of unique concepts. When the biomass retention mechanism is used to differentiate between digesters, the virtual countless number of designs are reduced to only four types. These are (1) attached biofilm systems, where the bacteria are immobilized in a biofilm on some sort of support such as in the anaerobic filter, the fixed-film digester and the fixed-bed digester; (2) systems where gravitational sludge settling takes place, such as the contact digester and the up-flow anaerobic sludge blanket digester; (3) systems where solids and liquids are separated by some mechanical means, such as the membrane anaerobic reactor system (MARS) and the anaerobic digestion - ultrafiltration (ADUF) system; and finally (4) completely mixed systems with no mechanism for biomass retention, such as the CSTR. Expanded bed digesters will actually be a hybrid form of (1) and (2), where the bacteria are immobilized on the carrier through biofilm formation and the carrier in its turn is retained in the digester by means of gravitational settling.

The specific nutritional requirements of many of the bacteria involved in anaerobic digestion have been established. However, each new application for anaerobic digestion demands a thorough evaluation of the requirements in order to obtain optimum treatment efficiency. This will include an assessment of the C:N:P ratio and the supply of sufficient trace nutrients.

Anaerobic digestion has been used very successfully for the full-scale treatment of dairy wastewater, achieving real reductions in disposal costs and environmental impact, while generating useful methane gas as a byproduct. However, the type of treatment intended for any particular dairy effluent will depend very much on the effluent itself and the results of preliminary laboratory trials on that specific effluent, using the type of digester configuration that is intended for the large scale treatment of the said effluent.

Concerning the possibility of erecting a full-scale treatment facility, the cited examples illustrate that not only is anaerobic digestion successful environmentally, it is also successful economically. The success achieved with anaerobic digestion on dairy effluent treatment elsewhere makes it a deserving proposition to investigate under South African conditions.

Such an investigation will ideally start with a survey to determine the effluent related needs of the dairy industry. From such a survey, it will be possible to determine what costs and environmental impacts are caused by the current lack of effluent treatment. Possibly, the reasons for the current lack of sophisticated effluent treatment measures will also emerge from such a survey. Concerning the laboratory-scale investigation of anaerobic digestion of dairy factory effluents, almost every type of digester was already used by other researchers. The exception is the hybrid anaerobic digester which combines an UASB and a fixed-bed design. Therefore, as far as laboratory-scale work is concerned, the use of the hybrid digester for the anaerobic treatment of dairy factory wastewater will contribute, without unnecessary duplication, to the existing body of scientific knowledge on the anaerobic digestion of dairy factory effluents.

CHAPTER 3

EFFLUENT PRODUCTION AND DISPOSAL IN THE SOUTH AFRICAN DAIRY INDUSTRY: A POSTAL SURVEY

3.1 Summary

In South Africa, where water has been identified as the country's most important natural resource, the dairy industry is significant, both from a water intake and discharge point of view. The requirements of the dairy industry in relation to on-site effluent treatment were thus determined by means of a postal survey. Of the 247 questionnaires sent out, 81 were returned. The data obtained indicated that the respondents from the survey receive and process 70 % of the total milk production in South Africa. A diverse range of effluents were described by the respondents. The larger factories generally dispose their effluents into municipal sewer treatment works resulting in high disposal costs. The majority of smaller factories and dairies dispose of their effluents by means of irrigation onto lands and pastures. A possible side-effect of this practice is of course ground-water pollution. Most of the respondents expressed a need for more information on the subject and a proposed project for the development of a biological effluent treatment procedure was supported by 49 % of the respondents. These respondents represent 40 % of the total milk volume processed in the country. The supportive respondents were also responsible for 84 % of the reported municipal levies.

3.2 Introduction

Ten years ago it was estimated that the South African dairy industry, with over 150 dairies, consumes approximately 4.5 x 10^6 m^3 water per annum (Water Research Commission, 1989). This makes the dairy industry a comparatively large water user. The specific water intake (water consumption : raw milk) ratio in the different dairy manufacturing sectors varies considerably and is dependent on the type of product and also on the individual management and cleaning practices. The overall range varies between 1.4 and 9.5 with an overall mean of 3.6 (Water Research Commission, 1989).

Milk buyers receive and process approximately 1.86 x 10^9 l of milk annually (Dairy Board, 1990). However, dairies also discharge large quantities of different effluents arising from milk processing, producing different milk products and from the cleaning processes. The ratios are dependent on the types of dairy products manufactured. It has been estimated that between 75% and 95% of the water intake emerges as effluent (Water Research Commission, 1989)

Milk and related products have exceptionally high chemical oxygen demand (COD) values (milk: 218 000 $mg.l^{-1}$; skimmed milk: 100 000 $mg.l^{-1}$; whey: 80 000 $mg.l^{-1}$). The inevitable wastage of milk and milk products can contribute greatly to the pollution loads discharged. It has been estimated by Jones (1974) that for the USA the average COD of dairy effluents is approximately 3 800 $mg.l^{-1}$. The average pollution load (as COD) for the South African dairy industry is not known but, since dairy practices in South Africa are similar to those practiced in the USA, it can be safely assumed that the average values would be similar.

Water management in the South African dairy industry for the purpose of effluent control is well documented (Funke, 1970; Water Research Commission, 1989). Significant recommendations have been made towards the in-house water

management in the South African dairy industry (Water Research Commission, 1989). However, the nature of dairy effluents7 changes significantly when the water usage of a factory is reduced.

Currently, another problem found in the dairy industry is the disposal of the effluents. Until fairly recently, the issue of effluent disposal or treatment did not receive any serious consideration in the dairy industry. It is thus important that before any studies on the treatment and disposal of dairy factory effluents can commence, the need for such a study has to be evaluated. A comprehensive questionnaire on this subject was thus compiled and sent to all registered milk buyers in South Africa. This country-wide postal survey was also used to determine the scope of other effluent related issues. These included the volumes of milk received, the products manufactured, the water usage, the expenditure associated with the effluent, the chemicals used in the factory, and the degree of effluent-awareness of the factory's management. This paper thus reports on the results from this national postal survey on dairy effluents.

3.3 Experimental

The questionnaire, sent to the 247 milk buyers registered during 1991 (Nell, 1991), covered the following aspects:

(i) Milk volume received
(ii) Products manufactured
(iii) Water usage
(iv) Chemicals used in the dairy or factory
(v) Effluent volume and strength
(vi) Effluent treatment prior to disposal
(vii) Effluent disposal
(viii) Economics related to effluent disposal
(ix) Interest in the intended future effluent treatment and/or disposal projects.

In the questionnaire, specific questions were used to determine figures on daily rates. These included daily water usage, milk reception volume and effluent discharge volume. The answers were converted, where applicable, to yearly rates by multiplying with a factor 264, assuming a month consisted of 22 workdays.

The 247 registered milk buyers included all the manufacturers of dairy products and fresh milk distributors, but not ice-cream as the manufacture of the latter does not involve fresh milk. All addresses were supplied by the Dairy Services Organization and pre-paid envelopes were included for the convenience of the respondents. The respondents were allowed 2 weeks to return the completed questionnaire. This deadline was extended in order to obtain as many replies as possible.

3.4 Results

Respondents

In response to the postal survey, 81 responses were received, of which 73 were found suitable for data processing. The remaining 8 were unsuitable due to insufficient answers to the questions - some even were returned completely blank. This represents a response of only 29.6% of the total sent out. However, these respondents

receive and process 70% of the total milk production in South Africa, calculated by using national figures published by the Dairy Board in 1990. This figure was also calculated using a 22 workday month. The respondents thus represent the largest and probably the most important members of the South African dairy industry.

The respondents manufacture the complete range of dairy products and therefore the returned questionnaires could be divided into 4 groups representing the: i) cheese manufacturers (17 respondents); ii) milk powder manufacturers (10 respondents); iii) fresh milk manufacturers - milk reception greater than 10 $kl.d^{-1}$ (21 respondents); and iv) the fresh milk manufacturers - milk reception less than 10 $kl.d^{-1}$ (25 respondents).

In Table 3.1 the results from the survey, relating to the management awareness of effluent volumes, pollution values and costs of effluent disposal, are shown for each individual group. Figures 3.1 and 3.2 respectively depict the specific water consumption and the product losses in relation to the milk volume received for each of the 4 respondent groups.

Cheese manufacturers

The cheese manufacturers produce soft and/or hard cheese varieties. Respondents in this group indicated that cheese was the only dairy product manufactured and thus, the volumes of milk received varied considerably. Milk reception volumes within this group, varied between 1.2 and 197.0 $kl.d^{-1}$, with an average of 48.0 $kl.d^{-1}$. The average water usage of the cheese manufacturers was 122 $kl.d^{-1}$, with the highest usage of 380 $kl.d^{-1}$ recorded by a milk buyer receiving 104 $kl.d^{-1}$ milk on average.

The effluent awareness of all 17 cheese producers was notable in that all the respondents indicated what their effluent volumes were. Two producers dispose of their effluent by land irrigation, the effluent volumes being 130 and 45 $kl.d^{-1}$, respectively. The rest of this group dispose of their effluent into local municipal sewers. Only 6 of the respondents reported expenditure associated with effluent disposal which, on average, represents a yearly total of R170 000 in terms of municipal levies and taxes.

Only 4 respondents indicated the pollution properties of their factory effluents, expressed as chemical oxygen demand (COD, as $mg.l^{-1}$), pH and temperature. Furthermore, only one respondent recorded the suspended solids content (230 $mg.l^{-1}$) of the specific factory.

The COD levels as recorded by the respondents varied from 1 000 to 2 100 $mg.l^{-1}$, with an average of 1 360 $mg.l^{-1}$. The effluent pH varied from pH 5.0 to pH 10.0 and the temperature, as recorded by the respondents, varied from 10 to 30 °C. Incidentally, one of the effluents used for irrigation had an average COD value of 1 150 $mg.l^{-1}$, pH 5.0 and suspended solids content of 230 $mg.l^{-1}$.

Milk powder producers

The 10 milk powder manufacturers indicated that milk powder was their only product of manufacture. As can be expected, all milk powder factories are fairly large operations, with milk reception volumes ranging from 32 to 200 $kl.d^{-1}$. The water usage ranged from 324 to 620 $kl.d^{-1}$.

Nine of the respondents of this group indicated their factories' estimated effluent volume. This ranged from 44 to 424 $kl.d^{-1}$. Three of the milk powder producers disposed of their effluents, which totalled 480 $kl.d^{-1}$, onto land or pastures. No

indication of the COD values was given by these respondents. In contrast, 5 of the remaining respondents clearly indicated the COD values of their effluents, which ranged from 641 to 9 700 $mg.l^{-1}$, with an average of 3 500 $mg.l^{-1}$. The pH of these effluents ranged from 4.5 to 9.5. Only 6 of the respondents recorded the costs associated with effluent disposal, these ranging from R 6 000 to R 60 000, with a total of R 207 000 per annum for the group as a whole.

Fresh milk producers (reception volumes greater than 10 $kl.d^{-1}$)

The 21 respondents included in this group produced pasteurized milk, UHT milk, sterilized milk, evaporated milk, condensed milk, milk powder, pasteurized cream, soft and hard cheeses, process cheese, butter, cultured buttermilk and buttermilk powder, evaporated whey and whey powder, custards, desserts and different varieties of yoghurt.

The milk reception volumes varied from 13.25 to 400.0 $kl.d^{-1}$. Their reported water usage varied from 2 to 1 514 $kl.d^{-1}$. All use municipal water, and 17 indicated that their effluents are received by their local municipal sewage treatment works. The remainder gave no indication as to how their effluents were disposed of.

Although only 4 respondents recorded their effluents' COD values, ranging from 1 000 to 7 000 $mg.l^{-1}$, 13 indicated the costs associated with the disposal of their effluents. The expenditure ranged from a mere R 900 to R 300 000 per year, with a total of R 1 171 000 for this group as a whole.

Fresh milk producers (reception volumes less than 10 $kl.d^{-1}$).

Even though groups 3 and 4 differed with regard to milk reception volumes, group 4 respondents indicated the production of only 4 dairy products, namely pasteurized milk, pasteurized cream, yoghurt and fruit juice blends.

Twenty-five respondents were grouped in this category, with milk reception volumes varying from 200 to 9 000 $l.d^{-1}$. Many of these respondents (10) receive milk in cans which are then washed on site. Furthermore, most respondents indicated substantial product losses ranging between 0.6 and 6.25%.

The water usage of the respondents of this group varied from 400 $l.d^{-1}$ to 20 $kl.d^{-1}$. Not one of these respondents indicated the pollution value of their effluents. For the group as a whole, the total reported effluent-related expenditure amounted to only R 9 200.

Support for the intended project

The interest expressed by the respondents in the intended project and their interest in a proposed seminar to be held on this subject are summarized in Table 3.2. Table 3.2 also shows which of the respondents are interested in having their factory's effluents analyzed.

Table 3.1 Management awareness of effluent volumes, pollution values and costs of effluent disposal as indicated in a postal survey representing 70 % of all milk received in South Africa

	Group* 1	Group 2	Group 3	Group 4
Number of respondents	17	10	21	25
Effluent volumes				
Awareness (%)**	100	90.0	81.0	80.0
Minimum ($kl.d^{-1}$)	2.5	44	0.25	0.04
Maximum ($kl.d^{-1}$)	519	424	3 000	20
Average ($kl.d^{-1}$)	134	212	401	3.5
Pollution values (chemical oxygen demand)				
Awareness (%)**	23.5	50.0	19.0	0
Minimum ($mg.l^{-1}$)	1 000	641	1 000	-
Maximum ($mg.l^{-1}$)	2 100	9 700	7 000	-
Average ($mg.l^{-1}$)	1 360	3 500	3 400	-
Disposal costs				
Awareness (%)**	35.3	60.0	61.9	16.0
Minimum ($R.a^{-1}$)	5 000	6 000	900	84
Maximum ($R.a^{-1}$)	8 000	60 000	300 000	8 000
Average ($R.a^{-1}$)	28 000	34 600	91 000	2 300

* Group 1: Cheese manufacturers; Group 2: Milk powder manufacturers; Group 3: Large fresh milk producers; Group 4: Small fresh milk producers.

** Number of group members, who indicated effluent volume, effluent COD and effluent disposal cost, expressed as a percentage of the total for each group.

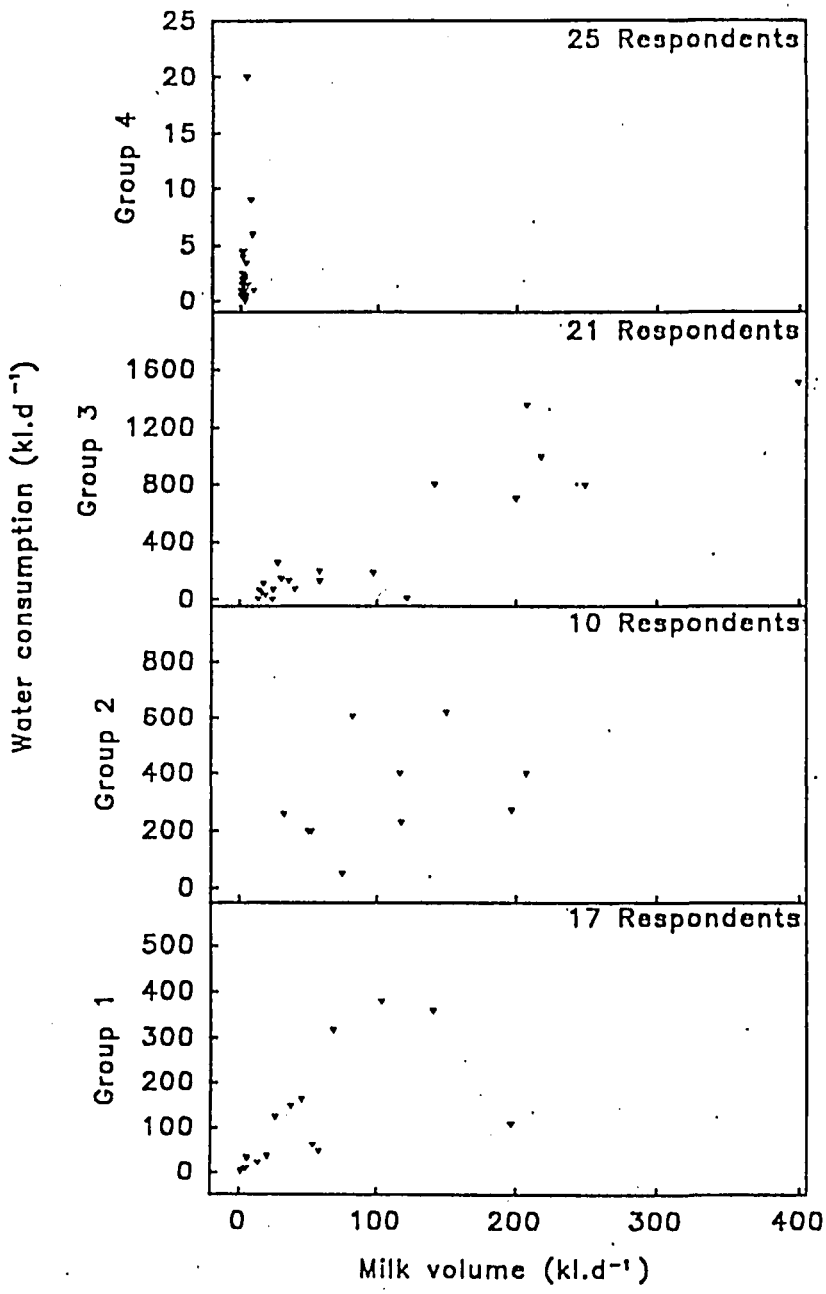

Figure 3.1 Comparison between the water consumption and raw milk volume ratios (v/v) of the four groups. (Group 1: Cheese manufacturers; Group 2: Milk powder manufacturers; Group 3: Large fresh milk producers; Group 4: Small fresh milk producers. ▼ = Individual respondent)

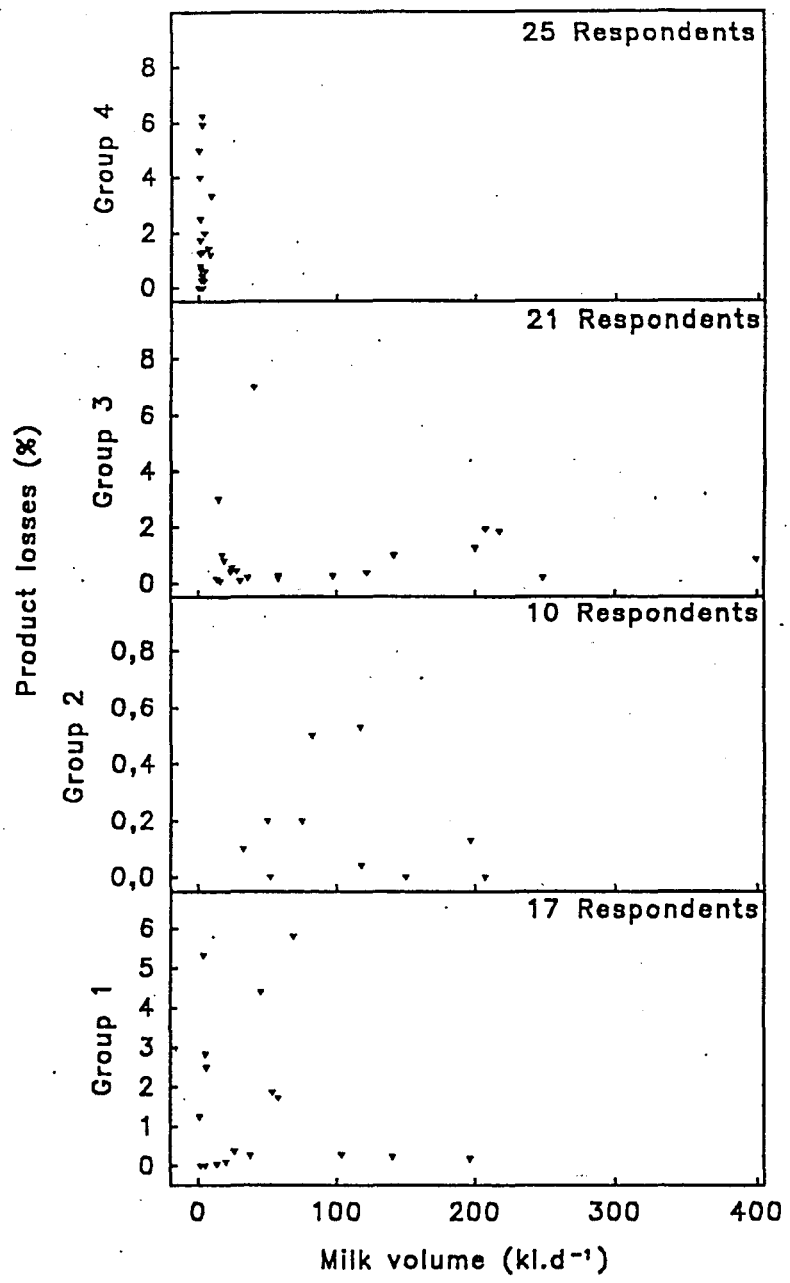

Figure 3.2 Product losses expressed as a percentage of the raw milk volume received. (Group 1: Cheese manufacturers; Group 2: Milk powder manufacturers; Group 3: Large fresh milk producers; Group 4: Small fresh milk producers. ▼ = Individual respondent)

3.5 Discussion

The frankness and co-operation of the respondents to the postal survey is indeed to the credit of the local dairy industry, bearing in mind the sensitivity of the subject. Even though only 81 of the 247 questionnaires were returned, the response is still significant since it represents 70% of all milk received and processed in South Africa. The results obtained from this survey give valuable insights into the situation of the South African dairy industry. It also highlighted several problems encountered by the industry. These include product losses, water usage, and effluent disposal.

Overall results

By dividing the respondents into 4 groups, data interpretation was simplified but certain important results are still obscured. These include the high cost of effluent disposal, an item on which the respondents annually spend R 1.5 million. Since this is only representative of 30% of the registered milk buyers, the total amount spent on effluent disposal by the dairy industry in South Africa would of course be much higher.

Moreover, by dividing the respondents into 4 groups, one or more groups might be singled out artificially. The four-group division, as presented in Table 3.1 representing 70% of the milk produced in South Africa, shows that the smaller fresh milk factories are not well informed on their factories' effluent situation and impact. However, when the data are presented differently (Figs. 3.1 and 3.2), it is obvious that equally alarming tendencies, such as high product losses and excessive water usage, are found, not only among the smaller factories but also at some of the large dairy factories. When the data are examined closely, ideal product loss and water usage values are obvious among some of the smaller dairy operations. However, as also found in the NATSURV 4 survey, larger dairies are generally more efficient in their water management than smaller ones although it is not only the smaller dairies who need to review their water management practices.

NATSURV 4 survey

In 1989 the Water Research Commission published the results of the "National Industrial Water and Waste-water Survey" on the water and waste-water management in the dairy industry (Water Research Commission, 1989), the fourth in the NATSURV series. This survey, representing 19 dairies, summarized the major steps involved in the production of the various milk products, the water intake, effluent and solid wastes produced by the South African dairy industry. In this survey several conclusions and recommendations were also made in terms of the water intake, as well as potential methods of reducing water intake, effluent volume and effluent load. Several recommendations were also made concerning effluent treatment and potential future research.

Table 3.2 The positive interest (as % of respondents) expressed by the South African dairy industry in terms of research and training options.

	Group *1	Group 2	Group 3	Group 4
Proposed project	59 %	50 %	60 %	32 %
Proposed seminar	65 %	90 %	55 %	24 %
Effluent analyses	35 %	20 %	35 %	24 %

* Group 1: Cheese manufacturers; Group 2: Milk powder manufacturers; Group 3: Large fresh milk producers; Group 4: Small fresh milk producers.

Table 3.3 Comparison of current data with data from the NATSURV 4 survey

	Current survey	NATSURV 4 survey
Survey year	1991	1986
Number of dairies surveyed	73	19
Total number of dairies	247	150 +
Total water consumption of respondents	3 700 000 $m^3.a^{-1}$*	4 500 000 $m^3.a^{-1}$
Milk volume represented by respondents**	70 %	53 %
Water intake : raw milk ratio (v/v)	0.01 – 9.5	1.4 – 9.5
Water intake emerging as effluent	13 % - 96 %	75 % - 95 %

* Assuming 22 workdays per month
** Calculated from 1990 Dairy Board figures

A comparison of the NATSURV 4 survey data and the data from this study is summarized in Table 3.3. The dairies which took part in the NATSURV 4 survey represented 53% of the raw milk produced in 1986. In contrast, the current survey represents 70% of the raw milk produced in 1990. However, the respondents to the current survey only make up 30% of the total number of dairies. This indicates that in both surveys a small number of very large dairies accounts for the vast majority of milk processed in South Africa. From Fig. 3.1, it is seen that only 14 of the 81 respondents receive more than 100 $kl.d^{-1}$ raw milk.

Further comparison between the current data and the NATSURV 4 data reveals an impossibly wide range (0.01 to 9.5) of specific water consumption values on the part of the current survey. This is probably due to an underestimation of water usage on the part of certain individual respondents. The diversity of the current survey's results

indicates doubts about the accuracy of the data submitted by the dairies. This must also be taken into consideration when comparing the current results to the NATSURV 4 survey. It also appears as if the effluent volumes are underestimated by some respondents in the current survey. It is, for example, highly unlikely that only 13% of a dairy factory's water usage would end up as effluent, as this means that the remaining 87% of the water consumed by the factory is either lost through evaporation or ends up in the final product sold to the public. It is however, possible that ice-cream manufacturers may have substantially lower effluent volume to water consumption values, since water is included in their final product. However, no ice-cream manufacturers were included in this survey since milk powder, instead of fresh milk, is used for the production of ice-cream. The NATSURV 4 survey did include ice-cream factories, and this might explain the wide reported range of water usage emerging as effluent.

Water consumption

The specific water consumption of a dairy factory is the amount of water used to process one liter of raw milk. In the literature, amounts are quoted ranging from 0.5 to 20 l per kg of milk processed, but according to Hiddink (1990) an amount of 0.5 to 3.0 l is generally acceptable. Recommendations on the specific water consumption of a factory in the NATSURV 4 survey (Water Research Commission, 1989), vary according to the type of product manufactured. In Fig. 3.1 the specific water consumption of the respondents from this survey is illustrated. Compared to the recommendations from the NATSURV 4 survey, the local dairy industry consumes excessive water. The target values in the NATSURV 4 survey vary from 1.1 l for milk packaged in sachets, to 6.3 l for cultured products. A value of 20 m^3 water per ton of cheese produced, is also recommended, and assuming a cheese yield of 10%, this translates to 2.0 l water per liter of milk used for cheese production. The high water consumption may explain why the local effluents have COD values lower than the average COD values reported by Jones (1974) for the USA. However, many respondents from this survey appear to have a very low specific water consumption value, and in several instances the indicated values are unrealistically low. This is either due to a misunderstanding or due to a deliberately low indication of their water consumption.

Product losses

Considering the reported product losses, it is obvious from Fig. 3.2 that the larger milk processors and factories appear to control product losses more successfully. Product losses should range between 0.5 to 2.0% (Hiddink, 1990), but many respondents reported losses of more than 5% and even as high as 7%. It must be taken into consideration that, should a reported product loss be a deliberate underestimation on the part of an individual respondent, that particular value will compare favourably with the rest of the data.

Clearly, the topic of product losses remains a sensitive one, since 39 respondents either failed or refused to state the product losses, or reported zero losses or losses below 0.5%. It is interesting to note from Fig. 3.2 that respondents from Group 2 reported very low and very similar product loss values. Since this group consists solely of milk powder manufacturers, these low values may be explained by

the advanced technology and high automation levels involved in large-scale milk powder manufacturing.

Water management

Due to the international tendency towards increased dairy plant sizes, effluents emanating from any single large-scale operation, will show corresponding increases in volume. From Figs. 3.1 and 3.2, it is evident that many dairies can benefit from better water management and product loss control. This will result in immediate savings especially where effluent is disposed, at high cost, into municipal sewers. Where effluents are used to irrigate pastures or lands, improved product-loss control will lessen the negative impact on soil condition. Even though dairy-generated effluents have some value as fertilizers and also do not contain serious toxic substances, land application is unacceptable since complexing agents and detergents are able to mobilize heavy metals in the soil and ground water (Hiddink, 1990).

The Presidents Council (Republic of South Africa, 1991) published an extensive report, with suggestions and recommendations on a national environmental management system. This environmental management system will have ecological, economic, social and legal implications. It is important to realize that the report reflects intended Government policy regarding the management of the environment and may soon find its way to actual legislation. A significant observation made is that in many parts of South Africa the re-use of water-borne effluents will become increasingly important. It is thus important that all industrial water users, not only in the food industry, should determine the true scope of their effluent situation.

3.6 Conclusions

Environmental problems are getting more and more attention world-wide. Though the dairy industry is not known as an industry causing severe environmental problems, it should nonetheless consider its environmental impact.

This postal survey has contributed to a better understanding of the effluent production and disposal in the South African dairy industry. Compared to the NATSURV 4 survey (Water Research Commission, 1989) where the emphasis was on industrial water consumption, this postal survey with emphasis on effluent production and disposal, covers more dairies and a greater proportion of the milk volume produced in the country. In the current survey, the dairy industry had the opportunity to assess the situation in their own factories, whereas the NATSURV 4 survey was personally conducted on-site by the surveying team.

It can also be concluded that the smaller dairies are experiencing less trouble with regard to effluent disposal than the bigger milk processors and factories. The disposal of effluent into municipal treatment systems is expensive, especially for the bigger dairies. High levies are not necessarily an indication of poor water management techniques, although it is clear that many dairies can benefit from improved water management techniques. Improving a factory's water management implies improved staff training, especially regarding attitudes towards efficient water use, water conservation and effluent treatment and management.

Considering that the respondents to this postal survey represent a significant portion of the milk processed in South Africa, it can be concluded that the dairy industry is optimistic in terms of pollution management research and training options. This optimism is also reflected by the high percentage of respondents seeking more

information on the subject and that many indicated that they would welcome a seminar on effluent management and treatment. This can be seen as a positive response and the need for more information must be met.

CHAPTER 4

ANAEROBIC TREATMENT OF A SYNTHETIC DAIRY EFFLUENT USING A HYBRID DIGESTER

4.1 Summary

A mesophylic laboratory-scale hybrid anaerobic digester, combining an upflow sludge blanket and a fixed-bed design, was evaluated for the anaerobic treatment of a synthetic dairy effluent. In the first experimental study, the chemical oxygen demand of the dairy effluent was increased stepwise from 3 700 to 10 300 $mg.l^{-1}$. In the second experimental study the COD of the synthetic dairy effluent was kept constant at 10 000 $mg.l^{-1}$ and the hydraulic retention time was shortened stepwise from 4.1 to 1.7 d. A COD removal of between 90 and 97 % was achieved at organic loading rates of between 0.82 and 6.11 kg $COD.m^{-3}.d^{-1}$. At an HRT of 1.7d, the digester achieved a methane yield of 0.354 m^3 CH_4 per kg $COD_{removed}$. The best results in terms of methane yield were at an HRT of 1.9 d. The data also showed that the maximum operational potential of the digester had been reached, as indicated by the drop in methane yield observed at the end of the second experimental study. The results clearly show that this particular type of digester would be suitable for the anaerobic treatment of dairy effluents. An important consequence of the data from this study is that a two-phase set-up will be required to protect the methanogens in the digester from inhibitively low pH values and high concentrations of VFAs produced during the acidogenic phase. The two-phase system will allow pH control in the acidogenic phase, should it be needed at full-scale or pilot-scale treatment plant.

4.2 Introduction

Water management in the dairy industry is well documented (Jones, 1974; Water Research Commission, 1989), but effluent production and disposal remain a problematic issue for the dairy industry. A survey in 1989 (Water Research Commission, 1989) concluded that South African dairies apply either very basic or very inefficient effluent treatment procedures. Moreover, a more recent survey of the South African dairy industry (Strydom *et al.*, 1993) revealed that effluent disposal is currently the most important water-related factor where improvement is desirable. Dairy effluent disposal in South Africa usually results in one of two problems: firstly, high treatment levies are charged by local authorities for industrial effluents; and secondly, further pollution can be caused when untreated effluents are either discharged into the environment or used directly as irrigation water. For the year 1991 (Strydom *et al.*, 1993), a total of R 1.5 m on effluent disposal was spent by dairies which represents 70% of the milk processed in South Africa. The second problem of disposal to the environment was more prevalent at large dairies situated near dairy farms in rural areas, where access to adequate waste-water treatment works is not available.

To enable the dairy industry to contribute to water conservation, an efficient and cost-effective effluent treatment technology has to be developed. To this effect, anaerobic digestion offers a unique treatment option to the dairy industry. Not only does anaerobic digestion reduce the chemical oxygen demand (COD) of an effluent, but little microbial biomass is produced. The biggest advantage is energy recovery in the form of methane and up to 95% of the organic matter in a waste stream can be converted into biogas (Weber *et al.*, 1984).

Many high-rate digester designs are currently available, and some have successfully been used for the treatment of dairy effluents. Lettinga and Hulshoff-Pol (1991) reported that of the 205 full-scale upflow anaerobic sludge blanket digesters in use world-wide in 1991, six were used to treat dairy effluents. The fixed-bed digester is another high-rate digester that has been used for the treatment of dairy effluents (De Haast *et al.*, 1983). A high-rate combination design, using the UASB and the fixed-bed digester types, was developed by Guiot and Van den Berg (1984). This design was successfully used to treat landfill leachate (Myburg and Britz, 1993) and baker's yeast factory effluent (Van der Merwe and Britz, 1993). Landfill leachate and yeast effluent both have high COD concentrations and both are difficult to degrade biologically. On the other hand, dairy effluents are fairly easily biodegradable, since they consist mainly of diluted dairy products. Thus, the aim of this study was to evaluate, on laboratory-scale under mesophilic conditions, the use of the hybrid digester as an option in the treatment of a synthetic dairy effluent.

4.3 Materials and methods

Digester design

A laboratory-scale hybrid anaerobic digester (Myburg and Britz, 1993) was used (Fig. 4.1). The digester had an operational volume of 5.0 l and combined an upflow anaerobic sludge blanket and a fixed-bed digester design with a gas/solids separator at the top of the digester. This high-rate hybrid digester successfully combines advantages of both systems while avoiding their drawbacks. It also facilitates an important phenomenon of microbial community acclimation to the elevated VFA concentrations. The gas exited through the top, while the substrate was introduced into the digester at the base. The overflow of the digester emptied through a U-shaped tube to prevent atmospheric oxygen from entering the system. The temperature of the digester was maintained at 35 °C using a heating tape and an electronic control unit (Meyer *et al.*, 1985) and the digester was insulated. The volume of the biogas was determined using a manometric unit equipped with an electronically controlled counter and a gas-tight valve. The biogas volumes were corrected to standard temperature and pressure (STP).

Substrate

The synthetic dairy effluent consisted of a mixture of 20 $g.l^{-1}$ plain yoghurt and 75 $ml.l^{-1}$ cottage cheese whey. This was diluted to the required COD concentration. Initially, the substrate was supplemented with 100 $mg.l^{-1}$ urea and 100 $mg.l^{-1}$ 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).

Digester start-up

The digester was originally seeded with a mixture of sewage sludge obtained from a municipal digester, as well as rumen fluid and digester effluents from other mesophilic digesters. This was done in order to supply the digester with a diverse mesophilic microbial community. The digester was then allowed to stabilize for 48 h in order to allow the bacterial community to acclimatize and attach to the polyethylene fixed-bed.

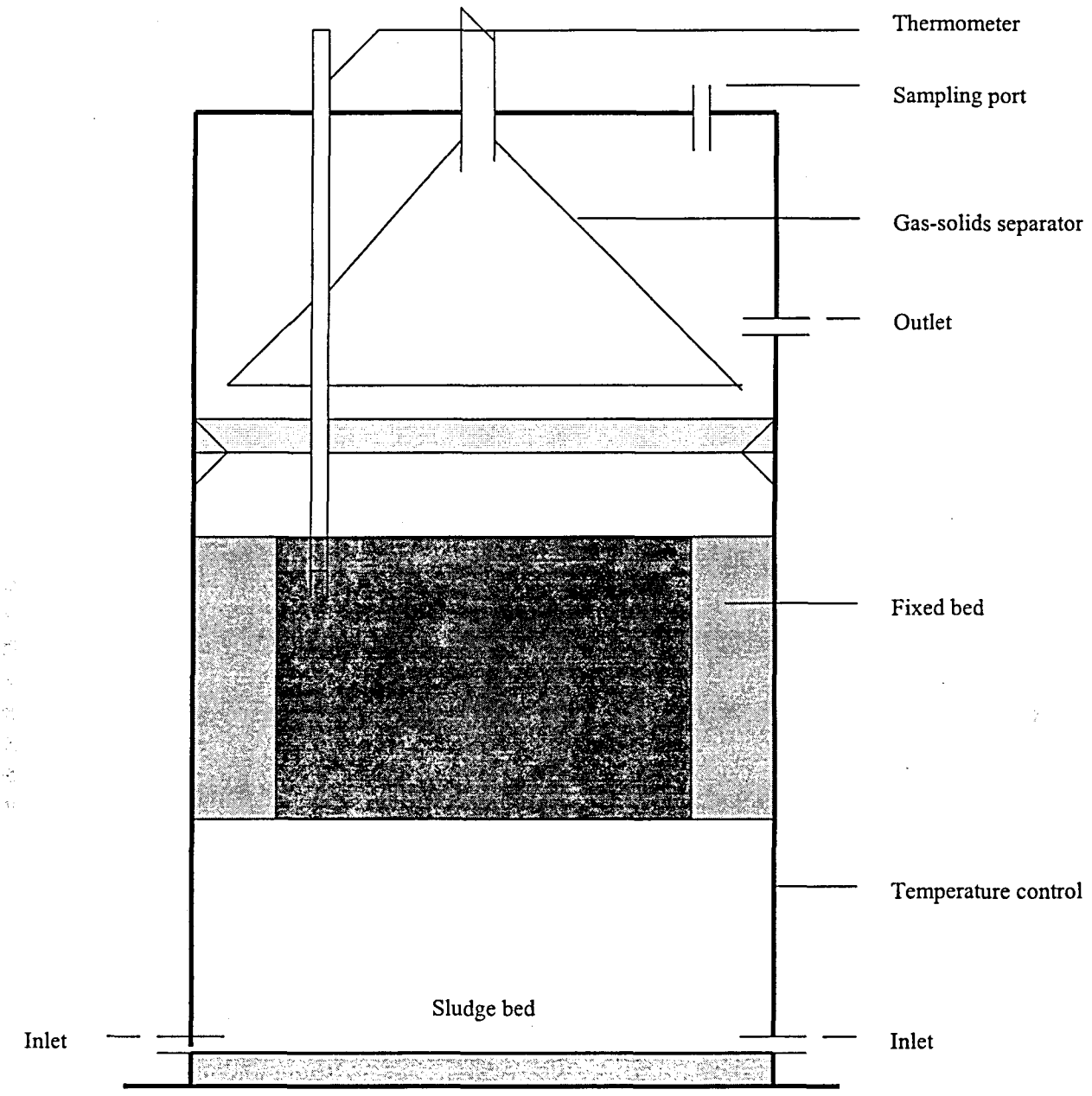

Figure 4.1 Schematic representation of the laboratory-scale anaerobic hybrid digester used in this study (after Myburg and Britz, 1993).

After the stabilization period, feeding was commenced with a diluted substrate (COD = 3 700 mg.l^{-1}). The substrate was semi-continuously fed to the digester by means of a peristaltic pump (Watson-Marlow 500) controlled by an electronic timer. After the start-up period, the HRT was set at 4.5 d.

Pre-acidification step

In order to counter persistent pH instability experienced during start-up, the feed was pre-acidified using plain yoghurt as inoculum and incubated at 30 °C for 24 h. The resultant acids were neutralized and the pH adjusted to 9.0 units with 6.0 N sodium hydroxide. This pre-acidified substrate was then fed to the digester. The use of yoghurt as inoculum was later discontinued and replaced with a *Klebsiella oxytoca* (strain A1) previously isolated from a digester treating a yeast factory effluent (Van Der Merwe and Britz, 1994).

Analytical procedures

The following parameters were analyzed according to Standard Methods (1985): pH, alkalinity, total Kjeldahl nitrogen (TKN), total solids (TS), volatile solids (VS) and total non-volatile solids (TNVS). COD and orthophosphate phosphorus (PO_4-P) were determined colorimetrically using a DR 2000 spectrophotometer (Hach Co. Loveland, CO) and standardized procedures (Standard Methods, 1985).

Volatile fatty acids (VFA) were determined using a Hewlett Packard (Avondale, PA) gas chromatograph, equipped with a flame ionization detector and a 30 m x 0.75 mm i.d. Nukol (Supelco, Inc., Avondale, PA) capillary column. The column temperature was initially held at 120 °C, then increased at a rate of 6°C.min^{-1} to 185 °C. The detector and the inlet temperatures were set at 250 °C and 160 °C respectively and nitrogen was used as carrier gas at a flow rate of 5 ml.min^{-1}.

The biogas composition was determined on a Varian 3300 gas chromatograph (Varian Ass., Walnut Creek, CA) equipped with a thermal conductivity detector and column (2.0 m x 0.3 mm i.d.) packed with Porapak Q (Waters Ass. Inc, Milford, MA), 80 - 100 mesh. The oven temperature was set at 55 °C and hydrogen was used as carrier gas at a flow rate of 40 ml.min^{-1}.

Experimental studies

The study comprised two experimental studies (I and II). In the first study (I), the synthetic dairy substrate COD concentration was increased stepwise from 3 700 to 10 300 mg.l^{-1} in five phases. In the second experimental study (II), the COD concentration was kept constant at 10 000 mg.l^{-1}, while the HRT was reduced stepwise from 4.1 to 1.7 d in nine phases. In both studies, the digester was allowed to reach stable state conditions before each HRT reduction. Stable state is defined as a state which can be maintained indefinitely without system failure (Cobb and Hill, 1990), during which the variation in digester performance parameters is less than 10%. Thus, the length of each phase was based on the stability of the digester effluent pH and the COD removal.

4.4 Results and discussion

Substrate

The average composition of the basic synthetic dairy effluent, after pre-acidification, as it was used as substrate for the digester, is shown in Table 4.1. The substrate for the first experimental study was a dilution of the substrate given in Table 4.1. The COD concentration of the substrate used in the different phases of the two experimental studies is given in Tables 4.2 and 4.3. The characteristics of the basic substrate used in the study were similar to values reported by other workers (Water Research Commission, 1989) for dairy effluents. The absence of VFA is also characteristic of dairy effluents. However, once microbial degradation of the effluent starts, the VFA concentrations will rapidly increase.

Table 4.1 Average composition of the synthetic dairy effluent substrate after pre-acidification

Parameter	Average	± s.d.
COD ($mg.l^{-1}$)	10 486	± 237
TOC ($mg.l^{-1}$)	2 090	± 49
pH	9.0	
TS ($mg.l^{-1}$)	7 240	± 400
TVS ($mg.l^{-1}$)	5 200	± 510
TNVS ($mg.l^{-1}$)	2 040	± 340
VFA ($mg.l^{-1}$)	212	
Acetic acid ($mg.l^{-1}$)	212	
Propionic acid ($mg.l^{-1}$)	0	
iso-Butyric acid ($mg.l^{-1}$)	0	
n-Butyric acid ($mg.l^{-1}$)	0	
iso-Valeric acid ($mg.l^{-1}$)	0	
n-Valeric acid ($mg.l^{-1}$)	0	
Caproic acid	0	
PO_4 * (as phosphorus, $mg.l^{-1}$)	60.6	
TKN* ($mg.l^{-1}$)	205.1	
Alkalinity (as $mg.l^{-1}$ $CaCO_3$)	612	± 86

*Mean values for three determinations

COD removal

Figure 4.2 depicts the percentage COD removal and COD removal rate (R) plotted as a function of OLR for both experimental studies (I and II). From Fig. 4.2 it is evident that the digester was operating efficiently in terms of COD removal, with the COD removal never below 90%. The best COD removal of 97% and was achieved in Phase 3 of the second experimental study (Table 4.3). The COD removal rate (R) for both experimental studies is also plotted in Fig. 4.2, as a function of the OLR. The linearity of the removal rate (Fig. 4.2) is a clear indication that the digester had not yet reached its maximum operational limit even though the final percentage COD removals show a decrease. When this limit is approached, the COD removal rate (R) normally reaches a plateau and will then start decreasing (Van Der Merwe and Britz, 1993).

Table 4.2 Operating conditions and digester performance during the first experimental study where the COD of the substrate was increased*

Parameter	Phase 1	2	3	4	5
Substrate COD ($mg.l^{-1}$)	3700	5095	6165	9200	10300
COD removal (%)	90	93	95	95	96
HRT (d)	4.5	4.5	4.5	4.5	4.5
OLR ($kgCOD.m^{-3}.d^{-1}$)	0.82	1.13	1.37	2.04	2.29
pH (digester effluent)	6.85	7.30	7.34	7.78	7.32
Biogas ($1.d^{-1}$)	n.d.	n.d.	3.32	4.25	4.71
Methane content (%)	n.d.	71	68	63	64
$Y_{CH4}(m^3.kg^{-1}\ COD_{removed})$	n.d.	n.d.	0.314	0.249	0.253
TS-in ($mg.l^{-1}$)	n.d.	3340	4390	5.820	7110
TS removal (%)	n.d.	57	63	67	68
VS-in ($mg.l^{-1}$)	n.d.	2200	2710	3.980	5660
VS removal (%)	n.d.	86	88	90	91
TNVS-in ($mg.l^{-1}$)	n.d.	1140	1680	1840	1450
TNVS removal (%)	n.d.	1.8	23	16	-22
Alkalinity-in ($mg.l^{-1}\ CaCO_3$)	n.d.	n.d.	n.d.	653	528
Alkalinity-out ($mg.l^{-1}\ CaCO_3$)	n.d.	n.d.	n.d.	1770	1793

*Data taken after stable state had been reached and mean values for three determinations
n.d. = not determined

Table 4.3 Operating conditions and digester performance during the second experimental study where the HRT was shortened*

Parameter	Phase 1	2	3	4	5	6	7	8	9
Substrate COD ($mg.l^{-1}$)	10360	10300	10800	10800	10485	10765	10220	10345	1038
COD removal (%)	96	95	97	96	94	95	95	93	92
HRT (d)	4.10	3.72	3.25	3.05	2.65	2.52	2.33	1.90	1.70
OLR ($kgCOD.m^{-3}.d^{-1}$)	2.53	2.77	3.32	3.54	3.96	4.27	4.39	5.44	6.11
pH (substrate)	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
pH (digester effluent)	7.54	7.56	7.70	7.25	7.55	7.70	7.54	7.12	7.56
Biogas ($1.d^{-1}$)	5.44	5.46	7.67	9.03	12.52	13.12	13.42	19.08	17.75
Biogas CH_4 content (%)	64	55	62	61	60	61	64	57	56
$Y_{CH4}(m^3.kg^{-1}\ COD_{removed})$	0.264	0.206	0.278	0.299	0.357	0.356	0.372	0.372	0.354
Substrate TS ($mg.l^{-1}$)	7020	6460	7560	6920	7250	7630	7540	7670	8570
TS removal (%)	67	70	68	73	70	69	70	68	76
Substrate VS ($mg.l^{-1}$)	5040	4640	5510	4400	4670	5750	5440	5690	6780
VS removal (%)	91	90	91	92	91	91	91	90	94
Substrate TNVS ($mg.l^{-1}$)	1980	1820	2050	2480	2580	1880	2100	1980	1800
TNVS removal (%)	6	19	5	35	33	-1	16	3	4
Substrate alkalinity ($mg.l^{-1}$)	562	543	640	778	560	725	583	593	633
Alkalinity-out ($mg.l^{-1}$)	1843	1718	2138	1778	2018	2213	2380	2178	2232

*Data taken after stable state had been reached and mean for three determinations

This is then indicative of insufficient digester capacity, or insufficient microbial biomass accumulation to handle the increased organic load.

The data also indicate that the digester effluent COD was not reduced sufficiently (Fig. 4.3) to allow direct disposal. In South Africa, the maximum COD concentration that is allowed for effluents which are discharged directly into rivers, is 75 $mg.l^{-1}$ (Republic of South Africa, 1962). With a COD concentration of 810 $mg.l^{-1}$ remaining in the digester effluent in the final phase of the second study, it will be necessary to use a final polishing step (Ross, 1991), to reduce the final effluent COD to an acceptable level. The lowest COD concentration achieved in the effluent, was 287 $mg.l^{-1}$ during Phase 3 of the first study. However, it must be stated that the COD values given are for total COD and not soluble COD. Thus, if a filtration or centrifugation step were to be added to the process, the microbial biomass or sludge would be removed, thereby further reducing the final effluent COD values.

pH and alkalinity

Initially, at the start of the study, the digester showed signs of pH instability, with a tendency towards pH values below 6.8 units, leading to the continuous failure of the anaerobic digestion system. As a result, a pre-fermentation step was introduced. In the first pre-fermentation step, a plain yoghurt culture was used to acidify the dairy substrate, and lactic acid was found to be the most prominent fermentation product. Since acetic acid is more desirable as a direct precursor for methanogenesis (Weber *et al.*, 1984), the inoculum for the synthetic dairy substrate used in the pre-fermentation was changed. With the use of the *Klebsiella oxytoca* (strain A1), acetic acid was produced as main fermentation product during the pre-fermentation. The pH of the substrate was then adjusted to 9.0 units after a 24 h pre-fermentation period. Although acetic acid was the main fermentation product of the *K. oxytoca* strain, the levels were comparatively low (Table 4.1). In spite of this, the pre-fermentation was still effective, reducing the pH of the effluent within 24 h at 30 °C, from 7.3 units to between 4.0 and 4.2 units (Fig. 4.4). However, as expected, the pre-fermentation continued after adjustment to pH 9.0, since the substrate was kept at room temperature while feeding. Thus, the pH of the remaining substrate was invariably much lower than 9.0 by the time it was replaced with new substrate. This was as a result of the lactose remaining in the substrate after pre-fermentation. At the end of the pre-fermentation period, only 22% of the original 1 370 $mg.l^{-1}$ lactose was fermented by the *K. oxytoca* strain. To minimize the effect of this variability, the substrate was prepared daily. However, complete conversion of a substrate during acidogenesis in a two-phase digester is not desirable (Lettinga and Hulshoff-Pol, 1991), and a maximum substrate utilization of only 20% is generally recommended.

The rapid fermentation of the effluent components necessitated the pre-fermentation step, since the methanogenic population was not adapted to the high flux of organic acids at the beginning of this study. After the pre-acidification step was introduced, the internal pH of the digester stabilized above pH 7.0 and the COD concentration could successfully be raised to 5 095 $mg.l^{-1}$ (Phase 2, first experimental study). During the remainder of the study, the pH of the digester effluent remained above 7.0 units, with one exception - at the start of the eighth phase of the second experimental study (Table 4.3) the pH of the digester effluent dropped to a low of 6.7 units, but the digester recovered and the pH stabilized again after four days at 7.12 units.

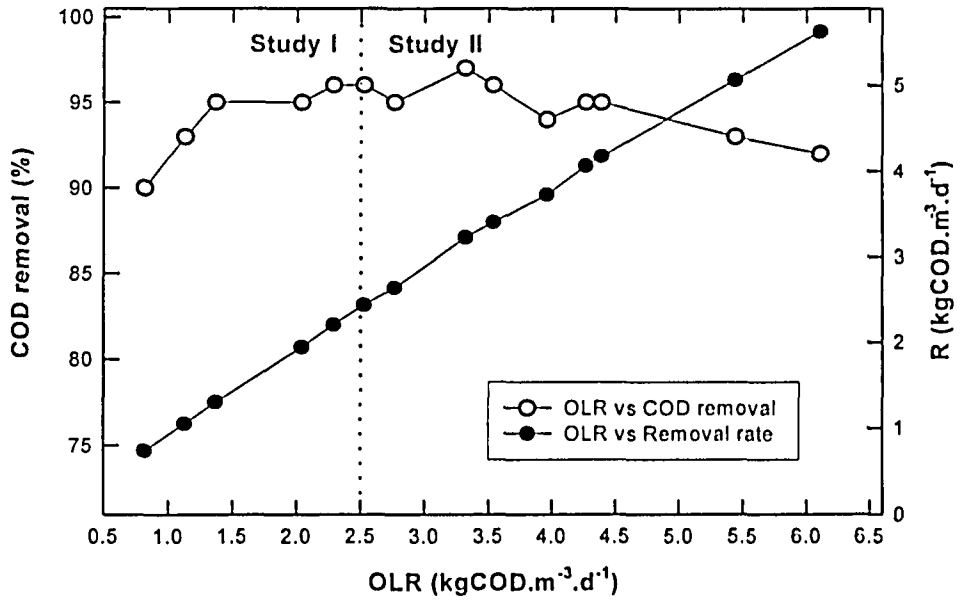

Figure 4.2 COD removal and COD removal rate (R) as a function of the OLR as found during experimental studies I and II

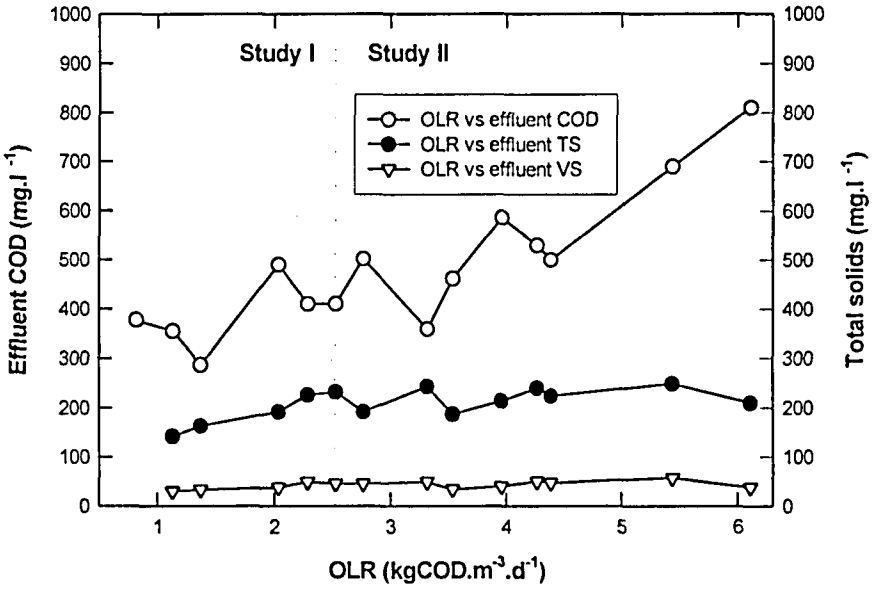

Figure 4.3 Digester effluent COD, total solids and volatile solids as a function of OLR as found during experimental studies I and II.

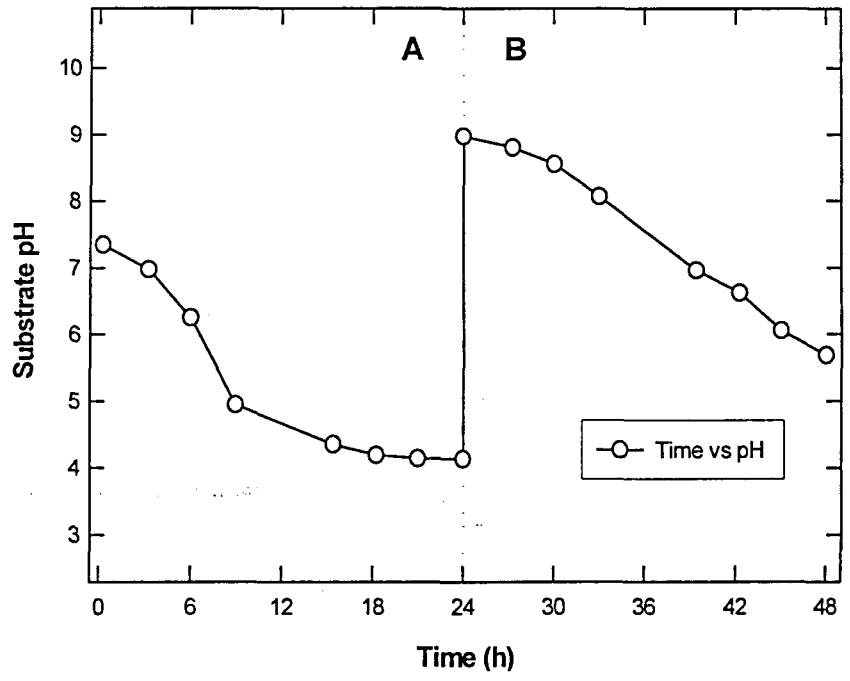

Figure 4.4 pH of the dairy substrate during the pre-fermentation period (A) and the subsequent period after neutralisation (B) to pH 9.0, at room temperature.

Thus, whenever changes are made to the environmental parameters of the digester, such as an increased OLR, the bacterial populations need time to adapt to the new conditions. During this time, the temporarily reduced pH is indicative of a less than optimal digester performance.

The alkalinity of the digester effluent throughout both experimental studies (Tables 4.2 and 4.3) showed that there was substantial buffering capacity available. This alkalinity, however, might have been due, in part, to the neutralization of the acids after the pre-fermentation step. Thus, the alkalinity might possibly be negatively affected if the amount of sodium hydroxide is reduced later on. The reduction of the amount of the neutralizing agent after the pre-fermentation, is important, in order to minimize the costs involved in the process.

Total solids, volatile solids and non-volatile solids

In Tables 4.2 and 4.3 the digester efficiency, in terms of the TS, VS and TNVS percentage removal values, is given. In Fig. 4.3 the effluent COD and the TS concentrations during both experimental studies are shown. The percentage VS removal shows a good correlation to the COD removal, with a correlation coefficient of 0.94 ± 0.004.

The TNVS concentration was reduced during the first three phases of the first experimental study, but thereafter it was found to be substantially higher. This is not

surprising, since the TNVS represent mainly minerals, which are utilized only to a very limited extent by the bacterial community in any biological system. These minerals may also have been precipitated in the digester and washed out at a later stage, thus explaining the removal efficiency of -22% which was found in Phase 5 of the first study (Table 4.2).

Volatile fatty acids

Throughout the study, the concentration of all the important fatty acids (acetic, propionic, *i*- and *n*-butyric, *i*- and *n*-valeric, and caproic acids) in the digester effluent at stable state, was below normal detection limits. This is indicative of a high methanogenic activity, and it is possible that the OLR for each phase could have been raised far more quickly without seriously affecting the operational efficiency of the digester.

Carbon:Nitrogen:Phosporus ratio

The carbon:nitrogen:phosporus (C:N:P) ratio has an important influence on digester stability and performance (Lettinga and Hulshoff-Pol, 1991). Since the optimal C:N:P ratio for anaerobic digesters is around 100:10:1, this particular effluent is very well suited to anaerobic digestion, with a C:N:P ratio of 100:9.8:2.89. This C:N:P ratio is for the substrate used in the second experimental study, without any urea or phosphate additions. During the first study the effluent was enriched with K_2HPO_4 and urea only to prevent nutrient limitations during start-up.

Biogas production and methane yield

Figure 4.5 illustrates the digester efficiency in terms of the biogas composition, as a function of the OLR. An overall decrease in the methane content of the biogas is evident for both experimental studies (Fig. 4.5). During the first experimental study, the methane yield ($Y_{CH4}/COD_{removed}$) showed a sharp decrease (Fig. 4.5). This was attributed to possible "dead space" in the digester, which was overcome during the second study where the HRT was continually shortened, resulting in a higher upflow velocity. The higher upflow velocity probably improved the mixing in the digester, thus reducing dead spaces. Thus, although the methane content of the biogas showed an overall decrease, the methane yield improved. In fact, the methane yield of the digester was slightly higher than the theoretical maximum yield of $0.35 m^3 CH_4.kg^{-1} COD_{removed}$, which can be obtained when glucose is used as carbon source.

Figure 4.6 illustrates the rate of both biogas ($m^3.d^{-1}$) and methane ($m^3.d^{-1}$) produced, plotted against the rate of COD removal rate (kg $COD_{removed}.d^{-1}$). The non-linearity of these plots is a clear indication that the digester microbial biomass was continuously adapting to the changing environmental conditions (increase in organic loading rate). However, the sudden downward trend at an OLR of 6.11 kg $COD.m^{-3}.d^{-1}$ (Phase 9 - Table 4.3) is indicative that the digester was probably reaching its maximum operational potential.

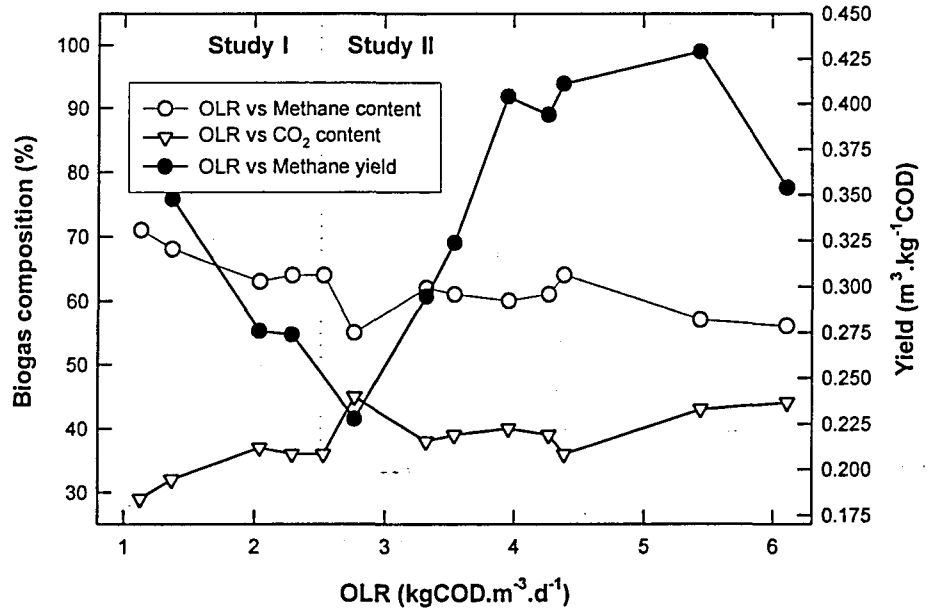

Figure 4.5 Biogas composition and methane yield per kg COD removed, as a function of the OLR as found during experimental studies I and II.

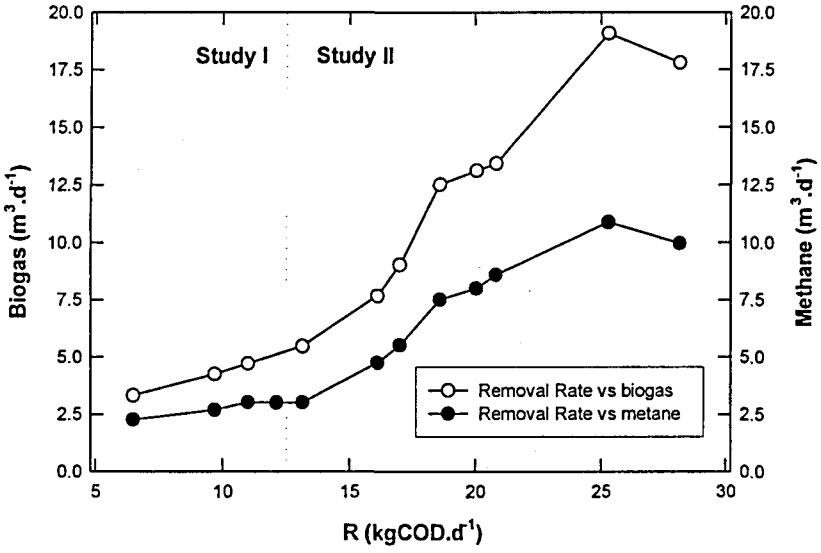

Figure 4.6 Biogas and methane production rates, as a function of the COD removal rate (R) during experimental studies I and II.

4.5 Conclusions

In conclusion, it is clear from the data obtained in this study that dairy effluents are suitable for treatment by means of the anaerobic digestion process and specifically with the use of a hybrid anaerobic digester. The anaerobically digested synthetic dairy effluent had a greatly reduced COD concentration (>90%) and a pH value around 7.2 units, making it more acceptable as a source of irrigation water or to a local water authority. Successful anaerobic digestion of dairy effluents will enable dairy companies to make a contribution to the conservation of South Africa's water resources.

The high biodegradability of the dairy effluent causes it to be easily hydrolyzed and fermented to organic acids. Thus, the rate-limiting step of the process is methane generation. The most important consequence of the data from this study is that a two-phase set-up will be required to protect the methanogens in the digester from inhibitively low pH values and high concentrations of VFAs produced during the acidogenic phase. The two-phase system will allow pH control in the acidogenic phase, should it be needed at a full-scale or pilot-scale treatment plant. Bearing in mind the extreme and often hourly fluctuation of dairy effluent quality, this is an important advantage. However, the acid neutralization requirement of the pre-fermentation step will have to be reduced. The effect of this reduction on the alkalinity and pH stability of the digester must still be evaluated. Another option is to make use of recirculation of the digester effluent, in order to utilize the high alkalinity in the digester effluent.

The economic feasibility of anaerobic treatment of dairy effluents using the hybrid digester is positively influenced by the high methane yield and the reduction in COD concentrations. Methane can possibly be utilized at dairy factories to supplement the use of coal as energy source for the generation of steam. Furthermore, the large reduction in total COD concentration should result in a reduction in effluent disposal expenditure, when the COD concentration and pH value of the digester effluent is used as basis for the calculation of trade effluent tariffs.

CHAPTER 5

TWO-PHASE ANAEROBIC DIGESTION OF THREE DIFFERENT DAIRY EFFLUENTS USING A HYBRID DIGESTER

5.1 Summary

The South African dairy industry is a major water user, and has to reconsider current effluent treatment and disposal methods. In this study, a mesophilic digester was used in conjunction with a pre-fermentation step to treat effluents from three different dairy factories which included a cheese, a fresh milk and a milk powder/butter factory. The effluents from these factories were analyzed and the chemical oxygen demand (COD), pH and effluent volumes were found to be highly variable over short time intervals. The pH was found to vary between 2.2 and 11.8 units, and the COD values ranged from 800 to 15 000 $mg.l^{-1}$ over a period of two hours. Significant differences were also found in the composition of effluents from the three factories. The average COD of effluents emerging from the three factories varied between 1 908 and 5 340 $mg.l^{-1}$. During the anaerobic treatment of these effluents using the hybrid digester, the COD of the effluents was reduced by between 91 and 97 %. The methane yield (per kg of $COD_{removed}$) varied between 73 and 91 % of the theoretical maximum yield. The data clearly indicated that anaerobic treatment of the different dairy effluents was successful. However, it was also clear that balancing tanks will be essential in full-scale treatment plants due to the high variations in effluent quality that were found over very short intervals. It was also found that the pre-fermentation step may be unnecessary when using highly diluted effluents as a digester substrate.

5.2 Introduction

Water is known to be South Africa's most limiting natural resource and the dairy industry is considered to be a major water user. In fact, the Water Research Commission (1989) estimated the total annual water usage of the South African dairy industry to be 4.5 million m^3. Generally, between 75 and 95% of this "process water" is discharged as effluent. In order to contribute to water conservation in South Africa, the dairy industry has to seriously reconsider present effluent treatment and disposal methods.

In a survey (Strydom *et al.*, 1993), it was reported that South African dairies were experiencing serious effluent-related problems. It was also found that dairies generally dispose of their effluents either to municipal sewage treatment works, or by means of irrigation onto pastures. Thus, dairy factories either run the risk of causing soil or ground water pollution, or they incur high financial obligations for the privilege of disposing effluents to nearby municipal sewage treatment works.

Anaerobic digestion, as an effluent treatment option, offers several attractive benefits to the dairy industry (Strydom *et al.*, 1995). Furthermore, successful treatment of dairy effluents not only offers pollution control in the short term, but can also serve as the starting point for the longer term development of a total water re-use biotechnology.

Since an anaerobic digester using a hybrid design was successfully used for the treatment of a synthetic dairy effluent (Strydom *et al.*, 1995), the aim of this study was to evaluate the hybrid digester as an option for the treatment of three different dairy wastewaters.

5.3 Materials and methods

Dairy Factories

Cheese factory

This factory handles up to 160 tons of milk per day for the production of various hard cheeses, notably Gouda, Edam and Cheddar. The whey produced is evaporated and transported by tanker to other factories for spray drying. The general factory effluent consists of diluted products and wash water as well as the initial rinse water from the silos and tankers. Presently, the final effluent is sprayed onto approximately 45 hectares of pasture.

Fresh milk factory

This factory produces mainly pasteurized milk, fruit juice blends, yoghurt and cottage cheese, and processes about 230 tons of milk per day. The cottage cheese whey is included in the general factory effluent. The effluent passes through a fat trap before being disposed to the local municipal sewage treatment works.

Milk powder/butter factory

The milk powder/butter factory produces up to 40 tons of butter per day. Roller dried milk powder and buttermilk are also produced by this factory. The effluent consists of highly diluted products and wash water without large quantities of buttermilk. After all possible butterfat has been reclaimed from the initial rinse water from the cream silos and tankers, the combined effluent is finally disposed of by irrigating onto pastures.

Effluents and sampling

The various dairy effluents were obtained by taking grab samples at half-hour intervals over the entire daily production cycle of each factory. The effluent flow rate, at each sampling interval, was determined by means of a bucket and stopwatch method. The resultant individual samples were then combined proportional to the flow rates, as measured for each respective interval. This resulted in flow-proportioned composite effluent samples. This is generally recommended as the most accurate method for sampling effluents of varying quality such as those produced by the dairy industry (Vernick, 1977).

Pre-acidification

The effluents from the various factories were pre-fermented using a *Klebsiella oxytoca* strain previously isolated from an anaerobic digester treating a yeast factory effluent (Van Der Merwe and Britz, 1994). The pH of the three flow-proportioned effluents were adjusted to 7.0 before inoculation with the *K. oxytoca* strain. The pH was measured at three-hour intervals, over a period of 24 hours.

Anaerobic digester

A five litre mesophilic hybrid digester, combining an up-flow anaerobic sludge blanket and a fixed-bed, was used. The digester was inoculated with digested sewage sludge, rumen fluid and effluent from two other mesophilic laboratory-scale digesters. The digester was, prior to this study, used to treat a pre-acidified synthetic dairy effluent (Strydom *et al.*, 1995), consisting of diluted whey and yoghurt, at organic loading rates of up to 6 kg $COD.m^{-3}.d^{-1}$. After several months of operation, the substrate was changed from the synthetic effluent to the actual cheese, fresh milk and milk powder/butter factory effluents. The hydraulic retention time (HRT) of the digester was reset to 1.90 days, which was found to yield the optimum results with the synthetic effluent as substrate.

Experimental phases

Effluent from the cheese factory was treated during the first phase. After stable state conditions were obtained, the substrate was changed in the second phase to fresh milk factory effluent, and again after stable state was reached, the substrate was changed in the third phase to the milk powder/butter factory effluent. Samples were taken at two-day intervals and stable state was assumed when there was less than 10% variation in results after 6 consecutive HRT's (Hill, 1991).

Chemical analyses

The following parameters were monitored, using methods described in Standard Methods (1985): pH, total solids (TS), volatile solids (VS) and non-volatile solids (NVS). COD was determined colorimetrically on a DR 2000 spectrophotometer (Hach Co. Loveland, CO) using an adapted method from Standard Methods (1985) and Merck Chemicals (E. Merck, Darmstadt).

Total volatile fatty acids (TVFA) and bicarbonate alkalinity were determined according to the five-point pH titration method of Moosbrugger *et al.* (1992). The biogas composition was determined using a Varian 3300 gas chromatograph (Varian Ass., Walnut Creek, CA) equipped with a thermal conductivity detector and column (2.0 m x 0.3 mm i.d.) packed with Porapack Q (Waters Ass. Inc, Milford, MA), 80 - 100 mesh. The oven temperature was set at 55 °C and hydrogen was used as carrier gas at a flow rate of 40 $ml.min^{-1}$. The biogas volume was determined using an electronically controlled manometer and counter. Each unit on the gas counter corresponded to 13.158 ml biogas at ambient temperature and pressure. Barometer readings were also taken, and the methane yield was then calculated using the universal gas equation, to obtain methane yield values, which are corrected for standard temperature and pressure (STP).

5.4 Results and discussion

Effluent production and composition

Effluent variability

The chemical composition and the flow rates of all three effluents were found to be highly variable over the sampling periods (Fig. 5.1). These variations were probably

due to the different manufacturing processes conducted during the different times of the various production cycles. The cleaning processes included tanker cleaning-in-place (CIP), CIP of various silo's, pipelines, plate pasteurizers and evaporators. Cheese vats and factory floors are manually washed with hoses. In all three factories, CIP caustic and acid chemicals were recycled to a large extent while initial rinse water was discharged immediately. The exception was the butter factory, where butterfat was recovered from the initial rinse water.

From the data it was also clear that there was no correlation between the three variables COD, flow rate and pH (Fig. 5.1). This was probably a direct result of the four-step CIP process (Romney, 1990; Bogh-Sorensen, 1992). With this process in mind, COD peaks could be expected to coincide with the higher pH values (up to 11.8 units) obtained after the caustic wash, since the alkaline detergent is designed to remove the bulk of the milk-soil in the equipment. The COD peaks (up to 15 000 mg.l^{-1}) which were found to coincide with low pH values (Fig. 5.1), would probably be due to either the simultaneous release of an acid-cycle rinse elsewhere in the factory, or to the initial rinse of equipment which was used to manufacture yoghurt, bulk starter or other types of low pH, fermented milk products.

Effluent composition

Results from the analyses of the three flow-proportioned effluents are summarized in Table 5.1. The three effluents were found to vary considerably in composition. Bearing in mind that between 75 and 95% of a dairy factory's initial water use (Table 5.1) ends up in the effluent (Strydom *et al.*, 1993; Water Research Commission, 1989), water usage volumes will give a reliable indication of potential effluent volume trends. A notable feature is the correlation between the specific water consumption and the average effluent COD values. The data obtained also shows that the milk powder/butter factory produced a fairly diluted effluent due to the much higher specific water consumption. It can be predicted that, should this factory's management establish tighter water-use control, the effluent COD value will approach those of the other two factories.

The predicted COD values, as given in Table 5.2, were determined by multiplying each individual sample's COD value with its corresponding flow-proportioning factor, and then adding-up all the COD values of the individual samples. The proportioning errors, as given in Tablel 5.2, show the excellent experimental accuracy during the mixing of individual samples.

Effluent treatment

The overall results for the combined pre-fermentation and anaerobic digestion treatment of all three effluents are presented in Tables 5.3, 5.4 and 5.5. The "raw effluent" columns contain results from the chemical analyses of the flow-proportioned composite effluents.

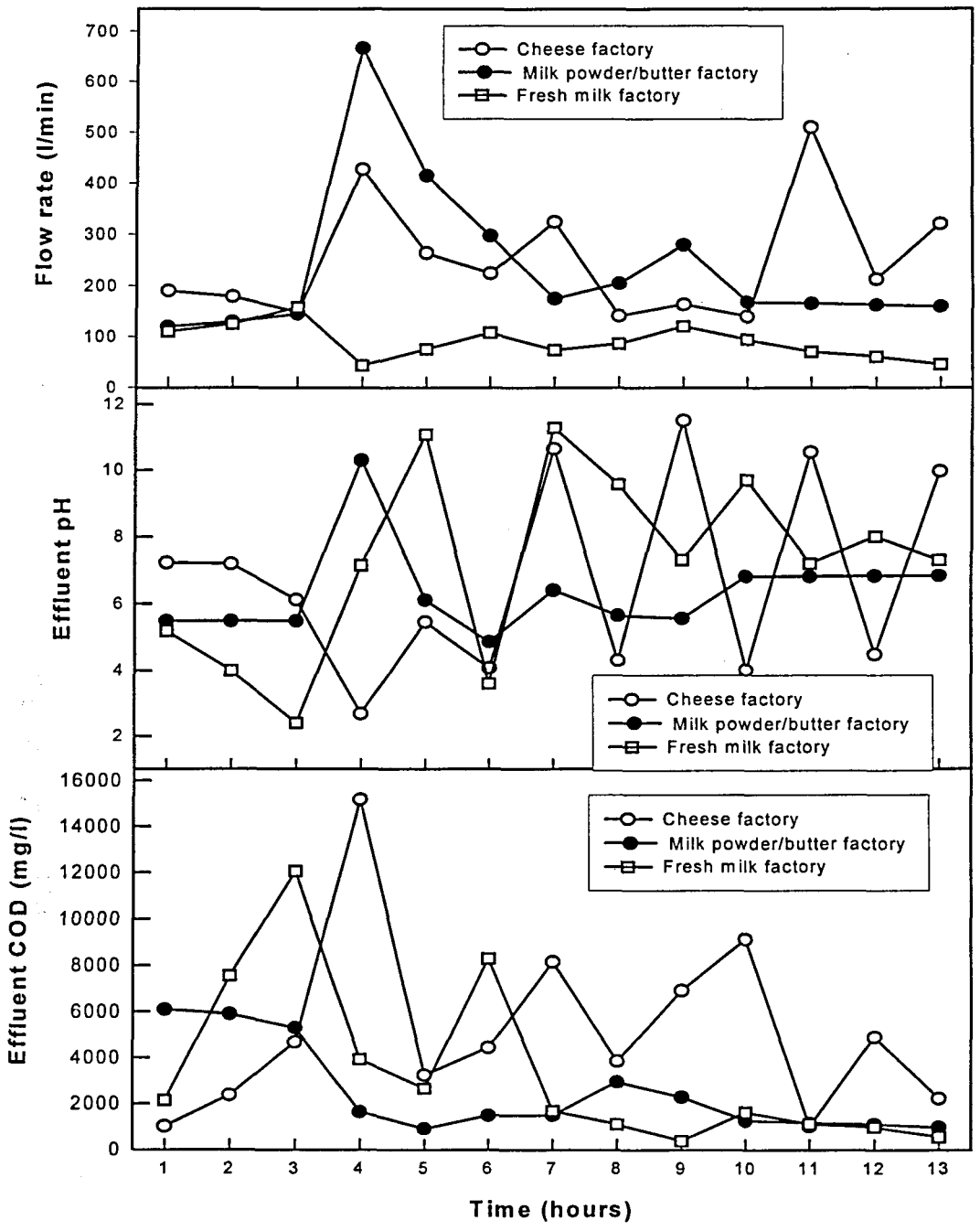

Figure 5.1 Effluent composition variations during the daily production cycles in terms of COD, pH and flow rates for a cheese factory, a fresh milk factory and a milk powder/butter factory.

Table 5.1 Comparison of flow proportioned effluents and specific water use from three different dairy factories (assuming a 22 workday month)

Parameter	Cheese factory	Fresh milk factory	Butter factory
pH	5.22	6.92	5.80
COD concentration ($mg.l^{-1}$)	5340	4656	1908
Water usage ($kl.d^{-1}$)	495	682	390
Milk intake ($kl.d^{-1}$)	168	223	86*
Specific water usage ($l.l^{-1}$)	2.94	3.06	4.54

*Figures for butter factory are in tons milk and cream

Table 5.2 Accuracy of flow-proportioning of three effluents, in terms of COD prediction

Parameter	Cheese factory	Fresh milk factory	Butter factory
Predicted COD ($mg.l^{-1}$)	5390	4735	1872
Measured COD ($mg.l^{-1}$)	5340	4656	1908
Proportioning error (%)	-0.93	-1.67	+1.92

Cheese factory effluent treatment

The overall treatment of the cheese effluent was successful. The pre-fermentation step was particularly successful with this effluent, as can be seen from the 85% increase in TVFA concentration, from 333 to 616 $mg.l^{-1}$ and subsequently, in the digester, all the TVFA's were completely utilized. The pH curve, as illustrated in Fig. 5.2, during the pre-fermentation of the cheese effluent, showed a similar pattern to that obtained by pre-fermentation of a synthetic effluent (Strydom *et al.*, 1995).

A COD reduction of 97% as well as a reduction in volatile solids of 92% were obtained. The final COD of 166 $mg.l^{-1}$ was the best COD removal achieved by this digester. This was even better than that achieved for the synthetic effluent (Strydom *et al.*, 1995). It should be pointed out that this COD level is still not low enough to allow direct disposal into a watercourse or river (Department of Water Affairs, 1986).

The treatment process was also successful in terms of pH neutralization. The raw effluent had a pH of 5.22, while the final treated effluent after the digestion step had a pH of 7.54. The alkalinity was similarly affected, being raised from 335 to 1 372 $mg.l^{-1}$.

A methane yield of 0.359 $m^3.kg^{-1}COD_{removed}$ (Table 5.3) was 90.9% of the theoretical maximum for glucose, with the biogas containing 66.6% methane.

Fresh milk factory effluent treatment

The overall result of treatment of the fresh milk effluent is presented in Table 5.4. Here, the pre-fermentation step yielded an 84% increase in TVFA concentration, to raise the TVFA concentration 200 to 368 $mg.l^{-1}$. Similarly, the overall anaerobic treatment resulted in a complete removal of TVFA's.

The COD removal (94%) was also very good for this effluent, with the final COD being 280 $mg.l^{-1}$. This does, of course, still necessitate a final polishing step before the effluent can legally be released into a river or other water body (Department of Water Affairs, 1986).

Regarding pH and alkalinity, it is clear that the standard pre-fermentation procedure developed with the synthetic effluent (Strydom *et al.*, 1995) was functioning entirely satisfactory (Fig. 5.2). The pH of the treated effluent after the digestion step was 7.82. This is, however, not unacceptably high, since the bicarbonate alkalinity, at 1 160 $mg.l^{-1}$, was slightly lower than that of the treated cheese effluent.

The methane yield was 0.327 $m^3.kg^{-1}COD_{removed}$ (Table 5.4) or 82.8% of the theoretical maximum for glucose, with a biogas methane content of 69.3%.

Powder milk/butter factory effluent treatment

The results of the combined effluent treatment for this factory's effluent are summarized in Table 5.5. The pre-fermentation of the milk powder/butter effluent, yielded an increase in TVFA's of 102%. However, the initial TVFA concentration of this effluent was so low (Table 5.5) that the pH was lowered to only 6.45 after 24 hours (Fig. 5.2). Thus, the pre-fermentation of this type of effluent is regarded as probably unnecessary, considering the results from the pre-fermentation of the other effluents. The digestion of the pre-fermented effluent was successful, with the TVFA concentration reduced to zero.

The COD removal was 91%, with an average final COD of 166 $mg.l^{-1}$. This is coincidentally, at the same level as that found for the digested cheese effluent.

The bicarbonate alkalinity of 533 $mg.l^{-1}$ (as $CaCO_3$) of this effluent was virtually unchanged by the digester treatment. The final pH of the effluent was 7.89, which was the highest final pH obtained during this study.

The biogas volume, and consequently the methane yield, was found to be low. The methane yield was 0.287 $m^3.kg^{-1}COD_{removed}$ (Table 5.5). However, the biogas was of high quality, with a methane concentration in the biogas of 80.7% (Table 5.5). This high methane content is probably due to the very low organic loading rate (Table 5.6). In a previous study (Strydom *et al.*, 1995), where a hybrid digester was used to treat synthetic dairy effluent, it appeared as if a correlation existed between organic loading rate and methane yield. This hypothesis still needs verification.

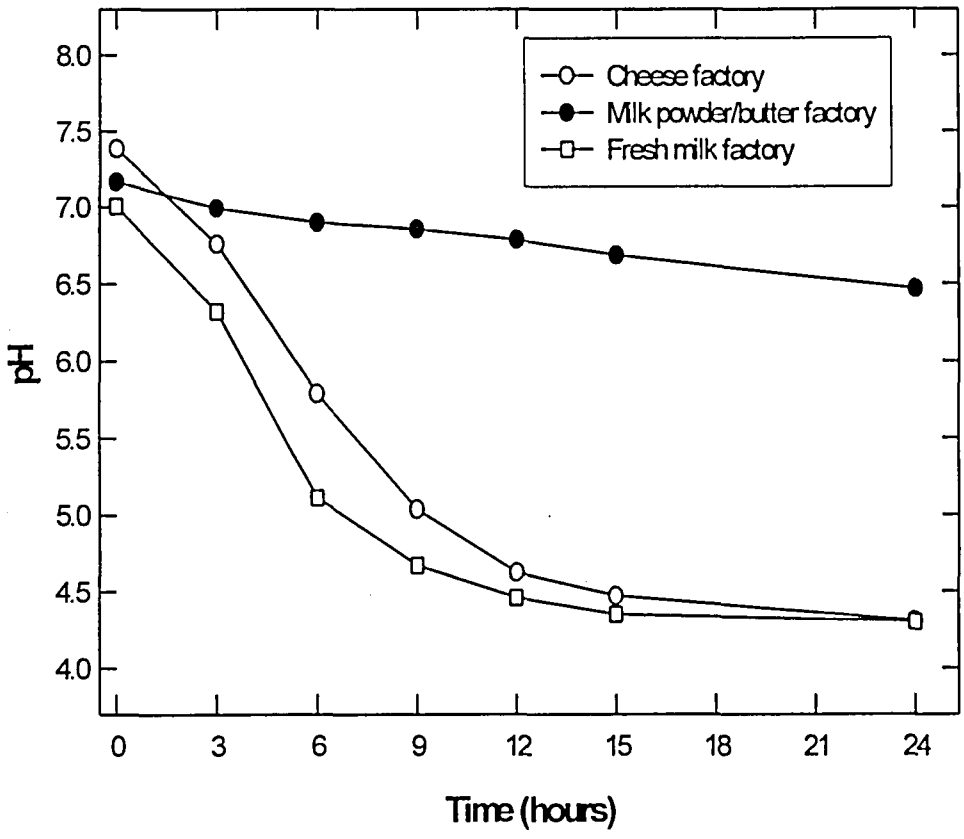

Figure 5.2 pH changes during the pre-acidification of the three raw effluents obtained from a cheese, a milk powder/butter and a fresh milk factory using *Klebsiella oxytoca* strain A1. Data are averages of duplicate runs

Table 5.3 Pre-fermentation and digester efficiency obtained during the two-stage anaerobic digestion of cheese factory effluent

Parameter	Raw effluent*	Pre-fermented effluent	Digested effluent
pH	5.22	9.00	7.54
COD($mg.l^{-1}$)	5340	5364	166
Total solids ($mg.l^{-1}$)	4210	4740	1535
Total volatile solids ($mg.l^{-1}$)	3110	3520	275
Non-volatile solids ($mg.l^{-1}$)	1100	1220	1260
Bicarbonate alkalinity ($mg.l^{-1}$)	335	355	1372
Total volatile fatty acids ($mg.l^{-1}$)**	333	616	0
Carbon dioxide (in biogas) (%)	n.a.	n.d.	33.4
Methane (in biogas) (%)	n.a.	n.d.	66.6
Methane yield ($m^3.kg^{-1}COD_{removed}$)	n.a.	n.d.	0.359

* Raw effluent = flow proportioned composite samples
** As acetic acid, determined by the five-point titration method
n.a. = not applicable
n.d. = not determined

Table 5.4 Pre-fermentation and digester efficiency obtained during the two-stage anaerobic digestion of fresh milk factory effluent

Parameter	Raw effluent*	Pre-fermented effluent	Digested effluent
pH	6.92	9.00	7.82
COD($mg.l^{-1}$)	4656	4644	280
Total solids ($mg.l^{-1}$)	2750	2850	1270
Total volatile solids ($mg.l^{-1}$)	2425	2120	365
Non-volatile solids ($mg.l^{-1}$)	325	730	965
Bicarbonate alkalinity ($mg.l^{-1}$)	546	193	1160
Total volatile fatty acids ($mg.l^{-1}$)**	200	368	0
Carbon dioxide (in biogas) (%)	n.a.	n.d.	30.7
Methane (in biogas) (%)	n.a.	n.d.	69.3
Methane yield ($m^3.kg^{-1}COD_{removed}$)	n.a.	n.d.	0.327

* Raw effluent = flow proportioned composite samples
** As acetic acid, determined by the five-point titration method
n.a. = not applicable
n.d. = not determined

Table 5.5 Pre-fermentation and digester efficiency obtained during the two-stage anaerobic digestion of butter factory effluent

Parameter	Raw effluent*	Pre-fermented effluent	Digested effluent
pH	5.80	9.00	7.89
COD($mg.l^{-1}$)	1908	1836	166
Total solids ($mg.l^{-1}$)	1720	2060	1450
Total volatile solids ($mg.l^{-1}$)	860	830	300
Non-volatile solids ($mg.l^{-1}$)	860	1230	1150
Bicarbonate alkalinity ($mg.l^{-1}$)	532	239	553
Total volatile fatty acids ($mg.l^{-1}$)**	137	278	0
Carbon dioxide (in biogas) (%)	n.a.	n.d.	19.3
Methane (in biogas) (%)	n.a.	n.d.	80.7
Methane yield ($m^3.kg^{-1}COD_{removed}$)	n.a.	n.d.	0.287

* Raw effluent = flow proportioned composite samples
** As acetic acid, determined by the five-point titration method
n.a. = not applicable
n.d. = not determined

Table 5.6 Comparison of results in terms of COD removal and methane yield

Parameter	Dairy factory		
	1	2	3
OLR (kg $COD.m^{-3}.d^{-1}$)	2.82	2.44	0.97
COD removal (%)	97	94	91
Y_{CH4} (% of the theoretical maximum)	90.9	82.8	72.7

* Dairy 1 = cheese factory, 2 = fresh milk factory, 3 = butter factory

5.5 Conclusions

The data from this study clearly shows that dairy effluents are highly variable in a two-fold way. Firstly, there are significant differences between average effluents from different types of factories. This is evident both from the results of the effluent analyses and the different effects the pre-fermentation has had on each specific effluent. Secondly, individual factories produce effluents, which display wide fluctuations in pH, flow rate and COD over very short time intervals. These variations are almost unpredictable at any given large factory, due to the complexities of the CIP processes in the factory. Considering the high variability of dairy effluent quality over such short time intervals, balancing tanks will be essential for the stable supply of substrate to any form of biological treatment system.

The pre-fermentation steps of the effluent from the cheese factory and the fresh milk/mixed product factories were successful. This simply means that these effluents were suitable as substrate for the particular strain of *Klebsiella oxytoca* which was used for acidogenesis. However, the pre-fermentation step of the milk powder/butter factory effluent was not as successful. This is attributed to the lack of fermentable sugars in

the milk powder/butter factory effluent. The small amounts of whey in the effluents, might explain the success of the pre-fermentation step, in the case of the cheese and the fresh milk/mixed product factories. This also underlines the fact that each type of factory produces an unique effluent.

The implications from the findings in this study are, that the pre-fermentation step might be unnecessary for the milk powder/butter factory effluent. Where a full-scale treatment facility is concerned, this will result in substantial savings in capital expenditure. Secondly, further research will have to include assessing the necessity of pre-fermentation for each type of effluent. Thirdly, it was seen that some factories have an excessively high specific water use. Proper water management in a dairy factory will reduce the water use, and therefore increase the effluent COD. This must be taken into consideration when a factory contemplates the installation of a water treatment facility. A higher COD concentration will, as mentioned earlier, increase the OLR and therefore, probably also the methane yield. Therefore, an evaluation and subsequent optimization of the specific water use must be conducted, before any dairy contemplates the treatment of its effluent.

Finally, the results from this study also show that the hybrid anaerobic digester is eminently suitable for the treatment of dairy factory wastewaters. When compared to results obtained with other types of digesters, as published in the literature, the hybrid digester is surpassed only by the anaerobic/aerobic lagoon combination (Anon., 1990), which removed 99% of the COD from a dairy effluent. This is due to the fact that the aerobic lagoon is capable of removing phosphorus and nitrogen. However, the hybrid digester requires much less space and its temperature is easier to control. As an option for the on-site treatment of dairy factory effluents, the hybrid digester offers a compact design and a stable treatment process. The problem of effluent variability can be resolved by using a balancing tank, and pilot-scale research should focus on the possibility of combining such a balancing tank with the pre-fermentation tank, in one single container.

CHAPTER 6

OPTIMIZATION OF ACIDOGENESIS OF DAIRY EFFLUENTS USING A SPECIALLY SELECTED *PEPTOSTREPTOCOCCUS PRODUCTUS* STRAIN

6.1 Summary

The optimization of acidogenesis of a synthetic dairy effluent was investigated in this study, using a continually operated mixed-culture acidogenic bioreactor. A population study was conducted and 47 isolates were obtained. These isolates were characterized and found to be very similar in terms of biochemical characteristics. By using both the Jaccard and the Sokal and Michener coefficient, a percentage similarity of above 80 % was obtained for 93 % of the isolates. The isolate with the highest volumetric lactic acid productivity (Q_p(max)), was subsequently identified. Three isolates had Q_p(max) values below 10.0g.l^{-1}.d^{-1}, while two isolates had Q_p(max) values above 30.0g.l^{-1}.d^{-1}. The dominant isolate, both in terms of Q_p and total counts in the mixed culture, was *Peptostreptococcus productus.* The strain (F06) with the highest Q_p(max) was subjected to a factorial design experiment, to determine the optimal levels of the factors temperature, COD and pH. With the exception of pH level, the optimal operational parameters as found in the factorial design, closely correlated with the levels as used in the acidogenic bioreactor. This underlines the highly selective pressure exerted by a chemostat on a bacterial population. In this study, an optimization procedure was developed which can possibly be used for the microbial and initial operational optimization of acidogenesis, even in existing full-scale acidogenic bioreactors. The optimal values of the vairous operational parameters must be verified and possibly adapted, when the pure cultures obtained in this study, are used for pilot or full-scale acidogenesis. However, the results obtained during the process optimization in this study will facilitate start-up and initial operation of such a full-scale acidogenic bioreactor. The use of a known isolate has yielded the unexpected bonus of odour control. Similar advantages, such as the elimination of unknown and possibly pathogenic bacteria are also implied.

6.2 Introduction

Effluent generated by the dairy industry in South Africa results either in high municipal levies when effluent is disposed to local sewage treatment works, or in a potentially polluting situation when untreated effluent is disposed to the environment (Strydom *et al.* 1993). Anaerobic digestion, as an option for dairy effluent treatment, was recently investigated (Strydom *et al.*, 1995) and shown to present a solution to both the above problems currently associated with dairy effluents.

Research (De Haast *et al.*, 1986; Kisaalita *et al.*, 1987 and 1990; Strydom *et al.*, 1995) has shown that the spatial separation of the acidogenic and methanogenic phases is essential when high strength, but easily degraded effluents such as dairy effluents are used as substrates. This spatial separation prevents rapid acidification of the digester contents, subsequent pH inhibition of the methanogenic population and ultimate digester failure. Due to the high levels of easily hydrolyzable lactose and milk proteins present in dairy effluents, pre-acidification is considered a pre-requisite for the successful high rate anaerobic digestion.

The two-phase anaerobic digestion of dairy effluents has been studied in the past by several workers. Burgess (1985) used a balancing tank simultaneously with an

acidogenic reactor for the acidification of a dairy effluent. No inoculum was used with the temperature merely being kept in the mesophilic range. This, together with a retention time of 24 hours, allowed the effluent to acidify spontaneously, after which it was fed to a conventional fixed-film anaerobic digester. In contrast, Kisaalita *et al.* (1987) used a heterogeneous inoculum, in this case digested sewage sludge, to start a continuous-flow acidogenic reactor. These workers, however, studied the kinetics of the lactose conversion process and not the microbial population which developed in the acidogenic reactor. De Haast *et al.* (1986) used undefined commercial lactic starter cultures for the batch acidogenesis of whey in a two-phase anaerobic treatment system. During previous work (Strydom *et al.*, 1995; Strydom *et al.*, 1997), a *Klebsiella oxytoca* strain was used in batch mode for the pre-fermentation of a synthetic effluent as well as three different dairy effluents. However, this specific strain of *K. oxytoca* (A1) was obtained during the microbial characterization of a yeast factory effluent (Van Der Merwe and Britz, 1994). In spite of this, the use of this particular *K. oxytoca* strain successfully facilitated a COD removal of up to 97% during two-phase anaerobic digestion of dairy effluents (Strydom *et al.*, 1995 and 1997).

The rigorous selection of specific microbial strains for the acidogenesis of "difficult" effluents, such as those produced by the bakers' yeast industry, has greatly improved the overall efficiency of the anaerobic digestion process (Van Der Merwe and Britz, 1994). In contrast to this approach, and as was shown by previous work (Burgess, 1985; De Haast *et al.*, 1986; Kissalita *et al.*, 1989; Strydom *et al.*, 1997), virtually any undefined, heterogeneous mix of bacteria can eventually produce satisfactory levels of organic acids from the lactose and milk proteins found in dairy effluents. The result of these circumstances is that continuous pre-acidification has never been studied with microbial strains selected specifically to optimize acidogenesis during two-phase anaerobic treatment of dairy effluents.

An optimized acidogenic process, utilizing specially selected microbes, offers the advantages that the optimum combination of operational parameters such as temperature, hydraulic retention time, pH and other process conditions, are known. Furthermore, the reactions of the system in response to overload shocks can be established beforehand and most importantly, the end products obtained during acidogenesis are known and controllable. Therefore, the aim of this study was to optimize the continuous acidogenesis of dairy effluents, firstly, by studying the microbial population in a continuously operated acidogenic reactor. Secondly, the aim was to select the most suitable acidogenic microbial strain, on the basis of product formation during the exponential growth phase in batch cultures and thirdly, to determine the optimum operational parameters using a factorial design experiment.

6.3 Materials and methods

General experimental layout

A synthetic dairy effluent (Strydom *et al.*, 1995) was used as substrate to continuously feed an acidogenic reactor. This bioreactor was temperature and pH controlled, and was inoculated with an undefined heterogeneous mix of bacteria. After six successive hydraulic retention times, a population study was conducted. The isolates were subsequently compared with each other in batch mode, and the strain with the highest volumetric acid production rate was identified. Finally, the optimum operational parameters of acidogenesis using this isolate was determined, using a factorial design experiment.

Acidogenic bioreactor

A New-Brunswick Scientific modular fermenter with a volume of 5.36 l was used. The temperature was set at 30 °C ± 0.5 °, and the pH was controlled to 6.0 units (Kissalita *et al.*, 1989) by means of a Digital Data Systems PHM302 pH meter/titrator equipped with a Radiometer combination electrode. The contents of the fermenter was stirred at 500 r.p.m. and fresh, sterile substrate was added by means of a Watson Marlow Du 505 peristaltic pump. An hydraulic retention time (HRT) of 20 hours was chosen, corresponding to a continuous feed rate of 4.46 $ml.min^{-1}$.

The substrate used in this study was a synthetic substrate which was found to be highly representative of dairy effluents in general (Strydom *et al.*, 1995; Strydom *et al.*, 1997). It consisted of unflavoured yoghurt (12.8 $g.l^{-1}$) and whole whey powder (2.2 $g.l^{-1}$), diluted with ordinary tap water.

Bioreactor inoculum

The acidogenic bioreactor as described above, was inoculated with 50 ml of effluent collected from a quiescent area in a dairy factory fat trap and 50 ml of final effluent from a two-phase anaerobic digester treating a synthetic dairy effluent (Strydom *et al.*, 1995). The acidogenic bioreactor was then left to reach stable state, which was assumed after total viable plate counts showed less than ten percent variation over six successive hydraulic retention times.

Population study

When stable state had been reached, 1.00 ml of a $1x10^{-7}$ dilution of the acidogenic reactor liquor was plated onto plate count agar (Biolab Diagnostics) containing an added 5.0 $g.l^{-1}$ whey powder (Table 6.1). From the highest dilution agar plates, 47 colonies were randomly selected using Harrison's disk (Harrigan and McCance, 1976). The first series of isolates (29 in total) were incubated aerobically while the second series of isolates (the remaining 18) were incubated under facultative conditions created by using the Gaspak (Oxoid) system. The isolates were then repeatedly streaked onto agar plates until pure cultures, verified by means of light microscopy, were obtained.

The isolates were characterized using the Gram stain and other primary biochemical tests, which included catalase and oxidase, as well as the standardized tests of the API 20 E and API 20 Strep test kits (BioMérieux, France). Final identification of the isolates were made using Bergey's Manual of Systematic Bacteriology (Sneath *et al.*, 1986). The similarity between the isolates were established using the Sokal and Michener coefficient as well as the Jaccard coefficient and average linkage clustering techniques (Lockhart and Lister, 1970).

Determination of acid volumetric productivity

All the isolates were subjected to a batch test, which was devised to give an indication of the ability of each test organism to produce organic acids from the synthetic dairy effluent substrate. To approximate the conditions as found in a continuous mode acidogenic bioreactor, the maximum volumetric lactate productivity ($Q_p max$) was determined during the logarithmic growth phase under batch culture conditions (Du Preez, 1990).

Isolates were streaked onto fresh agar plates (Table 6.1). After 18 h incubation, single loopfuls of cells were used to inoculate 50 ml of sterile, whey supplemented nutrient broth, as described in Table 6.1, but without the agar. After another 18 h incubation, 10 ml of the cell suspension was transferred to 500 ml of fresh, sterile synthetic dairy effluent substrate. This was done in duplicate sets and these 500 ml aliquots were then incubated at 30 °C in a water bath. At 90 minute intervals, 10 ml samples were aseptically withdrawn and used for the pH measurements. The experiment was stopped after 12 hours. pH measurements were made with a Radiometer PHM 83 Autocal pH meter equipped with a Radiometer combination electrode.

For this study, it was assumed that the drop in pH was the result of lactic acid (2-hydroxy-propanoic acid) production only. The Q_p(max) for each isolate was determined using the following equation (Du Preez, 1990):

$$Q_p = \frac{p_2 - p_1}{t_2 - t_1}$$

where Q_p = volumetric lactate productivity, p_1 = lactate concentration at time 1 (t_1) and p_2 = lactate concentration at time 2 (t_2). Each datum-point of the lactate curves that were obtained for each isolate, were substituted in the above equation. This method of using pH change to determine lactic acid production was verified by determining the degree of correlation between Q_p by using the pH method and the Q_p value obtained by using direct lactic acid chemical assays. The direct chemical assays were done according to a modified Barker-Summerson method (Pryce, 1969).

Strain selection and factorial design

The maximum volumetric acid production rate, Q_p(max), as determined above, was used as criterion for the selection of an isolate for further investigation. The isolate with the highest average Q_p(max) was subjected to the 3x3x3 factorial design experiment as set out in Fig. 6.1 (Box *et al.*, 1978). In this factorial design, the substrate pH, COD and temperature was varied according to the range of variation that was observed during the sampling of three different dairy factory effluents (Strydom *et al.*, 1997). The pH was varied between 5.0 and 9.0 units and the COD between 3 000 and 12 000 $mg.l^{-1}$. The temperature was varied between 25 °C, which represents the lower temperature limit observed in effluents at most factories (unpublished data) and 45 °C. This relatively high temperature was chosen since many *Lactococcus* species are thermophilic. Due to the fact that most isolates had coccoid morphology, a high probability that the selected strain could be thermophilic, was anticipated. The Q_p(max) of the isolate under each set of operational conditions, was determined in batch experiments as described above.

6.4 Results and discussion

Mixed culture acidogenesis

The results obtained during continuous acidogenesis are summarized in Table 6.2. For purposes of comparison, the results from a study by Kisaalita *et al.* (1987), where similar operating conditions were used, are included in Table 6.2. It is evident that very

similar results were obtained, especially in terms of volatile fatty acid concentrations. The exception is the concentration of *n*-butyric acid. There is no explanation for the high level of *n*-butyric acid obtained by Kisaalita *et al.* (1987), other than the fact that a different type of inoculum was used. Since the focus of this study was on the bacterial population, which developed in the bioreactor, it is not possible to compare the two studies in terms of kinetic results.

The bacterial counts eventually stabilized at an average of 152 x 10^7 viable colony forming units (cfu's) with a variation of less than 7.7% during six successive HRT's. This was taken as an indication that stable state had been reached and the population study was subsequently conducted. The particular method (Harrison's disk) which was used for the sampling of the population, was chosen because of its ability to ensure random sampling. Since the isolates were obtained from countable plates of greatest dilution, they can be considered as highly representative replicates of the microbial population, which was present in the acidogenic bioreactor (Atlas *et al.*, 1991).

Population study

The population study showed that the microbial population which developed during acidogenesis was fairly homogeneous (Table 6.3). On species level, only four different strains could be distinguished. Of these four strains, 93.3% were identified as *Peptostreptococcus productus*. One *Klebsiella* strain as well as two *Lactobacillus* strains were isolated, which accounted for the remaining 6.7% of the total. The *Lactobacillus* strains are both presumptive identifications, since it is highly unlikely that a *Lactobacillus* strain will be lactase negative (Table 6.3). The API test-kits that were used for the preliminary identification, were obviously not tailored to the general characteristics of this specific class of isolates. This was apparent from the low identification percentages - for example, the API 20 E test kit identified, with only 35.8% certainty, isolate F06 as *Lactococcus lactis* subsp. *lactis*. Therefore, care must be exercised to ensure that these standardized biochemical tests are not used beyond their intended applications.

Table 6.1 Growth medium used for culturing of inocula during determination of Q_p (max) for each isolate

Component	Concentration
Tryptone ($g.l^{-1}$)	5.0
Yeast extract ($g.l^{-1}$)	2.5
Dextrose ($g.l^{-1}$)	1.0
Whey powder ($g.l^{-1}$)	5.0
Agar ($g.l^{-1}$)	14.5
pH	7.0

Table 6.2 Composition of synthetic dairy effluent before and after continuous mixed-culture acidogenesis, compared with results obtained by Kisaalita *et al.* (1987)

Parameter	Before acidogenesis	After acidogenesis	Results obtained by Kisaalita *et al.* (1987)
COD ($mg.l^{-1}$)	6610 (±84.9)	6250 (± 495)	n.d.
Acetic acid ($mg.l^{-1}$)*	0	1792 (± 3.40)	1969
Propionic acid ($mg.l^{-1}$)*	0	391 (± 4.19)	446
i-Butyric acid ($mg.l^{-1}$)*	0	26 (± 1.25)	71
n-Butyric acid ($mg.l^{-1}$)*	0	50 (± 0.62	534
Total VFA ($mg.l^{-1}$)**	136.6 (±12.0)	2225 (± 60.7)	n.d.
Alkalinity ($mg.l^{-1}CaCO_3$)	78.0 (± 21.8)	180.0 (± 25.3)	n.d.
Total solids ($mg.l^{-1}$)	450	560	n.d.
Volatile solids ($mg.l^{-1}$)	393.7	274.5	n.d.
Non-volatile solids ($mg.l^{-1}$)	56.3	285.5	n.d.
Total viable plate count ($cfu.ml^{-1}$)	0	152×10^7 (± 7.7 %)	n.d.
pH	7.0	6.0	6.1

* Gas chromatographic method
** Five-point titration method
n.d = not determined
Values in brackets = s.d.

The results obtained during numerical analyses of the isolates confirmed the very high similarity. When using the Sokal and Michener coefficient, which takes into account both negative and positive characteristics, all the strains had an 85%, or higher, similarity (Fig. 6.2). When using the Jaccard coefficient, which takes into account only the positive characteristics, 43 of the isolates showed a percentage similarity above 80% (Fig. 6.3). This suggests that only four different species were present in the acidogenic reactor, which is in line with the results as obtained from Bergey's Manual (Table 6.3). However, the four different species in Table 6.3 do not correspond to the four different clusters, which can be observed at the 80% level in Fig. 6.2. This is due to the fact that characters such as Gram characteristic and cell morphology, which are crucial to distinguish isolates during initial identification, were not weighted during the numerical analyses.

The most important conclusion that can be derived from the numerical analyses, especially when using the Sokal and Michener coefficient, is that the microbial population which developed in the acidogenic bioreactor, was biochemically highly homogeneous. This is in spite of the fact that four species could be distinguished and it demonstrates the powerful selection pressure that a chemostat exerts on a microbial population.

Table 6.3 Selected characteristics of the 47 isolates obtained from the population study of an acidogenic reactor used for treatment of a synthetic effluent

Strain*	Presumptive identification	Gram stain	Amylase	Oxidase	Lactase	Q_p(max)(Γ s.d.) (g.l^{-1}.d^{-1} lactate)
A01	*Peptostreptococcus productus*	+	-	-	+	22.11 (2.70)
A02	*Peptostreptococcus productus*	+	-	-	+	18.39 (1.22)
A03	*Peptostreptococcus productus*	+	+	-	+	20.52 (0.11)
A05	*Peptostreptococcus productus*	var	+	-	+	17.50 (0.39)
A06	*Peptostreptococcus productus*	var	+	-	+	17.95 (0.67)
A07	*Peptostreptococcus productus*	+	+	-	+	20.54 (0.35)
A08	*Peptostreptococcus productus*	var	+	-	+	19.21 (1.10)
A09	*Peptostreptococcus productus*	var	+	-	+	19.90 (0.56)
A10	*Peptostreptococcus productus*	var	+	-	+	26.66 (2.24)
A11	*Peptostreptococcus productus*	var	+	-	+	16.28 (4.15)
A12	*Peptostreptococcus productus*	var	+	-	+	8.29 (3.20)
A13	*Peptostreptococcus productus*	var	+	-	+	12.72 (0.61)
A14	*Peptostreptococcus productus*	var	+	-	+	18.59 (4.21)
A15	*Lactobacillus* sp.	+	+	-	+	11.86 (0.15)
A16	*Peptostreptococcus productus*	+	+	-	+	0.02 (0.0007)
A17	*Peptostreptococcus productus*	var	+	-	+	12.99 (0.66)
A18	*Peptostreptococcus productus*	+	+	-	+	11.10 (2.61)
A19	*Lactobacillus* sp.	+	-	+	-	0.11 (0.02)
A20	*Peptostreptococcus productus*	var	+	+	+	21.53 (0.41)
A21	*Peptostreptococcus productus*	var	+	+	+	24.29 (0.38)
A22	*Peptostreptococcus productus*	var	-	-	+	25.90 (1.78)
A23	*Peptostreptococcus productus*	+	+	-	+	22.32 (0.83)
A24	*Peptostreptococcus productus*	+	-	-	+	19.32 (0.69)
A25	*Peptostreptococcus productus*	var	+	-	+	19.95 (2.61)
A26	*Peptostreptococcus productus*	var	-	-	+	23.13 (4.38)
A27	*Peptostreptococcus productus*	var	-	-	+	27.60 (3.68)
A28	*Peptostreptococcus productus*	var	-	-	+	19.93 (7.17)
A29	*Peptostreptococcus productus*	var	-	-	+	17.66 (0.28)
F01	*Peptostreptococcus productus*	var	+	-	+	24.91 (1.22)
F02	*Peptostreptococcus productus*	var	+	-	+	25.87 (0.89)
F03	*Peptostreptococcus productus*	var	-	-	+	24.94 (1.27)
F04	*Klebsiella* sp.	-	+	-	+	24.36 (2.09)
F05	*Peptostreptococcus productus*	var	+	-	+	27.72 (0.11)
F06	*Peptostreptococcus productus*	var	-	-	+	32.54 (0.21)
F07	*Peptostreptococcus productus*	var	-	-	+	32.47 (1.41)
F08	*Peptostreptococcus productus*	var	-	-	+	27.08 (0.81)
F09	*Peptostreptococcus productus*	var	-	-	+	26.16 (0.45)
F10	*Peptostreptococcus productus*	var	+	-	+	21.50 (1.03)
F11	*Peptostreptococcus productus*	var	-	-	+	19.23 (5.20)
F12	*Peptostreptococcus productus*	var	+	-	+	22.36 (0.76)
F13	*Peptostreptococcus productus*	var	+	-	+	21.42 (2.00)
F14	*Peptostreptococcus productus*	var	-	-	+	29.68 (1.27)
F15	*Peptostreptococcus productus*	var	-	-	+	28.62 (0.46)
F16	*Peptostreptococcus productus*	var	-	-	+	25.84 (0.54)
F17	*Peptostreptococcus productus*	var	-	-	+	21.68 (0.25)
F18	*Peptostreptococcus productus*	var	+	-	+	21.53 (0.92)

* Isolates with strain numbers Axx were incubated aerobically, while isolates with strain numbers Fxx were incubated under facultative conditions after selection with the Harrison disk. Strain A04 was lost during initial subculturing

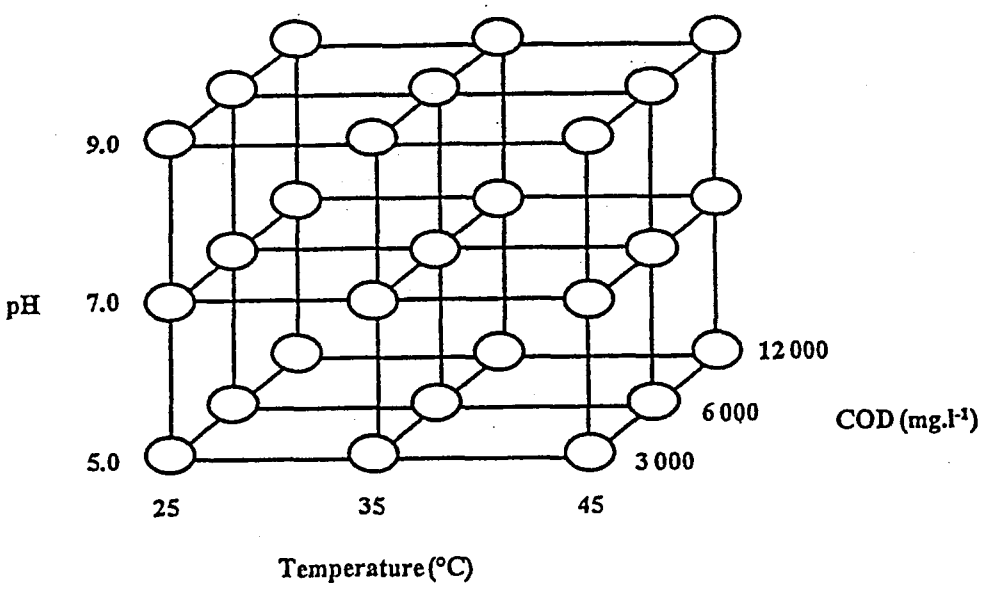

Figure 6.1 Factorial design used to determine the optimum values for the operational parameters used during acidogenesis of a synthetic dairy effluent

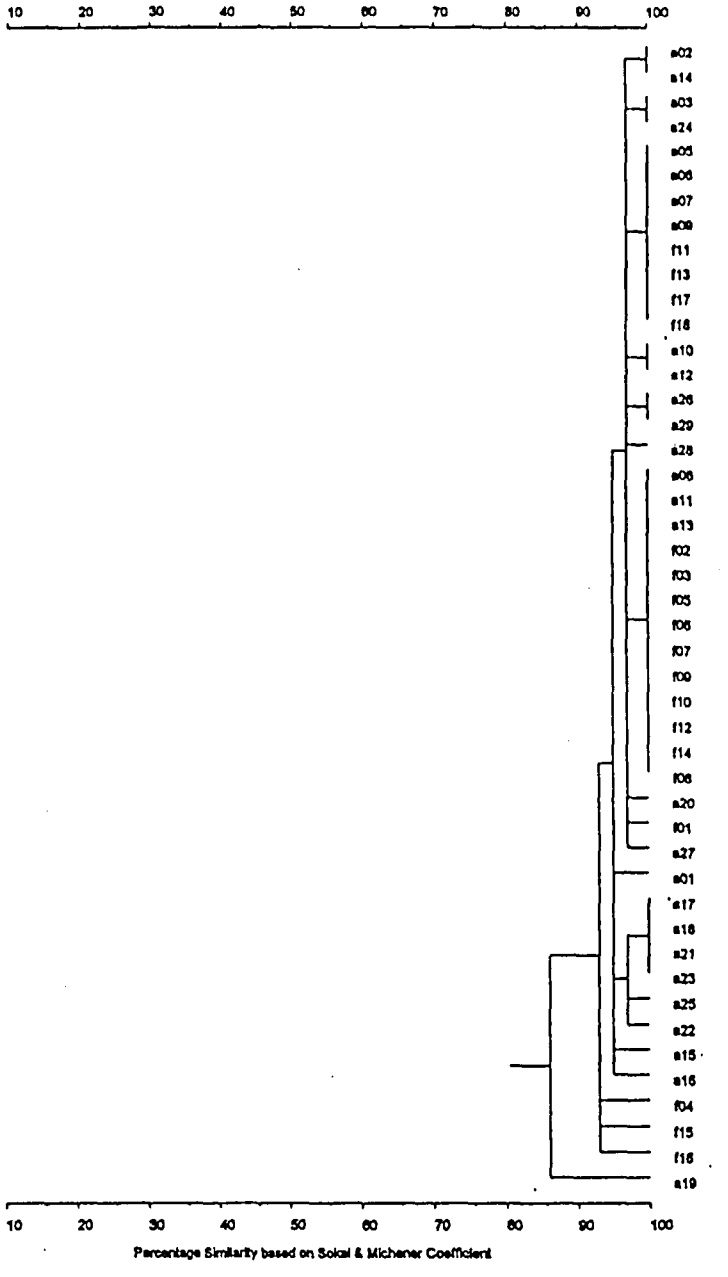

Figure 6.2 Percentage similarity of the isolates obtained during the population study, based on the Sokal and Michener coefficient

Strain selection

The maximum volumetric lactic acid productivity (Q_p(max) - Table 6.3) of the isolates varied between 0.02 and 32.54 $g.l^{-1}.d^{-1}$. Only three isolates had Q_p(max) values below 10.0 $g.l^{-1}.d^{-1}$, while only two isolates had Q_p(max) values above 30.0 $g.l^{-1}.d^{-1}$.

A type of diauxic acid production pattern (Du Preez, 1990) was observed with many isolates (Fig. 6.4). Initial acid production proceeded rapidly, followed by a reduction in acid production tempo, in its turn followed by a renewed acid production phase. The first acid production phase was used in all cases to determine Q_p(max) for each isolate. It is highly probable that this diauxic pattern is caused by the isolate using the primary metabolite as a substrate during the secondary phase. That this might indeed be the case is illustrated in Fig. 6.5. Here, it is obvious that the method used for determining the volumetric productivity is not valid when other types of acids are produced. Strain F06 produced only lactic acid, and a linear regression analysis between results obtained with the direct lactic acid assay and the pH method gave a correlation of 93.8%. In contrast, when other short chain fatty acids are produced, there is no correlation (Fig. 6.5, part B).

Optimization using the factorial design

The optimization of continuous acidogenesis by using a factorial design and specific microbial strains, implies that each individual strain must be evaluated during several runs in a continuous acidogenic reactor. This would be prohibitively time consuming and expensive. For this reason batch cultures were used. However, continuous acidogenic fermentation implies that the microbial strains which are present in the reactor, are perpetually in the exponential growth phase. Therefore, when batch cultures are used to simulate a process which in reality will have to run in continuous mode, the overall result of batch acidogenesis cannot be used to evaluate process efficiency. This is due to the fact that the overall result of batch acidogenesis is influenced by the lag phase, the exponential phase as well as the stationary growth phase. Therefore, to render batch culture results usable in continuous processes, the effect of the lag and stationary phases must be removed from the effect of the exponential growth phase. In order to achieve this objective, the same method which was devised for the determination of Q_p(max), was again used to determine the onset of the exponential phase during the factorial design experiments. This approach made it possible to perform a thorough factorial design experiment while removing the need to make use of time consuming and expensive replicated chemostat runs.

The data obtained in the factorial design experiment was analyzed by using the PROC-GL1 procedure of the Statistical Analysis System computer program (SAS). By utilizing a randomized block design, it was seen that the coefficient of variance between the replicated runs (CV) was 52.449, which is too high to use the data without transformation. The variance was stabilized by using the transformation $x = x^{0.1}$, which gave a CV of 3.809. The main effects of each factor were subsequently tested using linear and quadratic polinomial contrasts, as there were only quantitative and no qualitative variables.

The main effects of each individual factor is presented in Table 6.4. Analysis of this data showed that the effect of temperature is quadratic, i.e. there is a certain temperature at which a maximum Q_p value is obtained. The effects of pH and COD level were within the limits of both linear and quadratic response and no conclusion could be drawn regarding the specific type of response.

Among the individual factors, pH had the greatest effect, followed by the effect of temperature. The combined effect of pH and temperature (PxT) was the only significant two-factor interaction (Table 6.5). The effects of the two-factor interactions are illustrated in Figs. 6.6, 6.7 and 6.8. In each case, the Y-axis represents the predicted Q_p values. The three-factor interaction, PxTxC, had no statistical significance.

From the three-dimensional plots of the two-factor interactions, it is obvious that the range of the variable P (pH) should be extended before meaningful conclusions can be made regarding the optimum pH. This is surprising, as it was expected that the optimum pH of this isolate would be in line with the pH level which was used in the acidogenic bioreactor. However, the optimum temperature (35 °C) and the optimum COD level (3 000 $mg.l^{-1}$) as observed during the factorial experiment, are in line with the operating conditions used in the acidogenic bioreactor. These results suggest that the optimum pH for acidogenesis using this isolate, is probably below 5.0. This is in contrast with the results obtained by Kisaalita *et al.* (1987), who found that the optimum pH of mixed-culture acidogenesis was between 6.0 and 6.5 units. However, these authors found the nature of the acidogenic products to be pH dependent, and ascribed the change in end-products at different pH values, to a change in the bacterial population. The actual composition of the bacterial population, however, was not investigated. In this current study, pure cultures were used for process optimization. This implies that adaptation of a bacterial population by means of selection was impossible, and offers an explanation for the apparent conflict of results, as far as the effects of the pH level is concerned.

Table 6.4 Main effects of the three factors temperature (T), COD concentration (C) and pH (P) on the maximum volumetric lactic acid productivity (Q_p (max)) of *Peptostreptococcus productus* strain F06

Factor	Q_p (Transformed value)	± s.d.
Level of T (°C)		
25	0.961	0.193
35	1.056	0.254
45	0.911	0.167
Level of C ($mg.l^{-1}$ COD)		
3000	1.013	0.239
6000	0.988	0.216
12000	0.927	0.182
Level of P (pH)		
5.0	1.118	0.100
7.0	1.090	0.158
9.0	0.719	0.030

Table 6.5 Individual and combined effects of the three factors temperature (T), COD concentration (C) and pH (P) on the maximum volumetric lactic acid productivity (Q_p (max)) of *Peptostreptococcus productus* strain F06

Factor	Sum of squares (SS)	Contribution to overall effect (%)
T	0.194	8.06
C	0.071	2.95
P	1.786	74.42
TxC	0.015	0.62
TxP	0.187	7.80
CxP	0.043	1.79
TxCxP	0.069	2.85
Replication	0.00027	0.011
Total	2.40027	98.5*

* This value is equal to R^2, the regression coefficient, which indicates the extent to which the model fits the observed data

Products of acidogenesis

The method for strain selection and process optimization as described above, rests upon the important assumption that only lactic acid was produced by the isolates. This was indeed the case with many isolates, although some isolates capable of using lactate did produce other volatile fatty acids such as acetic acid (data not shown). In spite of the fact that up to 1 800 $mg.l^{-1}$ acetate was produced in the continuous acidogenic bioreactor, strain F06 produced only lactic acid during the optimization experiments. This is ascribed to the fact that batch cultures were used and the effect of continuous culture conditions on product distribution must still be investigated. However, the fact that only lactic acid was produced by strain F06, is not anticipated to present problems in an anaerobic digester. In the first instance, a low pH was the major causative factor of digester failure in experiments where a single digester was used for the treatment of high strength dairy wastewaters (De Haast *et al.*, 1986; Kelly and Switzenbaum, 1984; Kisaalita *et al.*, 1987). When lactic acid is produced in the acidogenic reactor, the second phase where methane is generated will receive a substrate containing neutralized organic acids with no lactose present. Thus, the root cause of a low pH is removed, and the methanogenic reactor is protected from inhibitively low pH values. In addition, lactic acid is utilized directly via pyruvate and acetyl-CoA to form acetate (Gottschalk, 1986). Furthermore, several other studies (Burgess, 1985; De Haast *et al.*, 1986) has shown that a pre-acidified, lactic acid containing wastewater, does not present a problem to the methane generating phase.

Finally, a recalcitrant odour problem was encountered during the mixed-culture continuous acidogenesis of the synthetic dairy effluent. This is ascribed to either secondary or syntrophic metabolite formation by bacteria present in small numbers in the mixed-culture acidogenic reactor. This odour problem was completely absent during the pure culture batch acidogenesis experiments, using strain F06. This demonstrates very clearly the advantages that may be derived by using defined inocula for process optimization.

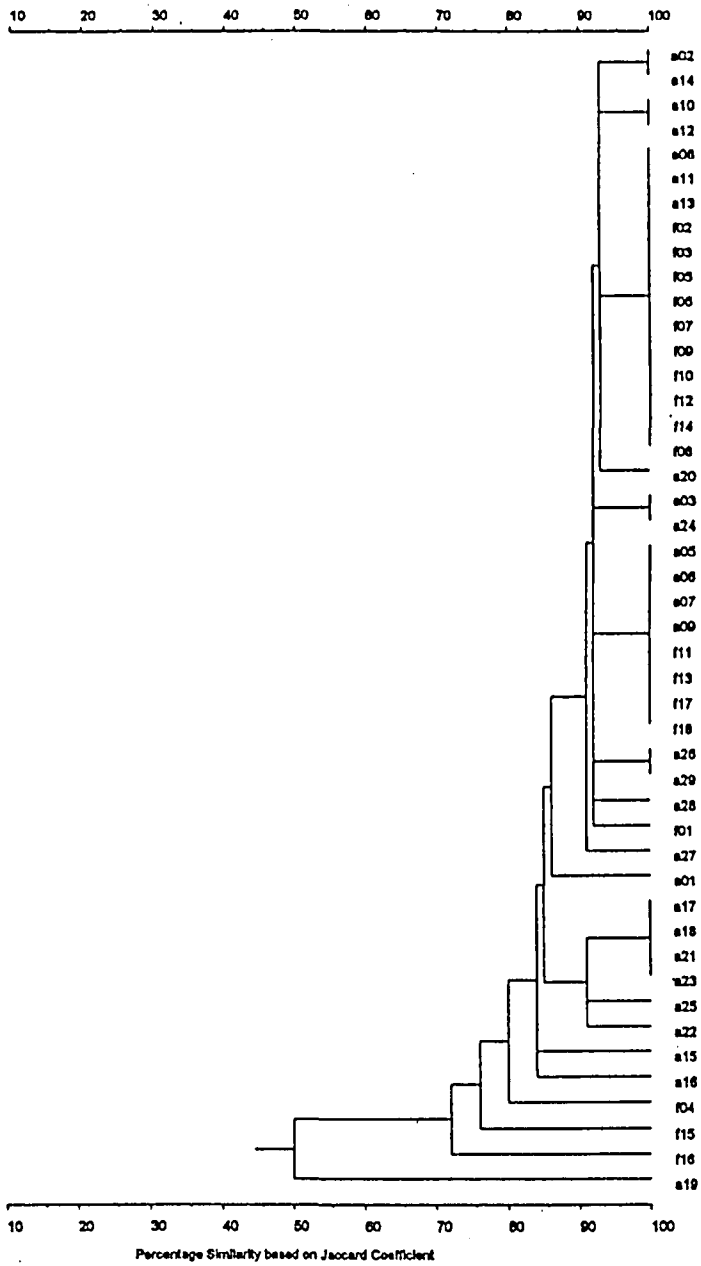

Figure 6.3 Percentage similarity of the isolates obtained during the population study, based on the Jaccard coefficient

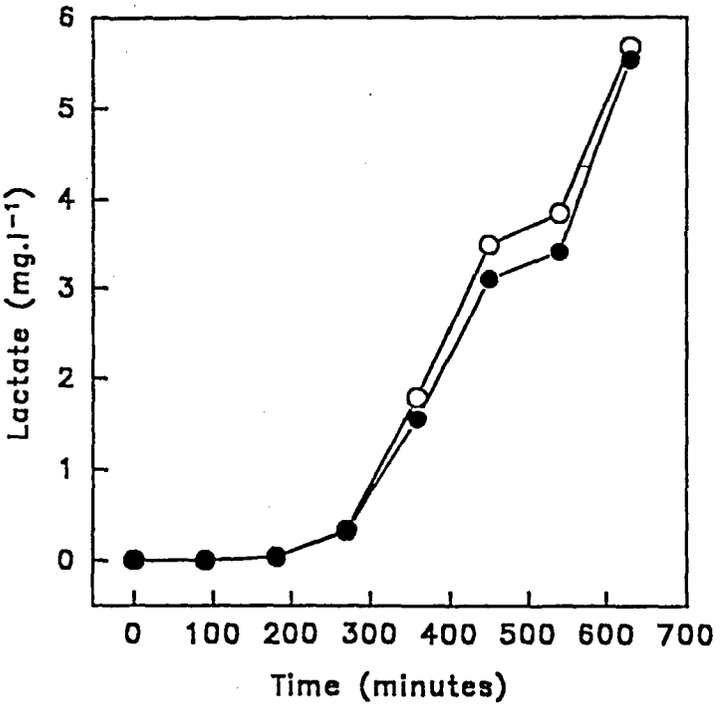

Figure 6.4 Diauxic pattern as observed with isolate A22 (O and ● represent duplicate runs using the same isolate)

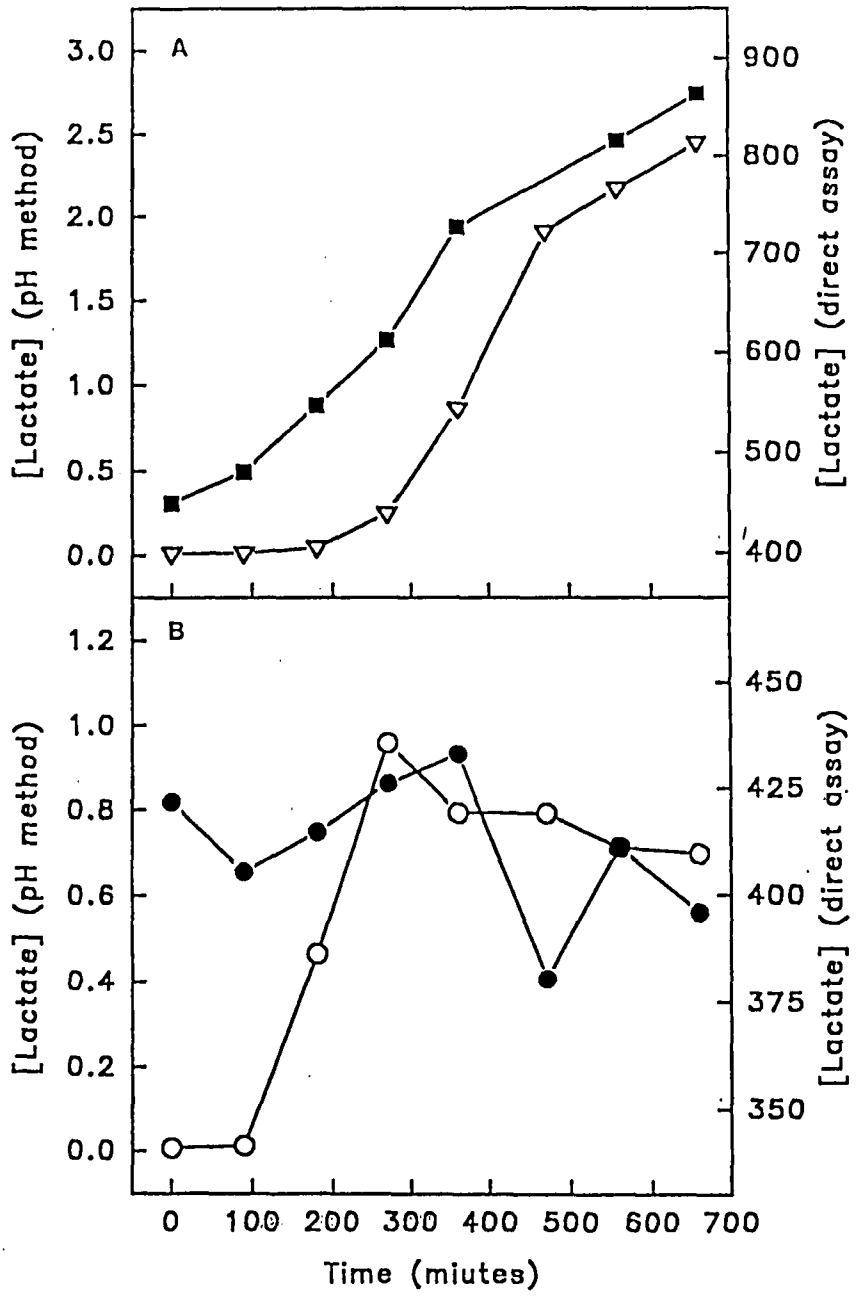

Figure 6.5 Correlation between the two methods used to determine the lactic acid concentration, where [Lactate] = lactic acid concentration in $mg.l^{-1}$, ■ and ● = direct assay result and and o = pH method result. (A) represent the results obtained with strain F06, and (B) those obtained with *Klebsiella oxytoca* strain A1

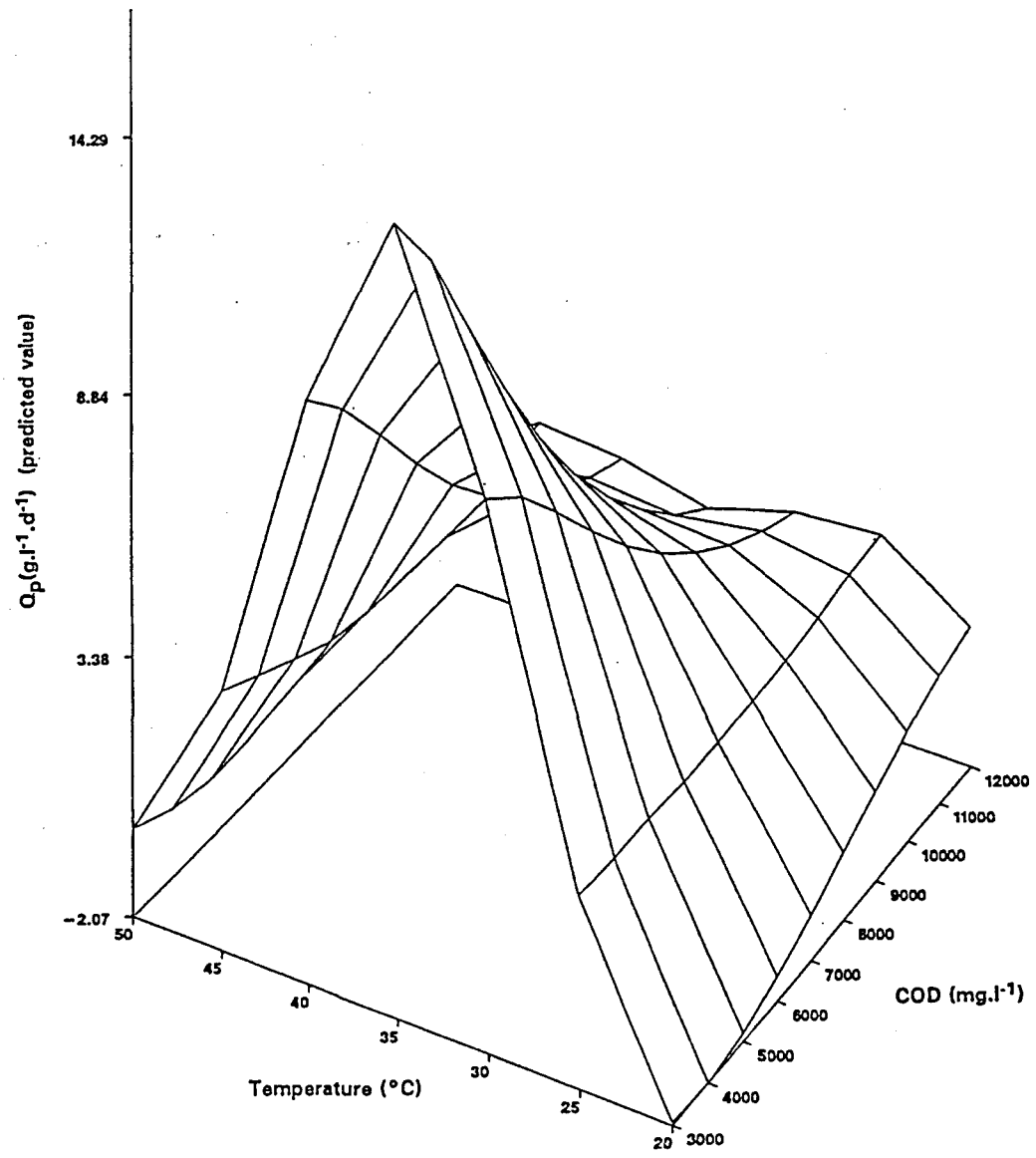

Figure 6.6 Two-factor interactions: Effects of COD and temperature levels on maximum volumetric lactic acid productivity

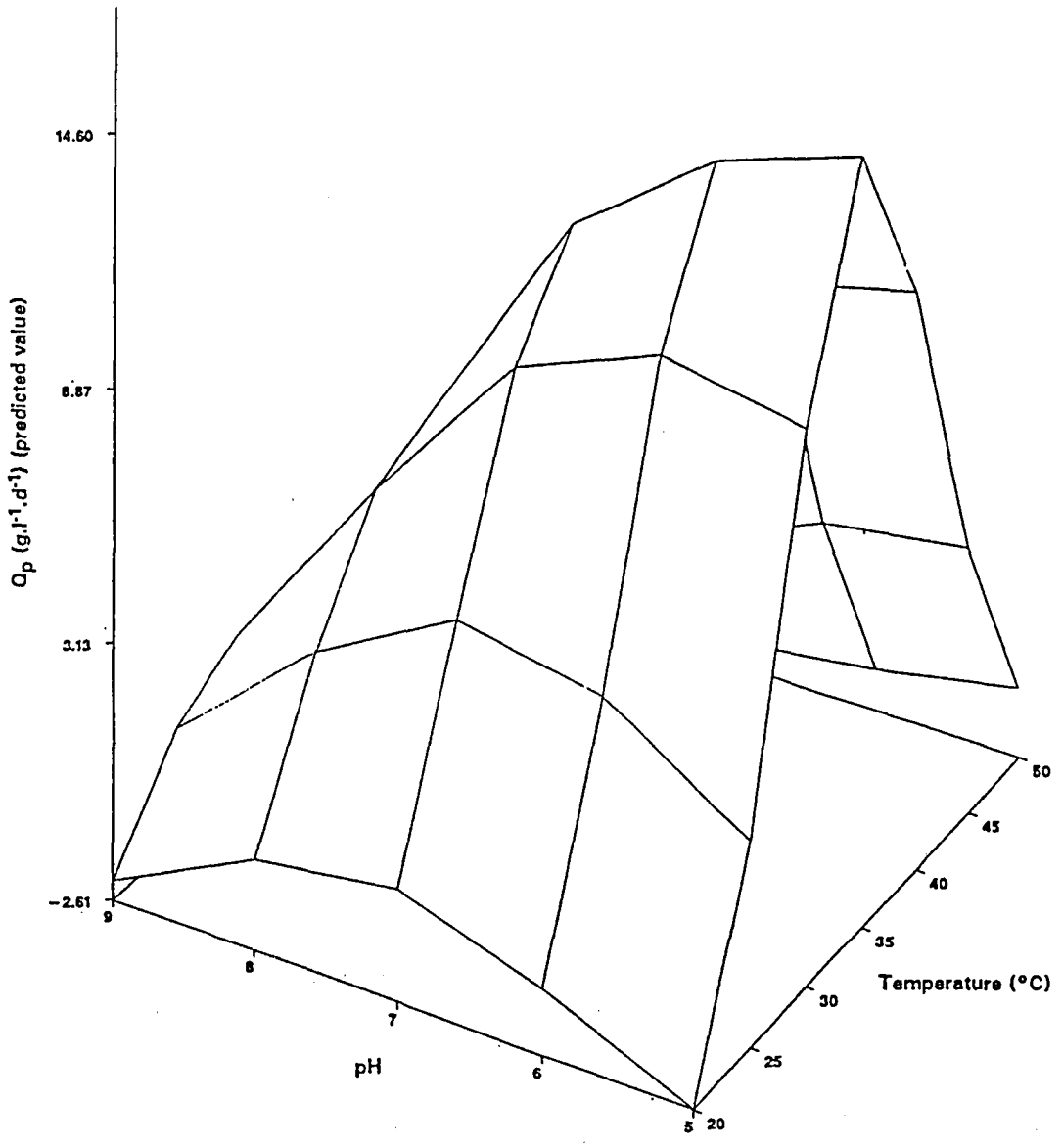

Figure 6.7 Two-factor interactions: Effects of pH and temperature levels on maximum volumetric lactic acid productivity (interaction of greatest significance)

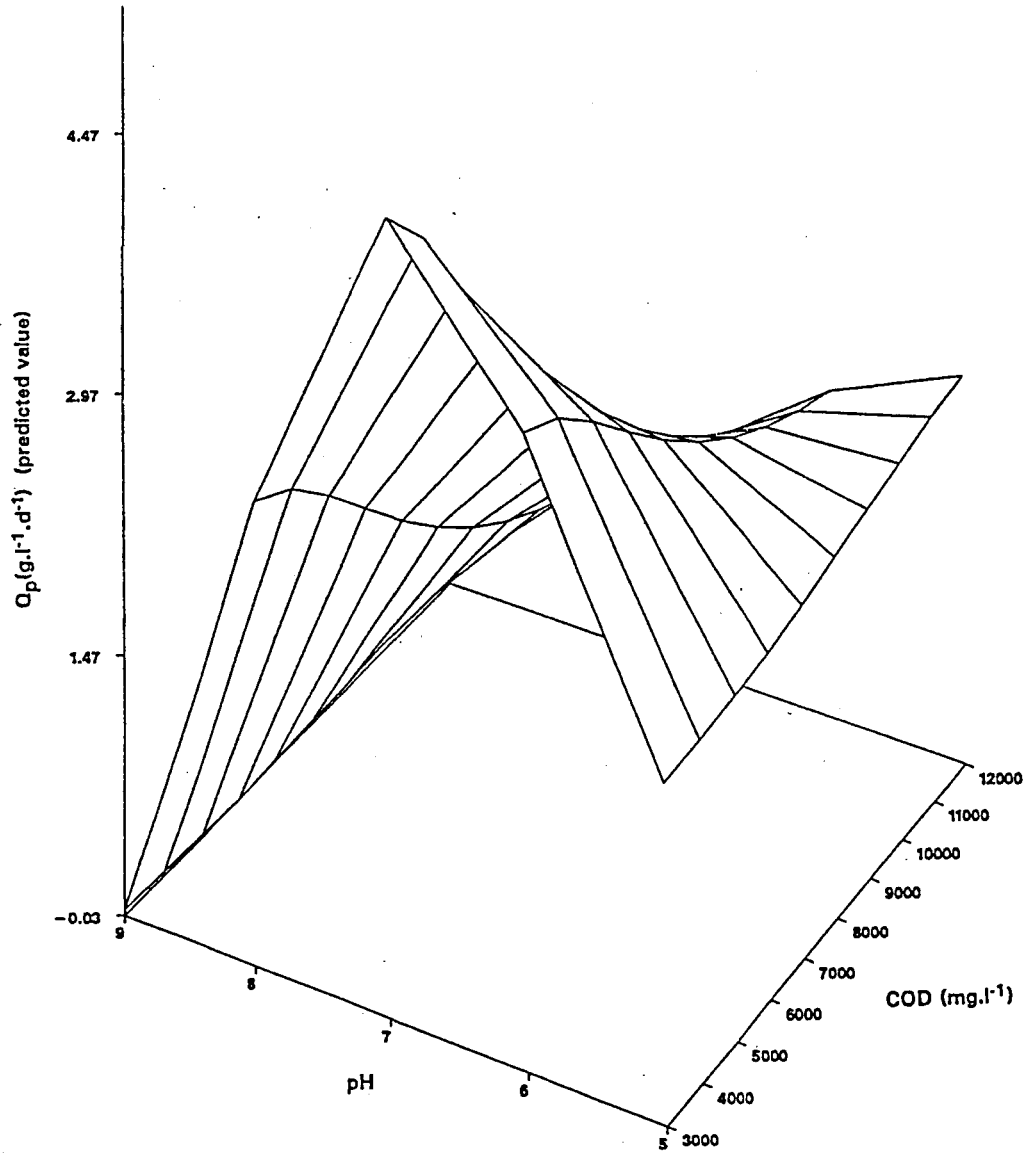

Figure 6.8 Two-factor interactions: Effects of pH and COD levels on maximum volumetric lactic acid productivity

6.5 Conclusions

The well known variability of dairy effluents as found between individual factories (Strydom *et al.*, 1997), complicates the procedure of optimizing acidogenesis of dairy effluents in general. However, the methanogenic phase of the two phase anaerobic hybrid digester, was shown to easily adapt to new types of dairy effluents after being started and operated with synthetic effluent as substrate. Very good results were obtained by using a synthetic dairy effluent substrate for anaerobic digestion. These results were eventually verified when actual effluents were used as substrates for the same two phase hybrid digester (Strydom *et al.*, 1997).

In this study, an optimization procedure was developed which can possibly be used for the microbial and initial operational optimization of acidogenesis, even in existing full-scale acidogenic bioreactors. The optimal values of the various operational parameters must be verified and possibly adapted, when the pure cultures obtained in this study, are used for pilot or full-scale acidogenesis. However, the results obtained during the process optimization in this study will facilitate start-up and initial operation of such a full-scale acidogenic bioreactor. Furthermore, it is anticipated that the optimization procedure that was successfully used for the acidogenesis of the synthetic effluent, will yield similar success when applied to other high strength dairy wastewaters.

The use of a known isolate has yielded the unexpected bonus of odour control. Similar advantages, such as the elimination of unknown and possibly pathogenic bacteria are also implied. Finally, the microbial optimization of acidogenesis might be the first step towards the microbial optimization of the overall anaerobic digestion process. This will ultimately enable engineers and process designers to develop treatment systems which are based on the extraordinary capabilities of naturally occurring but specially selected microbes.

ADDENDUM

ECONOMIC VIABILITY OF ON-SITE ANAEROBIC DIGESTION AT DAIRY FACTORIES

Two factors favourably influence the economic viability of implementing anaerobic digestion as an on-site effluent treatment option. These are (1) possible reduction in trade effluent tarriffs and (2), generation of biogas for use as coal or fuel oil replacement.

The overall effects of these two factors depend on several more factors. For example, a reduction in municipal tarriffs would depend on the policy of the said municipality, as well as the formulae used for the calculation of the tarriff. The type of dairy factory will also influence the economic viability of on-site effluent treatment. A factory where milk powder is produced may have an effluent with a temperature well in excess of 35°C, which will most probably remove the need to use the biogas for heating the digester.

Thus, each factory will have its own set of circumstances which will determine the economic viability of on-site effluent treatment.

In order to calculate possible cost-benefits which may be associated with on-site effluent treatment, the following constant factors must be taken into consideration:

* Successful anaerobic treatment may reduce the COD load by up to 95%
* Methane will be generated at a rate of up to 0.352 m^3 per kg of $COD_{removed}$.
* The digester must remain at a constant 35 °C.
* Methane has a calorific value of 35.8 MJ per m^3. It is roughly the equivalent of 0.4 kg diesel oil, 0.8 kg coal or 0.4 kg liquid petroleum gas (LPG).
* Methane liquefies at -82°C or 4.6 MPa. This makes storage and transport using pressurised containers unpractical, which implies that the gas should preferably be used while it is being generated.

Since virtually all dairy factories use steam on a continuous basis, only a small gas storage capacity should be necessary.

As an illustration, the cheese factory data as reported in Chapter 5, indicates that up to 46 000 MJ of nett heat energy per day, may be obtained in the form of biogas. If the said cheese factory were to dispose 14 000 litres of whey daily in addition to its normal effluent, this figure will rise up to 61 000 MJ of heat energy available to the factory. This may translate into considerable savings, in terms of coal or fuel oil expenditure.

It is clear from the above that anaerobic digestion as an effluent treatment option, has to be considered by each individual factory, and that no generalized conclusion in this regard can be drawn.

CHAPTER 7

REFERENCES

Anonymous, (1984). South Caernarvon Creameries converts whey into energy. *Dairy Industries International,* **49**(10), 16-17.

Anonymous, (1990). Anaerobic treatment of dairy effluents - The present stage of development. *Bulletin of the International Dairy Federation,* **252,** 3-23.

Atlas, R.M., Horowitz, A., Krichevsky, M. and Bej, A.K., (1991). Response of microbial populations to environmental disturbance. *Microbial Ecology*, **22,** 249-256.

Bekker, A.P., (1982). Water use in South Africa and estimated future needs. *The Civil Engineer in South Africa*, **24,** 653-659.

Bogh-Sorenson, T., (1992). *Dairy Technology* Published by APV Pasilac AS, Denmark.

Bonastre, N. and Paris, J.M., (1989). Survey of laboratory, pilot and industrial anaerobic filter installations. *Process Biochemistry*, **24,** 15-20.

Box, G.E.P., Hunter, W.G. and Hunter, J.S., (1978). *Statistics for Experimenters: An Introduction to Design, Data Analysis and Model Building* Published by John Wiley and Sons, New York, USA.

Britz, T.J., Venter, C.A. and Tracey, R.P., (1989). Anaerobic treatment of Coastal Park municipal landfill leachate using a hybrid digester. In *Proceedings of the Second Anaerobic Digestion Symposium* pp. 168-174. Published by the University of the Orange Free State, Bloemfontein, South Africa.

Burgess, S., (1985). Anaerobic treatment of Irish creamery effluents. *Probiotech - Wastes and Water* (*Supplement to Process Biochemistry* **20,** 6-7).

Cánovas-Diaz, M. and Howell, J.A., (1987a). Down-flow anaerobic filter stability studies with different reactor working volumes. *Process Biochemistry*, **22,** 181-184.

Cánovas -Diaz, M. and Howell, J.A., (1987b). Stratified ecology techniques in the start-up of an anaerobic down-flow fixed film percolating reactor. *Biotechnology and Bioengineering*, **10,** 289-296.

Carballo-Caabeira, J., (1990). Depuracion de augas residuales de centrales lecheras. Revista Española de Lechería **13**(12), 13-16 (As cited in Dairy Science Abstracts 1990 **52** abstract 5653).

Cayless, S.M., Da Motta Marques, D.M.L. and Lester, J.N., (1990). A study of the effects of methanol in start-up of UASB reactors. *Biological Wastes*, **31,** 123-135.

Cobb, S.A. and Hill, D.T., (1990). Using nitrogen ratio as an indicator of biomass retention and steady-state in anaerobic fermentation. *Transactions of the American Society of Agricultural Engineers*, **33,** 282-287.

Colin, F., Ferrero, G.L., Gerletti, M., Hobson, P., L'hermite, P., Naveau, H.P. and Nyns, E.J., (1983). Proposal for the definition of parameters and analytical measurements applicable to the anaerobic digestion process. *Agricultural Wastes*, **7,** 183-193.

Criddle, C.S., (1993). The kinetics of co-metabolism. *Biotechnology and Bioengineering*, **41,** 1048-1056.

Dairy Board, (1990). Annual Report for the year ended 28 February 1990. Published by the Dairy Board: Pretoria.

Daniel, F.B., Condle, L.W., Robinson, M., Stober, J.A., York, R.G., Olsen, G.R. and Wang, S., (1990). *Journal of the American Water Works Association,* **82,** 61-69 (As cited by Dietrich *et al.* 1992).

De Haast, J., Britz, T.J. and Novello, J.C., (1986). Effect of different neutralizing treatments on the efficiency of an anaerobic digester fed with deproteinated cheese whey. *Journal of Dairy Research*, **53,** 467-476.

De Haast, J., Britz, T.J., Novello, J.C. and Lategan, P.M., (1983). Anaerobic digestion of cheese whey using a stationary fixed-bed reactor. *New Zealand Journal of Dairy Science and Technology*, **18,** 261-271.

De Man, G. and De Bekker, P.H.A.M.J., (1986). New technology in dairy wastewater treatment. *Dairy Industries International* , **51**(5), 21-25.

Department of Water Affairs, (1986). Management of the water resources of the Republic of South Africa. Published by the Department of Water Affairs: Pretoria.

Dietrich, A.M., Ledder, T.D., Gallagher, D.L., Grabeel, M.N. and Hoehn, R.C., (1992). Determination of chlorite and chlorate in chlorinated and chloraminated drinking water by flow injection analysis and ion chromatography. *Analytical Chemistry,* **64,** 496-502.

Du Preez, J.C., (1990). Personal communication. Department of Microbiology and Biochemistry, University of the Orange Free State, Bloemfontein, South Africa.

Dunican, L.K., Newell, P.J. and McKeown, K.J., (1986). Anaerobic fixed-film fermentation of organic effluents from the food and drink industry as a means of biological energy production. *Annals of the New York Academy of Sciences*, **469,** 320-331.

Feilden, N.E.H., (1983). The theory and practice of anaerobic reactor design. *Process Biochemistry*, **18,** 34-37.

Funke, J.W., (1970). Industrial Water and Effluent Management in the Milk Processing Industry. *CSIR Technical guide K12.* Published by the CSIR, Pretoria.

Giraldo, E., Norgren, K. and Switzenbaum, M.S., (1990). Hydrogen and carbon monoxide as early warning indicators of toxic upsets in anaerobic digestion. *Proceedings: 44th Purdue Industrial Waste Conference.*

Goodwin, J.A.S., Wase, D.A.J. and Forster, C.F., (1990). Anaerobic digestion ice-cream wastewaters using the UASB process. *Biological Wastes*, **32,** 125-144.

Goodwin, J.A.S., Wase, D.A.J. and Forster, C.F., (1992). Pre-granulated seeds for UASB reactors: How necessary are they? *Bioresource Technology*, **41,** 71-79.

Gottschalk, G., (1986). Bacterial Metabolism. Second edition. Springer Verlag. New York.

Guiot, S.R. and Van Den Berg, L., (1984). Performance and biomass retention of an upflow anaerobic reactor combining a sludge blanket and a filter. *Biotechnology Letters,* **6**(3), 161-164.

Harrigan, W.F. and McCance, M.E., (1976). Laboratory Methods in Food and Dairy Microbiology Academic Press, New York, USA.

Hickey, R.F., Wu, W.M., Veiga, M.C. and Jones, R., (1991). Start-up, operation, monitoring and control of high-rate anaerobic treatment systems. *Water Science and Technology*, **24,** 207-255.

Hiddink, J., (1990). Subject E: Friends of the environment. Overview from a processor's perspective. In *Proceedings of the XXIII International Congress*, **2,** 803-813.

Hill, D.T., (1991). Steady state mesophilic design equations for methane production from livestock wastes. *Transactions of the American Society of Agricultural Engineers,* **35,** 2157-2163.

Ikonen, M., Latola, P., Pankakoski, M. and Pelkonen, J., (1985). Anaerobic treatment of waste water in a Finnish dairy. *Nordisk Mejeriindustri*, **12**(8), 81-82 (As cited in Dairy Science Abstracts 1987, **49** abstract 5647).

Iza, J., Colleran, E., Paris, J.M. and Wu, W.M., (1991). International workshop on anaerobic treatment technology for municipal and industrial wastewaters: Summary paper. *Water Science and Technology*, **24**(8), 1-16.

Jones, H.R., (1974). Pollution control in the dairy industry. Pollution Technology Review No. 7, Published in the USA by Noyes Data Corporation: Park Ridge, New Jersey.

Joubert, W.A. and Britz, T.J., (1987). The performance and mixing characteristics of an anaerobic hybrid reactor treating a synthetic fatty acid containing substrate. *Water SA*, **13**(2), 63-68.

Kelly, C.R. and Switzenbaum, M.S., (1984). Anaerobic treatment: Temperature and nutrient effects. *Agricultural Wastes*, **10,** 135-154.

Kisaalita, W.S., Lo, K.V. and Pinder, K.L., (1990). Influence of whey protein on continuous acidogenic degradation of lactose. *Biotechnology and Bioengineering,* **36,** 642-646.

Kisaalita, W.S., Pinder, K.L., and Lo, K.V., (1987). Acidogenic fermentation of lactose. *Biotechnology and Bioengineering*, **30,** 88-95.

Kissalita, W.S., Lo, K.V. and Pinder, K.L., (1989). Kinetics of whey-lactose acidogenesis. *Biotechnology and Bioengineering*, **33,** 623-630.

Lebrato, J., Perez-Rodriguez, J.L., Maqueda, C. and Morillo, E., (1990). Cheese factory wastewater treatment by anaerobic semicontinuous digestion. *Resources Conservation and Recycling*, **3,** 193-199.

Lettinga, G. and Hulshoff-Pol, L.W., (1991). UASB-process design for various types of wastewaters. *Water Science and Technology*, **24,** 87-107.

Lettinga, G., Van Velsen, A.F.M., Hobma, S.W., De Zeeuw, W. and Klapwijk, A., (1980). Use of the upflow sludge blanket (USB) reactor concept for biological wastewater treatment especially for anaerobic treatment. *Biotechnology and Bioengineering*, **22,** 699-734.

Li, A.Y. and Corrado J.J., (1985). Scale up of the membrane anaerobic reactor system. *Proceedings of the 40th annual Purdue Industrial Waste Conference* pp. 399-404.

Lin, C.Y., Noike, T., Furumai, H. and Matsumoto, J., (1989). A kinetic study on the methanogenesis process in anaerobic digestion. *Water Science and Technology,* **21,** 175-186.

Lo, K.V. and Liao, P.H., (1986). Digestion of cheese whey with anaerobic rotating biological contact reactor. *Biomass*, **10,** 243-252.

Lo, K.V. and Liao, P.H., (1988). Laboratory scale studies on the mesophilic anaerobic digestion of cheese whey in different digester configurations. *Journal of Agricultural Engineering Research*, **39,** 99-105.

Lockhart, W.R. and Liston, J., (1970). Methods for Numerical Taxonomy Published by the American Society for Microbiology, Washington DC.

McCarthy, P.L., (1981). One hundred years of anaerobic treatment. In: *Anaerobic Digestion 1981*, Eds. Hughes D.E. *et al.* pp. 2-22, *Elsevier Biomedical Press*, Amsterdam.

McCarty, P.L. and Mosey, F.E., (1991). Modelling of anaerobic digestion processes (a discussion of concepts). *Water Science and Technology*, **24**(8), 17-33.

Meyer, L.H., Britz, T.J. and Lategan, P.M., (1985). Temperature control for laboratory scale anaerobic digesters. *Water SA*, **9,** 79-80.

Monod, J., (1949). The growth of bacterial cultures. *Annual Reviews of Microbiology,* **3,** 371-376 (As cited by Pavlostathis and Giraldo-Gomez, 1991).

Moosbrugger, R.E., Wentzel, M.C., Ekama, G.A. and Marais, G. v R., (1992). Simple titration procedures to determine H2CO3 alkalinity and short chain fatty acids in aqueous solutions containing known concentrations of ammonium, phosphate and sulphide weak acid/bases. WRC report no. TT57/92. Published by the Water Research Commission, Pretoria, South Africa.

Myburg, C. and Britz, T.J., (1993). Influence of higher organic loading rates on the efficiency of an anaerobic hybrid digester while treating landfill leachate. *Water SA,* **19,** 319-324.

Nel, L.H., Britz, T.J. and Lategan, P.M., (1985). The effect of trace elements on the performance efficiency of an anaerobic fixed film reactor treating a petrochemical effluent. *Water SA,* **11,** 107-110.

Nell, F.J., (1991). Personal communication. Dairy Services Organization.

Odegaard, H. and Rusten, B., (1990). Upgrading of small municipal wastewater treatment plants with heavy dairy loading by introduction of aerated submerged biological filters. *Water Science and Technology*, **22**(7-8), 191-198.

Pavlostathis, S.G. and Giraldo-Gomez, E., (1991). Kinetics of anaerobic treatment. *Water Science and Technology,* **24**(8), 35-59.

Petrozzi, S. and Dunn, I.J., (1991). Gas measurement methods for laboratory-scale anaerobic reactors. *Biotechnology Techniques*, **5**(5), 355-358.

Pico, R.F., (1987). Dairy wastes. *Journal Water Pollution Control Federation,* **59,** 448-450.

Pryce, J.D., (1969). A modification of the Barker-Summerson method for the determination of lactic acid. *Analyst*, **94,** 1151-1158.

Republic of South Africa, (1962). Regional standards for industrial effluents. Government Gazette R.553, 1-5. Pretoria: Government Printer, South Africa.

Republic of South Africa, (1991). Report of the three committees of the President's Council on a national environmental management system. Published by Authority: The Government Printer: Cape Town.

Romney, A.J.D., (1990). CIP: Cleaning in place. *Published by the Society of Dairy Technology*, Huntingdon Cambridgeshire.

Ross, W.R., (1991). Anaerobic digestion of industrial effluents with emphasis on solids-liquid separation and biomass retention. Ph.D. Thesis, University of the Orange Free State, South Africa.

Ross, W.R., Barnard, J.P. and De Villiers, H.A., (1989). The current status of ADUF technology in South Africa. In *Proceedings of the Second Anaerobic Digestion Symposium* pp. 65-69. Published by the University of the Orange Free State, Bloemfontein, South Africa.

Ryder, D.N., (1985). Anaerobic digestion. *Bulletin of the International Dairy Federation*, **184,** 127-131.

Schröder, E.W. and De Haast, J., (1988). Anaerobic digestion in effluent treatment part 2: Microbiology and process control. *South African Journal for Dairy Science,* **20**(1), 9-15.

Schroepfer, G.J., Fuller, W.J., Johnson, A.S., Ziemke, N.R. and Anderson, J.J., (1955). The anaerobic contact process as applied to packinghouse wastes. *Sewage and Industrial Wastes*, **27,** 460-486 (As cited by Van Den Berg, 1984).

Schug, A., Schoberth, S.M. and Sahm, H., (1987). Conversion of lactose to methane by defined bacterial cocultures. *Acta Biotechnologica,* **7,** 337-345 (As cited in Dairy Science Abstracts 1988, **50** abstract 3284).

Senior, E., (1986). Wealth from Waste. In *Proceedings of the Anaerobic Digestion Symposium* pp. 19-30. Published by the University of the Orange Free State, Bloemfontein, South Africa.

Senior, E., (1990). Introduction. In *Microbiology of Landfill Sites*. pp. 1-15. ed. E. Senior, CRC press, New York.

Sneath, P.H.A., Mair, N.S., Sharpe, M.E. and Holt, J.G., (1986). *Bergey's Manual of Sytematic Bacteriology* (Volume 2), Williams and Wilkens, Baltimore USA.

Standard Methods, (1985). Standard Methods for the Examination of Water and Wastewater (16th edn.), American Public Health Association, Washington DC, USA.

Strydom, J.P., Mostert, J.F. and Britz, T.J., (1993). Effluent production and disposal in the South African dairy industry - a postal survey. *Water SA,* **19**(3), 253-258.

Strydom, J.P., Mostert, J.F.and Britz, T.J., (1995). Anaerobic treatment of a synthetic dairy effluent using a hybrid digester. *Water SA*, **21**(2), 125-130.

Strydom, J.P., Britz, T.J., and Mostert, J.F., (1997). Two-phase anaerobic digestion of three different dairy effluents using a hybrid bioreactor. *Water SA,* **23**, 151-156.

Toldrá , F., Flors, A., Lequerica, J.L. and Vall, S.S., (1987). Fluidized bed anaerobic biodegradation of food industry wastewaters. *Biological Wastes*, **21**, 55-61.

Van den Berg, L., (1984). Developments in methanogenesis from industrial waste water. *Canadian Journal of Microbiology*, **30**, 975-990.

Van Der Merwe, M. and Britz, T.J., (1994). Selection and optimisation of naturally occurring bacterial strains from raw bakers' yeast effluent for use in a pre-degradation step. *Mededelingen Faculteit Landbouwkundige en Toegepaste Wetenschappen*, **59**(4b), 2107-2120. Published by the University of Gent, Belgium.

Van Der Merwe, M. and Britz, T.J., (1993). Anaerobic digestion of baker's yeast factory effluent using an anaerobic filter and hybrid digester. *Bioresource Technology*, **43**, 169-174.

Van Der Merwe, M. and Britz, T.J., (1994). Characterization and numerical analysis of the microbial community in raw baker's yeast factory effluent. *Water SA*, **20**, 161-168.

Venkataraman, J., Kaul, S.N. and Satyanarayan, S., (1992). Determination of kinetic constants for a two-stage anaerobic up-flow packed bed reactor for dairy wastewater. *Bioresource Technology.* **40**, 253-261.

Vernick, A.S., (1977). How to conduct a wastewater survey, Part II: Developing waste stream profiles. *Plant Engineering,* **7**, 77-80.

Water Research Commission, (1989). Waste and Waste-water Management in the Dairy Industry WRC Project no. 145 TT #8/89. NATSURV series, no.4. Published by the WRC, P.O.Box 824, Pretoria.

Weber, H., Kulbe, K.D., Chmiel, H. and Trösch, W., (1984). Microbial acetate conversion to methane: kinetics, yields and pathways in a two-step digestion process. *Applied Microbiology and Biotechnology*, **19**, 224-228.

Weiland, P. and Rozzi, A., (1991). The start-up, operation and monitoring of high-rate anaerobic treatment systems: Discusser's report. *Water Science and Technology*, **24**(8), 257-277.

Yu, J. and Pinder, K.L., (1992). Build-up of symbiotic methanogenic biofilms on solid supports. *Biotechnology Letters*, **14**, 989-994.

Yu, J. and Pinder, K.L., (1993). Intrinsic fermentation kinetics of lactose in acidogenic biofilms. *Biotechnology and Bioengineering*, **41**, 479-488.