FACTORS INFLUENCING THE RATE AND STABILITY OF THE ANAEROBIC DIGESTION PROCESS

A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Biotechnology at Massey University

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1986

ABSTRACT

Three factors affecting the rate and stability of the methane fermentation of a readily-hydrolysable feedstock were investigated. The aim of this work was to develop improved processes and control strategies to facilitate economic treatment of industrial wastes by anaerobic digestion.

A comparison was made between the performance of a continuously-fed digester and semi-continuous digesters slug fed every second day. A semi-synthetic medium with glucose as the major carbon and energy source was used and seed material was transferred between the digesters, which were operated under similar loading conditions. The continuous digester repeatedly failed even when operated at dilution and loading rates much lower than the maximum values commonly reported. In contrast, the semi-continuous units provided satisfactory performance and could be easily and rapidly recovered from retarded operation. Failure of the continuous digesters was characterised by a steady fall in volatile suspended solids concentration followed by a rapid accumulation of acetate, and was attributed to a deficiency in the medium of one or more essential nutrients. These were thought to be provided in the semi-continuous digester by lysis of acidogenic bacteria or luxury uptake from the medium.

Degradation of acetic and propionic acids was investigated in batch culture. Increasing the concentration of either acid from low levels decreased the rate of utilisation of the acid, but the proposed inhibitory role of un-ionised acids was not conclusively supported. Increasing the initial acetate concentration above 1000 to 1500 $mg.l^{-1}$ significantly reduced the rate of degradation of propionate added at 500 $mg.l^{-1}$. When acetate was added at 2000 $mg.l^{-1}$ the rate of propionate utilisation was approximately half of that when acetate was present at 500 $mg.l^{-1}$ or lower.

In batch culture experiments, addition of up to 3.2 mM cysteine-hydrochloride or sodium sulphide, or 4.4. mM sodium thioglycollate did not inhibit total gas production from samples drawn from the continuous digester. However the rate of methane production in effluent samples from a semi-continuous digester was inhibited by 25 % to 30 % by addition of 3.2 mM cysteine or sulphide. Inhibition was attributed to the sulphide ion. Sodium thioglycollate did not inhibit methane production from acetate but propionate degradation was markedly reduced, with increasing inhibition noted with increasing incubation time.

The work adds to a considerable body of investigation into the factors influencing anaerobic digestion and the unresolved problem of process stability in long-term operation of conventional stirred tank digesters has again been highlighted. Indicators and possible causes of process failure have been suggested and further development of these should assist in the continuing increase in the rate of treatment while ensuring acceptable working margins of safety for the process.

ACKNOWLEDGEMENTS

I wish to acknowledge the following:

* Professor R.L. Earle, Dr V.F. Larsen and Dr G.J. Manderson, for their supervision and assistance.

* Mr John Alger and Mr Derek Couling, for their assistance in building and maintaining equipment.

* Fellow staff members and post-graduate students of the Biotechnology Department, for many useful discussions. I particularly wish to thank Dr Tony Paterson for his guidance on the use of various computer packages and his help with printing this thesis.

* The Department of Scientific and Industrial Research, for sponsoring this research.

* Nicky and Sally, for proof-reading at a critical time.

* Brenda and Isaac, and other friends and family, for their support and encouragement; this work is dedicated to them.

TABLE OF CONTENTS

TABLE OF FIGURES

* large diagram enclosed inside back cover.

TABLE OF TABLES

ABBREVIATIONS

Abbreviations of volatile fatty acid names:

Ac	acetic acid
Pr	propionic acid
iBu	iso-butyric acid
Bu	butyric acid
iVa	iso-valeric acid
Va	valeric acid

Acids will be reffered to by the suffixes "-ate" and "-ic acid" interchangeably.

A subscript "i" denotes the intial acid concentration.

Abbreviation of units:

atm	atmosphere
g	gramme
hr	hour
kcal	kilocalorie
l	litre
mg	milligramme
min	minute
ml	millilitre
mm	millimetre
mM	millimoles per litre
mol	mole
mV	millivolt
r.p.m.	revolutions per minute
µl	microlitre

Other abbreviations:

a	coefficient of the logistic equation (dimensionless)
ATP	adenosine triphosphate
b	coefficient of the logistic equation (day^{-1})
BOD_5	five-day biological oxygen demand ($mg.l^{-1}$)
CDR1	continuous digestion, run 1
CDR2	continuous digestion, run 2
CDR3	continuous digestion, run 3
COD	chemical oxygen demand ($mg.l^{-1}$)
COD_r	chemical oxygen demand removed ($mg.l^{-1}$)
$CODR_s$	specific rate of chemical oxygen demand removal ($g\ COD_r.g\ VSS^{-1}.day^{-1}$)
cyst	cysteine-hydrochloride
E_c	electrode potential relative to the saturated calomel electrode (mV)
E_h	electrode potential relative to the standard hydrogen electrode (mV)
HRT	hydraulic retention time (days)
i.d.	internal diameter
K	coefficient of the logistic equation ($g.l^{-1}$)
K_s	half-saturation constant for sustrate utilisation ($g.l^{-1}$)
NAD	nicotinamide adenine dinucleotide
NADH	reduced nicotinamide adenine dinucleotide
OLR_s	specific organic loading rate ($g\ COD.g\ VSS^{-1}.day^{-1}$)
OLR_v	volumetric organic loading rate ($g\ COD.l^{-1}.day^{-1}$)
ORP	oxidation-reduction potential
pCO_2	carbon dioxide partial pressure (bar)
r_s	rate of substrate utilisation ($g\ substrate.l^{-1}.hr^{-1}$)
$r_{s,max}$	maximum rate of substrate utilisation
r_x	rate of biomass growth ($g\ biomass.l^{-1}.day^{-1}$)
s	substrate concentration ($g.l^{-1}$)
S	total sulphur concentration (mM or $mg.l^{-1}$)
SCDR1	semi-continuous digestion, run1
SCDR2	semi-continuous digestion, run 2

s.d.	standard deviation
SRT	solids retention time (days)
sulp	sodium sulphide
t	time
thio	sodium thioglycollate
TSS	total suspended solids ($g.l^{-1}$)
TVFA	total volatile fatty acid concentration as acetate ($mg.l^{-1}$)
UVFA	un-ionised volatile fatty acid concentration as acetate ($mg.l^{-1}$)
VFA	volatile fatty acid (concentration) ($mg.l^{-1}$)
VSS	volatile suspended solids ($g.l^{-1}$)
Y_{xs}	biomass yield coefficient (g VSS.g COD_r^{-1})
μ	specific growth rate (day^{-1})
μ_{max}	maximum specific growth rate

CHAPTER ONE PREAMBLE

New Zealand has long been dependent on the export of primary products such as wool, meat, dairy and forest products for economic prosperity. The processing of these biological materials generates a significant volume of wastewater which must be treated to minimise pollution of the environment. This is essential not only for aesthetic, recreational and conservation reasons but also economically to preserve a desirable "clean, green" image as a tourist attraction.

Anaerobic digestion, in which bacteria degrade organic matter largely to methane and carbon dioxide, has many advantages to offer as a waste treatment method. The Biotechnology Department at Massey University has therefore been involved in a continuing programme investigating the application of this process to treatment of industrially important wastes, particularly those from the dairy and pulp and paper industries. However, as Speece (1983) has recently noted, the application of anaerobic digestion has been hindered by a widespread perception of the process as providing low treatment rates and having poor stability. To overcome this "hurdle of user confidence", a greater understanding of how environmental and operating conditions influence the activity of the methanogenic consortium is required. It is this problem that the current work addresses.

The research was commenced in March 1979. At that time a literature review indicated a need for investigation of the roles of fermentation intermediates and end-products and the frequency of feedstock addition in limiting the rate of the fermentation. During the course of the research new priorities emerged as it proved difficult to establish and maintain satisfactory performance in a continuous digester. Reasons for the failure of this unit were investigated and the perfomance of the continuously-fed digester was compared to that of semi-continuous digesters. These studies led to consideration of the degradation

of volatile fatty acid intermediates and the effect of various sulphur-containing reducing agents on methane production. Some implications for operation of large-scale systems have emerged both from this work and literature published during the ensuing period and these are also presented.

CHAPTER TWO LITERATURE REVIEW

2.1 INTRODUCTION

In the methane fermentation organic matter is degraded to methane and carbon dioxide by the co-ordinated activities of a large number of bacterial species acting in the absence of oxygen. The process plays a vital role in nutrient cycling in nature and occurs in such diverse environments as the rumen, fresh and salt-water sediments, soils and the rotting interiors of trees (Zeikus 1977). It is the basis of anaerobic digestion, a process now widely used in municipal sewage treatment plants for the stabilisation of waste sludges and increasingly being applied to the treatment of industrial wastes.

Cell growth is limited in the methane fermentation as about 90 % of the substrate energy is retained in the methane produced (Bryant 1979). This represents an advantage over aerobic waste treatment processes as disposal of waste sludge is minimised and nutrient requirements are much lower, an important factor in the treatment of many industrial wastes. McCarty (1964a) lists other advantages of anaerobic digestion as ;

(1) a high degree of waste stabilisation is possible with most of the degradable organic matter converted to easily-separable gaseous products,

(2) the power requirement for agitation is low as no oxygen is required and the degradation process is not limited by oxygen transfer,

(3) methane is a useful end-product which can provide energy for heating and other purposes.

Considerable development has taken place in anaerobic digester technology since its first deliberate application and a variety of process configurations are now available (McCarty 1981). These range from largely uncontrolled lagoons and septic tanks, through small community and farm-scale digesters widespread, for example

in India and China, to various sophisticated high-rate processes recently applied to treatment of industrial wastes in the developed countries. The last two decades in particular have seen major advances made in our understanding of the biochemistry and microbiology of methane production and this combined with the new process designs has considerably extended the range of substrates suitable for treatment. These developments have been spurred by increased pressure on industry and municipal bodies to minimise pollution and by the need for energy recovery and production to off-set rising energy costs.

The objectives of this literature review are to background the methane fermentation and to survey recent developments in the field particularly with regard to the environmental factors and operational parameters affecting the rate and stability of the process.

2.2 MICROBIOLOGY AND BIOCHEMISTRY OF THE METHANE FERMENTATION

2.2.1 Introduction

The methane fermentation has traditionally been viewed as a two-stage process involving catabolism of complex organic compounds via acidogenesis to volatile acids, carbon dioxide and hydrogen and subsequent methanogenesis i.e. methane formation (Barker 1957, McCarty 1964a). This approach still has utility but recent evidence now indicates at least three major bacterial groups are involved in the process (Bryant 1979). The functions and interrelationships of these are illustrated in Figure 2.1.

The fermentative bacteria represent a complex association of diverse microbial species including both obligate and facultative anaerobes. These initially hydrolyse biopolymers in the digester feedstock to simple molecules which are assimilated by the cells and converted to a variety of short-chain neutral and acidic compounds. Alcohols and fatty acids higher than acetate are then

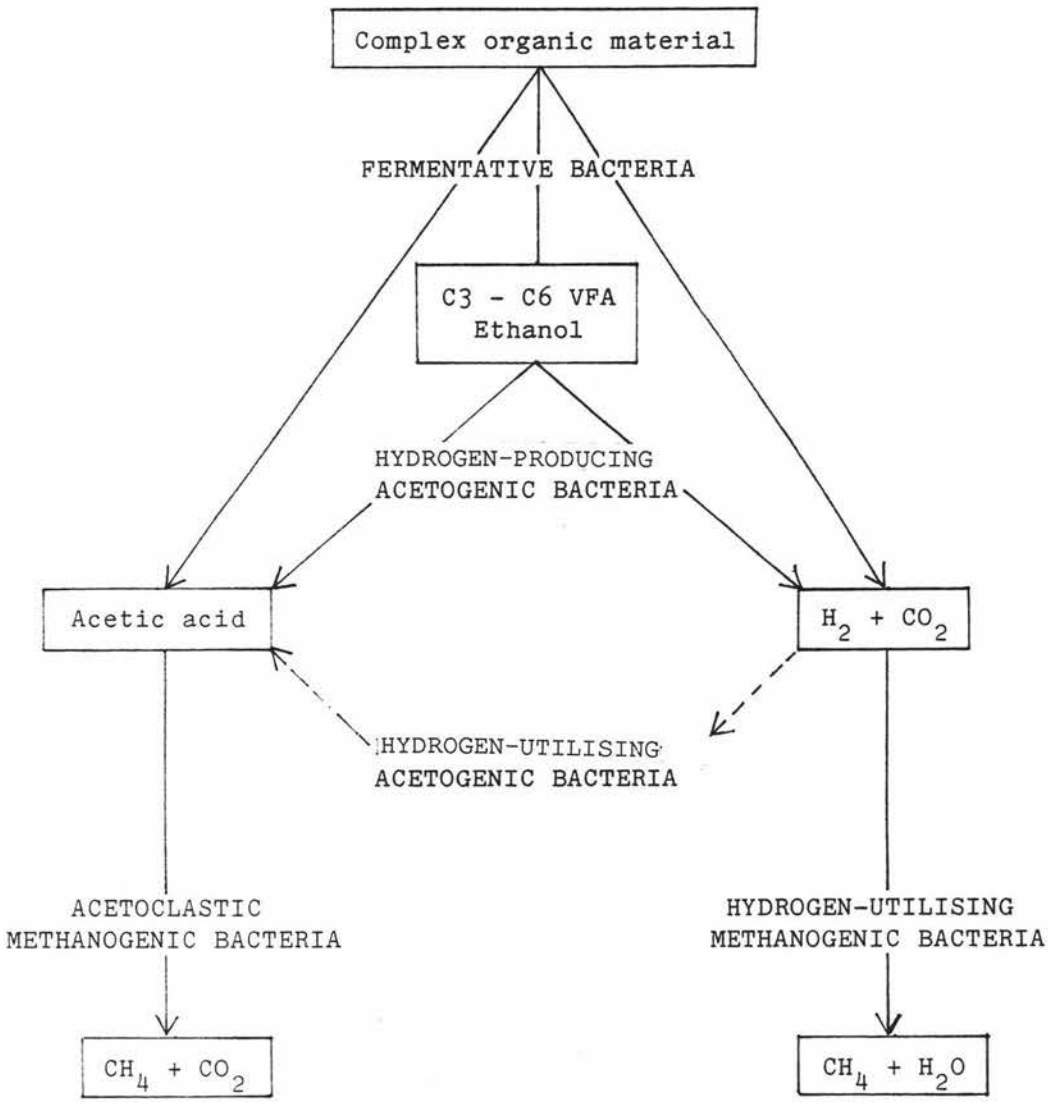


Figure 2.1: Outline of the major metabolic processes involved in the methane fermentation.

catabolised by the hydrogen-producing acetogenic bacteria to acetate, carbon dioxide and hydrogen from which methane is produced in the final step. Methane may be formed by reduction of carbon dioxide by hydrogen or via an acetoclastic reaction. A fourth metabolic group, the hydrogen-utilising acetogenic bacteria, have also been isolated from anaerobic digesters and their activity is also indicated in Figure 2.1. These microorganisms form acetate from hydrogen and carbon dioxide but their significance in the overall fermentation is not clear (Zeikus 1977, Braun et al. 1979).

The wastes treated by anaerobic digesters are usually complex and major components, especially carbohydrates, are often polymers (Hobson et al. 1981). The feedstocks may also vary in composition from day to day and in most instances are not sterile. These factors have a considerable influence on the bacterial flora and metabolic activities and make characterisation of the microbiology and biochemistry of the process very difficult. Much research is still required to complete our understanding of the methane fermentation as it occurs in anaerobic digestion, and in those areas where specific information is lacking analogy to the rumen ecosystem has been made (Hobson et al. 1974; 1981). This approach has proved useful primarily with regard to the role of the fermentative bacteria only as the methane fermentation in the rumen of a healthy animal is incomplete. The short retention time only permits growth of the acidogenic bacteria and the hydrogen-utilising methanogens so the major products are restricted to the the volatile acids which are absorbed and used as the energy source of the ruminants (Hungate 1966, Hobson et al. 1974).

Changes in the composition or rate of feeding of the waste or in the digester environment produce corresponding changes in the bacterial populations. The process of adaption to altered conditions is a very complex phenomenon and may occur over a considerable period of time (Hattingh et al. 1967). Providing all the metabolic groups are able to grow in the new environment

a new, stable association of microorganisms will result. The species composition may be very different to the original population but the trophic functions and interrelationships will be as described above (Zeikus 1977).

2.2.2 The fermentative bacteria

2.2.2.1 Hydrolysis of biopolymers

The spectrum of macromolecules present in the feed is very dependent on the origin of the waste. Agricultural wastes, municipal solid refuse and fruit and vegetable processing wastes are all likely to contain high levels of complex carbohydrates. Domestic sewage sludge and meat and fish processing wastes are all rich in proteinaceous material and may also have a significant lipid content. Additionally, in wastes containing a large bacterial population such as sewage and animal wastes, nucleic material and heterocyclic compounds resulting from its breakdown may also be present (Kotze et al. 1969, Hobson et al. 1974).

Activity of all major classes of hydrolytic bacteria has beeen detected in digesters treating a wide range of substrates. Typical population sizes and commonly isolated species have been reported by Toerien (1967), Kotze et al. (1968), Hobson and Shaw (1971), Hobson et al. (1974) and Iannotti et al. (1982a). When these are compared to typical rumen bacterial populations (Hungate 1966, Prins 1977) the only major differences appears to be in the proteolytic bacteria; Clostridium species predominate in anaerobic digesters but are found in only small numbers in the rumen (Hobson et al. 1974).

The mechanisms of degradation of carbohydrates and proteins are well established and details may be found in any standard text or review (e.g. see Barker 1961, Wood 1961, Prins 1977). Lipid degradation in anaerobic digesters involves hydrolysis of

esterified fatty acids followed by a partial yet rapid hydrogenation of the unsaturated acids (Heukelekian and Muller 1958) which are then degraded by β-oxiation (Jeris and McCarty 1965, Weng and Jeris 1976). The methanogens were originally thought to be responsible for this final step (Barker 1957) but it is now clear that this role is performed by the hydrogen-producing acetogenic bacteria (Boone and Bryant 1980, McInerney et al. 1981). Catabolism of nucleic acid and other nitrogenous compounds has been reviewed by Barker (1961) and McAllen and Smith (1973). Micrococcus and Clostridium species are able to degrade these compounds but the actual organisms invloved during anaerobic digestion have not been characterised (Hobson et al. 1974).

2.2.2.2 Production and utilisation of fermentation intermediates

The major products of acidogenesis are acetate, propionate, butyrate, carbon dioxide and hydrogen with higher and branched chain fatty acids occasionally produced to a minor extent. These fermentation intermediates are formed from the products of hydrolysis reactions of which the most important are the lower sugars and amino-acids (Hobson et al. 1974). A wide variety of fermentation pathways are available for the catabolism of sugars and amino acids by anaerobic bacteria (Barker 1961, Wood 1961, Prins 1977) and a significant feature of many of these pathways is that several products may be produced, with the distribution of these depending on energetic and environmental factors (Thauer et al. 1977). The presence of hydrogen in particular plays a very important role in anaerobic environments (see Section 2.2.4., Wolin 1974). Few detailed studies of intermediary metabolism of anaerobic digestion microorganisms have been published consequently reference is often made to the rumen ecosystem which has been more extensively studied (Hobson et al. 1974, Prins 1977).

Fermentation of lower sugars can proceed via the Embden-Meyerhof-Parnass metabolic pathway or via one of three pathways involving 6-phosphogluconate; the pentose-phosphate cycle, the pentose-phosphate phosphoketolase pathway or the Entner-Doudoroff scheme (Prins 1977). In the rumen, glycolysis is the favoured mechanism because of the greater yield of ATP per mole of glucose utilised (Prins 1977) and glycolysis also appears to play the major role in carbohydrate metabolism in anaerobic digestion (Jeris and McCarty 1965, Hattingh et al. 1967, Thiel et al. 1968, Kotze et al. 1969). The pentose-phosphate cycle is primarily used to convert pentoses into glycolytic intermediates and was shown to be active, but to a lesser extent, in digester microorganisms fed wastes rich in carbohydrate (Jeris and McCarty 1965, Kotze et al. 1969). The significance of the other pathways in carbohydrate catabolism by the fermentative bacteria has not been established (Kotze et al. 1969).

Pyruvate is formed from glycolysis and microorganisms show great diversity in the utilisation of this compound. Some important fermentation pathways are illustrated in Figure 2.2. Formation of acetate proceeds via acetyl CoA and acetyl phosphate with the formation of one mole of ATP per mole of pyruvate utilised (Wood 1961, Prins 1977). There are two mechanisms for pyruvate oxidation (Racker 1965). The pyruvate-lyase reaction is catalysed by pyruvate-ferredoxin oxidoreductase and yields acetate, carbon dioxide and reduced ferredoxin, a carrier of low potential electrons. This reaction is found in Clostridium species and some rumen bacteria (Prins 1977) and the electrons produced may be used for the production of reduced compounds such as propionate and butyrate or excreted as hydrogen in the reoxidation of ferredoxin (McInerney and Bryant 1981a). In the second reaction pyruvate is cleaved to acetyl-CoA and formate. This reaction is found to operate in Entereobacteriaceae, Lactobacillus and a number of rumen bacteria (Joyner and Baldwin 1966, Prins 1977) and has also been demonstrated in the methanogenic consortium (Chynoweth and Mah 1971).

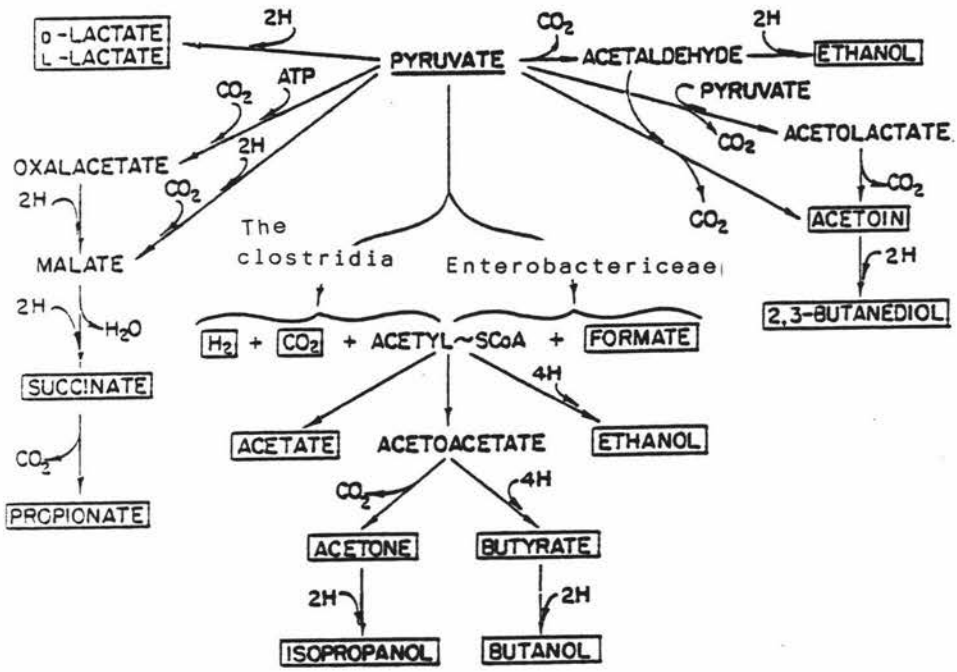


Figure 2.2: Major pathways for anaerobic utilisation of pyruvate (from Wood 1961).

There are two general pathways for propionate formation. In the direct reductive route, or acrylate pathway, pyruvate is first reduced to lactate. Propionate is then formed via lactyl CoA, phospholactyl CoA, acrylyl CoA and propionyl CoA, and the last reductive step may also involve ATP formation by electron transport phosphorylation (Prins 1977, Thauer et al. 1977). The second pathway involves carbon dioxide fixation into either pyruvate or phosphoenol pyruvate (PEP) to form oxaloacetate. This is reduced through malate and fumarate to succinate which is then decarboxylated to yield propionate. The conversion of PEP to oxaloacetate may generate ATP and this pathway appears the most important in the rumen bacteria except at high glucose or lactate concentrations (Baldwin et al. 1963, Wallnofer et al. 1966, Prins 1977).

Both propionate-forming pathways have been observed to operate in anaerobic digestion (Jeris and McCarty 1965, Runquist et al. 1981, Uribelarra and Pareilleux 1981, Schink 1985) and in batch digestion of glucose the acrylate pathway appears to be utilised (Runquist et al. 1981, Uribelarra and Pareilleux 1981). The importance of the succinate (or randomising) pathway (Wood 1961) in continuous culture where substrate concentrations are lower has not been clearly established but one study again indicates lactate as the more important precursor of propionate (Sinierez and Pirt 1977). However succinate is an end-product of some important fermentative bacteria (Bryant 1979) and Schink (1985) found that succinate-enriched digester liquors always resulted in an enrichment of the propionate-forming bacterial population.

Several recent studies of oxidation of propionate to acetate in anaerobic digesters have produced interesting results (Koch et al. 1983, Boone 1984b, Schink 1985). These studies suggest that propionate can be both formed and degraded via succinate indicating that, although not present as a significant extracellular intermediate, metabolism of succinate may be

overall more important than propionate metabolism (Schink 1985). These investigations confirm earlier work on propionate degradation by Buswell et al. (1951) and Stadtman and Barker (1951) and the observation of a randomising pathway in carbohydrate metabolism by Jeris and McCarty (1965). The oxidation of propionate through succinate may proceed by the methylmalonyl CoA pathway or the α-OH-glutarate pathway (Kaziro and Ochoa 1964). The current evidence suggests methymalonyl CoA is involved but the alternate pathway can not yet be conclusively excluded (Schink 1985).

Butyric acid is usually formed by obligate anaerobic organisms such as closridia and is never the sole end product of metabolism (Wood 1961, Prins 1977). Acetate, ethanol, butanol and iso-propanol may also be formed depending on the organism involved and environmental factors. Butyrate is formed by the condensation of two molecules of acetyl CoA in a reversal of β-oxidation and incurs a loss of potential ATP generation from acetyl CoA (Prins 1977).

Consideration can now be given to amino-acid catabolism. There are three main processes of amino-acid breakdown; transamination, combined transamination and deamination and the Stickland reaction (Barker 1961, Kotze et al. 1969). Transamination reactions are especially significant because of their close relationship with several important intermediates in carbohydrate metabolism including pyruvate, oxaloacetate and succinyl CoA (Lehninger 1975). The Stickland reaction is found in Clostridium species and involves the coupled oxidation and reduction of pairs of amino-acids. Branched chain volatile fatty acids which are sometimes detected in digesters are products attributed to deamination reactions (Hobson et al. 1974) and to Stickland reactions (Andrews and Pearson 1965). Detailed information on these reactions is provided by Barker (1957, 1961). Individual amino-acids may be degraded by different pathways depending on the organisms involved. Catabolic mechanisms used by rumen and

digester bacteria have been proposed for several amino-acids including leucine (Jeris and McCarty 1965) and glutamate (Weng and Jeris 1979). The metabolism of branched chain amino-acids has been discussed by Massey et al. (1976).

The biochemical mechanisms explaining the fate of the large molecules entering anaerobic ecosystems have been reviewed. The products of these catabolic processes provide substrates for the production of methane and carbon dioxide by the unique bacterial consortium found in these ecosystems. The role of these other microorganisms will now be considered.

2.2.3 The hydrogen-producing acetogenic bacteria

The hydrogen-producing acetogenic bacteria are a vital component of the methanogenic cosortium as they catabolise volatile fatty acids higher than acetate and other important intermediates formed by the fermentative bacteria. The existence of this metabolic group was postulated by Bryant et al. (1967) after it was established that methanogens were very limited in the substrates available for growth and metabolism. This discovery arose from a study of the ethanol-degrading Methanobacillus omelianski which was found to be a co-culture of a hydrogen-utilising methanogen and a symbiotic bacterium termed the S-organism, which catabolised ethanol to acetate and hydrogen. The S-organism was inhibited by high hydrogen partial pressures and neither bacterium would grow well in pure culture. It seemed plausible that the other organic compounds listed as substrates for M. omelianski were actually degraded by the S-organism and subsequent research has confirmed this. The following compounds are now listed as substrates for the hydrogen-producing acetogens; ethanol (Bryant et al. 1967), lactate (Bryant et al. 1977), propionate (Boone and Bryant 1980) and butyrate and higher fatty acids (McInerney et al. 1981).

Characterisation and identification of the acetogenic bacteria has proved difficult and to date only two organisms, Syntrophobacter wolinii (Boone and Bryant 1980) and Syntrophomonas wolfei (McInerney et al. 1981), have been classified, although several others are described in the literature (Sheldon and Tiedje 1984, Henson and Smith 1985).

2.2.4 The role of hydrogen in regulating the fermentation

The hydrogen-producing acetogens require a very low partial pressure of hydrogen to grow and can only be cultured in association with a hydrogen-utilising bacterium such as a methanogen or a Desulfovibrio species. An explanation for this arises from consideration of the proposed catabolic reactions. The stoichiometry and change in free energy for syntrophic associations of propionate and butyrate catabolising acetogens and hydrogen-utilising methanogens are listed in Table 2.1 . For both acids the catabolic reactions are endogonic and only become exogonic when combined with hydrogen removal by the methanogens.

Hydrogen also plays a very important role in controlling the product distribution of the fermentative bacteria. The reason for this lies in the energetics of utilising protons as the electron sink in the regeneration of reducing equivalents:

$$NADH + H^+ = NAD^+ + H_2 \qquad (E2.6)$$

Wolin (1974) has shown that at hydrogen partial pressures greater than about 10^{-3} atm the equilibrium does not favour hydrogen production. The methane bacteria have a high affinity for hydrogen; for example K_s for hydrogen utilisation in the rumen has been shown to be 10^{-6} atmosphere and the hydrogen partial pressure was also of this order (Hungate et al. 1970). In this situation proton reduction is favourable and pyruvate produced by the fermentative bacteria is largely degraded to acetate, carbon dioxide and hydrogen with the formation of an additional ATP.

Table 2.1: Stoichiometry and change in free energy values for propionate and butyrate degradation reactions (after Thauer et al. 1977, Bryant 1979).

A: Propionate-catabolising acetogen

$$CH_3CH_2COO^- + 3H_2O = CH_3COO^- + HCO_3^- + H^+ + 3H_2 \qquad (E2.1)$$

$\Delta G'_0$ = +18.2 kcal per reaction

B: Butyrate-catabolising acetogen

$$CH_3CH_2CH_2COO^- + 2H_2O = 2CH_3COO^- + H^+ + 2H_2 \qquad (E2.2)$$

$\Delta G'_0$= +11.5 kcal per reaction

C: Hydrogen-utilising methanogen

$$HCO_3^- + 4H_2 + H^+ = CH_4 + 3H_2O \qquad (E2.3)$$

$\Delta G'_0$ = -32.4 kcal per reaction

A + C:

$$CH_3CH_2COO^- + 3H_2O = 4CH_3COO^- + HCO_3^- + H^+ + 3CH_4 \qquad (E2.4)$$

$\Delta G'_0$ = -22.4 kcal per reaction

B + C:

$$2CH_3CH_2CH_2COO^- + HCO_3^- + H_2O = 4CH_3COO^- + CH_4 + H^+ \qquad (E2.5)$$

$\Delta G'_0$ = -9.4 kcal per reaction

In the case of high hydrogen partial pressure, proton reduction is unfavourable and other compounds must be used as the electron sink. Possible products are propionate, butyrate, ethanol and lactate and change in free energy values for these are listed in Table 2.2. The production of firstly propionate and then butyrate is most favoured and increased concentrations of these acids has been observed in digesters exposed to high hydrogen levels. Kaspar and Wuhrmann (1978b) observed propionate accumulated when hydrogen was introduced into a digester and propionate and butyrate are often the acids that predominate in overloaded digesters when the hydrogen concentration increases due to restriction of the methanogens (Pohland and Bloodgood 1963, Bryant 1979). As the degradation of these acids by the acetogenic bacteria is also only favoured at low hydrogen levels they can accumulate rapidly in the digester fluid. Under these conditions effective digestion has ceased as the fermentation products are not removed from the system and are of approximately the same energy content as the original material (Bryant 1979).

Overall, hydrogen plays a very important role in the methane fermentaion and controls the process at two major points. The success of the fementation depends on the efficiency of inter-species hydrogen transfer and the results of this transfer have been summarised by Zeikus (1977) as:

(1) increased substrate utilisation,
(2) different proportions of reduced end-products,
(3) more ATP synthesised by the methanogens,
(4) increased growth of both organisms, and
(5) displacement of unfavourable reaction equilibriums.

Table 2.2: Stoichiometry and change in free energy values for pyruvate reduction reactions (after McInerney and Bryant 1981a).

Products per mole pyruvate	$\Delta G'_0$ kcal.(mole pyruvate)$^{-1}$
propionate + $2NAD^+$	-20.9
butyrate + HCO_3^- + NAD^+	-18.5[a]
ethanol + HCO_3^- + NAD^+	-9.3
lactate + NAD^+	-6.0

a reactants are acetate and pyruvate

2.2.5 The methanogenic bacteria

The methanogenic bacteria occupy a vital role in nature allowing the effective mineralisation of organic matter which would otherwise accumulate in the environment (Zeikus 1977, Bryant 1979). Their action is due to their unique physiology which provides the ability to degrade substances in the absence of light and exogenous electron acceptors such as oxygen, nitrate and sulphate (Bryant 1979).

Barker (1957) recognised that although the methanogens were a morphologically diverse group of organisms they showed a marked physiological similarity and since this review, methane producing

bacteria have been classified together on this basis (Bryant 1974, Balch et al. 1979). Much knowledge has been added since 1957 and in 1979 at least thirteen organisms were available in pure culture and were described in a major review by Balch and co-workers. Their review was undertaken as the previous classification schemes (Barker 1957, Bryant 1974) were no longer regarded as satisfactory. The work was based on a comparative analysis of 16S ribosomal RNA composition supplemented by studies of cell wall structure and compostion, lipid distribution, intermediary metabolism and nucleic acid composition. In particular the methanogens were shown not to contain muramic acid in their cell walls (Kandler and Hippe 1977, Kandler and Konig 1978), to possess several unusual coenzymes (Keltjens and Vogels 1981) and to lack saponifiable lipids characteristic of typical bacteria (Balch et al. 1979).

This work has confirmed the methanogens as a unique and distinctive group of very ancient origin and they may constitute a third primitive group of microbial life, the archaebacteria (Woese and Fox 1977, 1978; Maugh 1977), or at least part of one of the three urkingdoms representing the highest phylogenic category in the procaryotic domain (Balch et al. 1979).

The substrates utilised by the methanogens are extremely limited. Acetate, hydrogen and carbon dioxide, formate, methanol, various methylamines and carbon monoxide have all been shown to be degraded under certain conditions (Daniels et al. 1977, Balch et al. 1979, Bryant 1979, Walther et al. 1980). Stoichiometric equations for some of these reactions are listed in Table 2.3.

The majority of species isolated to date are capable of the reduction of carbon dioxide (Zehnder et al. 1980) which is vital in maintaining the low hydrogen partial pressure required for the dissimilation of complex organics. Many species are also capable of using formate but only three have so far been isolated which are capable of using acetate as the sole source of carbon and

Table 2.3: Stoichiometry and change in free energy values for the major methane formation reactions (after McInerney and Bryant 1981a).

A: Hydrogen and carbon dioxide

$$4H_2 + HCO_3^- + H^+ = CH_4 + 3H_2O \qquad \text{(E2.7)}$$

$\Delta G'_0$ = -32.4 kcal per reaction

B: Formate

$$4HCOO^- + H_2O + H^+ = CH_4 + 3HCO_3^- \qquad \text{(E2.8)}$$

$\Delta G'_0$ = -31.2 kcal per reaction

C: Acetate

$$CH_3COO^- + H_2O = CH_4 + HCO_3^- \qquad \text{(E2.9)}$$

$\Delta G'_0$ = -7.4 kcal per reaction

D: Methanol

$$4CH_3OH = 3CH_4 + HCO_3^- + H^+ + H_2O \qquad \text{(E2.10)}$$

$\Delta G'_0$ = -75.2 kcal per reaction

---

energy. These are Methanosarcina barkeri (Mah et al. 1978), Methanothrix soehngenii (Zehnder et al. 1981, Huser et al. 1982) and Methanococcus mazei (Mah 1980)

Much research effort has been directed towards understanding the mechanisms and energetics of the methane fermentation. The development of knowledge in this area can be traced through the reviews of Barker (1957), Stadtman (1967), Wolfe (1971), Balch et al. (1979) and Wolfe (1980). The pathway of carbon dioxide reduction has now been largely elucidated and a common novel pathway appears to exist in all methanogens (Jones et al. 1985).

In the acetoclastic reaction the methyl group of acetate is transferred intact to methane and the carboxyl group is converted to carbon dioxide (Statdman and Barker 1949, Pine and Barker 1956). Similar methyl group transfers also occur for methanol and the methylamines (Pine and Vishniac 1957, Walther et al. 1980). The mechanism for methane and ATP generation from acetate is not known but is thought to converge with the carbon dioxide reduction pathway. The scheme proposed by Barker (1957) remains valuable and was based on the hypothesis that a single organism would not use several different mechanisms for generating one specialised product. A review of acetate catabolism has been published by Smith et al. (1980). A major difficulty remains in that the energy yield of the reaction of -7.4 kcal.mole^{-1} is not considered sufficient to meet the demand of 12 kcal.mole^{-1} for ATP formation (Thauer et al. 1977, Smith et al. 1980). The low energy yield is also reflected in low growth yields on acetate ranging from 1.1 to 2.7 g biomass per mole acetate utilised (Van den Berg et al. 1976, Smith and Mah 1978, Weimer and Zeikus 1978, Zehnder et al. 1980).

It is well known that 60 to 80 % of methane formed in digesters and other anaerobic environments is derived from acetate (Jeris and McCarty 1965, Smith and Mah 1966, Kaspar and Wuhrmann 1978a, Mackie and Bryant 1981, Boone 1982). It is therefore perhaps

surprising that so few species of methanogens able to use acetate as the sole carbon and energy source have been isolated. This appears to be due to the low energy yield of the reaction and the importance of optimising subculturing protocol. Zeikus et al. (1975) and Weimer and Zeikus (1978) suggested that growth could only occur in the presence of hydrogen and carbon dioxide on basal medium, or with acetate as the major carbon source on complex medium, but it is now accepted that this is not the case providing great care is taken in adapting the organism to acetate. However the organism grows faster and to a greater final density on basal medium with hydrogen and carbon dioxide or methanol than with acetate, and methane production is stimulated by the use of complex medium (Zeikus et al. 1975, Mah et al. 1978, Weimer and Zeikus 1978, Ferguson and Mah 1983). In contrast Methanothrix soehngenii has routinely been cultured on a mineral salts medium with acetate as the sole carbon and energy source and yeast extract, digester supernatant and rumen fluid did not significantly enhance methane production (Huser et al. 1982).

The acetoclastic reaction may be inhibited by high hydrogen partial pressures. Baresi et al. (1978) noted an age-dependent inhibition by hydrogen and formate which differed from the inhibition by chloroform and benzyl-viologen. In pure culture studies inhibition by hydrogen has been observed by McInerney and Bryant (1981b). This effect has recently been investigated extensively by Fergusson and Mah (1983) whose results indicate that acetate catabolism is inhibited in favour of hydrogen utilisation and that the acetoclastic reaction again becomes operative at low hydrogen levels but before hydrogen is completely exhausted. Inhibition was observed above 0.01 atm hydrogen but the significance of this effect in mixed culture studies is unclear. Short term exposure to hydrogen did not inhibit acetate degradation in an enrichment culture (Van den Berg et al. 1976) or sewage sludge digester (Kaspar and Wuhrmann 1978b) but inhibition in other mixed cultures has been observed by Van den Berg et al. (1980) and Boone (1982).

Typical population densities of methanogenic bacteria in anaerobic digesters have been quantified by several workers and their results have been extensively reviewed by Toerein and Hattingh (1969), Kirsch and Sykes (1971) and Hobson et al. (1974). All acetate-utilising methanogens have been isolated from digesters (Mah 1980, Smith et al. 1980, Zehnder et al. 1980) but the rod Methanothrix soehngenii appears to dominate at low acetate concentrations or when cell turnover rate is low. Hydrogen-utilising methanogens isolated from digesters include Methanobacterium formicicum, M. bryantii, M. thermautotrophicum, Methanobrevibacter ruminantum, M arboriphilus, M. smithii and Methanospirillum hungatii (Zehnder et al. 1981).

2.3 KINETIC ANALYSIS OF THE METHANE FERMENTATION

2.3.1 Introduction

In a multi-stage process such as anaerobic digestion the slowest reaction controls the rate of the whole fermentation (Lawrence and McCarty 1969). For process optimisation it is important to be able to identify the limiting reaction and the factors controlling the rate of this step. Several reactions have been proposed to be rate-limiting in anaerobic digestion including:

(1) solubilisation of particulate material (Pfeffer 1974, Ghosh et al. 1975),

(2) methane production from volatile acids (Novak and Carlson 1970, Chynoweth and Mah 1971, Ghosh and Pohland 1974), and

(3) mass transfer of product gases (Finney and Evans 1975)

For complex wastes the hydrolysis of particulate material and complex molecules is the controlling step (Pfeffer 1974, Ghosh and Klass 1978, Eastman and Ferguson 1981, Boone 1982). However many digester feedstocks, and particularly industrial wastes, contain largely soluble organics and for these wastes it appears

methane production from volatile acids controls the process (Ghosh and Pohland 1974). As several acid intermediates are formed in the fermentation, further research has been conducted to clearly identify the rate limiting step and this work is now reviewed.

Two approaches have been employed in attempting to define the control mechanism either;

(1) analysis of reaction rates, intermediate pool sizes and intermediate turnover times, or

(2) selection of a model of microbial growth and a determination and comparison of the growth parameters for the various organisms involved in the fermentation.

2.3.2 COD flux in the methane fermentation

Several schemes of varying complexity have been reported in the literature for flow of substrate (as COD) to methane (McCarty 1964a, Kaspar and Wuhrmann 1978a, Mackie and Bryant 1981, Gujer and Zehnder 1983). The scheme proposed by Kaspar and Wuhrmann (1978a) for degradation of complex wastes such as domestic sewage has found most favour and a modified version of this is shown in Figure 2.3. The essential features of the scheme are:

(1) approximately 70 % of the methane produced is derived from acetate (Jeris and McCarty 1965, Smith and Mah 1966, Cappenberg and Prins 1974, Mackie and Bryant 1981, Boone 1982),

(2) approximately 50 % of the COD flux through acetate is derived directly by fermentation of sugars and amino acids and the remainder from anaerobic oxidation of lipids, propionate and butyrate (Kaspar and Wuhrmann 1978a, Mackie and Bryant 1981), and

(3) approximately 15 % of the methane produced is derived from COD flux through propionate and aproximately 20 to 23 % derives from the combined degradation of propionate

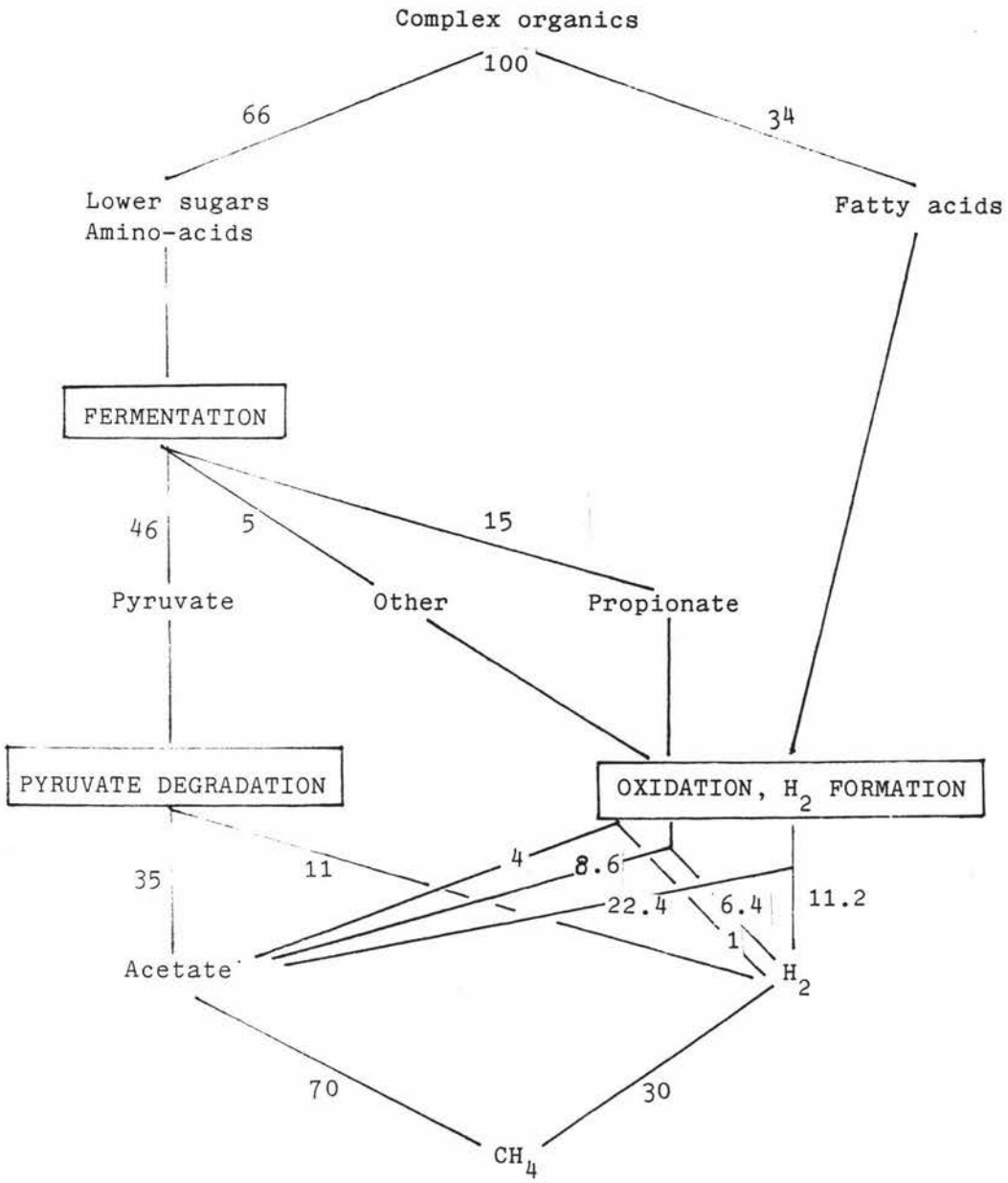


Figure 2.3: COD flux in the methane fermentation of complex waste (after Kaspar and Wuhrmann 1978a).

and butyrate (Kaspar and Wuhrmann 1978a, Mackie and Bryant 1981, Boone 1984a).

If this scheme is accepted as applicable to a wide range of substrates then for soluble wastes containing only fermentable sugars the contribution from lipid oxidation can be removed and a new scheme calculated as shown in Figure 2.4. This again predicts 70 % of the methane formed via acetate but the contributions from propionate degradation and combined propionate and butyrate degradation increase to 22.5 and 30 % respectively.

2.3.3. Reaction rate analysis of the rate-limiting step

Reaction rate analysis has been employed frequently to characterise the kinetics of the methane fermentation (Smith and Mah 1966, Kaspar and Wuhrmann 1978a, Boone 1982, Schink 1985). Volumetric reaction rates (e.g. g COD removed.l^{-1}.hr^{-1}) are usually reported because the data are easy to obtain and it is not necessary to determine the concentration of biomass responsible for the specific reaction. However this represents the major disadvantage of the approach because volumetric rates vary considerably from one system to another depending on such factors as the loading rate, nature of the feed and the digester operating conditions. In only a very few cases are specific reaction rates (g COD removed.g volatile suspended solids^{-1}.hr^{-1}) reported (Van den Berg 1977, Mackie and Bryant 1981). Although these offer a geater opportunity for comparison between systems the data are difficult to obtain.

A detailed reaction rate analysis of anaerobic digestion was undertaken by Kaspar and Wuhrmann (1978a). They investigated the initial steady-state degradation rates (V_0) for acetate, propionate and hydrogen in batch samples taken from a municipal sludge digester and then individually raised the concentrations of these compounds. The degradation rates under conditions of saturation (V_1) and concentration dependence were then monitored

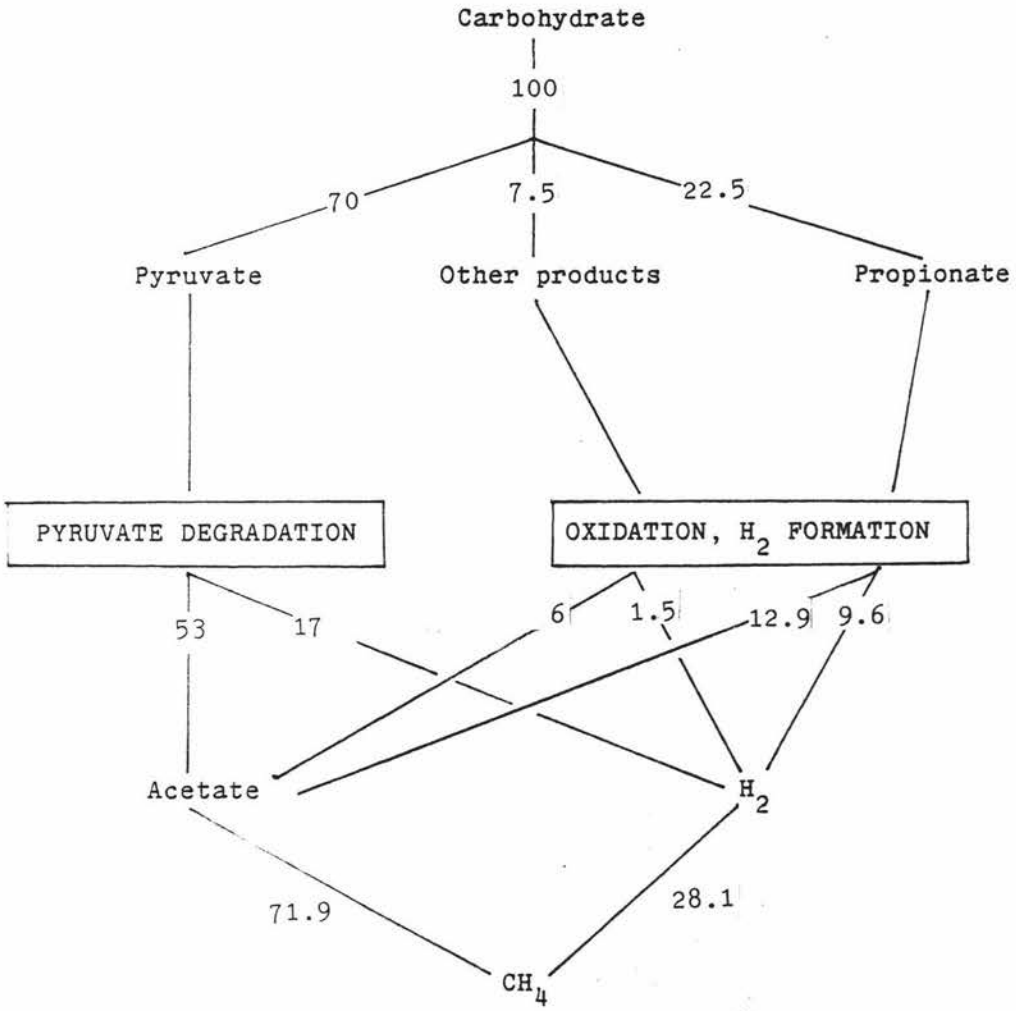


Figure 2.4: Proposed COD flux in the methane fermentation of carbohydrate waste.

and the kinetic parameters V_{max} and K_s calculated. While the actual reaction rates are not especially significant for the reasons noted above, the importance of this work lay in the discovery that the acetate-degrading system was slightly less than half saturated under natural conditions. In comparison the propionate and hydrogen degrading systems were only saturated to 10 to 15 % and 1 % respectively, strongly indicating acetate degradation was the rate-limiting step in the process.

This conclusion is supported by other results in the literature. Van den Berg et al. (1976) reported that in an acetate enrichment culture normally operating at a loading rate of 4 mM acetate. $l^{-1}.day^{-1}$ acetate utilisation could be doubled by raising the residual acetate concentration from less than 12 mM to 20 mM (720 to 1200 $mg.l^{-1}$). Similarly Shea et al. (1968) studied the utilisation of hydrogen in anaerobic digestion and concluded that the system operated at less than 3 % of the maximum possible rate.

2.3.4 Modelling of microbial growth in anaerobic digestion

Many mathematical models are available to describe microbial growth and substrate utilisation and several major reviews of the subject have been published recently (Fredrickson et al. 1970, Barford and Hall 1978, Roels and Kossen 1978). The most widely and successfully applied model is based on the Monod growth equations :

$$r_x = \mu x \qquad \text{(E2.11)}$$

$$\mu = \frac{\mu_{max} s}{K_s + s} \qquad \text{(E2.12)}$$

where r_x = biomass production rate (g biomass.$l^{-1}.hr^{-1}$)

μ = specific growth rate (hr^{-1})

x = biomass concentration (g biomass.l^{-1})

μ_{max} = maximum specific growth rate (hr^{-1})

s = substrate concentration (g substrate.l^{-1})

K_s = substrate concentration at which the specific growth rate is half the maximum value (g substrate.l^{-1})

The assumptions on which the model are based have been reviewed by Barford and Hall (1978) and include:

(1) balanced growth is assumed,

(2) the amount of cell material is described by the mass of viable cells,

(3) growth is occurring on a medium containing a known limiting substrate which stops growth when it is completely exhausted; no other substrate affects the growth of the organism before this occurs,

(4) growth is described as a single reaction step with constant yield and no dynamic lags, and

(5) biomass is not considered to form a separate phase.

The substrate utilisation rate (r_s) is related to the biomass production rate by the yield constant:

$$r_s = -Y_{xs}^{-1} r_x \qquad \text{(E2.12)}$$

where Y_{xs} = yield coefficient, expressed as g biomass. (g substrate used)$^{-1}$ and the maximum substrate utilisation rate $r_{s,max}$ can be calculated from:

$$r_{s,\,max} = \frac{\mu_{max}}{Y_{xs}} \qquad \text{(E2.13)}$$

Many modifications to the Monod model have been proposed to include concepts such as maintenance energy, endogenous metabolism or organism death, multiple substrates and substrate and product inhibition. Details of these and other modifications are considered in the review articles cited above and in the

anaerobic digestion literature (e.g. see McCarty 1965, Lawrence and McCarty 1969, Henze and Harremoes 1983).

The Monod model has been widely and extensively applied to anaerobic digestion studies. A comprehensive review of the kinetic data obtained from studies of wastes containing soluble organics was completed by Henze and Harremoes (1983) who found the data to be reasonably homogenous and proposed a representative set of parameters to describe the process (Table 2.4). Data obtained from other studies not included in the review (Hobson and MacDonald 1980, Zehnder and Koch 1983) for propionate and butyrate degradation and from major kinetic studies both in pure and enrichment cultures are also shown in Table 2.4 and are in agreement with the parameter values selected by Henze and Harremoes (1983). These data also indicate that for soluble wastewaters methane production from volatile acids is rate limiting. As the majority of COD flows through acetate and the maximum utilisation rates for the various acids are approximately the same in enrichment cultures, methane formation from acetate is again indicated as the rate-limiting step. Monod's model confirms the conclusions derived from reaction rate analyses.

The logistic equation is another model which has been applied to analysis of both chemical and microbiological systems (DeWitt 1943, Edwards and Wilke 1968). The form of the logistic equation is:

$$y = \frac{K}{1 + e^{a + bt}} \qquad \text{(E2.15)}$$

where y may be an estimate of the microbial biomass (x) or substrate (s). The rate of change of the parameter of interest is found from:

$$\frac{dy}{dt} = -yb(1 + y/K) \qquad \text{(E2.16)}$$

Table 2.4: Kinetic parameters describing the methane fermentation.

Metabolic group	μ_{max} day^{-1}	Y_{xs} g VSS.g COD_r^{-1}	K_s g $COD.l^{-1}$	k_d^a day^{-1}	r_s g COD_r.g VSS^{-1} .day^{-1}	Reference
acidogenic	2.0	0.15	0.2	-	$7-13^{b,c}$	1
methanogenic	0.4	0.03	0.05	-	$7-13^{b,c}$	
mixed	0.4	0.18	-	-	$1-2^{b,c}$	
acetate-degrading, mixed enrichment cultures	0.32	0.04	0.15	0.015	8^c	2
	0.49	0.28	4.5	-	1.75^c	3
	0.40	-	0.8-0.9	-	-	4
	0.237	0.043	0.075	0.022	5.56^c	5
	-	0.016	-	-	5.12	6
Ac-degrading pure culture	0.45	0.033	0.32	-	13.6^c	7
	0.11-0.21	0.018-0.023	0.05	-	$5.5-10.3^c$	8,9
Pr-degrading mixed enrichment cultures	0.31	0.04	0.03	0.01	7.8^c	2
	0.155	0.025	0.25	-	6.2^c	10
Bu-degrading mixed enrichment cultures	0.37	0.047	0.0005	0.027	7.9^c	2

References

1 Henze and Harremoes (1983)
2 Lawrence and McCarty (1969)
3 Ghosh and Klass (1978)
4 Hobson and MacDonald (1980)
5 Anderson and Duarte (1980)
6 Van den Berg (1977)
7 Smith and Mah (1978)
8 Zehnder et al. (1980)
9 Huser et al. (1982)
10 Gujer and Zehnder (1983)

Notes

a specific decay rate of cells
b for 50 % and 100 % active biomass respectively
c calculated from $r_s = \mu_{max}/Y_{xs}$

The equation has limited value in predicting kinetic parameters (Roels and Kossen 1978) and the major advantage of its use is the ease with which the equation may be used in computer-based non-linear regression algorithms to fit experimental data.

2.4 ANAEROBIC REACTOR DESIGNS

2.4.1 Introduction

The successful application of anaerobic digestion on a large scale requires careful selection of the reactor configuration and application of sound engineering priciples to the design of the system. Without meticulous preliminary consideration the desired process and economic goals will not be achieved (Hobson et al. 1981). The essential functions of the digester have been listed by Baader (1981) as:

(1) to continously provide the bacteria with nutrients and to remove the metabolic products from the biomass,

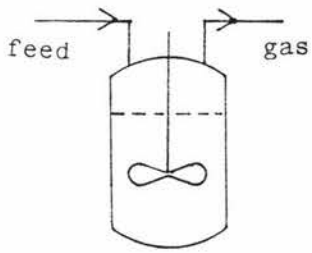
(2) to ensure a detention time for the organic matter adapted to the different digestion rates of the organic components,

(3) to prevent uncontrolled accumulation of solids in the digester and blockages in the material flowing through the digester, and

(4) to distribute equally the heat in the digester.

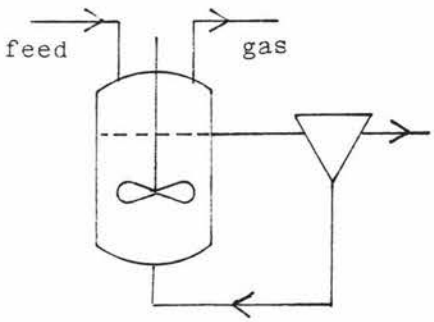
A number of reactor types are now available and the most important of these are listed in Figure 2.5. Characteristic features of the digesters are described in the following sections. The different configurations have been classified on whether biomass is suspended or attached to an inert surface and whether biomass is retained or not retained within the reactor. The ratio of solids retention time (SRT) to hydraulic retention time (HRT) is thus an important operational parameter distinguishing the reactor types and influencing their suitability for the treatment of various wastes. In the

A. Suspended biomass; biomass not retained

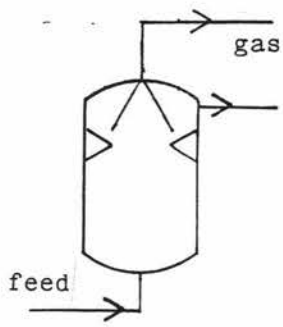


Conventional stirred tank

B. Suspended biomass; biomass retained

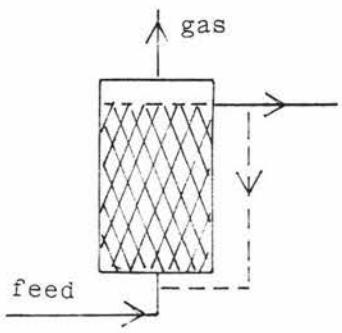


Contact process

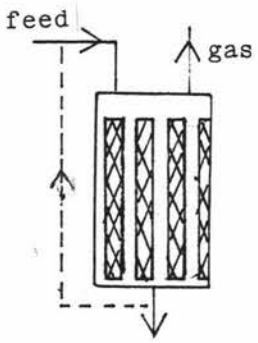


Upflow sludge blanket

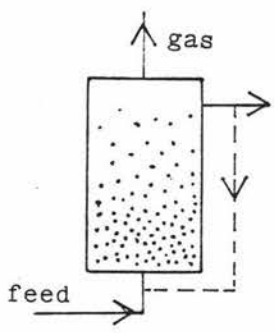
C. Attached biomass



Upflow filter



Downflow fixed-film



Fluidised/ expanded bed

Figure 2.5: Configurations for the major anaerobic reactor designs.

conventional stirred tank digester this ratio is approximately unity and the reactor is most suitable for the treatment of wastes containing particulate solids such as municipal sludge. This and similar feedstocks contain a range of often complex compounds which are degraded relatively slowly.

Industrial wastewaters by contrast contain predominantly soluble organics of very high digestability. For such wastes the SRT must be maintained as high as possible to allow a large bacterial population, particularly the slow-growing methanogens, access to the substrate. Conversely the HRT must be as low as possible to reduce the reactor volume and minimise capital costs. Retained biomass systems, where the SRT/HRT ratio may be in the order of 10 to 100: 1, are clearly favoured for these wastes (Baader 1981, Henze and Harremoes 1983).

2.4.2 Conventional single tank process

The most common digester system remains a single tank unit to which feed is introduced intermitantly or on a continuous basis. A constant liquid volume is usually maintained and retention times range from ten days to several months. Such digesters are most often used for the treatment of municipal sludge and animal wastes where the solids content is of the order of 2 to 10 % (w/v). They cover the whole size range from small on-farm units of about 2 m^3 up to the large, modern sewage digesters which can approach 10 000 m^3 in volume (Hobson et al. 1981).

The major design features of the single tank digester have been extensively reviewed by Hobson et al. (1981). With the exception of small or older units the digesters are generally heated and stirred. The usual operating temperatures are 30 to 40 °C but they may be operated at thermophilic temperatures of 55 to 65 °C (Garber 1977). In those cases where the contents are not heated, insulation is generally provided to maintain a constant temperature. This may be achieved by sinking the digester into

the ground or, for large digesters, using concrete as the construction material. Heating is generally by indirect hot-water heating using sludge recirculation through heat exchangers. The hot water may be obtained from burning the methane produced or may be recovered from heat exchangers fitted to plant engines and turbines (Hobson et al. 1981). The sludge recirculation provides some mixing but generally some other form of agitation, either mechanical or by gas recirculation, is also provided. This helps to maintain the homogeneity of the liquor and also ensures even heating. Gas is usually collected under a floating cover which provides storage for only a few hours of continuous gas production.

The major advanatages of the single tank digesters are the ease of their construction and operation, ensuring their continued use.

2.4.3 The anaerobic contact process

This process is similar in principle to the aerobic activated sludge system. Waste degradation occurs in a conventional stirred tank digester but a settling vessel subsequently separates the active biological solids from the waste stream for recycle to the digester. This represents the major advantage of the system as cell recycle gives a long SRT permitting the growth of the methanogens. However the HRT can be reduced allowing a greater volume of more dilute waste to be treated economically.

Successful large scale applications of this process have been reported for a range of substrates (Schroepfer et al. 1955, McCarty 1964d, Hobson et al. 1981, Baader 1985). However the application of the contact reactors has generally been limited by the failure of solids separation because of gas evolution in the settling vessel. In this situation the process becomes similar to the conventional digester and suffers the same limitations.

2.4.4 The upflow anaerobic sludge blanket reactor (UASBR)

The UASBR system (Lettinga et al. 1980) contains features of both the contact and upflow filter processes. Waste is introduced into the bottom of the reactor and passed through a blanket of active, granular sludge suspended by the upward liquid flow. Conical baffles are provided for separation of the gas, sludge and liquid and clarified effluent is withdrawn at the top of the reactor. The process has gained rapid acceptance because of its ability to operate efficiently at high loading rates with low suspended solids, its low construction costs and its ability to handle solids-containing feedstocks (Lettinga et al. 1980, Frostell 1981). Full scale plants in excess of 4000 m^3 have been constructed for the treatment of sugar, brewing and meat wastes (Lettinga et al. 1980, Pette and Versprille 1981).

2.4.5 The upflow anaerobic filter

In this reactor biomass is supported on a solid material in a tank through which waste is passed upwards. Although technically an attached film process, biomass trapped in the interstitial spaces is believed to contribute significantly to successful waste treatment (Henze and Harremoes 1983). The major advantages of the system have been high rates of treatment and an effluent low in suspended solids. However channelling, poor mixing, clogging by solids in the waste, loss of reactor volume and high capital costs have all been cited as disadvantages of the filter. To some extent these problems can be overcome by backwashing of the reactor; effluent recycling may also be necessary to neutralise acid wastes or to prevent acid conditions prevailing in the lower sections of the filter.

2.4.6 The downflow stationary fixed film reactor (DSFFR)

This process design was developed to solve the problems of channeling and solids accumulation found in anaerobic filters (Van den Berg and Lenz 1979, Van den Berg et al. 1981, Van den Berg and Kennedy 1983). All biomass is attatched as a film on the support material in relatively large diameter channels through which the waste passes downwards. Fired clays have proved particularly effective as supports and a range of wastes have been successfully treated (Van den Berg et al. 1981).

2.4.7 The attached film fluidised bed reactor (AFFBR) and attached film expanded bed reactor (AFEBR)

These reactors are characterised by growth of the biomass on small inert particles such as anthracite, sand, activated carbon and plastic. Beds of these particles are then either expanded or fluidised by flow of the waste stream combined with a significant degree of effluent recycle. Successful treament of a range of wastes has been reported at values of HRT below 1 day (Jewell 1981, Switzenbaum and Danskin 1982, Jeris 1983). Problems of plugging and solids accumulation are again avoided but at the expense of the energy needed to raise the bed (Speece 1983).

2.4.8 Novel anaerobic processes

Other process designs recently proposed for methane generation include the anaerobic rotating contactor (Tait and Freidman 1980) and the use of immobilised bacteria (Karube et al. 1980). Both systems have so far only been reported on a small scale and further investigation of their potential is required.

The use of two-phase systems in which the acid and methane forming stages are separated has also been proposed and evaluated over a lengthy period. Developments and performance

characteristics are discussed by Borchardt (1971), Ghosh and Klass (1978), Massey and Pohland (1978) and Cohen et al. (1979). Further development and wider acceptance of the two-phase system seems likely (Baader 1985) although in the immediate future single-phase systems are likely to remain the most important reactor types. Further development to combine features of existing processes into single units also appears to have considerable merit, an example would be mounting an anerobic filter over a UASB reactor.

2.5 ENVIRONMENTAL FACTORS AFFECTING THE RATE AND STABILITY OF THE METHANE FERMENTATION

2.5.1 Introduction

The methane fementation involves the interaction of many species of bacteria in a complex ecosystem where the bacterial growth rate is low and adaption to altered conditions is gradual. In particular the methanogens appear to respond more slowly to changes in environmental conditions than the other bacteria and this can lead to unbalanced digestion through the disruption of the metabolic interactions between the bacterial groups (McCarty 1964b, Bryant 1979). It is therefore important that the optimal environmental conditions are establisehed and maintained for the maximisation of fermentation rates and efficiencies and for anaerobic digestion to gain acceptance as an effective waste treatment system for a wide range of effluents (Speece 1983).

2.5.2 Temperature

Bacteria have no internal mechanism to maintain a constant favourable temperature and are markedly affected by changes in the ambient temperature. Every bacterial species exhibits growth over a defined temperature range and this is bounded by a temperature, or those temperatures, at which growth is just possible and contains a temperature at which growth is optimal.

A mixed population of bacteria may react to changes in temperature either at a biochemical level, exhibiting shifts in reaction rate, or at a cellular level by the selection of species growing most favourably at the new temperature (Kirsch and Sykes 1971).

Two temperature optima have been observed in the methane fermentation; one is in the mesophilic range of 35 to 40 °C and the other in the thermophilic range of 55 to 65 °C (McCarty 1964b). The effect of temperature on the process and the relative merits of operating in the mesophilic or thermophilic range have been extensively reviewed by Kotze et al. (1969), Kirsch and Sykes (1971), Cooney and Wise (1975) and Buhr and Andrews (1977). No substantive research of temperature effects on methanogenesis has been reported since these reviews and the reader may refer to these for further detail.

2.5.3 pH and alkalinity

Provision of sufficient alkalinity to buffer changes in pH and effective pH control have proved important to successful digester operation (McCarty 1964b). Digesters may be operated between pH 6.6 and 7.6 but optimum performance is usually observed in the range pH 7.0 to 7.2 (McCarty 1964b, Clarke and Speece 1970, Van den Berg et al. 1976, Karube et al. 1980).

In pure culture studies methane bacteria show a marked pH optimum between pH 6 and pH 8 with most species exhibiting highest activity near neutrality (Zehnder et al. 1981). However important differences between methanogens do exist especially for example between the two major acetate-degrading species. Methane production has been observed between pH 5.5 and pH 8 for a Methanosarcina species with an optimum close to pH 6 (Zinder and Mah 1979). Activity decreased only slowly from the optimum until about pH 7.5. In contrast, studies of the filamentous Methanothrix soehngenii show methane production only occurred

over the range pH 6.8 to 8.2 with an optimum in the range pH 7.4 to 7.8 (Huser et al. 1982). Beyond the optimum activity decreased rapidly and was only about 15 % of the maximum at pH 7 and pH 8. As this organism is thought to be the most widespread in anaerobic digesters (Cappenberg 1975, Huser et al. 1982) this behaviour could explain the observed narrow optimum range noted in some mixed culture studies (Van den Berg et al., Karube et al. 1980).

The pH of aqueous solutions is controlled by the interaction of weak and strong acid-base equilibria. In anaerobic digestion these arise from the presence of acids and bases in the influent or from their formation in the course of the fermentation. The carbonic, volatile fatty acid, ammonium, hydrosulphuric and orthophosphoric systems could contribute towards pH control. However generally only the first three equilibria are of importance as the concentration of phosphorous and sulphur are too low to provide sufficient buffering capacity (Pohland 1967, Capri and Marais 1975).

In the pH range 6.6 to 7.6 which is of interest in anaerobic digestion, McCarty (1964b) and Capri and Marais (1975) suggested the carbonic acid system can be simplified to:

$$CO_2(g) + H_2O = HCO_3^- + H^+ \qquad \text{(E2.17)}$$

Capri and Marais (1975) also demonstrated how the bicarbonate ion concentration, or bicarbonate alkalinity, may be calculated for a given pH and carbon dioxide partial pressure. The gas produced from a stable digester typically contains about 35 % carbon dioxide with a digester pH of 7 and under these conditions the bicarbonate alkalinity would be expected to be about 3800 $mg.l^{-1}$ (Capri and Marais 1975).

Alkalinity is a measure of the capacity of a solution to neutralise a strong acid at a given pH. The total alkalinity of

a system is the resultant of all the acid-base reactions occurring at that pH and in anaerobic digestion this is strongly influenced by the concentration of volatile fatty acids (Pohland and Bloodgood 1963, McCarty 1964b, Pohland 1967). When the acid concentration is low the bicarbonate alkalinity is approximately equal to the total alkalinity. At high acid concentrations bicarbonate alkalinity is neutralised with the formation of volatile acid alkalinity:

$$HCO_3^- + HAcid = Acid^- + CO_2 + H_2O \qquad (E2.18)$$

where HAcid represents the individual volatile fatty acids.

Stable digestion depends on a balance between bicarbonate and volatile acid alkalinities. The bicarbonate alkalinities obtained from theory are generally regarded as the minimum values from a process standpoint as they provide little margin for safety. In general a bicarbonate alkalinity at pH 4.0 of up to 5000 $mg.l^{-1}$ as $CaCO_3$ is recognised as desirable and to ensure optimal digester performance it is recommended that pH, total and bicarbonate alkalinities and volatile acid concentrations are monitored (McCarty 1964b). Monitoring of pH alone is insensitive to large changes in alkalinity and a low pH value only indicates that an imbalance has already occurred.

High acid concentrations are usually associated with unstable digestion. The effect of these can be reduced by lowering the loading rate to promote acid degradation or by adding alkaline materials to maintain the pH near neutrality. The various chemicals available and some control strategies have been outlined by McCarty (1964b), Capri and Marais (1975) and Barber (1977). Lime has commonly been used for this purpose as it is cheap and readily available. However it is limited by its relative insolubility and McCarty (1964b) recommends that it only be used to increase the pH to about 6.8. Beyond this point the most effective agent for pH control is sodium bicarbonate. It is

safe to use, does not react with carbon dioxide to form temporary vacuum conditions, is readliy soluble and can be dissolved proir to addition, does not cause scale or encrustation and is unlikely to be inhibitory to digestion. Although sodium bicarbonate is relatively expensive its advantages indicate that its use will only increase.

2.5.4 Oxidation-reduction potential (ORP) and anaerobiosis

Several investigators have shown that an optimum redox potential is established during anaerobic digestion. Dirasian et al. (1963) observed an optimum E_c of -520 mV to - 530 mV and estimated stable digestion was possible between -490 mV and -550 mV. Similar results have been obtained by Hartz and Kountz (1966), Blanc and Molof (1969, 1973) and Mosey and Hughes (1975) and it has been suggested that redox potential measurements can be useful for characterising the condition and activity of the bacterial flora (Pohland and Mancy 1969, Blanc and Molof 1969, 1973). However the observed ORP may be dependent on the nature of the waste being treated. The studies reported above were all of digesters treating complex wastes whereas Hansson and Molin (1981a) reported stable operation at an E_h of -220 mV, corresponding to an E_c of approximately -440 mV, for digestion of a glucose-based semi-synthetic medium.

The ORP is related to the degree of anaerobiosis and this can also be an important factor in control of the methane fermentation. Acetogenic and methanogenic bacteria behave as strict anaerobes in pure culture (Hungate 1966, Boone and Bryant 1980). For this reason reducing agents such as sodium sulphide, cysteine hydrochloride , sodium thioglycollate and dithionate are often added to the culture medium (Hungate 1966, 1969; Willis 1969, Boone and Bryant 1980) and care is taken to exclude all oxygen.

However studies in mixed culture indicate there may be a considerable degree of oxygen tolerance in digesters which is attributed to the extremely rapid utilisation of oxygen by facultative fermentative bacteria (Fields and Agardy 1971, Hobson and Shaw 1976, Van den Berg et al. 1976, Karube et al. 1980, Pirt and Lee 1983). Thus anaerobiosis may be achieved rapidly and process stability restored despite quite severe air leakage into a digester (Hobson et al. 1981).

Recently Scott et al. (1983) observed that methanogenesis was inhibited by 48 % by exposure to a gas phase of 10 % oxygen for 6 min although oxygen was not detectable (< 30 nM) in the sludge. Exposure to a 20 % oxygen gas phase for 5 min resulted in a further 34 % reduction in methane production and only a very limited recovery was observed over a short period (1 hr). The long term effects of oxygen exposure were not determined but the prospects for full recovery must be assumed to be good based on the other studies reported above. This is further supported by the observation of Huser et al. (1982) who were able to isolate Methanobacterium soehngenii from aerobic samples of pretreated raw sewage sluge and could demonstrate full recovery of methanogenic activity from oxygen sparged cultures after a few hours to three days.

2.5.5 Retention time and organic loading rate (OLR)

The organic loading rate may be expressed in many ways but the most commom is based on the reactor working volume. Typical units are g $COD.l^{-1}.day^{-1}$ and this volumetric OLR (OLR_v) is related to the hydraulic retention time (HRT) by:

$$OLR_v = \frac{\text{influent COD}}{\text{HRT}} \qquad \text{(E2.19)}$$

The specific OLR (OLR_s) based on biomass concentration is also an important parameter with typical units of g COD.(g VSS)$^{-1}$.day^{-1} where VSS is volatile suspended solids. The OLR, HRT and solids retention time (SRT) are all vital parameters in operation and control of anaerobic digesters. The relationship of SRT and HRT has already been considered in the previous discussion on anaerobic reactors (Section 2.4).

In a continuously mixed system with suspended growth the SRT and HRT are equal and the minimum limit is imposed by the growth rate of the slowest growing organisms within the reactor. In the case of soluble wastes for example, when the rate-limiting group is the acetate-degrading methanogens, the HRT must exceed 2.5 day assuming a μ_{max} of 0.4 day^{-1} (Henze and Harremoes 1983). For complex wastes a much higher HRT is required and frequently values of 10 to 40 day are selected (Hobson et al. 1981). If the minimum HRT is not exceeded then the limiting organisms will be washed out of the digester and treatment efficiency will rapidly decrease.

However although a minimum HRT can be established in this way, in practise this may not be achievable as the OLR for a given waste strength may exceed that which can be effectively treated. Equation 2.19 predicts that as the waste strength increases the OLR also increases for a given HRT. At low organic loading rates high conversion of substrate COD to methane is achieved but methane production per volume of reactor is low. At high loading rates the methane production rate per unit volume is high but less gas is generally produced per volume of feed as substrate utilisation is reduced (Bryant 1979, McInerney and Bryant 1981a). The maximum substrate utilisation rate, with units of g COD_r.(g VSS)$^{-1}$.day^{-1} where COD_r is COD removed, depends on the growth constants of the bacteria involved and as shown by Henze and Harremoes (1983) is probably between 1 and 2 g COD_r.g VSS^{-1}.day^{-1}.

Maintenance of optimal pH and alkalinity appears to be the most important strategy in controlling the effects of overloading. In cases where this is achieved the actual causes of failure at high loading rates do not appear to have been defintively characterised. The most recent evidence indicates that hydrogen production and consumption is localised within the digester and variations in the local concentration may be responsible for disruption of the delicate interactions between the groups involved in the fermentation (Gujer and Zehnder 1983, Boone 1984a, Conrad et al. 1985).

For retained biomass systems it is obviously easier to achieve the minimum SRT required for growth of the rate-limiting organism(s). The HRT may then be reduced to as low as a few hours and a very high OLR can be achieved. It is very difficult to predict a maximum loading rate for such systems because of the range of variables influencing the process. However as a rough guide the data of Van den Berg and Kennedy (1983) shown in Table 2.5 may be useful.

An important factor which does not appear to have been widely investigated in anaerobic digestion studies is the influence of the method and frequency of feeding on digester performance. Digesters may be operated in semi-continuous mode, with one or several medium additions per day, for five or seven days per week, or medium may be added continuously. It is well established that the metabolism of the various physiological groups varies significantly with time after daily batch feeding (Mountford and Asher 1978, Mackie and Bryant 1981) and this could be expected to result in differing performance between digesters operating with different feeding regimes. However there are few references in the literature to this.

Table 2.5: Comparison of typical organic loading rates and treatment efficiencies for various anaerobic reactor designs (after Van den Berg and Kennedy 1983).

Reactor type	COD loading rate $g.l^{-1}.day^{-1}$	COD removal %
contact	1 - 6	80 - 95
upflow filter	1 - 10	80 - 95
fluidised/expanded bed	1 - 20	80 - 87
downflow filter	5 - 15	75 - 88
sludge blanket	5 - 30	85 - 95

Hobson et al. (1981) reported results of Bousefield et al. (1974) which indicated digesters fed daily utilised less substrate than digesters fed every five minutes. Speece (1981) also suggested that continuously fed digesters could process more substrate than those fed at intervals, where substrate alternately becomes excessive and then limiting. No data was provided to support this view but Speece (1981) also noted that additional alkalinity may be required in semi-continuous digesters to buffer the expected surges in volatile fatty acid concentrations.

Assinari di san Marzano et al. (1981) operated digesters with a variety of substrates in semi-continuous mode and examined the importance of fatty acids in the regulation of methane production

and process stability. Their results suggested semi-continuous loading was more reliable than continuous loading. This was particularly true for retention times below 14 days when feeding on five days of the week was recommended, providing no more than 20 % of the digester mixed liquor was replaced at any time. The reason given for this behaviour was that fatty acids would be metabolised more effectively when they were the major nutrient; however supporting evidence was not provided.

2.5.6 Nutritional requirements and toxic compounds

2.5.6.1 Introduction

The provision of an adequate supply of nutrients and protection against inhibition by toxic compounds are important factors in the development and maintenance of an efficient digester population. Nutritional and toxic effects are interrelated phenomena as many compounds are essential nutrients at low concentrations but become toxic at high levels. The inhibitory concentration varies considerably for different compounds (McCarty 1964b, Speece 1983).

The importance of the nutritional requirements of the bacteria participating in the process has long been recognised with initial attention focussed primarily on the major nutrients such as nitrogen and phophorous. Speece and McCarty (1964) determined that the nitrogen requirement for acetate degradation to be approximately 3 kg per 1000 kg COD utilised. For carbohydrate wastes it was higher at about 20 kg per 1000 kg COD consumed and the phosphorous requirement in each case was about 15 % of the nitrogen requirement. However recent evidence suggests that trace amounts of heavy metals and other elements are equally as important for successful digestion (Speece 1983). Such compounds as iron, nickel, cobalt and possibly un-ionised hydrogen sulphide have been identified as critical in optimising fermentation rates and Speece has noted that failure or instability of many

digesters treating industrial wastes may have been soley caused by inadequate nutrition.

Operating conditions may also influence nutrient supply in waste treatment processes. For example, in the activated sludge plants uptake of phosphorous in excess of normal metabolic requirements (termed luxury uptake) has been observed under conditions of alternating oxygen supply and depletion (Levin and Shapiro 1965, Barnard 1983, Marais et al. 1983).

While the methanogens are nutritionally fastidious organisms, some fermentative bacteria have also been shown to have exacting nutritional requirements and may also influence the rate of treatment possible for particular wastes (Iannotti et al. 1981).

The role of several important compounds or classes of compounds in nutrition and toxicity of anaerobic systems is considered in detail in the following sections.

2.5.6.2 Ammonia

Ammonia species (NH_4^+, NH_3) may be present in the influent waste or may be formed from the degradation of organic nitrogenous compounds (McCarty 1964c). The species exist in a dynamic equilibrium and the dominant form depends on the pH. The ammonium ion is dominant below pH 7.2 and is the less toxic species. Early studies suggested inhibition occurs at about 1500 $mg.l^{-1}$ for the cation, especially if the pH was near the top end of the optimal range for methane production, and at about 3000 $mg.l^{-1}$ the ion was toxic regardless of the pH (McCarty 1964c, Melbinger and Donellon 1971). Toxicity was also subject to antagonism and synergism by alkali-earth metal cations. Free ammonia was found to be considerably more toxic and at 150 $mg.l^{-1}$ was severely inhibitory (McCarty and McKinney 1961).

Recent work contradicts some of these findings and suggests that acclimation of the biomass may considerably increase the threshold ammonium inhibition concentration (Anderson et al. 1982). Kroeker et al. (1979) reported staisfactory digestion at up to 7000 $mg.l^{-1}$ and 350 $mg.l^{-1}$ for the ammonium ion and free ammonia respectively and concluded that other factors, such as volatile fatty acid levels, played a more important role in determining methanogenic activity. Van Velsen (1979) has suggested that digesters could acclimate up to 5000 $mg.l^{-1}$ ammonium ion on the results of batch experiments and Parkin et al. (1983) observed only slight inhibition in batch and semi-continuous digesters at 2500 and 4000 $mg.l^{-1}$ ammonium ion respectively. An anaerobic digester also operated successfully at 6000$mg.l^{-1}$ ammonium after a period of acclimation. Anderson et al. (1982) reported rsults of Sathananthan who observed no inhibition at 7000 $mg.l^{-1}$ ammonium at pH 7 but inhibition at 2000 to 3000 $mg.l^{-1}$ at pH 7.5. Free ammonia was toxic above 80 $mg.l^{-1}$ regardless of pH.

2.5.6.3 Sulphur compounds

There are many reports in the literature on the nutritional and toxic effects of various sulphur-containing compounds on methanogenesis. The most important compounds studied are sulphide and sulphate salts, cysteine hydrochloride and sodium thioglycollate. Sulphides and sulphates are very important as they are often present in high concentrations in digester feedstocks or may be formed from other sulphur compounds in the waste by bacterial action. Sulphides, cysteine-hydrochloride and sodium thioglycollate are also important as they are commonly used as reducing agents in media for cultivating strict anaerobes, usually at of 0.05 % (w/v) concentration (Hungate 1969, Willis 1969).

A number of pure culture studies have established the importance of sulphide and cysteine in promoting the optimal growth of

methanogens. Sulphide has been shown to be the major sulphur source for methanogens and cannot be replaced by cysteine, methionine, coenzyme M, sulphate or other sources of sulphur (Bryant et al. 1971, Mountfort and Asher 1979, Ronnow and Gunnarson 1981). In some species growth is only possible if coenzyme M and sulphide are supplied (Balch and Wolfe 1976, Wellinger and Wuhrmann 1977) although cysteine may replace coenzyme M in some instances (Wellinger and Wuhrmann 1977). For Methanobacterium strain AZ cysteine was shown to be an essential amino acid (Wellinger and Wuhrmann 1977) and addition of cysteine in the presence of sulphide, or vice versa, considerably stimulated methanogenic activity (Wellinger and Wuhrmann 1977, Scherer and Sahm 1981). Optimum concentrations reported for sulphide in pure culture range from 0.1 to 3 mM (3.2 to 96 $mg.l^{-1}$) (Wellinger and Wuhrmann 1977, Mountfort and Asher 1979, Ronnow and Gunnarson 1981, Scherer and Sahm 1981) and for cysteine values of 0.5 and 0.85 mM (61 to 104 $mg.l^{-1}$) have been observed (Wellinger and Wuhrmann 1977, Scherer and Sahm 1981). In all cases these reports stress that the compounds were not required as reducing agents but as sources of sulphur or amino acids.

Toxic effects of both sulphide and cysteine have been observed in mixed culture studies. Sulphide is distributed between insoluble, soluble and gaseous chemical states and the concentration of each is dependent on such factors as pH, temperature, digester gas production rate, presence of heavy metals and mode of agitation (McCarty 1964c, Lawrence and McCarty 1965). The soluble chemical species is that which influences methanogenesis and the literature shows some variation on the concentration considered toxic in anaerobic digestion. Studies by Lawrence et al. (1964), Masseli et al. (1967) and Mosey (1971) established that 50 to 100 $mg.l^{-1}$ could be tolerated with little or no acclimation but that concentrations exceeding 200 $mg.l^{-1}$ were toxic. Digesters exposed to toxic concentrations typically exhibited an initial drop in gas production followed by a slow but significant increase in volatile acids which only became

apparent after a marked retardation of gas production had occurred (Lawrence et al. 1964). This was also confirmed by Khan and Trottier (1978) who reported that sulphide did not inhibit production of methane from cellulose up to 5 mM (160 $mg.l^{-1}$) but above this concentration methane production was extremely slow.

Other work suggests that lower slug doses may be toxic to unacclimated digesters. Van den Berg et al. (1976) observed inhibition of methanogenesis by an enrichment culture when cysteine or sulphide were added. Below 1 mM the effects of both were small and temporary but at 2.7 mM (329 $mg.l^{-1}$) cysteine inhibition was marked and sustained, especially in the presence of 1.7 mM (54 $mg.l^{-1}$) sulphide. Concentrations of sulphide alone up to 1.7 mM (54 $mg.l^{-1}$) showed significant temporary inhibition although no adverse long term effects were noted. Parkin et al. (1983) reported inhibiton of batch and semi-continuous digesters by slug doses of 50 and 100 $mg.l^{-1}$ sulphide but acclimation was also shown to occur and levels as high as 400 $mg.l^{-1}$ could be tolerated with no loss of process efficiency.

Measures suggested to remedy inhibition by sulphide include gas scrubbing to remove hydrogen sulphide, dilution of the waste, separation of the sulphur-containing waste stream and the addition of iron to precipitate sulphide (McCarty 1964c, Lawrence et al. 1964, Lawrence and McCarty 1965).

The effect of sulphate on methane formation depends in part on the concentration of other sulphur-containing compounds, particularly sulphide, present in the waste (Khan and Trottier 1978). Sulphate is reduced in anaerobic environments and can therefore satisfy nutritional requirements for sulphide or inhibition can result from the formation of excessive sulphide levels. However other factors are also important. In media containing sulphide or sulphide and cysteine, Patel et al. (1978) and Van den Berg et al. (1980) demonstrated stimulation of methanogenesis by the addition of sulphate which was further

enhanced by iron. It was postulated that sulphate may be incorporated more rapidly into the acetate-degrading methanogens than the sulphide and that at the high growth rates promoted by iron addition, sulphate was required to supplement growth. A major flaw is that sulphate has never been demonstrated as a sulphur source for methanogens (Bryant et al. 1971, Ronnow and Gunnarson 1981).

Sulphate has also been observed to inhibit methanogenesis in aquatic sediments (Cappenberg 1975, Winfrey and Zeikus 1977). This appears to result from competition betwen sulphate-reducing bacteria and methanogens for hydrogen and acetate under the conditions of nutrient limitation found in these environments and may also be due to elevated sulphide levels resulting from the action of the sulphate-reducing bacteria.

Literature on the effects of sodium thioglycollate and thioglycollic acid on anaerobic bacteria appears limited. Malin and Finn (1951) claimed thiogylcollate may be inhibitory to some organisms in the presence of carbohydrate and reported poor growth of several species of Clostridium on a thioglycollate broth. Hungate (1969) suggested inhibition may begin above 0.05 % (w/v) while Willis (1969) indicated thioglycollate could be added to media at 0.2 % (w/v) for anaerobes but cautioned that the compound could become gradually toxic with time and should be prepared fresh before use. In all cases no mechanism for inhibition was suggested.

2.5.6.4 Alkali and alkali-earth metal cations

The nutritive and toxic effects of the alkali and alkali-earth metal cations, viz Na^+, K^+, Ca^{2+} and Mg^{2+} on the activity of microorganisms in the anaerobic digester have been thoroughly investigated (McCarty and McKinney 1961, McCarty 1964c, Kugelmann and McCarty 1964, 1965) and this work was summarised by Kugelmann and Chin (1971). Both slug and daily-feed dose conditions were

studied with single cations, pairs of cations and combinations of three or more ions. Toxicity of the cations studied increased in the following order: sodium < potassium < calcium < magnesium. The upper limits suggested for slug doses are related to the relative toxicities and range from 0.2 M (4600 $mg.l^{-1}$) for sodium to 0.05 M (1215 $mg.l^{-1}$) for magnesium. Acclimation occurred when the cations were continuously introduced into the digesters and the upper limits in this case ranged from 0.3 M (6900 $mg.l^{-1}$) for sodium to 0.065 M (1580 $mg.l^{-1}$) for magnesium.

Toxicity of the cations was greatly affected by the presence of very low concentrations of antagonistic and synergistic cations. Antagonism was associated with the optimal nutritional requirements for each cation and levels of approximately 0.01 M for monovalent and 0.005 M for divalent ions were established. Antagonism by multiple cations was found to be superior to that of individual cations and in some cases stimulation of digesters above the activity of the control was observed. Overall, in the presence of anatagonists, the maximum tolerated dose of light metal cations was increased by 1.5 to 3 times.

A later study by Van den Berg et al. (1976) of the effect of alkali metal cations on acetate degradation by an enriched methanogenic population reached similar conclusions. Inhibition for sodium and potassium salts was noted at approximately 0.15 M in slug doses.

2.5.6.5 Heavy metals

The toxic effects of heavy metals on bacteria and other organisms are well known and several major studies have considered the effects of various metals on anaerobic digestion (Lawrence and McCarty 1965, Mosey and Hughes 1975, Sonada and Seiko 1977). The inhibitory concentrations reported by these and other studies vary considerably but there is agreement that toxicity increases in the following order: iron < cadmium < zinc < chromium < lead

< copper < nickle (Mosey and Hughes 1975, Hayes and Theis 1978). The digester pH is known to affect the tolerance of microorganisms to certain metals, particluarly zinc and cadmium, but usually an effect is only seen in or above the pH range of 7.2 to 7.6 (Mosey and Hughes 1975). Iron is generally recognised as the least toxic of the heavy metals and concentrations of over 1000 $mg.l^{-1}$ have been tolerated without adverse effects (Mosey and Hughes 1975). For this reason iron addition is recommended for controlling sulphide toxicity (Lawrence and McCarty 1965).

It is the ionic concentration of the metal that influences the toxicity (Barth et al. 1965, Hayes and Theis 1978) and Hayes and Theis (1978) also demonstrated that adsorption onto and later incorporation into, digester biomass was another important factor. The most effective control strategies are therefore those that reduce the solubility of the metals and their degree of association with the biomass. The preferred method is to use precipitating ligands, principally sulphide, at as high a pH as tolerable. However care is required as sulphide is itself toxic and other inhibitory effects could result from elevated pH. The sulphide salts of heavy metals are very insoluble and varying sulphide concentrations are usually cited as the cause of reported discrepancies in data regarding heavy metal toxicity in digesters.

However heavy metals are also functional components of many biological molecules and are essential nutrients in trace concentrations. The requirement of methanogens for iron and cobalt was identified over twenty years ago by Speece and McCarty (1964) but the crucial importance of these and other trace metals was not widely appreciated and has only recently been rediscovered. Speece and McCarty (1964) reported good performance with iron added at 103 $mg.l^{-1}$. Other studies in both pure and mixed methanogenic cultures have reported optimum values of 3.68 $mg.l^{-1}$ (Taylor and Pirt 1977), 11 to 112 $mg.l^{-1}$ (Hoban

and Van den Berg 1979), 10 $mg.l^{-1}$ (Speece 1981) and 150 $mg.l^{-1}$ (Kelly and Switzenbaum 1984). In bacteria, iron comprises approximately 0.25 % of the dry cell weight (Luria 1961).

Cobalt stimulation has been observed for Methanobacterium thermoautotrophicum by Schonheit et al. (1979) and in studies of anaerobic digestion of food industry wastes by Murray and Van den Berg (1981) and Kelly and Switzenbaum (1984). In addition requirements of anaerobic bacteria for molybdenum, selenium and tungsten have also been demonstrated (Jones and Stadtman 1977, Schonheit et al. 1979, Murray and Van den Berg 1981). The discovery of the nickel-containing factor F_{430} which appears unique to methanogens has also confirmed the importance of this metal to optimal growth of methanogens (Schonheit et al. 1979, Whitman and Wolfe 1980, Diekert et al. 1981). This is supported by mixed culture studies which have demonstrated dramatic improvements in acetate degradation and methane production rates as a result of nickel addition (Murray and Van den Berg 1981, Speece et al. 1983).

2.5.6.6 Other growth promoting factors

Many organic compounds have been shown to be stimulatory or essential nutrients for growth of methanogens in pure or mixed cultures. Examples are acetate, 2-methyl butyrate, coenzyme M, B vitamins and yeast extract (Bryant et al. 1971, Mah et al. 1978, Balch et al. 1979, Taylor 1982, Speece 1983). In studies of both methanogenic and fermentative bacteria additions of rumen fluid or supernatant digester fluid to the growth medium have significantly increased growth and recovery of the organisms (Bryant et al. 1971, Balch et al. 1979, Iannotti et al. 1981). The growth promoting agents present in these supplements include short-chain volatile fatty acids but not all the factors have yet been identified (Iannotti et al. 1981, 1982b; Taylor 1982). This is also true of the stimulatory action of yeast extract. Stimulation is undoubtably due in part to the vitamins and other

organic compounds contained in the extract but Mah et al. (1978) also demonstrated that yeast extract ash could be almost as stimulatory as the yeast extract itself. Yeast extract composition has been reported by Grant and Pramer (1962), BBL (1968), Pappelis and Schmid (1965) and Oxoid (1969).

Lysed cell material has also proved highly stimulatory to acetate degradation in mixed culture (Novak and Ramesh 1975) and reinoculation of digesters with active sludge has also been reported as helpful for maintaining optimum performance in digesters by Stander and Snyders (1950), Stander _ (1950) and McCarty and Vath (1963).

2.5.6.7 Other toxic compounds

Numerous studies of the toxic effects of a wide range of compounds are reported in the literature. Compounds studied include synthetic detergents (Swanwick et al. 1968), a wide variety of petrochemicals (Chou et al. 1978a, 1978b) and pharmaceuticals commonly added as supplements to animal feeds (Varel and Hashimoto 1982). Possibly the most significant result to emerge from these studies is that the toxic compounds generally exert a bacteriostatic or reversible growth inhibitory effect on the bacteria and significant acclimation can occur. Thus, providing safeguards are built into the design and operation of plants with the potential to receive toxic wastes and a suitably adapted population is used, anaerobic digestion remains an appropriate waste treatment process (Speece 1983, Parkin et al. 1983).

2.5.7 Fermentation intermediates and end-products

2.5.7.1 Introduction

The major fermentation intermediates are hydrogen, carbon dioxide, methane and the volatile fatty acids. Of these the

importance of hydrogen has already been described and methane apparently has no effect on the process (Hansson 1979, 1982). The remaining compounds are known to effect the methane fermentation and their roles are considered in the following sections.

2.5.7.2 Carbon dioxide

Carbon dioxide has long been recognised as inhibitory to many microorganisms at high concentrations (Valley and Retger 1927) and several studies on the effect of carbon dioxide on anaerobic digestion appear in the literature. The early studies of Keefer and Kratz (1933) and Hartz and Kountze (1966) were inconclusive because of inadequate control of the digester environment. Ort (1976) claimed that operating digesters at elevated pressures increased the availability of carbon dioxide as a hydrogen acceptor for methane production and patented a process to that effect. Similarly, Mangel et al. (1980) reported effectively constant gas production from digesters operated from 0 to 4 bar gauge pressure.

Hansson (1979) observed the effect of high and low carbon dioxide partial pressures on anaerobic digestion. These conditions were achieved by sparging oxygen-free nitrogen or carbon dioxide through a 2.5 l digester at 2 $l.hr^{-1}$. A semi-synthetic medium was used at 6 day retention time and a loading rate of approximately 3 g $glucose.l^{-1}.day^{-1}$.

In an initial experiment carbon dioxide sparging resulted in a sharp decrease in methane production which finally stabilised at about 30 % of the original rate. There was some adaption to the unfavourable condition but gas production did not increase again when gas sparging ceased. There was a distinct linear increase in acetate concentration during sparging to about 4000 $mg.l^{-1}$.

In a second experiment the carbon dioxide partial pressure (pCO_2) was changed rapidly by alternate nitrogen and carbon dioxide sparging. Methane production decreased again at high pCO_2 but could be restored again on nitrogen circulation. Rapid increases in the acetate concentration were also observed during carbon dioxide sparging but the propionate concentration remained constant throughout the experiments and the butyrate concentration was always low, although it clearly increased during periods of carbon dioxide sparging. The abrupt nature of the changes in methane production during alternate gas sparging suggested that carbon dioxide was directly inhibitory to the methanogens rather than causing a gradual accumulation of some toxic product or the washout of cells.

In a third experiment the effects of a 19 day period of high pCO_2 were examined. In this case adaption to the change in carbon dioxide level was apparent with a decrease in the usual accumulation of acetate. The acid concentration eventually stabilised at 800 $mg.l^{-1}$ and the concentration of butyrate was also very low. Methane production was clearly inhibited by the high pCO_2 being 33 % lower than that during nitrogen sparging.

Hansson and Molin (1981a, 1981b) further investigated the effect of pCO_2 on the various groups of bacteria involved in the methane fermentation. Working mainly with batch mesophilic enrichment cultures they demonstrated that carbon dioxide strongly inhibited acetate utilisation and reduced the yield of methane from acetate. At pCO_2 of 0 bar, acetate was degraded at an average rate of 1300 $mg.l^{-1}.day^{-1}$ and the yield was close to the theoretical value. At 1 bar pCO_2 acetate utilisation had dropped to 350 $mg.l^{-1}.day^{-1}$ and the yield was reduced by 20 to 30 %. Butyrate degradation was not inhibited by carbon dioxide but propionate degradation was reduced at both high and low pCO_2. An optimum pCO_2 of 0.2 bar was established for both propionate degradation and methane production. Glucose degradation was extremely fast and although slightly inhibited by carbon dioxide

was not considered rate-limiting. The major acid products of glucose degradation were acetate and propionate with a slight increase in the amount of butyrate formed evident at high pCO_2 conditions.

2.5.7.3 Volatile fatty acids (VFA)

The volatile fatty acid concentration in a digester is a useful measure of fermentation efficiency as it reflects the sum of all environmental factors acting on the bacteria mediating the process (McCarty 1964a). Low acid levels are indicative of stable operation while high acid levels are invariably associated with digester failure (Pohland and Bloodgood 1963). This is not to say that digesters operating with high acid levels will necessarily fail, as there are natural variations in the steady-state VFA concentration reflecting differences in reactor design and operation, substrates utilised and the composition of the microbial flora. The relative proportions of individual acids fluctuate with changes in the digester environment. Generally acetate is the most resistant to such changes while higher acids, and particularly propionate, are most sensitive. There are numerous reports in the literature detailing elevated concentration of C3 to C5 acids as indicative of digester failure (e.g see Andrews and Pearson 1965, Hobson et al. 1974, 1981; Speece 1981, 1983, Asinaro di san Marzano et al. 1981, Dohanyos et al. 1985) although similar increases in the rate of accumulation of propionate and acetate prior to failure have also been noted (e.g. see McCarty et al. 1963, Cohen et al. 1980).

There have been two schools of thought addressing the problem of toxicity of volatile acids to anaerobic digestion. Buswell and co-workers (1936, 1963) suggested that VFA levels of greater than 2000 to 3000 $mg.l^{-1}$ as acetate were toxic even at neutral pH and that propionic acid was more toxic than the other acids. In contrast McCarty and Brousseau (1963) and McCarty et al. (1963) demonstrated that additions of acetic, propionic and butyric

acids, individually and in combination, at concentrations of up to 8000 $mg.l^{-1}$ could be tolerated if pH and alkalinity were maintained at optimum levels. Successful digestion at high acid concentrations has also been reported by Van den Berg et al. (1976).

The current view combines elements of both theories. There is now strong evidence from both rumen and digester studies to support the view that un-ionised acids are more toxic to microorganisms than ionised acids (e.g. see Andrews 1968, Wolin 1969, Kroeker et al. 1979). As the un-ionised acid concentration increases with decreasing pH, both pH and total acid concentration are important in determining inhibition and this reinforces the need to maintain adequate alkalinity to buffer the pH (McCarty and Brousseau 1963, Pohland and Bloodgood 1963). The level of un-ionised acid at which inhibition occurs has been estimated as 10 to 25 $mg.l^{-1}$ (as acetate) by Duarte and Anderson (1982) and 30 to 60 $mg.l^{-1}$ by Kroeker et al. (1979). However exceptions to these figures have been reported; for example Clarke and Speece (1970) succesfully operated an anaerobic filter at lowered pH where these concentrations would have been exceeded.

There is also strong evidence that propionic acid is more inhibitory than other acids. Buswell and Morgan (1963) observed the effect of C1 to C5 acids on digestion and concluded that propionic acid was toxic and resulted in digester failure. McCarty and Brosseau (1963) observed that addition of 3000, 6000 and 8000 $mg.l^{-1}$ propionate to a sewage sludge digester initially decreased gas production for one to five days. In contrast addition of acetate and butyrate stimulated gas production. The effect of propionate addition was greater at the higher concentrations but the acid was eventually degraded at a rapid rate. Similarly Andrews (1968) reported a reduction of 50 % in the rate of methane production when 3200 $mg.l^{-1}$ propionate was added to a continuous digester. Hobson and Shaw (1976) added

varying amounts of acetate, propionate and butyrate to pure cultures of Methanobacterium formicicum. Up to 10 000 mg.l^{-1} of acetic and butyric acids did not inhibit methane production but inhibition by propionate was marked at 1000 mg.l^{-1}.

In addition, in many studies propionate degradation was observed to be slower than degradation of other acids (Schultze and Raju 1958, McCarty and Brouseau 1963, Andrews and Pearson 1965, Winter and Cooney 1980, Cohen et al. 1982, Duarte and Anderson 1982). Also there are several reports in the literature of no or very slow utilisation of propionate after a digester upset despite rapid utilisation of other acids (Andrews and Pearson 1965, Cohen et al. 1980, Asinaro di san Marzano et al. 1981, Duarte and Anderson 1982). These reports suggest that the integration of the propionate- degrading organisms in the overall fermentation scheme is the most critical requirement for successful digestion. It is also possible that the un-ionised propionate concentration may significantly influence the process and clearly there is still an urgent need for a more systematic study of the role of the volatile acids in anaerobic digestion.

Some evidence of product inhibition of propionate degradation by acetate has also been presented. Kaspar and Wuhrmann (1978b) reported that 40 mM calcium acetate (4800 mg acetate.l^{-1}) severely reduced propionate degradation in a mesophilic sewage sludge digester while addition of 40 mM calcium chloride or 4 mM calcium acetate (480 mg acetate.l^{-1}) had no significant effect. In a pure culture study, 15 mM sodium acetate (900 mg.l^{-1}) was shown to inhibit propionate degradation by an acetogenic bacteria by 15 % (Boone and Bryant 1980). Winter and Cooney (1980) added acetate, propionate and butyrate to a digester fed with cellulose and their results suggested that acetate and possibly butyrate affected the rate of utilisation of propionate. Zehnder and Koch (1983) also observed that 10 to 15 mM (600 to 900 mg.l^{-1}) acetate slowed degradation of 10 mM (740 mg.l^{-1}) propionate. There is thus sufficient evidence to suggest product inhibition of

propionate utilisation but again a systematic study is required to clarify the effect.

2.6 SUMMARY

The anaerobic digestion literature in recent years shows a rapid broadening and deepening of knowledge. This may in part be attributed to the development of both a "biotechnological", multi-disciplinary approach to problem solving (Cohen 1981) and , as Speece (1983) has noted, "a rather well balanced effort in the areas of basic microbiology, bench and pilot plant studies and full-scale installation evaluations".

Fundamental microbiological and biochemical studies have elucidated important mechanisms of the fermentation and have provided a substantial knowledge base for the operation of large-scale plants. In particular the recognition of the importance of hydrogen and the interrelationship of the hydrogen-producing and hydrogen-consuming bacteria in the process has contributed significantly to enhanced process control. Results of kinetic studies are now showing some cohesion and general agreement has been reached on the rate-limiting steps for degradation of soluble and chemically-complex wastes. Significant improvements in digestion rates have resulted from the recognition of the exacting nutritional requirements of some bacteria participating in the fermentation. Major advances have also been made in understanding the reversible nature of much toxicity, allowing the development of control measures to enhance the stability of digesters treating industrial wastes. Consequently new generation reactors have been developed and the range of wastes suitable for treatment greatly extended. Many of the new reactor designs have proved successful in lab and pilot plant studies and, especially the UASB reactor, are now increasingly acceptable for large scale waste treatment.

However challenges to our understanding still remain. The rate and stability of the methane fermentation have a marked influence on industry's acceptance of the process as a viable, economic waste treatment option. Anaerobic digestion has suffered in the past from a widespread perception of being difficult to control and therefore to be avoided. Further elucidation of the complex interactions of the various bacterial groups and the effect on these of manipulating the digester environment is required to combat this notion. This project was initiated (in 1979) to address this need and at that time the role of fermentation end-products and intermediates in limiting the rate of the fermentation, and the effect of the mode of operation (semi-continuous or continuous) on digester performance, were identified as important areas requiring further research. The results of the work performed are now reported.

CHAPTER THREE MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 General chemicals

Sodium thioglycollate and yeast extract were obtained from Difco Laboratories (Detroit, Michigan, U.S.A.). Food-grade sodium bicarbonate as used for pH control in the digesters was obtained from the National Dairy Association (Palmerston North, N.Z.). All other chemicals were obtained from B.D.H. Chemicals N.Z. Ltd (Palmerston North, N.Z.) or May and Baker N.Z. Ltd (Lower Hutt, N.Z.) and were of analytical reagent grade unless otherwise specified.

3.1.2 Gases

Bottles of methane, carbon dioxide and nitrogen of 99 % purity were obtained from New Zealand Industrial Gases Ltd (Palmerston North, N.Z.). Certified gas mixtures containing 20 %, 50 % and 80 % carbon dioxide in nitrogen were obtained from N.Z.I.G. Ltd (Petone, N.Z.). All gases were passed over heated copper at 320 ± 20 °C to remove traces of oxygen before being introduced into the digesters.

3.1.3 Chromatography materials

Free Fatty Acid Phase (FFAP) and Chromosorb G (100-120 mesh, AW-DMCS) were obtained from Varian Associates Inc. (Walnut Creek, California, U.S.A.). Poropak S (80-100 mesh) was obtained from Waters Associates Inc. (Milford, Massachusetts, U.S.A.).

3.1.4 Microbial growth medium

The growth medium used throughout this study for continuous and semi-continuous experiments was that of Hansson (1979) modified by the addition of resazurin as a redox indicator at 0.0001 % (w/v). This is a semi-synthetic medium containing glucose as the major carbon and energy source, yeast extract and mineral salts (Table 3.1). The medium is of high strength; the average COD was determined as 21,700 $mg.l^{-1}$. The components were dissolved in distilled water and autoclaved for 15 min at 121 °C in three parts: glucose solution, phosphates solution and the remainder. These were aseptically combined after sterilisation to give appropriate final concentrations. The medium was prepared in 2 l quantities and dispensed in 4 l flasks at normal strength. The medium was stored at 4 °C until required and was always used within one week of preparation. Here-after this modified medium will be called the standard growth medium.

Where indicated in the text, the standard growth medium was supplemented with cysteine-hydrochloride (0.025 or 0.05 % w/v final concentration) and/or sodium sulphide (0.025 or 0.05 % w/v final concentration) or vitamin solution (10 ml/l final concentration). The composition of the vitamin solution is shown in Table 3.2. Both reducing agent and vitamin solutions were prepared when required and filter-sterilised prior to aseptic addition to the growth medium.

Three substrates were used in the batch digestion experiments reported in Chapters Five and Six: standard growth medium, acetic acid and propionic acid. Detail of amounts added is given in the experimental method sections of these chapters.

Table 3.1: Composition of growth medium used in continuous and semi-continuous digestion experiments (after Hansson 1979).

Component	Concentration	
glucose	16	$g.l^{-1}$
yeast extract (Difco)	4	
NH_4Cl	2	
$Na_2HPO_4.2H_2O$	0.3	
KH_2PO_4	0.2	
$MgSO_4$	0.2	
$FeCl_3.6H_2O$	0.3	$mg.l^{-1}$
$CaCl_2.2H_2O$	0.33	
$ZnSO_4.7H_2O$	0.09	
$CoCl_2.6H_2O$	0.09	
$CuSO_4.5H_2O$	0.07	
$MnSO_4.4H_2O$	0.07	
resazurin	1	

Table 3.2: Composition of the vitamin solution used to supplement the standard growth medium.

Component	Concentration $mg.l^{-1}$
biotin	0.2
calcium pantothenate	40
pteroylglutamic acid (folic acid)	0.2
inositol	200
niacinamide	40
p-aminobenzoic acid	20
pyridoxine hydrochloride	40
riboflavin mononucleotide	20
thiamine hydrochloride	40

3.1.5 Glassware

Standard laboratory glassware obtained from commercial sources was used throughout this work. All glassware was routinely washed in hot water containing "Pyroneg" (Diversey-Wallace Ltd, Auckland, N.Z.), rinsed three times with distilled water and air-dried at about 50 °C.

3.2 ANALYTICAL PROCEDURES

3.2.1 Introduction

For continuous and semi-continuous experiments routine analyses were made of pH, volatile fatty acids, chemical and biological oxygen demand and solids composition of both the growth medium

and digester effluent samples. The gas phase composition was also routinely determined. These tests were performed three or four times per week and at least six determinations were made during one residence time at steady-state conditions. This condition was deemed to be reached when stable performance was observed for one retention time after two retention times had elapsed since a major change in operating conditions. As the above tests were used as indicators of digester stability and efficiency, selected performance characteristics were estimated for each procedure following the methodology of Cheeseman and Wilson (1978) and are reported below.

Alkalinity, ammonia nitrogen, dissolved sulphide and glucose concentrations and redox potential were measured but not on a routine basis.

Analyses performed on samples from batch digesters are described in the experimental method sections of Chapters Five and Six.

3.2.2 pH value

The pH of digester samples was measured using an E.I.L. model 7055 pH meter (Electronic Instruments Ltd, Chertsey, Surrey, England) equipped with manual temperature compensation and an E.I.L. series 1160 combination pH electrode. The electrode was calibrated twice weekly with standard commercial buffer solutions of pH 4.0 and pH 7.0 and pH values were recorded to the nearest 0.05 unit.

3.2.3 Volatile fatty acids (VFA) by gas chromatography

Volatile fatty acids were determined following the chromatographic procedure of Banfield et al. (1978). A Shimadzu GC-5A gas chromatograph (Shimadzu Seisakusho Ltd, Kyoto, Japan) fitted with a flame ionisation detector operated at 185 °C was used with a 2 m x 3 mm i.d. glass column packed with 10 % w/v

FFAP on Chromosorb G. The column temperature was 145 °C and the injector port temperature was 185 °C. The nitrogen flowrate was 80 $ml.min^{-1}$ and the air and hydrogen flowrates were 900 and 55 $ml.min^{-1}$ respectively. Digester effluent samples were centrifuged at 2700 x g for 10 min and 1 ml of formic acid (99.9 %) was added to 10 ml of the supernatant liquor. The sample volume of 1 µl was drawn from this solution and was delivered using an S.G.E. type 1B syringe (Scientific Glass Engineering Pty Ltd, Melbourne, Australia).

A Varian Chromatography Data System CDS 111 was used to evaluate the peak areas. A calibration curve was established from replicate determinations of a blank standard containing 1 ml formic acid in 10 ml distilled water and two standard acid solutions the composition of which are shown in Table 3.3. The stock solutions of these standards were prepared by accurately weighing and dispensing appropriate volumes of the individual AR grade acids. The stock solutions were stored at 4 °C and were replaced every three months. Calibration samples were drawn from this at weekly intervals and were also stored at 4 °C when not in use.

Precision and accuracy data for the procedure are listed in Tables 3.4 and 3.5. Precision was acceptable although decreased at low concentrations and good recovery was obtained from spikes of the three major acids; acetate, propionate and butyrate. These data compare favourably with the published method (Banfield et al. 1978). Limits of detection were not evaluated but were conservatively estimated at 10 $mg.l^{-1}$ and all concentrations reported were rounded to the nearest 10 $mg.l^{-1}$.

The total VFA concentration (TVFA) expressed as $mg.l^{-1}$ acetate was calculated as follows: the individual acid concentrations were multiplied by appropriate factors which related the COD of the acid to that of acetate, and these modified concentrations were then summed to give the TVFA concentration. Further details

of the procedure and values of the conversion factors appear in Appendix One.

Table 3.3: Composition of volatile fatty acid standard solutions.

Acid	Concentration	
	low standard $mg.l^{-1}$	high standard $mg.l^{-1}$
acetic	500	2000
propionic	500	2000
i-butyric	100	500
butyric	500	2000
i-valeric	100	500
valeric	200	1000

Table 3.4: Estimation of the precision of volatile fatty acid concentration measurement.

Acid	Sample	Mean concentration[a] $mg.l^{-1}$	Standard deviation[a] $mg.l^{-1}$	Relative s.d.[b] %
acetic	1	1040	26.3	2.5
	2	71.4	9.25	13
	low std	500	21.0	4.2
	high std	2000	29.3	1.5
propionic	1	421	17.1	4.1
	2	149	12.9	8.7
	low std	500	17.3	3.5
	high std	2000	43.3	2.2
i-butyric	1	49.2	11.5	23
	2	n.d.[c]	-	-
	low std	100	11.3	11
	high std	500	17.6	3.5
butyric	1	33.6	9.82	29
	2	n.d.	-	-
	low std	500	17.6	3.5
	high std	2000	42.8	2.1
i-valeric	1	31.8	11.9	30
	2	n.d.	-	-
	low std	100	11.9	12
	high std	500	12.8	2.6
valeric	1	n.d.	-	-
	2	n.d.	-	-
	low std	200	17.6	8.8
	high std	1000	35.3	3.5

a six measurements were made for each sample
b relative standard deviation = s.d. x 100/ mean concentration
c not detected

Table 3.5: Estimation of the accuracy of volatile fatty acid concentration measurement by gas chromatography.

Acid	Mean percent recovery of 300 $mg.l^{-1}$ spike[a]	Mean percent recovery of 3000 $mg.l^{-1}$ spike[a]
acetic	92	101
propionic	103	98
butyric	98	103

a six determinations were made for each sample

3.2.4 TVFA by direct titration

For a short period at the start of the first continuous digestion experiment the Shimadzu gas chromatograph described above was unavailable. During this period the TVFA concentration was estimated by direct titration of digester liquor samples following the method of DiLallo and Albertson (1963). Fifty ml of digester liquor was titrated to pH 3.3 with 0.1 N hydrochloric acid and boiled for 3 min to remove carbon dioxide. The sample was then titrated to pH 7.0 with 0.05 N sodium hydroxide and the titre value required to change the pH from 4.0 to 7.0 noted. The TVFA concentration was calculated from:

$$TVFA = (ml\ 0.05\ N\ NaOH \times 2500)/(ml\ sample) \qquad (E3.1)$$

3.2.5 Chemical oxygen demand (COD)

The chemical oxygen demand of samples was determined by the micro-technique of Jirka and Carter (1975). Total COD of both influent and effluent samples were measured and the COD of supernatant liquor from effluent samples centrifuged at 2700 x g for 10 min was also determined. This provided an estimate of the soluble COD and the approximate COD of biomass was determined from the difference of the total and soluble COD measurements.

The method follows the Standard Methods procedure (APHA-AWWA-WPCF, 1975; pg. 550); reagents and reagent concentrations remain the same but sample and reagent volumes are reduced by a factor of 25. One ml of potassium dichromate solution and 3 ml of sulphuric acid reagent were added to 2 ml of sample in a 16 mm x 100 mm screw-capped culture vial (Hach Chemical Co., Ames, Iowa, U.S.A.). The solution was refluxed at 140 °C for 2 hr and then the absorbance of the mixture was determined colorimetrically at 600 nm using an Hitachi model 101 spectrophotometer (Hitachi Ltd, Tokyo, Japan). Silver sulphate was added to the acid to ensure effective oxidation of straight-chain acids and excess mercuric sulphate was also added to samples as the chlorine content of the influent medium was high (APHA-AWWA-WPCF, 1975; pg. 551).

All samples were run in duplicate and distilled water blanks were included with each batch of analyses. The average blank reading was subtracted from the sample values and the COD determined from a previously prepared calibration curve. This was established using five potassium hydrogen pthalate standard solutions also following the method of Jirka and Carter (1975). The curve was linear in the range 0 - 750 $mg.l^{-1}$ COD and samples were diluted to bring the sample reading within this range. A regression coefficient of 0.9999 was calculated for the linear part of the curve and the COD was calculated from :

$$COD = \text{Absorbance at } 600 \text{ nm} \times 3283 \qquad (E3.2)$$

Replicates of typical influent and effluent samples were run to estimate the precision of the method and two recovery tests using potassium hydrogen pthalate, each comprising five replicates, were made to estimate the accuracy. Results of these analyses are shown in Table 3.6 and are very similar to those of Jirka and Carter (1975). After extensive testing they found the precision of the method to be as good as the Standard Methods procedure at low COD values and at high values the precision of the micro method was higher. This was attributed to improved recovery of volatile components through the use of capped tubes and this is claimed as a major advantage of the method.

3.2.6 Biochemical oxygen demand (BOD)

The biochemical oxygen demand of influent and effluent samples from all digesters was measured using Hach manometric apparatus following the method of Tool (1967). The BOD of duplicate samples was measured over the standard five-day period with samples incubated at 20 °C. The Hach BOD apparatus combines the operating features of the Sierp and Warburg methods and has the advantage over the standard dilution technique (APHA-AWWA-WPCF, 1975; pg. 543) of providing a continuous reading and using a larger sample, with the expectation of obtaining more representative results. While a manometric procedure is not included in Standard Methods (APHA-AWWA-WPCF, 1975), Tool (1967) reported that the method yielded results within 5 % of those using the standard dilution method.

Precision data for three effluent samples of varying BOD were estimated and the results appear in Table 3.7.

Table 3.6: Performance characteristics for chemical oxygen demand measurement.

Sample	Dilution factor	Precision data			Accuracy data
		Mean concentration[a]	Standard deviation[a]	Relative s.d.[b]	Mean percent recovery of 1000 $mg.l^{-1}$ spike[c]
		$mg.l^{-1}$	$mg.l^{-1}$	%	%
1	50	97.8	7.12	7.3	112
2	10	567	10.2	1.8	95

a six measurements were made for each sample
b relative stanadard deviation = s.d. x 100/ mean concentration
c five measurements were made for each sample

Table 3.7: Estimation of the precision of biological oxygen demand measurement.

Sample	Dilution factor	Mean concentration[a] $mg.l^{-1}$	Standard deviation[a] $mg.l^{-1}$	Relative s.d.[b] %
1	10	60.8	5.10	8.4
2	4	92.8	5.00	5.4
3	40	182	15.1	8.3

a six determinations were made for each sample

b relative standard deviation = s.d x 100/ mean concentration

3.2.7 Solids composition

Samples were analysed for the following:

(1) total solids at 103 °C,

(2) volatile and fixed component of total solids at 550 °C,

(3) filterable solids at 103 °C, and

(4) volatile and fixed component of filterable solids at 550 °C.

The first two tests were performed following Standard Methods (APHA-AWWA-WPCF, 1975; pp. 91 + 95) using 10 ml sample volumes. Modified Standard Methods tests were performed for the other two tests as the samples could not be easily filtered. Ten ml samples were centrifuged at 2700 x g for 10 min and the supernatant liquor was filtered using glass-fibre filter paper (GF/C, Whatman Ltd, Maidstone, Kent, England). The treated

samples were dried at 103 °C overnight to determine filterable solids and then ashed for 2 hr at 550 °C to determine the volatile and fixed solids composition. Suspended solids (non-filterable residue) and volatile suspended solids were determined from the difference of total and filterable solids data.

Precision of the solids tests was estimated by six replicate analyses of digester effluent. Each sample was divided into two parts for total and filterable solids determinations and the results are shown in Table 3.8. Standard Methods (APHA-AWWA-WPCF, 1975; pg. 98) quotes precision at three solids concentrations for an unspecified sample. At the highest concentration of 1707 $mg.l^{-1}$ the standard deviation of 40 analyses was 17 $mg.l^{-1}$. The coefficient of variation of 0.76 % was much lower than those obtained in this work. However considering the complex nature of the digester effluent and the small number of samples processed, the precision obtained appeared satisfactory (refer Table 3.8).

Table 3.8: Estimation of the precision of solids composition measurement.

Parameter	Mean concentration[a] $g.l^{-1}$	Standard deviation $g.l^{-1}$	Relative s.d.[b] %
total solids	4.38	0.14	3.2
volatile	2.06	0.06	2.9
fixed	2.32	0.11	4.7
filterable solids	2.00	0.06	3.0
volatile	1.07	0.08	7.5
suspended solids	2.38	0.19	8.0
volatile	0.99	0.1	10

a six determinations were made of an effluent sample from CDR2

b relative standard deviation = s.d. x 100/ mean concentration

3.2.8 Gas composition

Gas produced by the digesters was routinely analysed by gas chromatography for methane content. Carbon dioxide concentration was measured less frequently to ensure that methane and carbon dioxide represented the only significant components of the gas phase. Hydrogen could also be determined but was never detected in samples under normal operating conditions.

A Varian Aerograph model 920 gas chromatograph was used. This was fitted with a thermal conductivity detector and connected to a Sargent model SRG flat-bed recorder (E.H. Sargent Ltd, England). The 1.5 m x 6 mm i.d. stainless steel column was packed with Poropak S and operated at 75 °C. The detector temperature was 125 °C and the katharometer bridge current 120 mA. The carrier gas was nitrogen at 55 $ml.min^{-1}$ with a reference gas flowrate of 11 $ml.min^{-1}$. The sample volume was either 100, 200 or 500 µl and delivered by a gas-tight syringe (Types 1710RN and 1750RN, Hamilton Co., Reno, Nevada, U.S.A.).

Calibration curves for methane and carbon dioxide were established using peak heights obtained by injecting varying volumes of the pure gases. Usually four volumes of each gas were injected in duplicate. The calibration curve for methane was linear but for carbon dioxide the curve fell off markedly above 50 % (v/v). It was possible to construct a linear calibration curve for carbon dioxide but the sample volumes were so low that accuracy was very poor. This problem has been noted by other workers (Reddy et al. 1972, Rose and Pirt 1981) and could be overcome by using helium or hydrogen as the carrier gas as these have a much higher thermal conductivity than carbon dioxide (Mosey et al. 1978).

The carbon dioxide concentration was usually calculated from the difference between total gas and methane production. Separate carbon dioxide analyses were made once or twice per month to check the accuracy of these calculations and satisfactory agreement was always obtained. For ten separate, duplicate analyses of carbon dioxide and methane the mean combined total composition was 99.1 % with a standard deviation of 3 %. Assuming an average gas temperature of 20 °C, the water vapour content would be 2.3 % (Mosey et al. 1978) and therefore the gases should comprise 97.7 % of the total volume. Precision data and limits of detection were also estimated and are listed in

Table 3.9. All these data are comparable to the values obtained by Mosey et al. (1978).

3.2.9 Alkalinity

Alkalinity of 25 ml digester effluent samples was determined by potentiometric titration to pH 4.0 and pH 3.7 with 0.1 N hydrochloric acid (APHA-AWWA-WPCF, 1975; pg. 238). The end-point of pH 3.7 was that recommended by Standard Methods for complex wastes and the end-point of pH 4.0 was useful as it allowed the bicarbonate alkalinity to be calculated using the formula of McCarty (1964b).

3.2.10 Ammonia nitrogen

Total ammonia nitrogen concentration was measured by an E.I.L. model 8002-8 ammonia probe connected to an E.I.L. model 7055 pH meter operating on a direct-reading concentration scale. Effluent samples were centrifuged at 2700 x g for 10 min and 1 ml of 1.0 M sodium hydroxide added to 10 ml of supernatant liquor. The probe was calibrated using 0.1 and 0.01 M ammonium chloride standards which were prepared fresh from a 1.0 M solution. This stock solution was prepared every second week and stored at 4 °C. Probe performance was always within the manufacturers guidelines and the response time was about 2 min. The membrane was replaced and new filling solution added when the slope fell below 95 % of the optimum value of 58 mV per decade change in concentration.

3.2.11 Dissolved sulphide

Dissolved sulphide concentration was determined using the standard titrimetric method (APHA-AWWA-WPCF, 1975; pg. 501) following separation of soluble and insoluble sulphides and pretreatment to remove interfering compounds and to concentrate the sample.

Table 3.9: Performance characteristics of gas composition measurement.

Gas	Sample size	Volume of gas injected	Equivalent composition	Mean peak height	Standard deviation	Relative s.d.[a]	Limit of detection[b]
	µl	µl	% (v/v)	chart div.	chart div.	%	% (v/v)
Methane	500	500	100	85.7[c]	1.18	1.4	0.02
		400	80	71.7	0.66	0.9	
		300	60	55.3	1.14	2.1	
		200	40	36.8	1.59	4.3	
		100	20	19.5	0.80	4.1	
Carbon dioxide	50	50	100	67.9[c]	0.50	0.7	0.4
		40	80	65.0	0.55	0.8	
		30	60	59.4	0.80	1.4	
		20	40	49.7	0.90	1.8	
		10	20	25.7	1.73	6.7	
Hydrogen	500	1	0.2	22.4[d]	0.55	2.5	0.009

a relative standard deviation = s.d. x 100/ mean concentration
b calculation assumed the peak height was proportional to concentration and attenuation, and that the minimum detectable peak height was one chart division
c ten measurements were made for methane and carbon dioxide sample
d three measurements were made for the hydrogen sample

3.2.12 Glucose

Glucose concentration in digester liquor samples was determined using a Y.S.I. model 27 industrial sugar analyser (Yellow Springs Instrument Co. Inc., Yellow Springs, Ohio, U.S.A.) following the procedures recommended by the manufacturer.

3.2.13 Oxidation-reduction potential (ORP)

An E.I.L. series 1213 platinum electrode in combination with a series 1320 calomel reference probe was used at selected times for redox potential measurement. The platinum electrode was cleaned using the method described by Mountford and Asher (1979) and calibrated twice weekly using saturated quinhydrone buffer solutions (Radiometer 1966).

3.3 CONTINUOUS DIGESTION EXPERIMENTS

3.3.1 Equipment and instrumentation

A fermenter designed and constructed in the Biotechnology Department workshop was operated as a continuous stirred tank digester. The apparatus is illustrated in Figure 3.1. A 7 l pot fitted with a 10 m.m i.d. angled side arm was used and provided a working volume of 4.1 l. The glass pot and stainless steel headplate were obtained from New Brunswick Scientific Co. Ltd (New Brunswick, New Jersey, U.S.A.). The temperature was controlled at 37 ± 1 °C and the digester was continually stirred at 200 r.p.m..

Fresh medium was pumped into the digester using a Masterflex peristaltic pump incorporating a variable-speed drive (model 7546-00) fitted with interchangeable pump heads and silicone rubber tubing (Cole-Palmer International, Chicago, Illinois, U.S.A.). When operating at long retention times the flow was often too low to run the pumps continuously even using the

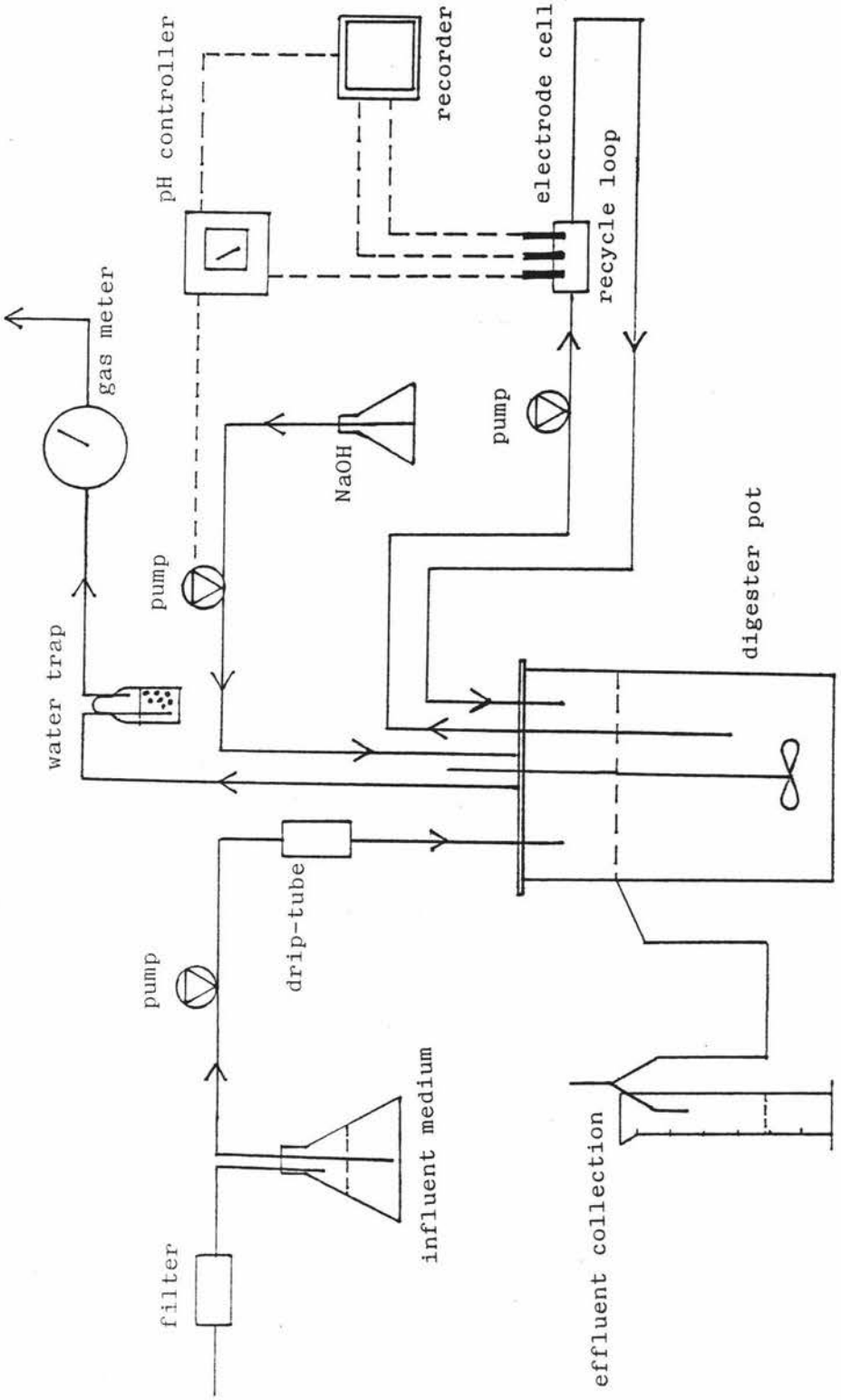


Figure 3.1: Experimental set-up for the continuous digestion experiments.

smallest diameter tubing available. In this case pump operation was interrupted for an appropriate period during a 15 min cycle using a camswitch. A drip-tube was incorporated into the feed line to prevent contamination of the feed reservoir and the fermenter was wrapped in aluminium foil to discourage wall growth of photosynthetic organisms. Effluent overflowed through the sidearm and was collected in a 250 ml measuring cylinder at ambient temperature.

The evolved gas was removed through thick-wall butyl rubber tubing to a condensate trap and a wet-type laboratory gas meter (model DM3A, Alexander Wright and Co. Ltd, London, England). The gas then bubbled through a water trap to preclude the entry of atmospheric oxygen and to maintain a slight positive pressure in the digester.

A second Masterflex pump recirculated digester liquor through an electrode cell construced in the Biotechnology Department workshop. The cell contained an E.I.L. series 1160 combination pH electrode connected to an Horizon pH controller (model 5997-20, Horizon Ecology Co., Chicago, Illinois, U.S.A.). One-way pH control at pH 7.0 was achieved by the addition of 4.0 N sodium hydroxide by a Masterflex fixed speed pump (1 r.p.m., model 7544-01). The electrode cell also contained the probes for redox potential measurement (see Section 3.2.13).

Digester temperature was measured by a copper-constantan thermocouple and, together with pH and redox potential, was recorded on a Honeywell Versaprint type 121-1 multipoint recorder (Honeywell S.A., Amiens, France).

3.3.2 Operating conditions

Cow manure and digested sewage sludge provided the original inocula for these experiments. Inoculum preparation, digester

start-up and sampling procedures and details of the experimental programme are described in Chapter Four.

3.4 SEMI-CONTINUOUS DIGESTION EXPERIMENTS

3.4.1 Equipment and instrumentation

3.4.1.1 Microferm digester

This unit comprised one side of a New Brunswick double-unit laboratory fermenter (model MF-214F) and is illusrated in Figure 3.2. The digester was equipped with a 14 l pot and a stainless steel headplate; the working volume was 5.0 l. The temperature was controlled at 37 ± 1 °C and the digester was continually stirred at 200 r.p.m..

Semi-continuous operation was achieved by removing digester liquor every second day using a Masterflex variable-speed pump and at the same time adding standard growth medium from a funnel. Gas production was measured by displacement of an acid salt solution (10 % w/v sodium chloride in dilute sulphuric acid, pH ca. 1.5) in a 2 l measuring cylinder. All gas tubing was butyl rubber and all other tubes were clamped off close to the headplate except during feeding. The pH was maintained above 7.0 by manual addition of sodium bicarbonate following measurement of the liquor pH.

3.4.1.2 Biogen digester

The Biogen laboratory fermenter fitted with a 2 l glass pot and butyl rubber headplate supplied by New Brunswick Scientific Co. Ltd was used in a further semi-continuous digestion experiment The working volume was 1.5 l, the temperature was maintained at 37 ± 1 °C and the digester was stirred at 300 r.p.m.. The apparatus layout was the same as for the Microferm digester (Figure 3.2).

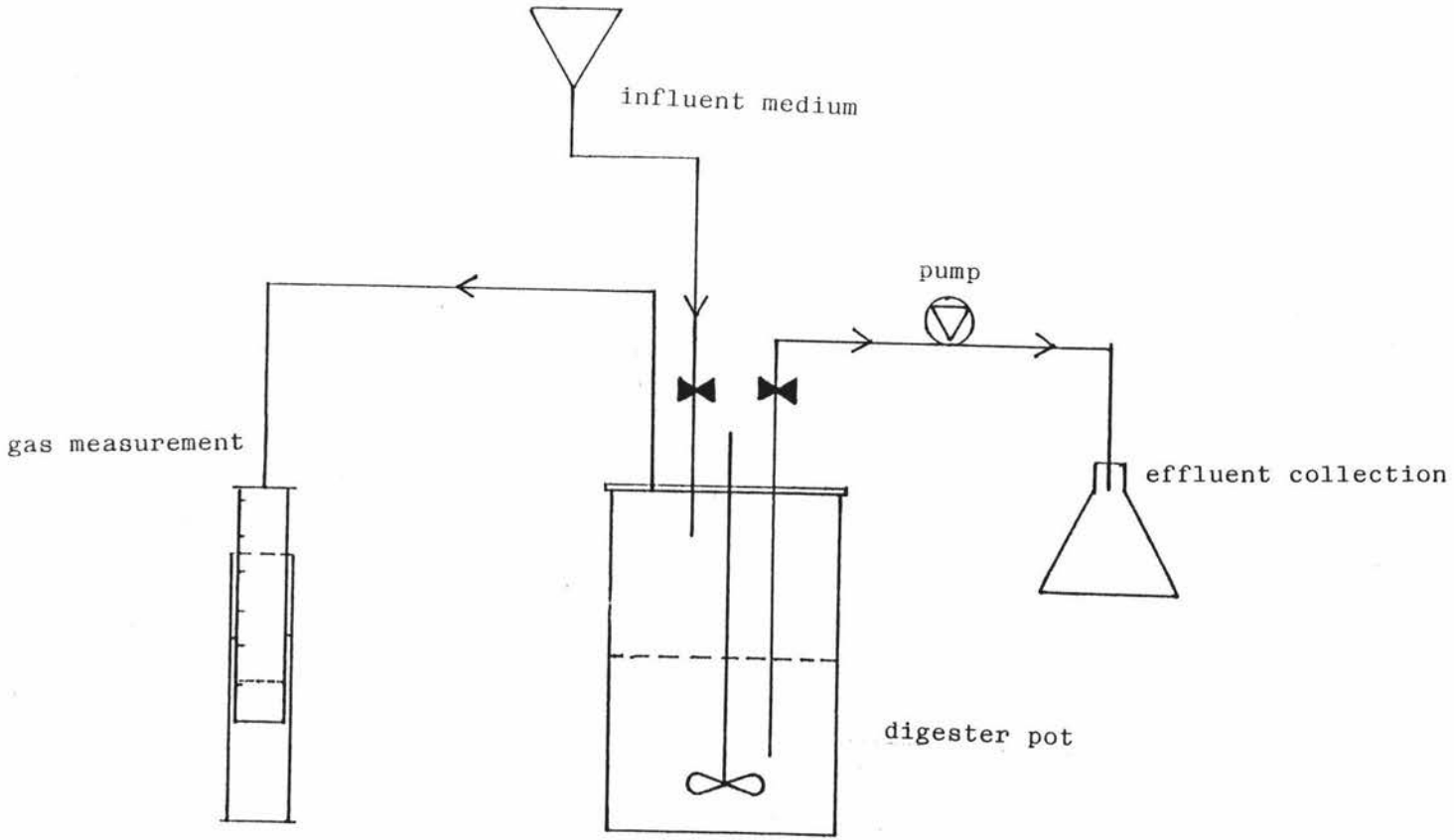


Figure 3.2: Experimental set-up for the semi-continuous digestion experiments.

Effluent was removed every second day using a 50 ml volumetric pipette and pipette-filler and standard growth growth medium was then added through a funnel. Gas production was measured by displacement of an acid salt solution in a 1 l measuring cylinder. All gas tubing was of butyl rubber and all other tubing was clamped off close to the headplate when not in use. The pH was maintained above pH 7.0 by manual addition of sodium bicarbonate following measurement of the liquor pH.

3.4.2 Operating conditions

Digested sewage sludge and effluent liquor fron the continuous digester provided the inocula for these experiments. Inoculum sources and preparation, digester start-up and sampling procedures and details of the experimental programme are described in Chapter Four.

3.5 BATCH DIGESTION EXPERIMENTS

3.5.1 Equipment and instrumentation

Two types of batch digester were employed during the course of this work and are described below. All batch cultures were incubated at 37 ± 1 °C in a temperature controlled room.

3.5.1.1 Serum bottle cultures

Serum bottles of 70 ml nominal volume (actual volume 69.0 ± 0.5 ml) stoppered with butyl rubber closures were used in a modification of the assay technique of Owen et al. (1979). The liquid volume was either 30 or 50 ml and the bottles were thoroughly mixed by hand before and after gas sampling. Gas production was measured by displacement of a 15 ml glass syringe. The barrel was lubricated with distilled water and the needle bent 90° to allow the gas volume to be read at atmospheric pressure (Owen et al. 1979).

3.5.1.2 Flask digesters

Flat-bottomed boiling flasks of 500 ml capacity stoppered with rubber bungs were used as flask digesters. The flasks were fitted with sample and gas outlet ports and the liquid volume was 250 ml. The volume of gas produced was not measured but vented to atmosphere through a water trap. The flasks were mixed by hand at least once per day and also immediately before sampling.

3.5.2 Operating conditions

Liquor from the continuous and semi-continuous digesters provided the inocula for these experiments. Inoculum sources and preparation, digester start-up and sampling procedures and details of the experimental program are described in Chapters Five and Six for the flask and serum bottle cultures respectively.

3.6 STATISTICAL ANAYSIS OF DATA

Linear regression analysis was performed using the MINITAB statistical package (version 82.1; The Pennsylvania State University, 1982) run on the university's Prime 750 computer. Non-linear regression analysis was performed using the GENSTAT statistical package (version 4.03; Lawes Agricultural Trust, 1980) which was also run on the Prime 750 computer. Further detail of the procedures used is given in the text.

CHAPTER FOUR CONTINUOUS AND SEMI-CONTINUOUS DIGESTION EXPERIMENTS

4.1 INTRODUCTION

As a prerequisite to anaerobic digestion studies simulating industrial-scale operation, it was essential to establish a fully functional digester receiving substrate on a continuous basis. The methodology and medium of Hansson (1979) were adapted for this purpose but early in the experiments difficulty arose in establishing a continuous digester operating at an organic loading rate comparable to that of Hansson (1979). The digester was unstable and the obvious strategy of increasing the retention time to improve performance was adopted with only limited success. In contrast a semi-continuous digester appeared to perform very well. The original objective was therefore broadened to encompass an investigation of the cause of failure of the continuous digester and to compare the performance of a continuously-fed digester with semi-continuous digesters batch fed every second day. These form the subjects of this chapter.

4.2 EXPERIMENTAL METHOD

4.2.1 Equipment

Three anaerobic digesters were used in these experiments. The continuous digestion runs were carried out with the 4.1 l working volume, stirred vessel described previously in Section 3.3.1. The two semi-continuous runs were performed using the 5.0 l Microferm and 1.5 l Biogen stirred fermentation vessels described in Section 3.4.1.

4.2.2 Experimental programme

A series of five experiments was performed over a period of approximately three years. At various times up to three

digesters were run concurrently and the major features of the programme are reviewed in Table 4.1. The runs are listed in chronological order and further details are elaborated in the following sections. Hansson (1979) reported on the effect of carbon dioxide at a volumetric organic loading rate (OLR_v) exceeding 3.2 g $COD.l^{-1}.day^{-1}$ and this was the maximum loading rate evaluated in continuous digestion, run 1 (CDR1). In subsequent experiments lower loading rates were employed.

Table 4.1: Overview of experimental programme for continuous and semi-continuous digestion experiments.

Run	Duration	Range of OLR_v studied[a]	Inoculum source
	days	g $COD.l^{-1}.day^{-1}$	
CDR1[b]	300	1.4 - 3	cow manure
CDR2	266	0.8 - 2.1	cow manure
SCDR1[c]	305	0.5	digested sewage sludge
CDR3	132	0.8 - 1.0	SCDR1
SCDR2	138	0.8 - 1.2	CDR3

a excluding start-up period

b CDR1 = continuous digestion, run 1

c SCDR1 = semi-continuous digestion, run 1

4.2.3 Growth medium

The standard growth medium (Section 3.1.4) was used throughout the semi-continuous studies and the early part of the continuous digestion experiments. From day 160 of CDR2 this was supplemented by the addition of 0.05 % (w/v) cysteine-hydrochloride and this modified medium was also used subsequently in CDR3.

4.2.4. Inoculum sources and preparation

Cow manure was obtained from cows milked at the No. 1 Dairy Unit, Massey University. The manure was slurried with an equal volume of tap water, strained through two layers of cheesecloth and added to the digester. Volumes used are described below.

Digested sewage sludge was obtained from a mesophilic digester at the Palmerston North City Corporation Sewage Treatment Plant. The sludge was strained through two layers of cheesecloth and added to the digester.

When effluent from one digester was used as seed material for establishing another run (refer Table 4.1), the effluent was collected and stored under an oxygen-free nitrogen atmosphere prior to use.

4.2.5 Start-up procedure

The start-up procedure adopted was to fill the digester to approximately 60 % with seed material and add a further 4 % of the digester volume as sterile medium. The vessel was then filled to the required level with tap water and sparged with oxygen-free nitrogen at 24 $l.hr^{-1}$ for 2 hr with continous agitation to remove all traces of air. The temperature was raised from ambient to 37 °C over a four to six hour period and the reactor was then operated in batch mode for several days.

After a period of sustained, vigorous gas production the digester was changed to continuous or semi-continuous operation at a low OLR_v. During this period of acclimation pH and alkalinity, volatile fatty acid (VFA) concentrations, gas production and gas composition were monitored. As acceptable performance, as based upon the reported results of Hansson (1979), was established the retention time was gradually shortened until the desired loading was reached.

4.2.6 Sampling

4.2.6.1 Continuous digestion experiments

Liquid effluent samples for most tests were taken using a 10 ml pipette and bulb pipette-filler attached to a sample tube on the digester headplate. The first 10 ml sample removed was always discarded ensuring a representative sample. When large volumes were required, as for the BOD_5 and solids tests, effluent collected overnight in the outlet measuring cylinder was thoroughly mixed and a sample taken from this. A gas sampling port equipped with a silicon rubber septum was fitted to the condensate trap and gas samples were removed using the precision micro-litre syringe employed for gas composition analysis.

4.2.6.2 Semi-continuous digestion experiments

Liquid samples were taken from the effluent displaced from the digesters during medium addition. Gas sample ports equipped with silicon rubber septa were incorporated in the gas vent line of the Microferm digester and on the headplate of the Biogen digester. Gas samples were taken using the syringe employed for gas analysis.

4.2.7 Analytical procedures

Analytical procedures used were as described in Section 3.2.

4.3 RESULTS AND DISCUSSION

4.3.1 Continuous digestion

4.3.1.1 Run 1 (CDR1)

Experimental data from start-up to day 103 are shown in Figure 4.1 (the figure is large to show the data and is enclosed inside back cover). The data for the start-up period are incomplete as experimental techniques were developed during this time and some equipment was still under construction, or awaiting delivery or commissioning. In particular, the Shimadzu gas chromatograph was under repair so the back-titration method of DiLallo and Albertson (1963) was used to estimate total volatile fatty acid (TVFA) concentration. A combination of the following data were therefore used to gauge process performance during this initial period: pH, alkalinity (not shown on Figure 4.1), TVFA by titration and effluent supernatant COD. Effluent supernatant COD data are reported as more frequent measurements of this parameter, rather than total effluent COD, were made during this period.

After a period of approximately one month operating at a very low loading rate the retention time was reduced to provide an OLR_v of 1.4 g $COD.l^{-1}.day^{-1}$. No marked change in pH or in effluent COD was observed following this reduction and on day 41 the loading rate was increased to 2.4 g $COD.l^{-1}.day^{-1}$. This resulted in a gradual rise in effluent supernatant COD and a rapid decrease in digester pH. The pH was controlled by manual addition of sodium bicarbonate until day 51, when automatic pH control was introduced (Section 3.3.1). Methane production dropped to 2 $ml.(ml\ feed)^{-1}$ from a stable level of between 5.0 and 5.3 ml.(ml

feed)$^{-1}$, and when VFA data were first obtained on day 51, increasing acid concentrations were noted. Acetate accumulated most rapidly and accounted for about two-thirds of the TVFA concentration.

For the period from day 41 to day 91 the OLR_v was maintained in the range 2.0 to 2.4 g $COD.l^{-1}.day^{-1}$. Initially during this period the trend to acid accumulation was reversed and increasingly stable digestion was indicated. By day 84 the TVFA concentration had fallen to 550 $mg.l^{-1}$ with acetate, propionate and butyrate present at concentrations of 130, 220 and 40 $mg.l^{-1}$ respectively. The effluent supernatant COD was reduced to 2500 $mg.l^{-1}$, comparable to values observed during start-up, and the volatile suspended solids (VSS) concentration appeared to stabilise at about 3 $g.l^{-1}$. Significantly, these data were similar to those achieved by Hansson (1979) during stable operation under non-inhibitory conditions. Methane production was erratic at this time and the possibility of errors in measurement associated with gas meter malfunction could not be discounted.

On day 92 the OLR_v was increased to 2.8 g $COD.l^{-1}.day^{-1}$ and on day 97 again raised to 3.0 g $COD.l^{-1}.day^{-1}$. This was done in an attempt to duplicate the loading conditions employed by Hansson (1979). Figure 4.1 clearly shows that this change in loading promoted a rapid deterioration in digester performance. There was a steady rise in effluent COD, a fall in VSS to below 2 $g.l^{-1}$ and a rapid increase in the TVFA concentration. The acetate concentration increased notably over ten-fold within 20 days, while propionate and butyrate concentrations did not change greatly. Methane content of the biogas remained at about 55 % while volumetric methane production continued to be erratic.

However examination of Figure 4.1 suggests that the onset of digester failure could have occurred much earlier (after day 82) and in particular, the fall in VSS after this time appeared

significant. On day 82 the biomass concentration was 2.95 $g.l^{-1}$ and the specific loading rate (OLR_s) had been reduced from 1.14 g COD.(g VSS)$^{-1}$.day^{-1} on day 68 to 0.55 g COD.(g VSS)$^{-1}$.day^{-1}. As a result of the decrease in VSS concentration after day 82, the OLR_s rose and exceeded 1 g COD.(g VSS)$^{-1}$.day^{-1} by day 90. This was higher than the average value of 0.85 g COD.(g VSS)$^{-1}$.day^{-1} calculated from the data of Hansson (1979) and was close to the maximum substrate removal rate indicated for simple stirred digesters by Henze and Harremoes (1983).

The reason for loss of biomass was not clear but the average retention time of 8 day was low compared to that usually employed in simple continuous tank digesters (Hobson et al. 1974, 1981). Subsequent to the period illustrated, the retention time was therefore increased to 12 and later to 16 day but cell washout and acid accumulation continued. During this period supplementation with a vitamin solution (Section 3.1.4) and trace elements, added to provide twice the concentration of those present in the influent medium, did not improve digester performance. Finally, 200 days after inoculation, the run was stopped.

4.3.1.2 Run 2 (CDR2)

CDR2 was initiated as per the start-up procedure (Section 4.2.5). The performance of this run is illustrated in Figures 4.2 and 4.3 (both figures enclosed inside back cover).

For the first 11 days the digester was operated at an OLR_v of 0.1 g COD.l^{-1}.day^{-1} (Figure 4.2). This was then increased to 0.6 g COD.l^{-1}.day^{-1} (on day 12) and later to between 0.8 to 0.9 g COD.l^{-1}.day^{-1} (day15). By day 33 satisfactory trends in performance were observed; acids which had accumulated rapidly since start-up fell to low levels with the TVFA concentration less than 400 $mg.l^{-1}$. Total effluent COD was also reduced rapidly to 5000 to 6000 $mg.l^{-1}$ as a result of acid utilisation

and washout of seed material and methane production and methane content of the gas were high.

From day 33 the OLR_v was gradually increased to 2.1 g $COD.l^{-1}.day^{-1}$ (day 40). Initially stable operation was maintained but after day 50 a trend to failure was observed. As in CDR1 this was characterised by a rapid accumulation of acetate with the butyrate concentration following a similar profile but at a lower concentration. The concentration of propionic acid rose only slowly from 120 $mg.l^{-1}$ on day 33 to 500 $mg.l^{-1}$ by day 57. Corresponding acetate and butyrate concentrations at this time were 1370 and 510 $mg.l^{-1}$. VSS and effluent COD increased slightly during this period. Volumetric methane production fell from a satisfactory level of about 5.5 ml.(ml feed)$^{-1}$ to between 4 and 4.5 ml.(ml feed)$^{-1}$, and the methane content of the gas also decreased slightly from 60 % to 55% by volume.

A slight reduction in OLR_v to 1.79 g $COD.l^{-1}.day^{-1}$ occurred on day 50 when fresh medium with a lower measured COD was introduced; the retention time remained unaltered at 12 days. However, from day 58 the OLR_v was reduced over a five day period to 1.4 g $COD.l^{-1}.day^{-1}$ by increasing the retention time from 12 to 16 days. This achieved a partial recovery in digester performance with a rapid fall in acetate, butyrate and TVFA concentrations, an increase in gas production to above 5.2 to 5.5 ml methane.(ml feed)$^{-1}$ and an average effluent COD of about 5000 $mg.l^{-1}$. However the propionate concentration fell only slowly from 500 to 330 $mg.l^{-1}$ (day 68). An attempt was then made to slowly raise the loading rate again, but at 1.6 g $COD.l^{-1}.day^{-1}$ (day 70) a gradual rise in acetate concentration was noted, with increasingly poor performance also indicated by other parameters.

When the acetic acid concentration exceeded 1500 $mg.l^{-1}$ and no reversal of the trend to failure was apparent, the OLR_v was reduced back to 1.2 to 1.5 g $COD.l^{-1}.day^{-1}$ (day 85) and maintained at this level for several months. However, although

acid accumulation was reversed, stable operation was approached only very slowly. Acetate and butyrate concentrations fell consistently but there was an initial rise in propionate concentration to a maximum of 1310 $mg.l^{-1}$ at day 115 followed by a gradual decline. The slight increase in VFA level from day 136 to 138 was attributed to contamination of the growth medium. This was replaced on day 138 and subsequent gradual improvement in performance was again observed.

No clear indication of the reason for digester failure was provided by Figure 4.2 but the data plotted in Figure 4.3 are more revealing. This figure shows TVFA and VSS concentrations, OLR_s and the specific rates of methane production (Met_s) and COD removal ($CODR_s$). These latter specific rate data were quite variable because of the high uncertainty associated with VSS measurement (Table 3.8) and in the case of $CODR_s$, both COD and VSS measurement (Table 3.6). Thus the overall trends in the parameters were more significant than individual values and to more clearly indicate these trends, smooth curves are drawn through the data on Figure 4.3.

VSS were first measured on day 42 after the OLR_v had been increased to 2.1 g $COD.l^{-1}.day^{-1}$ and the recorded concentration was 0.85 $g.l^{-1}$. The calculated specific loading rate was very high at 2.45 g $COD.(g\ VSS)^{-1}.day^{-1}$ but as the increased loading also permitted a gradual increase in biomass concentration, the OLR_s was subsequently reduced. The fall in OLR_v from day 50 further aided this trend, and by day 60 VSS had increased to over 2 $g.l^{-1}$ and the OLR_s was stablised in the range 0.5 to 0.7 g $COD.(g\ VSS)^{-1}.day^{-1}$ until about day 140. As noted above this reduced loading permitted a partial recovery in digester performance which clearly paralleled that observed in CDR1 from day 68 to day 82 (Figure 4.1) when a similar drop in OLR_s occurred. However, as in the initial experiment, the VSS concentration steadily decreased with further operation until about day 95. Significantly this loss of biomass occurred

despite relatively constant loading conditions. VSS again increased after day 95 to reach a maximum of approximately 3 $g.l^{-1}$ on day 121 after which time a steady decline in biomass level was again observed. The concentration of 3.45 $g.l^{-1}$ recorded on day 100 appeared to be an outlying point probably arising from the analysis of an unrepresentative sample.

The specific rates of methane formation and COD removal followed a very similar profile to OLR_s. On day 42 the initial calculated values of Met_s and $CODR_s$ were 0.54 l $CH_4.(g\ VSS)^{-1}.day^{-1}$ and 1.9 g $COD_r.(g\ VSS)^{-1}.day^{-1}$ and these fell steadily to 0.14 l $CH_4.(g\ VSS)^{-1}.day^{-1}$ (day 62) and to below 0.4 g $COD_r.(g\ VSS)^{-1}.day^{-1}$ (day 64). Accumulated acids were rapidly converted to methane during the period of partial recovery after day 60 and Met_s corresspondingly rose again to peak at 0.24 l $CH_4.(g\ VSS)^{-1}.day^{-1}$ on day 74. A gradual fall in Met_s was then observed to a low value of 0.12 l $CH_4.(g\ VSS)^{-1}.day^{-1}$ on day 121; thereafter the overall trend was again to increased biomass activity. A similar rise in $CODR_s$ to above 0.5 g $COD_r.(g\ VSS)^{-1}.day^{-1}$ was also evident after day 60 and biomass activity remained above this level until about day 84. $CODR_s$ then declined to vary between 0.3 and 0.5 g $COD_r.(g\ VSS)^{-1}.day^{-1}$ until about day 120 after which time a further increase was observed.

By day 160 however, only limited data was available to indicate this slow increase in biomass activity. Rather, the reduction in VSS and only slow decline in acid concentration suggested the correct environmental conditions for satisfactory operaton had not been achieved and possible reasons for the poor performance were reviewed. Checks made of the performance of the pH and temperature probes confirmed that these control systems were functioning well. Alkalinity was determined only infrequently but the available data confirmed pH control was satisfactory and provided adequate buffering capacity. For example, bicarbonate alkalinity on days 54, 75 and 152 was calculated as 2400, 1900 and 1400 $mg.l^{-1}$ as $CaCO_3$ respectively when the corresponding TVFA

concentrations were 1170, 1180 and 2780 $mg.l^{-1}$ as acetate. Effluent ammonium nitrogen was measured regularly from day 74 to day 121 and averaged 900 $mg.l^{-1}$. This was within the concentration range reported as having no adverse effect on digestion (McCarty 1964c) and at pH 7.0 free ammonia would also be present at only very low concentrations and would not affect the process.

The possibility of an inadequately reduced environment contributing to unstable digestion was also considered. Although this seemed unlikely, it was possible that air could be drawn into the recycle loop to the pH probe (Section 3.3.1) and introduced into the digester. However no platinum electrode for redox measurement was immediately available to test the hypothesis. An alternative approach was to add a reducing agent, so on day 165 a mixture was added to the digester to provide a mixed liquor concentration of 0.025 % (w/v) of each of sodium sulphide (as $Na_2S.9H_2O$) and cysteine-hydrochloride. At the same time the medium was replaced with one containing the two reducing agents at this same concentration. Addition of the mixture resulted in immediate and virtually complete cessation of gas production (Figure 4.2). From literature reports it was anticipated that these concentrations, respectively 1.6 and 1.05 mM for cysteine-hydrochloride and sodium sulphide, would not be inhibitory (Lawrence et al. 1964, Mosey 1971). Sodium sulphide was thought to exert the greater toxic effect so on the following day (day 166) fresh medium containing 0.05 % (w/v) cysteine only was introduced.

Recovery from the slug dose of reducing agents was rapid and during the ensuing period, digester performance appeared to be improved. In particular an increase in the rate of acetate utilisation was noted. This was not especially clear from the daily data recorded in Figure 4.2 but a plot of the average of

five days data as shown in Figure 4.4 illustrated the effect more clearly. From day 167 acetate degradation was increased from approximately 13 $mg.l^{-1}.day^{-1}$ to 43 $mg.l^{-1}.day^{-1}$; in contrast propionate degradation was not affected. A marked increase in methane production was also observed as shown on both Figure 4.2 and Figure 4.4. This appparent stimulation by cysteine addition could arise from the compound providing a more reduced environment (Hungate 1969, Willis 1969) or providing an essential nutrient (Wellinger and Wuhrmann 1977, Scherer and Sahm 1981), either acting as an amino-acid or as a sulphur source.

However, as noted above the specific rate data plotted in Figure 4.3 also indicated an overall rise in activity of the digester population after day 120 and the apparent stimulation observed following addition of the reducing agents may in part be attributed to this. Following the decline in VSS after day 121 the OLR_s increased and restabilised at about 1 g $COD.(g\ VSS)^{-1}.day^{-1}$ at about day 145. By day 180, $CODR_s$ had increased to approximately 0.8 g $COD_r(g\ VSS)^{-1}.day^{-1}$ and Met_s to about 0.29 l $CH_4.(g\ VSS)^{-1}.day^{-1}$ before both declined again.

From day 190 to day 210 the digester perfomance became more stable. TVFA concentration was consistently below 500 $mg.l^{-1}$, gas production was in the range 5.5 to 5.7 ml $methane.(ml\ feed)^{-1}$ and the average effluent COD was 4500 $mg.l^{-1}$. However, although no obvious change in environmental conditions occurred, performance again deteriorated markedly after day 207. Acetate and TVFA levels rose steadily and the propionate concentration also increased to between 400 and 500 $mg.l^{-1}$.

Possible causes of failure of the digester were again investigated. No evidence of failure of the temperature or pH control systems was observed either from the continuous record of these data or checks on probe calibration. Bicarbonate alkalinity was measured on days 201 and 214 and calulated as 2700

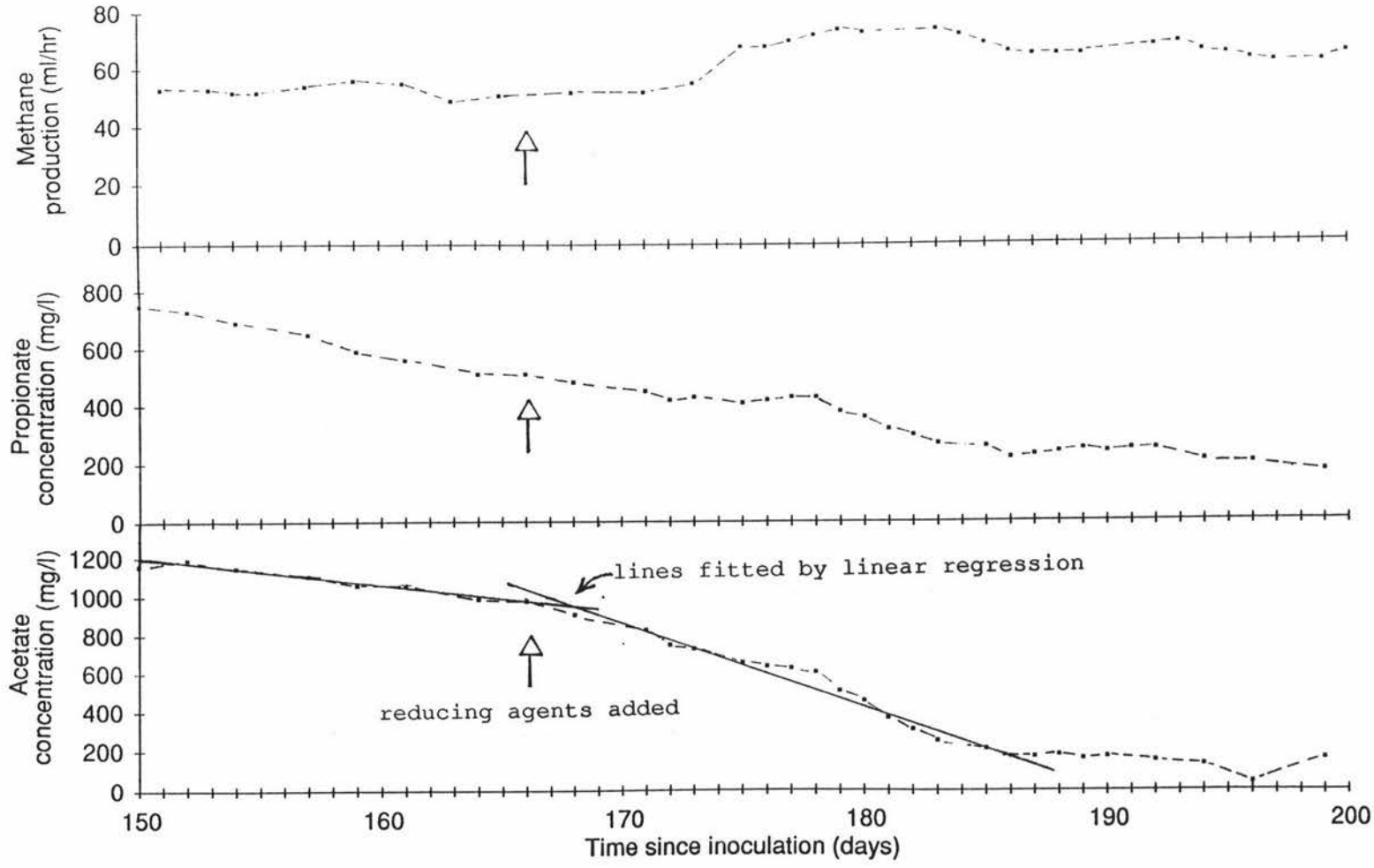


Figure 4.4: Effect of addition of reducing agents to CDR2: five-day average data.

and 2000 $mg.l^{-1}$ respectively, and ammonium nitrogen concentration remained in the the non-inhibitory region.

From day 195 a platinum electrode and calomel reference probe were fitted to the digester and measured ORP values (E_c) to day 216 are plotted in Figure 4.5 together with TVFA concentration. The average ORP of -470 mV was slightly higher than values observed in other anaerobic digestion studies (Dirasian et al. 1963, Hartz and Kountz 1966) but appeared sufficiently low to encourage active methanogenesis. It is also clear that the onset of retarded conditions was not accompanied by an increase in ORP. In more recent work, Hansson and Molin (1981a) have observed very similar ORP readings (E_h = -200 mV, E_c = -440 mV) using a similar medium. Additionally, these workers could demonstrate no relationship between acetate degradation and ORP in the range of E_h from -100 to -200 mV. Taken together these results offer no explanation for the change in stability of the digester, rather, they are all indicative of satisfactory operation. They also suggest the stimulation observed after cysteine supplementation was due to improved nutritional status of the medium rather than cysteine acting as a reducing agent.

However at the time of failure (day 208 to day 210), the VSS concentration had been reduced to only about 1 $g.l^{-1}$ and the OLR_s was correspondingly inceased to 1.3 g $COD.(g\ VSS)^{-1}.day^{-1}$. The VSS data were subject to much variation at the time (Figures 4.2 and 4.3) so values of Met_s and $CODR_s$ were difficult to estimate. $CODR_s$ appeared to average 0.7 g $COD_r.(g\ VSS)^{-1}.day^{-1}$ at the time of failure while Met_s varied considerably from 0.2 to 0.4 l $CH_4.(g\ VSS)^{-1}.day^{-1}$. However the 50 % reduction in VSS at day 207 in particular was not thought to accurately represent the true situation. Given the low growth rate, washout of solids must be determined by the residence time distribution for the completely mixed reactor. A decrease of the magnitude of that recorded in only 24 hr cannot be explained by washout when the average retention time was 16.1 day. Addition of a large slug of

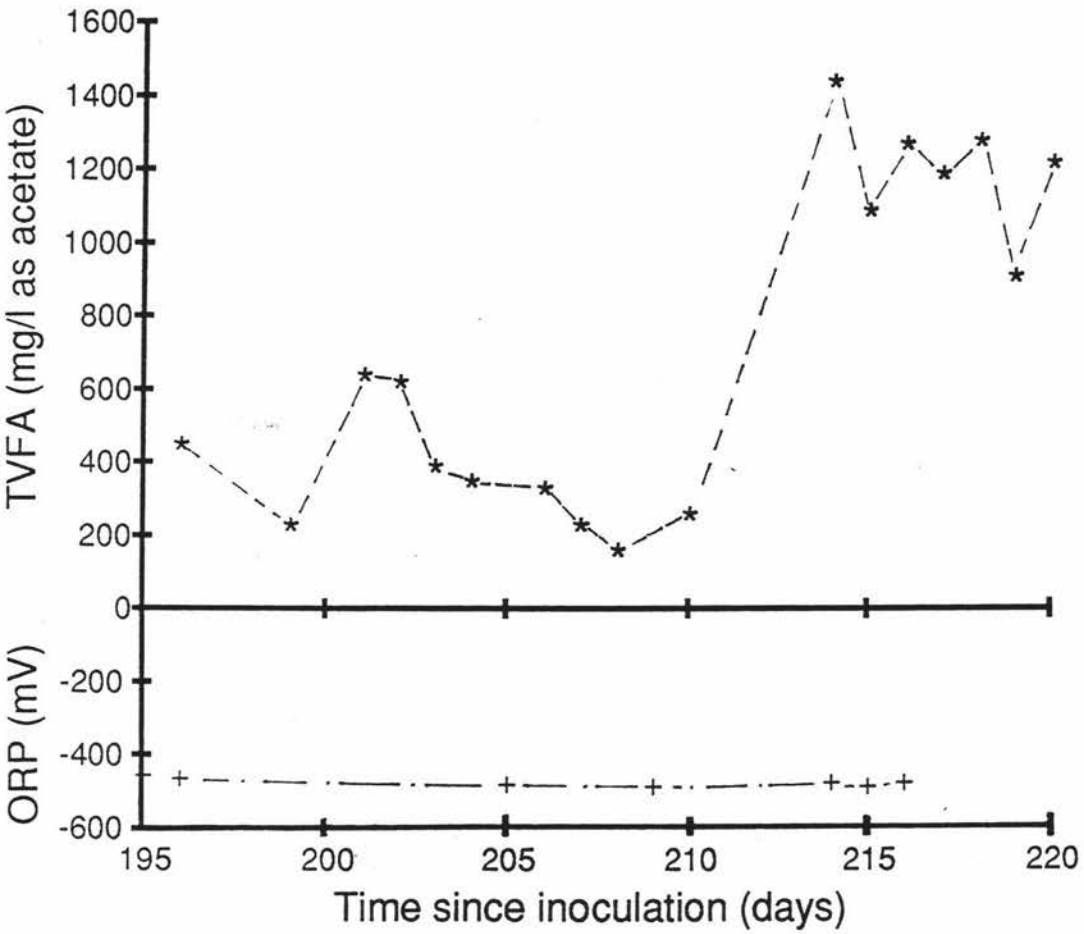


Figure 4.5: ORP and TVFA data for CDR2 during the onset of retarded digestion.

fresh medium to the digester was easily discounted by a check of the volume of medium consumed, leaving experimental error due to procuring an unrepresentative sample as the most likely cause of the observed behaviour.

The significance of these specific rate data at the time of failure is illustrated by a comparison with the results of Speece and McCarty (1964) and Hansson (1979) (Table 4.2). This indicates the digester population was very active, as $CODR_s$ and Met_s were comparable to the values of Hansson (1979) and the higest values reported by Speece and McCarty (1964). These literature data were obtained at low retention times i.e. at growth rates approaching the maximum value of 0.4 day^{-1} (HRT=2.5 day) postulated by Henze and Harremoes (1983). This suggested that methanogenesis was not inhibited; the digester population (HRT=16.1 day) was active to the maximum extent, but growth was limited to a very low value by some environmental constraint. Clearly the digester was overloaded but only because it was not possible to retain active biomass within the reactor. This is further confirmed by the biomass yield data shown in Table 4.2; the value calculated in this work of 0.07 g VSS.(g $COD_r)^{-1}$ was significantly lower than the yields calculated by other researchers (Henze and Harremoes 1983).

A slight increase in biomass concentration was observed as acetate accumulated but although the OLR_s was again reduced to about 0.8 g COD.(g VSS)$^{-1}$.day^{-1}, no improvement in process performance occurred. After a short period of batch operation (day 229 to 231), continuous feeding was resumed at a lower OLR_v of 1.1. g COD.l^{-1}.day^{-1} and this was reduced again to 0.9 g COD.l^{-1}.day^{-1} from day 244 to 252. However the OLR_s during these periods remained high as the VSS concentration had again declined to below 1 g.l^{-1}. Acetate accumulation was partially reduced by batch operation of the digester, but with continuous feeding the digester again failed, with $CODR_s$ and Met_s still

Table 4.2: Comparison of specific rate data for continuous digestion, run 2 and other studies using glucose-basd media.

Reference	Retention time day	Parameter[a] VSS	OLR_v	OLR_s	$CODR_s$	Met_s	Y_{xs}
This work (day 208)	16	1.3	1.3	1.1	0.8	0.29	0.08
Hansson (1979)	6-7	3.8	3.2	0.85	0.8[b]	0.28	0.19[c]
Speece and McCarty (1964)	5	0.76	0.67	0.88	0.56	0.22	0.35
	10	1.23	0.67	0.54	0.47	0.19	0.21
	20	2.03	0.67	0.33	0.31	0.12	0.16
	30	2.02	0.67	0.33	0.30	0.12	0.11

a VSS = volatile suspended solids ($g.l^{-1}$)

OLR_v = volumetric organic loading rate ($g\ COD.l^{-1}.day^{-1}$)

OLR_s = specific organic loading rate ($g\ COD.g\ VSS^{-1}.day^{-1}$)

$CODR_s$ = specific rate of COD removal ($g\ COD_r.g\ VSS^{-1}.day^{-1}$)

Met_s = specific rate of methane production ($1\ CH_4.g\ VSS^{-1}.day^{-1}$)

Y_{xs} = biomass yield coefficient ($g\ VSS.g\ COD_r$)

b estimated from $Met_s/0.352$ (McCarty 1964b)

c estimated from $1/(CODR_s \times \text{retention time})$

indicating the remaining biomass was very active. When the acetate concentration exceeded 2500 $mg.l^{-1}$ the run was stopped.

On reviewing the complete data for CDR2 two possible causes for the observed poor growth and biomass yield were considered; a deficiency of one or more essential nutrients or, secondly, an imbalance in the digester flora resulting in substrate utilisation via low energy-yielding pathways. Nutrient deficiency appeared to be increasingly possible in view of the frequent reports in the literature of 1980 to 1982 on the stimulatory effect of trace metals on methane production in both pure and mixed cultures. However additions of both a trace metals solution and a vitamin solution to CDR1 had not stimulated digester performance, and Hansson (1979) had obtained successful digestion using the medium employed in this study. Additionally a semi-continuous digester seeded with digested sewage sludge was operating well on the medium (SCDR1, refer Section 4.3.2.1). These observations indicated that the source of inoculum could be an important influence on stability, and this possibility was investigated in the final continuous digestion experiment.

4.3.1.3 Run 3 (CDR3)

CDR3 was seeded with effluent liquor from SCDR1 (Section 4.3.2.1) following the start-up procedure (Section 4.2.5) and the performance of the digester is illustrated in Figures 4.6 and 4.7 (both figures enclosed inside back cover).

A short start-up period was employed as the seed was well adjusted to the medium and the semi-continuous digester had been operating well at an OLR_v of 0.53 g $COD.l^{-1}.day^{-1}$. Within 12 days the OLR_v was stabilised in the range 0.8 to 1.0 g $COD.l^{-1}.day^{-1}$ and was then maintained at this value (Figure 4.6). Initially very satisfactory performance was obtained but, as in previous experiments, the digester failed after approximately one retention time (from day 35). As on all previous occasions this

was characterised most clearly by a substantial increase in the acetate concentration.

The initial VSS concentration was low at 0.95 $g.l^{-1}$ but increased gradually with the increase in OLR_v. Prior to the digester failure the OLR_s was approximately 0.8 g $COD.(g\ VSS)^{-1}.day^{-1}$ and during the period of acid accumulation was stabilised at about 0.7 g $COD.(g\ VSS)^{-1}.day^{-1}$ (Figure 4.7). Although this loading was lower than that employed during periods of improved performance noted in CDR2, retardation of digestion still occurred. The yield coefficient on day 31 was calculated as 0.07 g $VSS.(g\ COD_r)^{-1}$ and the specific rates of methane formation and COD removal at this time were 0.210 l $CH_4.(g\ VSS)^{-1}.day^{-1}$ and 0.83 g $COD_r.(g\ VSS)^{-1}.day^{-1}$ respectively. These data are very similar to those recorded in CDR2; methanogenic activity was high but cell growth was limited.

Feed addition was stopped on day 58 (Figure 4.6) to allow the digester to recover and recommenced on day 74 at a loading rate of 0.8 g $COD.l^{-1}.day^{-1}$. As the VSS concentration had again fallen to 1 $g.l^{-1}$ the OLR_s was 0.8 g $COD.(g\ VSS)^{-1}.day^{-1}$ (Figure 4.7). The biomass concentration slowly increased and OLR_s was then reduced to about 0.6 g $COD.(g\ VSS)^{-1}.day^{-1}$ by day 98. Digester performance was again satisfactory for about one retention time with very low acid levels (TVFA < 100 $mg.l^{-1}$) and high rates of methane production. On day 84 the yield coefficient, Met_s and $CODR_s$ were 0.06 g $VSS.(g\ COD_r)^{-1}$, 0.2 l $CH_4.(g\ VSS)^{-1}.day^{-1}$ and 0.67 g $COD_r.(g\ VSS)^{-1}.day^{-1}$. These values were all comparable to values recorded during periods of apparently satisfactory operation in both CDR1 and CDR2. During this period, from day 87 to 93, effluent was collected from the digester to establish the second semi-continuous digester described below. As before however, the performance of the continuous digester deteriorated after day 96, although the rate of retardation was significantly slower than on previous occasions. This was most likely due to the the low loading rate

employed. As no significant improvement in performance was observed compared to previous runs, the operation was halted after 132 days.

In summary, similar performance was obtained from from all three continuous digestion runs. These were characterised by instability; acetate utilisation and cell growth were retarded despite operation at low organic loading rates and long retention times. However at the time of failure biomass activity with respect to methane production and COD removal was high when compared to other values reported for comparable studies.

4.3.2 Semi-continuous digestion

4.3.2.1 Run 1 (SCDR1)

Hansson (1979) had used an inoculum derived from a sewage sludge digester in contrast to both the continuous digestion experiments reported above where cow manure was used. The purpose then in establishing this first semi-continuous digester was to provide an alternate, acclimated inoculum for use in further continuous experiments when required, to provide preliminary information on possible differences in performance arising from the change in inoculum, and to compare the performance of semi-continuous and continuous digesters operating at similar organic loading rates.

The run was commenced during the period of slow recovery experienced in CDR2 following day 82 (Figure 4.2). The Microferm digester was operated at a retention time of 40 day with a volume of 250 ml medium added every second day. This gave an average OLR_v of 0.53 g $COD.l^{-1}.day^{-1}$. The unit was operated for a total of 305 days and overall extremely good performance was obtained.

Start-up was accomplished rapidly and after a further 40 days (at day 66) stable digestion was achieved, although the effluent BOD_5 continued to increase gradually. After two retention times (day 106) all parameters were stable and data from day 126 to 202 were

used to characterise steady-state operation. The summarised results and derived data are shown in Table 4.3.

The pH was easily maintained between pH 6.7 and 7.0 by the addition of 1.5 g $NaHCO_3$ to the digester approximately every 20 days. Total alkalinity (to pH 4.0) ranged from 1600 to 2020 $mg.l^{-1}$ with an average value of 1770 $mg.l^{-1}$ as $CaCO_3$. Gas production was stable with a methane yield of 5.23 ml.(ml feed)$^{-1}$ and the average methane content of the biogas was 57 % by volume. The COD-specific methane production rate was 324 ml.(g COD removed)$^{-1}$, 92 % of the theoretical value (McCarty 1964a). Removal of COD was not particularly high at 77 % but the greater part of the effluent COD was contained in the cell fraction. The COD of the supernatant liquor averaging only 1010 $mg.l^{-1}$ and the average effluent BOD_5 was 250 $mg.l^{-1}$. VFA concentrations were extremely low with acetate and propionate the only acids detected.

The solids data were more variable than other parameters and over the whole period considered, VSS showed a slow but steady increase, ranging from 2.81 $g.l^{-1}$ to 3.39 $g.l^{-1}$, with an average of 2.97 $g.l^{-1}$. Values of g cell COD per g of total suspended solids (TSS) and g cell COD per g of VSS were calculated as 1.2 and 1.3 respectively. These were in approximate agreement with values for both anaerobic and aerobic waste treatment systems (McCarty 1965). The specific loading rate was 0.18 g COD.(g VSS)$^{-1}$.day^{-1} and the average values of Met_s and $CODR_s$ were calculated as 0.044 l CH_4.(g VSS)$^{-1}$.day^{-1} and 0.14 g COD_r.(g VSS)$^{-1}$.day^{-1}. These values were much lower than observed in the continuous digester, a reflection of the low loading engendered by the long retention time. The biomass yield coefficient of 0.18 g VSS.(g COD_r)$^{-1}$ was comparable to literature values (Speece and McCarty 1964, Henze and Harremoes 1983) and indicated bacterial growth was not limited under the fermentation conditions.

Table 4.3: Summary of steady-state data for the semi-continuous Microferm digester (SCDR1).

Parameter	Mean value	Standard deviation	Number of obs.
pH	6.80	0.07	34
Total gas production in 48 hr (ml)	2350[a]	160	32
Methane content of gas (%)	56	2	18
Methane production in 48 hr (ml)	1310		
Volumetric methane production ($ml.ml\ feed^{-1}$)	5.2		
COD influent ($mg.l^{-1}$)	21,100	800	8
effluent ($mg.l^{-1}$)	4940	240	13
eff. supernatant ($mg.l^{-1}$)	1010	180	12
COD removal (%)	77		
COD-specific methane production ($l\ CH_4.\ g\ COD_r^{-1}$)	0.324		
BOD_5 effluent ($mg.l^{-1}$)	250	30	7
TVFA ($mg.l^{-1}$)	20	-[b]	14
Total suspended solids ($g.l^{-1}$)	3.19	0.18	14
VSS ($g.l^{-1}$)	2.97	0.49	14
OLR_v ($g\ COD.l^{-1}.day^{-1}$)	0.53		
OLR_s ($g\ COD.g\ VSS^{-1}.day^{-1}$)	0.18		
Y_{xs} ($g\ VSS.g\ COD_r^{-1}$)	0.18		
g COD per g cell (TSS basis)	1.2[c]		
g COD per g cell (VSS basis)	1.3[c]		

a dry gas at STP

b s.d. could not be calculated as many observations were below the limit of detection

c for COD of cell fraction = effluent COD - eff. supernatant COD

An analysis of 32 days gas production data (Table A2.1, Appendix 2) from the steady-state period was made to produce the gas production profile shown in Figure 4.8. The figure shows that 4 hr after the addition of medium approximately one-third of the the total gas had been produced, while after 12 and 24 hr about 64 % and 90 % of the gas was evolved. Thus the fermentation was virtually complete within 24 hr and in the remaining 24 hr before feeding, gas production was limited by the availability of substrate. Dissimilation of glucose also appeared to be very rapid as glucose could not be detected in the digester liquor 2 hr after medium addition.

A period of instability was experienced from day 206 to 224 as a result of the failure of the temperature control unit on day 205. The temperature dropped from 37 °C to 22 °C overnight and gas production was reduced by about 35 %. On day 206 the temperature was restored and normal feeding was continued for eight days. However the pH fell to 6.5 and volatile acids accumulated, with propionate showing a dramatic increase to 750 $mg.l^{-1}$ by day 214. This was in marked contrast to stressed conditions in the continuous digester, where acetate accumulated rapidly and propionate concentration increased only slowly. Propionate accumulation was accompionied by an increase in effluent COD and a slight drop in the methane content of the gas from around 56 % to a low of 51 % on day 208. Hydrogen was not detected in the gas.

The usual feeding routine was stopped on day 214 and 250 ml of the medium was next added on day 220. A marked decrease in propionate concentration was noted and further medium was added on day 224. Normal feeding was then resumed as at this time the acid level was very low and the pH was 6.95. Stable digestion was then maintained at the same level of performance as before the upset.

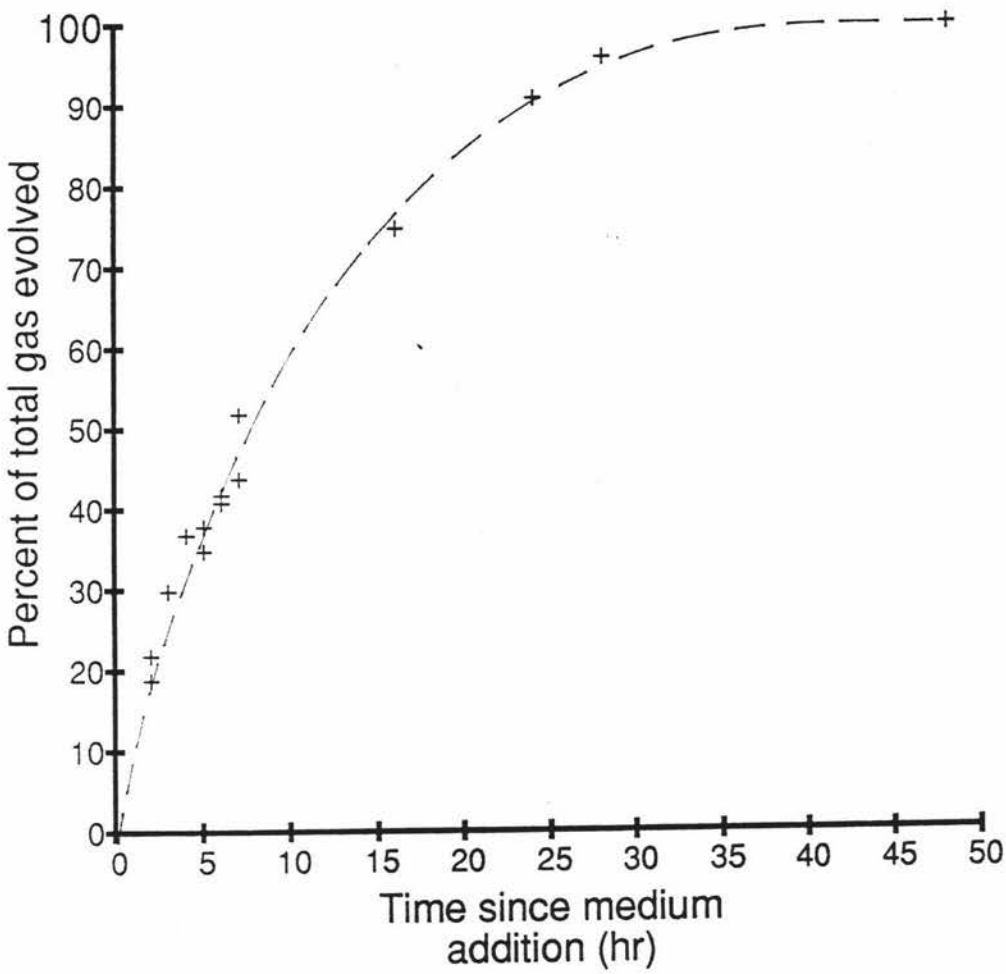


Figure 4.8: Average time course of total gas production from SCDR1.

4.3.2.2 Run 2 (SCDR2)

The Biogen digester was seeded with 1200 ml of effluent anaerobically collected from CDR3 as noted above. Feeding commenced on the following day and by day 10 the retention time of 24 day was achieved corresponding to an OLR_v of 0.86 g $COD.l^{-1}.day^{-1}$. The digester was run for a total of 138 days and experimental data are shown in Figure 4.9 (enclosed at back).

Total gas production, VFA concentrations, supernatant liquor COD and pH stabilised very quickly and were maintained until day 48. The VSS concentration showed a steady yet dramatic increase from an initial concentration of 0.85 $g.l^{-1}$ to 2.57 $g.l^{-1}$ on day 20 and to 3.79 $g.l^{-1}$ on day 40, where the level stabilised. Similar trends were reflected in the total effluent COD. As the seed material had been exposed to similar loading conditions in CDR3 for 24 days and the Biogen digester performance appeared very stable, steady state conditions were judged to have been reached on day 38. Data from day 38 to day 50 were then used to characterise the digester performance and the results are tabulated in Table 4.4.

A comparison of Tables 4.3 and 4.4 suggests the performance of the two semi-continuous digesters was very similar. The pH and alkalinity at 24 day retention time were slightly lower than that at 40 day, but both were stable and easily maintained. The gas produced at the lower retention time was slightly higher in methane and the volumetric methane yield and the COD-specific methane production rate were also increased. The latter value of 347 $ml.(g\ COD_r)^{-1}$ was very close to the theoretical value (McCarty 1964a).

The supernatant liquor COD and VFA concentrations were similar in both reactors. However at the lower retention time VSS averaged 3.69 $g.l^{-1}$ compared with 2.97 $g.l^{-1}$ in SCDR1 and this was reflected in both higher total effluent COD values and lower

Table 4.4: Summary of steady-state data for the semi-continuous Biogen digester (SCDR2).

Parameter	Mean value	Standard deviation	Number of obs.
pH	6.70	0.05	7
Total gas production in 48 hr (ml)	1110[a]	43	7
Methane content of gas (%)	59	3	6
Methane production in 48 hr (ml)	650		
Volumetric methane production ($ml.ml\ feed^{-1}$)	5.2		
COD influent ($mg.l^{-1}$)	20,700	300	3
effluent ($mg.l^{-1}$)	5660	130	5
eff. supernatant ($mg.l^{-1}$)	1050	50	5
COD removal (%)	73		
COD-specific methane production ($l\ CH_4.\ g\ COD_r^{-1}$)	0.347		
BOD_5 effluent ($mg.l^{-1}$)	440	-	1
TVFA ($mg.l^{-1}$)	10	-[b]	6
Total suspended solids ($g.l^{-1}$)	3.78	0.18	5
VSS ($g.l^{-1}$)	3.69	0.14	5
OLR_v ($g\ COD.l^{-1}.day^{-1}$)	0.86		
OLR_s ($g\ COD.g\ VSS^{-1}.day^{-1}$)	0.23		
Y_{xs} ($g\ VSS.g\ COD_r^{-1}$)	0.25		
g COD per g cell (TSS basis)	1.2[c]		
g COD per g cell (VSS basis)	1.2[c]		

a dry gas at STP

b s.d. could not be calculated as many observations were below the limit of detection

c for COD of cell fraction = effluent COD - eff. supernatant

percent COD removal. The effluent BOD_5 for SCDR2 was also higher at 440 $mg.l^{-1}$ but only one measurement was made. The OLR_s in SCDR2 was approximately 30 % higher than in SCDR1 at 0.233 g $COD_r.(g\ VSS)^{-1}.day^{-1}$ and the biomass yield coefficient was increased to 0.25 g $VSS.(g\ COD_r)^{-1}$. Values of Met_s and $CODR_s$ in SCDR2 were 0.059 l $CH_4.(g\ VSS)^{-1}.day^{-1}$ and 0.169 g $COD_r.(g\ VSS)^{-1}.day^{-1}$, increases of 34 % and 24 % respectively over the earlier semi-continuous run. The COD to cell solids ratios were similar in both digesters.

Twenty four days gas production data were analysed to produce the gas production profile shown in Figure 4.10. Raw data appears in Table 2.2 of Appendix Two. Four hours after medium addition approximately 27 % of the gas had been produced and after 12 and 24 hr this had increased to 58 % and 76 % respectively. The gas production profile for SCDR1 is also shown on the figure. It is apparent that gas production in SCDR2 proceeded at a slower rate as the fermentation proceeded than in the earlier run at the lower loading rate. As in SCDR1, acidogenesis was very rapid and glucose could not be detected in the digester liquor 2 hr after medium addition.

Major performance characteristics for SCDR2 at steady state and for CDR3 over the period from day 70 to day 96 are compared in Table 4.5. It was during this latter period (of CDR3) that the seed for SCDR2 was collected. The methane content of the gas and the methane yield were similar, although the continuous digester gave the higher values in each case. The percent COD removal was also higher in the continuous digester but the COD-specific methane yield of 305 $ml.(g\ COD\ removed)^{-1}$ was surprisingly low. This was only 87 % of the theoretical value and suggests that not all of the effluent COD was being measured. Acetate was the major acid present in both digesters but the average total acid concentration was 80 $mg.l^{-1}$ in the continuous digester compared with 10 $mg.l^{-1}$ in SCDR2. However both of these values represent very good performance.

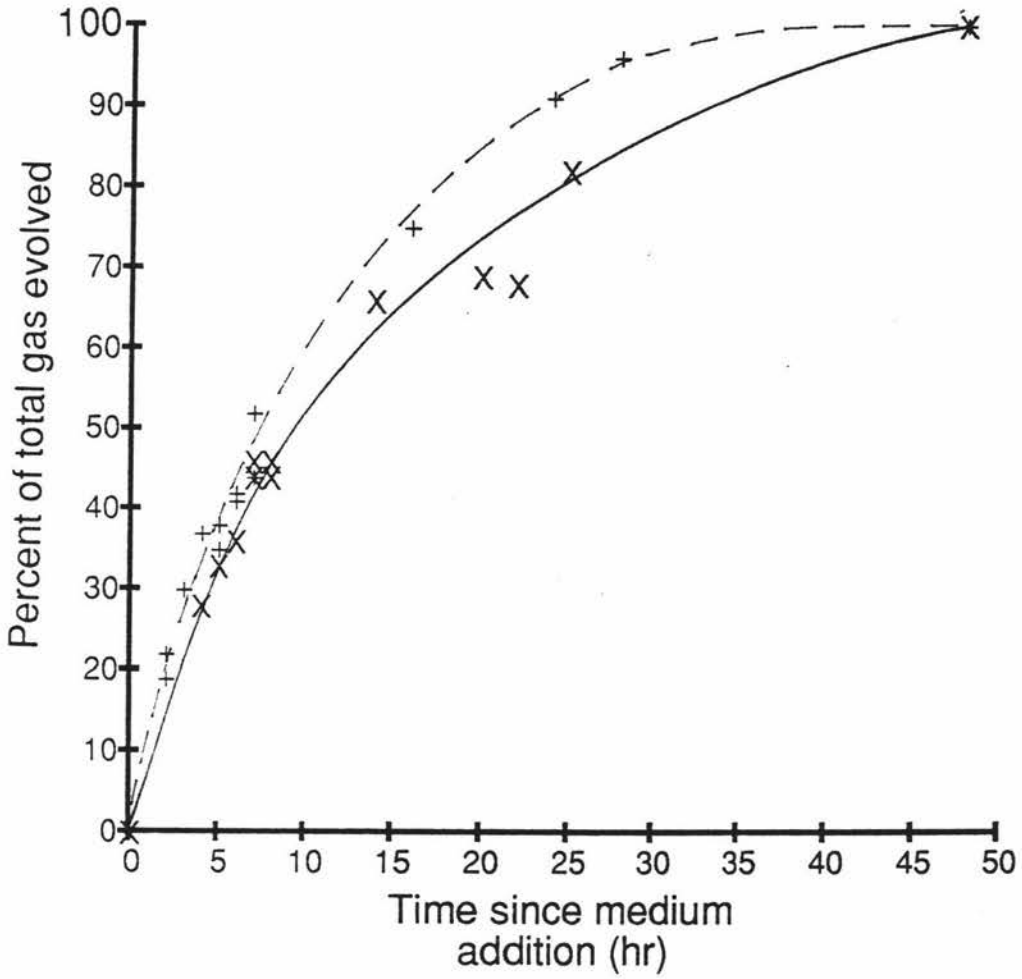


Figure 4.10: Average time course of total gas production from SCDR2. Data for SCDR1 also shown. SCDR1 (+); SCDR2 (x).

Table 4.5: Major performance characteristics for SCDR2 at steady-state and CDR3 from day 70 to day 96.

Parameter	Mean value	
	SCDR2	CDR3
Methane content of gas (%)	59	60
Volumetric methane production ($ml.ml\ feed^{-1}$)	5.2	5.7
COD removal (%)	73	85
COD-specific methane production ($l\ CH_4.g\ COD_r^{-1}$)	0.347	0.310
TVFA ($mg.l^{-1}$)	10	80
VSS ($g.l^{-1}$)	3.69	1.41
OLR_v ($g\ COD.l^{-1}.day^{-1}$)	0.86	0.81
OLR_s ($g\ COD.\ g\ VSS^{-1}.day^{-1}$)	0.23	0.4 - 0.9
$CODR_s$ ($g\ COD_r.g\ VSS^{-1}.day^{-1}$)	0.17	0.67[a]
Met_s ($l\ CH_4.g\ VSS^{-1}.day^{-1}$)	0.06	0.20[a]
Y_{xs} ($g\ VSS.g\ COD_r^{-1}$)	0.25	0.06

a on day 84

The most marked difference in performance was in the VSS concentration and the biomass yield coefficient, where the continuous run gave values only a quarter of that in the semi-continuous digester. While a lower value could be expected on the basis of more efficient substrate utilisation, the dramatic difference observed indicated cell growth in the continuous digester was limited and that this was the reason for the failure of these experiments.

After day 50 the retention time of SCDR2 was lowered from 24 to 17 days by increasing the volume of medium added during feeding (Figure 4.9). During the first four days the digester responded well to the altered conditions but retarded operation was evident after six days and the digester continued to fail over the next 22 days of operation. As in the first semi-continuous experiment stress on the bacterial population was characterised by an increase in propionate concentration, in marked contrast to the behaviour of the continuous digesters. Effluent COD rose and there was also a slight drop in VSS. Gas production fell and, for the first time in these experiments, there was a significant drop in the methane content of the gas; from 63 % on day 52 to a low of 48 % on day 74. Hydrogen was not detected however. The pH also tended to drop and high dosing of $NAHCO_3$ was required to maintain the pH above 6.6.

Feeding was discontinued on day 77 when the performance of the digester showed no improvement and the propionate concentration approached 3000 $mg.l^{-1}$. This produced an immediate and sustained drop in the accumulated acid. By day 92 the digester had fully recovered from the upset and feeding commenced again on day 100 at 24 day retention time. The digester was then operated for a further 38 days. A short period of instability occurred initially but stable operation was achieved rapidly and, by day 110, digester performance was comparable to that before the change in conditions.

The only major difference observed between the two periods of operation at 24 day retention time was in the solids data. After the change in loading the concentration of most solids components decreased and in particular there was a marked drop in VSS from 3.78 to 2.45 $g.l^{-1}$. As other performance characteristics were similar and stable over the period studied, this suggested a shift in bacterial populations towards more efficient substrate utilisation as a result of the temporarily increased loading. OLR_s was increased to 0.35 g $COD_r.(g\ VSS)^{-1}.day^{-1}$ and Met_s and $CODR_s$ to 0.091 l $CH_4.(g\ VSS)^{-1}.day^{-1}$ and 0.266 g $COD_r.(g\ VSS)^{-1}.day^{-1}$ respectively. The biomass yield coefficient was reduced to 0.15 g $VSS.(g\ COD_r)^{-1}$.

4.3.3 Evaluation of the nutritional status of the standard growth medium

The major conclusion to emerge from the latter three experiments was that failure of the continuous digester could not be attributed to the origin or composition of the microbial population. Effluent from SCDR1 did not promote successful digestion in CDR3, yet effluent from this continuous digester allowed very good performance in SCDR2. The growth medium used was therefore compared with other media successfully employed in digester studies or for pure cultivation of bacterial species involved in the methane fermentation. The aim of this exercise was to determine the adequacy of the medium used to satisfy the nutritional needs of the digester flora. The media selected for comparison were chosen from studies in which nutritional requirements of methanogens were specifically investigated (Speece and McCarty 1964, Mah et al.1978), from digester studies at approximately comparable loading rates with glucose as the major carbon and energy source (Speece and McCarty 1964, Cohen et al. 1979) and from a study of the acetogenic bacteria which play a key role in the degradation of higher acids (McInerney et al. 1981).

Before the media could be evaluated it was first necessary to characterise the yeast extract used in this work (Difco). Several analyses of yeast extracts are reported in the literature and data from three of these are shown in Table 4.6. There was general agreement between the compositons reported except for certain trace elements, particularly iron, cobalt and copper, which showed considerable variation between the various brands and even between batches (Grant and Pramer 1962). The only specific analysis of Difco yeast extract was that of Grant and Pramer (1962) and this was used as the primary data source. When data was not available from this reference the most conservative value from the other two analyses was used. Clarified rumen fluid was added in the medium of McInerney et al. (1981) but this was not considered in the comparison of the media compositions as a satisfactory analysis could not be found in the literature.

Table 4.7 shows the comparison of the media by components and this indicated that no major nutrients were lacking in the medium used in this work. Sodium was present at a lower concentration than in the other media but the analysis does not take into account the sodium added during automatic pH control of the digester. Considering the trace element composition, three of the other four media contained noteably higher concentrations of cobalt, iron and manganese. No vitamins were added to two of the media and a comparison of the others indicated only cyanocobalamin and pyridoxine as possibly deficient in the standard growth medium. As the two studies not supplemented with vitamins were both successful in maintaining stable operation for long periods and addition of a vitamin solution in CDR1 had no stimulatory effect, the possible trace element deficiencies appeared the only identifiable nutritional barrier to successful operation of the continuous digesters.

In addition to the various requirements for mineral nutrients and vitamins, one or more unidentified growth factors have been found necessary for, or highly stimulatory to, the methane

Table 4.6: Comparison of the composition of yeast extracts from various manufacturers.

Parameter	Difco[a]	BBL[b]	BBL[c]	NBCo[c]	Oxiod[d]
		% total weight			
Moisture	3.32[e]				4.1
Ash	12.87				11.5
Carbohydrates		16.6			
Total nitrogen		10.3			10.5
Ammonia nitrogen		5.5[f]			4.3
Salt		0.5[f]			1.3
		µg.(g total weight)$^{-1}$			
Al	3.2		<8	<8	
B			<4	<4	
Ba	1.3		<2	<2	
Ca		600	<1500	<1500	
Cd	1.6				
Co	3.6		0.3	0.3	
Cr	12.4				
Cu	73.7		9	8	
Fe	155	2000	44	34	
Ga	0.09				
K		34000	43500	41600	
Mg	1314	700	<900	<900	
Mn	2.4		<9	<9	
Mo	6.1		0.2	0.1	
Na			1300	1300	
Ni	18.8				
P		11600	14400	14500	14000
Pb	7.0				
Si			1600	1200	
Sn	0.09				
Sr	1.1		10	10	
Ti	3.1				
V	45.2				
Zn	76.5		116	23	
Biotin		4			
Choline		2000			
Cyanocobalamin		0			
Folic acid		20			
Niacin		400			
Pantothenic acid		100			
Pyridoxine		30			
Riboflavin		50			
Thiamine		100			
p-amino benzoic acid		24			

Table 4.6 (contd)

Notes:
a data from Grant and Pramer (1962)
b data from B.B.L. (1968)
c data from Pappelis and Schmid (1965)
d data from Oxoid (1979)
e determined by author (1982)
f as NaCl

Table 4.7: Comparison of the composition of the standard growth medium supplemented with cyseine-HCl and other media used in studies of the methane fermentation.

Reference	1	2	3[a]	4[a]	5[a]	6[a,b]
Component ($mg.l^{-1}$)						
C	6400	6666	6666	6666	6666	6666
N	520	932	1616	364	583	827
P	100	146	715	208	129	898
S	30	30	35	1.85	21.3	528
Na	80	88	7253	7453	126	5256
K	60	196	277	262	163	1127
Mg	20	25	196	3.32	12.5	315
Cl	1327	1339	3823	1714	1673	4657
B	-	<0.2	-[c]	-	0.2	0.42
Ca	0.09	2.49	-	10.0	0.32	110
Co	0.02	0.03	-	0.344	0.25	0.4
Cu	0.02	0.31	-	-	0.13	-
Fe	0.06	0.68	103	-	2.32	3.31
Mn	0.02	0.03	-	3.86	1.20	0.06
Mo	-	0.02	-	-	0.003	0.06
Ni	-	0.08	-	-	-	0.04
Zn	0.02	0.33	-	-	0.68	0.18
resazurin	1	1	-	1	-	1
cysteine-HCl	50	50	-	50	-	-
citrate	-	-	-	-	317	-
nicotinic acid	-	1.6	-	1.0	-	0.8
cyanocobalamin	-	-	-	0.01	-	0.8
thiamine	-	0.4	-	1.0	-	0.4
p.a.b.a[d]	-	0.096	-	-	-	0.4
pyridoxine	-	0.2	-	1.0	-	1.97
panothenic acid	-	0.4	-	1.0	-	0.20
biotin	-	0.016	-	0.025	-	-
choline	-	8	-	-	-	-
folic acid	-	0.08	-	0.013	-	-
riboflavin	-	0.2	-	1.0	-	-

Table 4.7 (contd)

References:

1 this work, analysis excluding yeast extract
2 this work, analysis including yeast extract (see text)
3 Speece and McCarty (1963)
4 Mah et al. (1978)
5 Cohen et al. (1979)
6 McInerney et al. (1979)

Notes:

a composition adjusted to equal carbon content with analysis 2
b analysis does not include 5 % (w/v) clarified rumen fluid
c tap water used in make-up was assumed to provide all trace elements required for growth
d p-amino benzoic acid

fermentation. Compounds of this type include; yeast extract (Bryant et al. 1971, Baresi et al. 1978, Mah et al. 1978), rumen fluid (Bryant et al. 1971, Balch et al. 1979, Iannotti et al. 1981), digester supernatant fluid (Bryant et al. 1971, Iannotti et al. 1981) and digester solids or effluent (Stander 1950, McCarty and Vath 1963, Bryant et al. 1971, Novak amd Ramesh 1975), and one of these is often added to media for the cultivation of digester microorganisms (McInerney et al. 1981, Iannotti et al. 1981).

4.3.4 Effect of medium additions on batch digestion of continuous digester liquor

In a preliminary attempt to establish if a deficiency of trace elements or some other unidentified nutrient influenced digester performance, three batch flask digesters (Section 3.5.1.2) were established using effluent collected from CDR3 under a nitrogen atmosphere. One digester acted as a control while a trace metal solution and a lysed cell solution (250 $mg.l^{-1}$) were added to each of the other digesters. The trace element solution was added to give twice the element's concentration as recommended in the medium used in this work. All digesters were initially flushed with an oxygen-free mixture of carbon dioxide in nitrogen (20:80) and incubated at 37 °C.

The digester contents were routinely analysed for volatile fatty acids and the data collected are shown in Table A2.3 of Appendix Two. Initially the propionate concentration in the reactor containing lysed cells increased, however there was little difference in the rates of degradation of the acids with the exception of acetate. The acetic acid concentration profiles for the batch digesters are shown in Figure 4.11 and indicate addition of trace elements had no effect on utilisation of this acid. However supplementation with the lysed cells solution decreased the time required to achieve a 95 % reduction in the acetate concentration from 22 to 14 days. It is not clear from

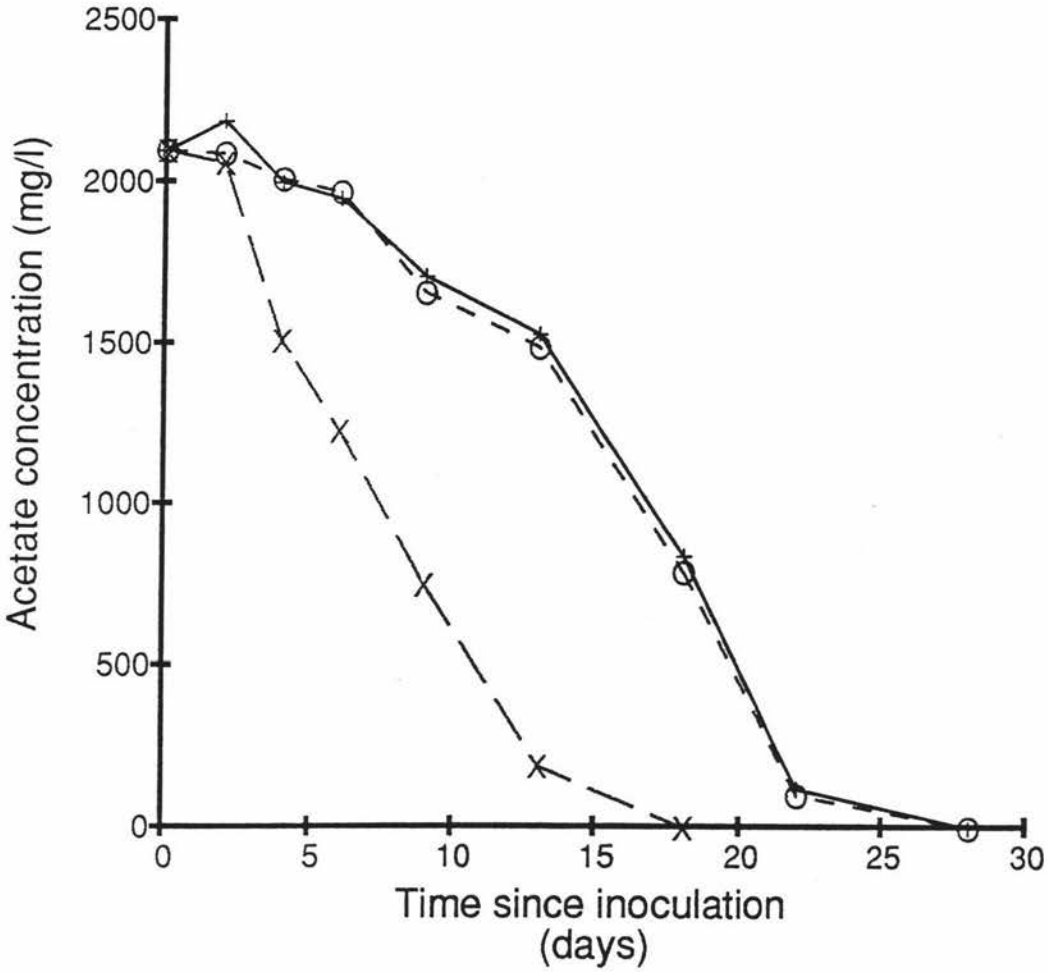


Figure 4.11: Acetate degradation in batch digesters supplemented with trace metals and lysed cells. Control (+), trace metals (o), lysed cells (x).

this single experiment if the stimulation was due to iron, some other growth factor, or a combination of growth factors. Iron was supplemented at 0.12 $mg.l^{-1}$ by addition of the trace metal solution and at approximately 0.54 $mg.l^{-1}$ by the lysed cell solution, assuming iron comprised 0.25 % of the dry cell weight (Luria 1961).

4.4 OVERALL DISCUSSION

There were several unusual features of the results of the continuous and semi-continuous digestion experiments and these indicated the reason for failure of the continuous runs.

Firstly the stability of the Biogen digester (SCDR2) when operated at the same volumetric loading rate as the final continuous run, CDR3, was surprising. The semi-continuous unit performed very well and could be successfully and easily recovered from severe retardation despite being slug fed every second day. In contrast satisfactory operation could not be obtained in CDR3. The semi-continuous feeding programme was expected to exert greater stress on the digester population compared to continuous medium addition, yet was apparently beneficial. As different equipment was used in the two experiments, the experimental protocol, digester construction and operation and the daily observations and recorded data were all examined carefully to check the possibility of some external, physical factor (e.g. temperature control or air infiltration) causing the poor performance of the continuous digester. No such explanation was offered by this analysis.

The second unusual feature of these runs was related to the first, namely, effluent from the first successful semi-continuous run (SCDR1) when used as seed for CDR3 did not provide stable operation; however when effluent from this continuous run was used as the inoculum for SCDR2, stable digestion resulted. Thus all organisms necessary for successful operation were present in

the continuous digester but good performance was prevented by some environmental imbalance. The presence of this imbalance was indicated by both the accumulation of acetate leading to failure of the continuous digesters, and the marked difference in biomass yield observed between the semi-continuous and continuous digesters.

The accumulation of acetic acid was unexpected. Literature reports suggested that when a digester is stressed by overloading or a change in environmental conditions, a rise in the concentration of propionate is the ususal indicator of poor operation (Pohland and Bloodgood 1963, Andrews and Pearson 1965, Hobson et al. 1974, Cohen et al. 1980, Speece 1981). This was observed in both semi-continuous digesters, in the first instance when the Microferm digester population (SCDR1) was subjected to thermal stress and later when the Biogen digester (SCDR2) was overloaded by shortening the retention time. In neither case did acetate accumulate.

This behaviour can be explained by the importance of interspecies hydrogen transfer in regulating the performance of the acetogenic bacteria and the product distribution of the fermentative bacteria. A healthy methanogenic population maintains a low hydrogen partial pressure in the digester which in turn ensures the degradation of propionate and higher acids by the hydrogen-producing acetogens (Wolin 1974, McInerney and Bryant 1981a). When methanogenic activity is reduced by a change in environmental conditions, the H_2 partial pressure is raised and propionate degradation is inhibited (Kaspar and Wuhrmann 1978b, Bryant 1979, McInerney and Bryant 1981a). In addition, there is a shift to the formation of more propionate and butyrate by the fermentative bacteria at the expense of acetate formation, and thus the accumulation of higher acids is accentuated. In contrast, acetate degradation appears less dependent on the hydrogen partial pressure, although the evidence is still

contradictory (Van den Berg et al 1976., Kaspar and Wuhrmann 1978a, Van den Berg et al. 1980, Boone 1982).

The inhibition of the acetoclastic reaction (Section 2.2.5) observed in the continuous digestion experiments therefore suggested another inhibitory mechanism specific to the acetate-utilising methanogens; this was believed to be nutrient limitation. Supportive evidence for this hypothesis was provided by the low biomass yield and the washout of cells observed in the continuous digesters. Prior to failure, Y_{xs} was calculated to be in the range 0.06 to 0.08 g VSS.(g COD_r)$^{-1}$ for both CDR2 and CDR3, significantly lower than values observed in other studies. In the semi-continuous digesters the biomass yield ranged from 0.16 to 0.25 g VSS.(g COD_r)$^{-1}$, Hansson's data (1979) indicated a value of 0.2 g VSS.(g COD_r)$^{-1}$, and Speece and McCarty (1964) observed yields in the range 0.11 to 0.35 g VSS.(g COD_r)$^{-1}$ for glucose-fed digesters operating with retention times ranging from 5 to 30 days. A review of kinetic parameters by Henze and Harremoes (1983) indicated an average value for Y_{xs} of 0.18 g VSS.(g COD_r)$^{-1}$.

For the greater part of the continuous digestion fermentations the biomass concentration also decreased, despite operation at retention times exceeding the minimum reported generation times for the methanogens (Lawrence and McCarty 1969). At various times, but particularly after reinoculation, an increase in VSS concentration was noted; this was attributed to the low loading rates employed during start-up and also to the rich supply of nutrients that the complex inocula used were expected to provide. The reason for the increase in VSS during CDR2 (from about day 82, Figure 4.2) is not clear but may have been due to growth of non-methanogenic organisms utilising acetate and other intermediates present at high concentrations.

Analysis of the specific rate data for COD removal and methane production also supports a nutrient limitation theory as the

cause of failure. Prior to acid accumulation, $CODR_s$ and Met_s were in the range 0.6 to 0.8 g COD_r.(g VSS)$^{-1}$.day^{-1} and 0.2 to 0.4 l CH_4.(g VSS)$^{-1}$.day^{-1} respectively. A comparison with the data in Table 4.2 clearly shows these rates are characteristic of cultures growing at close to their maximum growth rate, yet the retention times employed were very much greater than the minimum values normally reported for acetate degradation or methanogenesis of soluble substrates (Speece and McCarty 1964, Lawrence and McCarty 1969, Henze and Harremoes 1983).

Further supporting evidence for nutrient limitation was the observation by Speece and McCarty (1964) that acid accumulation was indicative of nutrient deficiency in their mixed-culture studies. This has since been reiterated in the recent review by Speece (1983). Unfortunately this important observation from the earlier paper was not noted until late in this project, when the differences between the two modes of operation became glaringly apparent and the batch experiment and comparison of various media led the author to the same conclusion. Significantly no other reports of this behaviour could be located in the literature yet the importance of rapidly identifiying causes for failure is vital to successful operation of digesters. Such observations must be disseminated more widely.

The success of the semi-continuous digesters indicated that the requisite growth factors were present in these experiments and the long interval between medium addition could be the reason for this. It was expected that acidogenesis would proceed very rapidly and the rapid conversion of glucose was confirmed experimentally for both digesters. The acidogenic bacterial population would increase with glucose utilisation and during the subsequent period of methanogenesis, lysis of a proportion of these organisms could have been the source of growth factors for the methanogenic bacteria. In the continuously-fed digesters cell lysis would be reduced and nutrients would be less available from this source unless a portion of the effluent biomass were

recycled back to the digester. Stander (1964) noted "the process of lysis in bacterial reactions is known to liberate nutrient elements and food material in suitable form for assimilation in the exogenous phase" and in the preliminary batch experiment reported above (Section 4.3.4), addition of lysed cell material increased the rate of acetate degradation in effluent from the continuous digester (CDR3).

Recycle of digester solids or supernatant to promote good digestion has been practised by Stander (1950), Stander and Snyders (1950) and McCarty and Vath (1963), among others. Stander (1950) believed reinoculation was the important mechanism, but in other work only growth factors were thought to be provided (McCarty and Vath 1963). The experience of both the author and these workers tends to confirm Stander's belief (1964) that "the establishment of a matured sludge seems to be the building up of a healthy balance between exogenous (cell synthesis) and endogenous (cell lysis i.e. breakdown) enzymic reactions in the breakdown of organic substrate". Stander (1964) further recommended that this could be achieved best by operating at extended retention times with a high organic loading rate. As these are typical operating conditions for such high rate systems as the UASBR and AFFBR processes, it would be interesting to determine to what extent cell lysis contributes to the observed stability of these systems.

Alternatively the improved nutritional status of the semi-continuous digesters may have arisen from luxury uptake of nutrients due to the pattern of excessive substrate available alternating with starvation (Speece 1983). Luxury uptake describes the uptake of a nutrient in excess of the rate indicated by the normal metabolic requirement of a microbial population (Levin and Shapiro 1965). This phenomenon has been the subject of much study in the context of phosphorous removal in activated sludge plants (Levin and Shapiro 1965, Barnard 1983, Marais et al. 1983) but has not been studied in anaerobic

digestion. In view of the results reported in this chapter, further research effort in this area would appear profitable

In summary, the results of the author's experiments suggested a deficiency in the medium of essential nutrients was the cause of failure of the continuous digester and that semi-continuous digestion may provide enhanced stability when operating with such media. After comparing the composition of the medium employed in these experiments with that of media used by other investigators (Speece and McCarty 1964, Mah et al. 1978, Cohen et al. 1979, McInerney et al. 1981) it seemed possible that iron, cobalt and manganese under some conditions may limit growth. However, growth of methanogens in pure culture was not dependent on manganese (Schonheit et al. 1979) while the requirement for cobalt can be satisfied by the yeast extract included in the formula (Mah et al. 1978, Schonheit et al. 1979). In contrast, iron was shown to stimulate acetate degradation in digesters by several workers (Speece and McCarty 1963, Van den Berg et al. 1980) and Speece and McCarty (1963) considered this discovery the turning point in their study on digester nutrition.

The preliminary batch experiment indicated the required nutrients were present in lysed cell material, but a distinction between the possible role of iron or other growth factors in stimulating acetate degradation could not be made. Further work is necessary to positively identify the nutrients limiting digestion of the modifed Hansson (1979) medium and also of methane fermentations in general. While this was within the scope of this project, mechanical failures subsequent to the experiments reported above resulted in the death of two of the three digester populations. Consequently, insufficient time was available to re-establish these populations and then pursue further investigations in this area.

The establishment of stable digestion with a reasonable biomass yield during the work of Hansson (1979) indicates that all

necessary growth factors were provided in those experiments. In the author's work the digesters were commissioned quite rapidly in contrast to Hansson (1979) who developed an acclimated population by maintaining the digester at a very low OLR_v for over a year. In most circumstances this would be impractical and unnecessary and certainly the semi-continuous experiments reported above indicate that successful digestion can be accomplished rapidly if the environment is adequate. However in a system stressed by an imbalance, such long acclimation periods may be necessary to develop a healthy population. This is supported by the gradual increase in VSS noted in CDR3 at retention times exceeding 25 days.

However it is still interesting to note the results of Hansson and Molin (1981a) who reported on a continuous digestion experiment at a low retention time (Figure 4.12). The medium employed was similar to that used by Hansson (1979) but acetate replaced glucose as the major carbon and energy source and, importantly, the concentrations of all other components except yeast extract and $MgSO_4.7H_2O$ were at least doubled. Propionate began to accumulate on day 8 when the retention time was 10 day, eventually stabilising at about 500 $mg.l^{-1}$. On day 9 the retention time was lowered to 6 to 7 day and on day 21 a gradual increase in acetate concentration was noted. The accumulation of acetate increased rapidly from day 32 but was alleviated on day 36 when pump failure resulted in a considerably reduced loading rate. On resumption of medium addition the digester failed within two days with an acetate concentration in excess of 2000 $mg.l^{-1}$. During this whole period of acetate accumulation the loading rate (OLR_v) was approximately 1.6 g $COD.l^{-1}.day^{-1}$, one half of that used by Hansson (1979).

This pattern of failure was very similar to that observed in the author's experiments reported above and suggests that a similar mechanism could be involved. Hansson and Molin (1981a) thought it possible that air leakage into the digester was responsible

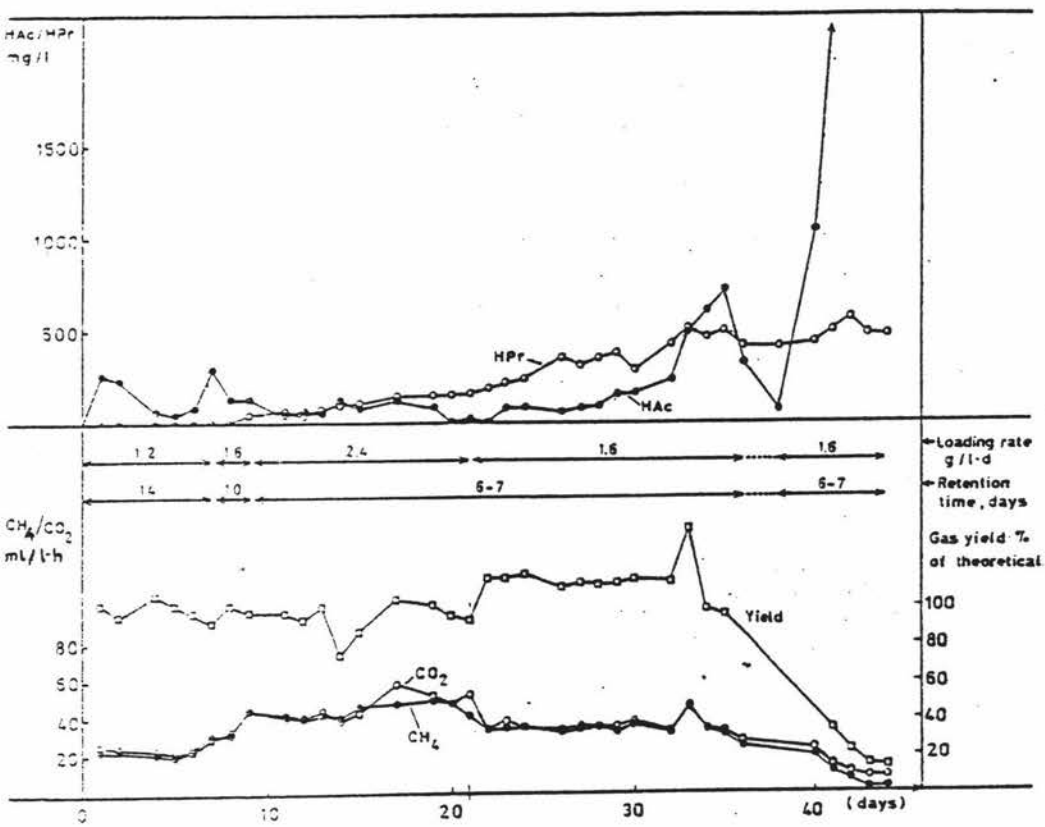


Figure 4.12: Data from Hansson and Molin (1981a) showing methane production from acetate under a nitrogen atmosphere.

for failure but this did not appear to be the case in the author's experiments. This belief was based on a thorough examination of procedures and equipment, the failure of added reducing agents to consistently stimulate digestion, and the continued low redox potential during the onset of acid accumulation. As the author's results suggest the inhibition of methanogenesis observed by Hansson (1979) and Hansson and Molin (1981a) could be attributed to nutrient limitation, it is desirable that further experiments be conducted to verify their hypothesis of the inhibitory role of carbon dioxide.

Three further important observations were made during this work. Firstly, during the initial 100 days of CDR2 it was apparent that the three periods of degradation of VFA from high concentration was characterised by increasingly slow utilisation of the accumulated acid (Figure 4.2). This trend was most pronounced for acetic acid and indicated that repeated stress by increased organic loading had an increasingly detrimental effect on methane formation. This behaviour contrasted with that observed by Cohen et al. (1982) who found successive shock loading of a one-phase digester by glucose resulted in an increase in the specific rates of acid degradation. The behaviour observed in CDR2 could thus be a further consequence of the apparent nutritional deficiency.

Knowledge of how a system responds to shock loads is important as improved process stability is of vital concern to encouraging the wider acceptance of anaerobic waste treatment (Speece 1983). Reports of such effects still appear relatively infrequently in the literature and given the marked differences observed between the author's work and Cohen et al. (1982), further study of the shock loading of digesters appears warranted.

Secondly, during periods of high acetate concentration, propionate degradation appeared retarded. This was particularly suggested by data from CDR2 (Figure 4.2). During the period 0 to 16 days and from day 26 when the acetate concentration was low,

propionate utilisation was significantly higher than during the intermediate period from day 16 to 26 when acetate was present at greater than 1500 $mg.l^{-1}$. Further, after day 82 when acetate was degraded only slowly from approximately 1700 $mg.l^{-1}$, propionate accumulated in the digester and was only utilised once the acetate concentration had fallen below 500 $mg.l^{-1}$. Product inhibition was indicated and a series of batch digestion experiments were performed to further investigate this phenomenum. The results of this work are reported in Chapter Five.

Finally the apparent stimulation of acetate utilisation by cysteine hydrochloride and inhibition of methane production by a mixture of cysteine and sodium sulphide was interesting. Although no lasting improvement in performance was observed in the continuous runs, a number of batch digestion experiments were run to further evaluate the effect of sulphur-containing reducing agents on the methane fermentation and the results of this work are presented in Chapter Six.

4.5 CONCLUSIONS

A comparison was made of a digester operating with continuous feeding and units slug fed every second day and possible causes of failure of the continuous digester were investigated. The semi-synthetic medium of Hansson (1979) was employed in these studies and two inoculum sources, cow manure and digested sewage sludge, were used.

For both inocula it was not possible to establish stable operation in a continuous digester even when the retention time exceeded 25 day, corresponding to an OLR_v of approximately 0.8 g $COD.l^{-1}.day^{-1}$. The digester repeatedly failed, with the onset of instability characterised by a steady accumulation of acetate. The temperature and pH control systems were operating well at the time of failure, redox potential was low and constant, and from a

comparison with other reported work, the biomass was observed to be very active. However the biomass yield coefficient of 0.08 g VSS.(g $COD_r)^{-1}$ was much lower than that reported in other comparable studies or observed in the semi-continuous work.

In contrast a semi-continuous digester operating at the same volumetric loading rate and using the same inoculum and medium performed very well. Stable operation was easily established and maintained and the digester could also be readily recovered from retarded conditions arising from a step increase in the loading rate. In this case retarded operation was characterised by an increase in the propionate concentration while acetate did not accumulate to any marked extent. The biomass yield coeffient of 0.18 to 0.25 g VSS.g $COD_r)^{-1}$ was similar to other values reported (Speece and McCarty 1964, Henze and Harremoes 1983).

These results suggested the continuous reactor failed as an active microbial population could not be retained within the vessel. The growth rates and biomass yield both appeared to be limited by a nutritional deficiency specific to the acetoclastic methanogens, however the limiting components could not be positively identified. The stability of the semi-continuous digesters was believed to arise either from cell lysis releasing nutrients or from luxury uptake of the required growth factors as a result of the pattern of excess substrate alternating with substrate depletion.

CHAPTER FIVE DEGRADATION OF VOLATILE FATTY ACIDS IN THE METHANE FERMENTATION

5.1 INTRODUCTION

Results from the continuous and semi-continuous digestion experiments reported in Chapter Four confirmed that acetate and propionate were the major volatile fatty acids present during anaerobic digestion of a semi-synthetic medium based on glucose. During periods of satisfactory digester performance the concentrations of acetate and propionate were low, suggesting utilisation of both acids, however propionate degradation appeared to be markedly reduced when the acetate concentration rose above 1000 to 1500 $mg.l^{-1}$. This suggested product inhibition of the acetogenic bacteria by acetate and a subsequent search of the literature also revealed several observations attesting to this possibility (Kaspar and Wuhrmann 1978b, Boone and Bryant 1980, Zehnder and Koch (1983), although no systematic study of the phenomenon had been reported. Tesing such a hypothesis may allow understanding of the methane fermentation in general and the behaviour of the continuous digester as reported in the Chapter Four. A series of batch experiments were therefore performed to provide preliminary information on acetate and propionate inhibition of anaerobic digestion. The results of this work are now reported.

5.2 EXPERIMENTAL METHOD

5.2.1 Equipment

Two experiments were performed involving a total of 19 individual batch runs. Flask digesters with a working volume of 250 ml were used in both experiments and these are described in Section 3.5.1.2.

5.2.2 Inoculum sources and preparation

Digester effluent was the seed material for the experiments and this was collected under a N_2-atmosphere to minimise contact of the microbial flora with air. The inoculum source for the initial experiment was effluent from the continuous digester, run 2 (CDR2). The digester appeared to be operating well at the time of seed collection but had recently experienced high concentrations of both acetic and propionic acids (1540 and 1260 $mg.l^{-1}$ respectively). Two 1 l effluent samples were taken and incubated at 37 °C until the individual acid concentrations had fallen to below 40 $mg.l^{-1}$. The samples were then combined to form the inoculum source for experiment 1.

Effluent from the semi-continuous Microferm digester (SCDR1) was used in experiment 2. As the VFA concentration was very low and stable in this effluent, further treatment prior to use was unnecessary.

5.2.3 Culture conditions and preparation

Standard solutions of each acid were prepared containing either 10 $g.l^{-1}$ of acetate or propionate or 100 $g.l^{-1}$ acetate, and aliquots of these were added to each flask digester by pipette to give the nominal concentrations listed in Table 5.1.

For experiment 1, the inoculum was used without dilution. The acid substrate was neutralised to pH 7.0 with 2 N sodium hydroxide and added to 250 ml of inoculum. The cultures were then flushed with a mixture of 5 % carbon dioxide in nitrogen (treated to remove all traces of oxygen) and incubated at 37 $\pm$ 1 °C. Run 7 was established with acid conditions duplicating those in run 4 to provide an indication of the reproducibility of the results.

Table 5.1: Experimental conditions for the batch digestion experiments.

Experiment	Run	Nominal initial acid concentration	
		Acetate	Propionate
		$mg.l^{-1}$	$mg.l^{-1}$
1	1	0	500
	2	500	500
	3	1000	500
	4	2000	500
	5	2000	0
	6	2000	250
	7	2000	500
	8	2000	1500
2	1	0	500
	2	500	500
	3	500	500
	4	1000	500
	5	2000	500
	6	2000	0
	7	2000	0
	8	2000	250
	9	2000	500
	10	2000	1500
	11	2000	1500

In the second experiment, the semi-continuous digester inoculum was diluted to allow more runs to be conducted at the same time. Fifty ml of the inoculum was added to approximately 150 ml of freshly distilled water, the appropriate volumes of substrates added, and then the digester was made up to volume with more water. The substrate was again neutralised, but in this experiment an equimolar mixture of 2N sodium hydroxide and potassium hydroxide was used to reduce potential inhibition arising from addition of the cations (Kugelman and Chin 1971). The flask digesters were flushed with 20 % carbon dioxide in nitrogen and again incubated at 37 $\pm$ 1 °C. The acid concentrations were duplicated in four instances - runs 2 and 3, runs 5 and 9, runs 6 and 7, and runs 10 and 11 (Table 5.1).

5.2.4 Sampling and analytical procedures

The digester liquor was sampled immediately after preparation and then either every second or fourth day. A 5 ml sample was removed and the VFA concentration determined by gas chromatography following the method outlined in Section 3.2.3. The pH value of each sample was also noted.

5.2.5 Analysis of results

Within each experiment two sets of runs were performed. In the first, propionate was added at a constant initial level (Pr_i) and the concentration of added acetate (Ac_i) was varied; in the second set of runs the procedure was reversed. The effect of the varying concentration of one acid on the utilisation of the other was of most interest and, as an aid to quantifying this effect, the data were analysed using a non-linear regression program run on the university's Prime 750 computer. The optimisation option of the general statistical package GENSTAT (Version 4.04B; Lawes Agricultural Trust 1980) was used for this and the model selected to describe substrate utilisation was the general logistic equation (E2.15) described in section 2.3.4.

This model was chosen because the very limited information available on the fermentation (i.e. substrate concentration only) made the exercise one of curve-fitting rather than prediction of kinetic parameters. Some consistency was apparent in the kinetic parameters reported in the literature for the methane fermentation (Section 2.3.4, Henze and Harremoes 1983) and average values from this source could have been substituted into more complex models such as the Monod equations. However in the absence of definite information on biomass yield and total biomass concentration, let alone the actual concentrations of the metabolic groups of interest (the acetogenic and methanogenic bacteria), this approach did not appear to offer advantages over that adopted.

However the value of the coefficient b was significant as this is equivalent to the maximum specific rate of substrate utilisation. Further the volumetric rate of substrate utilisation could also be predicted from equation 2.16 (Section 2.3.4) using the fitted model (Edwards and Wilke 1968).

In two runs acid degradation appeared linear with time and in these cases the data were analysed by simple linear regression using the MINITAB statistical package, again run on the Prime 750 computer.

5.3 RESULTS

5.3.1 Experiment 1

The concentrations of acetic and propionic acids observed during experiment 1 are listed in Table A3.1 of Appendix Three. Data on pH are not shown as the values recorded in all digesters were similar and do not add anything further to this discussion.

5.3.1.1 Effect of acetate on utilisation of propionic acid

For runs 1 to 4 and run 7 the inital propionate concentration was maintained approximately constant, ranging from 520 $mg.l^{-1}$ to 600 $mg.l^{-1}$, while the acetate concentration was varied from less than 10 $mg.l^{-1}$ (run 1, acetate not detected) to 1960 $mg.l^{-1}$ (run 7). The measured propionate concentrations for runs 1 to 4 are plotted in Figure 5.1 and for runs 4 and 7 in Figure 5.2. The coefficients estimated by fitting the logistic equation to these data are recorded in Table 5.2. An example GENSTAT program and the output listing from this are shown in Appendix Four. The predicted propionate concentration profile for runs 2 and 4 are plotted together with the observed data in Figure 5.3 and showed good agreement.

Both Figures 5.1 and 5.3 show that the rate of propionate utilisation was reduced at elevated acetate concentrations. When the initial acetate concentration was less than 600 $mg.l^{-1}$ about 8 to 10 days was required to achieve at least 90 % utilisation of the propionic acid; when Ac_i was increased to 1960 $mg.l^{-1}$, at least 15 days was required to achieve the same level of degradation. This is also confirmed by the trend in the value of the predicted specific rate coefficient, b (Table 5.2). This increased from 0.72 day^{-1} to 0.94 day^{-1} as Ac_i was raised from less than 10 $mg.l^{-1}$ (run 1) to 510 $mg.l^{-1}$ (run 2). Increasing Ac_i to 1240 $mg.l^{-1}$ reduced this to 0.49 day^{-1} (run 3) and further increasing Ac_i to approximately 2000 $mg.l^{-1}$ lowered the value of the coeffient to 0.37 and 0.41 day^{-1} for runs 4 and 7 respectively.

The volumetric rates of propionate degradation were also calculated from the fitted model and the data are listed in Table A3.2. The curves for runs 2 and 4 are plotted in Figure 5.4 and these provide further confirmation of the reduction in propionate utilisation as Ac_i was increased. The highest rate of utilisation observed in run 2 was 1330 $mg.l^{-1}.day^{-1}$ at day 5. In run 4 the

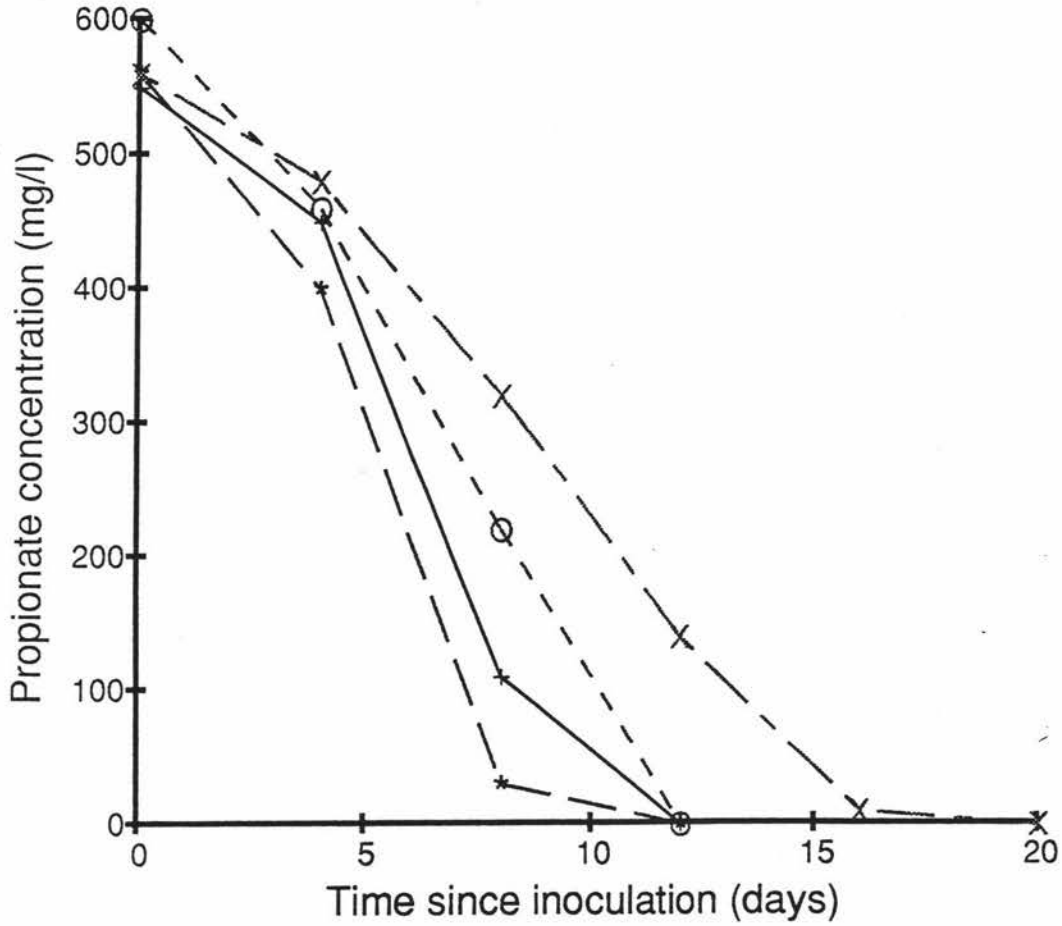


Figure 5.1: Propionate utilisation in selected runs with propionate added at 500 mg/l, experiment 1. Run 1 (+); run 2 (*); run 3 (o); run 4 (x).

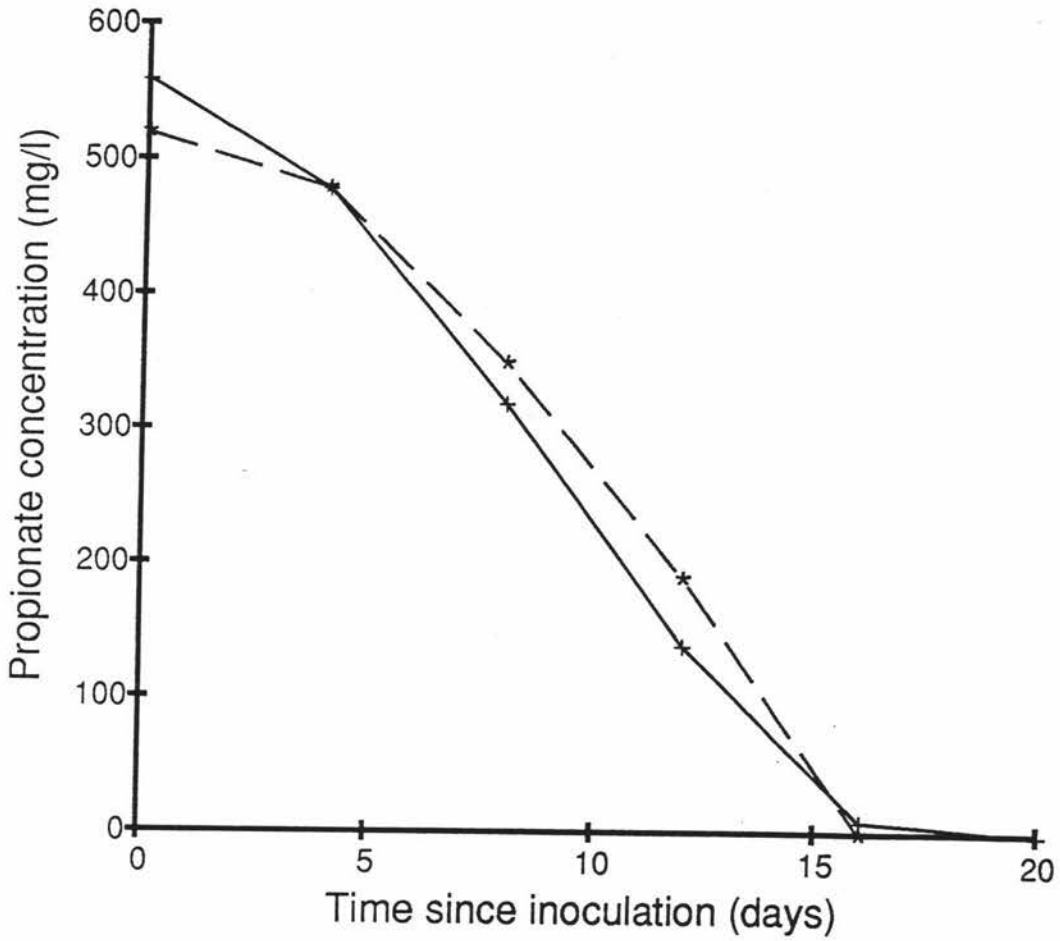


Figure 5.2: Propionate utilisation in duplicate runs with propionate added at 500 mg/l, experiment 1. Run 4 (+); run 7 (*).

Table 5.2: Coefficients of the logistic[i] equation for acid utilisation in experiment 1.

Acid	Run	K $g.l^{-1}$	a	b day^{-1}
acetic[ii]	1	0.45	-3.97	0.57
	2	1.10	-1.95	0.49
	3	1.86	-2.74	0.40
	4	2.62	-2.58	0.32
	5	4.09	0.10	0.30
	6	2.47	-2.00	0.32
	7	2.58	-2.54	0.28
	8	3.64	-2.73	0.27
propionic	1	0.56	-4.73	0.72
	2	0.57	-4.65	0.94
	3	0.62	-3.20	0.49
	4	0.58	-3.21	0.37
	5[iii]	-	-	-
	6	0.31	-2.57	0.49
	7	0.52	-4.14	0.41
	8	1.66	-4.77	0.37

i rounded to 2 decimal places. The program predicts the coefficients to 5 decimal places.
ii based on calculated potential acetate pool concentrations.
iii insufficient data for analysis.

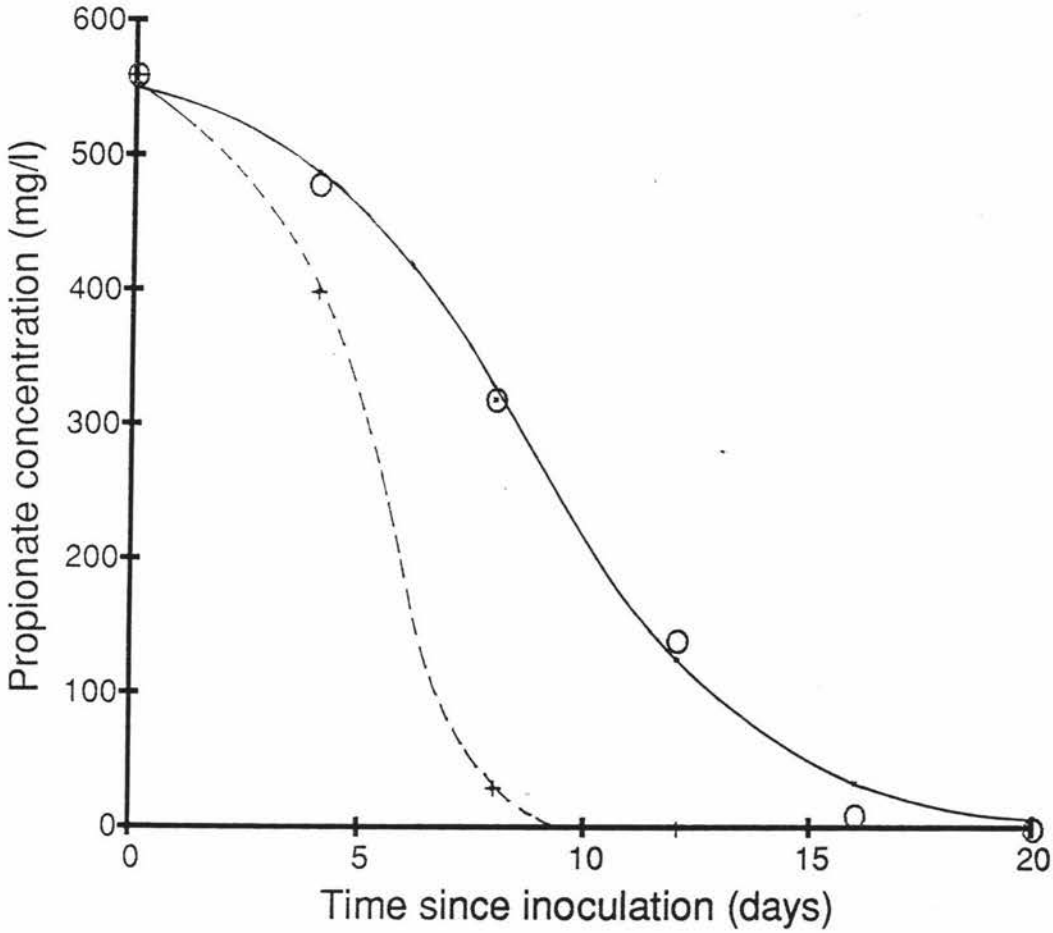


Figure 5.3: Observed and predicted propionate concentrations for selected runs, experiment 1. Run 2, obs. (+); run 2, pred. (---); run 4, obs. (o); run 4, pred. (—).

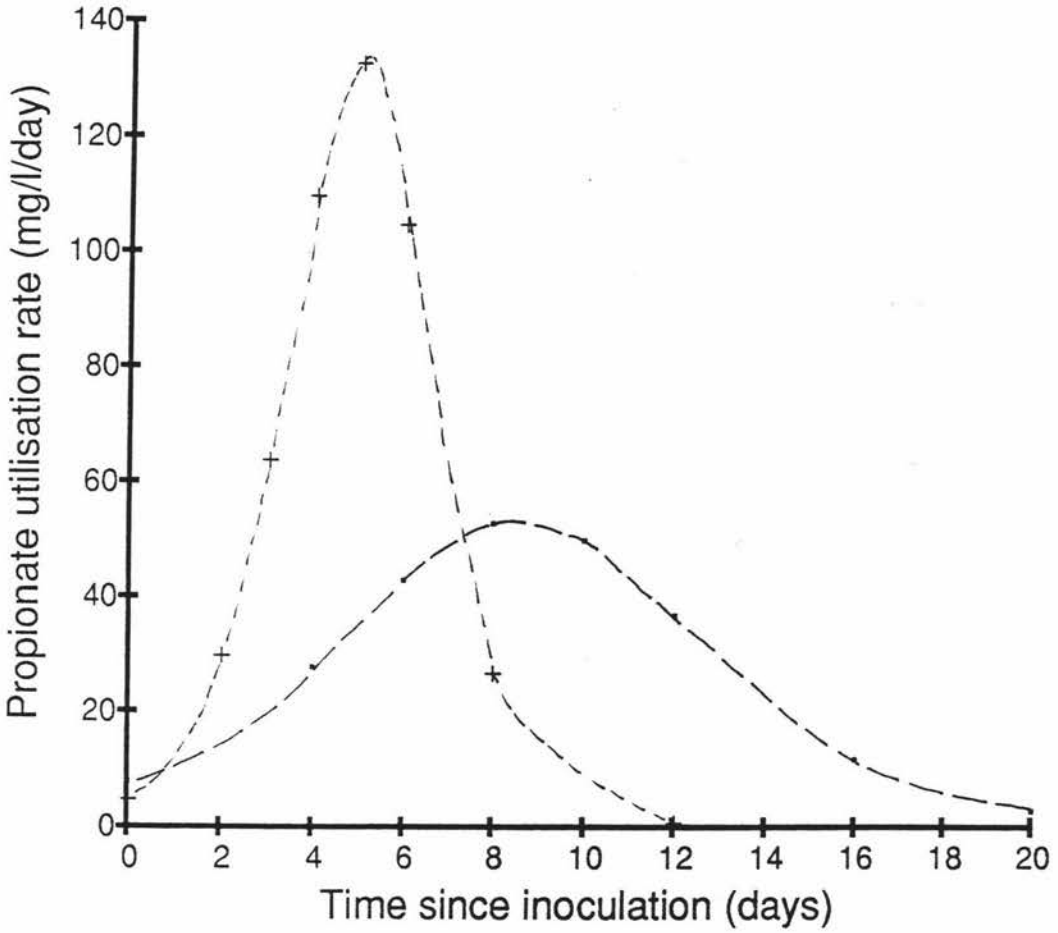


Figure 5.4: Volumetric rate of propionate utilisation in selected runs with propionate added at 500 mg/l, experiment 1. Run 2 (---); run 4 (- -).

maximum rate of utilisation had fallen to 54 $mg.l^{-1}.day^{-1}$ and this occurred later in the fermentation at day 9.

In Figure 5.5 the potential acetate pool during the course of digestion is shown for runs 1 to 4. The potential acetate pool at a given time was calculated as the sum of the actual acetate concentration and the acetate concentration that would result from the complete degradation of the propionate present at that time. This data is also recorded in Table A3.1 and coefficients of the model fitted to these data are listed in Table 5.2. These latter data indicate that as the acetate pool increased from 450 $mg.l^{-1}$ to over 2000 $mg.l^{-1}$ there was a drop in the maximum specific rate of utilisation from 0.57 day^{-1} to approximately 0.3 day^{-1}. The predicted acetate pool for runs 2 and 4 are compared with the observed concentrations in Figure 5.6.

Overall the data recorded in the tables and figures indicate both the fit of the model and the agreement between the duplicate runs was satisfactory.

5.3.1.2 Effect of propionate on utilisation of acetic acid

The acetate concentration was maintained at approximately 2000 $mg.l^{-1}$ in runs 4 to 8 while Pr_i varied from 80 $mg.l^{-1}$ (run 5) to 1700 $mg.l^{-1}$ (run 8). Runs 4 and 7 were duplicates with initial propionate concentrations of 560 and 520 $mg.l^{-1}$ respectively. The potential acetate pool for all these runs was calculated and the data are shown in Table A3.1. The fitted coefficients to the logistic model are listed in Table 5.2 and show that the specific rate of acetate utilisation was very similar in all runs.

The fitted coefficients for the propionate data are also shown in Table 5.2. At the lowest concentration for which sufficient data were available for analysis (290 $mg.l^{-1}$, run 6), the maximum specific rate of propionate utilisation was 0.49 day^{-1}. As the acid concentration was raised to 500 $mg.l^{-1}$ this was reduced to

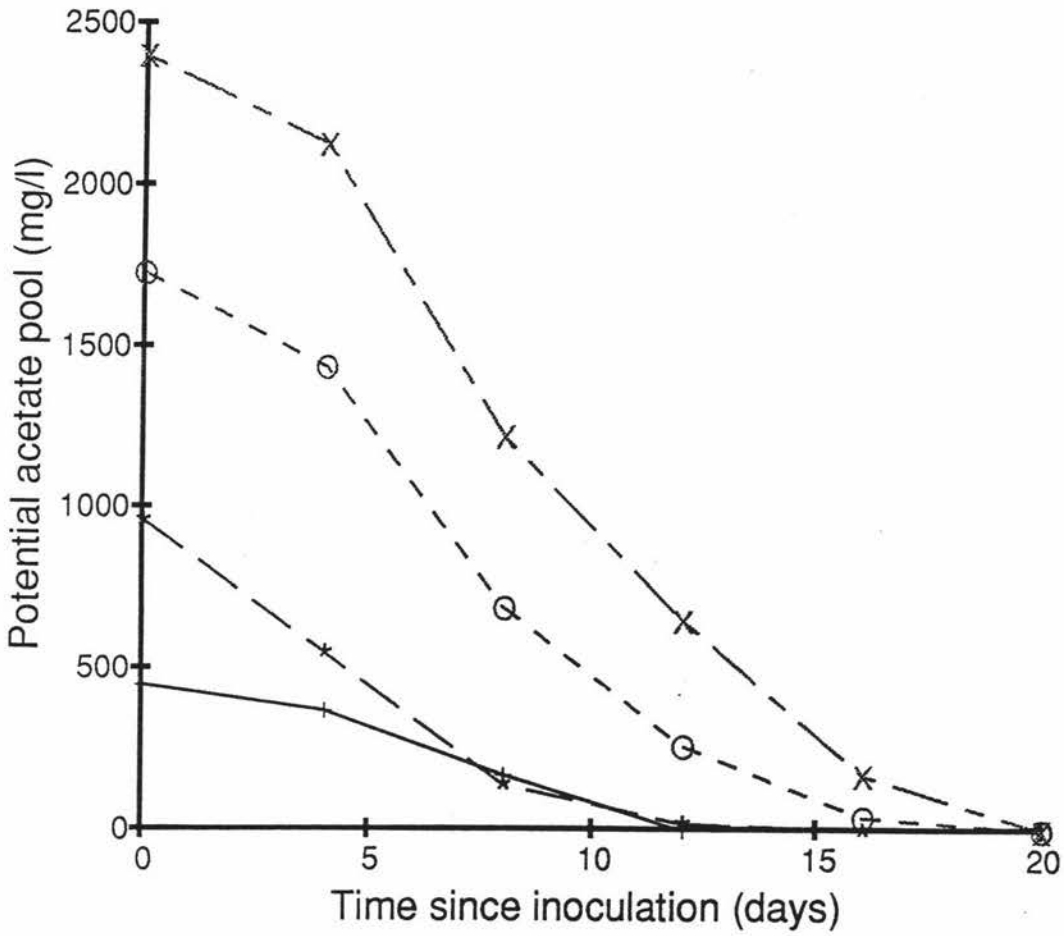


Figure 5.5: Acetate utilisation in selected runs with propionate added at 500 mg/l, experiment 1. Run 1 (+); run 2 (⊹); run 3 (o); run 4 (x).

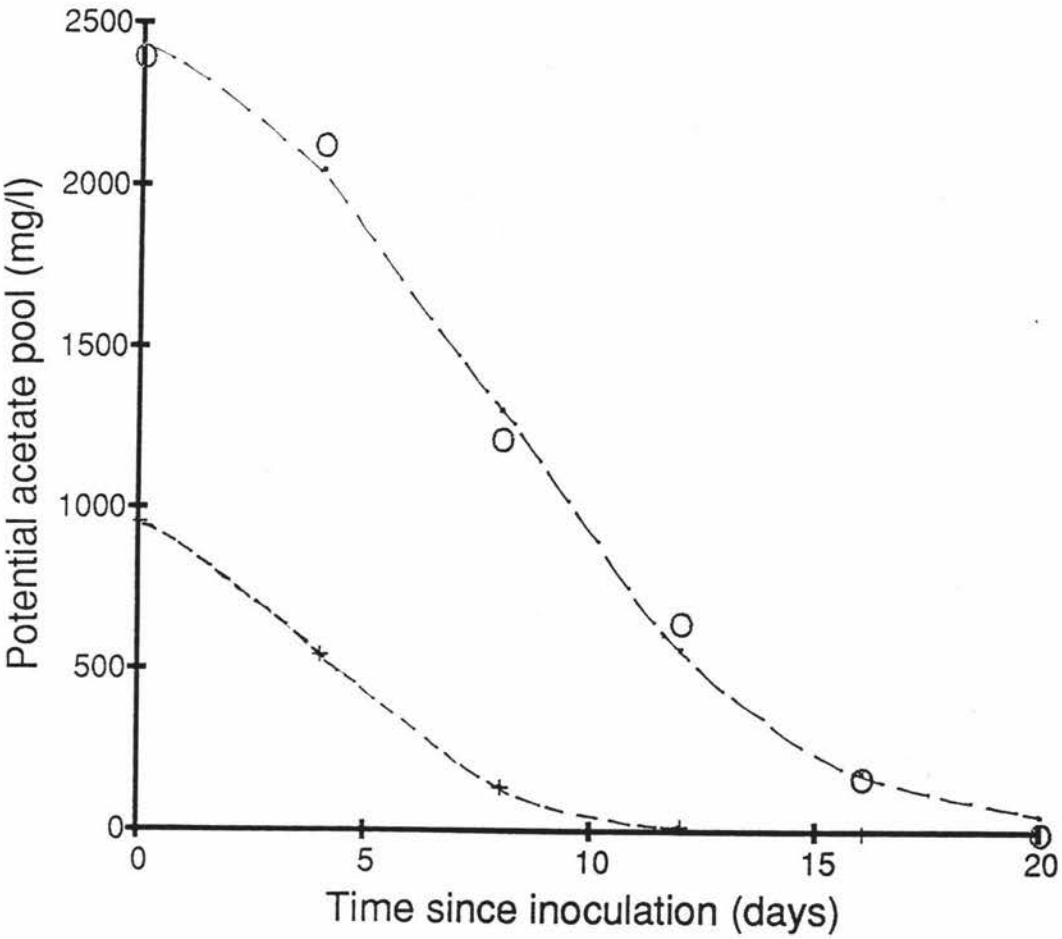


Figure 5.6: Observed and predicted potential acetate pool concentrations for selected runs, experiment 1. Run 2, obs. (+); run 2, pred. (---); run 4, obs. (o); run 4, pred. (- -).

approximately 0.4 day^{-1}, but further increasing the propionate concentration did not adversely affect the specific utilisation rate.

5.3.2 Experiment 2

The acid concentration data for experiment 2 are shown in Table A3.3. Data for pH are not shown but close agreement between runs was again observed.

5.3.2.1 Effect of acetate on utilisation of propionic acid

Propionate was added at 500 $mg.l^{-1}$ in runs 1 to 5 and run 9, with runs 2 and 3 and runs 5 and 9 established with duplicate acid concentrations. The propionate concentrations for runs 1, 2, 4 and 5 are shown in Figure 5.7 and the fitted coefficients for all the runs are listed in Table 5.3. No acetate was added in run 1 and propionate degradation proceeded at a fast rate (b = 1.12 day^{-1}) with a 90 % reduction in the initial level (Pr_i) achieved within 6 days after inoculation. Ac_i was increased in each subsequent run and it is clear from Figure 5.7 that this inhibited propionate degradation. The time required to achieve 90 % utilisaton increased to 7 days in run 2 (Ac_i = 520 $mg.l^{-1}$), to 8 days in run 4 (Ac_i = 1060 $mg.l^{-1}$) and to 11 days for run 5 (Ac_i = 2100 $mg.l^{-1}$). For runs 2 and 4 the maximum specific rates of propionate utilisation were similar at 0.8 day^{-1}, but this was lowered significantly in run 5 to 0.47 day^{-1}.

Data for the duplicate runs are shown in Figure 5.8 and the observed and predicted data for runs 2 and 5 are plotted in Figure 5.9. As in experiment 1 these confirm the fit of the model and the agreement between the duplicates was acceptable, although the propionate concentration in run 9 was always approximately 50 $mg.l^{-1}$ higher than that in run 5. Values of the fitted coefficients were also very similar in the duplicate runs. (Table 5.3)

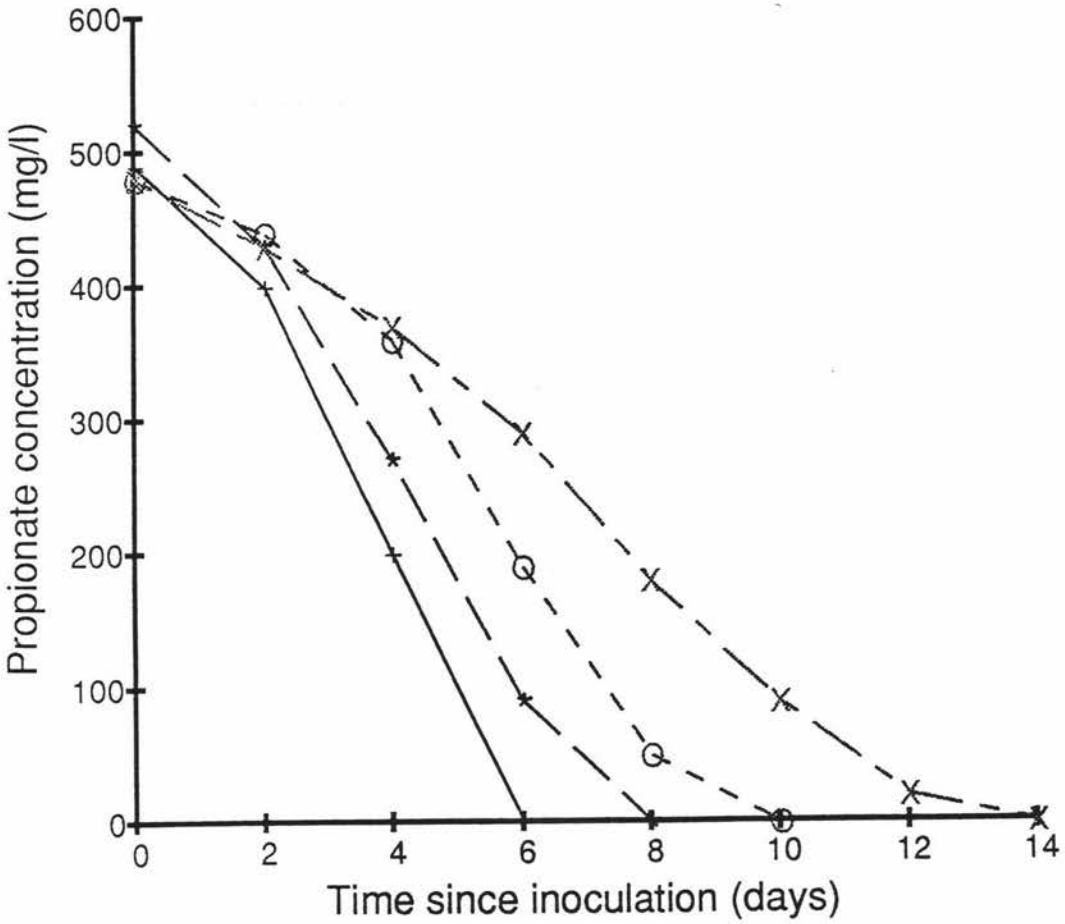


Figure 5.7: Propionate utilisation in selected runs with propionate added at 500 mg/l, experiment 2. Run 1 (+); run 2 (*); run 4 (o); run 5 (x).

Table 5.3: Coefficients of the logistic equation for acid utilisation in experiment 2.[i]

Acid	Run	K $g.l^{-1}$	a	b day^{-1}
acetic[ii]	1	0.41	-3.08	0.86
	2	1.04	-2.24	0.64
	3	1.10	-1.81	0.65
	4	1.52	-3.10	0.61
	5	2.61	-2.89	0.42
	6	2.36	-2.75	0.35
	7	2.49	-1.73	0.31
	8	2.54	-2.56	0.35
	9	2.71	-3.17	0.41
	10	4.08	-1.62	0.14
	11	4.12	-1.47	0.13
propionic	1	0.49	-3.99	1.12
	2	0.54	-3.15	0.80
	3	0.53	-2.97	0.82
	4	0.46	-6.30	0.84
	5	0.49	-3.19	0.47
	6[iii]	-	-	-
	7[iii]	-	-	-
	8	0.22	-3.47	0.64
	9	0.52	-3.35	0.45
	10[iv]	-	-	-
	11[iv]	-	-	-

i rounded to 2 decimal places. The program predicts the coefficients to 5 decimal places.
ii based on calculated potential acetate pool concentrations.
iii insufficient data for analysis.
iv data fitted by simple linear regression (see text)

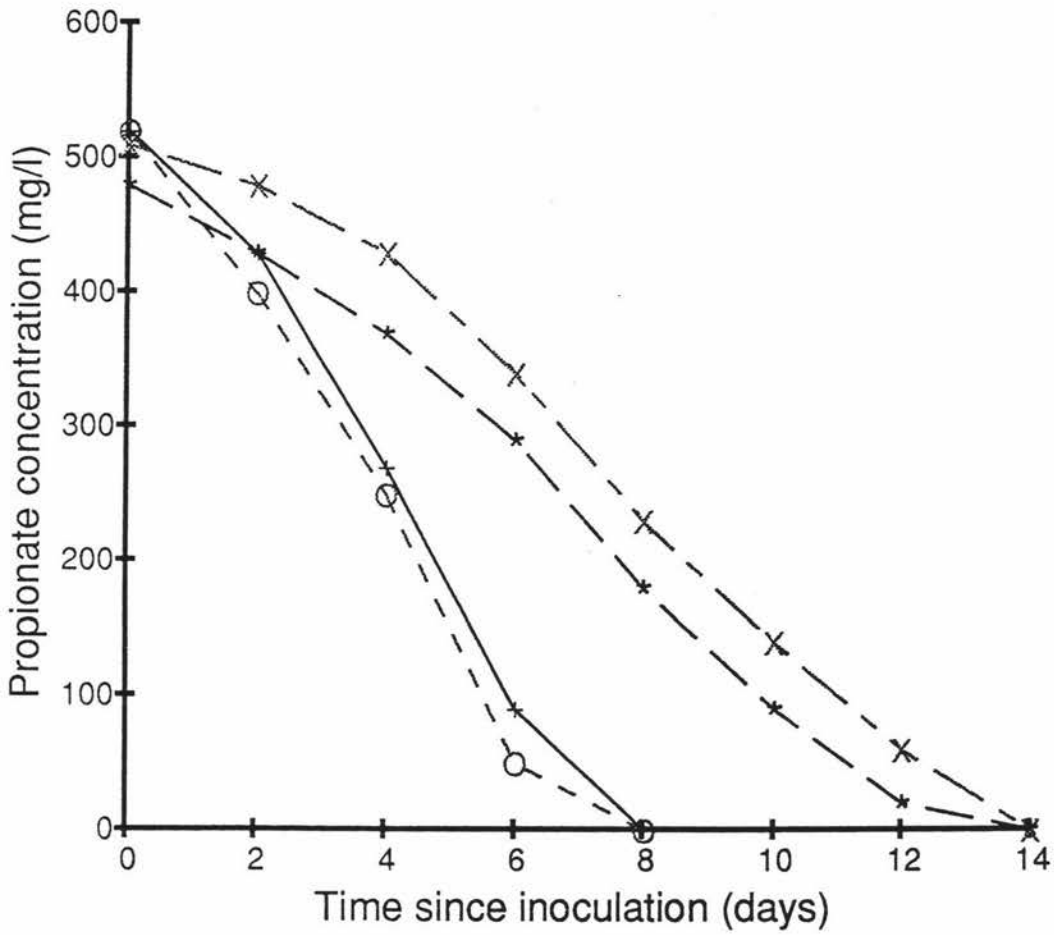


Figure 5.8: Propionate utilisation in duplicate runs with propionate added at 500 mg/l, experiment 2. Run 2 (+); run 3 (o); run 5 (*); run 9 (x).

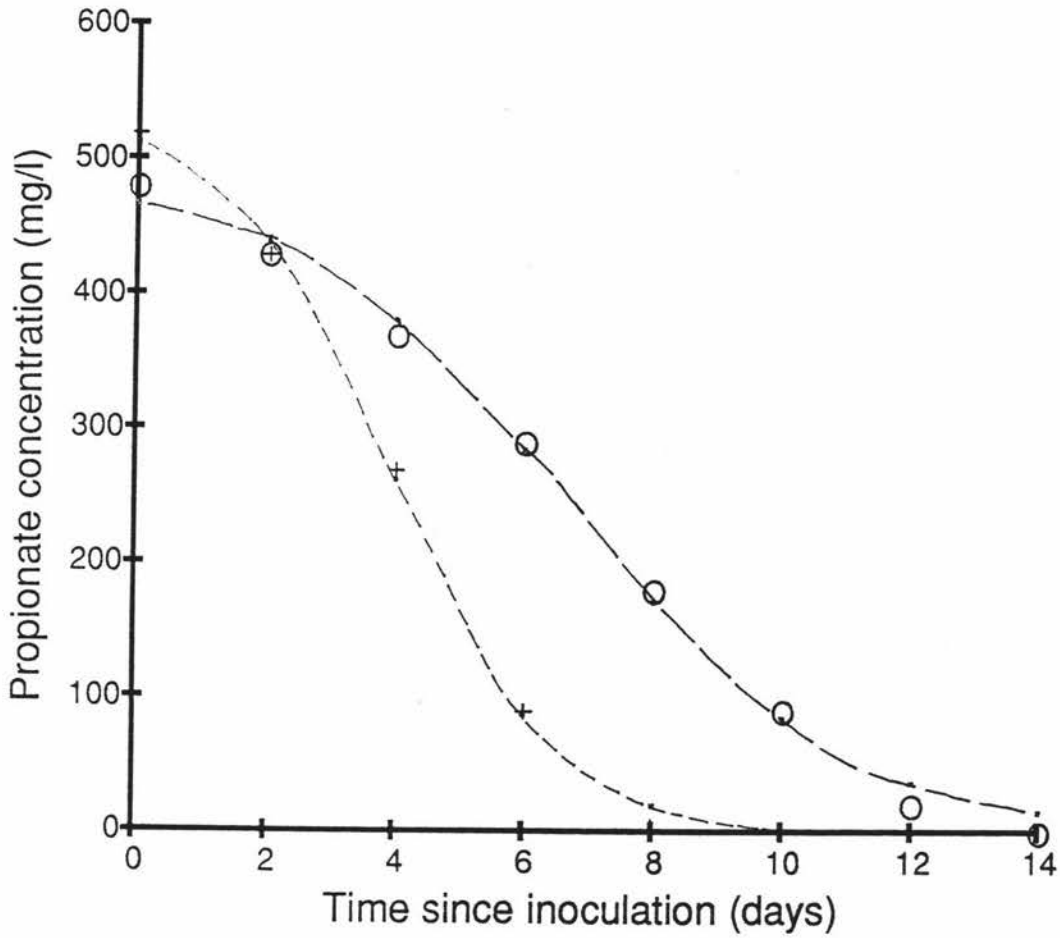


Figure 5.9: Observed and predicted propionate concentrations for selected runs, experiment 2. Run 2, obs. (+); run 2, pred. (---); run 5, obs. (o); run 5, pred. (- -).

The potential acetate pool during runs 1, 2, 4 and 5 are shown in Figure 5.10 and data for all runs are listed in Table A3.3; coefficients for the fitted model are listed in Table 5.3. The overall trend was similar to that observed in experiment 1: as the potential acetate pool increased from 400 $mg.l^{-1}$ (run 1) to 2500 $mg.l^{-1}$ (runs 5 and 9) the maximum specific rate of acetate utilisation was reduced from 0.86 day^{-1} to 0.41 day^{-1}. Observed and predicted data for both sets of duplicate runs are plotted in Figures 5.11 and 5.12 and show good agreement.

5.3.2.2 Effect of propionate on utilisation of acetic acid

Acetate was added at approximately 2000 $mg.l^{-1}$ in runs 5 to 11 while the propionate concentration was varied between less than 10 $mg.l^{-1}$ (runs 6 and 7) and 1500 $mg.l^{-1}$ (runs 10 and 11). The potential acetate pool concentrations for these runs are listed in Table A3.3 and the model coefficients appear in Table 5.3. Experimental data for runs 6, 8, 9 and 10 are plotted in Figure 5.13 and for the duplicate runs in Figure 5.14. Good agreement was observed between the duplicate runs (Figure 5.14, Table 5.3) and the fit of the model was also satisfactory.

For runs 5 to 9 the maximum specific rates of acetate utilisation were similar and in the range 0.35 to 0.41 day^{-1}, although a slightly lower value of 0.31 day^{-1} was estimated for run 7. The initial potential acetate pool for these runs ranged from 2200 $mg.l^{-1}$ to 2600 $mg.l^{-1}$. However when the acid concentration was increased to over 3000 $mg.l^{-1}$ the maximum rate parameter was reduced significantly to approximately 0.13 day^{-1} and acetate was not completely degraded even after 38 days fermentation (run 11).

The model coefficients for the propionate data are also shown in Table 5.3 and indicate a slight drop in the the maximum specific rate of utilisation from 0.64 day^{-1} (run 8, Pr_i = 220 $mg.l^{-1}$) to 0.45 day^{-1} in run 9 (Pr_i = 510 $mg.l^{-1}$). In runs 10 and 11

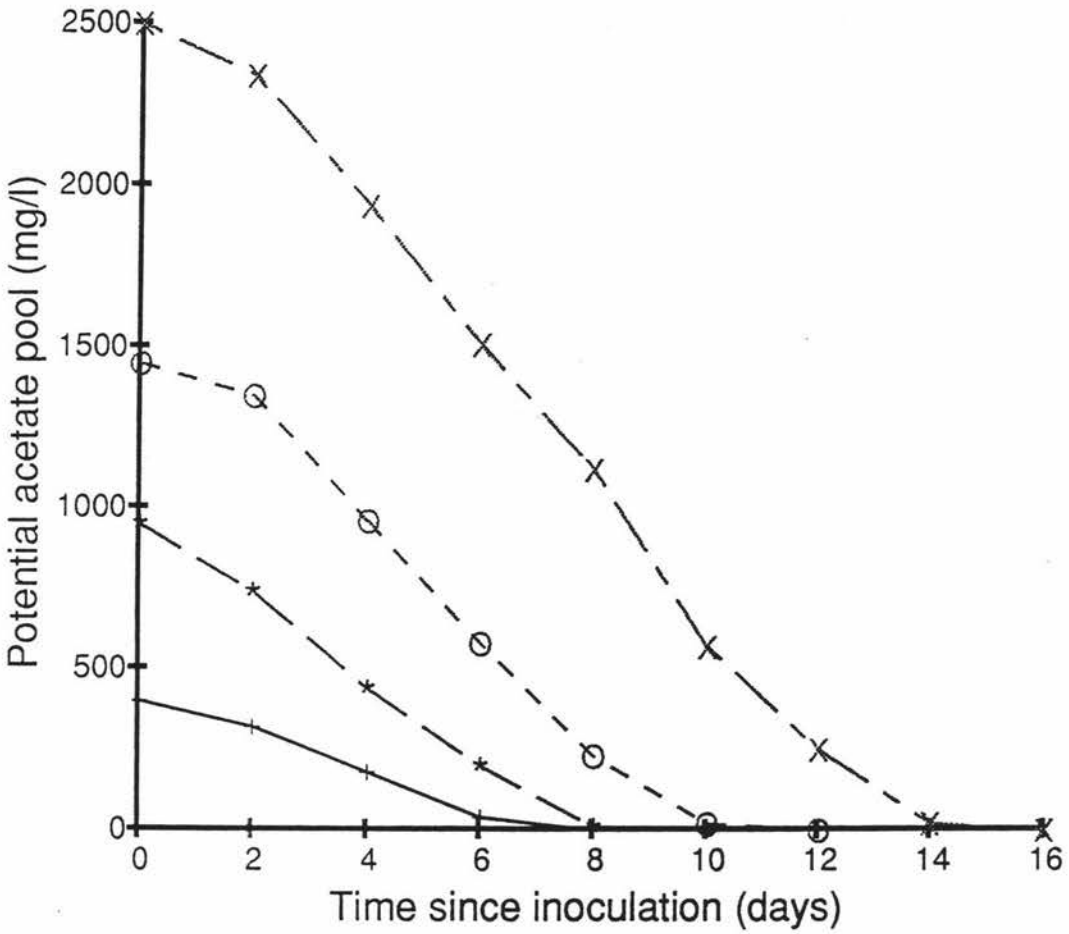


Figure 5.10: Acetate utilisation in selected runs with propionate added at 500 mg/l, experiment 2. Run 1 (+); run 2 (*); run 4 (o); run 5 (x).

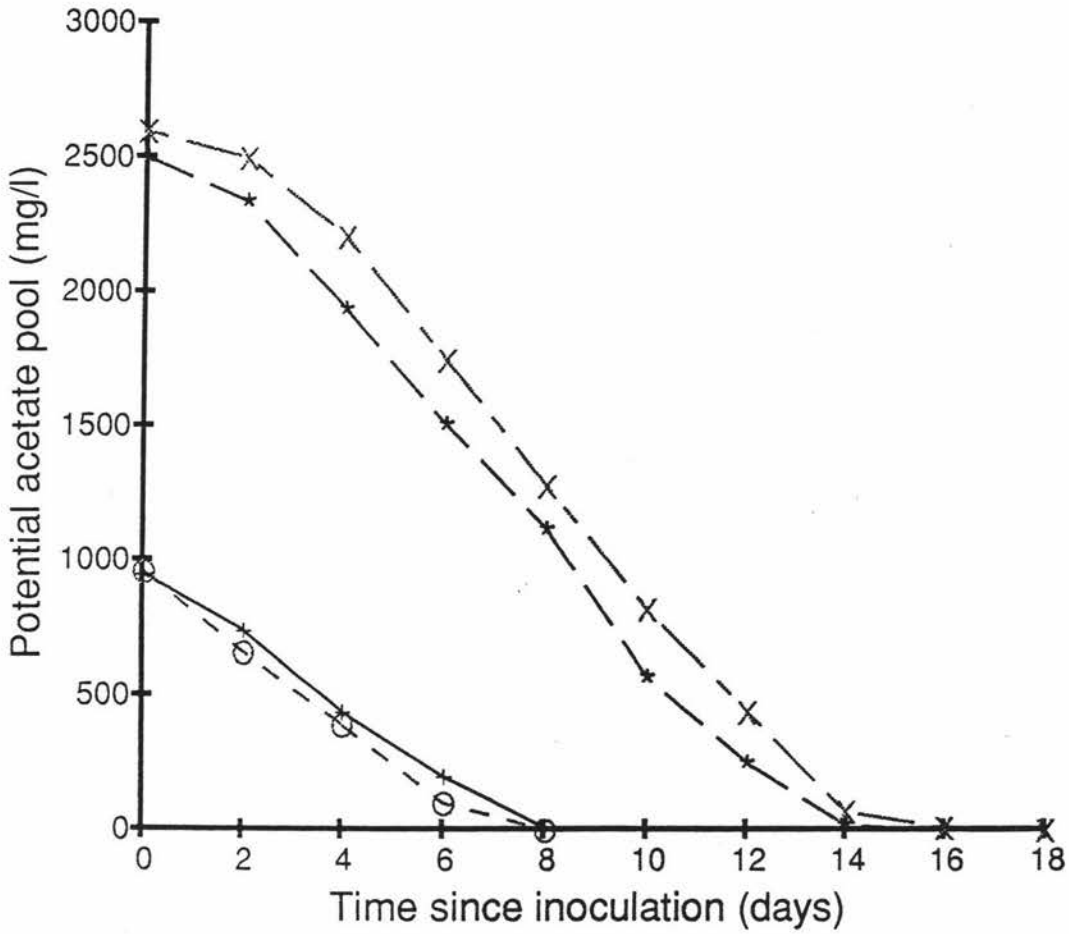


Figure 5.11: Acetate utilisation in duplicate runs with propionate added at 500 mg/l, experiment 2. Run 2 (+); run 3 (o); run 5 (*); run 9 (x).

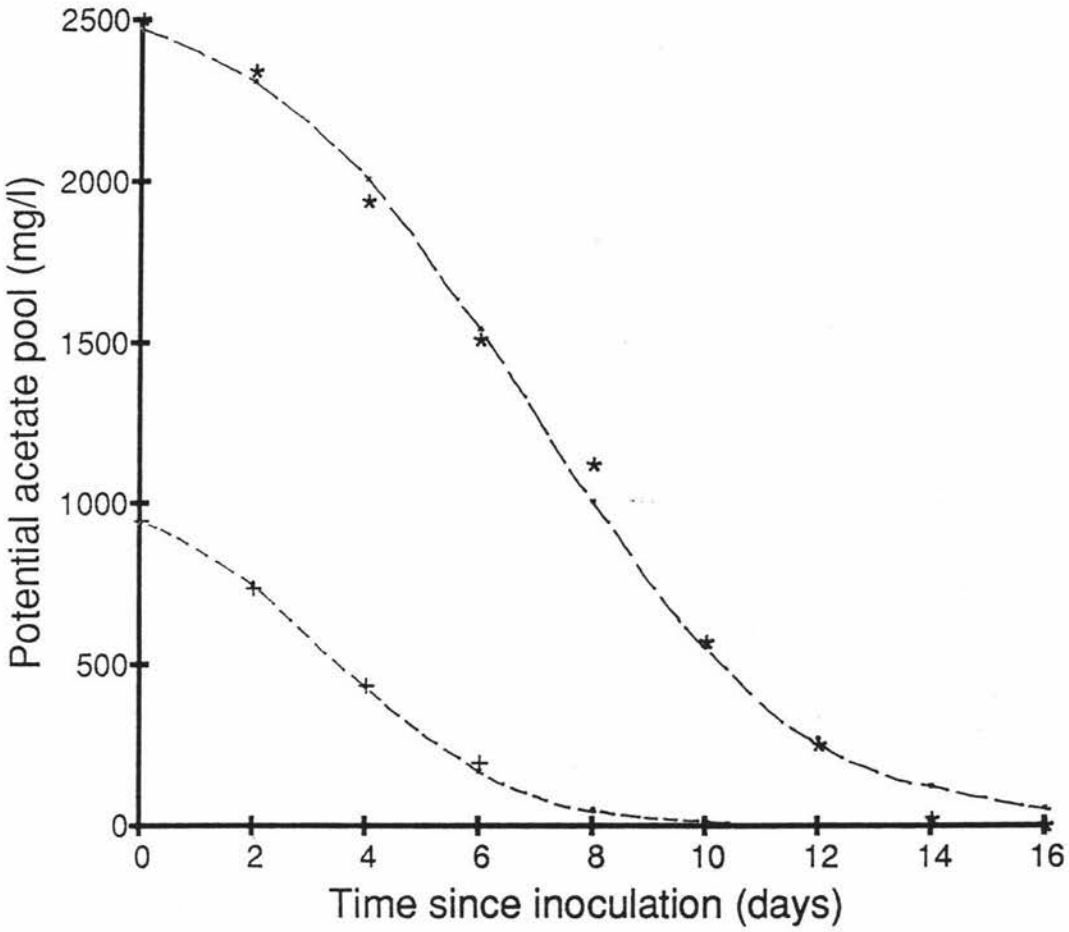


Figure 5.12: Observed and predicted potential acetate pool concentrations for selected runs, experiment 2. Run 2, obs. (+); run 2, pred. (---); run 5, obs. (*); run 5, pred. (- -).

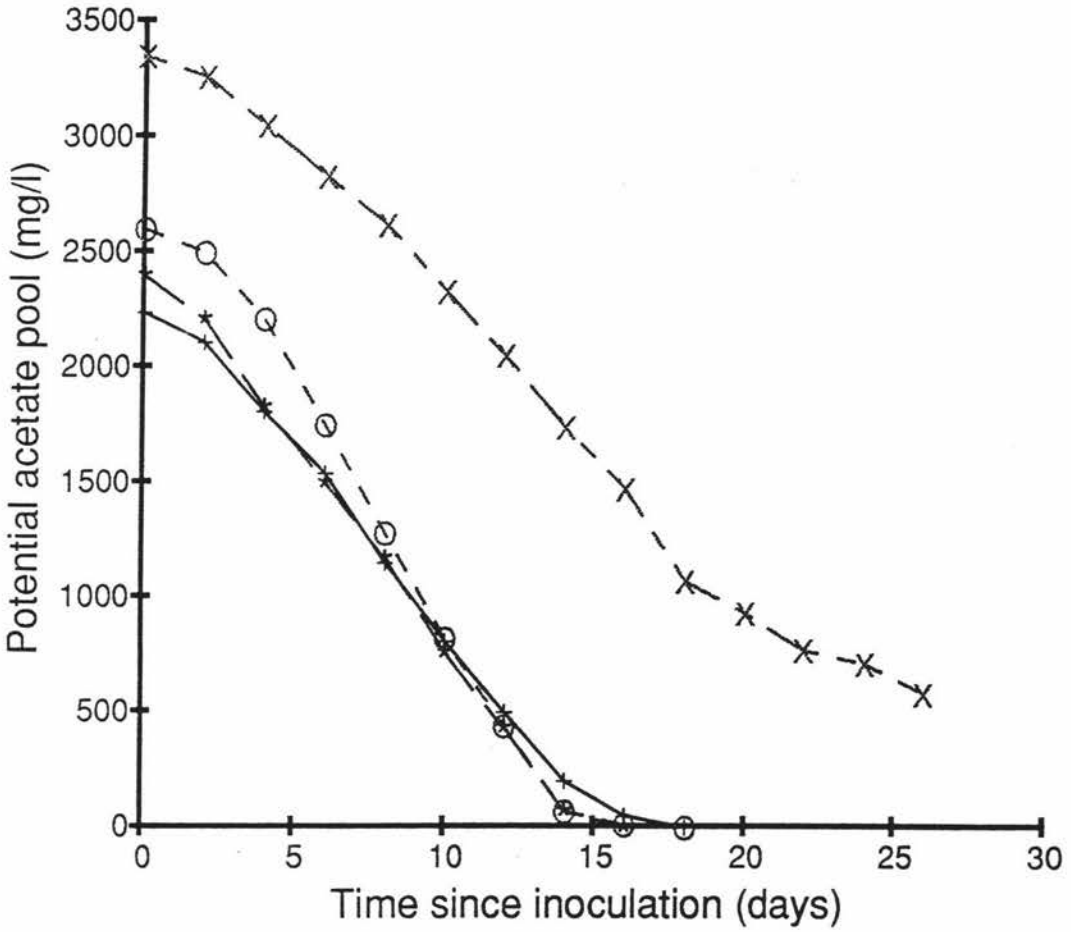


Figure 5.13: Acetate utilisation in selected runs with acetate added at 2000 mg/l, experiment 2. Run 6 (+); run 8 (*); run 9 (o); run 10 (x).

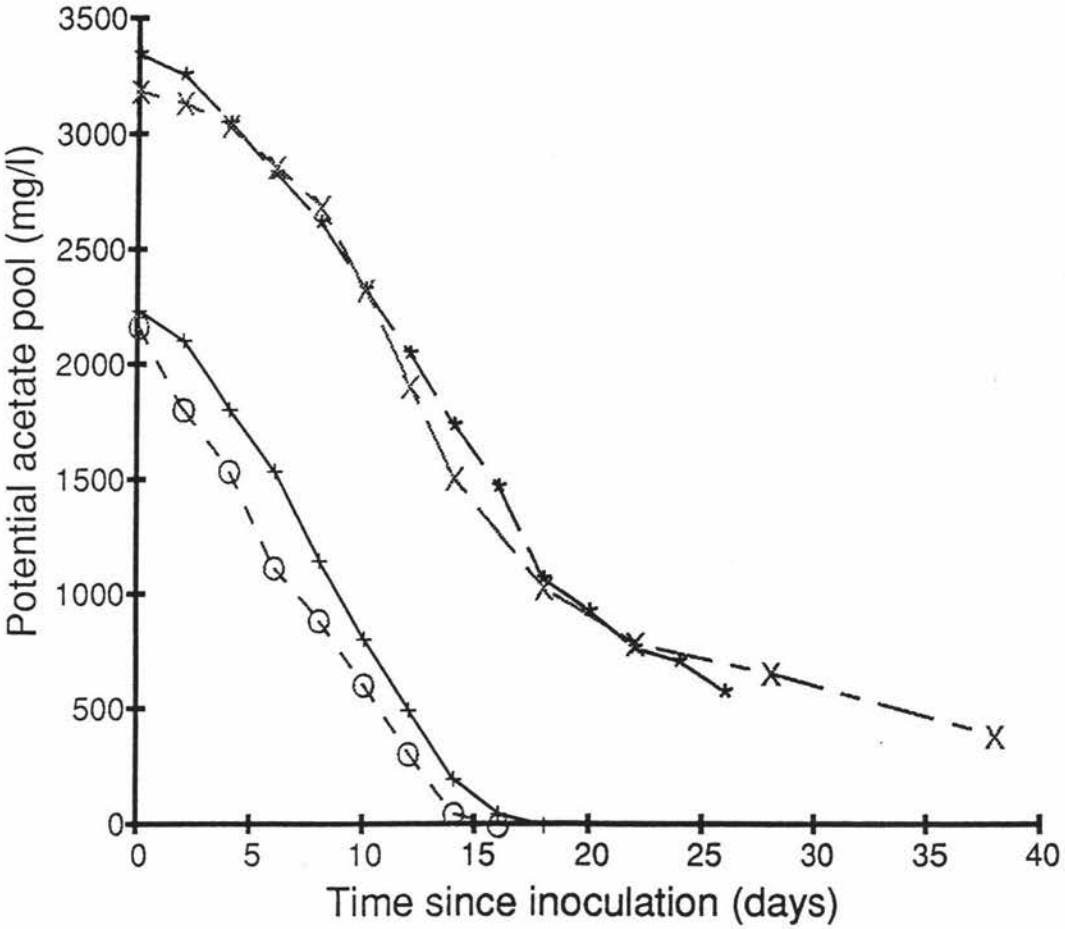


Figure 5.14: Acetate utilisation in duplicate runs with acetate added at 2000 mg/l, experiment 2. Run 6 (+); run 7 (o); run 10 (*); run 11 (x).

however, propionate degradation appeared linear with time. The data were analysed by simple linear regression using the MINITAB package and the fitted equations were:

run 10 $Pr = 1500 - (39.1 \times time)$

run 11 $Pr = 1550 - (30.7 \times time)$

The value of R^2 in each case was 99.2 % confirming that the data strongly conformed to the linear model.

5.3.3 Estimation of the maximum specific growth rate

Values of μ_{max} could be calculated for each metabolic group from the predicted maximum rate of substrate utilisation using equation 2.13 (Section 2.3.4). The yield coefficient for acetate utilisation has been estimated at 0.03 $g.g^{-1}$ by Henze and Harremoes (1983) and for propionate utilisation a value of 0.04 $g.g^{-1}$ was reported by Lawrence and McCarty (1969). Substituting these values into equation 5.5 with the rate coeficients from Tables 5.2 and 5.3 gave values of μmax in the range 0.004 to 0.026 day^{-1} and 0.015 to 0.045 day^{-1} for acetate and propionate degradation respectively.

5.4 DISCUSSION

In these experiments the interaction of acetate utilisation with propionate utilisation was studied. The effect of acetate on propionate degradation was monitored by adding propionate at a constant initial level of 500 $mg.l^{-1}$ and varying the acetate concentration from less than 10 $mg.l^{-1}$ to 2000 $mg.l^{-1}$. In the other runs Ac_t was maintained at 2000 $mg.l^{-1}$ and Pr_t varied from 0 to 1700 $mg.l^{-1}$. These concentrations were selected after consideration of the results of the continuous digestion experiments reported in Chapter Four. The propionate concentration usually varied within a narrow range around 500 $mg.l^{-1}$ and Hansson (1979) also noted a similar concentration in

his work with the same media. The concentration of acetate varied widely in the continuous digester, but periods of satisfactory operation were noted when the level did not exceeed 500 $mg.l^{-1}$, while 2000 $mg.l^{-1}$ was characteristic of the concentration during retarded operation. Also, as noted in the introduction, when acetate exceeded about 1000 to 1500 $mg.l^{-1}$, propionate degradation appeared to be inhibited. The range of propionate concentrations examined reflected the range observed in the continuous digestion runs.

The results of both batch experiments demonstrated that the acid concentration within a digester markedly influences the performance of the various metabolic groups participating in the methane fermentation. It is clear from Figures 5.1 and 5.7 and the trends in the maximum specific rate parameter reported in Tables 5.2 and 5.3 that increasing Ac_i to 2000 $mg.l^{-1}$ significantly reduced the utilisation of propionate. In experiment 1 the effect became significant when Ac_i was added at 1240 $mg.l^{-1}$, while in experiment 2 a reduction in propionate utilisation only occurred when Ac_i was increased from 1000 to 2000 $mg.l^{-1}$. In both experiments the rate of propionate utilisation when acetate was added at 2000 $mg.l^{-1}$ was approximately half of that at 500 $mg.l^{-1}$ or lower.

In these same runs, as Ac_i was increased to 2000 $mg.l^{-1}$ with propionate added at a constant level, a progressive reduction in the rate of acetate degradation was observed (Tables 5.2 and 5.3). However further increasing the potential acetate pool did not result in a further reduction of acetate utilisation in experiment 1. Similarly, increasing Pr_i when acetate was added at a constant initial level reduced the rate of propionate degradation in both experiments. In experiment 1 this effect was noted when propionate was added at up to 500 $mg.l^{-1}$, but further increasing Pr_i did not affect the specific rate parameter.

These trends noted in experiment 1 were not verified in experiment 2, as acid utilisation in runs 10 and 11 was severely retarded. This may have been due to the use of effluent from the semi-continuous digester in this experiment. This digester was slug-fed every second day and the bacterial population should have been adapted to rapidly changing acid concentrations. However the overall variation in both acetic and propionic acid concentrations observed in the semi-continuous digester was much lower than in the continuous digester (Chapter Four). Thus the large initial slug dose of acid in the batch experiments may have been very toxic in comparison with the effect of the same acid dose on the continuous digester inoculum. Also the continuous digester population had recently been exposed to high concentrations of both acids. An alternative explanation for the slow rate of acid utilisation may have been that the five-fold dilution of the effluent reduced the concentration of an essential nutrient to a limiting value.

The maximum specific growth rates for acetate utilisation estimated from the data (Section 5.3.3) are particularly low, even compared to the low growth rates noted in the continuous digestion experiments, and indicate that fermentaton conditions within the flask digesters were far from optimum. This was true even at low acid concentrations and may again have been due to nutrient limitation. At the time these experiments were run the possible controlling role of nutrient availability in the continuous digestion experiments had not been identified and so no additional nutrients were added with the acid substrates.

However the results noted in these batch experiments were still believed to accurately portray the effects of increasing acid concentrations on the methane fermentation. Within each experiment the effects observed were consistent between the two inocula used, between the duplicate runs performed in each experiment and between the measured data and the trends in the fitted logistic equation; and overall the results support and

extend other observations in the literature (Kaspar and Wuhrmann 1978b, Boone and Bryant 1980, Zehnder and Koch 1983).

The slight differences in performance observed between the duplicate runs could be attributed to uncertainty in the VFA determination and the variation typically observed in all biological processes. In experiment 2 the flask digesters were established in random order on three separate days using separate effluent samples, and this could also have introduced a further source of variation.

The reason for the choice of the logistic model was outlined above in Section 5.2.5. While less sensitive than other available models of microbial metabolism (e.g. Monod's), the equation has been successfully applied to the fitting of batch culture data for other fermentations. In the current work a good fit to most experimental data was obtained although the agreement was sometimes poor at acid concentrations below 50 $mg.l^{-1}$. This occured because the logistic equation became asymtotic as the concentration approached zero, whereas the acids were often utilised at a relatively constant rate at low concentrations. Additionally, concentrations below 10 $mg.l^{-1}$ could not reliably be analysed and such data were entered in the program as a concentration of 0 $mg.l^{-1}$. Accurate quantification of these low residual acid concentrations may have improved the fit of the model but would not have altered the overall trends observed.

Another unsatisfactory aspect of the modelling exercise was the limited experimental data available for some runs. This was particularly evident in experiment 1 when the digesters were only sampled every fourth day. In some cases this required the three model parameters to be fitted using only four data points, although again, this did not apppear to adversely affect the overall trends noted from application of the model. In hindsight, samples should have been taken more frequently in

those runs where the potential acetate pool was less than 1000 $mg.l^{-1}$ and this was the reason for the change to sampling every second day in experiment 2. However the inoculum derived from the semi-continuous digester proved as active as that employed in experiment 1 despite the five-fold dilution, and the overall time course for the fermentations was similar in the two experiments. Consequently only one extra data point was obtained in those runs with low acid concentrations in experiment 2 and even more frequent sampling would have been desirable.

The model as used was appropriate to the level of investigation described here. However, for a more sensitive analysis of digester performance it would not only be necessary to have a considerably wider data base but also to have available the means to process the data. For such work, a more detailed model than the logistic equation is required (Roels and Kossen 1978). It was not the purpose of the current programme to pursue such an investigation.

The toxicity of high acid concentrations, regardless of the actual species present, has been documented in many studies (e.g. Buswell 1962, Kroeker et al. 1979, Duarte and Anderson 1982). The inhibition of methanogenesis has been attributed to the action of un-ionised acids so both the pH and the total acid concentration are important in determining the effect of acids on the fermentation. Kroeker et al. (1979) reported inhibition when the UVFA concentration was in the range 30 to 60 $mg.l^{-1}$, while Duarte and Anderson (1982) observed 50 % inhibition of methane formation when the UVFA concentration exceeded 10 $mg.l^{-1}$ in acetic and glucose-fed digesters.

In this work, approximately 50 % inhibition of both acetic and propionate degradation was observed in batch digesters with initial concentrations of 2000 $mg.l^{-1}$ acetate and 500 $mg.l^{-1}$ propionate, equivalent to a TVFA concentration of 2700 $mg.l^{-1}$ as acetate. At pH 7.0, the corresponding UVFA concentration is about

14 $mg.l^{-1}$ (Kroeker et al. 1979) so in this respect the results are in general agreement with the other studies reported above. However in both experiments increasing the TVFA concentration from 1200 $mg.l^{-1}$ to between 1700 and 1900 $mg.l^{-1}$ (runs 2 and 3 in experiment 1; runs 2 and 4, experiment 2) did not further inhibit the rate of acetate utilisation. The rate of acid degradation was also lower at 2000 to 2200 $mg.l^{-1}$ TVFA than at 2500 to 3000 $mg.l^{-1}$ TVFA in both experiments, and in experiment 1, further increasing the acid concentrations did not further reduce the rate of substrate utilisation. As noted above, this latter effect may have been due to acclimation of the inoculum to high acid concentrations; in experiment 2, increasing the TVFA concentration to over 4000 $mg.l^{-1}$ (2000 $mg.l^{-1}$ acetate and 1500 $mg.l^{-1}$ propionate) dramatically reduced utilisation of both acids.

Taken overall these results do not conclusively support the inhibitory role of un-ionised acids in the fermentation and several other reports in the literature also contradict this hypothesis. McCarty and Brosseau (1963), Clarke and Speece (1970), Hobson and Shaw (1976) and Van den Berg et al. (1976) have all successfully operated digesters at elevated acid concentrations without adverse effects. The differences between these studies and those cited above may be explained by acclimation or variation in other important parameters such as pH or alkalinity. Given the importance of acid degradation in the digestion process, further investigation of the role of un-ionised acids still apears warranted.

In contrast, a number of other researchers have published results which show a marked similarity to the effect of acetate on propionate utilisation observed in the batch experiments. Some of these specifically investigated product inhibition, but in single experiments; in other cases the effect was observed during research into other aspects of the methane fermentation and was not expressly discussed in the original paper.

McCarty and Brosseau (1963) added acetic,propionic and butyric acids individually and in combination to investigate the inhibitory action of high acid concentrations on the methane fermentation. Their results indicated that when acetate exceeded about 1000 $mg.l^{-1}$ the rate of propionate degradaton was reduced. In one particular instance, after addition of propionate to 6000 $mg.l^{-1}$, propionate was initially degraded rapidly with the formation of acetate. Three days after inoculation the acetate concentration reached 2000 $mg.l^{-1}$ and for a further five days the utilisation of propionate was significantly reduced. Acetate was then rapidly degraded and this was followed by a marked increase in the degradation of propionate. Similar trends were evident following the addition of 3000 and 8000 $mg.l^{-1}$ propionic acid.

Slight inhibition of propionate utilisation was reported at acetate concentratons of up to 900 $mg.l^{-1}$ by Boone and Bryant (1980) and Zehnder and Koch (1983). Kaspar and Wuhrmann (1978b) observed marked inhibtion at 4800 $mg.l^{-1}$ acetate but none at 480 $mg.l^{-1}$. The results of Winter and Cooney (1980) suggested that pulse addition of acetate (and possibly butyrate) at 1200 to 4200 $mg.l^{-1}$ inhibited propionate utilisation.

It is clear from the work reported in this chapter that the presence of acetate at up to 500 $mg.l^{-1}$ has little effect on propionate utilisaton. Some inhibition is apparent as acetate is increased beyond 1000 $mg.l^{-1}$ and at 2000 $mg.l^{-1}$ the rate of propionate utilisation may be reduced to one half of that at low acetate concentrations. These results are very significant with regard to digester operation and control and also help to explain the behaviour of the continuous digester noted in Chapter Four.

Digester failure is invariably associated with an increase in the TVFA concentration and this has been clearly shown to be detrimental to degradation of both acetic and propionic acids. Hence recovery of a digester from a period of retarded operation will necessarily require adequate time for the accumulated acids

to be degraded at the lower rate. Additionally, the un-ionised acid concentration appears to play a role in the reduction of methanogenic activity and therefore the provision of adequate alkalinity is also important to minimise the effect of an increase in acid concentration. In digesters operating near the lower limit of alkalinity normally observed, increasing acid concentrations can significantly lower the pH, with a resultant increase in the UVFA concentration and the potential for significant inhibition to occur (Kroeker et al. 1979).

Further problems arise when digesters are poorly provided with nutrients. As discussed in Chapter Four, acetate can accumulate rapidly under these conditions and significantly retard propionate utilisation as a consequence. In such situations the time required for digester recovery will be prolonged. This appears to explain the results of the second continuous digestion experiment, CDR2 (Figure 4.2) from day 75, when a gradual increase in acetate concentration was noted. The OLR_v was reduced to allow the digester to recover and from day 82 the concentration was slowly reduced, but at a much slower rate than had been previously observed. During this time propionate accumulated and was only utilised when the acetate concentration had fallen below 500 $mg.l^{-1}$. The biochemical mechanisms which might explain the observed phenomenon remain to be elucidated.

5.5 CONCLUSIONS

Two batch digestion experiments were conducted to examine the effect of increasing either acetic or propionic acid on the utilisation of a constant initial level of the other acid. In the first experiment the inoculum was obtained from the second continuous digestion experiment (CDR2) and in the second, effluent from the semi-continuous digester (SCDR1) was used.

It was found that increasing the total acid concentration from low levels reduced the rate of utilisation of both acids and that

increasing the acetate concentration to 2000 $mg.l^{-1}$ or greater significantly inhibited propionate degradation. This inhibition may also occur at acetate concentrations exceeding 1000 $mg.l^{-1}$. These effects were observed in both experiments, and good agreement was obtained from fitting the logistic equation to the measured data and between duplicate runs performed within the experiments. The role of acetate in inhibiting propionate degradation also confirmed several earlier reports in the literature and offered a partial explanation for the poor operation of the continuous digester. The role of un-ionised acids in regulating the fermentation was not clarified however and further research in this area is recommended.

CHAPTER SIX EFFECT OF SULPHUR-CONTAINING REDUCING AGENTS ON THE METHANE FERMENTATION

6.1 INTRODUCTION

During the second continuous-culture experiment reported in Section 4.3.3 it was observed that addition of a mixture containing 0.025 % (w/v) of each of cysteine-hydrochloride and sodium sulphide resulted in a temporary, but vitrually complete, cessation of gas production. Fresh growth medium supplemented with 0.05 % (w/v) cysteine-hydrochloride only was then introduced and subsequently this enhanced the degradation of acetate in the culture, while propionate utilisation remained unaffected. The concentrations of cysteine and sulphide in the original slug dose, approximately 50 and 34 $mg.l^{-1}$ as total sulphur (S) respectively, were low compared with the commonly reported concentrations producing inhibition in mixed culture digesters (Lawrence et al. 1964, Mosey 1971). However enhancement of cysteine toxicity by sulphide, and inhibition by both compounds at similar concentrations, has been observed in acetate enrichment cultures by Van den Berg et al. (1976) and Parkin et al. (1983).

In view of these observations the batch experiments reported in this chapter were performed to provide further information on the effect of slug doses of cysteine-hydrochloride and sodium sulphide on mixed digester populations. The role of sodium thioglycollate was also investigated. This is another sulphur-containing reducing agent which may be added to media for cultivation of strict anaerobes (Hungate 1969) but its effect on the methanogenic consortium has not previously been reported. Standard growth medium, acetate and propionate were used as substrates so that the effect of the reducing agents on all the metabolic groups participating in the fermentation could be evaluated. Additionally two inocula were used. One was obtained from the continuous digester and was possibly acclimated to the

reducing agents, following their addition to the digester in the modified standard medium. The other was obtained from a semi-continuous digester (SCDR1) to which reducing agents had never been added.

6.2 EXPERIMENTAL METHOD

6.2.1 Equipment and general procedure

A modification of the bioassay technique of Owen et al. (1979) was employed. Volumes of substrate solution, redox indicator-buffer solution (0.001 % w/v resazurin + 2.0 % w/v sodium bicarbonate) and inoculum were added to 70 ml serum-bottle batch digesters (Section 3.5.1.1). These were then flushed with an oxygen-free gas mixture containing 20 % carbon dioxide in nitrogen and incubated at 37 ±1 °C. A total of eight experiments involving 42 individual batch digesters were run and experimental conditions for these are shown in Tables 6.1 and 6.2.

Table 6.1 Batch digester protocol showing component volumes.

Experiment	Total volume ml	Inoculum added[a] ml	Indicator-buffer added ml
1	30	15	2
2	50	25	5
3 to 8	50	25	5

a inoculum 1 - experiment 1 and 2; inoculum 2 - experiments 3 to 8

Table 6.2: Experimental conditions for the batch digestion experiments

Experiment	Substrate	Substrate COD added	Run	Reducing agent[a]	Concentration	
		mg			$mg.l^{-1}$	mM
1	SGM[b]	31.1	1,2[c]	-	-	-
			3,4	cyst	500	3.2
			5,6	thio	500	4.4
			7,8	sulp	500	2.1
2	SGM	62.2	1[c]	-	-	-
			2	cyst	500	3.2
			3	thio	362	3.2
			4	sulp	756	3.2
3	SGM	-	1[d]	-	-	-
		103.5	2[c]	-	-	-
			3	cyst	25	0.16
			4	cyst	500	3.2
			5	thio	18	0.16
			6	thio	362	3.2
			7	sulp	38	0.16
			8	sulp	756	3.2
4	SGM	-	1[d]	-	-	-
		103.5	2[c]	-	-	-
			3	cyst	10	0.06
			4	cyst	100	0.64
			5	cyst	250	1.6
5	Ac[e]	80.0	1,2[c]	-	-	-
			3,4	cyst	25	0.16
			5,6	cyst	500	3.2
6	Ac	80.0	1,2[c]	-	-	-
			3,4	thio	362	3.2
7	Ac	80.0	1,2[c]	-	-	-
			3,4	sulp	756	3.2
8	Pr[f]	75.7	1[c]	-	-	-
			2	thio	18	0.16
			3	thio	362	3.2

a cyst = csyteine-hydrochloride, thio = sodium thioglycollate, sulp = sodium thiosulphide
b SGM = standard growth medium
c control
d blank
e Ac = acetic acid
f Pr = propionic acid

6.2.2 Inoculum source and medium composition

Two sources of inocula were used. In experiments 1 and 2 the inoculum was obtained from an effluent sample removed on day 223 from the continuous digester, run CDR2. The sample was flushed with the gas mixture and incubated in a flask digester (Section 3.5.1.2) until all gas production had ceased (approximately two weeks). The resultant culture was then used as the inoculum for these experiments. The total volume used in experiment 1 was 30 ml. In experiment 2 this was increased to 50 ml and the amount of substrate COD added was also raised to increase the volume of gas produced and to improve the accuracy of gas volume determination. In experiment 1 the reducing agents were added at 0.05 % (w/v) or 500 $mg.l^{-1}$. In all subsequent experiments the reducing agents were added on the basis of equal initial sulphur content in all cultures. Distilled water was added when required to make up the final volume. Control runs were included in each experiment. These contained a substrate for methane production, inoculum and redox indicator-buffer solution, but no reducing agents were added.

In experiments 3 to 8 effluent from the semi-continuous digester (SCDR1) operating at steady-state was used as the inoculum without further treatment. The total volume was 50 ml and 25 ml inoculum and 5 ml redox indicator-buffer solution were added. Three substrates were added in different experiments as shown in Table 6.2. Distilled water was added to all cultures to make up the final volume. Control runs containing all components except reducing agents were also included in each experiment. In addition blanks were run in experiments 3 and 4. These contained 25 ml inoculum, 5 ml redox indicator-buffer solution and 20 ml distilled water.

For all experiments stock solutions of the three reducing agents were prepared fresh before each experiment was conducted.

6.2.3 Sampling and analysis

The gas volume was sampled using a 15 ml glass syringe as described in Section 3.5.1.1. Measurements were taken frequently during the first two to four days following inoculation and once or twice per day thereafter. During experiments 3 to 8 the methane content was also analysed by gas chromatography (Section 3.2.7) at approximately atmospheric pressure after the gas production rate had been determined. The contents of all digesters were thoroughly mixed by hand before and after sampling. Where appropriate, selected digester liquor samples were analysed for pH, COD, VFA and residual sulphide as described in Section 3.2.

6.3 RESULTS

In experiments 1 and 2 total gas production only was monitored and the data are plotted in Figures 6.1 and 6.2 respectively. In experiment 1 the reducing agents tested were all added at 0.05 % (w/v) while in experiment 2 supplementation was on the basis of an initial sulphur concentration of 3.2 mM, or approximately 100 $mg.l^{-1}$. Similar trends were observed in all runs. Gas production was initially rapid for approximately 48 hr and subsequently rates decreased to a low value by 100 hr. Total gas production was greatest when cysteine was added to the culture but the rate of gas production was similar to the other runs. In both experiments little difference in amount or rate of gas production was observed between the control and those cultures supplemented with sodium sulphide or sodium thioglycollate.

In experiment 1, 31.1 mg of substrate COD was added and total gas production ranged from 10.7 ml in the presence of sodium sulphide to 14.2 ml in the culture with added cysteine. In experiment 2 the COD added was doubled and the gas production increased slightly more than proportionally. The control, sodium sulphide and sodium thioglycollate cultures yielded approximately 27 ml

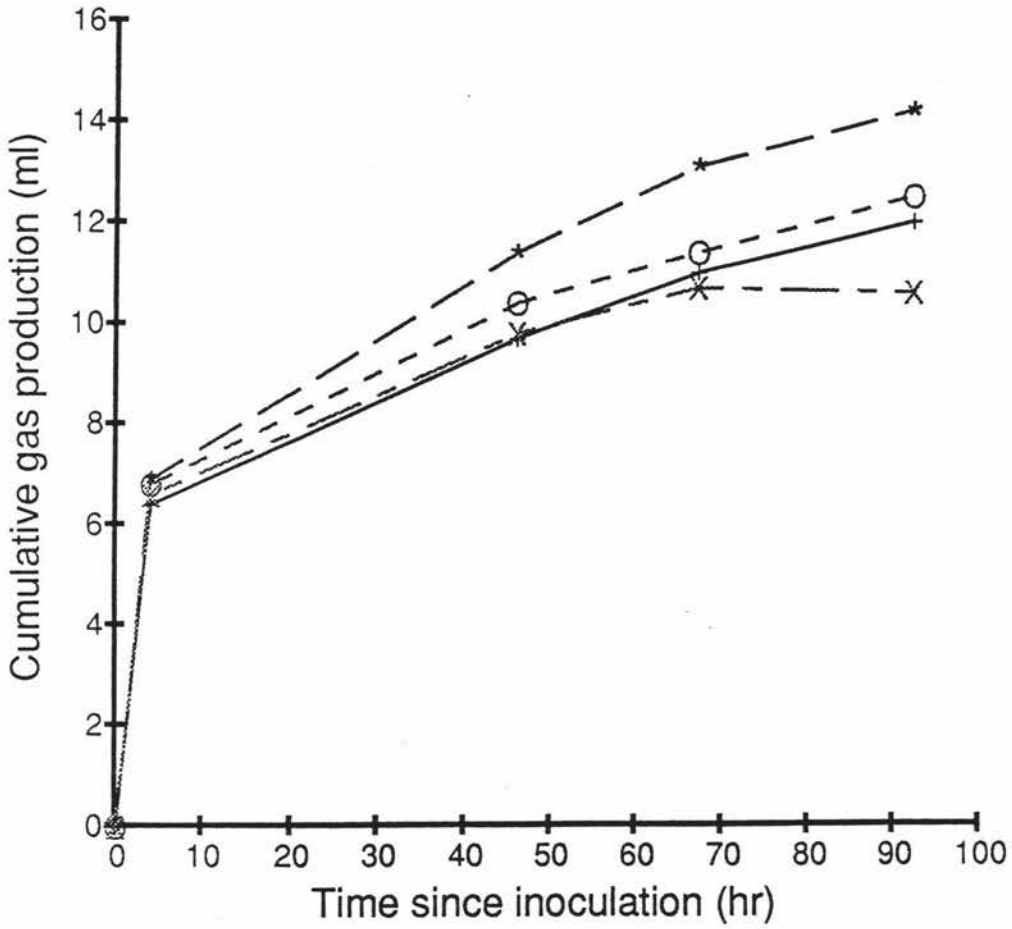


Figure 6.1: Total gas production in batch digesters, experiment 1. Control (+); 3.2 mM cysteine (*); 4.4 mM thioglycollate (o); 2.1 mM sulphide (x).

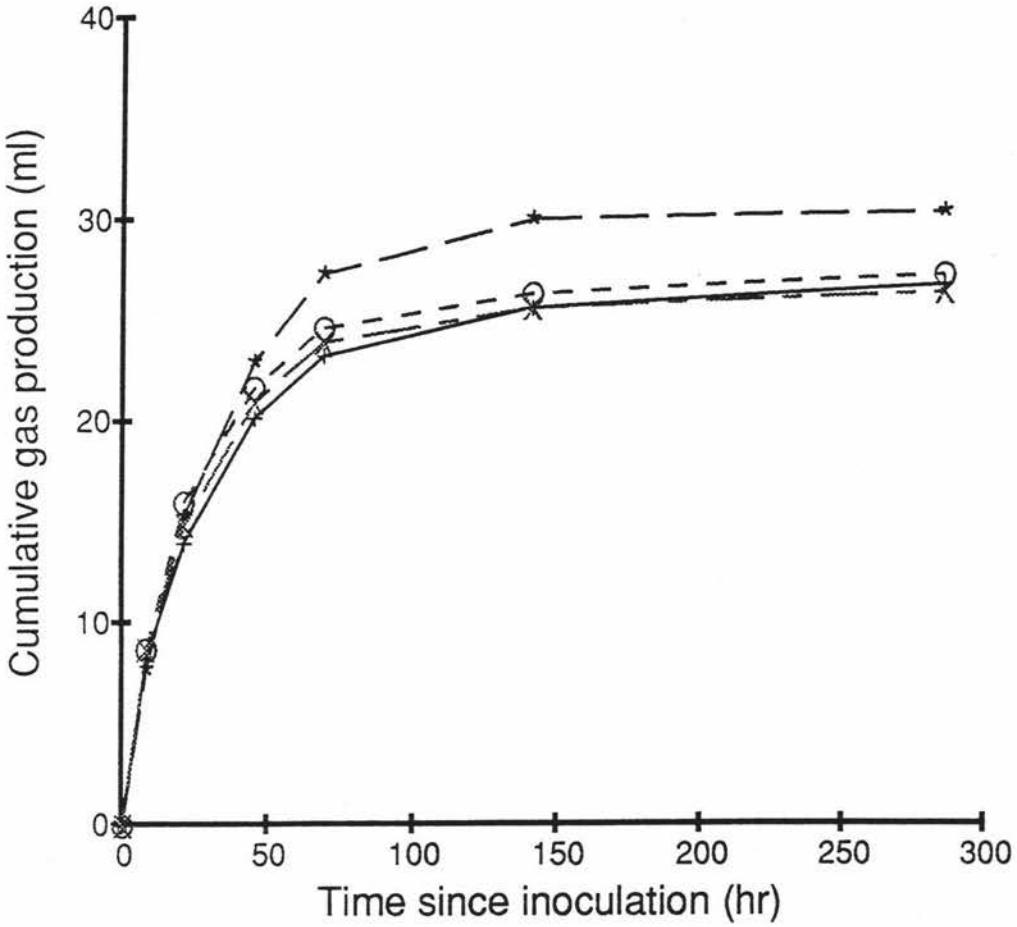


Figure 6.2: Total gas production in batch digesters, experiment 2. Control (+), 3.2 mM cysteine (*), 3.2 mM thiioglycollate (o), 3.2 mM sulphide (x).

total gas while addition of cysteine slightly enhanced gas production to 30.5 ml, but the stimulatory effect was less marked than in experiment 1.

Data from experiment 3 are plotted in Figures 6.3 and 6.4. Figure 6.3 indicates that in those runs with a low initial reducing agent concentration (0.16 mM or 5 mg $S.l^{-1}$), the reducing agent had little effect on gas production. After a short lag of about 4 hr methane production was linear with time to about 90 hr. The average rate of methane production, corrected for gas production from the inoculum without added substrate (run 1), was calculated by regression analysis as 0.42 $\pm$ 0.01 $ml.hr^{-1}$ (at 95 % level of confidence). The yield of methane based on added COD was also calculated and the results are reported in Table 6.3. Good recovery was obtained from all cultures with addition of cysteine (run 3) again resulting in slighly enhanced gas production.

When the reducing agents were added at 3.2 mM (100 mg $S.l^{-1}$) very different results were obtained as shown in Figure 6.4. Cysteine supplementation did not increase the initial lag phase but the rate of methane production was deceased to 0.29 $ml.hr^{-1}$ (31 % inhibition) during the period 20 to 92 hr and to 0.19 $ml.hr^{-1}$ (55 % inhibition) over the following 99 hr. Addition of sulphide increased the lag period before the onset of methane production to approximately 15 hr and the rate of methane production was also reduced by 24 % to 0.32 $ml.hr^{-1}$. In the presence of thioglycollate, methane production initially followed that of the control but slowed markedly after 44 hr and had effectively ceased at 92 hr.

Methane yields for these runs are recorded in Table 6.3. Recovery of added substrate COD was in the range 54 to 105 % and as in previous experiments, cysteine addition gave the highest value. Sulphide addition had no significant effect but the yield for the thioglycollate culture was very low at 54 %. The

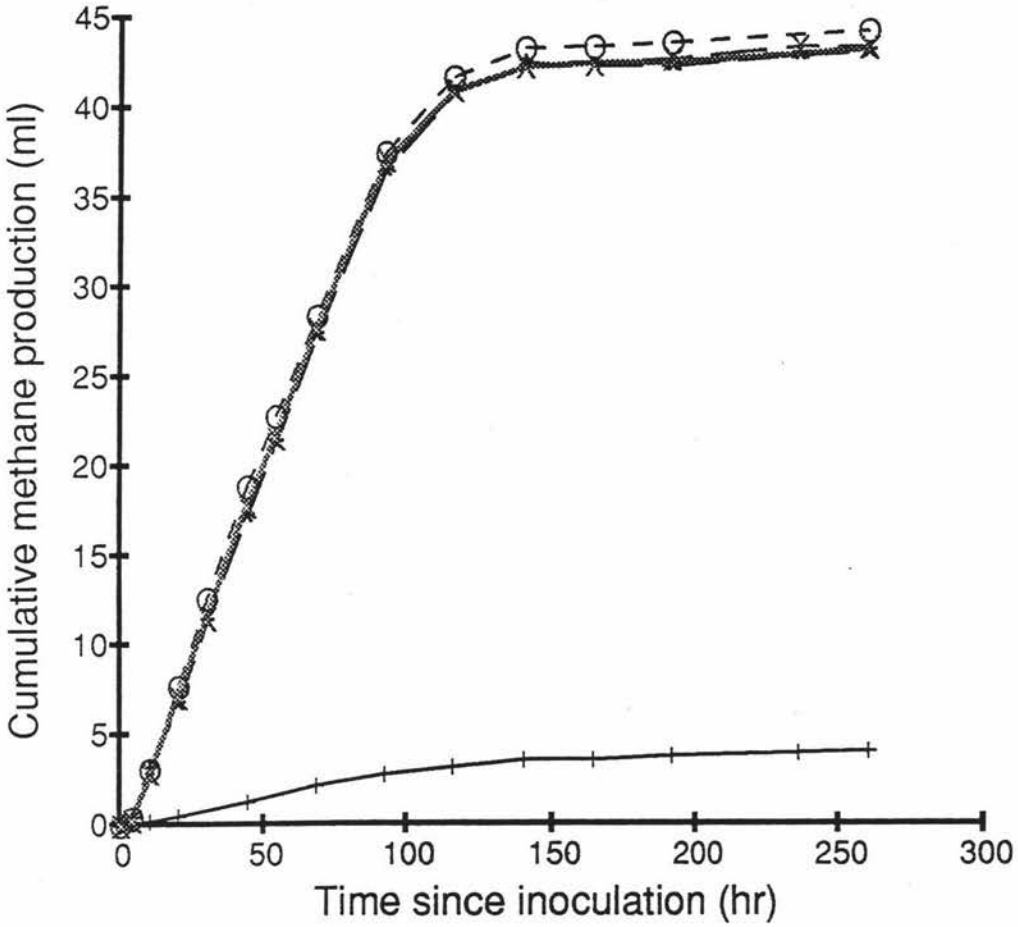


Figure 6.3: Methane production in batch digesters, experiment 3. Reducing agents added at 0.16 mM. Blank (+); control (x); cysteine (o); thioglycollate (x); sulphide (•).

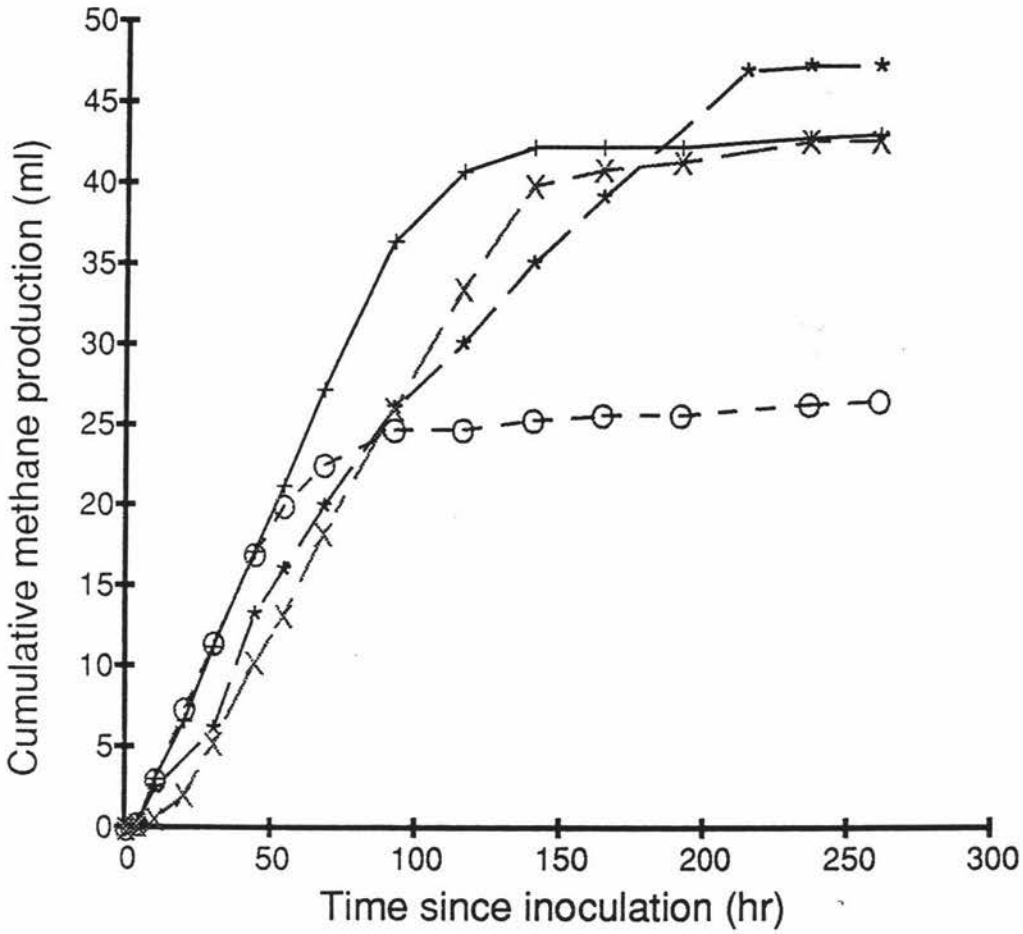


Figure 6.4: Methane production in batch digesters, experiment 3. Reducing agents added at 3.2 mM. Control (+); cysteine (*); thioglycollate (o); sulphide (x).

Table 6.3: Actual and theoretical yields of methane from batch digesters for experiments 3 to 8.

Experiment	Run	Substrate COD added	Theoretical yield[a]	Actual yield	Percent recovery
		mg	ml	ml	%
3	2	103.5	41.3	39.0[b]	94
	3			40.2	97
	4			43.2	105
	5			39.3	95
	6			22.5	54
	7			39.1	95
	8			38.6	93
4	2	103.5	41.3	28.4[b]	69
	3			28.1	68
	4			29.8	72
	5			31.6	77
5	1,2	80.0	31.9	29.6[c,d]	93
	3,4			31.2	98
	5,6			33.8	106
6	1,2	80.0	31.9	27.4[c,d]	86
	3,4			25.9	81
7	1,2	80.0	31.9	28.7[c,d]	90
	3,4			28.7	90
8	1	75.7	30.2	25.9	86
	2			25.3	84
	3			7.4	25

a theortical yield = substrate COD x 0.351 x (273 + 37)/ 273
b all values within experiment corrected for gas production from the blank run
c all values within experiment corrected for gas production from the blank run in experiment 3
d the average of the duplicate runs is reported

digester liquor for this run was analysed for volatile fatty acids at the completion of the experiment and the results are listed in Table 6.4. Acetate could not be detected but propionate was present at a high concentration; low concentrations of higher acids were also observed.

Table 6.4: Residual volatile fatty acids in a batch digester with added thioglycollate; experiment 3, run 6.

Acid	Concentration $mg.l^{-1}$
acetic	n.d.[a]
propionic	490
i-butyric	20
butyric	10
i-valeric	40
valeric	n.d.

a not detected

In experiment 4 the effect of addition of cysteine at concentrations intermediate to those of previous runs was evaluated and the data obtained are recorded in Figure 6.5. After a short lag the rate of methane production was linear at 0.37 $\pm$ 0.03 $ml.hr^{-1}$ (at 95 % level of confidence). This was slightly less than observed in experiment 3 while the calculated yields (Table 6.3) were markedly lower. The yield for the control was only 69 % and was increased by cysteine supplementation to a maximum of 77 % for run 5 (1.6 mM added cysteine).

Acetate was used as substrate for experiments 5, 6 and 7 and data for these runs are shown in Figures 6.6, 6.7 and 6.8 respectively. In experiment 5, cultures were supplemented with 0.16 mM and 3.2 mM (5 and 100 mg $S.l^{-1}$) cysteine and similar trends to previous experiments were observed. At the low concentration methane production followed that of the control and was produced at a linear rate of 0.51 $ml.hr^{-1}$. At the high concentration the rate was reduced by 24 % to 0.39 $ml.hr^{-1}$. Methane yields corrected for gas production by the inoculum (experiment 3, run 1) were calculated and are listed in Table 6.3. Methane recovery exceeded 90 % in all cultures with enhanced methane production apparent at the high cysteine concentration.

In experiment 6 sodium thioglycollate was added at 3.2 mM and experimental data indicated this had no effect on methane production (Figure 6.7) or yield (Table 6.3). However addition of sodium sulphide at the same concentration markedly inhibited methane production from acetate (experiment 7, Figure 6.8). Gas production in this experiment was less clearly linear than in previous runs but the results suggest the rate of methane formation was reduced from approximately 0.53 $ml.hr^{-1}$ to 0.39 $ml.hr^{-1}$ (26 % inhibition) by addition of the reducing agent. The corrected methane yield was constant at 90 % of theoretical.

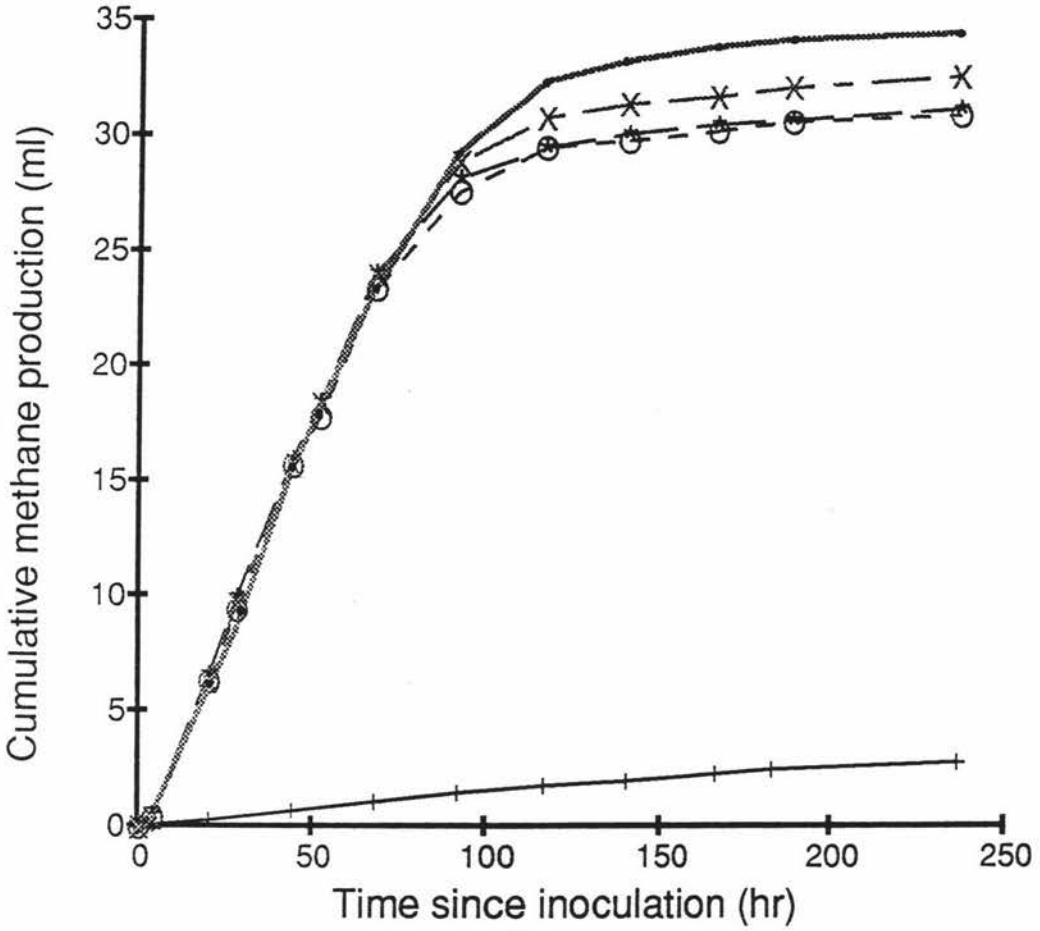


Figure 6.5: Methane production in batch digesters, experiment 4. Blank (+); control (*); 0.06 mM cysteine (o); 0.64 mM cysteine (x); 1.6 mM cysteine (•).

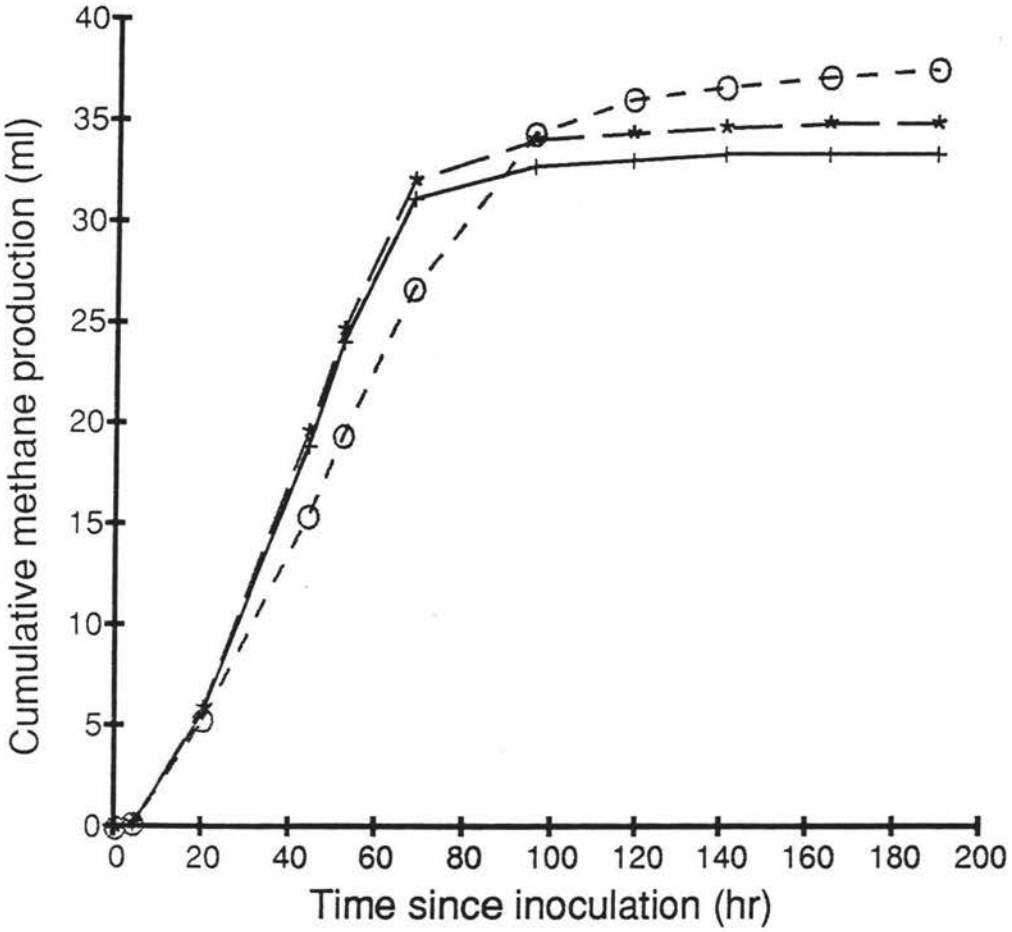


Figure 6.6: Methane production in batch digesters, experiment 5. Points represent average of duplicate runs. Acetate as substrate. Control (+); 0.16 mM cysteine (*); 3.2 mM cysteine (o).

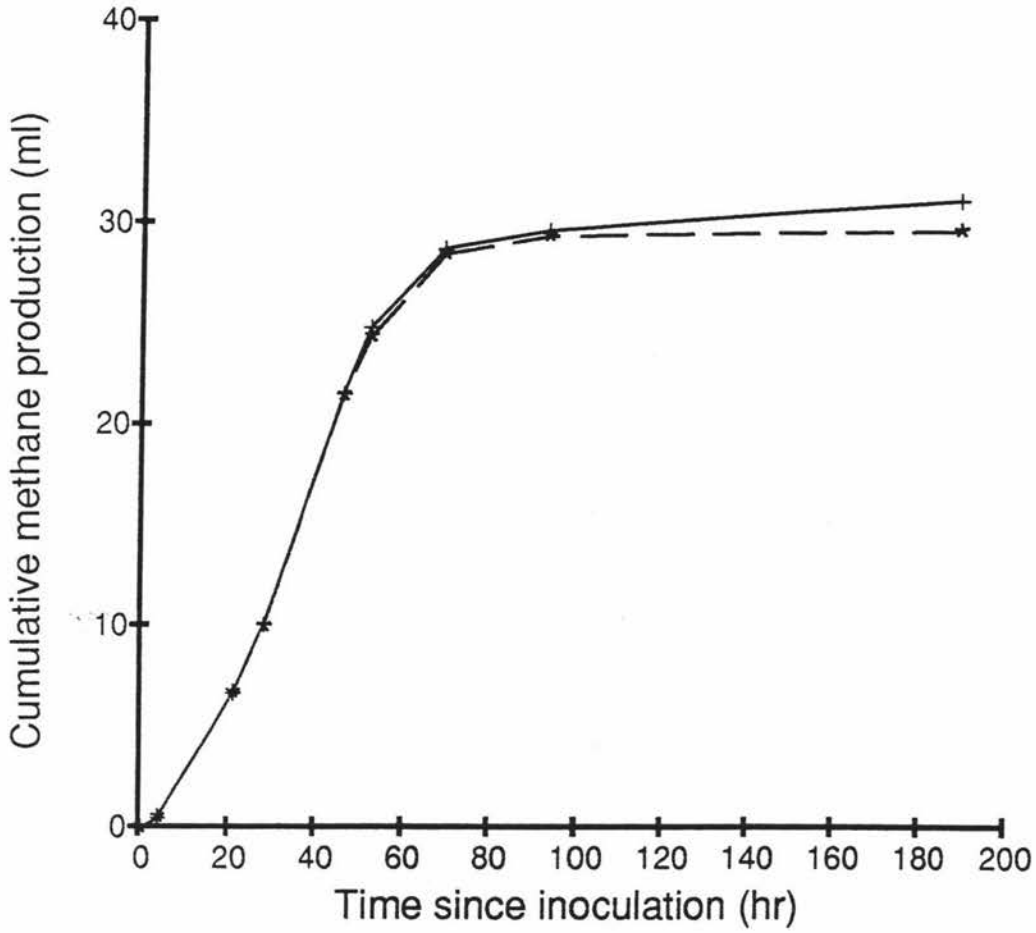


Figure 6.7: Methane production in batch digesters, experiment 6. Points represent average of duplicate runs. Acetate as substrate. Control (+); 3.2 mM thioglycollate (*).

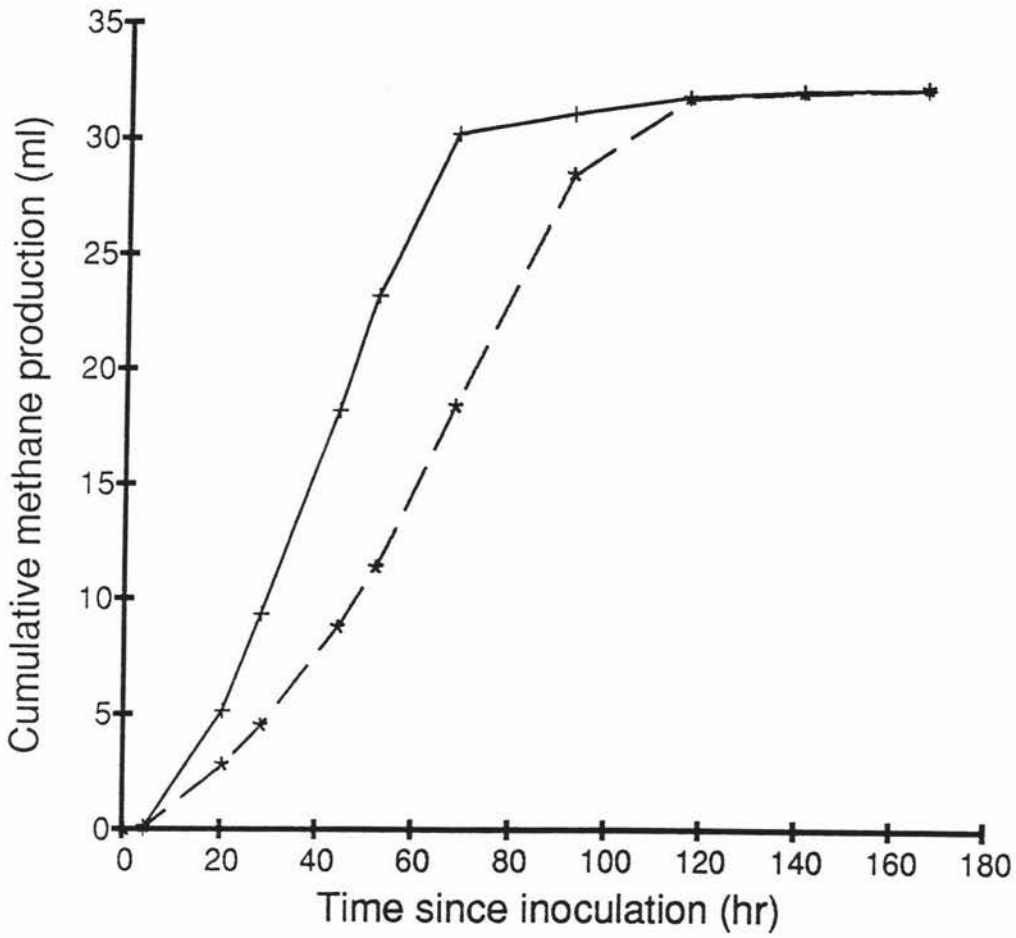


Figure 6.8: Methane production in batch digesters, experiment 7. Points represent average of duplicate runs. Acetate as substrate. Control (+); 3.2 mM sulphide (*).

The effect of sodium thioglycollate supplementation on propionate degradation was investigated in experiment 8 (Figure 6.9). At the low concentration (0.16 mM), total methane production and yield were very similar to the control at 0.27 $ml.hr^{-1}$ and 84 %. However when added at 3.2 mM, thioglycollate severely inhibited gas production after 20 hr and the final methane yield after 188 hr (corrected for gas production by the inoculum) was only 7.4 ml, 25 % of that expected. The digester contents for this run were analysed for acids and the results are reported in Table 6.5. As for the corresponding run in experiment 3 (run 6; Table 6.4) a high concentration of propionate was detected with some C_5 acids also present. Acetate was not detected.

Table 6.5: Residual volatile fatty acids in a batch digester with added thioglycollate; experiment 8, run 3.

Acid	Concentration $mg.l^{-1}$
acetic	n.d.[a]
propionic	630
i-butyric	n.d.
butyric	n.d.
i-valeric	70
valeric	20

a not detected

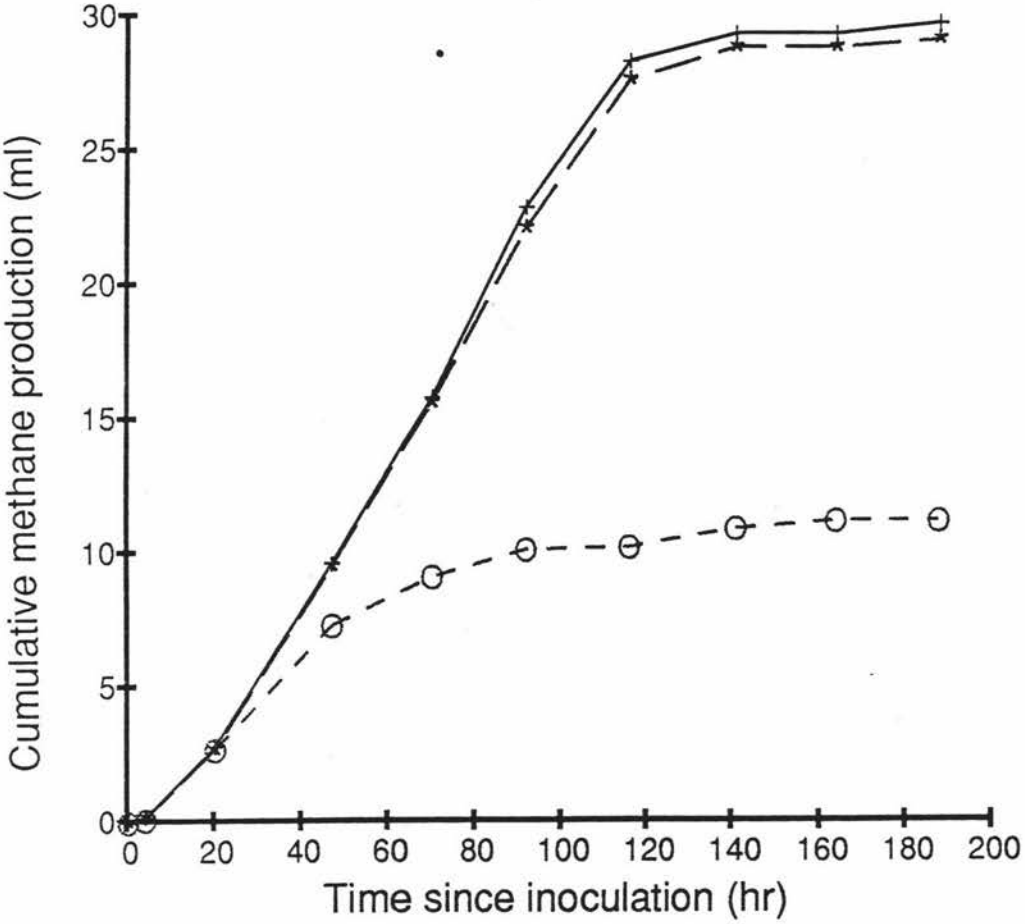


Figure 6.9: Methane production in batch digesters, experiment 8. Propionate as substrate. Control (+); 0.16 mM thioglycollate (*); 3.2 mM thioglycollate (o).

Residual dissolved sulphide concentrations were measured for several runs at the completion of the experiments and are reported in Table 6.6. The concentration in a control (experiment 5, run 1) and in cultures with added sodium thioglycollate (experiment 6, run 4 and experiment 8, run 3) were low while much higher concentrations were observed when cysteine or sulphide were added (experiment 5, run 5 and experiment 7, run 4).

Table 6.6: Residual dissolved sulphide concentrations in selected batch digesters.

Experiment	Run	Reducing agent[a]	Reducing agent concentration mM	Dissolved sulphide mM
5	1	-	-	0.05
	5	cyst	3.2	0.61
6	4	thio	3.2	0.08
7	4	sulp	3.2	0.96
8	3	thio	3.2	0.11

a cyst = cysteine-hydrochloride; thio = sodium thioglycollate; sulp = sodium sulphide

6.4 DISCUSSION

Cysteine and sulphide can be important nutrients in the methane fermentation (Wellinger and Wuhrmann 1977, Scherer and Sahm 1981) but inhibitory action has also been observed (Van den Berg et al. 1976, Parkin et al. 1983) and is confirmed by the results of these experiments. Data from experiments 3 to 8 utilising a mixed population from a semi-continuous digester fed a semi-synthetic medium clearly demonstrates the three sulphur-containing reducing agents are inhibitory when dosed at 3.2 mM (100 mg initial $S.l^{-1}$). However the mode of action of the compounds appeared to differ.

The degree of inhibition resulting from supplementation at 3.2 mM was similar for cysteine and sulphide. For growth on the standard medium (experiment 3), cysteine and sulphide inhibited methane production by 31 % (during the first linear phase of gas production) and 24 % respectively. Using acetate (experiments 5 and 7), inhibition of 24 % and 26 % respectively was observed and residual dissolved sulphide concentrations in each of these latter experiments were high compared to the control and cultures with added thioglycollate. These results implicate the sulphide ion as the inhibitory agent. This is supported by two further observations. Firstly cysteine addition above 0.16 mM (5 mg $S.l^{-1}$) always enhanced total methane production and in two runs resulted in yields greater than those predicted by theory. Together with the high residual sulphide level this indicates cysteine was degraded during batch digestion and the additional carbon converted to methane. Similar behaviour was noted by Van den Berg et al (1976). Secondly, in experiment 3 it was noted that sulphide addition increased the lag period prior to methane formation while inhibition was not apparent until 20 hr after cysteine addition. This is consistent with the release of an inhibitory compound (sulphide) as the cysteine was metabolised. A similar trend was also noted after cysteine addition in experiment 5.

The separate linear phases of methane production observed in run 5, experiment 3 were atypical and indicated sequential utilisation of fermentation intermediates with the different pathways varying in their susceptibility to inhibition. However this behaviour was not observed in other runs and may have simply been an artefact of the experiment.

Beyond these observations caution must be exercised in interpreting the data and a definite statement on minimum toxic concentrations cannot be made. The concentration of dissolved sulphide would have varied in the digesters depending on the equilibrium established with hydrogen sulphide in the gas phase as a result of the prevailing temperature, pressure and pH. The soluble sulphide concentration is also markedly affected by chelation and formation of insoluble salts with heavy metals and some sulphide would also be removed in the gas wasted after determination of the volume produced. The results are therefore indicative only. However as rapid acclimation has been observed in other systems with similar concentrations, it is most likely the effect of the initial shock dose that determines the behaviour of the culture. The final residual sulphide concentrations recorded of 0.61 and 0.96 mM (approximately 20 and 31 mg $S.l^{-1}$) for cysteine and sulphide addition and the degree of inhibition observed are consistent with the results of Van den Berg et al. (1976) and Parkin et al. (1983) obtained from studies of acetate-enriched cultures.

Results for those runs supplemented with sodium thioglycollate indicate the acetogenic bacteria responsible for the degradation of propionate were strongly inhibited by the reducing agent or some product arising from its transformation. Addition of thioglycollate at 3.2 mM had no effect on methane production from acetate (experiment 6) but supplementation when standard growth medium (experiment 3) or propionate (experiment 8) was used as substrate resulted in markedly lower gas yields. In each

case the the digester liquor contained high concentrations of propionate and C_5 acids which would be degraded via propionate (Boone and Bryant 1980). The theoretical methane yield from these residual acids was calculated as 17.4 ml for run 6, experiment 3 and 22.6 ml for run 3, experiment 8. This satisfactorily accounts for the difference in gas production between these runs and the appropriate control runs.

In experiment 8 inhibition of propionate degradation began at 20 hr and increased with time, suggesting the toxic compound was formed from thioglycollate. However the residual sulphide levels recorded in the cultures, although double those of control runs, were low (Table 6.6) and suggest no significant degradation of the reducing agent to sulphide occurred. Age-dependent inhibition by thioglycollate has previously been reported by Malin and Finn (1951) and Willis (1969) but neither author proposed a mechanism for this action, nor has any mechanism been reported in the literature since these reports. Further investigation may be useful in clarifying the biochemistry and ecology of the acetogenic bacteria and it is clear that uncritical use of this reducing agent in studies of the methane fermentation cannot be recommended.

When data from all experiments are considered it is evident that the two inocula employed responded differently to the presence of the reducing agents. The population from the continuous digester was not affected by concentrations of cysteine or sulphide up to 3.2 mM or thioglycollate up to 4.4 mM, in marked contrast to the semi-continuous digester population. The performance of the first culture (experiments 1 and 2) with cysteine and sulphide supplementation could be explained by acclimation; the inoculum used had already been exposed to low slug doses of the reducing agents and then maintained in batch mode prior to these experiments. However the absence of inhibition by thioglycollate cannot be explained in this way. Based on the observations from

the latter experiments, this suggests either different acetogenic bacteria were present in the inoculum for the first two experiments or a different mechanism for propionate degradation was employed or, alternatively, bacteria or process conditions responsible for conversion of thioglycollate to an inhibitory compound were absent.

The possibility of a difference in bacterial flora between the digesters was not unexpected as the first inoculum was derived originally from cow manure and therefore of rumen origin while the second inoculum was derived from a sewage sludge digester. The change in inoculum was made primarily to observe the response of an unacclimated mixed digester population. Additionally, the composition of the the semi-continuous digester effluent was stable in contrast to the performance of the continuous digester at that time (Chapter Four).

A further observation from all experiments was that in no case was stimulation of the rate of methane production by cysteine addition observed. This was in apparent contrast to the results from the continuous digester experiment (Figure 4.2) and a clear and satisfactory explanation of this earlier behaviour was not offered by the experimental data.

Overall the batch assay technique employed in these experiments proved satisfactory for preliminary investigation of toxicity phenomena in the methane fermentation. Excellent reproducibility of gas production was obtained from the serum bottle digesters for replicate runs within experiments and the varying rates of methane production observed between experiments presumably reflected natural variations in the inocula used. Similar performance was noted by Parkin et al. (1983) who also used a technique modified from Owens et al. (1979).

The major points of concern with the method are the difficulty in controlling the concentration of volatile components, such as hydrogen sulphide, and the variable yields experienced. Good recovery of methane was obtained in experiments 3, 5, 6, 7 and 8 but recovery was low, although approximately constant, in experiment 4. This could be due to errors in measurement or to losses, or it could also reflect on differences in the inoculum used with, in this case, an increased amount of carbon diverted to cell production.

6.5 CONCLUSIONS

A systematic study was made of the effect of adding three sulphur-containing reducing agents to batch anaerobic digesters charged with different inocula and substrates.

Using an inoculum derived from the continuous digester reported in Chapter Four (CDR2), addition of cysteine-hydrochloride and sodium sulphide to 3.2 mM or sodium thioglycollate to 4.4 mM produced no inhibition when standard growth medium was used as the substrate. However cysteine addition produced a significant increase in the total gas yield.

For the semi-continuous digester inoculum (SCDR1, Chapter Four), supplementation with cysteine and sulphide at 3.2 mM reduced the rate of methane production from both the standard growth medium and acetate by 25 to 30 %. Sulphide was indicated as the inhibitor in each case and in cultures containing cysteine this appeared to be released by degradation of the reducing agent. This process also contributed to the higher yield of methane observed in these cultures. No inhibition was noted for either reducing agent at 0.16 mM or for intermediate cysteine concentrations up to 1.6 mM.

Addition of thioglycollate with the semi-continuous digester inoculum did not affect methane formation from acetate but

markedly inhibited gas production from standard growth medium and propionate. In each case degradation of propionate and C5 acids by the acetogenic bacteria appeared to be retarded and the severity of inhibition increased with further incubation. The nature of the inhibitory compound was not determined.

Overall the results for the semi-continuous digester inoculum were similar to those reported by Van den Berg et al. (1976) and Parkin et al. (1983) for acetate enrichment studies. However the different response observed between the two inocula used highlights the complexity and variability of the eco-physiology of the methane fermentation and the inherent difficulty of estimating the effects of potential process inhibitors without conducting screening experiments. These must be conducted using a representative sample of the digester population. Methods based on the technique of Owen et al. (1979) are clearly appropriate for this purpose.

CHAPTER SEVEN FINAL DISCUSSION AND CONCLUSIONS

A number of experiments have been performed to provide information on factors affecting the rate and stability of the methane fermentation. This research was seen as an integrated part of ongoing programmes, both in this and many other laboratories, with the aim of developing processes and operating stategies which will allow industrial wastes to be economically treated by anaerobic digestion.

A number of compelling reasons exist for the use of anaerobic digestion over other waste treatment technologies; low energy and nutrient inputs are required, biological sludge production is low and methane is a useful fuel which is easily recovered. However a high rate of substrate conversion and consistent fermentation performance over long periods, during which the process may be exposed to marked variations in organic loading, toxicant concentrations and environmental conditions, must also be demonstrated. As Speece (1983) has noted, wider acceptance of anaerobic digestion has been hindered by a perception that treatment rates are low and the process difficult to control. This view has arisen because of past digester failures resulting from a limited understanding of the mechanism of the fermentation and the factors which influence it. It is this deficiency which this research has attempted to address.

The starting point for this project was an investigation of the performance of continuous and semi-continuous digesters fed a semi-synthetic medium (Chapter Four). A detailed comparison of digesters operating with different feeding regimes had not been reported in the literature, and this became of interest when marked differences in behaviour and process parameters were noted between the two modes of digester operation although similar loading conditions and inoculum sources were employed. The continuous digester performed erratically and repeatedly failed, while the semi-continuous digesters provided satisfactory

treatment despite being slug-fed with medium every second day. They could also be easily and rapidly recovered from retarded operation resulting from step changes in loading or environmental conditions. The comparison was also useful as the medium was analogous to easily-hydrolysable industrial wastewaters, such as produced from dairy and other food industries, and for which stable digestion has often been difficult to achieve because of the rapid rate of acid production compared to methane formation.

In the continuous runs, digester failure was characterised by a gradual decline in biomass concentration followed by a rapid accumulation of acetate. The specific rates of methane production and COD removal (based on VSS) were high however, and comparable to methanogenic cultures growing at close to the maximum reported growth rate for these organisms (Speece and McCarty 1963, Hansson 1979, Henze and Harremoes 1983), but the biomass yield coefficient was much lower than typically observed (Henze and Harremoes 1983). Redox potential at the time of failure was stable and low (E_c = -470 mV), and the change in performance could not be attributed to failure of the pH or temperature control systems or other faults in digester construction or operation.

In contrast, the semi-continuous digesters exhibited elevated propionate concentrations during periods of retarded operation following failure of the temperature control system or organic overloading. This was typical of the pattern of digester failure most commonly reported (Pohland and Bloodgood 1963, Bryant 1979) and can be explained on the basis of the importance of interspecies hydrogen transfer in regulating the various metabolic groups participating in the fermentation (McInerney and Bryant 1981a). The biomass yield coefficient observed in these runs was always within the normal range.

After detailed analysis of all the experimental data from five runs, the failure of the continuous digestion experiments was

attributed to a deficiency in the medium of one or more essential nutrients. The concentration of iron in particular appeared low, but a requirement for other growth factors could not be discounted. Speece and McCarty (1964) noted that nutrient deficiency was characterised by a gradual accumulation of acids in the digester and the current work confirmed their view. This is an important observation because rapid identification of the cause of digester failure so that corrective action may be taken is crucial for successful digester operation.

The success of the semi-continuous digesters was believed due to release of nutrients by lysis of the acidogenic bacteria or luxury uptake of nutrients due to the pattern of excess substrate alternating with starvation. The importance of unidentified organic growth factors or recycle of cell material in promoting methanogenic activity has frequently been reported, and luxury uptake is recognised as an important mechanism in phosphorus removal in activated sludge. The complexity of the methane fermentation renders investigation of the nutritional requrements of the process difficult, but as noted in Chapter Four, research in this area has contributed significantly to improved treatment rates and continued research effort is recommended.

The stability of the semi-continuous digesters compared to that of the continuous digesters has significance to the design and operation of full-scale plants. Firstly the importance of providing a correct balance of nutrients is obvious. This is of particular importance in the treatment of many industrial wastes and the experimental results reported suggest that semi-continuous operation may offer an alternative to nutrient supplementation and/or biomass recycle. Many small-scale digesters such as those found on farms or treating effluent from small industries or communities, are already run on a semi-continuous basis and few problems arise from this. However on a large scale semi-continuous operation poses some difficulties. In many industries, large volumes of wastes are required to be

processed daily and the factories often operate continuously. In these cases the high-rate, retained-biomass processes, such as the UASBR or AFFBR systems, appear to offer the best performance. However for wastes with an appreciable solids content or in situations where the waste is produced at varying rates or for only a portion of the day, semi-continuous operation could be feasible. Large-scale balance tanks or ponds would be required to regulate the flow or alternatively, several smaller digesters fed intermitantly could be used to process the waste. The feasibility of the operation would to a great extent depend on the the required interval between feeding. It may be, for example, that continuous feeding but with a varying flowrate could offer similar advantages to intermitant slug dosing.

Such a feeding regime could also offer economic benefits other than potentially increased stability of the process. If digester loading could be more closely matched to a factory's effluent output, savings in the use of balancing tanks could be achieved. Even greater advantage could result if loading were varied in such a way as to make the energy supply (methane production) more closely coincide with the energy demand within the plant. This would reduce the cost of gas storage, which has been shown to significantly affect the economics of energy substitution by anaerobic digestion for a major food industry (Monteith 1986).

As has been noted, there is little practical information in the literature on the effect of varying loading regimes on digester operation. Reviews such as those by Speece (1981) tend to favour the view that semi-continuous operation does not offer advantages over continuous loading. However based on experimental work with a variety of agricultural wastes, Asinari di san Marzano et al. (1981) reported that semi-continuous operation could be at least as efficient in terms of substrate removal as continuous feeding, so long as no more than 20 % of the digester contents were removed at any one time. The experiments reported in the present work appear unique in the literature in providing a detailed

comparison of the effect of different feeding regimes on digester performance but further research in this area to investigate some of the issues raised above is warranted.

The results of this comparative study also emphasised the importance of careful and complete monitoring of the methane fermentation. Without an awarness of trends in all major process parameters, but particularly of the trends in the specific rate parameters, it is very difficult to accurately assess the status of the digester population and the reasons for process instability that might develop. Much of the current work remains purely descriptive with process parameters reported solely on a volumetric basis and is consequently of limited value. The results reported above indicate that even an approximate analysis based on VSS provides very useful data. Determination of the actual methanogenic biomass concentration via measurement of factor F_{420} (Asinari di san Marzano et al. 1981) now offers the hope of more sophisticated analysis of the effects of various environmental factors on the fermentation and this should in turn permit the formulation of enhanced control strategies and processes.

The results of the second continuous digestion experiment also indicated that the relative levels of acetate and propionate, the two major acids present in the methane fermentation, could significantly affect the recovery of the digester after process failure. This was further investigated in batch culture using effluent from both types of digester and the results were reported in Chapter Five. It was found that increasing the concentration of either acid from low levels markedly reduced the rate of utilisation of the acid. The batch experiments also clearly demonstrated that increasing the concentration of acetate above about 1000 to 1500 $mg.l^{-1}$ significantly inhibited propionate degradation. This had been indicated by many observations in the literature but this work appears to be the

first systematic study of the phenomenon over a range of concentrations of the acids.

These results, combined with the findings from the feeding studies, offer useful insights into the recovery of digesters from high acid conditions. Clearly, elevated acid concentrations will reduce the activity of the acetogenic and methanogenic bacteria and this will persist even once the cause of the original failure (e.g. a temperature shock) has been remedied. The loading rate must be reduced and sufficient time allowed to permit the acid concentration to fall to normal levels before the previously used loading regime is resumed. In some cases an upset may result in accumulation of acetate, and this is particularly likely if the digester feedstock is low in nutrients. In these situations, the reduction in acetogenic activity resulting from the high acetate concentration can be expected to further prolong the recovery process. The results in Chapter Four also indicated that repeated shocks were increasingly detrimental to digester operation so every effort must be made to protect the digester from further upset.

The proposed role of un-ionised acids in limiting the fermentation (Kroeker et al. 1979, Duarte and Anderson 1982) was not conclusively supported by these results and several other reports in the literature appear to contradict this hypothesis. Further work in this area is desirable to clarify how process variables, such as loading rate, alkalinity and the previous history of the culture, influence the effect of the total acid concentration on the fermentation.

In the second continuous digestion experiment apparent stimulation of methanogenesis was noted following addition of cysteine to the medium, while addition of cysteine and sodium sulphide together appeared inhibitory. The effect of slug doses of these compounds and also of sodium thioglycollate, another sulphur-containing reducing agent, were examined in batch culture

(Chapter Six). Inocula drawn from both the continuous and semi-continuous digesters were used and differences in performance were noted. No inhibition of gas production was observed with the continuous-culture inoculum when up to 3.2 mM cysteine or sulphide, or 4.4 mM of thioglycollate, were added. However for the semi-continuous digester population additions of cysteine and sulphide at 3.2 mM reduced the rate of methane formation by 25 % to 30 %, although the final yield of methane was not adversely affected. Thioglycollate added at 3.2 mM was observed not to inhibit the acetoclatic methanogens but propionate degradation was markedly inhibited, with increased toxicity noted with increasing time of exposure.

Sulphide was implicated as the inhibitory agent when cysteine or sodium sulphide were added and the effects noted were very similar to those observed in studies of acetate-enrichment cultures by Van den Berg et al. (1976) and Parkin et al. (1983). Sulphide appeared to be released by degradation of cysteine, as the inhibitory effect was noted after a short lag period, whereas direct addition of sulphide produced immediate inhibition of methane production. The methane yield was also higher in those cultures supplemented with cysteine, indicating carbon released from the reducing agent was converted to methane. Determination of the mechanism of thioglycollate inhibition was considered beyond the scope of this project, but further research into this may illuminate aspects of the biochemistry of the acetogenic bacteria.

The difference in behaviour observed between the two inocula could be explained by acclimation of the continuous digester population to sulphide released from the addition of cysteine to the medium. However the absence of a toxic effect of thioglycollate in the continuous-digester inoculum also indicated that the bacterial flora of the two populations were different.

In summary, the influence of different loading regimes, volatile fatty acids, and sulphur-containing reducing agents on the rate and stability of the methane fermentation have been investigated. Given the length of time required to obtain data, the complexity of the fermentation and the diversity of process designs and feedstocks, it was optimistic to expect that a single research project of this type would achieve dramatic advances in process performance or understanding. Rather, the development of anaerobic wastewater technologies relies heavily on the gradual gathering and synthesis of knowledge across a very broad front, and in this respect, this study is perhaps typical of much recent research effort (Cohen 1981, Speece 1983). Although the research approach has been largely "technological" it has also touched on aspects of the biochemistry and microbiology of the process. The results have provided insights into the methane fermentation which may led to improved stability in industrial digesters, but these must also be further developed to realise the potential of enhanced process control and performance.

REFERENCES

Anderson, G.K.; Donnelly, T. and McKeown,K.J. (1982). Identification and control of inhibition in anaerobic treatment of industrial wastewaters. Process biochemistry 17 (4), 28-33.

Anderson,G.K. and Duarte, A.C. (1980). Research and applications of anaerobic processes. Environmental technology letters 1, 484-493.

Andrews, J.F. (1968). A dynamic model of the anaerobic digestion process. Industrial wastes conference, Purdue University 23, 285-310.

Andrews, J.F. and Pearson, E.A. (1965). Kinetics and characteristics of volatile acid production in anaerobic fermentation processes. International journal of air and water pollution 9, 439-461.

APHA-AWWA-JWPCF (1975). Standard methods for the examination of water and wastewater, 14th edition. Washington, D.C.: APHA-AWWA-JWPCF.

Asinari di san Marzano, C-M.; Binot, R.; Bol, T.; Fripiat, J-L.; Hutschemakers, J.; Melchior, J-L.; Perez, I.; Naveau, H. and Nyns, E-J. (1981). Volatile fatty acids, an important state parameter for the control of the reliability and the productivities of methane anaerobic digestions. Biomass 1, 47-59.

Baader,W. (1981). Design characteristics of anaerobic digestion systems. pp. 71-87. In Anaerobic digestion 1981. Edited by Hughes, D.E. et al.. Amsterdam: Elsevier Biomedical Press.

Baader, W. (1985). Personal communication.

Balch, W.E.; Fox, G.E.; Magrum, L.J.; Woese, C.R. and Wolfe, R.S. (1979). Methanogens: re-evaluation of a unique biological group. Microbiological reviews 43, 260-296.

Balch, W.E. and Wolfe, R.S. (1976). New approach to the cultivation of methanogenic bacteria: 2-mercaptoethane-sulfonic acid (HS-CoM)-dependent growth of Methanobacterium ruminantium in a pressurized atmosphere. Applied and environmental microbiology 32, 781-791.

Baldwin,R.L.; Wood, W.A. and Emery, R.S. (1963). Conversion of glucose-C^{14} to propionate by the rumen microbiotica. Journal of bacteriology 85, 1346-1349.

Banfield, F.S.; Meek, D.M. and Lowden, G.F. (1978). Manual and automated gas chromatographic procedures for the determination of volatile fatty acids. Stevenage: Water Research Centre. (Water Research Centre. Technical report no. 76).

Barber, N.R. (1977). Keep your digester in good shape. Water and wastes engineering 14 (9), 55 + 59.

Baresi, L.; Mah, R.A.; Ward, D.M. and Kaplan, I.R. (1978). Methanogenesis from acetate: enrichment studies. Applied and environmental microbiology 36, 186-197.

Barford, J.P. and Hall, R.J. (1978). An evaluation of the approaches to the mathematical modelling of microbial growth. Process biochemistry 13 (8), 22-26 + 29.

Barker, H.A. (1957). Bacterial fermentations. New York: John Wiley and Sons.

Barker, H.A. (1961). Fermentation of nitrogenous organic compounds. pp. 151-207. In The bacteria, volume 2. Edited by Gunsalus, I.C. and Stanier, R.Y.. New York: Academic Press.

Barnard,J.L. (1983). Background to biological phosphorus removal. Water scince and technology 15, 1-14.

Barth, E.F.; Ettinger, M.B.; Salotto, B.V. and McDermott, G.N. (1965). Summary report on the effects of heavy metals on the biological treatment processes. Journal Water Pollution Control Federation 37, 86-96.

BBL (1968). BBL manual of products and laboratory procedures. Cockeysville, Ma.: BBL.

Blanc, F.C. and Molof, A.H. (1969). Electrode potentials and electrolytic control in the anaerobic digestion process. Industrial wastes conference, Purdue University 24, 1040-1059.

Blanc, F.C. and Molof, A.H. (1973). Electrode potential monitoring and electrolytic control. Journal Water Pollution Control Federation 45, 655-667.

Boone, D.R. (1982). Terminal reactions in the anaerobic digestion of animal wastes. Applied and environmental microbiology 43, 57-64.

Boone, D.R. (1984a). Mixed-culture fermentation for simulating methanogenic digesters. Applied and environmental microbiology 48, 122-126.

Boone, D.R. (1984b). Propionate exchange reactions in methanogenic ecosystems. Applied and environmental microbiology 48, 863-864.

Boone, D.R. and Bryant, M.P. (1980). Propionate degrading bacterium Syntrophobacter wolinii sp. nov. gen. nov., from methanogenic ecosystems. Applied and environmental microbiology 40, 626-632.

Borchardt, J.A. (1971). Anaerobic phase separation by dialysis technique. pp. 108-125. In Anaerobic biological treatment processes. Edited by Pohland, F.G.. Washington, D.C.: American Chemical Society.

Bousefield, S.; Hobson, P.N. and Summers, R. (1974). Pilot plant high-rate digestion of piggery and silage wastes. Journal of applied bacteriology 37, xi. (Title only).

Braun, M; Schoberth, S. and Gottschalk, G. (1979). Enumeration of bacteria forming acetate from H_2 and CO_2 in anaerobic habitats. Archives of mocrobiology 120, 201-204.

Bryant , M.P. (1974). Methane-producing bacteria. pp. 472-477. In Bergey's manual of determinative bacteriology, 8th edition. Edited by Buchanan, R.E. and Gibbons, N.E.. Baltimore: Williams and Wilkins.

Bryant, M.P. (1979). Microbial methane production - theoretical aspects. Journal of animal science 48, 193-201.

Bryant,M.P.; Campbell, L.L.; Reddy, C.A. and Crabill, M.R. (1977). Growth of Desulfovibrio in lactate or ethanol media low in sulphate in association with H_2-utilising methanogenic bacteria. Applied and environmental microbiology 33, 1162-1169.

Bryant, M.P.; Tzeng, S.F.; Robinson, I.M. and Joyner, A.E. (1971). pp. 23-40. In Anaerobic biological treatment processes. Edited by Pohland, F.G.. Washington D.C.: American Chemical Society.

Bryant, M.P.; Wolin, E.A.; Wolin, M.J. and Wolfe, R.S. (1967). Methanobacillus omelianski a symbiotic association of two species of bacteria. Archiv fur mikrobiologie 59, 20-31.

Buhr, H.O. and Andrews, J.F. (1977). The thermophilic anaerobic digestion process. Water research 11, 129-143.

Buswell, A.M. (1936). Anaerobic fermentations. Urbana: Department of Registration and Education, State of Illinois. (Bulletin no. 32).

Buswell, A.M.; Fina, L.R.; Mueller, H. and Yahiro, A. (1951). Use of C^{14} in mechanism studies of methane formation. II. Propionic acid. Journal of the American Chemical Society 73, 1809-1811.

Buswell, A.M and Morgan, G.B. (1963). Paper chromatography for volatile acid determinations. III. Paper chromatography method for volatile acids: toxicity of propionic acid. Engineering progress at the University of Florida, Technical paper 16 (9), 10-20. Chemical Abstracts (1963) 58, 4293d.

Cappenberg, Th.E. (1975). A study of mixed continuous cultures of sulphate-reducing and methane-producing bacteria. Microbial ecology 2, 60-72.

Cappenberg, Th.E. and Prins, R.A. (1974). Interrelations between sulphate-reducing and methane-producing bacteria in bottom deposits of a freshwater lake. 3. Experiments with C-14-labelled substrates. Antonie van Leeuwenhoek 40, 457-469.

Capri, M.G. and Marias, G.v.R. (1975). pH adjustment in anaerobic digestion. Water research 9, 307-313.

Cheeseman, R.V. and Wilson, A.N. (1978). Manual on analytical quality-control for the water industry. Stevenage: Water Research Centre. (Water Research Centre. Technical report no. 66).

Chou, W.L.; Speece, R.E. and Siddiqi, R.H. (1978a). Acclimation and degradation of petrochemical wastewater components by methane fermentation. Biotechnology and bioengineering symposium 8, 391-414.

Chou, W.L.; Speece, R.E.; Siddiqi, R.H. and McKeon, K. (1978b). The effect of petrochemical structure on methane fermentation toxicity. Progress in water technology 10, 545-558.

Chynoweth, D.P. and Mah, R.A. (1971). Volatile acid fomation in sludge digestion. pp. 41-54. In Anaerobic biological treatment processes. Edited by Pohland, F.G.. Washington : American Chemical Society.

Clarke, R.H. and Speece, R.E. (1970). The pH tolerance of anaerobic digestion. pp. II.27/1-II.27/14. In Advances in water pollution research. Edited by Jenkins, S.H.. London: Academic Press.

Cohen, A. (1981). Optimisation of anaerobic digestioon of soluble carbohydrate containing wastewaters by phase separation. Thesis, Ph.D., University of Amsterdam.

Cohen, A.; Zoetemeyer, R.J.; Van Deursen, A. and Van Andel, J.G. (1979). Anaerobic digestion of glucose with separated acid production and methane formation. Water research 13, 571-580

Cohen,A.; Breure, A.M.; Van Andel, J.G. and Van Deursen, A. (1980). Influence of phase separation on the anaerobic digestion of glucose - I maximum COD-turnover rate during continuous operation. Water research 14, 1439-1448.

Cohen, A.; Breure, A.M.; Van Andel, J.G. and Van Deursen, A. (1982). Influence of phase separation on the anaerobic digestion of glucose - II stability, and kinetic responses to shock loadings. Water research 16, 449-458.

Conrad, R.; Phelps, T.J. and Zeikus, J.G. (1985). Gas metabolism evidence in support of the juxtaposition of hydrogen producing and methanogenic bacteria in sewage sludge and lake sediments. Applied and environmental microbiology 50, 595-601.

Cooney, C.L. and Wise, D.L. (1975). Thermophilic anaerobic digestion of solid waste for fuel gas production. Biotechnology and bioengineering 17, 1119-1135.

Daniels, L.; Fuchs, G.; Thauer, R.K. and Zeikus, J.G. (1977). Carbon monoxide metabolism by methanogenic bacteria. Journal of bacteriology 132, 118-126.

DeWitt C.C. (1943). Correlation of rate data. Industrial and engineering chemistry 35, 695-700.

Diekert,G.; Konheiser, U.; Piechulla, K. and Thauer, R.K. (1981). Nickel requirement and factor F_{430} content of methanogenic bacteria. Journal of bacteriology 148, 459-464.

DiLallo, R. and Albertson, O.E. (1963). Volatile acids by direct titration. Journal Water Pollution Control Federation 33, 356-365.

Dirasian, H.A.; Molof, A.H. and Borchardt, J.A. (1963). Electrode potential developed during slugdge digestion. Journal Water Pollution Control Federation 35, 424-439.

Dohanyos, M.; Kosova, B.; Zabranska, J. and Grau, P. (1985). Production and utilisation of volatile fatty acids in various types of anaerobic reactors. Water science and technology 17, 191-205.

Duarte, A.C. and Anderson, G.K. (1982). Inhibition modelling in anaerobic digestion. Water science and technology 14, 749-763.

Eastman, J.A. and Fergusson, J.F. (1981). Solubilisation of particulate organic carbon during the acid phase of anaerobic digestion. Journal Water Pollution Control Federation 53, 352-366.

Edwards, V.H. and Wilke, C.R. (1968). Mathematical representation of batch culture data. Biotechnology and bioengineering 10, 205-232.

Fergusson, T.J. and Mah, R.A. (1983). Effect of H_2-CO_2 on methanogenesis from acetate or methanol in Methanosarcina spp.. Applied and environmental microbiology 46, 348-355.

Fields, M. and Agardy, F.J. (1971). Oxygen toxicity in digesters. Industrial wastes conference, Purdue University 26, 284-293.

Finney, C.D. and Evans, R.S. (1975). Anaerobic digestion: the rate limiting process and the nature of inhibition. Science 190, 1088-1089.

Fredrickson, A.G.; Megee, R.D. and Tsuchiya, H.M. (1970). Mathematical models for fermentation processes. Advances in applied microbiology 13, 419-465.

Frostell, B. (1981). Anaerobic treatment in a sludge bed system compared with a filter system. Journal Water Pollution Control Federation 53, 216-222.

Garber, W.F. (1977). Certain aspects of anaerobic digestion of wastewater solids in the thermophilic range at the Hyperion treatment plant. Progress in water technology 8, 401-406.

Ghosh,S.; Conrad, J.R. and Klass, D.L. (1975). Anaerobic acidogenesis of wastewater sludge. Journal Water Pollution Control Federation 47, 30-45.

Ghosh, S. and Klass, D.L. (1978). Two-phase anaerobic digestion. Process biochemistry 13 (4), 15-20 + 22-24.

Ghosh,S. and Pohland, F.G. (1974). Kinetics of substrate assimilation in anaerobic digestion. Journal Water Pollution Control Federation 46, 748-759.

Grant,C.L. and Pramer, D. (1962). Minor element composition of yeast extract. Journal of bacteriology 84, 869-870.

Gujer, W. and Zehnder, A.J.B. (1983). Conversion processes in anaerobic digestion. Water science and technology 15, 127-167

Hansson, G. (1979). Effects of carbon dioxide and methane on methanogenesis. European journal of applied microbiology and biotechnology 6, 351-359.

Hansson, G. (1982). End product inhibition in methane fermentations. Process biochemistry 17 (6), 45-49.

Hansson, G. and Molin, N. (1981a). End product inhibition in methane fermentations: effects of carbon dioxide and methane on methanogenic bacteria utilising acetate. European journal of applied microbiology and biotechnology 13, 236-241.

Hansson, G. and Molin, N. (1981b). End product inhibition in methane fermentations: effects of carbon dioxide on fermentative bacteria. European journal of applied microbiology and biotechnology 13, 242-247.

Hartz, K.E. and Kountz, R.R. (1966). Effects of CO_2 and N_2 on anaerobic digestion. Journal of the Sanitary Engineering Division, American Society of Civil Engineers 92, 83-95.

Hattingh, W.H.J.; Kotze, J.P.; Thiel, P.G.; Toerien, D.F. and Siebert, M.L. (1967). Biological changes during the adaption of an anaerobic digester to a synthetic substrate. Water research 1, 255-277.

Hayes, T.D. and Theis, T.L. (1978). The distribution of heavy metals in anaerobic digestion. Journal Water Pollution Control Federation 50, 61-72.

Henson, M.J. and Smith, P.H. (1985). Isolation of a butyrate-utilising bacterium in coculture with Methanobacterium thermoautotrophicum from a thermophilic digester. Applied and environmental microbiology 49, 1461-1466.

Henze, M. and Harremoes, P. (1983). Anaerobic treatment of wastewater in fixed film reactors - a literature review. Water science and technology 15, 1-101.

Heukelekian, H. and Mueller, P. (1958). Transformation of some lipids in anaerobic sludge digestion. Sewage and industrial wastes 30, 1108-1120.

Hoban, D.J. and Van den Berg, L. (1979). Effect of iron on conversion of acetic acid to methane during methanogenic fermentations. Journal of applied bacteriology 47, 153-159.

Hobson, P.N.; Bousefield, S. and Summers, R. (1974). Anaerobic digestion of organic mater. Crtical reviews in environmental control 4, 131-191.

Hobson, P.N.; Bousefield, S. ans Summers, R. (1981). Methane production from agricultural and domestic wastes. London: Applied Science.

Hobson, P.N. and McDonald, I. (1980). Methane production from acids in piggery-waste digesters. Journal of chemical technology and biotechnology 30, 405-408.

Hobson, P.N. and Shaw, B.G. (1971). The role of strict anaerobes in the digestion of organic material. pp 103-122. In Microbial aspects of pollution. Edited by Sykes, G. and Skinner, F.A.. London: Academic Press.

Hobson, P.N. and Shaw, B.G. (1976). Inhibition of methane production by Methanobacterium formicicum. Water research 10, 849-852.

Hungate, R.E. (1966). The rumen and its microbes. New York: Academic Press.

Hungate, R.E. (1969). A roll tube method for cultivation of strict anaerobes. pp 117-132. In Methods in microbiology, volume 3B. Edited by Norris, J.R. and Ribbons, D.W.. London: Academic Press.

Hungate, R.E.; Smith, W.; Bauchop, T.; Yu, I. and Rabinowitz, J.C. (1970). Formate as an intermediate in the bovine rumen fermentation. Journal of bacteriology 102, 389-397.

Huser, B.A.; Wuhrmann, K. and Zehnder, A.J.B. (1982). Methanothrix soehngenii gen. nov. sp. nov., a new acetotrophic non-hydrogen-oxidising methane bacterium. Archives of microbiology 132, 1-9.

Iannotti, E.L.; Wulfers, M.K.; Fischer, J.R. and Sievers, D.M. (1981). The effect of digester fluid, swine manure extract, and rumen fluid on the growth of bacteria from an anaerobic swine manure digester. Developments in industrial microbiology 22, 565-576.

Iannotti, E.L.; Fischer, J.R. and Sievers, D.M. (1982a). Characterisation of bacteria from a swine manure digester. Applied and environmental microbiology 43, 136-143.

Iannotti, E.L.; Fischer, J.R. and Sievers, D.M. (1982b). Medium for enhanced growth of bacteria from a swine manure digester. Applied and environmental microbiology 43, 247-249.

Jeris, J.S. (1983). Industrial wastewater treatment using anaerobic fluidized bed reactors. Water science and technology 15, 169-176.

Jeris, J.S. and McCarty, P.L. (1965). The biochemistry of methane fermentation using C^{14} tracers. Journal Water Pollution Control Federation 37, 178-192.

Jewell, W.J. (1981). Development of the attached microbial film expanded-bed process for aerobic and anaerobic waste treatment. pp. 251-271. In Biological fluidised bed treatment of water and wastewater. Edited by Cooper, P.F. and Atkinson, B.. Chichester: Ellis Horwood Ltd.

Jirka, A.M. and Carter, M.J. (1975). Micro semi-automated analysis of surface and waste-waters for chemical oxygen demand. Analytical chemistry 47, 1397-1402.

Jones, J.B. and Stadtman, T.C. (1977). Methanococcus vannelii: culture and effects of selenium and tungsten on growth. Journal of bacteriology 130, 1404-1406.

Jones, W.J.; Donnelly, M.I. and Wolfe, R.S. (1985). Evidence of a common pathway of carbon dioxide reduction to methane in methanogens. Journal of bacteriology 163, 126-131.

Joyner, A.E. and Baldwin, R.L. (1966). Enzymatic studies of pure cultures of rumen microorganisms. Journal of bacteriology 92, 1321-1330.

Kandler, O. and Hippe, H. (1977). Lack of peptidoglycan in the cell walls of Methanosarcina barkeri. Archives of microbiology 113, 57-60.

Kandler, O. and Konig,H. (1978). Chemical compostion of the peptidoglycan-free cell walls of methanogenic bacteria. Archives of microbiology 118, 141-152.

Karube, I.; Kuriyama, S.; Matsunaga, T. and Suzuki, S. (1980). Methane production from wastewaters by immobilized methanogenic bacteria. Biotechnology and bioengineering 22, 847-857.

Kaspar, H.F. and Wuhrmann, K. (1978a). Kinetic parameters and relative turnovers of some important catabolic reactions in digesting sludge. Applied and environmental microbiology 36, 1-7.

Kaspar, H.F. and Wuhrmann, K. (1978b). Product inhibition in sludge digestion. Microbial ecology 4, 241-248.

Kaziro, G. and Ochoa, S. (1964). The metabolism of propionic acid. Advances in enzymology 26, 283-433.

Keefer, C.E and Kratz, H. (1933). The effect of gases on sewage sludge digestion. Sewage works journal 4, 247-251.

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

Keltjens, J.T. and Vogels, G.D. (1981). Novel coenzymes in methanogens. pp. 152-159. In Microbial growth on C_1 compounds. Edited by Dalton, H.. London: Heyden and Sons Ltd..

Khan, A.W. and Trottier, T.M. (1978). Effect of sulphur-containing compounds on anaerobic degradation of cellulose to methane by mixed cultures obtained from sewage sludge. Applied and environmental microbiology 35, 1027-1034.

Kirsch, E.J. and Sykes, R.M. (1971). Anaerobic digestion in biological waste treatment. Progess in industrial microbiology 9, 155-237.

Koch,M.; Dolfing, J.; Wuhrmann, K. and Zehnder, A.J.B. (1983). Pathways of propionate degradation by enriched methanogenic cultures. Applied and environmental microbiology 45, 1411-1414.

Kotze, J.P.; Thiel, P.G.; Toerien, D.F.; Hattingh, W.H.J. and Siebert, M.L. (1968). A biological and chemical study of several anaerobic digesters. Water research 2, 195-214.

Kotze, J.P.; Thiel, P.G. and Hattingh, W.H. (1969). Anaerobic digestion II: the characterisation and control of anaerobic digestion. Water research 3, 459-493.

Kroeker, E.J.; Schulte, D.D.; Sparling, A.B. and Lapp, H.M. (1979). Anaerobic treatment process stability. Journal Water Pollution Control Federation 51, 718-727.

Kugelmann, I.J. and Chin, K.K. (1971). Toxicity, synergism, and anatagonism in anaerobic waste treatment processes. pp 55-90. In Anaerobic biological treatment processes. Edited by Pohland. F.G.. Washington, D.C.: American Chemical Society.

Kugelmann, I.J. and McCarty, P.L. (1964). Cation toxicity and stimulation in anaerobic waste treatment. 2: daily feed studies. Industrial wastes conference, Purdue University 19, 667-686.

Kugelmann, I.J. and McCarty, P.L. (1965). Cation toxicity and stimulation in anaerobic waste treatment. Journal Water Pollution Control Federation 37, 97-116.

Lawrence, A.W. and McCarty, P.L. (1965). The role of sulphide in preventing heavy metal toxicity in anaerobic treatment. Journal Water Pollution Control Federation 37, 392-406.

Lawrence, A.W. and McCarty, P.L. (1969). Kinetics of methane fermentation in anaerobic treatment. Journal Water Pollution Control Federation 41, R1-R7.

Lawrence, A.W.; McCarty, P.L. and Guerin, F.J.A. (1964). The effects of sulphides on anaerobic treatment. Industrial wastes conference, Purdue University 19, 343-357.

Lehninger, A.L. (1975). Biochemistry. New York: Worth Publishers.

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

Levin, G.V and Shapiro, J. (1965). Metabolic uptake of phosphorous by wastewater organisms. Journal Water Pollution Control Federation 37, 800-821.

Luria, S.E. (1961). The bacterial protoplasm: composition and organisation. pp. 1-34. In The bacteria, volume 1. Edited by Gunsalus, I.C. and Stanier, R.Y.. New York: Academic Press.

Mackie, R.I. and Bryant, M.P. (1981). Metabolic activity of fatty acid-oxidising bacteria and the contribution of acetate, propionate, butyrate, and CO_2 to methanogenesis in cattle waste at 40 and 60 C. Applied and environmental microbiology 41, 1363-1373.

Mah, R.A. (1980). Isolation and characterisation of Methanococcus mazei. Current microbiology 3, 321-326.

Mah, R.A.; Smith, M.R. and Baresi, L. (1978). Studies of an acetate-fermenting strain of Methanosarcina. Applied and environmental microbiology 35, 1174-1184.

Malin, B. and Finn, R.K. (1951). The use of a synthetic resin in anaerobic media. Journal of bacteriology 62, 349-350.

Mangel, G.; Villermaux, J. and Prost, C. (1980). Methane production under pressure by fermentation of waste materials. European journal of applied microbiology and biotechnology 9, 79-81.

Marais, G.v.R.; Lowenthal, R.E. and Siebritz, I.P. (1983). Observations supporting phosphate removal by biological excess uptake - a review. Water science and technology 15, 15-42.

Masselli, J.W.; Masselli, N.W. and Burford, M.G. (1967). Sulphide saturation for better digester performance. Journal Water Pollution Control Federation 39, 1369-1373.

Massey, M.L. and Pohland, F.G. (1978). Phase separation of anaerobic stabilisation by kinetic controls. Journal Water Pollution Control Federation 50, 2204-2222.

Massey, L.K.; Sokatch, J.R. and Conrad, R.S. (1976). Branched-chain amino acid catabolism in bacteria. Bacteriological reviews 40, 42-54.

Maugh, T.H. (1977). Phylogeny; are the methanogens a third class of life. Science 198, 812.

McAllan, A.B. and Smith, R.H. (1973). Degradation of nucleic acid derivatives by rumen bacteria in vitro. British journal of nutrition 29, 467-474.

McCarty, P.L. (1964a). Anaerobic waste treatment fundamentals. Part one: chemistry and microbiology. Public works 95 (9), 107-112.

McCarty, P.L. (1964b). Anaerobic waste treatment fundamentals. Part two: environmental requirements and control. Public works 95 (10), 123-126.

McCarty, P.L. (1964c). Anaerobic waste treatment fundamentals. Part three: toxic materials and their control. Public works 95 (11), 91-94.

McCarty, P.L. (1964d). Anaerobic waste treatment fundamentals. Part four: process design. Public works 95 (12), 95-99.

McCarty, P.L. (1965). Thermodynamics of biological synthesis and growth. pp. 169-199. In Advances in water pollution research. Edited by Baars, J.K.. Oxford: Pergamon Press.

McCarty, P.L. (1981). One hundred years of anaerobic treatment. pp. 3-22. In Anaerobic digestion 1981. Edited by Hughes, D.E et al.. Amsterdam: Elsevier Biomedical Press.

McCarty, P.L. and Brosseau, M.H. (1963). Effect of high concentrations of individual volatile acids on anaerobic treatment. Industrial wastes conference, Purdue University 18, 283-296.

McCarty, P.L.; Jeris, J.S. and Murdoch, W. (1963). Individual volatile acids in anaerobic treatment. Journal Water Pollution Control Federation 35, 1501-1516.

McCarty, P.L. and McKinney, R.E. (1961). Salt toxicity in anaerobic digestion. Journal Water Pollution Control Federation 33, 399-415.

McCarty, P.L. and Vath, C.A. (1963). Volatile acid digestion at high loading rates. Journal of the Instute of Air and Water Pollution 6, 65-73.

McInerney, M.J. and Bryant, M.P. (1981a). Review of methane fermentation fundamentals. pp. 19-46. In Fuel gas production from biomass, volume 1. Edited by Wise, D.L.. Boca Raton, Fa.: CRC Press.

McInerney, M.J. and Bryant, M.P. (1981b). Anaerobic degradation of lactate by syntrophic associations of Methanosarcina barkeri and Desulfvovibrio species and effect of H_2 on acetate degradation. Applied and environmental microbiology 41, 346-354.

McInerney, M.J.; Bryant, M.P.; Hespell, J.W. and Costerton, J.W. (1981). Syntrophomonas wolfei gen. nov. sp. nov., an anaerobic, syntrophic, fatty acid-oxidising bacterium. Applied and environmental microbiology 41, 1029-1039.

Melbinger, N.R. and Donnellon, J. (1971). Toxic effects of ammonia nitrogen in high-rate digestion. Journal Water Pollution Control Federation 43, 1658-1668.

Monteith, W. (1986). Personal communication.

Mosey, F.E. (1971). The toxicity of cadmium to anaerobic digestion: its modification by inorganic ions. Water pollution control 70, 584-598.

Mosey, F.E.; Foulkes, M. and Jago, D.A. (1978). Chromatographic analysis of digester gases. Stevenage: Water Research Centre. (Water Research Centre. Technical report no. 85).

Mosey, F.E. and Hughes, D.A. (1975). The toxicity of heavy metal ions to anaerobic digestion. Water pollution control 74, 18-39.

Mountford, D.O. and Asher, R.A. (1978). Changes in proportions of acetate and carbon dioxide used as methane precursors during the anaerobic digestion of bovine waste. Applied and environmental microbiology 35, 648-654.

Mountford, D.O. and Asher, R.A. (1979). Effect of inorganic sulphide on the growth and metabolism of Methanosarcina barkeri strain DM. Applied and environmental microbiology 37, 670-675.

Murray, W.D. and Van den Berg, L. (1981). Effects of nickel, cobalt and molybdenum on performance of methanogenic fixed-film reactors. Applied and environmental microbiology 42, 502-505.

Novak, J.T. and Carlson, D.A. (1970). The kinetics of anaerobic long-chain fatty acid degradation. Journal Water Pollution Control Federation 42, 1932-1943.

Novak, J.T. and Ramesh, M.S. (1975). Stimulation in anaerobic digestion. Water research 9, 963-967.

Ort, J.E. (1976). High quality methane gas through modified anaerobic digestion. U.S. Patent no. 3 981-000.

Owen, W.F.; Stuckey, D.C.; Healy, J.R.; Young, L.Y. and McCarty, P.L. (1979). Bioassay for monitoring biochemical methane potential and anaerobic toxicity. Water research 13, 485-492.

Oxoid (1969). The oxoid manual, 4th edition. Basingstoke:Oxoid Ltd

Pappelis, A.J. and Schmid, W.E. (1965). Comparative nutrient element content of two yeast extracts. Mycologia 57, 974-975.

Parkin, G.F.; Speece, R.E.; Yang, C.H.J. and Kocher, W.M. (1983). Response of methane fermentation systems to industrial toxicants. Journal Water Pollution Control Federation 55, 44-53.

Patel, G.B.; Khan, A.W. and Roth, L.A. (1978). Optimum levels of sulphate and iron for the cultivation of pure cultures of methanogens in synthetic media. Journal of applied bacteriology 45, 347-356.

Pette, K.C. and Versprille, A.I. (1981). Application of the UASB concept for wastewater treatment. pp. 121-133. In Anaerobic digestion 1981. Edited by Hughes, D.E. et al.. Amsterdam: Elsevier Biomedical Press.

Pfeffer, J.T. (1974). Temperature effects on anaerobic fermentation of domestic refuse. Biotechnology and bioengineering 15, 771-787.

Pine, M.J. and Barker, H.A. (1956). Studies on the methane fermentation: 4 - the pathway of hydrogen in the acetate fermentation. Journal of bacteriology 71, 644-648.

Pine, M.J. and Vishniac, W. (1957). The methane fermentation of acetate and methanol. Journal of bacteriology 73, 736-742.

Pirt, S.J. and Lee, Y.K. (1983). Enhancement of methanogenesis by traces of oxygen in bacterial digestion of biomass. FEMS microbiology letters 18, 61-63.

Pohland, F.G. (1967). High rate digestion control: 2 - techniques for evaluating acid-base equilibrium. Industrial wastes conference, Purdue University 22, 353-365.

Pohland, F.G. and Bloodgood, D.E. (1963). Laboratory studies on mesophilic and thermophilic anaerobic sludge digestion. Journal Water Pollution Control Federation 35, 11-42.

Pohland, F.G. and Mancy, K.H. (1969). Use of pH and pE measurements during methane biosynthesis. Biotechnology and bioengineering 11, 683-699.

Prins, R.A. (1977). Biochemical activities of gut microorganisms. pp. 73-184. In Microbial ecology of the gut. Edited by Clarke, R.T.J. and Bauchop, T.. London: Academic Press.

Racker, E. (1965). Mechanisms in bioenergetics. New York: Academic Press.

Radiometer (1966). Redox measurements; their theory and technique. Copenhagen: Radiometer.

Reddy, C.A.; Bryant, M.P. and Wolin, M.J. (1972). Characterisation of S-organism isolated from Methanobacillus omelianski. Journal of bacteriology 109, 539-545.

Roels, J.A. and Kossen, N.W.F. (1978). On the modelling of microbial metabolism. Progress in industrial microbiology 14, 95-203.

Ronnow, P.H. and Gunnarsson, L.A.H. (1981). Sulphide-dependent methane production and growth of a thermophilic methanogenic bacterium. Applied and environmental microbiology 42, 580-584.

Rose, C.S. and Pirt, S.J. (1981). Conversion of glucose to fatty acids and methane: role of two mycoplasmal agents. Journal of bacteriology 147, 248-254.

Runquist, E.A.; Abbott, E.H.; Armold, M.T. and Robbins, J.E. (1981). Application of C^{13}-nuclear magnetic resonance to the observation of metabolic interactions in anaerobic digesters. Applied and environmental microbiology 42, 556-559.

Scherer, P. and Sahm, H. (1981). Influence of sulphur-containing compounds on the growth of Methanosarcina barkeri in a defined medium. European journal of applied microbiology and biotechnology 12, 28-35.

Schink, B. (1985). Mechanisms and kinetics of succinate and propionate degradation in anoxic freshwater sediments and sewage sludge. Journal of general microbiology 131, 643-650.

Schonheit, P.; Moll, J. and Thauer, R.K. (1979). Nickel, cobalt and molybdenum requirement for growth of Methanobacterium thermoautotrophicum. Archives of microbiology 123, 105-107.

Schroepfer, G.J; Fullen, W.J.; Johnson, A.S.; Ziemke, N.R. and Anderson, J.J. (1955). The anaerobic contact process as applied to packing house wastes. Sewage and industrial wastes 27, 460-486.

Schultze, K.L. and Raju, B.N. (1958). Studies on sludge digestion and methane fermentation II. Methane fermentation of organic acids. Sewage and industrial wastes 30, 164-184.

Scott, R.I.; Williams, T.N. and Lloyd, D. (1983). Oxygen sensitivity of methanogenesis in rumen and anaerobic digester populations using mass spectrometry. Biotechnology letters 5, 375-380.

Shea, T.G.; Pretorius, W.A.; Cole,R.D. and Pearson, E.A. (1968). Kinetics of hydrogen assimilation in the methane fermentation. Water research 2, 833-848.

Sheldon, D.R. and Tiedje, J.M. (1984). Isolation and partial characterisation of bacteria in an anaerobic consortium that mineralises 3-chlorobenzoic acid. Applied and environmental microbiology 48, 840-848.

Sineriz, F. and Pirt, S.J. (1977). Methane production from glucose by a mixed culture of bacteria in the chemostat: the role of Citrobacter. Journal of general microbiology 101, 57-64.

Smith, M.R. and Mah, R.A. (1978). Growth and methanogenesis by Methanosarcina strain 227 on acetate and methanol. Applied and environmental microbiology 36, 870-879.

Smith, M.R.; Zinder, S.H. and Mah, R.A. (1980). Microbial methanogenesis from acetate. Process biochemistry 15, 34-39.

Smith, P.H. and Mah, R.A. (1966). Kinetics of acetate metabolism during sludge digestion. Applied microbiology 14, 368-371.

Sonoda, Y. and Seiko, Y. (1977). Effects of heavy metal compound, inorganic salts, hydrocarbon compounds, and antibiotics in methane fermentation. Hakkokogaku kaishi 55, 22-29.

Speece, R.E. (1981). Fundamentals of the anaerobic digestion of municipal sludges and industrial wastewaters. Presented at Seminar on Anaerobic Wastewater Treatment and Energy Recovery, November 3-4, Pittsburgh, Pa..

Speece, R.E. (1983). Anaerobic biotechnology for industrial wastewater treatment. Environmental science and technology 17, 416A-427A.

Speece, R.E. and McCarty, P.L. (1964). Nutrient requirements and biological solids accumulation in anaerobic digestion. pp. 305-333. In Advances in water pollution research, volume 2. Edited by Eckenfelder, W.W.. London: Pergamon Press.

Speece, R.E.; Parkin, G.F. and Gallagher, D. (1983). Nickel stimulation of anaerobic digestion. Water research 17, 677-683.

Stadtman, T.C. (1967). Methane fermentation. Annual review of microbiology 21, 121-142.

Stadtman, T.C. and Barker, H.A. (1949). Studies on the methane fermentation IV. Tracer studies. Archives of biochemistry 21, 256-264.

Stadtman, T.C. and Barker, H.A. (1951). Studies on the methane fermentation IX. The origin of methane in the acetate and methanol fermentations by Methanosarcina. Journal of bacteriology 61, 81-86.

Stander, G.J. (1964). Discussion to Speece and McCarty (1964).

Stander, G.J. (1950). Effluents from fermentation industries. IV. Method for increasing and maintaining efficiency in the anaerobic digestion of fermentation effluents. Journal of the Insitute of Sewage Purification 1950, 438-447.

Stander, G.J. and Snyders, R. (1950). Re-inoculation as an integral part of the anaerobic digestion method of purification of fermentation effluents. Journal of the Institute of Sewage Purification 1950, 447-458.

Swanwick, J.D.; Bruce, A.M and Vandyke, K.G. (1968). Inhibition of sludge digestion by synthetic detergents. Water pollution control 67, 91-99.

Switzenbaum, M.S. and Danskin, S.C. (1982). Anaerobic expanded bed treatment of whey. Agricultural wastes 4, 411-426.

Tait, S.J. and Friedman, A.A. (1980). Anaerobic rotating biological contactor for carbonaceous wastewaters. Journal Water Pollution Control Federation 52, 2257-2269.

Taylor, G.T. (1982). The methanogenic bacteria. Progess in industrial microbiology 16, 231-329.

Taylor, G.T. and Pirt, S.J. (1977). Nutrition and factors limiting the growth of a methanogenic bacterium (Methanobacterium thermoautotrophicum). Archives of microbiology 113, 17-22.

Thauer, R.K.; Jungermann, K. and Decker, K. (1977). Energy conservation in chemotrophic bacteria. Bacteriological reviews 41, 100-180.

Thiel, P.G.; Toerien, D.F.; Hattingh, W.H.J.; Kotze, J.P. and Siebert, M.L. (1968). Interrelationships between biological and chemical characteristics in anaerobic digestion. Water research 2, 391-408.

Toerien, D.F. (1967). Enrichment culture studies on aerobic and facultatively anaerobic bacteria found in anaerobic digesters. Water research 1, 147-155.

Toerien, D.F. and Hattingh, W.H. (1969). Anaerobic digestion: 1. The microbiology of anaerobic digestion. Water research 3, 385-416.

Tool, H.R. (1967). Manometric measurement of the biochemical oxygen demand. Water and sewage works 114, 211-218.

Uribelarrea, J.L. and Pareilleux, A. (1981). Anaerobic digestion: microbial and biochemical aspects of volatile acid production. European journal of applied microbiology and biotechnology 12, 118-122.

Valley,G. and Rettger, L.F. (1927). The influence of carbon dioxide on bacteria. Journal of bacteriology 14, 101-137.

Van den Berg, L. (1977). Effect of temperature on growth and activity of a methanogenic culture utilising acetate. Canadian journal of microbiology 23, 893-902.

Van den Berg, L. and Kennedy, K.J. (1983). Comparison of advanced anaerobic reactors. pp. 71-89. In Proceedings of the Third International Symposium on Anaerobic Digestion.

Van den Berg, L.; Kennedy, K.J. and Hamoda, M.F. (1981). Effect of type of waste on performance of anaerobic fixed-film and upflow sludge blanket reactors. Industrial wastes conference, Purdue University 36, 686-692.

Van den Berg, L.; Lamb, K.A.; Murray, W.D. and Armstrong, D.W. (1980). Effects of sulfate, iron and hydrogen on the microbiological conversion of acetic-acid to methane. Journal of applied bacteriology 48, 437-447.

Van den Berg, L. and Lentz, C.P. (1979). Comparison between up- and downflow fixed-film reactors of varying surface-to-volume ratios for the treatment of bean blanching waste. Industrial wastes conference, Purdue University 34, 319-324.

Van den Berg, L.; Patel, G.B.; Clark, D.S. and Lenz, C.P. (1976). Factors affecting rate of methane formation from acetic acid by enriched methanogenic cultures. Canadian journal of microbiology 22, 1312-1319.

Van Velsen, A.F.M. (1979). Adaption of methanogenic sludge to high ammonia-nitrogen concentrations. Water research 13, 995-1000.

Varel, V.H. and Hashimoto, A.G. (1982). Methane production by fermenter cultures acclimated to waste from cattle fed monsein, salinomycin, or avoparcin. Applied and environmental microbiology 44, 1415-1420.

Wallnofer, P.; Baldwin, R.L. and Stagno, E. (1966). Conversion of C^{14} labeled substrates to volatile fatty acids by the rumen microbiotica. Applied microbiology 14, 1004-1010.

Walther, R.;Fiebig, K.; Fahlbusch, K.; Caspari, D.; Hippe, H. and Gottschalk, G. (1980). Growth of methanogens on methylamines. pp. 146-151. In Microbial growth on C_1 compounds. Edited by Dalton, H.. London: .Heyden and Sons Ltd..

Weimer, P.J. and Zeikus, J.G. (1978). Acetate metabolism in Methanosarcina barkeri. Archives of microbiology 119, 175-182.

Wellinger, A. and Wuhrmann, K. (1977). Influence of sulphide compounds on the metabolism of Methanobacterium strain AZ. Archives of microbiology 115, 13-17.

Weng, C-N. and Jeris, J.S. (1976). Biochemical mechanisms in the methane fermentation of glutamic and oleic acids. Water research 10, 9-18.

Whitman, N.B. and Wolfe, R.S. (1980). Presence of nickel in factor F_{430} from Methanobacterium bryantii. Biochemical and biophysical research communications 92, 1196-1201.

Willis, A.T. (1969). Techniques for the study of anaerobic spore-forming bacteria. pp. 79-115. In Methods in microbiology, volume 3B. Edited by Norris, J.R. and Ribbons, D.W.. London: Academic Press.

Winfrey, M.R. and Zeikus, J.G. (1977). Effect of sulfate on carbon and electron flow during microbial methanogenesis in freshwater sediments. Applied and environmental microbiology 33, 275-281.

Winter, J.U. and Cooney, C.L. (1980). Fermentation of cellulose and fatty acids with enrichments from sewage sludge. European journal of applied microbiology and biotechnology 11, 60-66.

Woese, C.R. and Fox, G.E. (1977). Phylogenetic structure of the procaryotic domain: the primary kingdoms. Proceedings of the National Academy of Science of the United States of America 74, 5088-5090.

Woese, C.R. and Fox, G.E. (1978). Methanogenic bacteria. Nature 273, 101.

Wolfe, R.S. (1971). Microbial formation of methane. Advances in microbial physiology 6, 107-146.

Wolfe, R.S. (1980). Respiration in methanogenic bacteria. pp. 161-186. In Diversity of bacterial respiratory systems, volume 1. Edited by Knowles, C.J.. Boca Raton, Fa.: CRC Press.

Wolin, M.J. (1969). Volatile fatty acids nd the inhibition of Escherichia coli growth by rumen fluid. Applied bacteriology 17, 83-87.

Wolin, M.J. (1974). Metabolic interactions among intestinal organisms. American journal of clinical nutrition 27, 1320-1328.

Wood, W.A. (1961). Fermentation of carbohydrates and related compounds. pp. 59-150. In The bacteria, volume 2. Edited by Gunsalus, I.C. and Stanier, R.Y.. New York: Academic Press.

Zehnder, A.J.B.; Huser, B.A.; Brock, T.D. and Wuhrmann, K. (1980). Characterisation of an acetate-decarboxylating, non-hydrogen oxidising methane bacterium. Archives of microbiology 124, 1-11.

Zehnder, A.J.B.; Ingvorsen, K. and Marti, T. (1981). Microbiology of methane bacteria. pp. 45-68. In Anaerobic digestion 1981. Edited by Hughes, D.E. et al.. Amsterdam: Elsevier Biomedical Press.

Zehnder, A.J.B. and Koch, M.E. (1983). Thermodynamic and kinetic interactions of the final steps in anaerobic digestion. pp 86-96. In Proceedings of the European Symposium on Anaerobic Waste Water Treatment.

Zeikus, J.G. (1977). The biology of the methanogenic bacteria. Microbiological reviews 41, 514-541.

Zeikus, J.G.; Weimer, P.J.; Nelson, D.R. and Daniels, L. (1975). Bacterial methanogenesis: acetate as a methane precursor in pure culture. Archives of microbiology 104, 129-134.

Zinder, S.H. and Mah, R.A. (1979). Isolation and characterisation of a thermophilic strain of Methanosarcina unable to use H_2-CO_2 for methanogenesis. Applied and environmental microbiology 38, 996-1008.

APPENDIX ONE PROCEDURE USED TO CALCULATE THE TOTAL VOLATILE FATTY ACID (TVFA) CONCENTRATION

The procedure used was as follows: the individual acid concentrations were multiplied by appropriate conversion factors, and the modified concentrations were then summed to obtain the TVFA concentration as $mg.l^{-1}$ acetate.

The conversion factors related the COD of the individual acids to that of acetate. For example, the conversion factor for propionate was calculated as follows.

For acetate,

$$CH_3COOH + 2O_2 = 2CO_2 + 2H_2O$$

Complete oxidation of 60 mg acid requires 64 mg oxygen (COD), so the COD conversion factor for acetate is:

$$CF_{ac} = 64/60 = 1.067 \text{ mg COD.(mg acetate)}^{-1}$$

For propionate,

$$CH_3CH_2COOH + 3.5O_2 = 3CO_2 + 3H_2O$$

Complete oxidation of 74 mg acid requires 112 mg oxygen (COD), so the COD conversion factor for propionate is:

$$CF_{pr} = 112/74 = 1.514 \text{ mg COD.(mg propionate)}^{-1}$$

The overall conversion factor to convert the propionate concentration to an equivalent acetate concentration is then given by:

$$\text{Equivalent } mg.l^{-1} \text{ acetate} = mg.l^{-1} \text{ propionate} \times CF_{pr}/CF_{ac}$$
$$= mg.l^{-1} \text{ propionate} \times 1.419$$

Overall conversion factors calculated in the same way for the other acids are:

i-butyric acid	1.704
butyric acid	1.704
i-valeric acid	1.911
valeric acid	1.911

APPENDIX TWO ADDITIONAL EXPERIMENTAL DATA PERTAINING TO THE CONTINUOUS AND SEMI-CONTINUOUS DIGESTION EXPERIMENTS

Table A2.1: Gas production from SCDR1 at varying times from feed addition.

Day	Time since feed addition	Percent of total gas production evolved
	hr	%
152	5	38
154	5	35
156	7	44
158	7	44
162	7	52
166	16	75
168	6	44
170	6	42
172	6	42
174	2	19
	28	96
182	24	91
184	4	37
186	3	30
188	2	22
190	7	48
192	6	41

Table A2.2: Gas production from SCDR2 at varying times from feed addition.

Day	Time since feed addition	Percent of total gas production evolved
	hr	%
26	8	44
28	7	44
30	14	66
32	7	46
36	22	68
38	7	44
40	8	46
42	25	82
44	5	33
46	6	36
48	20	69
50	4	28

Table A2.3: Acid concentrations in batch digesters supplemented with trace metals or lysed cells.

Run	Time	Acid concentration mg.l^{-1}					
	days	Ac	Pr	iBu	Bu	iVa	Va
Control	0	2100	220	110	80	90	n.d.[a]
	2	2190	230	100	20	80	n.d.
	4	2000	210	100	10	80	n.d.
	6	1950	110	90	10	90	n.d.
	9	1710	90	80	20	110	n.d.
	13	1530	10	20	n.d.	90	n.d.
	18	840	n.d.	n.d.	n.d.	60	n.d.
	22	120	n.d.	n.d.	n.d.	10	n.d.
	28	n.d.	10	n.d.	n.d.	n.d.	n.d.
Trace metals	0	2100	220	110	80	90	n.d.[a]
	2	2090	220	110	10	80	30
	4	2010	210	100	10	80	n.d.
	6	1970	90	90	10	90	n.d.
	9	1660	40	50	10	80	n.d.
	13	1490	10	10	n.d.	80	n.d.
	18	790	n.d.	n.d.	n.d.	60	n.d.
	22	100	n.d.	n.d.	n.d.	10	n.d.
	28	n.d.	10	n.d.	n.d.	n.d.	n.d.
Lysed cells	0	2100	220	110	80	90	n.d.[a]
	2	2060	320	80	10	90	n.d.
	4	1510	290	90	10	80	n.d.
	6	1230	150	80	n.d.	90	n.d.
	9	750	70	20	n.d.	90	n.d.
	13	190	n.d.	n.d.	n.d.	20	n.d.
	18	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

a not detected

APPENDIX THREE EXPERIMENTAL DATA FOR BATCH DIGESTION EXPERIMENTS INVESTIGATING DEGRADATION OF VOLATILE FATTY ACIDS

Table A3.1: Data for experiment 1.

Run	Time	Acetate concentration	Propionate concentration	Potential acetate pool
	days	$mg.l^{-1}$	$mg.l^{-1}$	$mg.l^{-1}$
1	0	n.d.[a]	550	450
	4	10	450	370
	8	80	110	170
	12	n.d.	n.d.	n.d.
2	0	510	560	960
	4	230	400	550
	8	120	30	140
	12	20	n.d.	20
	16	n.d.	n.d.	n.d.
3	0	1240	600	1730
	4	1070	460	1440
	8	510	220	690
	12	260	n.d.	260
	16	40	n.d.	40
	20	n.d.	n.d.	n.d.
4	0	1950	560	2400
	4	1740	480	2130
	8	960	320	1220
	12	540	140	650
	16	160	10	170
	20	n.d.	n.d.	n.d.
5	0	1890	80	1950
	4	850	20	870
	8	350	n.d.	350
	12	50	10	60
	16	20	n.d.	20
	20	n.d.	n.d.	n.d.
6	0	1940	290	2170
	4	1520	200	1680
	8	770	70	820
	12	420	n.d.	420
	16	20	n.d.	20
	20	n.d.	n.d.	n.d.

Table A3.1 (contd)

Run	Time	Acetate concentration	Propionate concentration	Potential acetate pool
	days	$mg.l^{-1}$	$mg.l^{-1}$	$mg.l^{-1}$
7	0	1960	520	2380
	4	1740	480	2130
	8	1130	350	1410
	12	710	190	860
	16	360	n.d.	360
	20	n.d.	n.d.	n.d.
8	0	2040	1700	3420
	4	1820	1580	3100
	8	1040	1390	2170
	12	660	1000	1470
	16	270	470	650
	20	50	n.d.	50
	24	n.d.	n.d.	n.d.

a not detected

Table A3.2: Predicted volumetric rates of propionate degradation for batch digesters, experiment 1.

Time	Propionate utilisation						
days	$mg.l^{-1}.day^{-1}$						
Run	1	2	3	4	6	7	8
0	5	5	11	8	10	3	5
4	62	110	53	28	35	15	5
8	63	27	66	53	25	45	72
12	5	1	18	37	5	46	147
16				12		16	115
20				3			41

Table A3.3: Data for experiment 2.

Run	Time	Acetate concentration	Propionate concentration	Potential acetate pool
	days	$mg.l^{-1}$	$mg.l^{-1}$	$mg.l^{-1}$
1	0	n.d.[a]	490	400
	2	n.d.	400	320
	4	20	200	180
	6	40	n.d.	40
	8	n.d.	n.d.	n.d.
2	0	530	520	950
	2	390	430	740
	4	220	270	440
	6	130	90	200
	8	10	n.d.	10
	10	n.d.	n.d.	n.d.
3	0	540	520	960
	2	340	400	660
	4	190	250	390
	6	60	50	100
	8	n.d.	n.d.	n.d.
4	0	1060	480	1450
	2	990	440	1350
	4	670	360	960
	6	430	190	580
	8	190	50	230
	10	20	n.d.	20
	12	n.d.	n.d.	n.d.
5	0	2110	480	2500
	2	1990	430	2340
	4	1640	370	1940
	6	1280	290	1510
	8	970	180	1120
	10	500	90	570
	12	230	20	250
	14	20	n.d.	20
	16	n.d.	n.d.	n.d.

Table A3.3 (contd)

Run	Time	Acetate concentration	Propionate concentration	Potential acetate pool
	days	$mg.l^{-1}$	$mg.l^{-1}$	$mg.l^{-1}$
6	0	2240	n.d.	2240
	2	2110	n.d.	2110
	4	1810	n.d.	1810
	6	1540	n.d.	1540
	8	1150	n.d.	1150
	10	810	n.d.	810
	12	500	n.d.	500
	14	200	n.d.	200
	16	50	n.d.	50
	18	n.d.	n.d.	n.d.
7	0	2170	n.d.	2170
	2	1810	n.d.	1810
	4	1540	n.d.	1540
	6	1120	n.d.	1120
	8	890	n.d.	890
	10	610	n.d.	610
	12	310	n.d.	310
	14	50	n.d.	50
	16	n.d.	n.d.	n.d.
8	0	2220	220	2400
	2	2060	190	2210
	4	1670	160	1830
	6	1430	90	1500
	8	1140	40	1170
	10	760	n.d.	760
	12	430	n.d.	430
	14	70	n.d.	70
	16	n.d.	n.d.	n.d.
9	0	2190	510	2600
	2	2110	480	2500
	4	1860	430	2210
	6	1470	340	1750
	8	1100	230	1280
	10	710	140	820
	12	390	60	440
	14	70	n.d.	70
	16	10	n.d.	10
	18	n.d.	n.d.	n.d.

Table A3.3 (contd)

Run	Time	Acetate concentration	Propionate concentration	Potential acetate pool
	days	$mg.l^{-1}$	$mg.l^{-1}$	$mg.l^{-1}$
10	0	2140	1490	3350
	2	2080	1460	3260
	4	1990	1310	3050
	6	1810	1260	2830
	8	1670	1180	2620
	10	1440	1100	2330
	12	1220	1020	2050
	14	960	960	1740
	16	740	900	1470
	18	490	710	1070
	20	350	720	930
	22	250	640	770
	24	250	570	710
	26	180	490	580
11	0	1960	1510	3190
	2	1950	1470	3140
	4	1910	1400	3040
	6	1760	1360	2860
	8	1610	1330	2690
	10	1290	1280	2330
	12	930	1210	1910
	14	540	1160	1510
	18	200	1030	1030
	22	50	910	790
	28	110	680	660
	38	110	340	390

a not detected

APPENDIX FOUR SAMPLE GENSTAT PROGRAM USED TO FIT THE LOGISTIC EQUATION TO EXPERIMENTAL DATA FROM STUDIES ON DEGRADATION OF VOLATILE FATTY ACIDS.

Two computer listing are shown on the following pages. The example uses propionate concentration data from run 4, experiment 1.

The input program (LOG.GEN) contains GENSTAT commands followed by the experimental data. Comments, which are enclosed within double primes (’’ ’’), have been included to explain the structure of the program and the purpose of the commands statements.

In the output listing (E1R4PR.LIST) the input GENSTAT commands are printed first, then the convergence of the optimisation program is monitired with each iteration. When the convergence criterion has been staisfied estimates of the model parameters (K, a, b) are listed. The observed and fitted data are then printed and plotted.

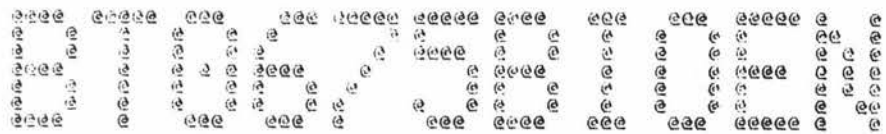
```
User: BT0675BIDEN                              -at AGHORT

<USER2>BT0675BIDEN>AJM.82>E1R4PR.LIST
```

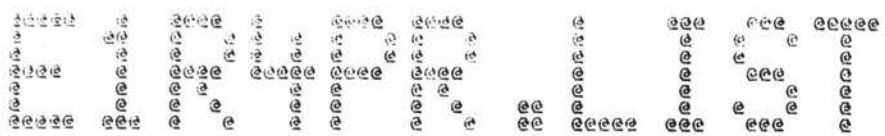
```
Label: PRT009 -form

Pathname: <USER2>BT0675BIDEN>AJM.82>LOG.GEN
File last modified: 86-12-05.14:48:12.Fri

Queued:   86-12-05.14:53:40.Fri    [Print rev 19.0.]
Started:  86-12-05.14:57:08.Fri    on: AMLC     by: AGHORT

Ag. Hort. Building Remote Printer.
```

```
'REFERENCE' LOGISTIC


'REFERENCE' LOGISTIC
'UNITS' $ 6
''UNITS SPECIFIES THE NUMBER OF DATA POINTS''

'READ' TIME, SUBSTR

'SCALAR'  K=1
  :       A=-2
  :       B=0.5
''SCALAR VALUES ARE THE INTIAL ESTIMATES OF THE MODEL PARAMETERS''

'VARIATE' STEPSIZE = 0.1, 0.05, 0.01
''THIS SPECIFIES THE STEPSIZE FOR EACH PARAMETER IN THE OPTIMISATION ALGORITHM''

'MODEL' DIGEST $ EXPSUB = K/(1+EXP(A+B*TIME))
''SPECIFIES THE MODEL TO BE FITTED TO THE DATA''

'OPTIMIZE/NPAR=3, LIK=2, ITER=30, PRINT=PFM' MODEL=DIGEST; PARAM=K,A,B;
           STEPS = STEPSIZE ; Y = SUBSTR ; Z = EXPSUB ;
           FVAL = FITSUB
''INITIATES THE NON-LINEAR REGRESSION ALGORITHM''

'HEADING' GTYPE = ''LL''
  :       SYMBS = ''+*''
'GRAPH' SUBSTR, FITSUB ; TIME, TIME $ GTYPE ; SYMBS
''HEADING AND GRAPH STATEMENTS PROVIDE THE FORMAT FOR THE PLOT IN THE OUTPUT''

'RUN'
0 0.56
4 0.48
8 0.32
12 0.14
16 0.01
20 0

'EOD'
''THE EXPERIMENTAL DATA ARE READ''
'CLOSE'
'STOP'
```

User BT0675BIOEN -at AGHORT

<USER2>BT0675BIOEN>AJM.82>E1R4PR.LIST





Label: PRT010 -form

Pathname: <USER2>BT0675BIOEN>AJM.82>E1R4PR.LIST
File last modified: 86-12-05.14:48:44.Fri

Queued: 86-12-05.14:54:40.Fri [Print rev 19.0.]
Started: 86-12-05.14:57:56.Fri on: AMLC by: AGHORT

Ag. Hort. Building Remote Printer.

1

```
GENSTAT V    RELEASE 4.04B
COPYRIGHT 1984 LAWES AGRICULTURAL TRUST (ROTHAMSTED EXPERIMENTAL STATION)

  1  'REFERENCE' LOGISTIC
  2  'UNITS' $ 6
  3  ''UNITS SPECIFIES THE NUMBER OF DATA POINTS''
  5  'READ' TIME, SUBSTR
  8  'SCALAR'  K=1
  9  :       A=-2
 10  :       B=0.5
 11  ''SCALAR VALUES ARE THE INTIAL ESTIMATES OF THE MODEL PARAMETERS''
 13  'VARIATE' STEPSIZE = 0.1, 0.05, 0.01
 14  ''THIS SPECIFIES THE STEPSIZE FOR EACH PARAMETER IN THE OPTIMISATION ALGORITHM''
 16  'MODEL' DIGEST $ EXPSUB = K/(1+EXP(A+B*TIME))
 17  ''SPECIFIES THE MODEL TO BE FITTED TO THE DATA''
 19  'OPTIMIZE/NPAR=3, LIK=2, ITER=30, PRINT=PFM' MODEL=DIGEST; PARAM=K,A,B;
 20                 STEPS = STEPSIZE ; Y = SUBSTR ; Z = EXPSUB ;
 21                 FVAL = FITSUB
 22  ''INITIATES THE NON-LINEAR REGRESSION ALGORITHM''
 24  'HEADING' QTYPE = ''LL''
 25  :         SYMBS = ''+*''
 26  'GRAPH' SUBSTR, FITSUB ; TIME, TIME $ QTYPE ; SYMBS
 27  ''HEADING AND GRAPH STATEMENTS PROVIDE THE FORMAT FOR THE PLOT IN THE OUTPUT''
 30  'RUN'


  IDENTIFIER    MINIMUM       MEAN    MAXIMUM     VALUES    MISSING
        TIME      0.000     10.000     20.000          6          0
      SUBSTR     0.0000     0.2517     0.5600          6          0
```

*** CONVERGENCE MONITORING ***

ITER	FNO.	MOVE	FUNC VALUE	CURRENT PARAMETERS		
0	2	0	0.15857443	1.000000	-2.000000	0.500000
			STEPS	0.100000	0.050000	0.010000
			STEPS	0.018634	0.093171	0.028799
1	27	2	0.03436943	0.877419	-1.381435	0.309994
			STEPS	0.027827	0.100721	0.017840
2	51	2	0.02089413	0.483135	-2.798946	0.309328
3	66	0	0.00096733	0.577044	-3.218643	0.372108
4	83	0	0.00095651	0.574230	-3.277190	0.378341
5	100	0	0.00095648	0.574105	-3.279936	0.378648
			STEPS	0.020222	0.151867	0.016281
6	126	0	0.00095648	0.574105	-3.279936	0.378648
			STEPS	0.005051	0.038006	0.004069
7	143	1	0.00095607	0.573729	-3.287512	0.379593
8	158	0	0.00094583	0.576066	-3.215662	0.373983
9	176	6	0.00094583	0.576115	-3.213878	0.373832
			STEPS	0.002459	0.046927	0.004163
1	191	0	0.00094583	0.576115	-3.213878	0.373832

***** NON-LINEAR REGRESSION ANALYSIS *****

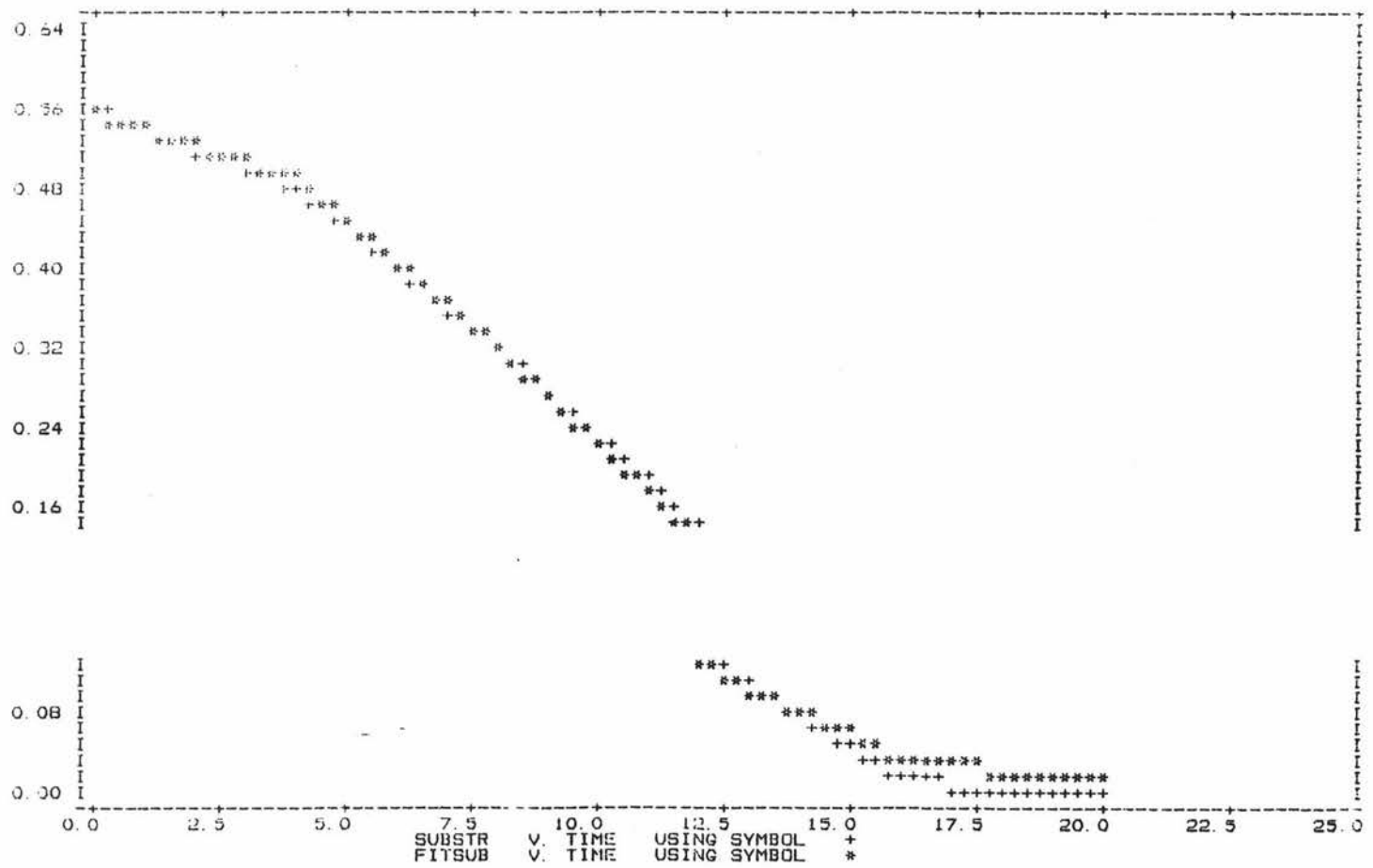
*** PARAMETER ESTIMATES ***

	ESTIMATE	S.E.	CORRELATIONS		
K	0.57612	0.02457	1.0000		
A	-3.21388	0.46834	0.8163	1.0000	
B	0.37383	0.04155	-0.7113	-0.9622	1.0000

	DF	SS	MS
RESIDUAL	3	0.000946	0.000315

*** FITTED VALUES AND RESIDUALS ***

UNIT	OBSERVED	FITTED	RESIDUAL
1	0.5600	0.5539	0.0061
2	0.4800	0.4885	-0.0085
3	0.3200	0.3201	-0.0001
4	0.1400	0.1261	0.0139
5	0.0100	0.0341	-0.0241
6	0.0000	0.0080	-0.0080




```
 39  ''THE EXPERIMENTAL DATA ARE READ''
 40  'CLOSE'

******** END OF LOGISTIC.  MAXIMUM OF 2645 DATA UNITS USED AT LINE 26 (30122 LEFT)
```

APPENDIX FIVE EXPERIMENTAL DATA FOR BATCH DIGESTION EXPERIMENTS INVESTIGATING THE EFFECT OF SULPHUR-CONTAINING REDUCING AGENTS ON THE METHANE FERMENTATION.

Table A5.1: Data for experiment 1.

Time	Methane production							
hr	ml							
Run	1	2	3	4	5	6	7	8
4	6.6	6.2	7.1	6.7	6.7	6.9	6.6	6.6
46	9.8	9.5	11.3	11.4	10.3	10.5	9.8	9.8
67	11.2	10.7	13.2	13.0	11.4	11.4	10.8	10.6
92	12.0	12.0	14.4	14.0	12.5	12.5	10.8	10.6

Table A5.2: Data for experiment 2.

Time	Methane production			
hr	ml			
Run	1	2	3	4
8	8.3	7.7	8.7	8.7
21	14.0	15.3	16.0	14.9
45	20.2	23.0	21.7	21.0
69	23.3	27.4	24.7	24.0
141	25.7	30.1	26.4	25.7
285	26.9	30.5	27.4	26.5

Table A5.3: Data for experiment 3.

Time	Methane production							
hr	ml							
Run	1	2	3	4	5	6	7	8
4	0.1	0.3	0.4	0.3	0.3	0.3	0.3	0.2
10	0.2	3.1	3.1	2.5	2.9	3.0	2.5	0.6
20	0.5	6.7	7.7	6.2	7.1	7.4	7.1	2.1
30		11.3	12.6	8.9	11.5	11.5	11.4	5.3
44	1.3	17.2	18.9	13.3	17.8	17.0	17.7	10.3
54		21.3	22.8	16.1	21.6	20.0	21.6	13.2
68	2.2	27.3	28.4	20.0	27.7	22.6	27.6	18.3
92	2.8	36.5	37.5	26.1	37.1	24.8	36.8	26.1
116	3.2	40.8	41.7	30.1	41.0	24.8	40.8	33.5
140	3.6	42.3	43.3	35.1	42.3	25.4	42.2	39.9
164	3.6	42.3	43.4	39.2	42.4	25.7	42.4	40.9
191	3.8	42.3	43.6	44.3	42.7	25.7	42.4	41.4
236	4.0	42.9	44.1	47.0	43.4	26.4	42.9	42.7
260	4.1	43.1	44.3	47.3	43.4	26.6	43.2	42.7

Table A5.4: Data for experiment 4.

Time	Methane production				
hr	ml				
Run	1	2	3	4	5
4	0.1	0.5	0.4	0.4	0.4
20	0.3	6.6	6.3	6.3	5.8
28		10.0	9.4	9.7	8.5
44	0.7	16.0	15.7	15.7	15.4
52		18.5	17.8	18.4	18.1
68	1.1	24.1	23.4	24.0	23.3
92	1.5	28.2	27.6	28.9	29.3
116	1.8	29.5	29.5	30.8	32.3
140	2.0	30.1	29.8	31.4	33.2
166	2.3	30.5	30.2	31.7	33.8
188	2.5	30.7	30.6	32.1	34.1
236	2.8	31.2	30.9	32.6	34.4

Table A5.5: Data for experiment 5.

Time	Methane production					
hr	ml					
Run	1	2	3	4	5	6
4	0.2	0.2	0.2	0.1	0.2	0.2
20	5.6	5.8	5.9	5.8	5.3	5.2
44	18.8	18.9	19.6	19.6	15.7	15.1
52	23.8	24.3	24.8	24.6	19.8	19.0
68	30.8	31.5	32.1	32.1	27.2	26.2
95	32.4	33.1	34.2	34.0	34.4	34.4
118	32.8	33.4	34.4	34.4	35.8	36.3
140	32.9	33.8	35.0	34.7	36.4	36.9
164	32.9	33.8	35.0	34.9	36.9	37.5
189					37.3	37.8
212					37.3	37.8

Table A5.6: Data for experiment 6.

Time	Methane production			
hr	ml			
Run	1	2	3	4
4	0.4	0.5	0.5	0.5
21	7.0	6.7	6.6	6.7
28	10.4	10.1	10.0	10.0
46	21.9	21.6	21.4	21.5
52	25.1	24.9	24.4	24.4
69	28.9	28.8	28.4	28.5
93	29.7	29.7	29.2	29.4
189	31.1	31.2	29.5	29.7

Table A5.7: Data for experiment 7.

Time	Methane production			
hr	ml			
Run	1	2	3	4
4	0.1	0.1	0.1	0.1
20	4.7	5.2	2.9	2.8
28	8.8	9.4	4.7	4.5
44	17.7	18.3	9.0	8.8
52	22.8	23.3	11.3	11.4
68	30.3	30.0	18.5	18.4
92	31.2	31.2	28.6	28.5
116	32.0	31.9	31.9	31.8
140	32.2	32.2	32.3	32.1
166	32.3	32.3	32.4	32.3

Table A5.8: Data for experiment 8.

Time	Methane production		
hr	ml		
Run	1	2	3
4	0.2	0.2	0.1
20	2.8	2.7	2.7
47	9.6	9.5	7.3
70	15.8	15.6	9.1
92	22.9	22.1	10.1
116	28.3	27.6	10.2
141	29.3	28.8	10.9
164	29.3	28.8	11.2
188	29.7	29.1	11.2

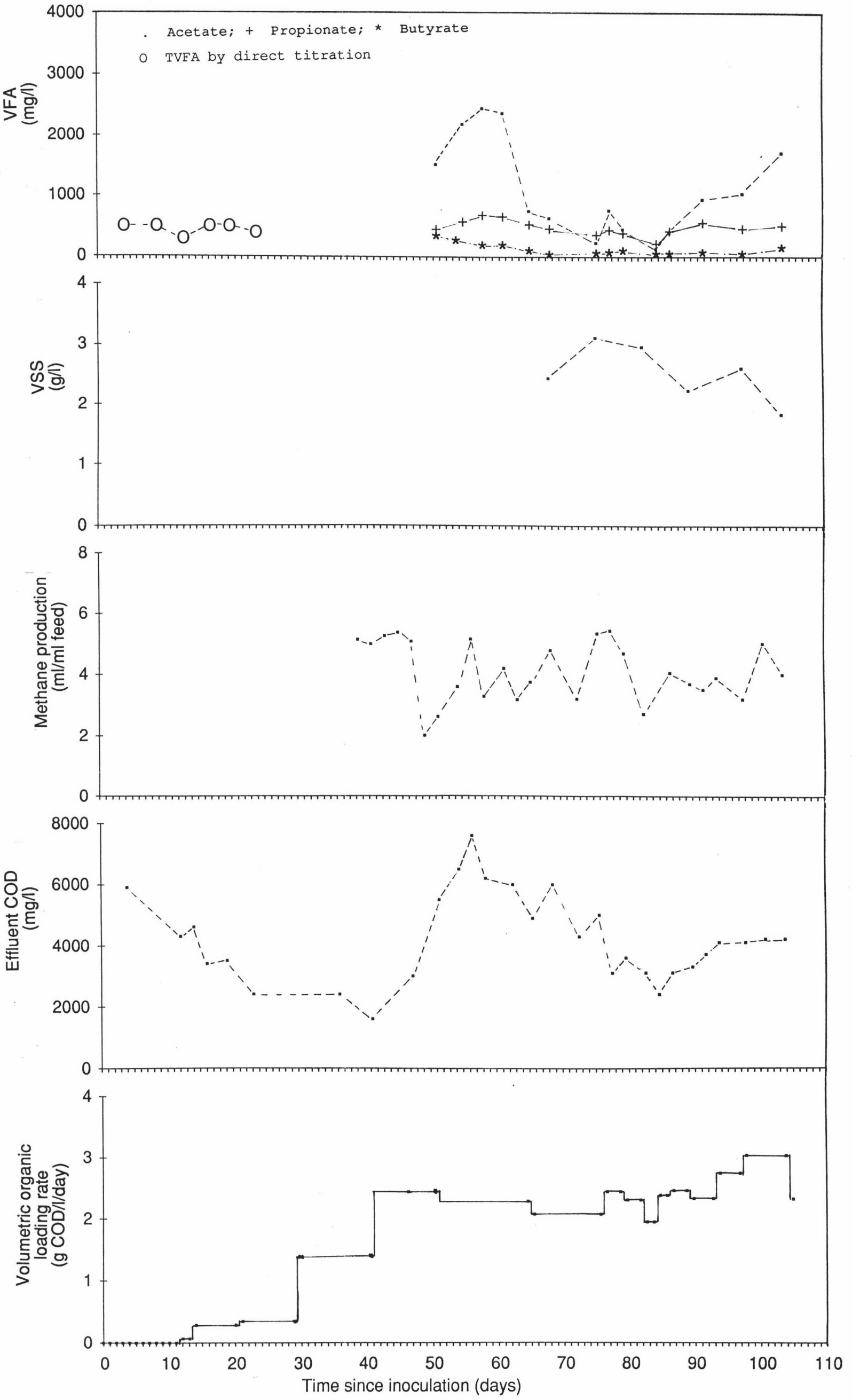


Figure 4.1: Performance of the continuous digester, run 1 (CDR1), from inoculation to day 103.

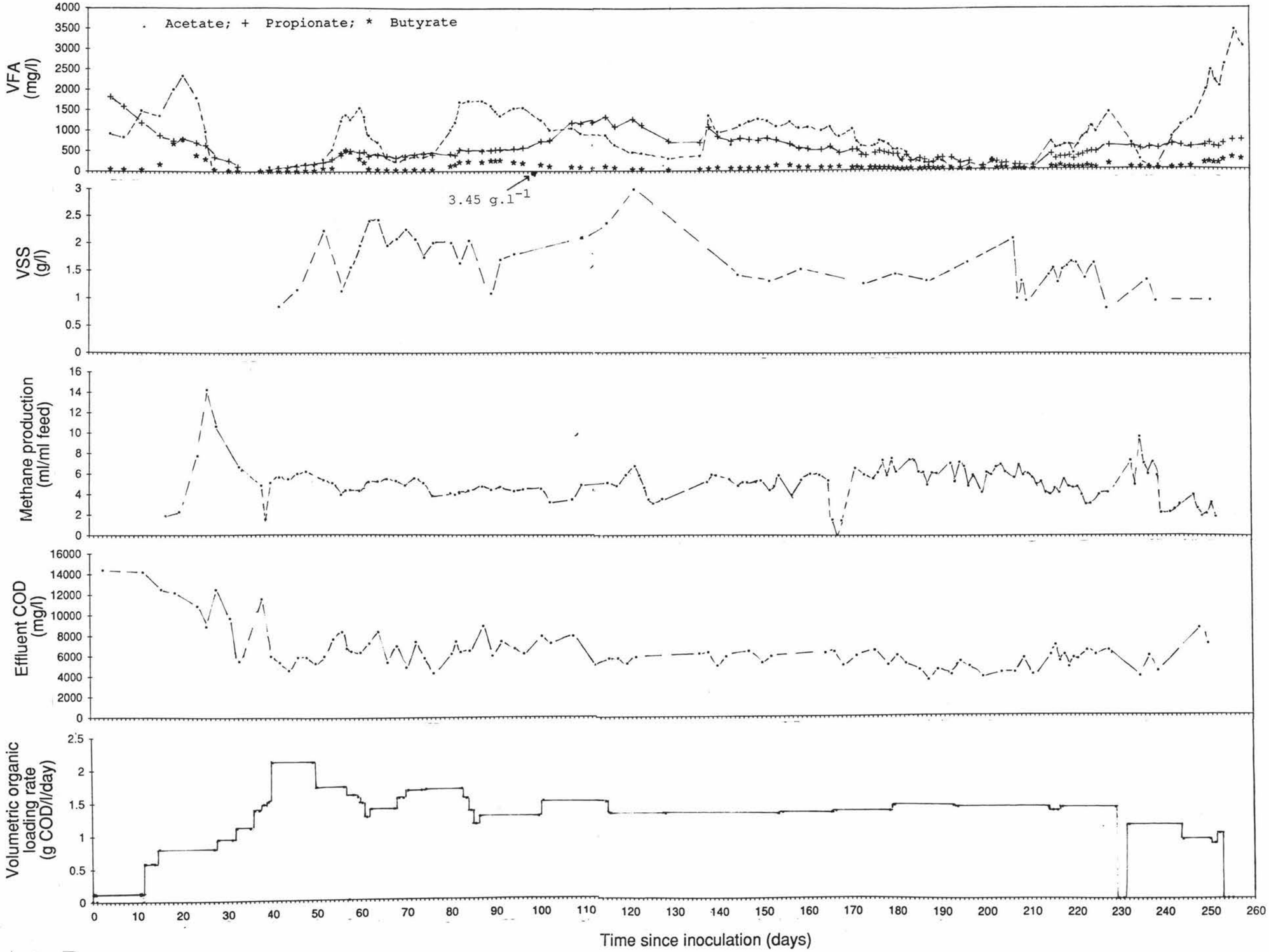


Figure 4.2: Performance of the continuous digester, run 2 (CDR2).

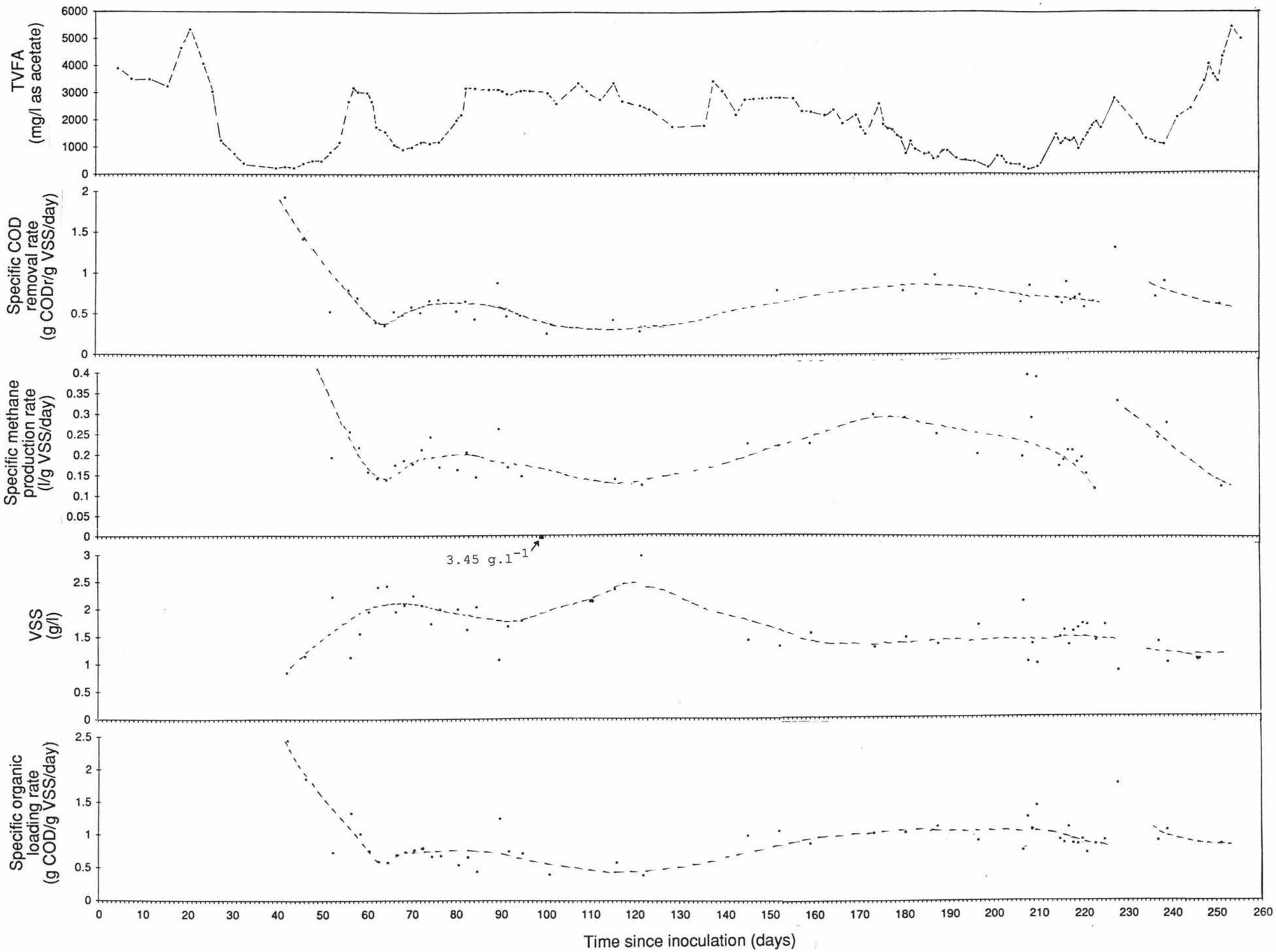


Figure 4.3: Specific rate data for CDR2

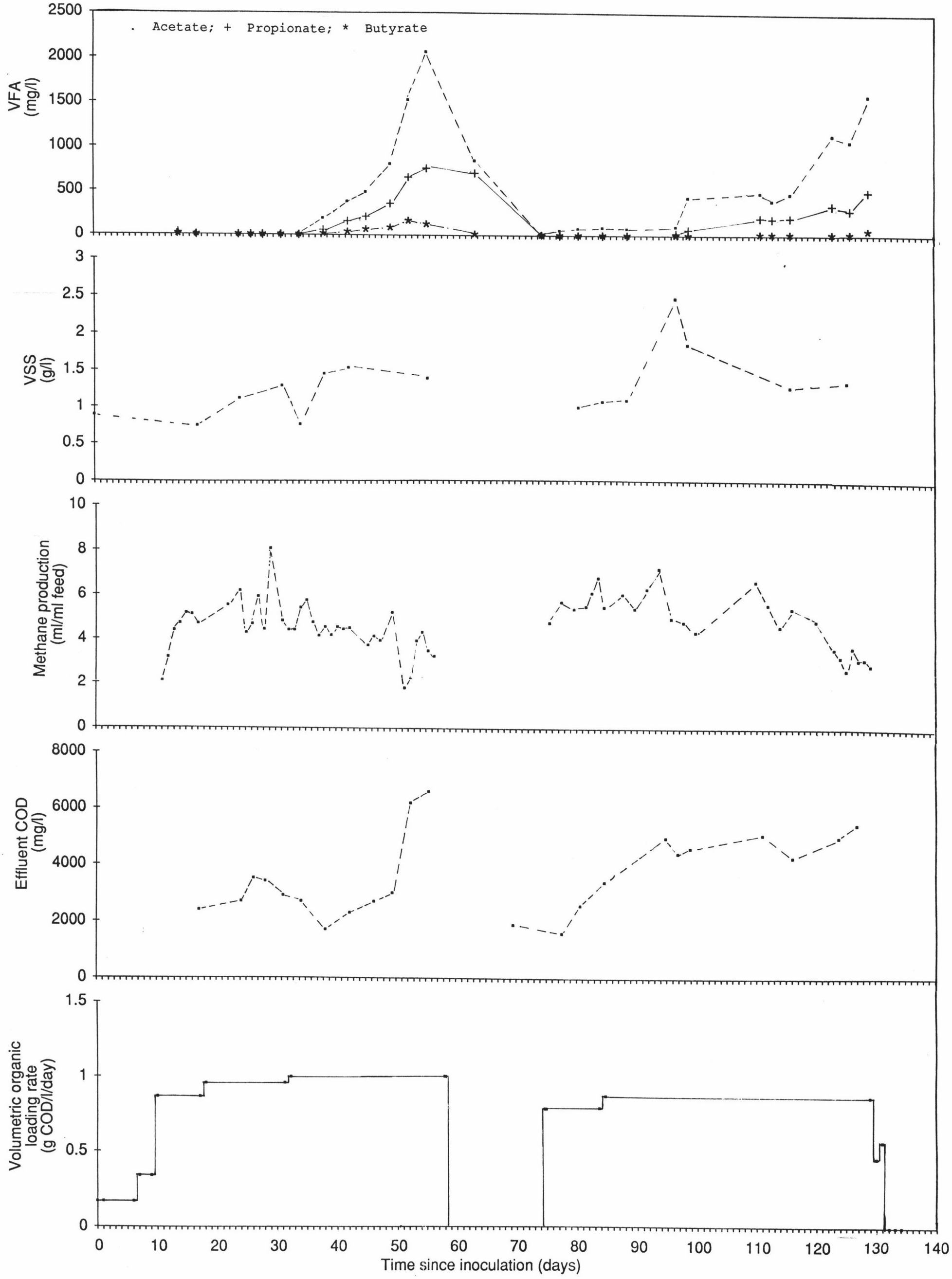


Figure 4.6: Performance of the continuous digester, run 3 (CDR3).

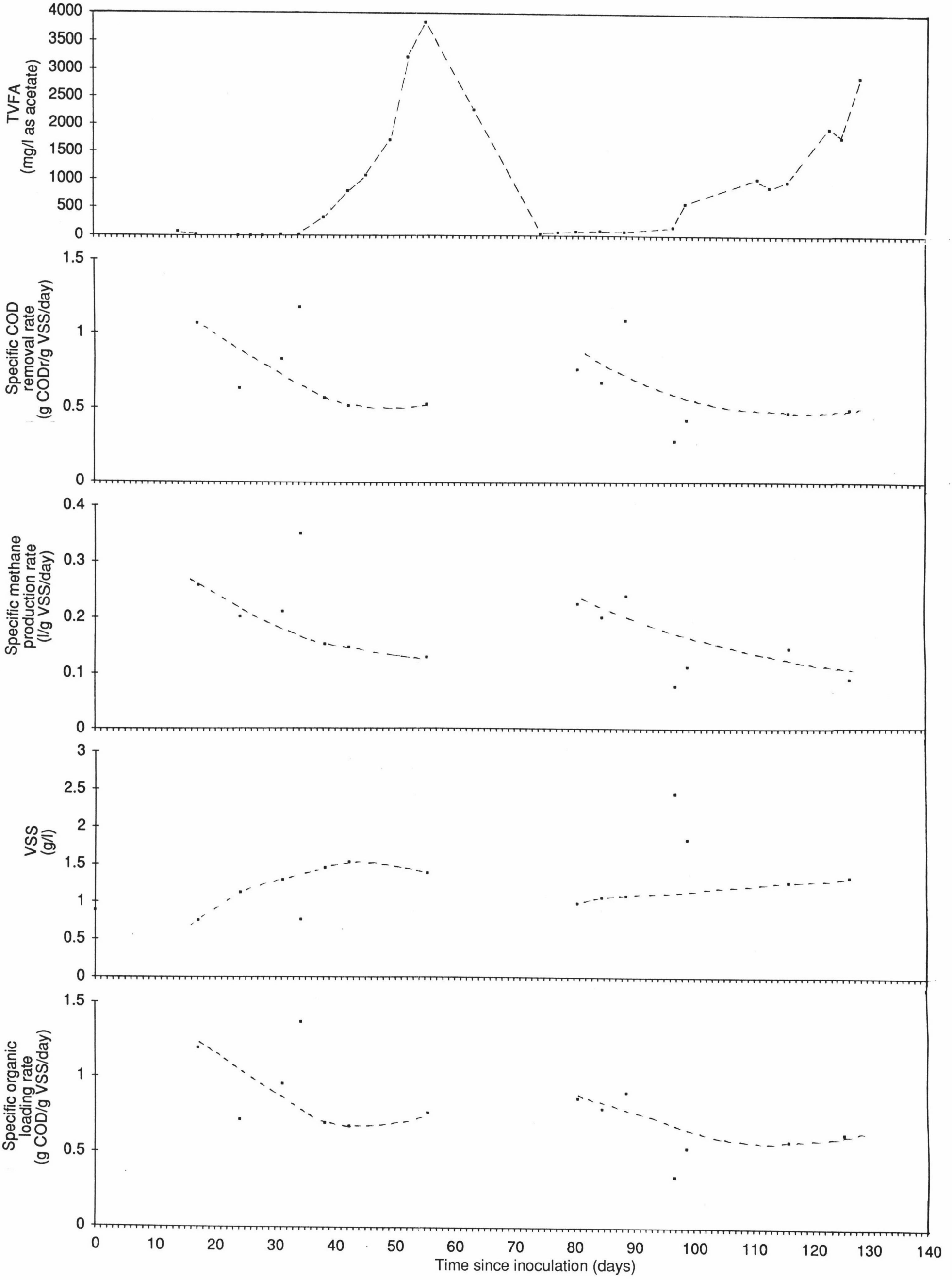


Figure 4.7: Specific rate data for CDR3.

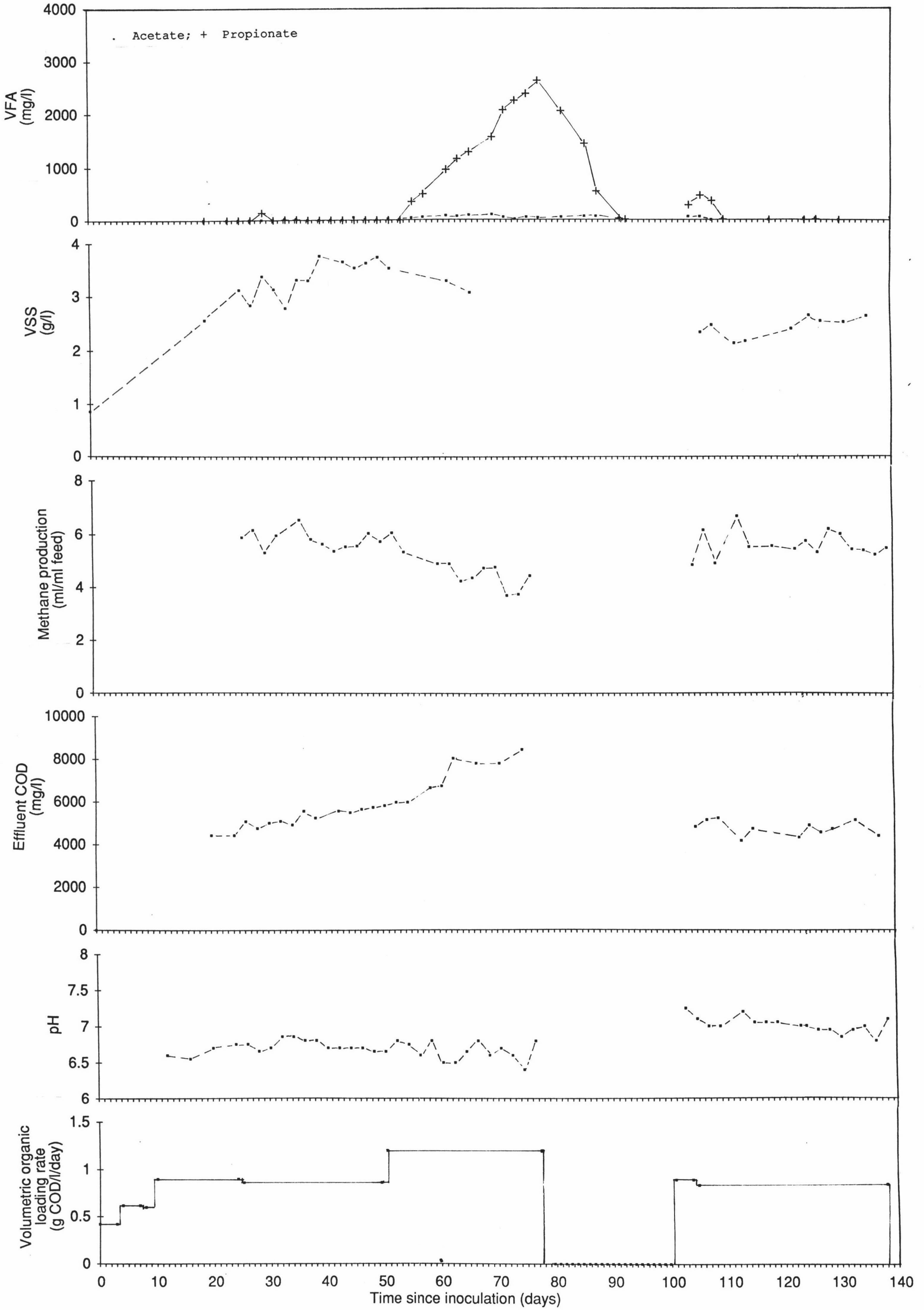


Figure 4.9: Performance of the semi-continuous Biogen digester (SCDR2).