A LABORATORY SCALE STUDY TO INVESTIGATE THE EFFECTS OF SOLIDS CONCENTRATION ON THE EFFICIENCY OF ANAEROBIC DIGESTION

by
Valerie Naidoo BSc(Hons)

Submitted in fulfilment of the requirements for the degree of Master of Science of the University of Natal, Durban.

Department of Microbiology and Plant Pathology

in co-operation with

Pollution Research Group

Department of Chemical Engineering

March 1995

Declaration of Candidate

I, Valerie Naidoo, declare that this dissertation is my own work and that it has not been submitted for a degree at another university or institute.

.....

March 1995

Acknowledgements

I would like to thank:

Water Research Commission(WRC) for the funding of this project.

The staff of Northern Waste Water Treatment Works for their co-operation and assistance during the collection of samples.

Professor E. Senior for his supervision and advice on the microbiological aspects of this project.

Professor C.A. Buckley for his supervision and encouragement throughout this project.

Dr. G. Tivchev for his advice during the planning of the project.

Dr. V.L. Pillay for his assistance and for highlighting the lighter side of life at a sewage works.

Ms C. Carliell for showing me around when I arrived at the Department of Chemical Engineering and for her assistance during the project.

Mrs D. Harrison for her advice on laboratory equipment.

Miss B. Duvel for her assistance with samples in the laboratory.

Members of the Pollution Research Group of 1993-1994 for their warm conversation.

Mom, Dad, and sisters for their encouragement and support during this period of study.

Abstract

With the exceptions of mixing and heating mechanisms, and the recycling of settled solids, no radical changes or improvements have been made to conventional anaerobic digesters treating municipal sewage. These digesters usually function with a hydraulic retention time of 30 to 60 days and at a total solids concentration of 2.6%(m/v). Volumetric loading is limited since high loadings effect the displacement of the slow growing methanogens. Thus, the hydraulic retention time is coupled to the solids retention time.

A crossflow microfiltration unit has been constructed at Northern Waste Water Treatment Works, Durban, to concentrate sludge from a conventional anaerobic digester and, thus, facilitate operation with a higher solids concentration. In addition, this process should result in the retention of the active biomass which would otherwise be lost as a waste product of the treatment process. The solids retention time is, thus, decoupled from the hydraulic retention time. The net result could be higher volumetric loadings, increased microbial activity and increased volatile solids destruction and, hence, improvement in the efficiency of anaerobic digestion of sewage sludge. To test these, different experiments were conducted to specifically determine the effect of higher solids loads.

Preliminary experiments were undertaken to determine the biodegradability of primary sludge from the Northern Waste Water Treatment Works. Results showed that primary sludge of 76% VS could be reduced to approximately 48 to 50% VS during an experimental period of 85 days. Reduction of the first 20% VS was rapid if conditions were optimum but subsequent reduction from 55 to 50% VS was slow. It was calculated that approximately 0.88 ℓ gas was produced for every g volatile solids catabolised.

Further experiments were conducted to investigate the effects of different solids concentrations on microbial activity. The results showed that the volume of gas produced increased as the solids

concentration increased from 2 to 6%(m/v). Digesters with solids concentrations of 6 to 13%(m/v) produced similar volumes of gas. Digesters with solids concentrations of 6 to 13%(m/v) TS produced approximately 300 ml more gas than the control during the 20 days experimental period. The rate of gas production also increased as the solids concentration increased. However, digesters containing 11%(m/v) and 13%(m/v) TS produced similar rates. These results indicate that the introduction of concentrated sludge into the digester improves digestion efficiency.

Finally, a semi-continuous digester was operated at a 30 days retention time and at optimum temperature to investigate the efficacy of digesters with increased solids concentrations. The results showed that the rate of gas production increased as the solids concentration increased from 2%(m/v)(control) to 3.8%(m/v). However, the digester operated with 4.7%(m/v) TS produced gas at a rate lower that the digester with 3.8%(m/v) TS. The volatile solids concentrations of all four digesters were similar, indicating neither favourable nor unfavourable effects from increased solids concentrations. The digesters operated with 3.8%(m/v) and 4.7%(m/v) TS produced higher concentrations of volatile acids than the control. The alkalinity concentrations ($\geq$ 4000 mg ℓ^{-1}) were similar for all four digesters.

Table of Contents

List of Figures

List of Tables

Glossary

acetogen	A microorganism capable of producing acetic acid
acidogenesis	The production of acids through the biological action of microorganisms
adenosine triphosphate	A compound containing a purine, a pentose and three phosphate groups, regarded as the energy currency in biological systems
aerobe	A microorganism which is capable of growth and metabolism in the presence of free oxygen
anaerobe	A microorganism which is capable of growth and metabolism in the absence of free oxygen
archaebacteria	Heterogeneous group of bacteria with a cell chemistry that is different from that of eubacteria. They are believed to be evolutionary relics
bactericidal	Capable of killing bacteria
bacteriostatic	Capable of retarding bacterial growth but not killing bacteria
bioconversion	Biological transformation of substances
biodegradation	Biological breakdown of substances by microorganisms
biomass	Mass of microorganisms
catabolism	That part of metabolism concerned with the breakdown of large protoplasmic molecules and tissues, often with the liberation of energy
cellulolytic enzymes	These are exoenzymes produced by cellulose decomposing bacteria to destroy cellulose
coenzyme	The non-protein portion of an enzyme which functions as an acceptor of electrons or functional groups

dewatering	Removal of water from solid material by solid-liquid separation techniques
effluent	The liquid waste of sewage and industrial processing
facultative anaerobes	An organism that grows well under aerobic and anaerobic conditions
fermentation	The breakdown of organic compounds in the absence of oxygen to obtain energy i.e., fermentation is life without air; the organic substrate undergoes a series of oxidative and reductive reactions
glycolysis	The enzymatic breakdown of glucose or other carbohydrates with the formation of lactic or pyruvic acid and the release of energy in the form of ATP
Gram negative	Gram negative bacteria are rapidly and completely decolourized and appear pink under the microscope
hydrolysis	Decomposition of a chemical substance by or in the presence of water
influent	An input stream of a fluid e.g., the primary sludge into an anaerobic digester
inoculum	Material (containing microorganisms) used to inoculate medium to initiate a biological conversion process.
interspecies hydrogen transfer	The continual removal of hydrogen by methanogenesis which shifts the equilibrium and thus, the fermentation balance towards more oxidized end products, with higher yields of ATP and biomass
lipolytic enzymes	These are exoenzymes secreted by bacteria to break down the lipid structure
mesophile	Bacteria that grow well in the midrange of temperature (20 to 45 °C)

methanogen	Bacteria capable of generating methane from their simple substrates
methanogenesis	Production of methane by methanogens
mineralization	Decomposition of plant and animal remains by microorganisms to its inorganic form
nitrate-reducing bacteria	Bacteria that reduce nitrate to ammonia by electrons derived from an organic substrate
non-biodegradable	Substances that cannot be broken down through the biological actions of microorganisms
obligate anaerobes	Microrganisms capable of growth only in the absence of oxygen
organotroph	A microorganism that requires carbon in the organic form for growth and metabolism
permeate	Atoms, molecules or ions that have moved through a porous or permeable membrane
phosphorylation	The formation of ATP by ADP by the transfer of a high energy phosphate group from an intermediate of a fuelling pathway
proteolytic enzymes	Exoenzymes secreted by bacteria to degrade proteins
psychrophilic	Organisms that grow well at 0 °C
scouring	An erosion process resulting from the action of the flow of e.g., water or slurry
sludge	A semi-solid waste from a chemical or biological process
slurry	A free flowing, suspension of fine solid material in liquid that can be pumped
sour or stuck digesters	These are digesters that have failed owing to toxic shock, organic or hydraulic overload

substrate(feed)	Substances that microorganisms are able to utilize for growth and metabolism
sulfate-reducing bacteria	These are strict anaerobes that are capable of utilizing a wide variety of oxidized compounds of sulphur as electron acceptors
symbiotic association	An inter-relationship between two different organisms in which the effects of the relationship is harmful or beneficia
thermophilic	Organisms that grow well at elevated temperatures (above 55 °C)

Abbreviations

Al		Aluminium
Alk		Alkalinity
atm		atmosphere
ATP		Adenosine triphosphate
BA		Bicarbonate alkalinity
BOD		Biochemical oxygen demand
CFMF		Cross-flow microfiltration
COD		Chemical oxygen demand
U.S EPA		United States Environmental Protection Agency
g		gram
HA		Homoacetogens
HAc		Acetic acid
HOM		Hydrogen-oxidizing methanogens
HRT		Hydraulic retention time
m		mass
MCRT		Mean cell residence time
N		Nitrogen
NRB		Nitrate-reducing bacteria

NWWTW		Northerm Waste Water Treament Works
OHPA		Obligate hydrogen-producing acetogens
P		Phosphorus
SRB		Sulphate-reducing bacteria
SRT		Solids retention time
TA		Total alkalinity
TS		Total solids
TVA		Total volatile acids
v		volume
VA		Volatile acids
VFA		Volatile fatty acid
VS		Volatile solids
UASB		Upflow anaerobic sludge blanket

Chapter One

Introduction and Literature Survey on Anaerobic Digestion of Wastewater Sludges

1.1 INTRODUCTION

Anaerobic digestion originated from the rather crude septic tank systems and the Imhoff Tank for waste stabilisation and has since evolved through a series of modifications into the high rate processes presently employed at many water pollution control plants. Whatever the process configuration, organic materials are sequentially and continuously transformed into simpler intermediates and final end mineralisation products with the concomitant synthesis of biomass. Initially anaerobic digestion was considered to be diphasic, relying on two separate groups of microorganisms(Canale, 1971; Pohland, 1971). However, further research has revealed that complete digestion is dependent on three phases of digestion viz: hydrolysis (non-methanogenic phase), acidogenesis (non-methanogenic phase) and methanogenesis (Gray, 1989; Malina, 1992). The capacity of each group of microorganisms is different, specific to its respective phase and primary products, and responsive to varying environmental stresses. In conventional practice, these phases coexist within the same physical and chemical environment and process efficiency and control requirements are, therefore, determined by the sensitivity and kinetic characteristics of the rate-limiting phase (Canale, 1971; Pohland, 1971; Gray, 1989; Malina, 1992).

1.1.1 Recent Developments

Initially, anaerobic digestion was in open lagoons at ambient temperatures with sludge retention periods of several months. The heated mesophilic digesters were developed in the late 1920's. By raising the temperature of digesting sludge to 25 to 35 ^{0}C an increased rate of treatment resulted, enabling shorter retention times. By the 1930's the digester tanks were installed with gas collection, sludge heating and mixing systems (Bruce, 1986). More recently, anaerobic digestion has been applied to chemically treated primary sludge, which contains the chemicals required for phosphorus removal , biological sludge, produced by the activated sludge or trickling filter processes, and sludge mixtures containing significant industrial waste components which exhibit markedly different chemical and physical characteristics (Parkin and Owen, 1986).

Thus, over the years, a number of design configurations have been used in anaerobic treatment (Figure 1.1). The completely-mixed reactor (CSTR) and anaerobic contact processes are systems which have been in operation for years. They are generally considered dispersed-growth systems. Upflow and downflow packed beds have been gaining popularity since 1970. They tend to function as biofilm reactors. Over the past decade, however, the upflow anaerobic sludge blanket (UASB), fluidized bed, expanded bed, and baffled reactors have been introduced. All are capable of exceptionally high volumetric rates of treatment (6-20 kg COD m^{-3}.day) for relatively dilute industrial wastewater (1000 mg l^{-1} COD). All offer the possibility of maintaining a large bacterial biomass within the reactor system as required for high decomposition rates per unit volume. In addition, they tend to act more like biofilm than dispersed growth systems. The success of the UASB reactor is the result of the development of a granular sludge of nearly spherical particles approximately 10.5 cm in diameter. These particles consist of a dense mixture of microorganisms responsible for the specific anaerobic treatment. In fluidized and expanded bed systems, an active biofilm develops on small, dense,

inert support material. Granular particles also develop within baffled reactors. Thus , most of the newer anaerobic reactors which offer stable and reliable treatment of industrial wastewater can be modelled mathematically and conceptually as biofilm systems. The presence of biofilms appears to be one of several reasons for their operating stability (McCarty and Smith, 1986).

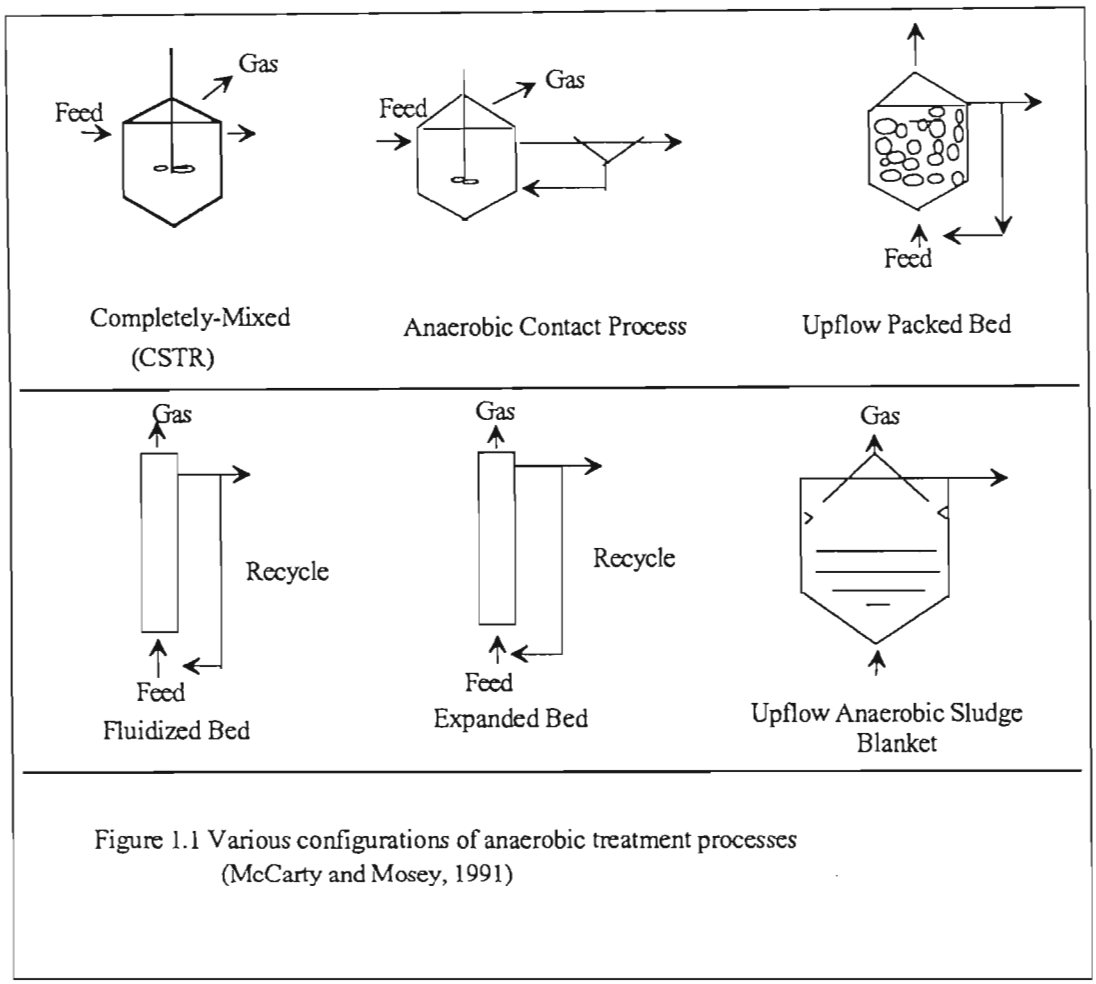

Figure 1.1 Various configurations of anaerobic treatment processes
(McCarty and Mosey, 1991)

Apart from the introduction of mixing and heating facilities and better control measures, anaerobic digestion of municipal sludge has undergone no significant improvements. Anaerobic digestion of sewage sludge can be divided into two systems : the standard rate digester; and the high rate digester. The standard rate digester has no heating facilities and mixing is sometimes provided. In the unmixed digester three zones develop, the scum layer on the top followed by the supernatant and the sludge zones. The scum layer has an actively

decomposing layer and a relatively stabilised bottom layer. The contents of the high rate digester, however, are well mixed and heated in the mesophilic range (32 ^{0}C to 37 ^{0}C). Furthermore, the system operates at lower retention times (15 to 25 days) and at higher loading rates (Ross *et al.*, 1992). A general comparison of the two systems is shown in Table 1.1.

The principles of high rate anaerobic reactors are based on 3 fundamental aspects:

1. Accumulation within the reactor of biomass by means of settling, attachment to solids or by recirculation. Such systems allow the retention of slow growing microorganisms by ensuring that the mean solids residence time becomes much longer than the mean hydraulic residence time;

2. Improved contact between biomass and wastewater overcoming problems of diffusion of substrates and products from the bulk liquid to biofilms or granules; and

3. Enhanced activity of the biomass due to adaptation and growth (Iza *et al.*, 1991).

Table 1.1 Comparison of loading data between standard and high rate digester (Ross *et al.*, 1992)

PARAMETERS	STANDARD RATE DIGESTER	HIGH RATE DIGESTER
Heating	no	yes
Mixing	sometimes	yes
Loading (kg VS m^{-3} d^{-1})	0.5 - 1.5	1.5 - 3.0
Retention Time (days)	30 - 60	15 - 25

Digester performance is dictated by the relative balance of viable populations among the major types of bacteria. Thus, the key to efficient anaerobic digestion is to develop and maintain a

large, stable and viable population of methanogens. In order to accomplish this goal it is necessary to provide: adequate contact between the bacterial population and appropriate nutrient sources in the substrate (i.e. efficient mixing); a suitable, uniform environment; and sufficient bacterial retention time. Most problems encountered in anaerobic digestion are associated with non-uniform, unstable or other unusual conditions in the reactor (Parkin and Owen, 1986).

However, if there are any doubts on the process of anaerobic digestion they centre around process efficiency and reliability since many potential residues for bioconversion are relatively recalcitrant and many materials are toxic or bacteriostatic to the highly sensitive methanogenic population. Anaerobic processes have been demonstrated to treat wastewater efficiently. However, some missing aspects of technology include: recognition of process potential, a policy which encourages development of the technology; and evaluation of the effects of environmental field variations on the processes. Finally, there are two general approaches to improving a biological process:

1. Select faster growing organisms or use processes that have a large number of slow growing organisms; and
2. Since the selection of better organisms is of limited value in treatment systems, concentrating the existing organisms holds greater promise. (McCarty and Smith, 1986).

1.1.2 Cross-Flow Microfiltration : An Emerging Technology

In recent years the importance of membrane technology, both technically and commercially, in many industrial applications is becoming increasingly popular. Membranes for sewage treatment, which many believe are unsuitable for the task, are currently being developed for improving the quality of effluents and clearly hold great promise (Gosling and Brown, 1993). Cross-flow microfiltration (CFMF) is an emerging process for concentrating sludge and removing suspended and colloidal matter. The process is capable of concentrating sludge more effectively than the gravity process and at the same time produces a suspended solids-free overflow (Treffry-Goatley *et al.*, 1986).

Cross-flow microfiltration can be defined as a process which retains undissolved particulate material by the filtration barrier with tangential suspension flow (Bindoff *et al.*, 1988).The process operates by pumping slurry, tangentially, through a flexible woven fabric tube under pressure. This action causes the suspended matter to be deposited on the inside of the filter tube (Figure 1.2). The slurry permeates through the filter cake depositing more suspended matter, leaving a clear filtrate which is collected for additional processing and the concentrate is recycled. The continuous cross-flow velocity of slurry through the 25 mm diameter tubes scours the dynamic membrane and prevents the accumulation of fouling matter (Bindoff *et al.*, 1988; Rencken *et al.*, 1989; Gosling and Brown, 1993). An equilibrium (steady-state) is eventually reached where the rate of deposition is equal to the rate of scouring (Hunt, 1987; Bindoff *et al.*, 1988; Pillay, 1991). Cross-flow microfiltration is a concentrating process and does not produce a low moisture content cake. This process operates on a high recycle and a low water recovery basis to produce a clear permeate and a concentrated slurry (Bindoff *et al.*, 1988).

For waste stabilization, the anaerobic digestion process has several advantages over the aerobic process. These include a significantly lower operating cost and sludge production rate per

kilogram of organics oxidized, the potential for chemical energy production through methane and the generation of a sludge which is relatively odour free and easy to dewater. The process, however, is limited by a low bacterial growth rate which, until fairly recently, has restricted the use of the process for the treatment of low volume streams such as raw sewage sludge (Treffry-Goatley *et al.*, 1986).

Conventional anaerobic digesters, which are single pass reactors with no selective solids recycle, are currently used for primary sludge digestion (Malina, 1992; Pillay *et al.*, 1994). The concentration of solids (biomass) within such a system is relatively low (ca. 1.5 to 3% TS). In conventional systems the solids retention time is the same as the liquid retention time. Since anaerobic digestion of sewage sludge requires longer retention times for effective volatile solids destruction, the hydraulic loading rate of the digester is restricted to prevent washout of the slow growing bacteria (Pillay *et al.*, 1994). Thus, CFMF, using a flexible woven hose, has been suggested as a process that may be used in conjunction with an anaerobic digester either as the sludge concentrator prior to sludge dewatering or for the side stream concentration and recycle of digester solids (Figure 1.3) (Treffry-Goatley *et al.*, 1986). Laboratory-scale cross-flow microfiltration units have been used to concentrate both activated sludge solids and anaerobic digester solids. Potential advantages for concentrating digested sludge are:

1. Increased solids loading in the digester, thus improving the digester performance;
2. Increased solids concentration to the dewatering equipment; and,
3. A suspended solids-free permeate to return to the head of the works (Bindoff *et al.*, 1988).

Pilot-scale studies by Bindoff *et al.* (1988) (Table 1.2), using a locally developed woven fabric cross-flow microfilter, indicated that anaerobic digester solids concentration may be increased to between 45 and 80 g ℓ^{-1} using this process i.e., a doubling of the total solids in the digester from 2.6% (m/v) to 5.5% (m/v). This effectively decoupled the solids retention time from the

hydraulic retention time, enabling the volumetric loading rate to be increased. The HRT was decreased from 26 days to 14 days while the SRT remained constant at 26 days. The volumetric flow-rate was almost doubled from 70 $\ell\ d^{-1}$ to 130 $\ell\ d^{-1}$. This implies that the volumetric and solids loads to, and within, the digester can be increased without decreased volatile solids destruction. Furthermore, the need for thickening the sludge by secondary digestion can be eliminated and the capacity of the installed dewatering equipment is increased. The solids in the supernatant liquor are reduced thus reducing the recycle of solids within the sewage works (Bindoff *et al.*, 1988; Pillay, 1994)

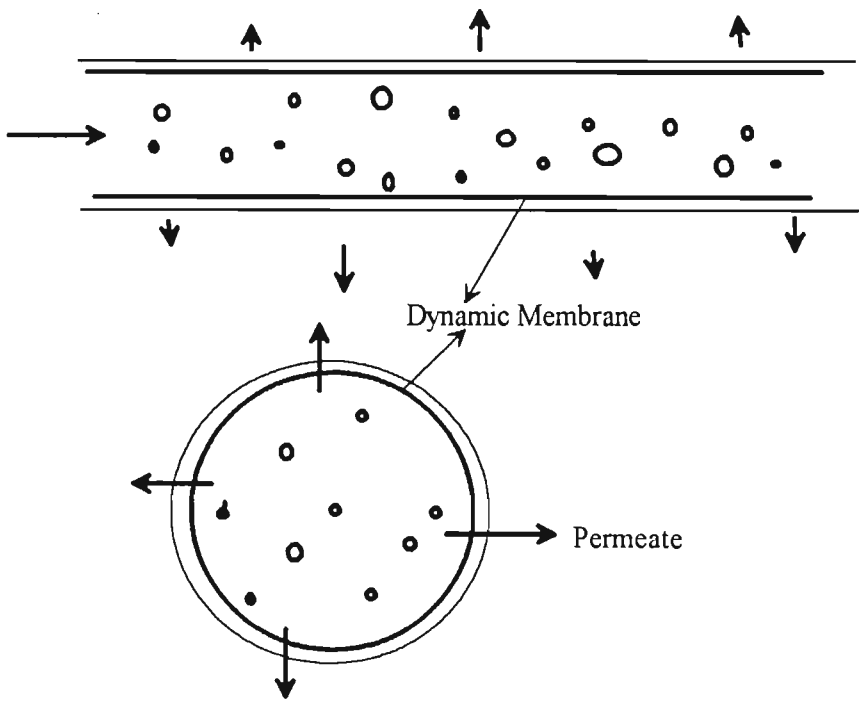

Figure 1.2 Diagrammatic representation of the crossflow microfiltration process (Gosling and Brown, 1993).

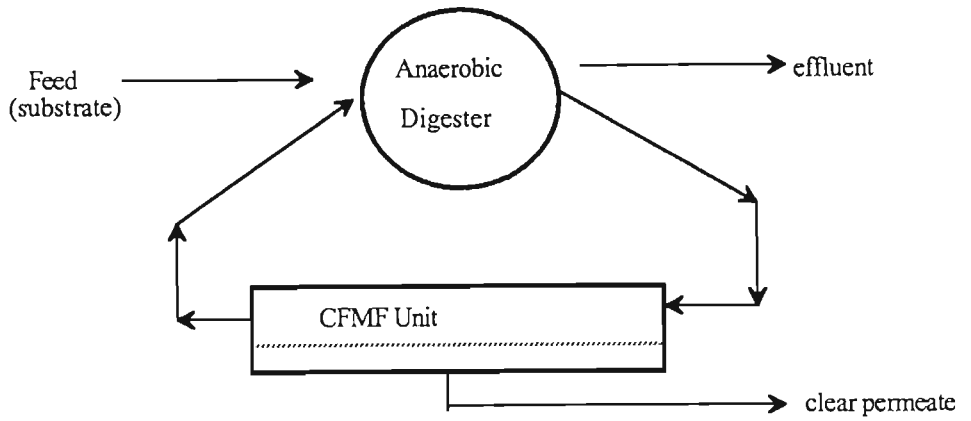

Figure 1.3 Diagrammatic representation of Coupled CFMF / Anaerobic Digestion Process (Pillay *et al.*, 1994)

Table 1.2 Digester operating conditions before and after cross-flow microfiltration (Bindoff *et al.*, 1988).

	Before CFMF	After CFMF
Digester solids concentration (g l^{-1})	26	55
Digester loading (kg VS m^3 d^{-1})	1.8	3.1
Feed to digester (l d^{-1})	70	130
Hydraulic retention time (d)	26	14
Solids retention time (d)	26	26

Further studies on membrane treatment for sewage sludge revealed that CFMF with a woven polyester tube resulted in a considerable reduction in bacteria and viruses in the permeate samples. Suspended solids and BOD levels were also reduced substantially after passing through the cross-flow microfilter (Table 1.3). Phosphorus concentrations decreased from 8.0 mg ℓ^{-1} to 0.64 mg ℓ^{-1} (Gosling and Brown, 1993). A comparitive study between the qualities of the CFMF permeate and the secondary digester overflow was conducted by Bindoff *et al.* (1988)(Table 1.3). The chemical oxygen demand (COD) and permanganate values of the CFMF permeate were almost 50% lower than those of the secondary digester overflow while the suspended solids concentration was almost 5 times lower. These results indicate that a digester coupled to a cross-flow microfilter unit could not only operate at a higher solids concentration and volumetric loading but also produce permeate of high quality. Thus, the solids in the supernatant are reduced thereby reducing the recycle of solids within the wastewater treatment works. An economic feasibility study undertaken by Pillay *et al.*, 1994 showed that a CFMF unit coupled to an anaerobic digester an economically feasible improvement to conventional anaerobic digestion. The volatile solids destruction of 55.7% was projected, while the final effluent fed to the dewatering equipment had a total solids concentration of 4.5% TS (Pillay *et al.*, 1994).

In summary, cross-flow microfiltration unit aims to improve the process by increasing the solids retention time, thus retaining the active biomass (Treffry-Goatley *et al.*, 1986). Therefore, CFMF can be used for retaining and concentrating solids in the reactor. Its use could potentially improve the performance of the anaerobic digestion process for treating both high strength industrial effluents and sewage sludge (Treffry-Goatley *et al.*, 1986).

Table 1.3 Comparison of permeate quality from the CFMF process and that of digester effluent and supernatant of secondary digester overflow (Bindoff *et al.*, 1988; Gosling and Brown, 1993)

Chemical analysis ($mg\ell^{-1}$)	Influent	Effluent	Permeate- CFMF	Supernatant*
	Values from Gosling and Brown (1993)		Values from Bindoff *et al.* (1988)	
Phosphorus P	8	0.64		
BOD	19	1		
Suspended solids	38	2	122	570
Al	0.84	0.11		
COD			700	1,300
Permanganate value			86	160
* Supernatant from secondary digester overflow				

1.2 MICROBIOLOGY OF ANAEROBIC DIGESTION

Anaerobic digestion of organic matter to carbon dioxide and methane requires the concerted action of various metabolic groups of bacteria, whose biochemical behaviour can be dynamic (i.e. short term changes in metabolic rates while populations remain constant) or transient (i.e. long term shifts in microbial ecology). These organisms can broadly be classified as hydrolytic fermentative bacteria, syntrophic acetogenic bacteria and methanogenic bacteria (Wu *et al.*, 1990; McCarty and Mosey, 1991; Harper and Suidan, 1991). The overall conversion process is illustrated in Figure 1.4 and involves both direct and indirect symbiotic associations between the different groups of microorganisms. Methane is generated as a result of nine recognizable steps (represented numerically in Figure 1.4), each linked by a specific group of microorganism and their enzyme complements:

(1) Enzymatic hydrolysis of organic polymers to intermediate monomers such as sugars, fatty acids and amino acids (Pohland, 1992);

(2) Fermentation of organic polymers to hydrogen (or formate), bicarbonate, pyruvate, alcohols and lower fatty acids such as acetate, butyrate and propionate;

(3) Oxidation of reduced organics to hydrogen, bicarbonate and acetate by obligate hydrogen-producing acetogens (OHPA);

(4) Acetogenic respiration of bicarbonate by homoacetogens (HA);

(5) Oxidation of reduced organic products to bicarbonate and acetate by nitrate-reducing bacteria (NRB) and sulphate-reducing bacteria (SRB);

(6) Oxidation of acetate to bicarbonate by NRB and SRB;

(7) Oxidation of hydrogen by SRB and NRB;

(8) Aceticlastic methane formation;

(9) Methanogenic respiration of bicarbonate.

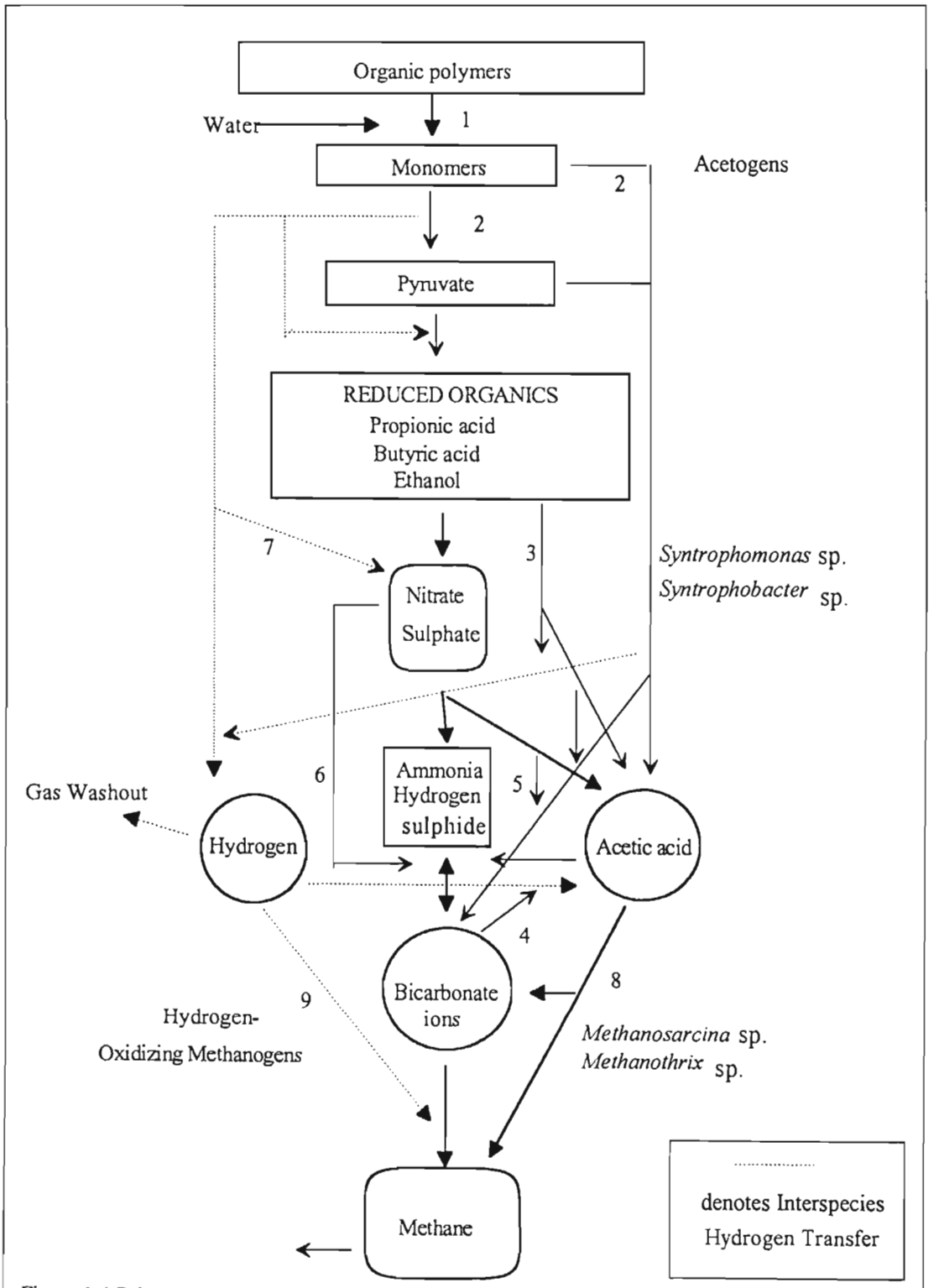

Figure 1.4 Substrate conversion pathway associated with anaerobic digestion of municipal and industrial wastewaters (Pohland, 1992).

1.2.1 Hydrolytic Phase

Hydrolysis (non-methanogenic phase) is the first stage of anaerobic digestion during which complex substrates are hydrolyzed to their basic components viz. proteins to amino acids, fats to glycerol and long-chained fatty acids, and polysaccharides to mono- and disaccharides with the aid of extracellular proteolytic, lipolytic and several cellulolytic enzymes, respectively (Bailey and Ollis, 1986; Gray, 1989). Proteins are catabolized to smaller units such as polypeptides, oligopeptides or amino acids by the enzyme, protease, produced by a small proportion of bacteria. These smaller peptides and amino acids permeate through the cell wall to be broken down intracellularly by the majority of the bacteria. The protease-producing bacteria form a small percentage of the total bacterial population but produce an excess of the enzyme protease. The most active proteolytic bacteria are *Clostridium* spp (spore-formers). It is estimated that 65% of the proteolytic bacteria are spore formers, 21% are cocci and the remaining 14% are non-sporing rods and bifid-like bacteria. The cellulolytic bacteria present in anaerobic digesters are predominantly Gram-negative coccobaccilli, with *Bacteriodes ruminicola* a common species. The ability to hydrolyze starch is the most common activity of these bacteria (Gray, 1989).

1.2.2 Acidogenesis

This second acid forming stage (non-methanogenic phase) is responsible for the microbial conversion of the hydrolyzed substrate to organic acids and alcohols, with the production of new bacterial cells (Corbitt, 1989). In anaerobic digester systems propionate and butyrate are thought to be the major intermediates of the hydrolysis-fermentation stage. This heterogeneous group of bacteria are responsible for hydrolysis and acid formation (Corbitt, 1989; Gray, 1989; Wu *et al.*, 1990). The acid-forming organisms are generally facultative, although some are strict anaerobes. The acid formers are relatively tolerant to pH changes, and grow more rapidly than the methanogens. Facultative organisms can use, and in fact prefer, molecular oxygen during metabolism. These microorganisms, therefore, protect the obligate anaerobic bacteria from small

concentrations of free dissolved oxygen that may be introduced into the system with the influent substrate (Malina, 1992).

The butyrate-forming bacteria are obligate hydrogen-producing bacteria (e.g., clostridia). They have adapted to the low substrate concentrations and low hydrogen pressures of the anaerobic digester (McCarty and Mosey, 1991). Propionibacteria and enterobacteria produce propionate, acetate and formate in fixed ratios. Butyrate degraders can utilize butyrate, valerate, and other higher molecular weight fatty acids as substrates. Some of these acetogens can utilize branched volatile fatty acids such as *iso*butyrate and 2-methylbutyrate. Known butyrate degraders such as *Syntrophomonas* sp. and *Clostridium* sp.8 do not utilize propionate. *Syntrophomonas wolfei* is a versatile anaerobic bacterium capable of fermenting octanoic, heptanoic, caproic, valeric, and butyric acids into a mixture of acetic and propionic acids which results in hydrogen gas generation. Butyrate catabolism proceeds by β-oxidation and *S. wolfei* relies on the presence of hydrogen scavenging bacteria such as *Methanobacterium bryantii* to allow it to ferment these acids (Figure 1.5) (Wu *et al.*, 1990; McCarty and Mosey, 1991; Pohland, 1992).

Propionic acid is decarboxylated to acetic acid and can also be reductively carboxylated to butyric acid or propionyl CoA. Since most biochemical reactions are reversible, it is not surprising that butyric acid can be decarboxylated to propionic acid. This finding may be significant. For example, if all the glucose in carbohydrate degradation was fermented via a pathway through butyric acid first, followed by propionic acid ,and finally acetic acid, then two-thirds of the methane generated would come from hydrogen or formate, and only one-third from acetic acid. With the differences in substrate utilization rates among these two groups of methanogenic reactions, this could result in a two-fold or higher increase in the rate-limiting reaction rate (Harper and Suidan, 1991). The propionic acid produced is fermented to acetic acid by the slow growing bacterium *Syntrophobacter wolinii* which specializes in this reaction (Figure 1.5) (McCarty and Mosey, 1991).

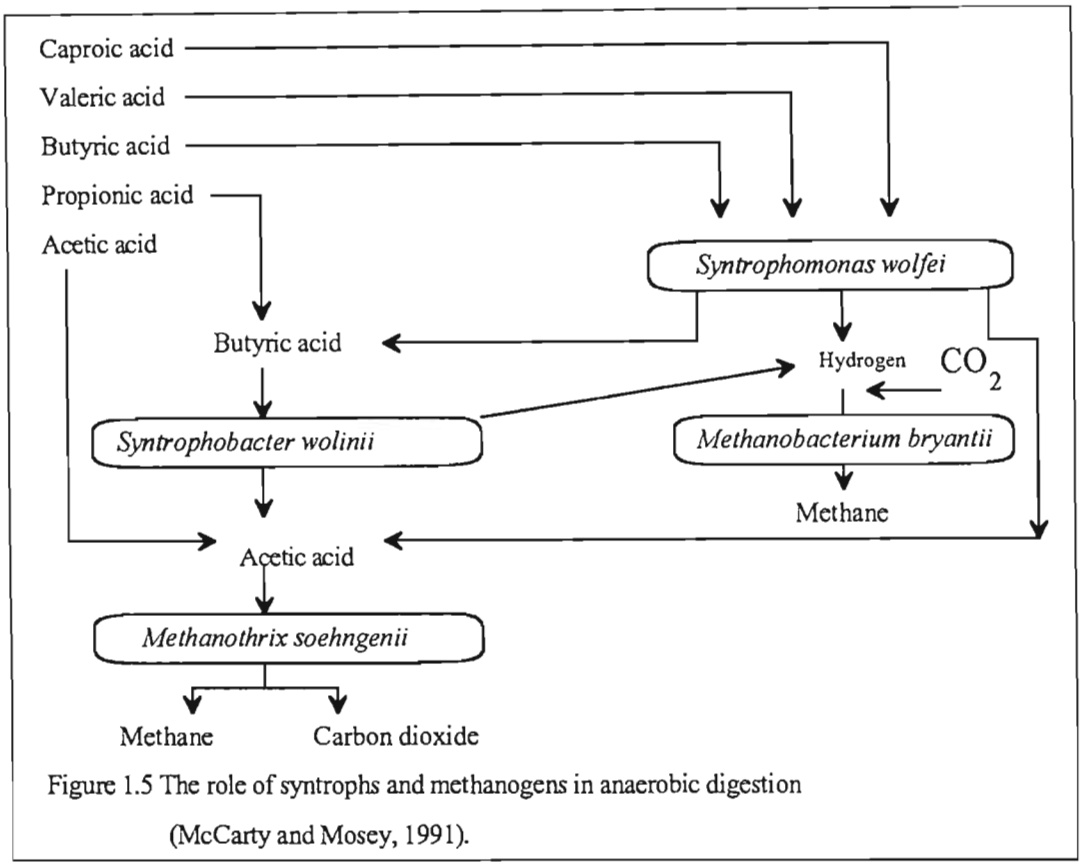

Figure 1.5 The role of syntrophs and methanogens in anaerobic digestion (McCarty and Mosey, 1991).

The generation time for *Syntrophomonas wolfei* oxidizing butyrate is about three days and *Syntrophobacter wolinii* growing on propionate is about seven days. Thus, the complete conversion of these substrates may require longer solids retention times, a feature of many conventional anaerobic digestion systems with enhanced biomass retention (Pohland, 1992). Another group of acid-producing bacteria, the homoacetogens produce acetic and sometimes other acids (Gray, 1989). Some acid is produced directly from pyruvate in the glycolysis pathway. Acetic acid may not always be produced independent of butyric or propionic acids. Harper (1989) estimated that 60% of the glucose converted during soft-drink wastewater treatment in packed bed anaerobic filters was channelled through butyric acid, and 40% through propionic acid. Therefore, all the acetic acid was produced from these acids, with one-third arising from propionic acid and the rest from butyric acid (Harper and Suidan, 1991).

The activities of fatty acid oxidizing bacteria are important for methanogenesis to proceed since low molecular weight fatty acids are common intermediate products of microbial conversion and cannot be catabolized by the methanogens. These reactions are thermodynamically possible only when the reduced products (hydrogen or formate) are maintained at low concentrations by the "scavenging" activities of the methanogenic bacteria. The accumulation of acids, particularly propionic acid, is a common indication of stress within the digester (Pohland, 1992).

1.2.3 Methanogenesis

The methanogenic bacteria are responsible for the major final step in the transfer of electrons from a number of donor species (Gray, 1989; Pohland, 1992). Most methanogenic bacteria belong to the genera *Methanobacterium*, *Methanosarcina*, *Methanospirillum*, and *Methanococcus*. Methanogens are composed of many species with very different cell morphologies. They require a strict anaerobic environment for growth with a redox potential -300 mV (Gray, 1989; Hespell, 1990). Methanogenic species have simple nutritional requirements of ammonia and sulphide as their nitrogen and sulphur sources, respectively. The synthesis of ATP occurs via electron transport linked to phosphorylation. Methanogens are considered by some workers to be a primitive group of bacteria (Archaebacteria) owing to their ultrastructure and cellular composition being different from typical bacteria. Their unique coenzymes and oligonucleotide sequences of the 16S ribosomal molecule and the absence of muramic acid in their cell wall structure has motivated the reclassification of this group (Gray, 1989).

In anaerobic digesters and high rate systems, *Methanothrix* sp. are the major acetate utilizing methanogens. *Methanobacterium*, *Methanospirillium* and *Methanobrevibacter* species are the H_2-CO_2 and formate utilizing methanogens while *Methanosarcina* sp. utilizes both acetate and H_2-CO_2 . The acetotrophic methanogens usually grow slower than the H_2 -CO_2 or formate utilizing methanogens (Wu *et al.*, 1990). Methanogens are crucial in the stabilization of a narrow array of simple substrates. Approximately two-thirds of the methane generated during

anaerobic microbial conversion is derived from the methyl moiety of acetate and about one-third is derived from carbon dioxide reduction (Pohland, 1992). Microorganisms producing methane as the end product of metabolism benefit by the production of cellular energy during the catabolism of extremely simple substrates at low reduction potential. When it was shown that the substrates for methanogenesis were limited to carbon dioxide , hydrogen, formate, methanol, *iso*-propanol, methylamines, acetate and carbon monoxide it became clear that the central metabolic pathway in methanogens involved the stepwise reduction of a one-carbon unit which was derived from the growth substrate. Methanogens are limited to simple growth substrates (Jones *et al.*, 1987).

Fixation and initial reduction of carbon dioxide are not well understood but central to the process are three coenzymes, unique to the methanogens, which are the one carbon carriers during the sequential reduction of carbon dioxide to methane. The coenzymes are MFR, H_4MPT, and coenzyme M. The terminal reduction of CH_3-S-CoM to methane by hydrogen involves two additional cofactors, component B and factor F_{430} whose functions have still to be resolved (Jones *et al.*, 1987). Methanogenesis from methanol or methylamines in the absence of hydrogen requires that the methyl carbon is dismutated to carbon dioxide and methane. Organisms of the family *Methanosarcinaceae* are capable of this reaction. Aceticlastic methanogenesis is dependent upon the cell to cleave the acetate molecule , reduce the methyl equivalent and oxidize the carboxyl equivalent (Jones *et al.*, 1987). Thus, methane is formed by a disproportionation reaction whereby some of the substrate is oxidized to generate reducing equivalents for methyl group reduction (Pohland, 1992). Coenzyme M has been identified as a carrier of the methyl equivalent from acetate and the methyl coenzyme M reductase is involved in the conversion of the methyl moiety to methane (Jones *et al.*, 1987). In addition, secondary alcohols, including 2-propanol and 2-butanol as well as primary alcohols, are partially oxidized and serve as electron donors for the reduction of carbon dioxide to methane (Pohland, 1992).

Acetic acid cleavage is a single step process carried out by one group of bacteria whereas propionic acid fermentation is a two step process involving two groups of methanogenic

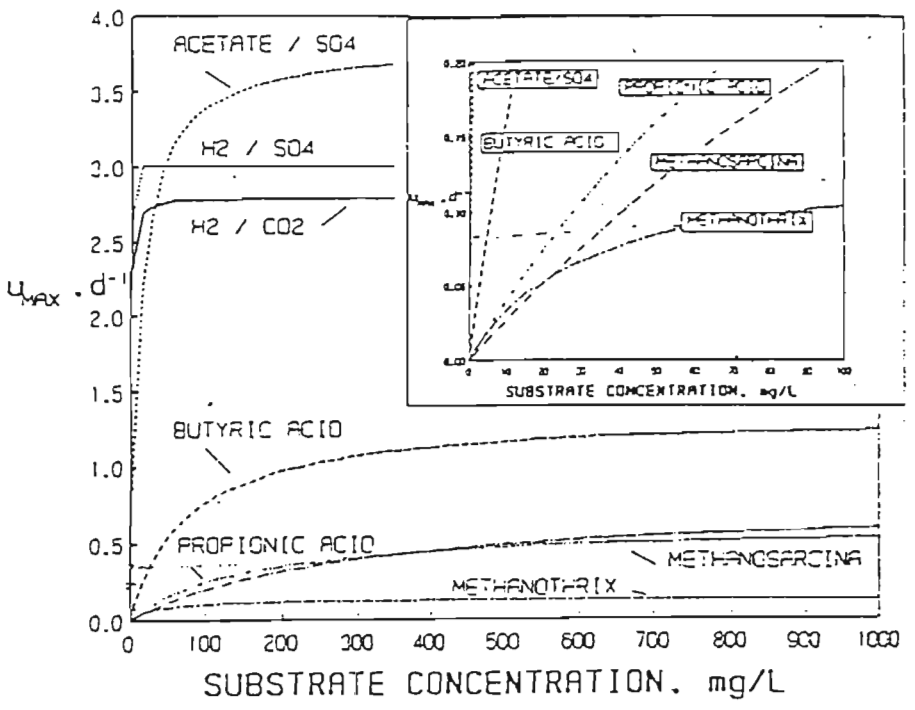

Figure 1.6 Growth rates of various anaerobic bacteria as a function of substrate COD (Harper and Suidan,1991)

bacteria, with acetic acid as the intermediate step (Gray, 1989). Methanogenesis is facilitated by various methanogenic species with specific substrate preferences (Table 1.4). The methane-forming bacteria are strict anaerobes that utilize simple substrates to produce methane. Each species of methane-forming bacteria can ferment only a narrow array of simple compounds to methane (Parkin and Owen, 1986; Gray, 1989; Malina, 1992). Thus, the complete stabilization of waste requires the concerted action of several species of methanogens. For example, the complete fermentation of valeric acid requires the interaction of three species of methane bacteria. *Methanobacterium suboxydans* oxidizes valeric acid to acetic and propionic acids, but cannot break these compounds any further. The propionic acid is converted by *Methanobacterium propionicum* to acetate, carbon dioxide, and methane. A third species, *Methanococcus mazei*, is necessary to cleave acetate to methane and carbon dioxide (Malina, 1992). This final stage of anaerobic digestion converts the end-products of the acid fermentation to gases, particularly, methane and carbon dioxide. Since the end-products of methanogenesis

are only gases it is a more efficient waste minimization process than complete aerobic biodegradation (Gray, 1989).

In anaerobic digesters and high rate reactor systems, *Methanothrix* spp. are the major acetate utilizing methanogens. *Methanobacterium*, *Methanospirillium* and *Methanobrevibacter* species are the most frequently isolated H_2-CO_2 and formate utilizing methanogens. *Methanosarcina* sp. utilizes both formate and acetate. *Methanothrix* spp. and *Methanosarcina* sp. ferment acetate to carbon dioxide and methane but they have very different morphology and growth kinetics (Figure 1.6) (McCarty and Mosey, 1991).

Table 1.4 Classification of methanogens by substrate specificity (McCarty and Mosey, 1991; Pohland, 1992)

Rod-shaped cells	Substrates
Methanobacterium formicum	formic acid, carbon dioxide, hydrogen
Methanobacterium propionicum	propionic acid
Methanobacterium sohngennii	acetic acid, butyric acid
Methanobacillus omelianski	primary and secondary alcohols, hydrogen
Methanothrix spp.	acetic acid
Spherical cells	
Methanococcus mazei	acetic acid, butyric acid
Methanococcus vannielii	formic acid, hydrogen
Methanosarcina barkeri	methanol, acetic acid, carbon monoxide, hydrogen
Methanosarcina methanica	acetic acid, butyric acid

Methanothrix spp., are sheathed rods, sometimes growing as long filaments. They grow slowly with minimum doubling times of approximately 4 days at 35 °C. They survive because they have a high affinity for acetate (k_s= 30 mg ℓ^{-1} at pH 7.0). *Methanosarcina* spp. are coccoid bacteria which grow together in discrete clumps. They grow faster with minimum doubling times of 1.5 days but they have a lower substrate affinity (k_s= 400 mg ℓ^{-1} at pH 7.0) (McCarty and Mosey, 1991). Pohland (1992) reported that these methanogens have 24h doubling times. Both

Methanosarcina sp. and *Methanothrix* sp. constitute the primary aceticlastic methanogens. They are, however, relatively slow growing organisms and uncompetitive with the more rapidly growing hydrogenotrophs (hydrogen-oxidizing methanogens) with one to four hours doubling times. Moreover, the aceticlastic methanogens are adversely affected by the accumulation of hydrogen and low hydrogen concentrations are important if these species are to contribute effectively to overall substrate conversion and mineralization (Pohland, 1992).

1.2.4 Interspecies Hydrogen Transfer

When a particular metabolic pathway dominates a specific substrate conversion sequence it is frequently regulated by hydrogen or formate production and its potential for accumulation to inhibiting levels (Pohland, 1992). Excessive accumulations of hydrogen or intermediate products can result if there is a lack of syntrophy between the hydrogen-producing acidogens and the hydrogen-consuming methanogens, sulphate-reducing bacteria and nitrate-reducing bacteria unless other hydrogen sinks (e.g., Fe^{3+}, Mn^{4+}, oxygen, unsaturated compounds etc.) are available (Pohland, 1992). Among the first 50 species of methanogens isolated, 38 species utilized hydrogen as their substrate. The minimum generation times of these organisms is between 4 and 11 hours (Zhang and Noike, 1991). Oxidation of organic compounds coupled to reduction of various electron acceptors decreases in the order $O_2 > NO_3 > MnO_2 > FeOOH > SO_4 > CO_2$ (Pohland, 1992).

Hydrogen inhibition of anaerobic microbial conversion usually requires both ultimate cleavage of acetate and reduction of carbon dioxide (Pohland, 1992). In addition, degradation of higher fatty acids such as butyric and propionic acids is facilitated by organisms which grow only when hydrogen is used by the hydrogenotrophs, a process termed "interspecies hydrogen transfer". Even at low concentrations, hydrogen has an effect upon the pathway of flow of carbon during mineralization of organic matter. Hydrogen concentrations in the biogas of anaerobic sludge digesters range from 36 to 220 x 10^{-6}, with an average of 73 x 10^{-6}. These values are all less than the thermodynamically calculated inhibitory value of 4 x 10^{-4} kPa (Mosey, 1982).

At hydrogen pressures below 10^{-4} atm, the continuous production of acetic acid from influent and intermediate organics such as ethanol, butyric and propionic acids is assured in stable anaerobic digesters (Mosey, 1983). Consequently, the efficient degradation of organic waste becomes a function of the acetate utilization capacity provided by either *Methanothrix* or *Methanosarcina* spp., the aceticlastic methanogens (Harper and Pohland, 1987). The redox potential of the process is regulated by the very rapid growing hydrogen utilizing bacteria which convert hydrogen and carbon dioxide to methane. The rapidly growing hydrogen utilizing bacteria use the hydrogen, thus keeping hydrogen concentrations low and allowing acid formation to continue, until the acid concentrations are high enough to effect product inhibition (Mosey, 1983; Dohanyos *et al.*, 1985). However, imbalance between the organotrophic proton-reducing bacteria and hydrogenotrophic methanogens may lead to accumulation of hydrogen. High hydrogen partial pressures have been shown to inhibit the formation of acetate from reduced intermediate metabolites by obligate syntrophic acetogenic bacteria (Mosey, 1982). One of the effects of high hydrogen concentrations involves the oxidation of alcohols and 3-carbon, or longer chain acids to acetate by proton reducers such as *Syntrophomonas* and *Syntrophobacter*. Increases in hydrogen concentrations lead to alcohol or acid degradation inhibition (Harper and Pohland, 1987). As much as 30% of the electrons associated with methane production flow through propionic acid and hydrogen. Thus, hydrogen is an important intermediate, and the bacteria responsible for its conversion must be present in sufficient numbers for the process to operate efficiently. Propionic acid has a relatively high Gibbs free energy value for its oxidation and requires the concentration of either acetate or hydrogen, or both, to be sufficiently reduced to provide a favourable free energy change. Therefore, it is hydrogen that tends to be most important in the control of the process (McCarty and Smith, 1986). Thus, propionic acid catabolism leads to its accumulation, causing pH reductions, amplifying process instability and reducing treatment efficiency.

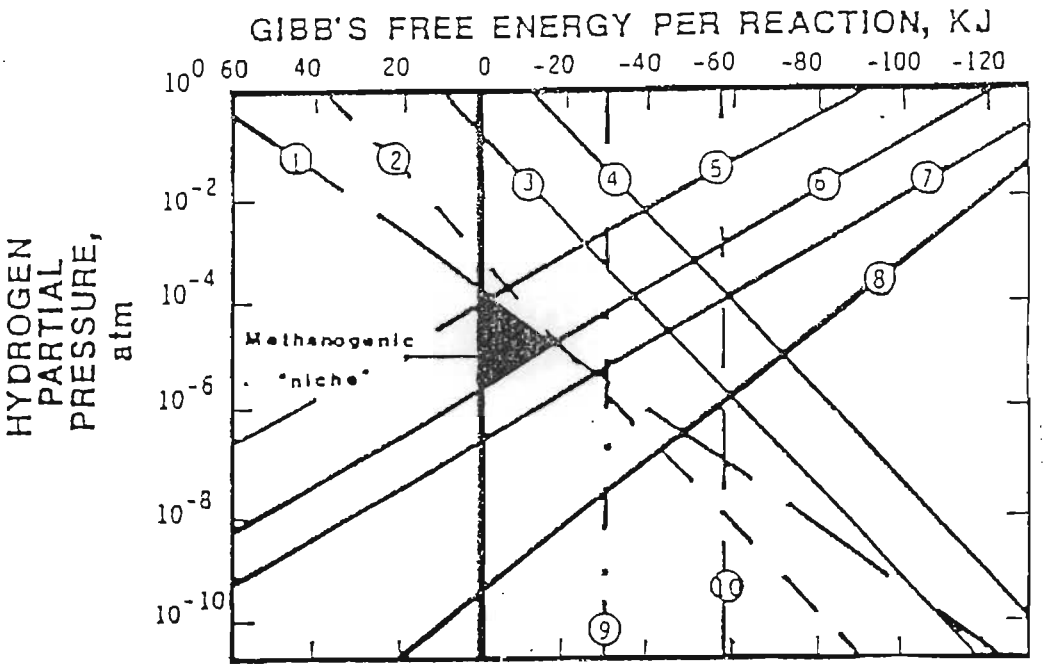

Figure 1.7 Graphical representation of the hydrogen dependent thermodynamic favourability of anaerobic reactions (Harper and Pohland, 1987).

It is imperative for hydrogen partial pressures to be maintained as low as possible to facilitate continuous and efficient oxidation of propionic and higher acid analogues. This problem may be overcome by increasing either the biological hydrogen removal capacity (methanogens, sulphate-reducing bacteria, nitrate-reducing bacteria) or the physical hydrogen removal of hydrogen (i.e. gas phase washout). Such control must recognize the substrate requirements of the other syntrophic bacteria, particularly the aceticlastic methanogens. At elevated hydrogen concentrations, hydrogen oxidation becomes more energetically favourable than acetate cleavage. Hydrogen partial pressures above 10^{-4} atm are undesirable because they promote metabolism by *Methanosarcina* spp.. This in turn limits acetate removal capacity and increases acetogenesis through homoacetogenesis (Harper and Pohland, 1987). Continuing acidogenic activity results in a decrease in pH and, ultimately a reduction or inhibition of methane production (Mosey, 1982; Kidby and Nedwell, 1991).

Thus, obligate interspecies hydrogen transfer requires syntrophy between the production of hydrogen from the acids and its utilization by methanogens to permit reactions which yield energy for the growth of both species. The "thermodynamic barrier" to the reduction of protons to hydrogen is characteristic of such syntrophic associations and such a barrier can be overcome by coupling the formation of hydrogen to the reduction of carbon dioxide to methane (Pohland, 1992). When the thermodynamic calculations for typical reactions in anaerobic digestion are plotted against hydrogen partial pressure, a methanogenic "niche" can be established (Figure 1.7). Identification of such thermodynamically favourable niches for syntrophic associations can be used to establish possible process configurations as well as control options. Propionic acid oxidation (1) to acetate becomes favourable only at hydrogen partial pressures below 10^{-4} atm while butyric acid oxidation (2) is favourable below 10^{-3} atm and ethanol and lactate oxidation are favourable below about one atmosphere (Figure 1.7). Similarly bicarbonate respiration is favoured to acetic acid cleavage at hydrogen partial pressures above 10^{-4} atm. Furthermore, sulphate reduction (7) is favoured to bicarbonate respiration and the favourability of acetate cleavage by sulphate-reducing bacteria compared with cleavage by methanogens (9 and 10) is shown in Figure 1.7. Similarly, thermodynamic calculations illustrate the favourability of nitrate reduction to ammonia (and to nitrogen gas) is an order of magnitude greater than that of methanogenic reactions. This has important process implications such as redox incompatibility of methanogenesis and dissimilatory nitrogen reduction (Harper and Pohland, 1987; Pohland, 1992).

1.2.5 The Kinetics Of Anaerobic Digestion

Operational models are derived to describe the consequences of growth-limiting substrates, essential nutrients and /or environmental conditions on microbial metabolism and growth. Such models are used in defining process configurations and control options. The hydrolysis of complex organic molecules is generally described by models which follow first order kinetics. The Monod model on the other hand is used to simulate growth on labile substrates. The Grau, Contois, and Chen and Hashimoto models contend that the predicted effluent substrate concentration, S, is a function of the influent substrate concentration, S_o. This differs from the Monod expression where S is independent of S_o and substrate loading effects are not addressed (Pohland, 1992).

Initial hydrolysis is an important reaction involving a wide variety of complex substrates such as sludges, animal wastes, refuse and biomass as well as a broad spectrum of bacteria. These solubilization reactions are facilitated by a number of extracellular enzymes (McCarty and Mosey, 1991). The reactions need to occur fast enough to prevent rate limitation of the overall conversion sequence. The hydrolysis rate constants can vary considerably due to the type of substrate and the experimental conditions. Hydrolysis rate constants of complex biopolymers range from 0.04 to 0.13, 0.54, 0.08 to 0.17, 0.02 to 0.03, 3.0, and 0.1 day^{-1} for cellulose, hemicellulose, lipids, proteins, sewage sludge, and wheat straw and corn stover , respectively . Anaerobic digestion of activated sludge is a slow and rate limiting process (Bailey and Ollis, 1986; Pohland, 1992).

Kinetic data for anaerobic fermentation of carbohydrates, oxidation of long- and short-chained fatty acids, aceticlastic methanogenesis and carbon dioxide reduction can be compiled using Monod type kinetics. There are significant variations in the kinetic results reported. The ranges of kinetic parameters obtained are substrate specific and are dependent on the microorganism culturing and selection process applied. Although the kinetic data obtained are largely dependent on the method of measurement, the kinetic factors are informative for process selection, design

and control. Table 1.5. highlights some representative values for overall process kinetics (Pohland, 1992).

Table 1.5 Representative kinetic data for anaerobic digestion at 35^0 C (after Pohland, 1992).

Process	k mgCOD mg VSS^{-1} d^{-1}	Y mgVSS mg COD^{-1}	K_s mgCOD ℓ^{-1}	μ_{max} ℓ d^{-1}
Acidogenesis	13	0.15	200	2
Methanogenesis	13	0.03	50	0.4
Overall	2	0.18	-	0.4

1.2.6 Environmental Factors Influencing Microbial Behaviour

a. pH

Anaerobic digestion systems operate efficiently at near neutral pH. Change from this optimum is usually introduced with the influent substrate or the high production and accumulation of acidic or alkaline conversion products which include organic fatty acids or ammonia, repectively (Gray, 1989; Pohland, 1992). Thus, if the acids are not oxidized as fast as they are produced their concentrations will increase. Consequently, the buffering capacity of the system will be exceeded and the pH will drop (Schroeder, 1977). Displacement of the neutral pH bicarbonate buffer system is considered to be more inhibitory to the methanogens than fermentative bacteria, which will continue to grow until a pH of 4.5 to 5.0 is reached. The continuation of acid production by the fermentative bacteria aggravates the environmental conditions within the digester (Pohland, 1992). Any continuous downward trend in pH is an important warning sign and requires immediate control measures (Gray, 1989).

Methanogenesis is known to occur in both acidic and alkaline environments, suggesting that methane production is not exclusively limited to a neutral pH. *Methanosarcina barkeri* and

Methanosarcina vacuolata are two well known acetate-degrading methanogens which grow well at low pH with an optimum of pH 5.0 when cultured on hydrogen and methanol as the catabolic substrates. Similarly, hydrogen-oxidizing methanogens and methylotrophic methanogens have been found at very alkaline pH values. No aceticlastic methanogens have been found (Pohland, 1992). It has been suggested that pronounced inhibition of aceticlastic methanogens is probably not experienced at pH values above 8.0 (Ghosh, 1987).

b. Temperature

Methanogenesis is strongly temperature dependent with reaction rates generally increasing with temperatures up to 60 °C. The warmer the reactor temperature , the faster the substrate removal rate and the faster the cell decay rate resulting in a shorter retention time for complete digestion. Two optimal temperature ranges, mesophilic (about 35 °C) and thermophilic (55°C to 60 °C) have been cited (Figure 1.8) (Corbitt, 1989; Gray, 1989; Pohland, 1992). Most anaerobic digesters are operated in the mesophilic temperature range, usually between 30 - 32 °C, with a residence time of 20 to 40 days (Corbitt, 1989; Ross *et al.*, 1992).

The drop in gas production rates at temperatures above 35 °C and 55 °C for mesophilic and thermophilic digestion, respectively, suggests different populations are responsible for thermophilic and mesophilic digestion. Erratic gas production occurs between the optima of the two temperature ranges. The initial stage of anaerobic digestion is not adversely affected by temperature since a number of different bacteria are involved. However, the acetogenic and methanogenic bacteria are sensitive to to even 2-3 °C falls in temperature in mesophilic digesters (Gray, 1989). Thermophilic systems require a smaller reactors than the mesophilic reactors. However, thermophilic reactors are very slow to startup and cannot tolerate variations in loading or substrate characteristics, or toxic compounds (Corbitt, 1989).

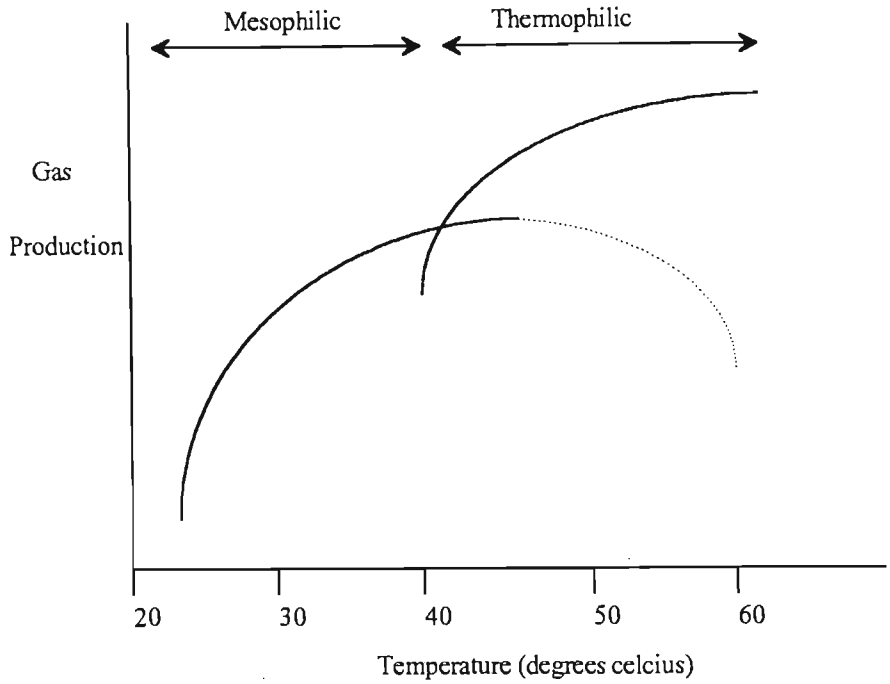

Figure 1.8 The effects of temperature on gas production (Gray, 1989).

c. Nutrients

Since many anaerobes are unable to synthesize some essential vitamins or amino acids, supplementation with specific nutrients for growth and metabolism is necessary. The gross level of essential nutrients can be evaluated if the biomass yield is known. The C:N ratio is frequently used to describe this micronutrient requirement but is occasionally affected by substrate specificity. COD:N ratios of about 400:7 and 1000:7 have been estimated at high and low substrate loadings, respectively. A ratio requirement of 7:1 for nitrogen and phosphorus, respectively, has been reported for anaerobic digestion (Pohland, 1992). Typical COD:N:P ratios of 1000:10:2 have also been cited. The specific nitrogen and phosporus requirements depend on the nature of the organic compounds to be catabolized and the sludge age (Corbitt, 1989).

Trace elements such as nickel, iron, cobalt, magnesium, calcium, sodium, barium, tungstate, molybdate and selenium are necessary for active methanogenesis. Selenium, tungsten and nickel

have been implicated in the enzyme systems of acetogenic and methanogenic bacteria. The formate dehydrogenase and hydrogenase of *Methanococcus vanielii*, the formate dehydrogenase of *Clostridium thermoaceticum*, and the hydrogenase of *Desulfovibrio desulfuricans* require the presence of selenium, tungsten and nickel, respectively. Generally, the mixed substrate systems have an abundance of essential nutrients, unless the waste is from a process which negates such introduction (Pohland, 1992).

d. Toxicity

Volatile fatty acid toxicity: Methanogenic bacterial growth is restricted in the presence of excessive amounts of volatile fatty acids, particularly when propionate accumulates. Such inhibition is manifested by deviations in environmental conditions brought about by pH and buffering capacity changes due to high acid build up. The overall inhibitory effect of the volatile fatty acids is related to the pH established by the prevailing buffer system, and may involve an increase in the concentration of the un-ionized or undissociated species. Un-ionized species exert a greater internal cellular effect as they migrate more readily across the bacterial cell membrane. Although some end production repression has been observed for acidogens, the more toxic effects of such accumulations are manifested on the methanogenic populations (Pohland, 1992).

Sulphide and ammonia toxicity: As with volatile fatty acids, hydrogen sulphide and ammonia, the products of sulphate and nitrate reduction are also capable of forming weak acid and weak base systems. These systems are usually less intensive and less likely to exert principal control on the pH unless the influent concentration contains high levels of sulfate and nitrate (Pohland, 1992). In the presence of sulphate, the sulphate-reducing bacteria can use the same substrates as methanogens viz., hydrogen and acetate. The sulphate ions act as the electron acceptors in the process. More energy is generated from the reduction of sulphate to sulphide than methanogenesis, making the latter noncompetitive. Methanogenesis can, thus, be competitively inhibited by the SRB or by direct inhibition of the cell functions by soluble sulfides. Ueki *et al.* (1988) however, reported that while sulphate reduction and methanogenesis share the role of hydrogen scavengers, they do not practically compete with each other. Electron flow to sulphate

reduction in the sludge with sulphate in elevated concentrations can be supported, apparently, without retarding electron flow to methanogenesis. The total reducing power distributed to methanogenesis is, however, finally reduced when compared with that in the absence of sulphate (Ueki *et al.*, 1988).

Sulphide toxicity to methanogens is directly proportional to its concentration in the substrate and hydrogen sulphide in the gas phase (Karhadkar *et al.*, 1987). The concentration of sulphides and ammonium species are pH dependent and the former can be rendered insoluble by association with cations. The formation of sulphide precipitates, as in the case of iron sulphide, effectively eliminates the potential toxic effects. Similarly, at elevated pH values, free ammonia might exist at concentrations sufficient to exert a toxic or inhibitory effect. Sulphide concentrations ranging from 200 to 1500 mg ℓ^{-1} are cited as toxic to the microorganisms (Pohland, 1992).

Corbitt (1989) reported that 200-300 mg ℓ^{-1} of total dissolved sulphide was toxic to the methanogens. However, the toxicity concentration is dependent upon the pH of the wastewater. High pH values allow the less toxic sulphide form, HS^- to predominate, while low pH values result in the formation of the more toxic H_2S. At neutral pH there will be equal concentrations of both species. Hydrogen sulphide toxicity can be reduced or eliminated by preventing hydrogen sulphide or sulphates from entering the wastewater , by diluting the wastewater below the toxic threshold, or by purging hydrogen sulphide from the wastewater. In addition, iron or aluminium salts can be introduced to the anaerobic reactor to form insoluble complexes with the sulphide ions (Corbitt, 1989).

In the case of the weak base, ammonia, microbial acclimation is important and is linked to the presence of volatile fatty acids and the effect of the acid-neutralizing capacity of ammonia on pH. Free ammonia is considered to be more toxic than the ionized ammonium species. However, the effect of high ammonia concentration is only bacteriostatic and methanogens are known to quickly adapt to moderately high concentrations (Van Velsen, 1979). *Methanobacterium formicum* has been reported to be partially inhibited at a total ammonia concentration of 3000

mg ℓ^{-1} and a pH of 7.1, whereas 4000 mg ℓ^{-1} ammonia caused complete inhibition. Non-methanogenic populations are functional at ammonia concentrations in excess of 6000 mg ℓ^{-1} and a pH of 8 (Pohland, 1992).

Heavy metal toxicity: Another factor influencing digester stability is heavy metal toxicity which affects microbial conversion processes. Primary sludge contains about 30% of the incoming metals, with an average concentration factor of metals in the sludge of about 30-40 times (Kouzeli-Katsiri *et al.*, 1988). This factor is influenced by oxidation-reduction potential, pH and ionic strength, and the resultant speciation of the metals or metal complexes. Free metals exert a toxic threshold above which inhibition or failure of the process occurs. Furthermore, the actual availability and fate of the heavy metals determine the intensity of the toxic effect, whether as a result of interference with certain enzyme systems or metabolic precursors (Pohland, 1992).

Sewage sludge treated with inhibitory concentrations of heavy metals produce a predictable response. Since the more sensitive methanogenic bacteria are affected, the volatile acids accumulate and ammonia concentrations increase, with a decrease in the percentage reduction of volatile solids, rate of gas production and the percentage methane generated. The pH also shows a slight decline. It was shown that the pH in digesters exhibiting strong inhibition did not drop during digestion indicating no significant accumulation of organic acids (Nasr and Abdel-Shafy, 1992). The pH varied between 7.0 and 6.6 with increasing metal concentration but the reduction in pH was caused by the acidity of the metallic salt itself. The experimental response was similar for both step feeding and pulse feeding of metals, except the magnitudes of the volatile acids and ammonia concentrations were less. This was possibly due to the rapid poisoning of all active bacteria in the digester. It was also reported that the volume of gas produced per g of volatile solids destroyed was independent of metal concentration (Kouzeli-Katsiri *et al.*, 1988; Nasr and Abdel-Shafy, 1992).

Research has shown that between 30 to 60% of the added metals in anaerobic digestion accumulated in the intracellular fraction of the sludge (Kouzeli-Katsiri *et al.*, 1988). This

indicates that microbial uptake competes actively with precipitation in the removal of heavy metals from solution. It was also noted that toxic effects occur when a large buildup of metals exists in close association with the cell mass. It has been suggested that toxic effects may be initiated before all sulphide or other precipitants in the digester have been exhausted (Kouzeli-Katsiri *et al.*, 1988). The order of decreasing toxicity of the heavy metals of most frequent concern has been recorded as Ni > Ca > Pb > Cr > Zn (Pohland, 1992). However, Nasr and Abdel-Shafy (1992) reported the general ranking for heavy metal toxicity of the metals investigated to be Cu > Cr > Pb > Zn.

Iron is considered to be more beneficial than detrimental because of its mediating effects on sulphide toxicity. Cyanide, which is often associated with heavy metals, displays toxic effects towards microorganisms, depending on exposure time and concentration. Cyanide has been reported to prevent methane generation from acetate but does not prevent the organism *Methanosarcina barkeri* from generating methane from either carbon dioxide or methanol (Parkin and Owen, 1986; Pohland, 1992).

1.3 ANAEROBIC DIGESTION OF WASTEWATERS

The full scale process of anaerobic digestion was employed nearly 70 years ago for sewage sludge stabilization (Bruce, 1986; McCarty and Smith, 1986; Parkin and Owen, 1986). The primary advantage of anaerobic digestion of wastewater sludge is for the stabilization of organic matter, with a concomitant removal of pathogens, conditioning for solids dewatering, and removal of offensive odours and grease, thereby reducing the risk of nuisance and organic contamination during disposal. Stabilization is brought about by the partial degradation of sludge solids and a reduction in sludge volume (Pfeffer, 1968; Bruce, 1986; Parkin and Owen, 1986). Anaerobic digestion of wastewater sludges involves the sequential and simultaneous degradation of volatile solids and other compounds by an association of bacteria into simpler intermediates, in the absence of oxygen, resulting in the generation of gases, particularly methane and carbon dioxide, with the concomitant synthesis of biomass (Canale, 1971; Pohland, 1971; Corbitt, 1989; Malina, 1992).

1.3.1. Factors Affecting Anaerobic Digestion

Anaerobic biological destruction of a portion of volatile solids in the sludge is applied to enhance the dewaterability of the sludge and to minimize the putrescibility of the sludge. Several factors need to be considered to start up and optimize the process of anaerobic digestion (Figure 1.9) viz., the temperature of sewage; the concentration and varying characteristics of the wastewater; the state of degradation of the wastewater; fluctuations in the flow; concentration of sulphate; and the characteristics of the suspended solids. In addition, design criteria for anaerobic digesters must take the time dependency factor of volatile solids destruction into consideration. Such systems are based on the hydraulic retention time required to achieve a specific reduction in the volatile solids content of digested sludge (Lettinga and Hulshoff Pol, 1991; Malina, 1992). Thus, the design and operational parameters to be monitored and controlled must include hydraulic retention time, solids retention time, mixing, wastewater characteristics and heating.

a. Wastewater Characteristics

Wastewater generally is a complex mixture of many different compounds and it is not possible to represent the chemical composition of one type of sludge by a single chemical formula (Brunetti *et al.*, 1988; Kidby and Nedwell, 1991; Iza *et al.*, 1991). Complex wastewaters are defined as wastewaters containing insoluble or partially soluble compounds which may give rise to scaling, foaming, inhibition and /or scum formation. It is important to evaluate the biodegradability of the wastewater prior to the application of a biological treatment process. The sludge is a mixture of compounds all of which are subject to different degradation rates which require different hydraulic retention times. Sewage sludge contains a high percentage of refractory compounds requiring hydrolysis. More than two-thirds of the organic material in municipal wastewaters is suspended particulates or colloidal matter. It has been reported that 35% of the dry weight of municipal sewage sludge was composed of cellulose (Jewell, 1987; Brunetti *et al.*, 1988; Kidby and Nedwell, 1991; Iza *et al.*, 1991; Lettinga and Hulshoff Pol, 1991).

Primary sludge has a sludge concentration ranging from 1.95 to 4.6% (m/v) TS. Pre-thickened sludge has a total solids concentration of 4-6% (m/v) TS and only in relatively fewer cases are thicker sludges fed to digesters (Bruce, 1986; Brunetti *et al.*, 1988). The upper limit of sludge solids content in raw sludge is 10% (m/v). The percentage solids in raw sludge also dictates the solids concentration of digested sludge in conventional anaerobic digesters (Kapp, 1984). The influent substrate has a pH of 5.0-5.4 and a volatile fatty acids concentration of 1.2-2.0 kg m^{-3} (as acetic acid). The gas productions of primary sludge range from 0.71 to 1.01 m^3 kg^{-1} of organic matter destroyed (Brunetti *et al.*, 1988).

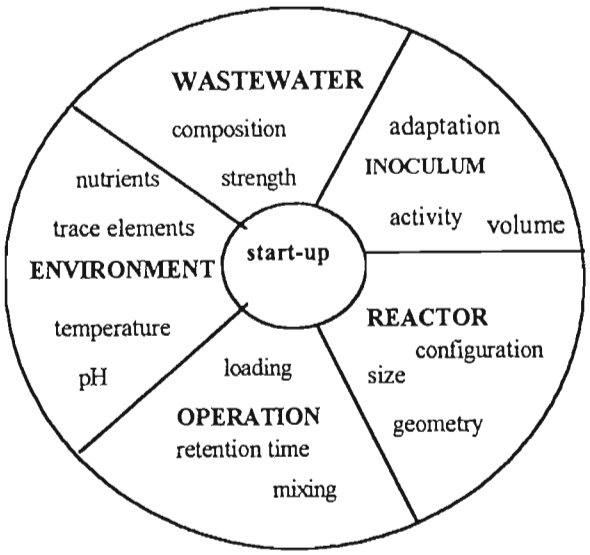

Figure 1.9 Important parameters for digester start up (Weiland and Rozzi, 1991)

Approximately 60% of the organic content of wastewater is biodegradable under mesophilic conditions and this percentage may increase under thermophilic conditions (Bruce, 1986; Brunetti *et al.*, 1988). Thereafter, the recalcitrant fraction of municipal sludge ranges from 35-40% to 70-80% of the volatile material, depending on the wastewater type and previous treatment. The rate-limiting step in anaerobic digestion depends on the type of wastewater to be treated. For soluble substrates, methanogenesis is considered to be the rate-limiting step, while for partially soluble wastewaters the hydrolysis step is rate limiting. Therefore, the total volatile solids destruction will vary from plant to plant because of variations in feed sludge characteristics as well as upstream processing, irrespective of the efficiency of the digestion process (Parkin and Owen, 1986; Iza *et al.*, 1991; Lettinga and Hulshof Pol, 1991).

It is important to have sufficient buffering capacity in the influent substrate to maintain a neutral pH within the digester as well as sufficient micro- and macronutrients. The C:N:P ratio of the feed (substrate) should be in the range of 100:1-10:1-5. The wastewater should also be screened for potential inhibitors such as high salt concentrations (Na, K, etc.), ammonia (>1000 mg l^{-1}) and sulphide. Dolfing and Bloemen (1985) reported a 50% inhibition of methanogenesis at a

concentration of NaCl of about 150mM. Thus, optimal wastewaters for anaerobic digestion are warm, readily biodegradable, neutral pH, low salinity and lacking toxic or inhibitory compounds (Iza, 1991).

b. Hydraulic Retention Time

The growth rate of the methanogens is much slower than those values reported for the facultative anaerobes or aerobic organisms. The generation times for methanogens range from less than two days to more than twenty days at a temperature of 35 °C. Therefore, typical hydraulic retention times are fifteen to twenty days. Hydraulic retention times <10 days may be employed for systems which have a high level of operational control. For conventional anaerobic digesters the solids retention time (i.e., the mean cell residence time) is the same as the hydraulic retention time(Lettinga and Hulshof Pol, 1991; Malina, 1992; Ouyang and Lin, 1992). Hydraulic retention times which are lower than the minimum growth rate of the slowest growing microorganisms will result in washout of these microorganisms. In anaerobic digestion the methanogens are the slowest growing population and are also the most significant group with regard to waste stabilization (Song *et al.*, 1992).

Hydraulic or volumetric retention time affects the volatile solids reduction, and the rate (if solids concentration is low) and the extent of methane production. This in turn is affected by the environmental conditions within the anaerobic digester, the temperature maintained, and the solids and volatile solids concentrations of the influent sludge (Figure 1.10). The volatile solids content controls the rate and volume of gas production. The total solids concentration affects the ability to mix the sludge effectively to eliminate the pockets of raw sludge and equalise the sludge temperature. Volatile solids conversion to gaseous products is controlled by the hydraulic retention time. Therefore, the design retention time is a function of the final disposition of the digested sludge, i.e., land application or incineration (Malina, 1992).

c. Solids or Organic Loading

If loading is selected as the design parameter, the retention time becomes a function of the feed sludge solids concentration. The concentration of solids in the feed sludge controls the loading to and the size of the digester (Pfeffer, 1968; Malina, 1992). The ability to thicken the sludge becomes an important design and operating consideration and may be a major limitation to digester loadings. Consequently, pretreatment options may include blending of primary sludge with thickened excess activated sludge or thickening the blended primary and biological sludges to maintain the organic loading to the digester. In addition, high solids loading reduces the required digester volume for a given retention time (Malina, 1992).

The solids loading to anaerobic digesters should be between 3.2 to 7.2 kg VS m^{-3} d^{-1}. Operating data for hydraulic retention time and percentage volatile solids of full scale anaerobic digesters are illustrated in Figure 1.11. The solid triangles represent data collected from full scale plant operations by Topey, who continued to add concentrated sludge until the process failed, while the solid squares represent data collected by Estrada (Malina, 1992). These data indicate a wide variation in the concentration of volatile solids at the same hydraulic retention time. These variations in the digested sludge are reflective of the effects of variations in the composition of raw sludge. Longer hydraulic retention times may be required depending on the level of operational expertise exercised at the treatment facilities (Malina, 1992).

Therefore, the hydraulic retention time influences the effectiveness of the volatile solids destruction and the size of the digestion tank required. Consequently, the size of the digester and the concentration of the solids in the influent substrate dictate the solids loading. A digester operating at a 10-days hydraulic retention time, with a volatile solids loading of 3.2 kg $m^{-3}d^{-1}$, must introduce to the system a feed sludge concentration of 3.2%(m/v) based on volatile solids and 4.5%(m/v) total solids. These loadings would require thickening of the biological and primary sludges to 2%(m/v) and the solids concentration of primary sludge would have to

approach 7%(m/v) (Malina, 1992).

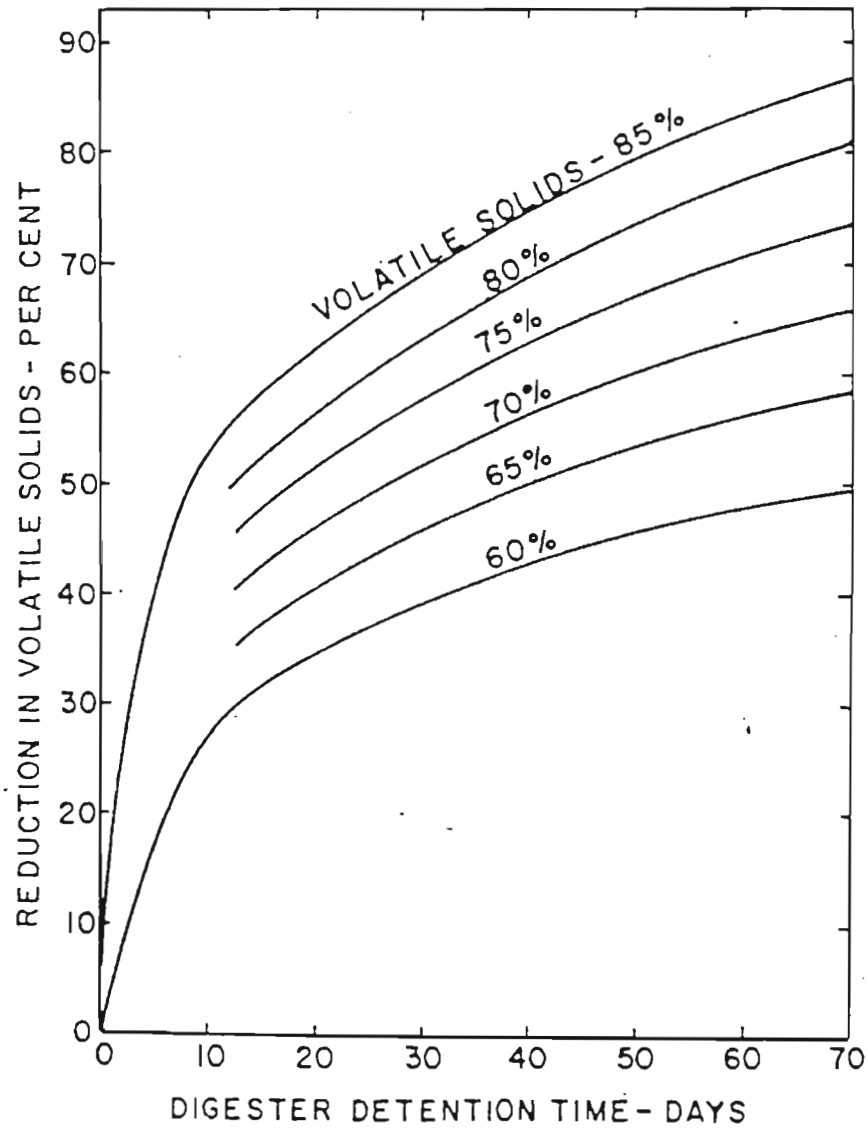

Figure 1.10. The reduction of volatile solids is dependent on retention time (from Pfeffer, 1968).

d. Solids Retention Time

Solids retention time (SRT) is defined as the mass of solids contained in the reactor divided by the mass of solids discharged and/or wasted from the system per day. Solids retention time is recognized as a key parameter for successful design and operation of an anerobic digester because it most accurately expresses the relationship between the bacterial system and operating conditions. The degree of waste stabilization is a function of both retention time and waste characteristics (Pfeffer, 1968; Parkin and Owen, 1986). The more time the sludge spends in the digester , the greater the volatile solids destruction.

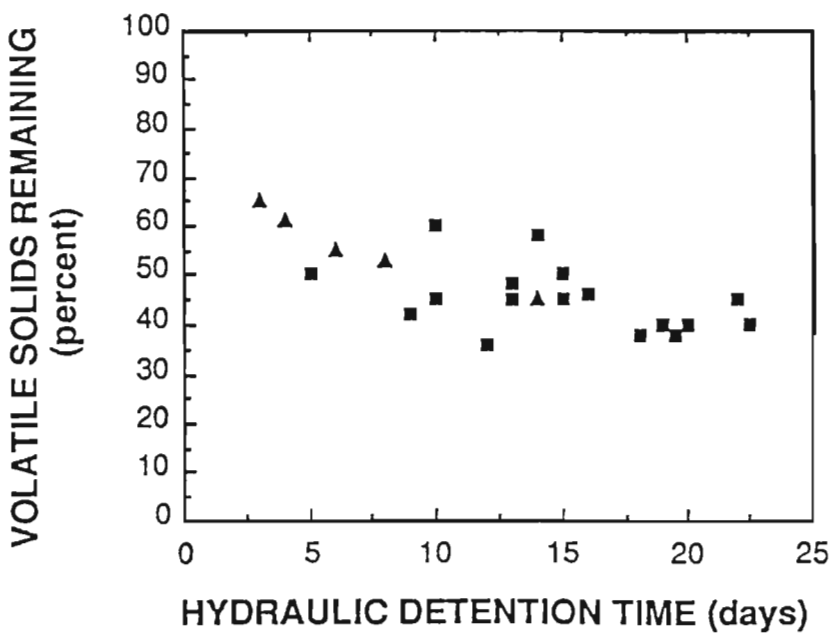

Figure 1.11. Relationship between volatile solids destruction and retention time (from Malina, 1992).

Methanogenesis is the rate-limiting step in systems which operate at a solids retention time of 10 days, while hydrolysis becomes rate limiting in systems with a solids retention time >10 days. To obtain substantial reduction on volatile solids, retention times ranging from 20 to 60 days are required for primary and waste activated sludge (Pfeffer, 1968). The operation of digesters at optimum temperature is vital even at long solids retention times. It was shown that at 15 °C the lipid fraction of sludge was not readily degraded even at a SRT of 60 days. At 35 °C there was no significant improvement in performance once the solids retention time was greater than 10 days (Parkin and Owen, 1986). Thus, the mean cell residence time (MCRT) is dependent on temperature (Table 1.6) (Gray, 1989).

Solids are lost from the digester in the effluent and by the biological conversion to gas. It is possible to increase the solids retention times by incorporating the use of digested solids recycle. Higher solids retention times prevent the washout of the slow growing bacteria (Pfeffer, 1968; Ouyang and Lin, 1992). Zhang and Noike (1991) showed that a decrease in the solids retention time in an acidogenic reactor resulted in washout of methanogens utilizing HAc, HCOOH and CH_2 OH, while the population sizes of the hydrogenotrophs, such as the hydrogen-utilizing methanogens, homoacetogens and sulphate-reducing bacteria increased rapidly, with no apparent washout. These workers also showed that with shorter solids retention times, when the hydrogenotrophs were 10 to 100 fold less than the fermentative and acetogenic bacteria of a single phase reactor, there was not enough hydrogenotrophs to grow with the hydrogen-producing acetogens. Since the interspecies hydrogen transfer was not sufficiently efficient, an accumulation of hydrogen beyond the assimilative capacity of these hydrogenotrophs occurred. This caused the conversion of propionic and butyric acids to acetate to be seriously inhibited. Solids retention time is vital since the energy available for bacterial growth in anaerobic digesters is low (Lema *et al.*, 1991).

Ouyang and Lin (1992) reported that digesters operating with a recyle ratio of 0.5 and thus a recycle sludge concentration of about 28 g ℓ^{-1} were found to have higher dehydrogenase concentrations and methane yields. This was attributed to higher biomass activity at increased solids concentrations . Parkin and Owen (1986) also recorded higher overall methane yields per unit organic matter destroyed at longer solid retention times but the gas production increased by only 4% while the reactor volume increased by about 570%. The recycle ratio is a function of the concentration of solids in the recycled sludge. Other factors which influence the recycle ratio are the ratio of solids retention time to liquid retention time in the primary digester and the amount of solids wasted from the digester. The wasted solids are determined by the difference between the influent concentration and the volatile solids destroyed (Pfeffer, 1968).

About 40% of the components of wastewater are nonbiodegradable (Ouyang and Lin, 1992). In most wastewaters the organic fraction is largely biodegradable. The rate of hydrolysis and the solids retention time will determine if there will be an accumulation of organic solids. The accumulation of refractory organic material within the digester reduces the available digester volume and leads to inefficient digester performance. Such solids contribute to the volatile suspended solids measurements and interfere with microbial biomass measurements (Iza *et al.*, 1991). The accumulation of inerts and inorganic solids can be minimized by selecting a point of recycle withdrawal such that the very coarse inorganics are not recycled but are withdrawn from the digested sludge (Pfeffer, 1968).

Thus, an adequate solids retention time enhances digester performance in the following ways:

1. The effects of temperature fluctuations are minimized by a high SRT;

2. Longer SRT provides a buffer for inefficient mixing systems;

3. The correct SRT can allow for acclimation or metabolism of a potential toxicant; and

4. Increasing the SRT increases the organic material removal efficiency (Parkin and Owen, 1986).

Table 1.6 Suggested Mean Cell Retention Times (MCRT) for anaerobic digestion of sewage sludge at various temperatures (Gray, 1989).

Temperature (^{0}C)	MCRT (days)
18	28
20	22
25	18
30	14
35	10
40	8-10

e. Mass Transfer Influences

The transport of substrate to the microorganisms and its potential energy for growth and metabolism determine the success or failure of anaerobic digestion. External mass transport is dependent on the contact opportunity provided at the microorganism-substrate interface. This is influenced by individual specificities as well as the flow dynamics. It may be concluded that the apparent saturation constant, k_s , should increase as mass transfer limitations become more severe. This dependence on mass transfer limitations should also extend to microbial population density which is also related to the saturation constant as suggested by the Contois model. The effects of gas production and transfer often influence the intrinsic hydrodynamics of the digestion process and may determine whether the system is plug-flow or completely-mixed (Figure 1.1). The substrate mass transport potential is also affected by the relative characteristics of the substrate (soluble, semi-soluble, particulate) and the indigenous microbial populations (dispersed,

agglomerated, attached). In particulate-type substrate systems, particle deposition, entrapment and sorption may effectively limit substrate utilization and microbial activity. In such sytems, consideration has to be given to convection and diffusion related mechanisms, as the substrate is solubilized, as well as sedimentation and interception. These mechanisms are, however, difficult to evaluate (Pohland, 1992).

f. Feeding Mode

There are two modes of feeding viz; continuous and intermittent (fed-batch) feeding. Intermittent feeding of sludge occurs once a day or once each shift, which is normally 2-3 times a day (Parkin and Owen, 1986; Gray, 1989). During fed-batch feeding the bacteria are alternatively nutrient limited and exposed to excess substrate substrate. This causes surges in acid and hydrogen production with potential detrimental decreases in the pH if sufficient alkalinity is not present to neutralize the excess acid. The negative effects of such fluctuations in the digester can be minimized by a longer solids retention time. However, intermittent feeding is less expensive and more convenient. Continuous feeding is considered more advantageous since it promotes a uniform and relatively constant environment for the bacteria. This mode of loading is recommended for optimum performance. Pumping of dilute sludge to the digester results in inefficient use of the digester volume and should be avoided (Parkin and Owen, 1986).

g. Temperature

Constant optimal operating temperatures need to be maintained in anaerobic digesters since the methanogenic bacteria are highly sensitive to any sharp or frequent fluctuations. Therefore, process temperature fluctuations must be kept to a minimum. Methane production is inhibited at temperatures above 40 °C and below 50 °C. These methane forming bacteria are most active in two temperature zones, the mesophilic and thermophilic range. Anaerobic digestion can also be

successfully applied at temperatures as low as 20 °C as long as sufficient residence time for the methanogens is provided (Malina, 1992).

Digesters operating in the mesophilic range need to be maintained at 35 °C, while thermophilic digesters function effectively at 55 °C. Gas production is lower for those temperatures in the intermediate range of mesophilic and thermophilic digestion. The biomass activities are 25-50% higher in thermophilic than mesophilic digesters. Thermophilic operation produces low bacterial biomass and high endogenous death rates. Up to 2 times higher volatile acid concentrations are observed in the thermophilic systems. However, thermophilic digestion is more effective in the destruction of pathogenic bacteria than mesophilic digestion (Malina, 1992; Hall, 1992).

Anaerobic digestion is also possible under psychrophilic conditions. However, at temperatures of $\leq$20 °C the methane production rates decrease. Acetate appears to be the main precusor of methane at low temperatures. At 6 °C the maximum specific growth rate of the microorganisms is 0.057 d^{-1} which is about 4 times less than that for microorganisms growing at 35 °C. Microbial growth rates are low and result in prolonged lag phases (Nozhevnikova and Kotsyurbenko, 1994). An observed maximum growth rate of 0.37 d^{-1} and a decay rate of 0.1 d^{-1} was estimated for anaerobic mesophilic digestion at pH 7.1 (Siegrist *et al.*, 1993). For every 10 °C drop in temperature, the growth rate decreases by 50%. Thus, a digester may require a solids retention time of approximately 200 days to achieve high efficiencies at 10°C (Jewell, 1987).

Digesters are maintained at constant temperatures with the aid of external heat exchangers which are used to heat the raw sludge and maintain the temperature of the sludge undergoing digestion. In some cases the biogas produced during anaerobic digestion is used as fuel and is converted to mechanical or electrical energy. The cooling water that is discharged during this process is used to preheat the sludge. Some digesters are equipped with an internal heat exchanger which is incorporated in the draft tube of the digestion system mixed by recirculation gas. Water jackets

are placed around the periphery of the draft tubes through which the sludge is pumped. Another method of heating sludge involves directly injecting steam into the system. This method, however, has the disadvantage of introducing more water to the sludge thereby diluting the concentration of digested sludge and increasing the volume of the supernatant (Malina, 1992).

Heating requirements are influenced by the concentrations of the solids within the digester. As the solids content increases the amount of sludge solids remains constant but the volume of water associated with the sludge is reduced. Consequently, the heating requirements per pound of solids decreases. Heating requirements for a sludge at 2%(m/v) solids can be up to 4 times greater than for sludge at 8%(m/v) solids. The heating requirements of an anerobic digester. Heat losses from the digester to the surrounding environments depends on the shape of the digester as well as the materials of construction (Malina, 1992).

h. Mixing

Initially, mixing was used to displace the pockets of biogas from the sludge or to break up the scum. Mixing the contents of anaerobic digesters is now considered imperative if the entire digester volume is to be utilized. Mixing anaerobic tanks eliminates thermal stratification and promotes uniform temperature throughout the tank by maintaining chemical and physical uniformity throughout the digesting sludge. Mixing of the feed sludge and digesting sludge also promotes intimate contact between the active biomass, bacterial enzymes and the substrate, thus improving sludge digestion (Parkin and Owen, 1986; U.S. EPA Report, 1987; Malina, 1992). Three factors which adversely affect biomass/substrate contact are short circuiting of raw sludge through the digester, channelling, formation of dead zones and clogging of poorly designed and maintained systems. Channelling affects the mass transfer of substrates while dead zone formation results in sludge compaction. These factors are eliminated in systems which are efficiently mixed (Bruce, 1986; Iza *et al.*, 1991).

In addition, mixing minimizes the inhibitory and toxic effects on the microorganisms by rapidly distributing metabolic end products produced during sludge digestion. Mixing also serves to disperse any toxic materials entering the system in the feed sludge. Adequate mixing also discourages scum formation and the settlement of grit and dense solids. Inefficient mixing decreases the available system volume, thereby decreasing the SRT, and pushes the system closer towards failure. Inefficient mixing may reduce the volume of a digester by as much as 70%. The major disadvantages of mixing are high costs and the need for further facilities to enhance separation of the digested solids from the liquid phase (Parkin and Owen, 1986; U.S. EPA Report, 1987; Gray, 1989; Malina, 1992).

A limited amount of mixing occurs naturally in the digester by recirculation of heated sludge, which causes some thermal convection currents, and by gas pockets breaking loose causing boiling and rolling. Natural mixing is, however, inefficient so auxilliary mixing is essential to optimize the advantages of complete mixing (U.S. EPA Report, 1987; Osborne, 1992; Malina, 1992).Various methods of mixing the sludge, such as pumping sludge from one digester to another, recirculating supernatant to keep the scum layers moist, mechanical mixing devices and recirculating biogas, have been employed (U.S. EPA Report, 1987; Gray, 1989). Digesters are also equipped with wall-mounted baffles to prevent vortexing by mechanical stirring systems. Vigorous mixing within a digester may lead to foaming and poor sludge settlement and thickening (U.S. EPA Report, 1987; Malina, 1992).

1.3.2 Indicators of Digester Performance

Owing to the sensitivity of particularly, the methanogens to changes in the environment of the anaerobic digester, it is essential for conditions within the system to be maintained optimally for the microorganisms. The optimum conditions for maximum volatile solids destruction and methane generation during anaerobic digestion are listed in Table 1.7.

Table 1.7 Environmental and operating conditions for optimal methane production during anaerobic digestion of wastewater sludges (after Malina, 1992).

Variable	optimum	extreme
pH	6.8 to 7.4	6.4 to 7.8
Redox potential (mV)	-520 to -530	-490 to -550
VFA's (mg ℓ^{-1} as HAc)	50-500	>2000
Alkalinity (mg ℓ^{-1} as $CaCO_3$)	1500-3000	1000-5000
Temperature		
Mesophilic	30-35 °C	20-40 °C
Thermophilic	50-56 °C	45-60 °C
Hydraulic retention time(d)	10-15	7-30
Gas composition		
Methane (%v/v)	65-70	60-75
Carbon dioxide (%v/v)	30-35	25-40

a. pH

The optimal pH range for acidogenic bacteria is between 5.0 and 6.5. Methanogenic bacteria function best at a pH above 6.8. In an actively digesting single phase anaerobic system a balance between acid production, acid utilization and methane formation needs to be maintained. The pH of such a system will range from 6.8 to 7.4 with no continuous upward or downward trend (Hall,

1992; Malina, 1992). A digester functioning at pH 7.0 is considered ideal (Osborne, 1992). As the influent substrate is pumped in the anaerobic digester the pH of the system will initially decrease. This decrease will subsequently be buffered by the alkalinity produced in the digester by the bicarbonate ions. At hydraulic retention times of five days or more, the methanogens convert the volatile acids produced during the acid formation stage to methane and carbon dioxide. Methane production results in an observable reduction in organic material, measured as COD (Malina, 1992).

At pH values $\leq$6.0 inhibition of the methanogens is observed. The volatile acids in the system accumulate and eventually gas production will cease (Malina, 1992). It has been reported that complete digester recovery from a drop to pH 5.0 can be prompt if the duration of the instability is less than 12 hours (Parkin and Owen, 1986). In addition to the exertion of direct microbial effects, low digester pH can increase the toxic or inhibitory characteristics of a number of organic and inorganic inhibitors (Hall, 1992). The conversion of volatile solids to methane is substantially complete at hydraulic retention times of ten days or more (Malina, 1992).

When insufficient buffering capacity is present in acidic wastewater, the digester pH can be controlled by the addition of alkaline chemicals such as caustic soda, lime, ammonia and bicarbonates (Hall, 1992; Malina, 1992; Osborne, 1992). In a single phase system, alkalinity is used to neutralize dissolved carbon dioxide. Lime is one of the cheapest forms of alkali but as the concentration of bicarbonate alkalinity approaches 500 to 1000 mg ℓ^{-1}, continued addition results in the precipitation of calcium carbonate which causes scaling and solids accumulation difficulties. Ammonia assists in the dissolution of the scum layer. Ammonia reacts with the water and carbon dioxide in the digester to form ammonium carbonate which provides alkalinity to the system. The ammonium carbonate reacts with the free volatile acids which are present in an unbalanced fermentation. Anhydrous carbonate must be added carefully to the digester since indiscriminate addition could lead to ammonia toxicity. Furthermore, addition of large quantities

of a single cation e.g. Na^+ for pH control can contribute to metal cation toxicity (Hall, 1992; Malina, 1992).

Buffering in digesters is determined by the carbon dioxide-bicarbonate system and is normally assayed off-line by titration to pH 5.75 for bicarbonate alkalinity and to pH 4.3 for total alkalinity (Jenkins *et al.*, 1983). Such analyses are usually made once a day and thus any deterioration in digester performance may not be detected for many hours. Hawkes *et al.* (1994) developed an on-line instrument for measuring bicarbonate alkalinity with a response time of 30 minutes. This instrument indirectly allows one to follow variations in volatile fatty acid concentration and the pH of the effluent to be followed.

The pH of a system cannot be considered as a very sensitive parameter and is neither ideal as a stability indicator or for feedback process control. The effectiveness of pH is not constant but varies according to the waste characteristics. As volatile acid production increases, the pH variation in the wastewater will depend on the bicarbonate buffer and will decrease as the bicarbonate buffer increases (Weiland and Rozzi, 1991).

b. Volatile Acids

The major volatile acids present during anaerobic digestion are acetic and propionic and their concentrations provide a useful measure of digester performance. Low concentrations of volatile acids indicate stable operating conditions while high acid concentration are invariably associated with digester imbalance (Parkin and Owen, 1986; Mawson *et al.*, 1991). Propionic acid degrading bacteria appear to be particularly sensitive to changes in the digester environment although rapid accumulation of both acetic and propionic acid has been noted during stress conditions prior to system failure. The biodegradation of these accumulated volatile acids is essential for the recovery and control of the digestion process (Mawson *et al.*, 1991).

The activity of the metabolic groups participating in the methane fermentation is markedly influenced by the volatile acid concentration within the digester. It has been reported that increasing the initial acetate concentration to 2000 mg ℓ^{-1} significantly reduced the utilization of propionate added at 500 mg ℓ^{-1} (Mawson *et al.*, 1991). This resulted in propionate utilization decreasing to approximately half of that when acetate was present at 500 mg ℓ^{-1} or lower. Similarly, increasing propionate when acetate was added at a constant initial concentration reduced the rate of propionate degradation. Eventually degradation of both acids was severly retarded at the highest propionate concentration. These inhibition effects could not be attributed to pH which varied by only 0.1 to 0.3 pH units across all digesters, or to all the salts added (Mawson *et al.*, 1991).

The inhibition of methanogenesis has been attributed to the action of un-ionized acids. Thus, both the pH and the total acid concentration are important in determining the gross effect. Approximately 50% methane inhibition was reported when the un-ionized volatile fatty acid concentration exceeded 10 mg ℓ^{-1} in acetic acid and glucose fed digesters. Satisfactory digester activity is observed for acetate concentrations of $\leq$50mM (Mawson *et al.*, 1991). Duarte and Anderson (1982) investigated the effects of low pH and un-ionized acid accumulation on methane production. Table 1.8 illustrates the downward trend in methane production as the pH decreases and un-ionized acids increase. The methane produced at pH 5.0 was due to methanogenesis taking place in the wall growth.

Table 1.8 Effects of low pH and high un-ionized acid concentration on methane production (Anderson and Duarte, 1982).

pH	Methane production $l\,d^{-1}$	pH Inhibition factor*	Un-ionized acetic acid content, mg l^{-1}
7	6.3	1	3
6	0.3	0.05	30
5.5	0.19	0.03	91
5	0.04	0.01	240
*pH inhibition factor = methane produced/ methane produced at pH 7.0			

Propionate appears to be more toxic to methanogenesis and significant substrate inhibition has been observed at concentrations of ≥1000 mg l^{-1}. Product inhibition of propionate degradation has been noted in digesters treating sewage sludge and a mixed acid feed, in propionate enrichment cultures and in defined co-cultures. Moderate inhibition was reported at acetic acid concentrations of 900-1800 mg l^{-1} for initial propionic acid concentrations of 740-1850 mg l^{-1} (Mawson *et al.*, 1991). More than 50% of failing digesters had acetate concentrations in excess of 800 mg l^{-1} and a propionate to acetate ratio greater than 1.4 (Mawson *et al.*, 1991). In order to ensure the development of the different microbial populations, particularly the methanogenic bacteria, it is important to observe a non-accumulation of volatile fatty acids (Lema *et al.*, 1991).

c. Alkalinity

Alkalinity is derived from the breakdown of organic molecules in the digester and is present primarly in the form of bicarbonates,which are in equilibrium with the carbon dioxide in the gas at a given pH. The relationship between alkalinity, the carbon dioxide in the gas and the pH is illustrated in Figure 1.12. Effective pH control requires sufficient alkalinity. At a pH between 6.6 and 7.4 and a carbon dioxide content in the gas of 30 to 40% by volume, the bicarbonate

alkalinity will range between 1000 and 5000 mg ℓ^{-1} as $CaCO_3$ (Malina, 1992). When bicarbonate alkalinity drops to about 500 mg ℓ^{-1} and carbon dioxide content of the reactor is approximately 38%, the pH of the reactor will decrease to 6.0 (Corbitt, 1989). The bicarbonate alkalinity (BA) is approximately equal to total alkalinity (TA) of the anaerobic system at lower volatile acid concentrations. As total volatile acids (TVA) increase, however, the bicarbonate alkalinity is much lower than total alkalinity. About 83.3% of volatile acids concentration contributes to alkalinity as "volatile acid salts" alkalinity and the following equation (eqn. 1.1) may be used to estimate the concentration of bicarbonate alkalinity:

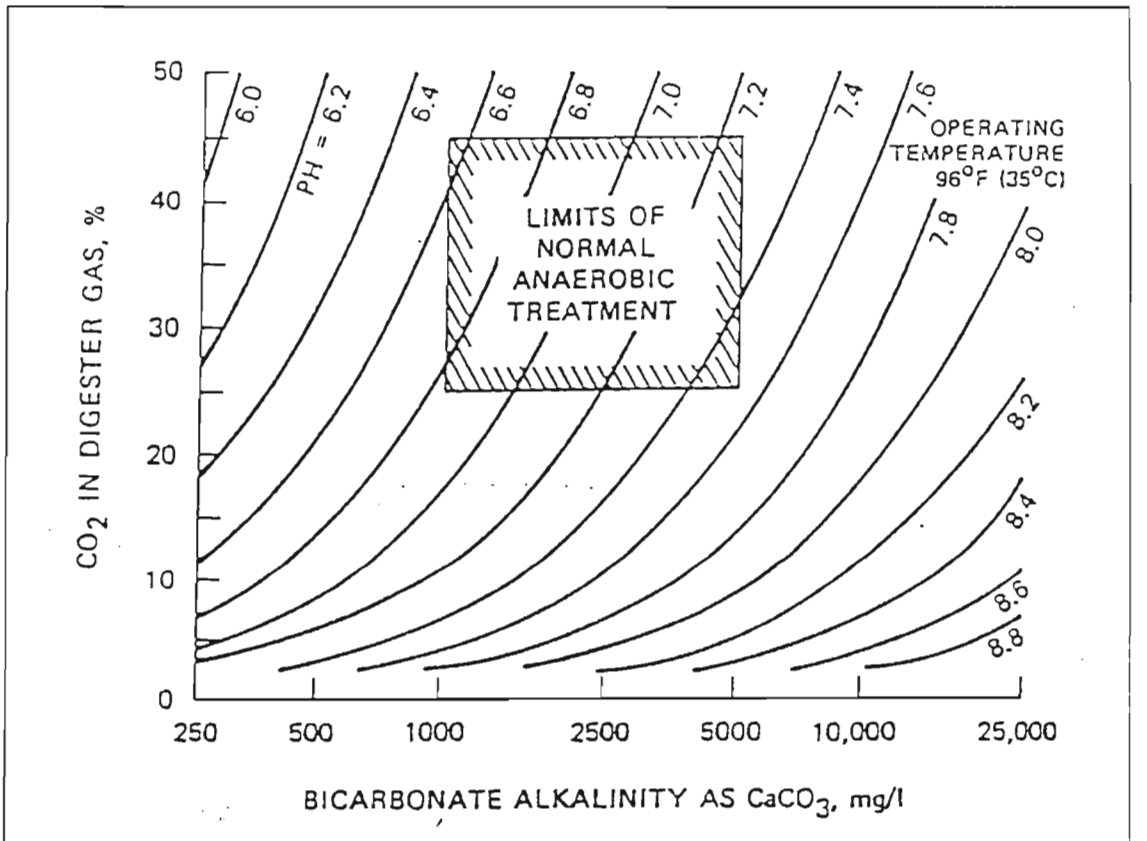

Figure 1.12 The relationship between pH, carbon dioxide and bicarbonate alkalinity (McCarty, 1975)

$$BA = TA - (0.85)(0.833)TVA \quad \text{.....(1.1)}$$

The factor 0.85 accounts for the fact that 85% of the "volatile acid salts" alkalinity is measured by titration to pH 4.0 (Malina, 1992).

The ratio of volatile acids to alkalinity (VA/ALK) is a good indicator of fermentation balance. A VA/ALK ratio of 0.1 indicates normal conditions within the digester. As the ratio increases to about 0.5, the carbon dioxide concentration within the digester starts to increase and the digester gas will not burn well. At a VA/ALK ratio of 0.8 the pH decreases and the methanogens are inhibited. The methanogens may eventually be killed if control measures are not administered (Osborne, 1992). During an organic overload neither the digester off-gas nor the pH change quickly at the onset of digester instability. The two parameters most frequently used to monitor digester stability are alkalinity and total volatile acids concentration (Ripley *et al.*, 1986). Thus, the volatile acid in the system can be kept low by avoiding organic and volumetric overloading (Osborne, 1992).

1.3.3. Products of Anaerobic Digestion

a. Liquid effluent

The waste liquor from anaerobic digesters has a suspended solids concentrationof 500 mg ℓ^{-1} and BOD concentration of 400-800 mg ℓ^{-1}, due to the soluble organic compounds present. The liquid effluent may also have high concentrations of soluble nitrogen present. The characteristics and strength of the liquid make it difficult to dispose of or treat separately, and it is returned to the head of the works to be mixed with the incoming sewage and treated in admixture (Gray, 1989).

b. Biogas

Biological waste stabilization of sludge results in the generation of biogas, particularly, methane and carbon dioxide as well as trace amounts of hydrogen, water vapour, carbon monoxide and hydrogen sulphide. A typical biogas contains between 65 and 70% methane and approximately 30 to 35% carbon dioxide (Bailey and Ollis, 1986; Parkin and Owen. 1986; Gray, 1989). Hydrogen is produced by the fermentative and hydrogen producing acetogenic bacteria and has been shown to play a significant role in regulating organic acid production and consumption. If the hydrogen partial pressure exceeds 10^{-4} atm, methane production is inhibited. Thus, the presence of a large, stable population of carbon dioxide reducing methanogens will ensure maintenance of low hydrogen partial pressures and, consequently, higher methane yields. Hydrogen sulphide which is present in small amounts is produced by sulphate-reducing bacteria (Bailey and Ollis, 1986; Parkin and Owen, 1986).

Methane is essentially insoluble in water and readily escapes from the sludge while carbon dioxide either escapes in the gaseous phase or is converted to bicarbonate alkalinity (Parkin and Owen, 1986). Approximately 72% of the methane generated comes from acetate cleavage and 28% comes from the reduction of carbon dioxide. Of the 28% of methane generated, 13% flows via propionic acid and 15% from other intermediates, with hydrogen as the energy source (McCarty, 1975). With the aid of stoichiometry it can be calculated that for each mole of sewage sludge 0.195 mole of new cells is produced and 5.75 moles of methane are released. Approximately 1.0 m^3 of biogas is produced per kg of organic matter destroyed whereas 0.35 m^3 of biogas is produced per kg of COD removed at standard temperature and pressure (Parkin and Owen, 1986; Gray, 1989). Removal of biogas from the early stages of microbial conversion improves degradation in the final stages and provides overall process stability and treatment efficiency. The concentration of hydrogen affects the substrate conversion potential of most major anaerobic groups (Harper and Pohland, 1987). Biogas produced from anaerobic microbial

conversion is usually burned on site or is used to produce heat to maintain the temperature within the digester. Thus, biogas can be a valuable resource which is used to curtail the expenses of the overall waste treatment system (Gray, 1989; Iza *et al.*, 1991).

c. Solids

The conversion of volatile solids to stable innocuous products is represented in Figure 1.13 which is based on 100 kg of total solids, containing 70% volatile solids. Approximately 30 to 40% by weight of the initial volatile solids added to the digester remains after anaerobic digestion. Thus, not all the volatile solids in the sludge are labile and a percentage of the volatile solids remaining after anaerobic digestion may be nonbiodegradable or recalcitrant compounds. It is assumed that 50% by weight of the digested sludge is volatile solids, which is the same as the fixed solids (noncombustible solids). It is cited in literature that digested sludge has 45-50% volatile solids but this may vary from plant to plant depending on process efficiency and composition of substrate(Brunetti *et al.*, 1988). Thus, the composition of the influent sludge introduced into the anaerobic digester dictates the final products of digestion (Malina, 1992; Brunetti *et al.*, 1988).

Digested sludge is different from primary and secondary sludge since it is pathogen free, stabilized and far less odourous. It is normally dried to an inert friable condition and applied onto agricultural land (Gray, 1989). Typically, anaerobic digestion systems have a low sludge yield. This makes it necessary to operate a unit which can retain microbial biomass in the system. Conversely, the low sludge yields can produce a net zero sludge production because biomass can be lost in the effluent below the required suspended solids discharge limit (Jewell, 1987). However, solids can accumulate within the digester due to biomass production,the accumulation of non-biodegradables solids and chemical precipitation of heavy metals. The quantity and nature of solids are dependent on the composition of wastewater and the type and design of the reactor. These solids need to be disposed of safely or re-used effectively (Iza *et al.*, 1991). It is necessary

to incorporate mechanisms for the removal of excess sludge from the digester into the design of digesters (Lettinga and Hulshoff Pol, 1991).

1.3.4 Anaerobic Digester Failure

Digester imbalance can be attributed to overloading, toxic substances and sudden changes in the digester environment. Digester imbalance results in a decrease in microbial activity which leads to reduced conversion of organic material, lower methane yield, reduced methane production rate, and an accumulation of volatile organic acids and other fermentation products, which results in a reduction in the pH and alkalinity. If such changes in the digester environment are not detected early they may lead to "sour" or "stuck" digesters which may require several months to recover to normal activity (Chynoweth *et al.*, 1994; Moletta *et al.*, 1994).

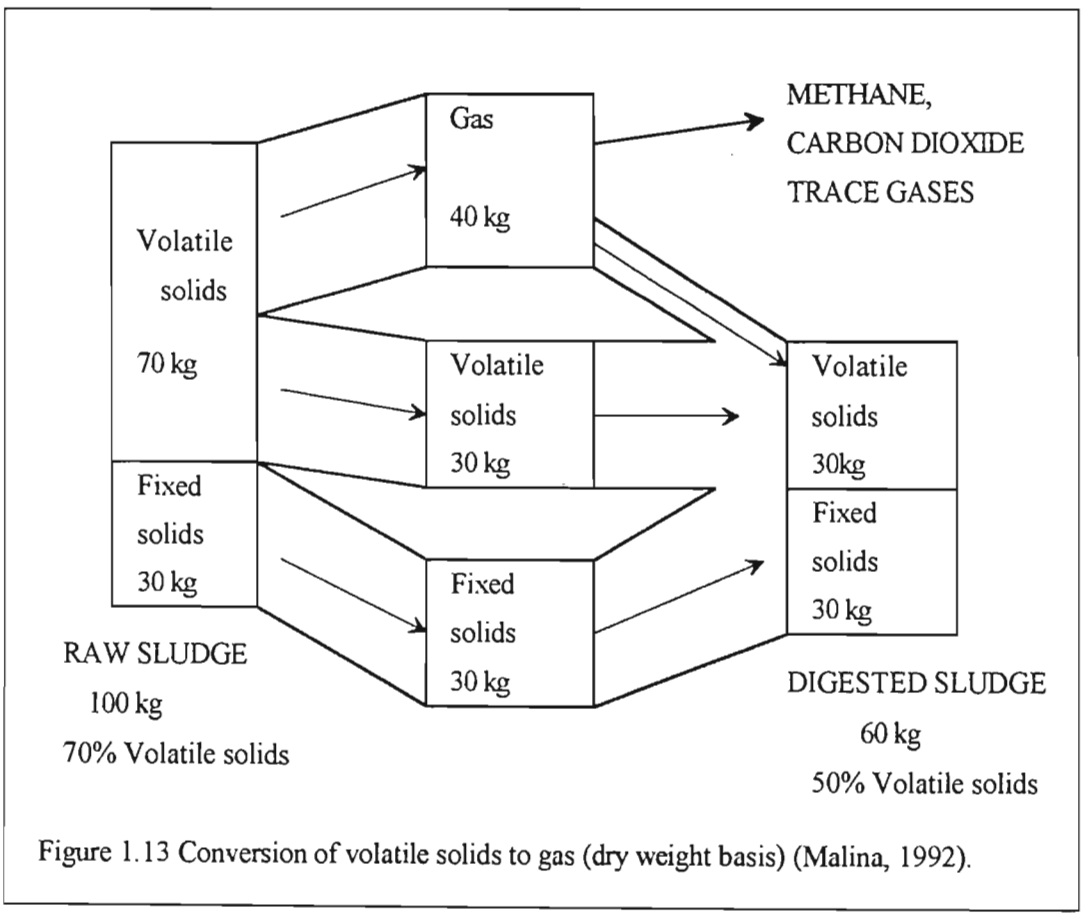

Figure 1.13 Conversion of volatile solids to gas (dry weight basis) (Malina, 1992).

Fluctuations in loading rates are a result of poor control of feed volume or concentration. Organic overloading is characterised by rapid accumulation of volatile fatty acids, followed by a drop in pH to ≤6.7. The concentration of hydrogen gas peaks at 600 ppm. An increase in the carbon dioxide fraction of the digester off-gas or a decrease in the digester pH results from the destruction of bicarbonate buffering and volatile acid build-up (Moletta *et al.*, 1994; Chynoweth *et al.*, 1994; Ripley *et al.*, 1986). These results correlate with earlier work by Mosey and Fernandes (1989) who showed that hydrogen accumulated in laboratory scale digesters when they were subjected to pulse loadings of easily available carbohydrates such as glucose(Moletta *et al.*, 1994). This was accompanied by an accumulation of organic acids in the digester. Organic overloading can be controlled by decreasing the feed until the digester stabilizes (Moletta *et al.*, 1994). This allows adequate time for the accumulated acids to be degraded at the lower rate. Since the un-ionized acids appear to play an important role in the reduction of methanogenic activity, provision of sufficient alkalinity to buffer the pH is important to minimize the high acid concentrations (Mawson *et al.*, 1991). Thus, impending failure can be averted by cessation of feeding, neutralization of acids and by allowing for a period of recovery (Bailey and Ollis, 1986).

Volumetric overloading (i.e., changes in feed volume rate) may lead to washout of the slow growing methanogens. Digester failure under volumetric stress may be microbiologically different from failure under conditions of organic overload. Furthermore, under conditions of volumetric overloading, hydrogen does not accumulate prior to digester failure. Toxic compounds usually enter the digester via the feed and may be bacteriostatic or bactericidal to the microorganisms (Kidby and Nedwell, 1991; Chynoweth *et al.*, 1994). Further difficulties may also arise when digesters are poorly provided with nutrients as volatile fatty acids and particularly acetate can accumulate rapidly under these conditions. This could lead to prolonged recovery periods (Mawson *et al.*, 1991).

Chynoweth *et al.* (1994) used a methane yield of 0.38 ℓ CH_4^{-1} g VS^{-1} as a performance parameter to monitor overloading, underloading and toxicity symptoms. If the rate of methane production increased above the set point, it was assumed that an overloading was occurring and the dilution rate was decreased. When conditions returned to normal the dilution rate was increased to maintain a constant methane production. A decrease in methane production below the set point was countered by increasing the dilution rate. A response of increased methane production indicated an underloading was occurring and the dilution rate was increased up to a washout retention time constraint. If, however, the response was a decrease in methane production, the presence of an inhibitor was indicated. In this case the operator would have to decrease the dilution rate to batch operation for an interval to facilitate recovery of microorganisms (Chynoweth *et al.*, 1994).

1.4 PROJECT OBJECTIVES

A microfiltation unit was coupled to one of the full-scale anaerobic digesters at Northern Waste Water Treatment Works, Durban. The purpose of the microfiltration unit is to concentrate the digested sludge and recycle the sludge to the digesters. This will result in an increase in solids concentration within the digester and will effectively decouple the hydraulic retention time from the solids retention time. Solids will, therefore, remain within the digester for longer periods and could result in higher volatile solids reduction. Furthermore, an increase in digested solids concentration should result in an increase in biomass and, thus, an improvement in anaerobic digestion. Therefore, the primary objective of this project was to determine if an increase in solids concentration would influence microbial activity and anaerobic digestion efficiency. To achieve this goal it was necessary to design and conduct experiments to explore the trends in microbial activity and anaerobic digestion efficiency with increased solids concentrations. The significance of the experiments is explained, below.

The Biodegradability Study (Chapter 2) was conducted to determine the biodegradation potential of the primary sludge in the substrate. Since the characteristics of primary sludge vary from plant to plant, it was important to establish what percentages of the substrate were biodegradable and recalcitrant. These experiments were carried out in batch digesters which were analysed routinely for volatile solids, total solids, pH, volatile acids/alkalinity and gas production. These experiments determined the minimum volatile solids concentration obtainable after 90 days of digestion and produced an estimate of the ratio of gas produced per g volatile solids destroyed.

Further experiments investigated **the effects of higher solids concentrations on microbial activity (Chapter 3)**. Such activity was estimated by measuring the volume of gas produced and the rate of biogas production in batch digesters. The microbial activities of seven different concentrations of solids were investigated. The aim of this experiment was to determine if increased solids concentrations produced different volumes of gas at different rates. This study

identified which solids concentrations resulted in increased microbial activity. It also identified which concentrations could be maintained in semi-continuous or continuous anaerobic digesters to improve the efficiency of the process.

The final experiment **(Chapter 4)** involved the **operation of semi-continuous digesters with different solids concentrations at optimum temperatures**. The concentrations selected were based on the results and observations of the second experiment (Chapter 3). Digesters were operated with constant organic and volumetric loads and their efficacies were monitored. Changes in gas production, volatile solids destruction, alkalinity, pH, volatile acids and volatile acids/alkalinity ratios were monitored. The specific objective of this experiment was to determine if a digester with increased solids performed more or less efficiently than a digester with 2-2.6% TS (control). A digester with increased solids which performed as well as the control was also considered as a positive result.

Chapter Two

Experimental Results : The Biodegradability of Primary Sludge from NWWTW

The results of the biodegradability investigations with batch cultures are analysed in this chapter. Batch cultures often provide acid production and acid consumption imbalances within a digester which can be disadvantageous. However, batch tests are easier to set up and operate and do provide useful information such as the upper and lower limits of volatile solids destruction and rate of gas production.

The composition of sewage sludge varies from plant to plant. Since the composition of sewage sludge determines the hydrolysis rate constant and, thus, the overall efficiency of anaerobic digestion, it is important to ascertain the biodegradation potential of the substrate. Sewage sludge used in this experiment was collected from Northern Waste Water Treatment Works (NWWTW), Durban, which also treats a small percentage (about 5%) of paper mill wastes with the sewage. Paper wastes are high in lignin and cellulosic compounds which are not readily degradable and may require more time for degradation. It was, therefore , decided to conduct a biodegradation study to determine the lower volatile solids limits and the volume gas produced per gram volatile solids removed.

2.1. Experimental Procedure

Two biodegradability trials using batch digesters were undertaken. The only difference between Trial 1 and 2 were the ratio of substrate to inoculum. More inoculum was used in Trial 2 to prevent the long lag period which was prevalent in Trial 1.

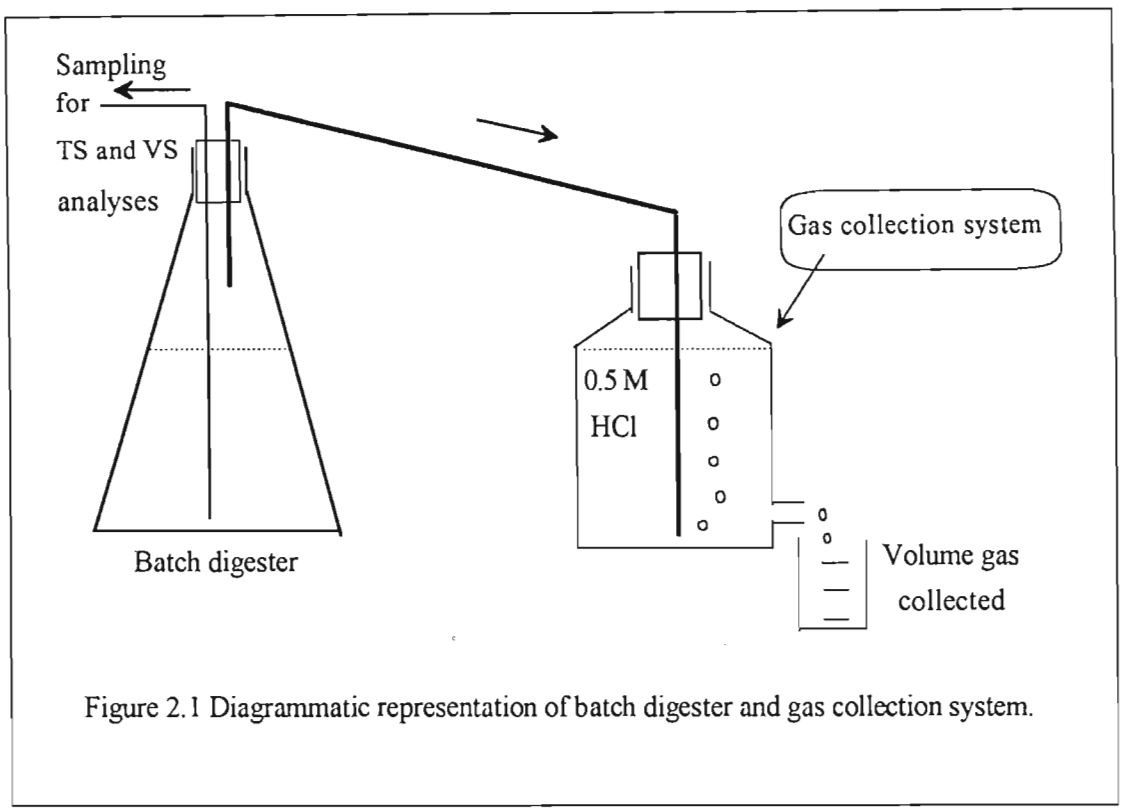

Figure 2.1 Diagrammatic representation of batch digester and gas collection system.

2.1.1 Batch Digester Configuration

An Erlenmeyer flask (3 ℓ)was used as a digester. The port of each digester was closed with a rubber stopper containing two openings and vaseline seal was applied to the edges to make the digester airtight (Figure 2.1). A long glass tube was fitted through the first opening to facilitate the sampling of solids. Silicon tubing was fitted onto the glass tube and clamped with a surgical clamp to prevent air from entering the digester. The second opening contained a shorter glass tube well above the sludge meniscus which was connected to the gas collection system by silicon tubing. Silicon tubing was used since it minimizes air ingression. Gas generated from waste stabilization was bubbled through a vessel (2 ℓ) which contained 0.5 M HCl (Ross *et al.*, 1992).

This solution prevents carbon dioxide solubilization and thus, facilitates accurate measurement of the gas generated. The gas produced during anaerobic digestion was measured by displacement of the liquid solution in the gas collection vessel. The displaced liquid was transferred to 100 ml and 1 ℓ measuring cyclinders and measured to the nearest 2.5 and 10 ml.

Substrate and Inoculum : Substrate (primary sludge) and Inoculum (digested sludge) was collected from NWWTW. The total solid concentration of the primary sludge was approximately 5 to 6%(m/v) and contained 80 to 85% VS. The digested sludge was collected from the bottom draw-off point with a total solids concentration of about 2 to 2.6%(m/v) and a volatile solids concentration of 60%. Each digester had a working volume of 2500 ml. A substrate:inoculum ratio of 4:1 and 2:3 was used for Trial 1 and 2, respectively. Two different ratios were used since the ratio of substrate to inoculum select for Trial 1 resulted in an acid generation/consumption imbalance in the digester. To counteract this imbalance a larger volume of digested sludge was added in Trial 2 with a smaller volume of substrate.

2.1.2. Analyses

Volatile Solids and Total Solids : Since volatile solids destruction of sewage sludge is not a rapid process, the contents of the digester were sampled weekly for volatile solids and total solids content. The samples were treated according to the method outlined in Standard Methods (APHA, 1985). Approximately 50 ml were removed from the digester for analysis in Trial 1 while 100ml were removed in Trial 2.

pH and Volatile Acids/Alkalinity Ratio (Ripley Ratio) : The pH was measured weekly with an Orion pH meter which was calibrated with pH buffer solutions 7.0 and 4.0 prior to use. Readings were taken immediately after sampling to prevent carbon dioxide solubilisation from the atmosphere. Exposure to air would possibly increase the bicarbonate ion concentration within the sample, thus effecting a higher but inaccurate pH reading. The volatile acids/alkalinity ratio was calculated according to the method described by Ross *et al.* (1992).

2.1.3. pH Control

The batch experiments resulted in acidogenesis/acidotrophy imbalances which led to unfavourable environmental variations. Once the pH dropped below 6.8 chemicals such as lime and sodium bicarbonate were added as solids to neutralize acid accumulation and to aid the digester in its recovery. Thus, overdosing the digester with chemical additions was avoided.

2.2. Results and Discussion

2.2.1. Monitoring and Control of the Digesters

A pH of 7.0 is optimum for gas and methane production in anaerobic digestion (McCarty, 1975; Pohland, 1992). Most digesters, however, operate efficiently within the range 6.8 to 7.4. Control of pH with chemicals is administered when the pH falls outside this range. At the start of Biodegradability Trial 1 the pH dropped, initially, to 5.5 which is well below the minimum acceptable pH of 6.8 in anaerobic digestion. Similarly, the volatile acids/alkalinity ratio increased from about 0.4 to 1.1. The high ratio calculated is representative of unbalanced fermentations with high acid concentrations, low methane yield, a higher carbon dioxide yield and low gas production. Prolonged periods of low pH is not rate limiting to the acetogenic or fermentative stages of anaerobic digestion but are detrimental to the sensitive methanogenic bacteria. It was, therefore, necessary to assist the system in its recovery by adding sodium bicarbonate to counteract high acid concentrations.

After about 20 days the pH of the digester did increase to about pH 6.4 but this was still too low for effective anaerobic digestion. Thus, lime was added to the digester to neutralize the accumulated acids. Care was taken not to overdose the digester with lime since calcium bicarbonate is soluble up to a concentration of 1 mg ℓ^{-1}. The addition of large quantities of sodium bicarbonate and lime can lead the chemicals precipitating out of solution and increasing the total solids concentration of the sludge. Further addition of lime was avoided and the digester was allowed to recover with time. A recovery period of approximately 23 days was

required for the pH to stabilize to 7.1. During this period the volatile acids/alkalinity ratio decreased to 0.5. This ratio was still too high but continued its downward trend over the next few weeks until it stabilized below the permissable level of 0.3. The VA/ALK ratio levelled off at about 0.2 units.

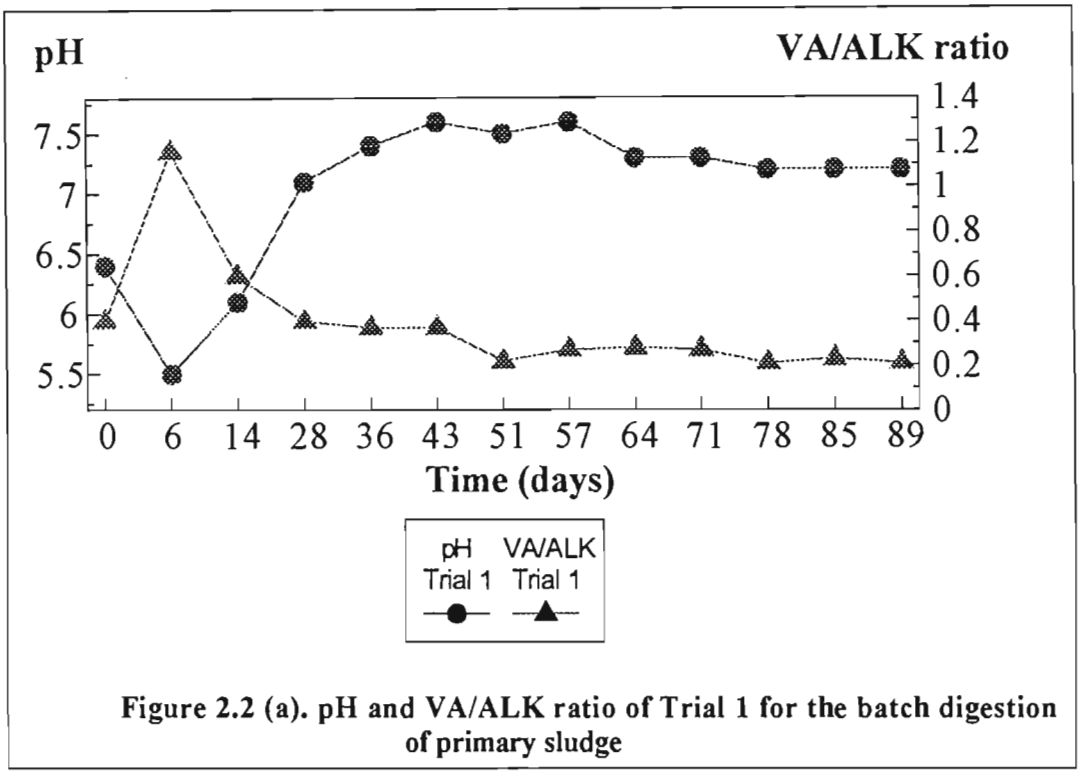

Figure 2.2 (a). pH and VA/ALK ratio of Trial 1 for the batch digestion of primary sludge

The initial pH of Trial 2 was 7.6 since a higher proportion of inoculum to substrate was used to counteract the previously long lag period encountered in Trial 1. On day 6 the pH dropped to 6.8 which is the lower limit for anaerobic digestion. Sodium bicarbonate was added to increase the alkalinity, thereby neutralizing the acids produced during the hydrolysis-fermentation stage of digestion. This low pH and unfavourable conditions was further emphasized by the high VA/ALK ratio which peaked above 0.3. Nine days later the pH stabilized around 7.0 and 7.3 which is considered to be within the optimum pH range required for methane production. The VA/ALK ratio ranged from 0.2 to 0.3.

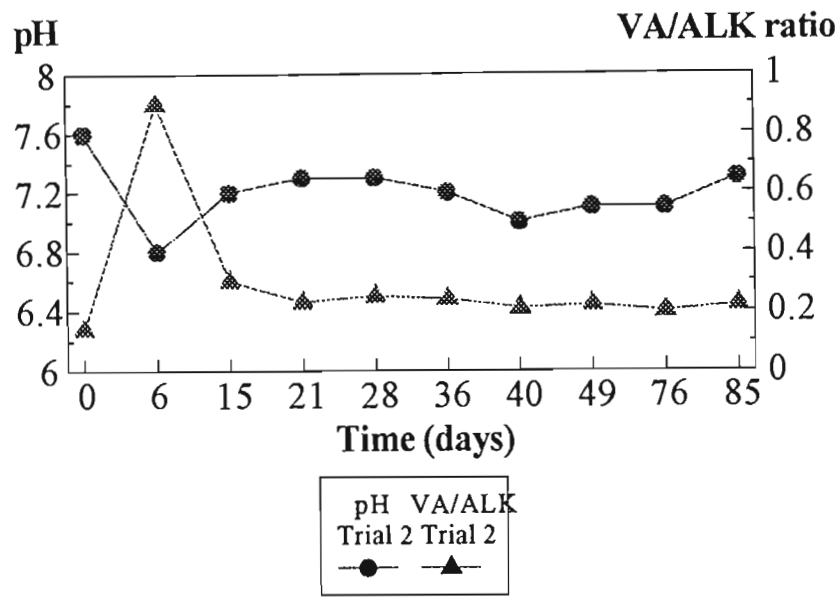

Figure 2.2 (b). pH and VA/ALK ratios of Trial 2 for the batch digestion of primary sludge

2.1.2. Volatile Solids Destruction and Total Gas Production

The initial volatile solids content for Trial 1 was 76%(m/m) (Figure 2.3(a)). Over the next four weeks the volatile solids content (%) dropped to 74% which is indicative of a long lag period and poor volatile solids destruction or consumption.Furthermore, the total gas produced after about 40 days was only 8330 ml (0.3266 moles at STP). The low volatile solids consumption and gas production were probably due to the unfavourable environmental conditions in the digester which were due to the low initial pH. The long recovery period was followed by a rapid VS destruction stage and resulted in the volatile solids content decreasing from 74% to about 63% within 30 days. The total gas production also increased substantially from 8 330 ml to 23 490 ml (0.9229 moles) by day 70. Thus, the total gas production tripled within 30 days of the initial lag stage. After day 70 little or no gas was produced. Thereafter, the volatile solids (%) in the samples decreased to about 50% and levelled off with no further significant decreases in the volatile solids content after 85 days.

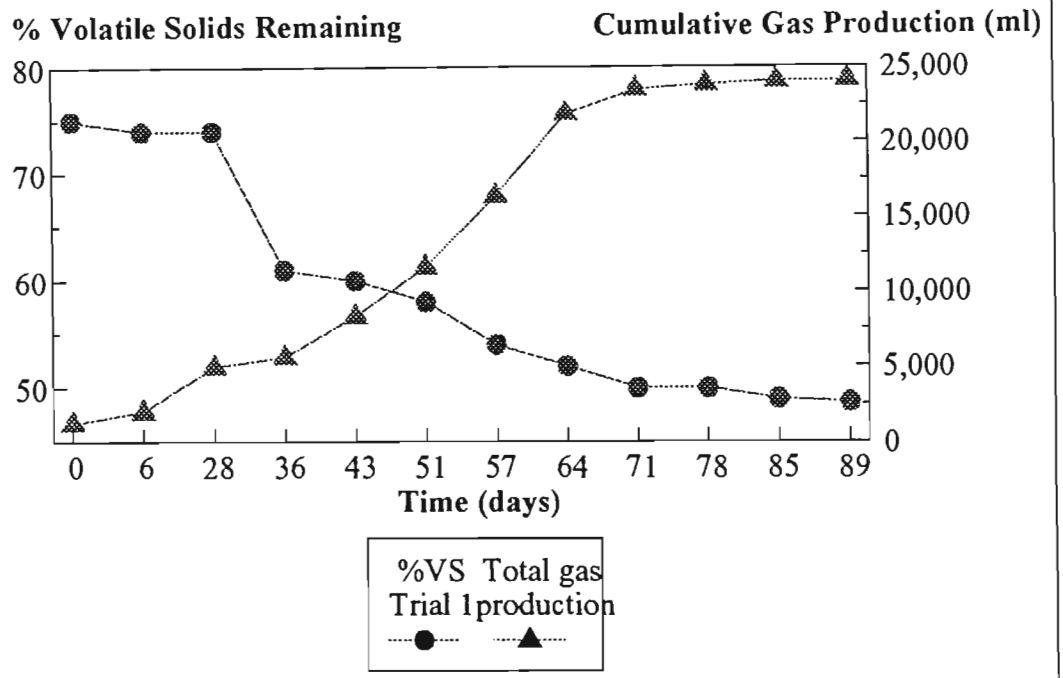

Figure 2.3 (a). Changes in volatile solids and cumulative gas production with time

Trial 2 produced a shorter lag period, owing to the change in the substrate/inoculum ratio (Figure 2.3(b)). The initial lag period lasted only 10 days for Trial 2 compared to the 28 day lag period of Trial 1. Similarly, the gas produced during this period was minimum (1 280 ml, 0.2 moles). In Trial 2 the unfavourable environmental conditions did not last as the pH and VA/ALK ratio was quickly reinstated by the addition of sodium bicarbonate. The volatile solids content (%) fell from 70% to 54% and the total gas generated increased from 1 280 ml (0.2 moles) to 16 949 ml (0.8 moles) in about 30 days. Trial 2 produced less gas than Trial 1 because the initial volatile solids concentration was 70% which implies that less readily or esily biodegradable substrate was made available to the bacterial population. The initial volatile solids concentration in Trial 1 was about 75%. The volatile solids concentration then levelled off at about 50% VS with a concomitant decrease and, finally, a cease in gas production. In Trial 2 the lowest concentration of volatile solids attained after 80 days was 50%.

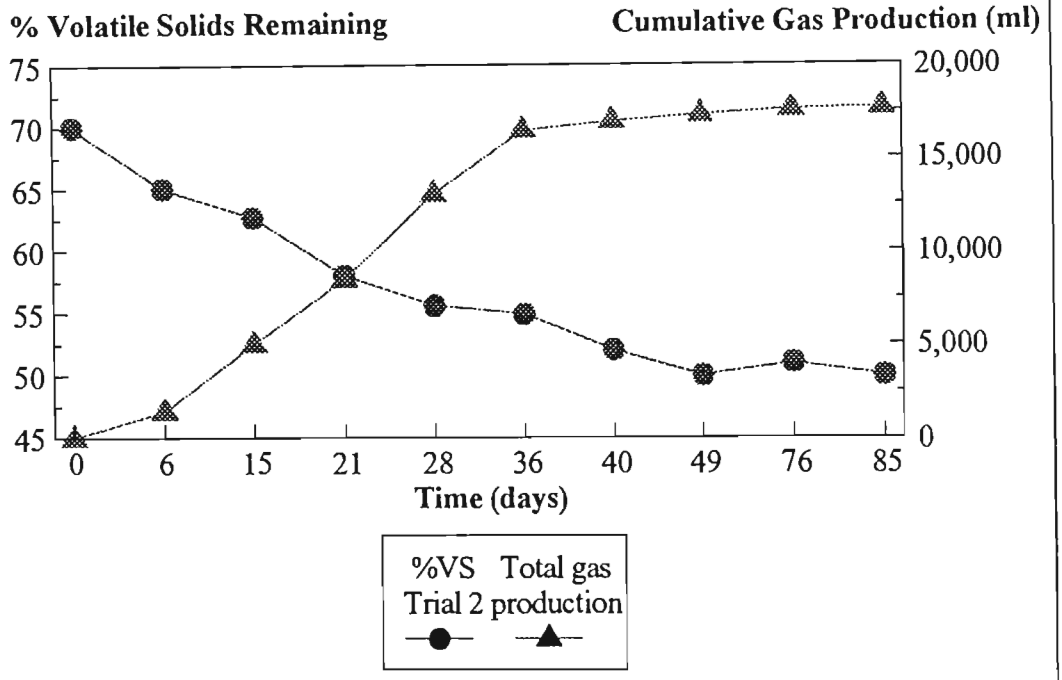

Figure 2.3 (b). Changes in volatile solids and cumulative gas production with time

2.3. Summary

1. Low pH and high volatile acids/alkalinity ratios resulted in unfavourable environmental conditions which required the use of alkaline chemicals such as lime and sodium bicarbonate to correct the pH variations. A large pH drop to 5.5 necessitated the use of these chemicals and a recovery period to enable the digesters to stabilise to a neutral pH. When the digester operated out of the optimum pH and VA/ALK ratio range there was negligible volatile solids removal and gas production was minimum.

2. It was evident from the volatile solids and total gas production data that efficient volatile solids removal is necessary for high gas production since as the volatile solids content in the digester decreased, the gas production increased.

3. The final volatile solids content in Trial 1 and Trial 2 were 48% and 50%, respectively. These were regarded as the lower VS (%) limits for sewage treated at Northern Waste Water Treatment Works.

4. The volatile solids reduction was calculated as 57% and 68% for Trial 1 and 2, respectively.

5. At the end of each trial the volume of gas produced per gram volatile solids removed was calculated.The anaerobic digestion norm is $1m^3$ gas produced g VS destroyed^{-1}. In Trial 1 and Trial 2 this figure was calculated to be 0.92 ℓ gas produced g VS destroyed^{-1} and 0.85 ℓ gas produced g VS removed^{-1}, respectively. Thus the average volume of gas produced g VS removed^{-1} was 0.885 ℓ for the primary sewage treated at NWWTW. (Appendix A)

6. The mass balances of the sludges were conducted after the completion of each experiment and found to be 97.7% for trial 1 and 94.8% for trial 2 (Appendix A)

Chapter Three

Experimental Results : The Effects Of Increased Digested Sludge Concentrations On Microbial Activity

Concentrating digested sludge produces two advantages to the process of anaerobic digestion: increasing the biomass concentration; and reducing the unit volume required for a given quantity of solids. Most conventional digesters are operated with a total solids concentration of 2% to 2.6%. This study was made to determine the differences in gas production volumes and rates in the presence of different digested sludge concentrations. It is important to know if cumulative gas production increases with higher digested sludge concentrations or if concentrating digested sludge produces a negative effect on the process of anaerobic digestion. Increased solids should give greater volumes of gas at increased rates. This study examined seven different total solids concentrations, their respective gas production volumes and the gas production rates.

3.1 Experimental Procedure

Serum bottles (125 ml) were used as batch digesters. Each bottle was filled with 25 ml of substrate (primary sludge) (ca. 6% TS; 80% VS) and 45 ml digested sludge (with different total solids concentrations; 60% VS). The sludge was concentrated in a Beckman centrifuge at 10 000 rpm xg for 25 minutes to 13% TS. The centrate was used to prepare digested sludge solutions of 3%, 4%, 5%. 6.5% and 11% TS. The digested sludge with 2%TS was used as the control. The digesters contained a working volume of 70 ml and a gas headspace volume of about 50 ml. Sufficient headspace was necessary to prevent high gas pressures from breaking the serum bottle seal. Each bottle was then overgassed with oxygen-free nitrogen to displace the air from the bottle and promote the onset of anaerobiosis. The bottles were sealed with a butyl rubber septum and an aluminium cap. and placed in an incubator at 35 ^{0}C and a disposable syringe and

hypodermic needle were inserted through the septum to measure the gas produced daily. All experiments were conducted in quadruplicate.

3.2 Results and Discussion

Figures 3.1 (a), (b) and (c) illustrate cumulative gas productions with time of primary sludge obtained from NWWTW, supplemented with varying concentrations of digested sludge. Each figure shows the average cumulative gas production results of the control and two higher concentrations of digested sludge and their respective maximum and minimum standard deviations (SD). Figure 3.1 (a) shows the effect of digested sludge concentrations of 2% (X0-control), 3% (X1) and 4-4.5% (X2) TS on cumulative gas production. Theoretically, increased biomass concentrations should shorten the lag phase and thus, improve the efficiency of the anaerobic digestion process. There was, however, little difference in the total gas produced after 580 hours for X0 and X1, since X1 produced about 30 ml more than X0. Initially, X1 produced gas at a slower rate than X0 but the gas production rate of X1 increased with time. Within the first five days(100 hours) there was little difference in gas production in all three sets of digested sludge concentrations. Thenceforth, however, the volume of gas produced per day by the bottles which contained digested sludge concentrations >3% TS increased.

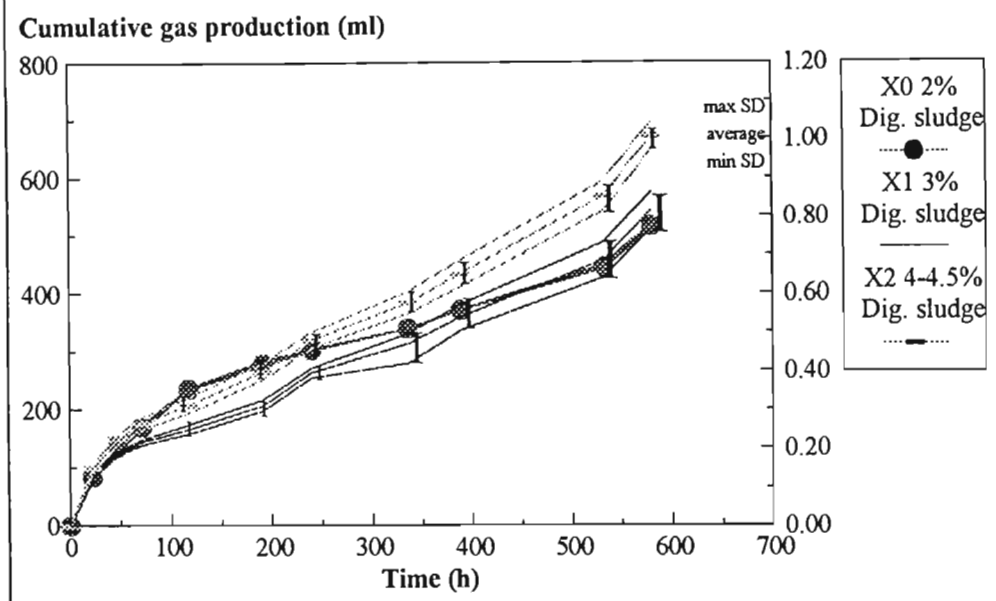

Figure 3.1 (a). Cumulative gas production with time of digested sludge with 2%, and 4-4.5% TS concentration

Figure 3.1 (b) shows a difference in gas production between the control bottles and the digesters with 5.3-5.6% Ts and 6.4-6.6% TS. Digesters with 5.3-5.6% TS produced gas at a similar trend to the control for the first 117 hours. Thereafter, the digesters with 5.3-5.6% TS produced gas at a faster rate. Concentrating up digested sludge to a TS concentration of 6% resulted in both a greater volume of gas and a higher rate of production than the control. The rate of gas production was relatively constant throughout the experimental period. Digesters with sludge concentrations of 11% TS and 12.8-13% TS produced similar gas production curves. These digesters produced gas faster than the control digesters (Figure 3.1 (c)). Thus, from the Figures 3.1 (a), (b) and (c) it can be seen that concentrating sludge produces higher volumes of gas at faster rates. Table 3.1 shows the average cumulative gas production values and their standard deviations.

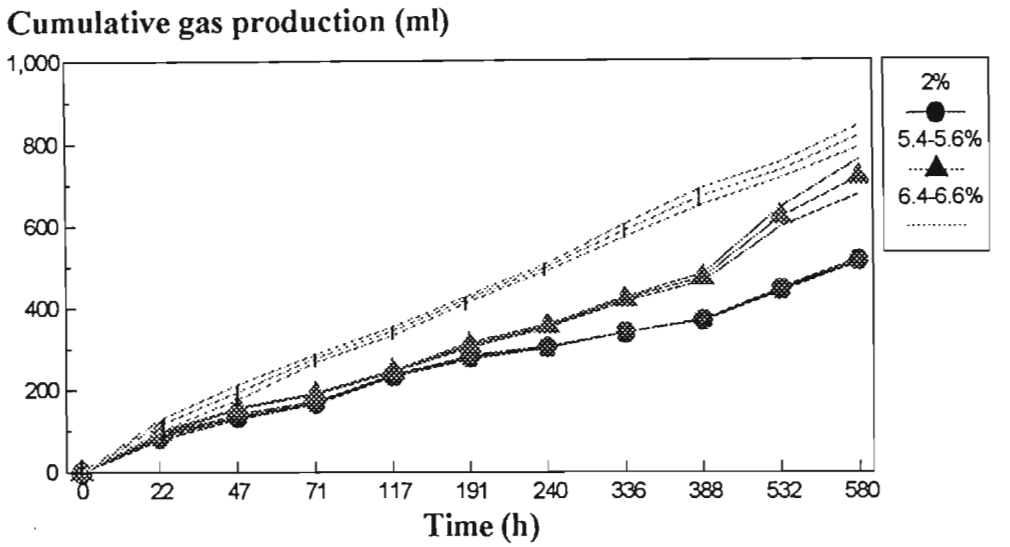

Figure 3.1 (b). Cumulative gas production with time of digested sludge with 2%, 5.1-5.6% and 6.4-6.6% TS concentrations

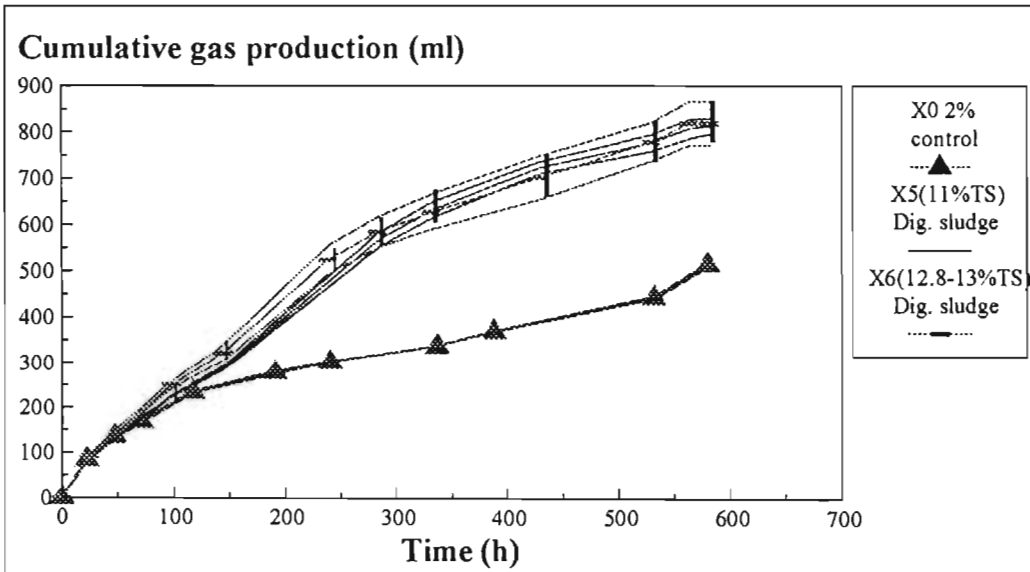

Figure 3.1 (c). Cumulative gas production with time of digested sludge with 2%, 11% and 12.8-13% TS concentrations

Table 3.1 List of average gas production values and their respective standard deviations of each set of total solid concentrations.

Total solids (%)	Time (h)										
	0	22	47	71	117	191	240	336	388	532	580
	Cumulative gas production (ml)										
X0 (2%)											
ave	0	84	135	169	235	279	303	339	370	444	515
+	0	89.52	141.58	173.18	237.6	283.49	304.87	339.71	371.3	448.03	520.85
-	0	78.48	128.42	164.82	232.4	274.51	301.13	338.29	368.7	439.97	509.15
X1 (3%)											
ave	0	82	124	143	165	206	263	314	357	456	543
+	0	84.06	126.95	148.17	172.56	215.97	271.15	330.66	379.39	486.53	575.07
-	0	79.94	121.05	137.83	157.44	196.03	254.85	297.34	334.61	425.47	510.93
X2 (4-4.5%)											
Ave	0	98	150	175	207	265	319	383	433	567	671
+	0	104.22	158.26	184.84	220.81	279.15	332.95	402.11	456.38	592.97	695.72
-	0	91.78	141.74	165.16	193.19	250.85	305.05	363.89	409.62	541.03	646.28
X3 (5.3-5.6%)											
mean	0	95	156	191	246	309	355	421	473	621	717
+	0	98.74	158.24	193.38	249.08	315.61	359.39	426.7	482.6	644.82	759.01

–	0	91.26	153.76	188.62	242.92	302.39	350.61	415.3	463.4	597.18	674.99
X4 (6.4-6.6%)											
Ave	0	114	195	277	343	423	499	590	672	734	815
+	0	127.44	213.12	286.12	353.89	430.63	508.23	605.85	692.94	753.6	841.39
-	0	100.56	176.88	267.88	332.11	415.37	489.77	574.15	651.06	714.4	788.61
X5 (11%)											
Ave	0	89	225	289	476	566	632	723	777	807	813
+	0	92.74	227.6	294.2	487.56	581.64	648.96	735.52	794.09	827.21	830.01
-	0	85.26	222.4	283.8	464.44	550.36	615.04	710.48	759.91	786.79	795.99
X6 (12.8-13%)											
Ave	0	91	251	323	524	584	628	702	779	819	819
+	0	97.98	258.48	340.63	554.54	618	665.84	749.65	820.97	865.68	866.63
-	0	84.02	243.52	305.37	493.46	550	590.16	654.35	737.03	772.32	771.37

Figure 3.2. shows the differences in total gas volumes produced by the batch digesters with different digested sludge concentrations over 24 days. The total gas produced from the digestion of about 1.2 g of volatile solids (primary sludge) increased as the digested sludge total solids concentration increased (Table 3.2). The batch digesters produced similar volumes of gas for digested sludge concentrations of 6% TS. It was evident that concentrated digested sludge produced more gas which suggested that volatile solids destruction was greater with the higher solids concentrations. This may be equated to improved anaerobic digestion of primary sludge.

By using the anaerobic digestion results from the biodegradability study made prior to this experiment, the mass of volatile solids destroyed can be estimated. Table 3.2 lists the values calculated from Figures 3.1 (a) , (b) and (c).

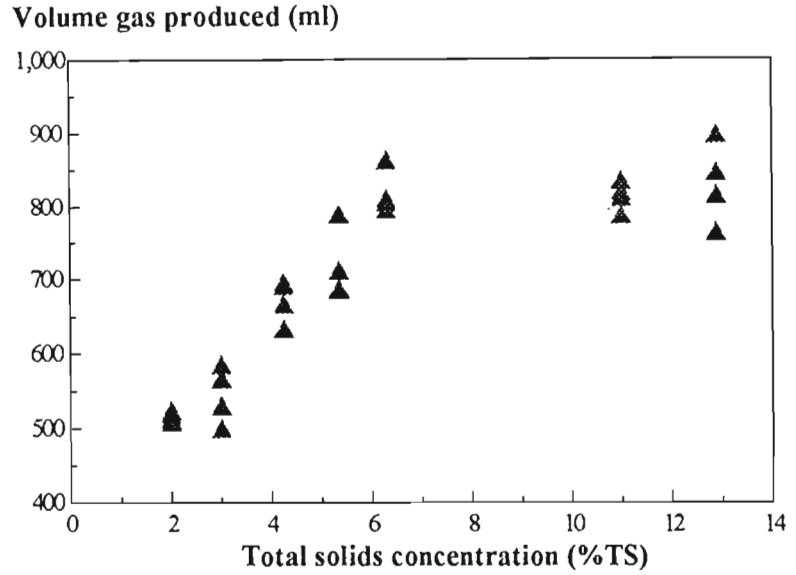

Figure 3.2. The total gas produced by different concentrations of digested sludge over 24 days.

An increase in digested sludge concentration resulted in an increase in the volatile solids catabolised up to 6% TS. Any further increase in the TS concentration did not, however, result in improved volatile solids destruction. There was, however, an increase in the rate at which the gas was produced at concentrations above 6% TS. Figure 3.3 shows the rates of gas production of the different digested sludge concentrations. X5 and X6 generated gas at about 3 times the rate of the controls. Initially, digested sludge concentrations of 11% and 13% TS produced gas at a rapid rate and, although this rate decreased during the 24 day experimental period, the overall rate of gas production per day was still higher than that of the control and the other four concentrations of digested sludge tested. However, setting up anaerobic digesters in the laboratory with solids concentrations greater than 6% proved problematic.

Table 3.2 Total gas produced, rate of gas production and volatile solids destruction of substrate subjected to different concentrations of digested sludge.

	TS	Total Gas produced ml	Rate (ml d^{-1})	Volatile solids destroyed (A)		Volatile solids destroyed (B)	
				g	%	g	%
X0	2	515	6.22	0.59	49	0.52	43
X1	3	543	8.37	0.62	51	0.54	45
X2	4-4.5	671	9.93	0.76	64	0.67	56
X3	5.3-5.6	717	10.44	0.81	68	0.71	59
X4	6.4-6.6	815	16.07	0.93	77	0.82	68
X5	11	813	21.86	0.92	76	0.81	67.5
X6	12.8-13	819	20.84	0.93	78	0.82	68

A= 0.88 ℓ gas produced g volatile solids destroyed^{-1} (Calculated from the biodegradability study)

B= 1.0 ℓ gas produced g volatile solids destroyed^{-1} (Standard anaerobic digestion value for primary sludge)

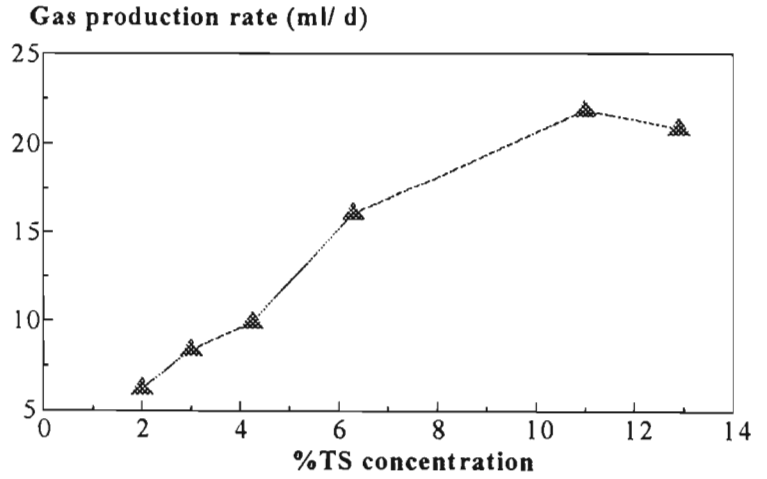

Figure 3.3. Changes in the rate of gas production in response to different total solids concentrations

3.3 Summary

1. Increased digested sludge concentrations produced greater volumes of gas during the anaerobic digestion of primary sludge. The digesters operated with initial digested sludge concentrations of 6% produced up to 300 ml more gas.

2. Increased digested sludge concentrations also produced gas at a faster rate since the more concentrated sludge degraded the primary sludge more rapidly than the control (2% TS).

3. Using the anaerobic digestion figures of 0.88 ℓ (experimental value) and 1.0 ℓ (standard anaerobic digestion value for primary sludge) gas produced per g volatile solids destroyed, the mass of volatile solids accounted for by the bacterial population was estimated (Table 3.2). The percentage volatile solids catabolised increased as the concentration of digested sludge increased up to 6% TS, after which there appeared to be little difference in the efficacy of the batch digesters with 6%, 11% and 13% TS.

Chapter Four

Experimental Results : Operation Of Four Semi-Continuous Anaerobic Digesters With Different Solids Concentrations

Although semi-continuous systems are more time consuming to set up and operate, they have the advantage of utilizing actively growing and metabolizing microbial cells to biodegrade substrates. These systems operate within the exponential phase of bacterial growth thus avoiding the lag, stationary and autolytic phases. Thus, under optimum and constant environmental conditions a steady state of anaerobic digestion can be achieved. However, the systems have to be carefully monitored and controlled to negate toxic shock and/or, organic or volumetric overloads.

4.1 Experimental Procedure

Digester Configuration: Four sequencing batch reactors (semi- continuous digesters) were operated simultaneously to determine the efficiency of digestion with different solids concentrations. The total volume of each digester was 2 ℓ, with a working or available volume of 1.5 ℓ, resulting in a headspace volume of about 500 ml. These were standard rate digesters with no mechanical mixing device, and thus, a time of 30 days was selected. The digesters were, however, shaken daily during sampling and maintained in a waterbath at a constant temperature of 35 °C. Each digester (Figure 4.1) was connected to the gas collection system by silicone

tubing. An effluent and influent channel allowed for waste from the system to be removed and for the substrate (primary sludge) to be added daily, respectively.

Digester Operation: Conventional digesters usually operate with solids concentrations of about 2-3%(m/v) TS, with 3%TS(m/v) the maximum solids concentration attainable. The four digesters were operated with 2% (Run 1), 3% (Run 2), 3.8% (Run 3) and 4.7% (Run 4) TS. Higher solids concentrations (i.e. > 5% TS) proved difficult to handle on a laboratory-scale, with sampling almost impossible in a 2 ℓ digester. Solids concentrations > 5% TS could have resulted in many sampling errors. Run 1 was the control with no recycle of solids. To maintain concentrations of total solids > 2% TS, the effluent wasted per day had to be centrifuged and the solids recycled to the digester. The sludge was concentrated in a Beckman centrifuge at 10,000 rpm xg for 25 minutes and the centrate was used to prepare solutions of 3%, 3.8% and 4.7% TS daily.

Approximately 50 ml of sludge from Run 1 were removed daily and replaced with 50 ml of primary sludge. Primary sludge of 5% total solids and 78-80% volatile solids concentration was added daily. Care was taken to maintain a constant concentration. Approximately 150 ml of digested sludge were removed daily from Runs 2, 3 and 4. A small volume of 20 to 50 ml (depending on type of analysis) digested sludge was stored for analysis and the rest were centrifuged and, thus, concentrated to solids thicknesses of 3%, 3.8% and 4.7% TS. A total of 150 ml, consisting of 50 ml substrate and 100 ml concentrated sludge was recycled to the digesters 2, 3 and 4. Since Runs 2, 3 and 4 were initiated with solids concentrations of 2% TS, this procedure of removal and recycling was continued until the solids concentration within the digesters reached a steady state of operation.

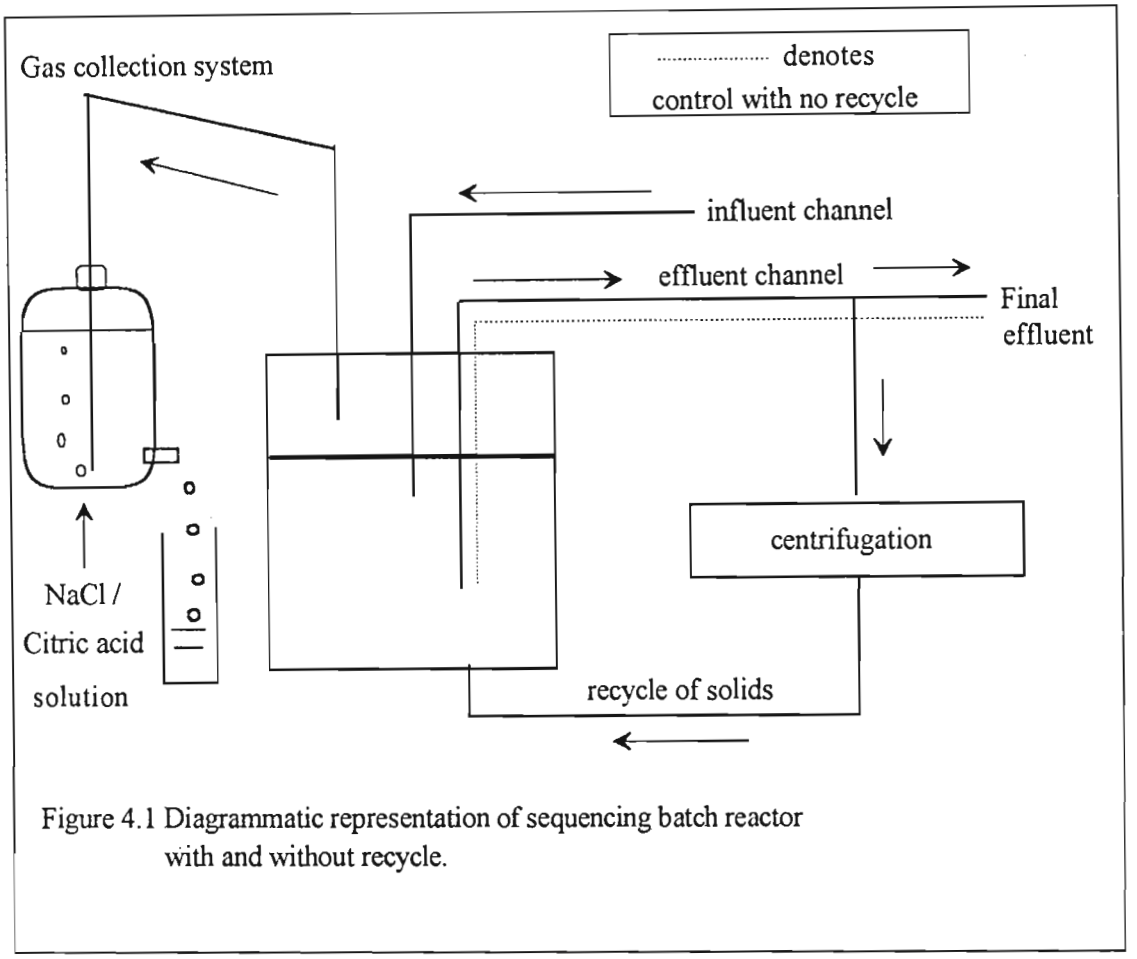

Figure 4.1 Diagrammatic representation of sequencing batch reactor with and without recycle.

Subsequently, the wasted sludge was subjected to various analyses such as volatile solids and total solids concentration, volatile acid concentration, alkalinity, pH, and gas measurements. With the exception of volatile acids and alkalinity measurements, all of these analyses were conducted according to Standard Methods (APHA, 1985). The method of analyses of the latter two are detailed in Appendix D.

4.2 Results and Discussion

4.2.1 Gas Measurements

The gas productions per day in Runs 1,2 3 and 4 was erratic (Figure 4.2). Run 1(2%TS) produced a maximum of 857 ml on day 7 and a minimum of 422 ml on day 1. Throughout the 30 days sampling and analysis period the gas production fluctuated, reaching no uniform rate of production. Runs 2, 3 and 4 produced similar fluctuations in gas production. The initial gas measurements from Run 3 were disregarded owing to a leak in the silicone tubing of the gas collection system.

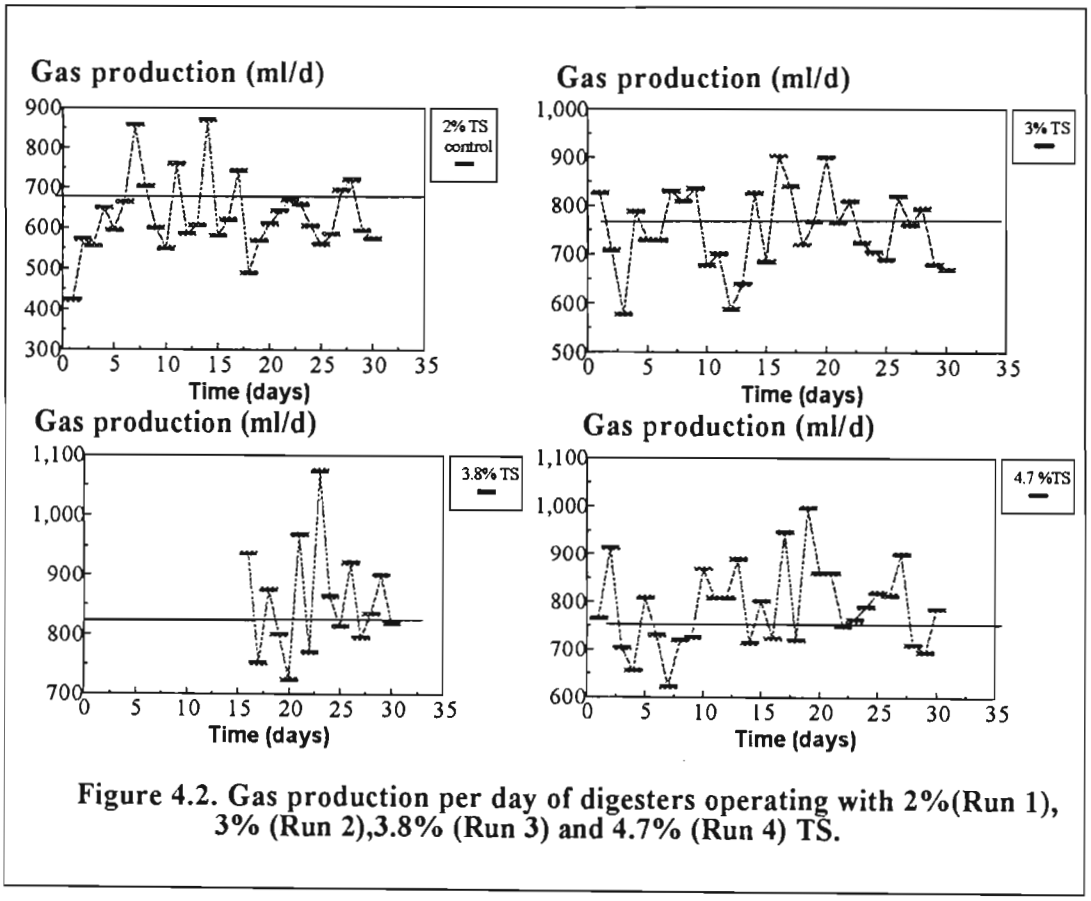

Figure 4.2. Gas production per day of digesters operating with 2%(Run 1), 3% (Run 2),3.8% (Run 3) and 4.7% (Run 4) TS.

Table 4.1 lists the average volumes of gas produced per day and the minimum and maximum values recorded over the 30 days sampling and analysis period. For each of the solids concentrations there was a difference of approximately 300 ml between the maximum and minimum values recorded. The rate of gas production increased from 631 ml d^{-1} for 2%(m/v) TS to 856 ml d^{-1} for 3.8%(m/v) TS, an increase of 26%. Thus, a doubling in solids concentration did not effect a doubling in gas volume production. The digester which was operated with a solids concentration of 4.7%(m/v) TS produced gas at a lower rate (784 ml d^{-1}) than Run 3.This could indicate that digesters with concentrations of 4.7% TS affect microbial activity. However, gas could have been trapped within the viscous sludge since the digester contained no efficient mixing mechanism. This could have lead to inaccurate gas measurements. Inefficient mixing could have also resulted in a non-uniform distribution of substrate to the microorganisms which could have influenced microbial activity. The lack of homogeneity within the digester could have led to the concentration of inhibitory compounds produced during anaerobic digestion.

4.2.2. Volatile and Total Solids Analyses

Total solids analysis of digested sludge indicated a relatively stable total solids concentration. during the 30 days experimental period (Figure 4.3). During Run 1 the total solids fluctuated from 2% to 2.5%, with a maximum of 2.7% TS recorded only once on day 30. The volatile solids of the digested sludge ranged between 60 and 65%, decreasing below 60% only twice during Run 2. The total solids concentration remained fairly constant for the initial 15 days during Run 2 but increased to 3.4% and then levelled off at 3.25% TS. The total solids concentration of Run 3 started at 3.8% TS and decreased to 3.6% before rising again to 3.8%. During Run 4 the total solids percentage ranged between 4.8 and 4.6%. The fluctuations in total solids concentrations could be attributed to the inability to ensure complete mixing of the contents of the digester. Although the digesters were shaken prior to sampling, homogeneity in

the digesters could not be guaranteed. The volatile solids concentrations for Runs 3 and 4 ranged between 60 and 64%, with little change. Thus, most of the digested sludge analysis of the four digesters revealed a volatile solids percentage between 60% and 65% , while operating with relatively stable total solids concentrations.

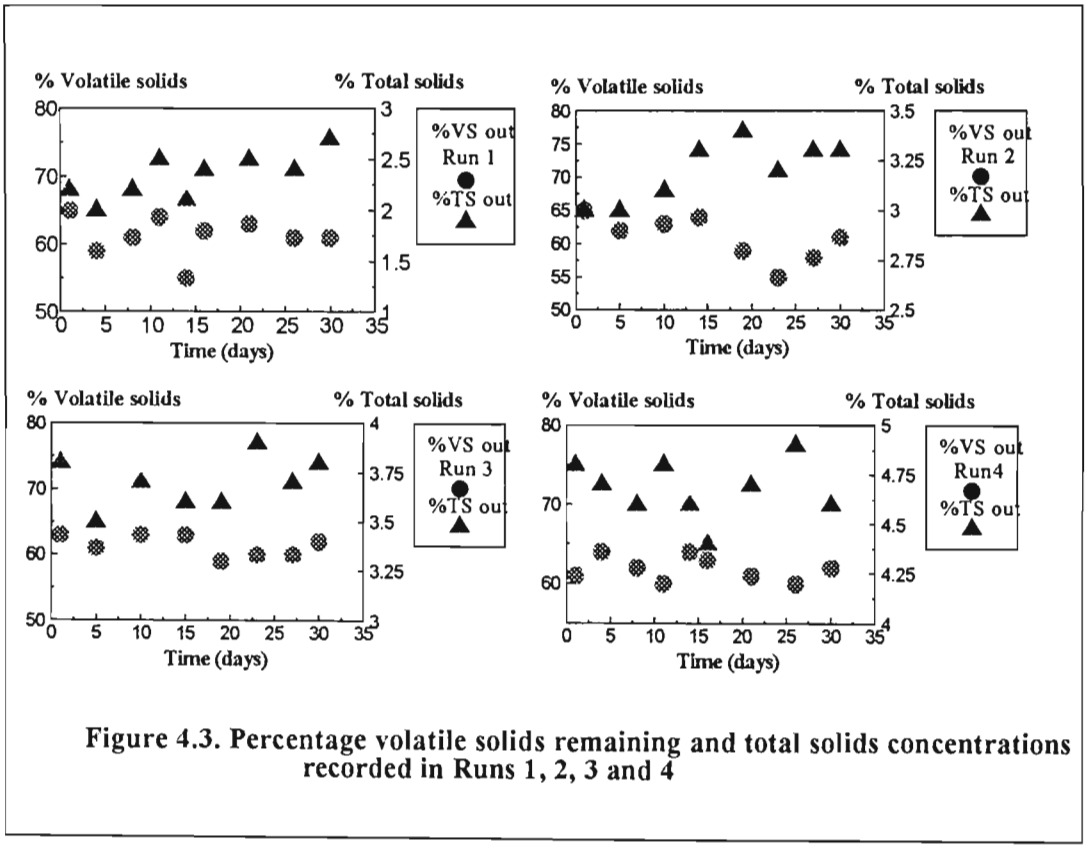

Figure 4.3. Percentage volatile solids remaining and total solids concentrations recorded in Runs 1, 2, 3 and 4

Table 4.1 The average, minimum and maximum volume of gas produced per day by digesters loaded with different solids concentrations.

Run	Concentration % TS	Gas produced per day (ml d^{-1})		
		Average	Minimum	Maximum
1	2%	631	422	857
2	3%	742	578	904
3	3.8%	856	752	1,075
4	4.7%	784	622	998

4.3.3 Volatile Acids, Alkalinity, pH and Volatile Acids/Alkalinity Ratio

During the continuous or semi-continuous operation of anaerobic digesters there is always, potentially, the threat of toxic, organic or volumetric overload. Overloading is manifested in various ways such as pH,volatile acids and alkalinity changes. Thus, stress and impending digester failure or "souring" can be averted by monitoring the changes within the chemical environment of the digester.

Figure 4.4 illustrates the changes in volatile acid and alkalinity concentrations. The volatile acid concentration fluctuated between 400-600 mg ℓ^{-1} in Runs 1 and 2. Higher volatile acid concentrations were measured in Run 3 when the initial volatile acid concentration of 600 mg ℓ^{-1}, decreased to 400 mg ℓ^{-1} and, subsequently, increased to >800 mg ℓ^{-1}. During Run 4 the initial volatile acid concentration of 264 mg ℓ^{-1}, increased to about 874 mg ℓ^{-1}. Subsequent analyses revealed volatile acid concentrations between 700 and 800 mg ℓ^{-1}. Thus, the digesters which were operated under increased solids concentrations produced higher volatile acid concentrations.

Figure 4.5 shows the pH and volatile acids/alkalinity (VA/ALK) ratio results of all four digesters. The pH of each digester remained steady throughout the sampling period although increases in volatile acid concentrations for Runs 3 and 4 were recorded. High accumulations of acids within the digesters are required to effect pH changes. Thus, pH measurements do not provide a rapid warning of imminent stress or failure. Therefore, reliance on pH alone as a tool for process control of continuous and semi-continuous systems is inadequate.

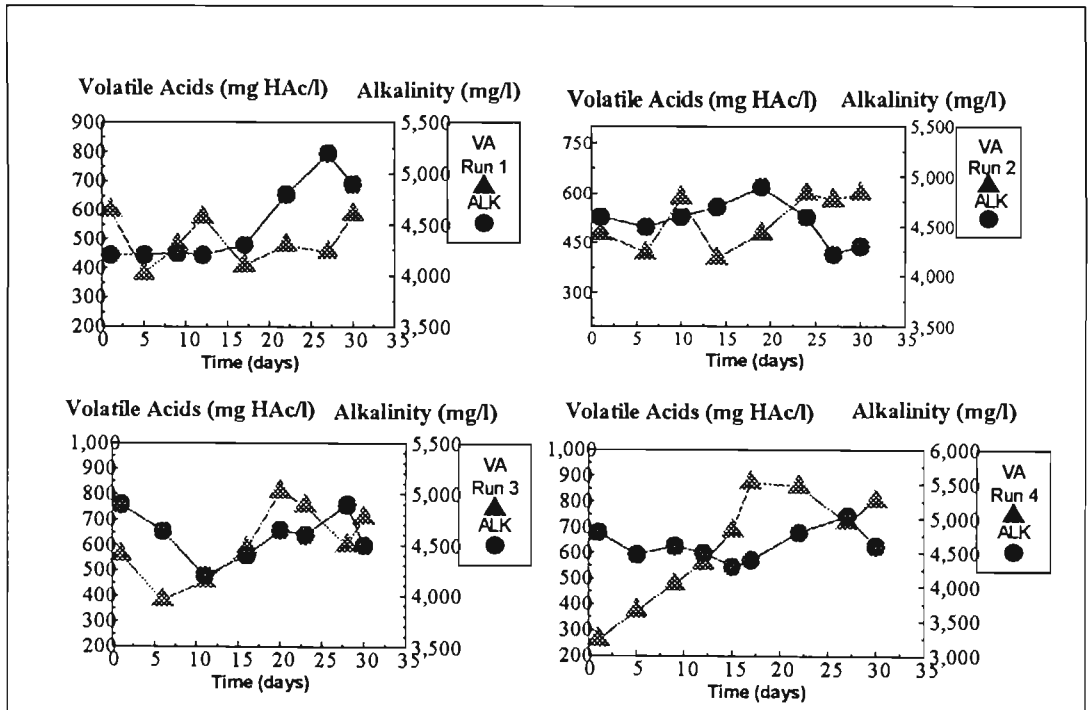

Figure 4.4. Changes in concentrations of volatile acids and alkalinity of sequencing batch digesters operated with different solids concentrations for 30 days

The VA/ALK ratio is an important analytical criterion for monitoring digester behaviour owing to its senstivity to changes in the volatile acid concentration and buffering capacity. The VA/ALK ratios for Runs 1 and 2 ranged between 0.1 and 0.14. Digester monitoring revealed

higher VA/ALK ratios (> 0.15) in Runs 3 and 4 towards the end of the sampling and analysis period. However, these values were still below the permissible limit of 0.3.

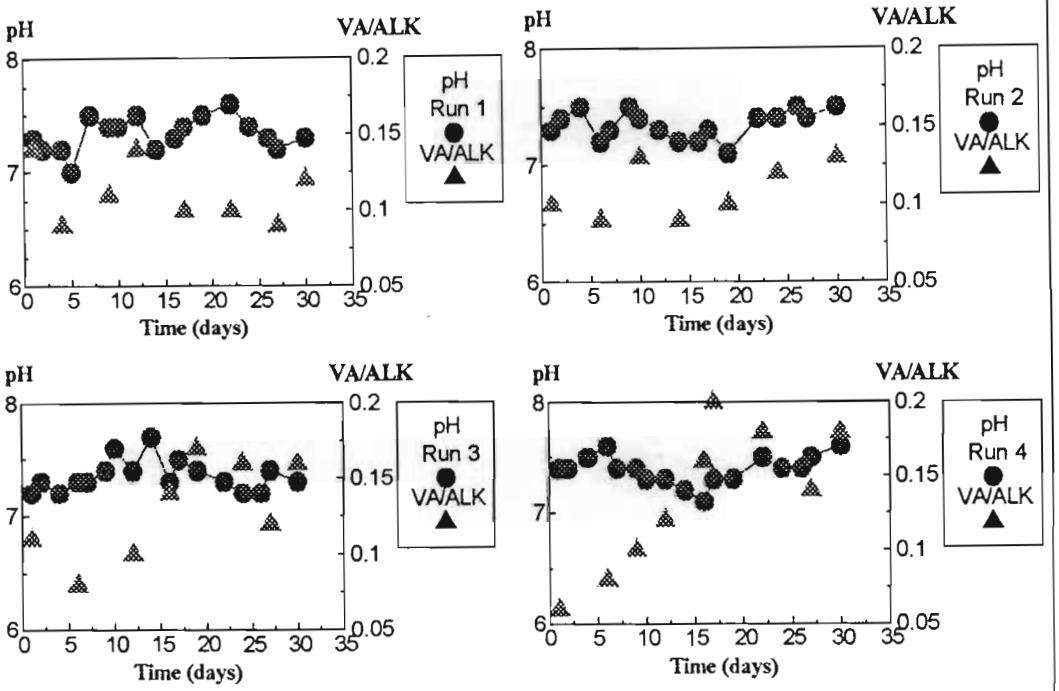

Figure 4.5. Changes in pH and volatile acids/alkalinity ratios of digesters operating with different solids concentrations

4.2.4 Digested Sludge Observations

The digested sludge wasted daily from the four digesters varied in characteristics. The digested sludge from Run 1 can be described as a free flowing slurry which could be removed from the digester and measured with little difficulty. Runs 2, 3 and 4 produced sludge which was black and relatively odourless. Run 4 produced sludge which had a high viscosity and was difficult to sample and recycle to the digester. Shaking of the digesters operating with solids concentrations of 3.8% and 4.7% TS was problematic because of the sticky nature of the sludge.

4.3 Summary

1. The gas produced per day (rate) for Runs 1, 2 and 3 increased as the solids concentration of the digester increased. Run 4, however, produced gas at a lower rate than Run 3. From the four solids concentrations tested it appeared that the rate of gas production increases up to a concentration of about 4%TS. Any further increase in total solids results in a lower rate.

2. All four digesters produced digested sludge with a % volatile solids concentration ranging between 60% and 65%.

3. The volatile acids concentrations recorded for Runs 1 and 2 fluctuated between 400 and 600 mg ℓ^{-1}, while Runs 3 and 4 produced volatile acids concentrations > 800 mg ℓ^{-1}.

4. The alkalinity of all four digesters was > 4000 mg ℓ^{-1} and there were no downward trends in the buffering capacities of the digesters.

5. The pH measurements taken were not sensitive indicators of changes in the volatile acid concentrations, especially for Runs 3 and 4.

6. The volatile acid/alkalinity ratios changed as the volatile acid concentrations changed and, thus, provided useful information of when the volatile acid concentrations were becoming critically high.

Chapter Five

Summary

One of the primary objectives of this study was to determine if increasing the concentration of digested sludge in digesters has the potential to improve anaerobic digestion. However, it was equally important to determine if thickened sludge would reduce microbial activity. The conclusions from the three experiments described in Chapters 2, 3 and 4 are discussed below.

The Biodegradability Study was an essential preliminary experiment which assessed the biodegradation potential of primary sludge from the Northern Waste Water Treatment Works, Durban. Since the solids will remain in the digester for longer time periods, the microorganisms have the potential to increase the volatile solids reduction of the substrate. The lower volatile solids limits of 48%(m/m) and 50%(m/m) can, therefore, be used to assess the efficiency of volatile solids destruction of the anaerobic digester/CFMF unit. Furthermore, the ratio of gas production per g volatile solids catabolised can be used to assess the biodegradation potential of the full-scale digester coupled to the microfiltration unit at the Northern Waste Water Treatment Works.

The second experiment evaluated the effects of seven different digested solids concentrations on microbial activity. It was concluded that operating a full-scale anaerobic digester at solids concentrations >2% should improve anaerobic digestion efficiency. The results also indicated that an increase in solids concentration to > 6.6%(m/v) was not favourable due to mass transfer and mixing difficulties. However, it is highly improbable that the anaerobic digesters would be operated at such high concentrations due to the viscosity of the sludge and problems associated

with pumping concentrated sludge. Thus, microbial activity will not be reduced in full-scale digesters with 3-6%(m/v) TS.

The results of four semi-continuous anaerobic digesters with different solids concentrations showed that increased solids concentrations did not significantly change volatile solids destruction or gas production. Digesters with higher solids concentrations may, however, require expert process control and monitoring due to the higher concentrations of volatile acids and volatile acid/alkalinity ratios. By linking a full-scale anaerobic digester to a cross-flow microfiltration unit greater quantities of solids per unit volume could be treated with concomitant higher gas production rates. Thus, it can be concluded that an increase in solids should not benefit or impede volatile solids destruction and microbial activity. Since increased solids concentrations did not reduce anaerobic digestion efficiency, it can be concluded that it would be beneficial to operate a full-scale digester with increased solids concentrations.

The conventional anaerobic digestion process at the Northern Waste Water Treatment Works consists of a primary thickener, three primary digesters and a secondary digester. Based on loading considerations a CFMF/anaerobic digestion process (see Figure 1.3) would consist of a primary thickener and two primary digesters. Since the capital costs, calculated during an economic feasibility study of a CFMF/anaerobic digester system, were 27% lower than those of the conventional process equipment the new linked process would be economically feasible (Personal Communication, V.L. Pillay, 1994). Furthermore, existing digesters could be operated at hydraulic loading rates which exceed their present maximum values without compromising the extent of volatile solids destruction. This would, therefore, delay the need for construction of additional digesters. There would also be advantages to upstream and downstream processing. The effluent from the digester should have higher solids concentrations, thus reducing the volumetric load to the sludge concentration and dewatering equipment. The permeate from the cross-flow microfiltration unit should have a negligible suspended solids content, thus reducing the recirculating solids load.

References

Ahring, B.K. (1994) **Status on Science and Application of thermophilic anaerobic digestion,** *Proceedings of Seventh International Symposium on anaerobic digestion.* 23-27 January, Cape Town, South Africa. pp. 328-337.

American Public Health Association. (1985) **Standard Methods for the Examination of Water and Wastewater,** 16th edition. APHA. Washington.

Bailey, J.E. and Ollis, D.F. (1986) **Biochemical Engineering Fundamentals**. McGraw-Hill, N.Y.

Bindoff, A.M., Treffry-Goatley, K., Fortmann, N.E., Hunt, J.W. and Buckley, C.A. (1988) **Application of CFMF technology to the concentration of sewage works sludge streams,** *J. IWEM.* 2:513-522.

Bruce, A.M. (1986) **Anaerobic Sludge Digestion,** In: *Anaerobic Digestion of Sewage Sludge and Organic Agricultural Wastes.* Elsevier Appl. Sci. Publ., Barking, U.K.

Brunetti, A., Lore, F. and Lotito, V. (1988) **Methanogenic Potential of Substrate in Anaerobic Digestion of Sewage Sludge,** *Environ. Tech. Let.* 9: 753-762.

Canale, R.P. (1971) **Biological Waste Treatment,** pp. 1-5 and 85-107.

Chynoweth, D.P., Svoronos, S.A., Lyberatos, G., Harman, J.L., Pullammanappillil, P., Owens, J.M. and M.J. Peck. (1994) **Real-time expert system control of anaerobic digestion,** *Proceedings of Seventh International Symposium on anaerobic digestion.* 23-27 January, Cape Town, South Africa. pp. 22-31.

Corbitt, R.A. (1989) **Wastewater Disposal Standard Handbook of Environmental Engineering.** pp. 6.125-6.129. McGraw-Hill, N.Y.

De Baere, L.A., Rozzi, A. and Verstraete, W. (1984) **Solubilization of particulate organic matter as the rate limiting step in anaerobic digestion,** *Trib. Cebedeau*. 34(484): 75-81.

Dohanyos, M., Kosova, B., Zabranska, J. and Grau, P. (1985) **Production and Utilization of Volatile Fatty Acids in various types of Anaerobic reactors,** *Wat. Sci. Tech.* 17: 191-205.

Dolfing, J., and Bloemen, W.G.B.M. (1985) **Activity measurements as a tool to characterize the microbial composition of methanogenic environments,** *J. Micro. Methods.* 4: 1-12.

Duarte, A.C., and Anderson, G.K. (1982) **Inhibition Modelling in Anaerobic Digestion**. *Wat. Sci. Tech.* 14: 749-763.

U.S. EPA Report. (1987) **EPA Design Information: Anaerobic Digester Mixing Systems,** *J. Wat. Poll. Contr. Fed.* 59(3): 162-170.

Ghosh, S. (1987) **Improved Anaerobic Gasification by two-phase anaerobic digestion,** *J. Environ. Eng. Div. ASCE.* 113(6): 786-791.

Gosling, P., and Brown, D. (1993) **Membranes for Sewage Treatment : The Reality,** *Wat. Sci. Tech.* 27(5-6): 439-447.

Gray, N.F. (1989) **Biology of Wastewater Treatment**. pp. 208-217. University Press, Oxford.

Hall, E.R. (1992) **Anaerobic Treatment of Wastewaters in Suspended Growth and Fixed Film Processes,** In: *Design of Anaerobic Processes for the Treatment of Industrial and Municipal Wastes,* J.F. Malina and F.G. Pohland (eds.). pp.41-65. ISBN No. 87762-942-0

Harper, S.R., and Pohland, F.G. (1987) **Enhancement of Anaerobic Treatment Efficiency through process Modification,** *J. Wat. Poll. Contr. Fed.* 59(3): 152-161.

Harper, S.R., and Suidan, M.T. (1991) **Anaerobic Treatment Kinetics,** *Wat. Sci. Tech.* 24(8): 61-78.

Hawkes, F.R., Guwy, A.J., Hawkes, D.L. and Rozzi, A.G. (1994) **On-line monitoring of anaerobic digestion: Application of a device for continuous measurement of bicarbonate alkalinity,** *Proceedings of Seventh International Symposium on anaerobic digestion*, 23-27 January, Cape Town, South Africa. pp. 2-11.

Hespell, R.B. (1990) **Isolation of Anaerobic Microorganisms,** In: *Isolation of Biotechnological Organisms from Nature,* D.P. Labeda (ed.). McGraw-Hill, N.Y. pp. 117-122.

Hunt, J.W. (1987) **Mathematical Modelling of Cross-flow Microfiltration,** M.Sc. Thesis. University of Natal, Durban.

Iza, J. (1991) **Fluidized bed reactors for Anaerobic Wastewater Treatment,** *Wat. Sci. Tech.* 24(8): 109-132.

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,** *Wat. Sci. Tech.* 24(8): 1-16.

Jenkins, S.R., Morgan, J.M. and C.L. Sawyer, C.L. (1983) **Measuring anaerobic sludge digestion and growth by a simple alkalimetric titration,** *J. Wat. Poll. Contr. Fed.* 55: 448-453.

Jewell, W.J. (1987) **Anaerobic sewage treatment,** *Environ. Sci. Tech.* 21(1): 14-20.

Jones, W.J., Nagle, D.R. and Whitman, W.R. (1987) **Methanogens and the Diversity of Archaebacteria,** *Micro. Rev.* 513: 154-163.

Kapp, H. (1984) **Sludge thickening prior to anaerobic digestion,** *Wat. Sci. Tech.* 16:419-432.

Karhardkar, P.P., Audic, J.M., Faup, G.M. and Khanna, P. (1987) **Sulfide and Sulfate Inhibition of Methanogenesis,** *Wat. Res.* 21(9): 1061-1066.

Kidby, D.W., and Nedwell, D.B. (1991) **An Investigation into the Suitability of Biogas Hydrogen Concentration as a performance monitor for Anaerobic Sewage Sludge Digestion,** *Wat. Res.* 25(8): 1007-1012.

Kouzeli-Katsiri, A., Kartsonas, N. and Priftis, A. (1988) **Assessment of the Toxicity of Heavy Metals to the Anaerobic Digestion of Sewage Sludge,** *Environ. Tech. Let.* 9: 261-270.

Lettinga, G. and Hulshoff Pol, L.W. (1991). **UASB - Process Design for Various types of Wastewaters,** *Wat. Sci. Tech.* 24(8): 87-107.

Lema, J.M., Mendez, R., Iza, J., Garcia, P. and Fernandez-Polanco, F. (1991) **Chemical Reactor Engineering Concepts in Design and Operation of Anaerobic Treatment Processes,** *Wat. Sci. Tech.* 24(8):79-86.

Malina, J.F. (1992) **Anaerobic Sludge Digestion,** In: *Design of Anaerobic processesfor the Treatment of Industrial and Municipal Wastes,* J.F. Malina and F.G. Pohland (eds.). pp. 167-189. ISBN No. 87762-942-0

Mawson, A.J., Earle, R.L. and Larsen, V.F. (1991) **Degradation of Acetic and Propionic Acids in the Methane Fermentation,** *Wat. Res.* 25(12): 1549-1554.

McCarty, P.L. (1975) **Anaerobic Processes,** *Wat. Res.* 9:307.

McCarty, P.L. and Mosey, F.E. (1991) **Modelling of Anaerobic Digestion Processes,** *Wat. Sci. Tech.* 24: 8-c.

McCarty, P.L. and Smith, D.P. (1986) **Anaerobic Wastewater Treatment,** *Environ. Sci. Tech.* 20(12): 1200-1206.

Miyahara, T. and Noike, T. (1994) **Behaviour of suspended solids and anaerobic bacteria in an anaerobic fixed bed reactor,** *Proceedings of Seventh International Symposium on anaerobic digestion.* 23-27 January, Cape Town, South Africa. pp. 64-73.

Molleta, R., Escoffier, Y., Ehlinger, F., Coudert, J.P. and Leyris, J.P. (1994) **On-line automatic control system for monitoring an anaerobic fluidized-bed reactor: Response to organic overload,** *Proceedings of Seventh International Symposium on anaerobic digestion.* 23-27 January, Cape Town, South Africa. pp. 12-21.

Mosey, F.E. (1982) **New Developments in the Anaerobic Treatment of Industrial Wastes,** *J. Wat. Poll. Contr. Fed.* 81: 540-549.

Mosey, F.E. (1983) **Mathematical Modelling of the Anaerobic Digestion Process: Regulatory Mechanisms for the Formation of Short Chain Volatile Fatty Acids from Glucose,** *Wat. Sci. Tech.* 15: 209-232.

Nasr, F.A. and Abdel-Shafy, H.I. (1992) **Biodegradation of sewage Sludge: Toxic Effects of Heavy Metals,** *Environ. Man. Health.* 3(4): 18--25.

Nozhevnikova, A.N., and Kotsyurbenko, O.R. (1994) **Anaerobic digestion under psychrophilic conditions,** *Proceedings of Seventh International Symposium on anaerobic digestion.* 23-27 January, Cape Town, South Africa. pp. 90-100.

Osborn, F.D. (1992) **Anaerobic Digestion: What's Going on Here?,** *Operations Forum.* 9(8): 28-30.

Ouyang, C.F., and Lin, H.Y.(1992) **A Study of Controlled Recirculation of Anaerobic Activated Sludge digestion Reactors,** *Wat. Sci. Tech.* 26(9-11): 2449-2452.

Parkin, G.F., and Owen, W.F. (1986) **Fundamentals of Anaerobic Digestion of Wastewater Sludges,** *J. Environ.Engin. Div. ASCE.* 112(5): 867-914.

Pfeffer, J.T. (1968) **Increased loadings on digesters with recycle of digested solids,** *J. Wat. Poll. Contr. Fed.* 40(11): 1920-1933.

Pillay, V.L. (1991) **Modelling of Turbulent CFMF of Particulate Suspension,** Ph.D. Thesis. University of Natal, Durban.

Pillay, V.L., Townsend, B. and Buckley, C.A. (1994) **Improving the Performance of Anaerobic Digesters at Wastewater Treatment Works : The Coupled Cross-flow Microfiltration / Digester Process,** *Seventh International Symposium on Anaerobic Digestion.* 23-27 January, Cape Town, South Africa. pp. 577-586.

Pohland, F.G. (1971) **Anaerobic Biological Treatment Processes**. pp. 126-190.

Pohland, F.G. (1992) **Anaerobic Treatment: Fundamental Concepts, Applications and New Horizons,** In: *Design of Anaerobic Processes for the Treatment of Industrial and Municipal Water,* J.F. Malina, and F.G. Pohland (eds). pp. 2-37. ISBN No: 87762-942-0.

Rencken, G.E., Buchan, M.I., Treffry-Goatley, K. and Buckley, C.A. (1989) **The Tubular filter press: A locally developed alternative for sludge dewatering,** *ChemSA*, August. pp. 260-263.

Ripley, L.E., Boyle, W.C. and Converse, J.C. (1986) **Improved alkalimetric monitoring for anaerobic digestion of high strength wastes,** *J. Wat. Poll. Contr. Fed.* 58(5): 406-411.

Ross, W.R., Novella, P.H., Pitt, A.J., Lund, P., Thomson, B.A., King, P.B. and K.S. Fawcett, K.S. (1992) **Anaerobic Digestion of Wastewater Sludge: Operating Guide,** WRC Project No. 390.

Schroeder, E.D. (1977) **Water and Wastewater Treatment**. pp. 313-337. McGraw-Hill, N.Y. ISBN No: 0-07-055643-1.

Siegrist, H., Renggli, D. and Gujer, W. (1993) **Mathematical Modelling of Anaerobic Mesophilic Sewage Sludge Treatment,** *Wat. Sci. Tech.* 27(2): 25-36.

Song, J.J., Takeda, N. and Hiraoka, M. (1992) **Anaerobic Treatment of Sewage Sludge Treated by Catalytic Wet oxidation Process in Upflow Anaerobic Sludge Blanket Reactors,** *Wat. Sci. Tech.* 26(3-4): 867-875.

Treffry-Goatley, K., Bindoff, A.M. and C.A. Buckley, C.A. (1986) **The potential of CFMF for improving anaerobic digester performance,** *Anaerobic Digestion Symposium Proceedings, Bloemfontein, South Africa.* pp. 126-134.

Ueki, K., Ueki, A. and Simogoh, Y. (1988) **Terminal Steps in the Anaerobic Digestion of Municipal Sewage Sludge: Effects of Inhibitors of Methanogenesis and Sulfate Reduction,** *J. Gen. Appl. Micro.* 34: 425-432.

Van Velsen, A.F.M. (1979) **Adaptation of Methanogenic Sludge to High Ammonia-Nitrogen Concentrations,** *Wat. Res.* 13: 995-999.

Weiland, P. and Rozzi, A. (1991) **The start-up, operation and monitoring of high-rate anaerobic treatment systems: Discussers Report,** *Wat. Sci. Tech.* 24(8): 257-269.

Wu, M. M., Hickey, R.F., Bhatnagar, L., Jain, M.K. and Zeikus, J.G. (1990) **Fatty Acid Degradation as a Tool to Monitor Anaerobic Sludge Activity and Toxicity,** *44th Purdue Industrial Waste Conference Proceedings.* pp. 225-232.

Zhang, T.C. and Noike, T. (1991) **Comparison of One-phase and Two-phase Anaerobic Digestion Processes in Characteristics of Substrate Degradation and Bacterial Population levels,** *Wat. Sci. Tech.* 23:1157-1166.

Appendix A

CALCULATIONS : THE BIODEGRADABILITY STUDY

A1. *Ratio of Volume Gas Produced Per Gram Volatile Solids Removed*

Table A1: Initial and final volatile solids and gas production values for Trials 1 and 2.

	Trial 1 error = 0.01	**Trial 2** error = 0.02
Initial mass of volatile solids (g)	93.75	64.2
Final mass of volatile solids (g)	40.22	19.94
Mass volatile solids removed during sampling (g)	27.32	23.22
Mass volatile solids removed (g)	26.21	21.04
Total gas produced (ml)	24 110	17,800
R* (vol. gas produced g VS removed^{-1}) (ℓ g^{-1})	0.85	0.92

*The following equation was used to calculate R : $R = \frac{\text{total gas produ } \ell}{\text{VS remove } g}$ (A1.1)

Trial 1: $R = \frac{24.110}{26.21} = 0.92\ \ell\ g^{-1}$

Trial 2: $R = \frac{17.799}{21.04} = 0.85\ \ell\ g^{-1}$

A2: *Mass Balances of Trials 1 and 2*

Table A2: Initial and final volatile solids and total solids percentages.

	Trial 1 error = 0.01	**Trial 2** error = 0.02
(%) VS in	75	69.5
(%) TS in	5	3.7
(%) VS out	48	52
(%) TS out	4.9	2.95
g VS in	93.75	64.25
g VS out	40.22	19.94
g VS removed as sample	27.32	23.22

Eqn: **VS in should be = VS out + VS removed as sample + Gas produced+ error** (A2.1)

Trial 1: 93.75 = 40.22 + 27.32 + 24.11+ error (0.58 + 1.52)

93.75 = 91.65 + 2.10

$$\text{Relative \% accuracy} = \frac{91.65}{93.75} = 97.76\%$$

Trial 2: 64.25 = 19.94 + 23.22 + 17.799+ error (0.58 + 2.71)

64.25 = 60.96 + 3.29

$$\text{Relative \% accuracy} = \frac{60.96}{64.25} = 94.89\%$$

Appendix B

DATA OBTAINED FROM THE BATCH DIGESTER EXPERIMENTS

B1:*Data Obtained from the Biodegradability Study*

Table B1:Data recorded during Trial 1 of the anaerobic digestion of primary sludge collected from NWWTW.

Time (d)	Pressure (atm)	Total Gas vol. (ml)	Total Gas vol. $x10^{-1}$ (moles)	% TS	% VS	Sample VS removed (g)	pH	VA/ALK ratio
0	1.01	0	0	5	75	0	6.4	0.54
6	1	1 250+0.25	0.5	4.9	74	5.44	5.5	0.9
28	0.99	2 025+0.01	1.94	6.7	74	3.5	7.1	0.65
36	0.99	4 945+0.03	2.2	6.7	61	2.9	7.4	0.2
43	1	5 620+0.01	3.27	6.4	60	2.69	7.3	0.21
51	0.98	8 330+0.03	4.55	5.9	58	2.44	7.3	0.23
57	1	11 640+0.04	6.42	5.6	54	2.12	7.25	0.23
64	1	21 870+0.11	8.59	5.3	52	1.93	7.2	0.21
71	0.99	23 490+0.02	9.23	5.1	50.2	1.66	7.3	0.27
78	0.99	23 825+0.01	9.36	4.95	50.3	1.49	7.3	0.22
85	0.99	24 048+0.025	9.44	4.95	49.5	1.59	7.2	0.24

89	1	24 110+0.025	9.47	4.9	48.5	1.56	7.15	0.22

Table B2: Data recorded during Trial 2 of the anaerobic digestion of primary sludge collected from NWWTW

Time (d)	Pressure (atm)	Total Gas vol. (ml)	Total Gas vol. x10^{-1} (moles)	% TS	% VS	Sample VS removed (g)	pH	VA/ALK ratio
0		0	0.05	3.7	69.5	3.08	7.6	0.14
6	1 012	1 400+0.12	1.94	3.65	65.2	2.86	6.8	0.85
15	1 007	5 020+0.04	3.37	3.55	62.7	2.71	7.25	0.3
21	0 999	8 490+0.04	4.74	3.3	58	3.25	7.3	0.23
28	1 001	13 045+0.06	6.53	3.25	55.6	2.17	7.25	0.25
36	1 008	16 450+0.03	7.88	3	54.9	1.98	7.2	0.24
40	1 003	16 950+0.01	8.07	2.85	52	1.78	7	0.21
49	1 010	17 380+0.075	8.21	2.8	52.1	1.75	7.15	0.22
76	1 010	17 660+0.1	8.32	2.9	51.8	1.8	7.1	0.2
85	1 009	17 800+0.075	8.36	2.95	52	1.84	7.3	0.24

B2: Cumulative Gas Productions of The Anaerobic Digestion of Primary Sludge With Different Concentrations of Digested Sludge as Inoculum.

Table B3: Cumulative gas productions of serum bottles supplemented with different concentrations of digested sludge.

Digested sludge concentration	Time (h)										
	0	**22**	**47**	**71**	**117**	**191**	**240**	**336**	**388**	**532**	**580**
X0(2%TS)											
1	0	93	145	175	235	275	301	338	370	447	521
2	0	84	134	166	231	271	298	337	369	439	506
3	0	79	132	167	237	282	302	337	367	438	510
4	0	80	127	164	231	281	303	336	367	446	517
Mean	**0**	**84**	**135**	**169**	**235**	**279**	**303**	**339**	**370**	**444**	**515**
SD	0	5.52	6.58	4.18	2.6	4.49	1.87	0.71	1.3	4.03	5.85
X1(3%TS)											
1	0	84	127	141	163	200	253	289	323	413	497
2	0	83	125	141	167	213	272	335	386	497	582
3	0	80	119	137	154	193	257	311	359	446	536
4	0	79	124	151	175	218	270	320	359	466	564
Mean	**0**	**82**	**124**	**143**	**165**	**206**	**263**	**314**	**357**	**456**	**543**
SD	0	2.06	2.95	5.17	7.56	9.97	8.15	16.66	22.39	30.53	32.07
X2(4-4.5%TS)											
1	0	94	146	166	193	250	298	351	396	526	631
2	0	95	145	176	214	270	332	397	445	599	689
3	0	109	164	190	225	284	329	398	458	567	664

4	0	95	144	166	193	251	310	377	425	569	693
Mean	0	98	150	175	207	265	319	383	433	567	671
SD	0	6.22	8.26	9.84	13.81	14.15	13.95	19.11	23.38	25.97	24.72
X3 (5.3-5.6%TS)											
1	0	99	157	189	242	304	353	421	478	653	786
2	0	97	159	192	244	307	352	412	458	623	683
3	0	95	155	188	249	304	351	419	468	586	684
4	0	89	153	194	249	320	362	428	483	616	709
Mean	0	95	156	191	246	309	355	421	473	621	717
SD	0	3.74	2.24	2.38	3.08	6.61	4.39	5.7	9.6	23.82	42.01
X4 (6.4-6.6%TS)											
1	0	135	220	292	360	436	506	617	708	768	860
2	0	98	171	267	330	419	501	582	657	720	792
3	0	115	203	274	344	416	484	576	664	726	807
4	0	109	188	277	339	423	507	586	659	723	802
Mean	**0**	**114**	**195**	**277**	**343**	**423**	**499**	**590**	**672**	**734**	**815**
SD	0	13.44	18.12	9.12	10.89	7.63	9.23	15.85	20.94	19.6	26.39
Digested sludge	**Time (h)**										
concentration	**0**	**24**	**98**	**144**	**238**	**283**	**331**	**429**	**529**	**563**	**582**
X5(11%TS)											
1	0	85	222	285	490	583	646	723	776	803	808
2	0	95	228	297	482	576	645	738	802	831	831
3	0	89	226	285	460	543	604	703	754	774	784
4	0	87	222	285	469	558	630	725	773	806	816

Mean	0	89	225	289	476	566	632	723	777	807	813
SD	0	3.74	2.6	5.2	11.56	15.64	16.96	12.52	17.09	20.21	17.01

Digested sludge concentration	Time (h)										
	0	24	98	144	238	283	331	429	529	563	582
X6 (12.8-13%TS)											
1	0	80	251	314	532	575	608	659	728	749	761
2	0	90	255	338	553	633	700	784	844	879	894
3	0	94	259	342	539	589	618	678	796	826	832
4	0	99	239	299	473	538	613	710	771	803	812
Mean	**0**	**91**	**251**	**323**	**524**	**584**	**628**	**702**	**779**	**819**	**819**
SD	0	6.98	7.48	17.63	30.54	34	37.84	47.65	41.97	46.68	47.63

SD = standard deviation

Appendix C

EXPERIMENTAL METHODS

C 1. Gas Collection System

Gas, consisting predominantly of 60-70%(v/v) methane and 30-40%(v/v) carbon dioxide, was channelled through silicone tubing to a 2 ℓ vessel filled with citric acid acidified NaCl solution. The solution consisted of 20% (m/v) NaCl and 0.5% (m/v) citric acid. This solution prevents carbon dioxide, which is highly soluble in water, from dissolving and thus facilitates correct measurement of the total gas generated by the bacterial population. The gas was measured by using the gas bubbles to displace the liquid.

C 2. Alkalinity

The digested sludge sample was centrifuged with a Beckman centrifuge at 5000 rpm xg for about 5 minutes. A 50 ml volume of supernatant was transferred to a beaker and the pH was determined with an Orion pH meter. The sample was then titrated to pH 4.0 with 0.1N H_2SO_4. The volume of acid titrated x100 gave the total alkalinity which was reported as mg $CaCO_3$ ℓ^{-1}. When the volume of supernatant was insufficient a smaller volume was diluted to give a 50 ml sample.

C 3. Volatile Acid Concentration

Once the alkalinity was determined the pH of the solution was reduced to 3.5 with the aid of sulphuric acid (0.1N). The supernatant was then boiled for 3 minutes and left to cool to room temperature. The volatile acid concentration was determined by titrating the solution to pH 4.0 (a) and pH 7.0 (b) with 0.1N NaOH. The volatile acid concentration was calculated as follows:

$$\text{Volatile acids concentration(VA)} = (b-a) \times 120 \qquad \text{(C3.1)}$$

$$= \text{mg HAc } \ell^{-1}$$

Appendix D

DATA COLLECTED FROM SEMI-CONTINUOUS OPERATION OF ANAEROBIC DIGESTERS LOADED WITH DIFFERENT SOLIDS CONCENTRATIONS

Table D 1. Process control and monitoring results taken during Run 1(i.e. digester operated with 2%(m/v) TS loading).

Day	Gas produced (ml d^{-1})	%VS out	% TS out	Volatile acids (mgHAc l^{-1})	Alkalinity (mg l^{-1})	VA/ALK ratio	pH
1	422	65	2.2	600	4,200	0.14	7.2
2	571						7.3
3	556						7.4
4	650	59	2				7.1
5	596			380	4,200	0.09	7.4
6	665						7.3
7	857						7.3
8	705	61	2.2				7.4
9	600			480	4,220	0.11	7.2
10	550						7.3
11	760	64	2.5				7.2
12	585			576	4,200	0.14	7.5
13	607						7.6

14	870	55	2.1				7.5
15	582						7.4
16	622	62	2.4				7.3
17	743			410	4,300	0.1	7.5
18	490						7.2
19	570						7.3
20	610						7.4
21	643	63	2.5				7.4
22	670			480	4,800	0.1	7.3
23	660						7.2
24	605						7.3
25	560						7.3
26	585	61	2.4				7.3
27	695			456	5,200	0.09	7.3
28	720						7.2
29	595						7.1
30	575	61	2.7	588	4,800	0.12	7.3

Table D 2. Process control and monitoring results of Run 2 (i.e. digester operated with 3%(m/v) TS loading).

Day	Gas produced (ml d^{-1})	%VS out	%TS out	Volatile acids (mgHAc l^{-1})	Alkalinity (mg l^{-1})	VA/ALK ratio	pH
1	827	65	3	480	4,600	0.1	7.2
2	709						7.3
3	578						7.3
4	788						7.4
5	730	62	3				7.2
6	729			420	4,500	0.09	7.1
7	830						7.2
8	810						7.4
9	835						7.2
10	678	63	3.1				7.3
11	702			588	4,600	0.13	7.3
12	589						7.2
13	640						7.1
14	828	64	3.3				7.3
15	686			404	4,500	0.09	7.2
16	904						7.2
17	840						7.4
18	720						7.3
19	768	59	3.4				7.2
20	900			480	4,900	0.1	7.4
21	765						7.4
22	810						7.6
23	725	55	3.2				7.4

24	705			600	4,900	0.12	7.3
25	690						7.3
26	820						7.2
27	760	58	3.3				7.3
28	795			582	4,600	0.13	7.3
29	680						7.2
30	670	61	3.4	580	4,400		7.3

Table D 3. Process control and monitoring results of Run 3 (i.e. digester operated with 3.8%(m/v) TS loading).

Day	Gas production (mls d^{-1})	% VS out	% TS out	Volatile acids (mgHAc l^{-1})	Alkalinity (mg l^{-1})	VA/ALK ratio	pH
1	D	63	3.8	560	4,900	0.11	7.3
2	I						7.3
3	S						7.2
4	R						7.1
5	E	61	3.5				7.4
6	G			384	4,640	0.08	7.3
7	A						7.4
8	R						7.5
9	D						7.4
10	E	63	3.7				7.3
11	D			456	4,400	0.1	7.2
12							7.2

13							7.2
14							7.3
15	63	3.6					7.4
16	937			588	4,200	0.14	7.4
17	752						7.4
18	876						7.3
19	800	59	3.6				7.3
20	725			810	4,650	0.17	7.6
21	969						7.4
22	770						7.3
23	1,075	58	3.9				7.4
24	864			756	4,600	0.16	7.3
25	815						7.3
26	920						7.3
27	795	60	3.7				7.3
28	835			600	4,900	0.12	7.2
29	900						7.2
30	820	61	3.8	710	4,500	0.16	7.1

Initial gas production volumes of Run 3 were disregarded owing to a leak in the gas collection apparatus which led to inconsistent measurements.

Table D 4. Process control and monitoring results of Run 4 (i.e. digester operated with 4.7%(m/v) TS loading).

Day	Gas production (ml d^{-1})	%VS out	%TS out	Volatile acids (mgHAc l^{-1})	Alkalinity (mg l^{-1})	VA/ALK ratio	pH
1	766	61	4.8	264	4,800	0.06	7.3
2	914						7.4
3	703						7.2
4	655	64	4.7				7.2
5	810			374	4,480	0.08	7.2
6	730						7.3
7	622						7.4
8	720	62	4.6				7.4
9	725			480	4,600	0.1	7.4
10	890						7.3
11	790	60	4.8				7.3
12	810			560	4,600	0.1	7.2
13	920						7.2
14	684	64	4.6				7.1
15	852			688	4,300	0.16	7.4
16	674	63	4.4				7.3
17	670			874	4,400	0.2	7.3
18	998						7.2
19	859						7.2
20	860						7.4
21	750	61	4.7				7.5
22	762			860	4,800	0.18	7.4
23	790						7.4

24	820						7.3
25	815						7.3
26	900	60	4.9				7.3
27	710			720	5,000	0.14	7.3
28	695						7.5
29	785						7.3
30	870	62	4.6	805	4,600	0.18	7.4