ANAEROBIC DIGESTION OF SEPARATED CATTLE SLURRY

by

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ABSTRACT

Passing whole cattle slurry (WCS) through a mechanical separator removed a large proportion of fibrous solids and produced separated cattle slurry (SCS). SCS gave a higher gas yield (per VS added) at 35°C and a 10 day, 25 day and infinite retention time (RT) than WCS, and three contributing factors were identified. Firstly, the higher solids concentration of WCS (39% greater) resulted in digester overloading at a 10 day RT but had little effect at longer retention times. Secondly, holocellulose represented a majority of destroyed solids in WCS, whereas in SCS over half the degraded solids were attributed to lipids, VFA and crude protein. These differences accounted for the higher gas yield (per VS destroyed) and richer quality of biogas from SCS, and the more rapid fall in the gas yield from WCS as the retention time was dropped. A third factor, particle size did not significantly influence the digestion process.

Shock treatments were performed by lowering the temperature of digesters operated at a 10 and 25 day RT on SCS from 35°C to 3-6°C over several days, and employing different recovery strategies. Measurement of gas composition, gas volume, pH, individual and total VFA concentrations revealed that steady-state conditions were re-established more rapidly when the temperature was restored to 35°C in a single day rather than over a longer period. The most sensitive indicator of instabilty was a large but unequal rise in the VFA concentration, particularly of branched VFA and propionate.

A higher gas yield (per VS added) was produced at a 5 day RT (35°C) from SCS in an upflow anaerobic filter than a conventional digester. This was attributed to the retained biomass preventing overloading in the upflow anaerobic filter.

CONTENTS

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ABBREVIATIONS

ATP	Adenosine 5'-triphosphate
CSTR	Continuously stirred tank reactor
DSFF	Downflow stationary fixed film reactor
DWCS	Diluted whole dairy cattle slurry
RCS	Reconstituted whole dairy cattle slurry
RT	Retention time
SEM	Scanning electron microscope
SCS	Separated dairy cattle slurry
TS	Total solids
UASB	Upflow anaerobic sludge blanket
VFA	Volatile fatty acids
VS	Volatile solids
WCS	Whole dairy cattle slurry

PUBLICATIONS

Two scientific papers have originated from this work, and are enclosed in a back folder:

Peck, M.W., Hawkes, F.R. and Hawkes, D.L. (1985). Effect of mechanical separation on the anaerobic digestion of cattle slurry. In 'Advances In Fermentation 2', pp 34 - 42. Turret-Wheatland, Rickmansworth, UK.

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CHAPTER ONE

INTRODUCTION

1.1 THE ANAEROBIC DIGESTION OF DAIRY CATTLE SLURRY

1.1.1 The Anaerobic Digestion of Whole Cattle Slurry (WCS) and Separated Cattle Slurry (SCS)

Dairy cattle slurry has been frequently considered a suitable substrate for anaerobic digestion, although poor degradability has led to low rates of biogas production compared with other animal wastes (Badger et al., 1979; Hashimoto et al., 1979; Hill, 1982, 1983). This is presumably attributed to the poorly degraded large fibrous particles in the waste, and to the digestion of a significant proportion of the more degradable solids in the cow's rumen. The original whole cattle manure (approximately 20-30% total solids) has been successfully treated in an anaerobic digester (eg. Hills, 1980; Hall et al., 1985a,b), although the faeces generally became diluted with washing water to produce a slurry of 7-8% total solids (termed whole cattle slurry). The high solids content of whole dairy cattle slurry (WCS) and its particulate nature often cause handling problems such as pipe blockages, scum and crust formation, and the settling of solids within the digester (Staley et al., 1971; Bartlett et al., 1977; Hawkins, 1979; Kiely, 1984). In an attempt to alleviate such handling problems and improve the efficiency of the digestion process, WCS has been passed through a mechanical separator. The two products formed are

both more manageable than the initial waste. The dry, friable high solids fraction (now more concentrated and less odourous) is suitable for composting, stacking, spreading on the land or for sale as a soil conditioner/horticultural fertilizer, whilst the free running liquid, termed separated cattle slurry (SCS), may be pumped freely for anaerobic digestion. A number of basic machine types are available commercially for separation, and include vibrating or inclined screens, roller or belt presses and centrifuges (Pain and Hepherd, 1981).

In addition to improving the handling properties of the waste, mechanical separation also leads to improved biogas production and more efficient digestion (Table 1.1). Higher gas yields (per VS added), have been obtained from the mesophilic anaerobic digestion of SCS than WCS at all retention times tested; and at retention times of less than 16 days, more methane (per m^3 digester) has also been produced. Furthermore, the reduced production of methane (per m^3 digester) from SCS at longer retention times may be offset by a fall in capital and operating costs, and by improved system reliability. Therefore, mechanical separation may permit both smaller digesters and shorter retention times to be employed, and hence make anaerobic digestion more economically viable.

It is generally assumed that the microbial flora present under any one set of steady-state conditions represents that most capable of degrading the particular waste, and it is therefore anticipated that the microbial

flora/physiology of the organisms present will vary considerably with retention time, temperature and type of waste. Large variations in the gas yields have been obtained in previous studies. This is not an uncommon feature of such studies, and must be partly attributed to the different temperatures employed and variations in the manner of slurry collection, the cattle diet and bedding, the amount of foreign material (eg. hair, bedding, diet) entering the slurry, the initial solids content of the waste, the age of the slurry, the method of slurry storage and digester design and mode of operation. Furthermore, variations in the size of mesh employed in the separator may also contribute to differences in the gas yield (per VS added), for significant variations have been found between the gas yields obtained from slurry passed through a 2.0mm and 2.4mm mesh (Lo et al., 1983).

1.1.2 The Enhancement of Digestion After Mechanical Separation

Apart from suggesting that the removal of large fibrous particles may improve the digestibility of the slurry, previous work has, to a large extent, failed to explain this enhancement in digester efficiency. Four routes by which separation may affect the efficiency of the digestion of dairy cattle slurry will be considered: the lowering of the solids content of the waste, the reduction

in particle size, the compositional changes effected, and the removal of surfaces for attachment.

EFFECT OF SOLIDS CONCENTRATION

Mechanical separation generally reduced the solids concentration of cattle slurry by approximately 40% (from perhaps 7% TS (70 kgTS m^{-3}) for WCS to 4% TS (40 kgTS m^{-3}) for SCS). The effect of the solids concentration on the digestion process has been predicted from equations produced by Hashimoto (1982) based on Monod kinetics. It has been proposed that increases in the solids concentration of the waste will initially raise the gas yield (per VS added) until overloading occurs, and the gas yield then falls. The solids concentration at which the optimal gas yield (per VS added) is achieved depends upon the operating temperature, retention time and digestibility of the slurry. When long retention times (20-25 days, 30-35°C) have been employed with dairy cattle slurry, the gas yield (per VS added) reached a peak at 70-100 kgVS m^{-3} (Figs. 1.1 and 1.2), but fell away with further increases in the solids concentration. In these circumstances lowering the solids concentration from 70 kgVS m^{-3} to 40 kgVS m^{-3} brought about a fall of approximately 8% in the gas yield (per VS added). However, at shorter retention times lowering the solids concentration by dilution had little effect or enhanced the gas yield (Bryant *et al.*, 1977; Hashimoto, 1982; Lo *et al.*, 1983; Liao *et al.*, 1984; Liao and Lo, 1985;

Lo et al., 1985). Hashimoto (1982) found that the methane yield fell with increasing solids concentration at 35°C and an 8-15 day RT (Fig. 1.2), and higher gas yields have been obtained from diluted whole cattle slurry (DWCS - 40 kgVS m^{-3}) than whole cattle slurry (WCS - 70 kgVS m^{-3}) at all retention times tested (5-16 days) at both 30°C (Fig. 1.3a) and 35°C (Fig. 1.3b,c). Therefore, at short retention times lowering the solids concentration of WCS from 70 to 40 kgVS m^{-3} had little effect or increased the gas yield (per VS added). These studies also revealed that the overloading of digestion became so severe at very short retention times that more methane was liberated per m^3 of DWCS than from an equivalent volume of the original WCS (Lo et al., 1983; Liao et al., 1984; Liao and Lo, 1985).

However, although the solids concentration of SCS and DWCS are similar (approximately 40 kgVS m^{-3}), a much greater proportion of SCS solids have been destroyed than DWCS solids (Lo et al., 1983; Liao et al., 1984; Liao and Lo, 1985). Thus the reduction in the solids concentration brought about by separation had a smaller effect on the digestion process than the reduction brought about by dilution. It is therefore anticipated that the removal of solid material by the separator will have a negligible effect on the gas yield (per VS added) at long retention times (eg. 20-25 days), and that relief from overloading at short retention times (due to a fall in solids concentration) will be less than that found with DWCS.

EFFECT OF PARTICLE SIZE

Several studies have been concerned with an examination of the effect of particle size on the batch digestion of waste material. The grinding or chopping of particulate wastes such as wheat straw (Nelson et al., 1939; Hashimoto, 1983), cornstalks (Nelson et al., 1939) and a variety of soft and hard woods (Millet et al., 1975; Jerger et al., 1983) all reduced the average particle size without affecting the composition of the waste. Such treatments did not affect the ultimate digestibility of the waste but did in some cases lead to an increase in the rate of solids degradation. Thus, at finite retention times, reductions in the mean particle size of these wastes may lead to an increase in the gas yield (per VS added). Indeed, this has been the case with landfill waste (De Walle et al., 1978) and tomato solids waste (Hills and Nakano, 1984). However, it should be noted that treatments such as ball milling also influence the nature of the cellulose fibres in addition to particle size (see section on α-cellulose degradation), and that the enhancement in the rate of solids degradation may also, therefore, be attributed to other changes in the waste material.

The effect of mechanical separation on dairy cattle slurry is, however, different to chopping or grinding. Mechanical separation reduces the particle size by removing some of the larger particles and thereby also brings about a change in the composition of the slurry. Previous studies

only tell us that smaller particles are degraded more rapidly than larger particles of a similar composition. Thus, further work is required to determine whether the smaller particle size fractions of dairy cattle slurry are, in fact, degraded more rapidly than the larger size fractions.

EFFECT OF COMPOSITIONAL CHANGES

The passage of WCS through a mechanical separator has chiefly led to the removal of ∝-cellulose and hemicellulose, with smaller amounts of lignin and ash also being lost (Rorick et al., 1980; Kiely, 1984). It is anticipated that the selective removal of ∝-cellulose and hemicellulose may have several effects on the digestion process and the reader is referred to section 1.2.1, where the degradation of ∝-cellulose, hemicellulose and other proximate constituents of cattle slurry are considered.

SURFACES FOR BACTERIAL ATTACHMENT

It is anticipated that particles may provide a microenvironment for bacteria, in which the concentration of metabolites may differ from that in the bulk phase (see section 1.2.4). Although many bacteria are found free in digesters or as 'conglomerates', it has been demonstrated that the presence of a surface for bacterial attachment does play an important role in the digestion process. This has

been observed with asbestos addition to digesters (De Renzo, 1977), and it has been demonstrated that bacteria frequently adhere to calcium carbonate granules (Kirsop et al., 1984) and other particulate matter (Hobson, 1981). It might be anticipated therefore that the removal of a large amount of particulate material during separation might effect the intimacy of bacterial associations, and hence the efficiency of digestion. However, as a large amount of particulate material remained after separation, it is likely that this would prove sufficient for bacterial attachment and that digestion would not be greatly effected.

1.2 THE MICROBIOLOGY OF ANAEROBIC DIGESTION

Anaerobic digestion may be considered as a three stage process with all three stages occurring simultaneously (Fig. 1.4). In the first stage organic polymers (eg. protein, carbohydrates and lipids) are hydrolysed to their constituent monomers such as amino acids, sugars and long chain fatty acids (Fig. 1.4, reaction 1), and then degraded further (Fig. 1.4, reactions 2 and 3) to acetate, hydrogen and other intermediate products (essentially VFA (volatile fatty acids) longer than acetate). Gujer and Zehnder (1983), have calculated that for sewage sludge approximately 20% of the methane is derived from intermediate products, 58% from acetate and 22% from hydrogen formed during stage 1 of digestion (Fig. 1.4). However, not all of the

intermediate products can be directly converted to methane, and in the second stage of digestion they are catabolised to acetate and hydrogen (Fig. 1.4, reaction 4). In this stage, hydrogen is also oxidized to acetate with concomitant carbon dioxide reduction (Fig. 1.4, reaction 5). The third and final stage of digestion involves the formation of methane from acetate (Fig. 1.4, reaction 6) and hydrogen (Fig. 1.4, reaction 7) by methanogenic bacteria. The rate limiting step in the digestion of a particulate waste such as dairy cattle slurry is believed to be the initial hydrolysis of particulate organic matter (Hobson, 1981; Archer, 1983; Gujer and Zehnder, 1983).

1.2.1 The Destruction of Polymers

Bacteria responsible for the hydrolysis and fermentation of polymers (Fig. 1.4) are believed to represent a significant fraction of the digester flora, and it has been estimated that there are 10^8-10^9 such organisms cm^{-3} (Zeikus, 1980a). Dairy cattle slurry contains many organic polymers suitable for anaerobic digestion, the respective concentrations of which are estimated by proximate constituent assays (Table 1.2). Volatile fatty acids (VFA) and ash are not organic polymers, but are frequently included in proximate constituent assays for the sake of completeness. Starch is sometimes found in municipal sewage waste, but is absent from dairy cattle

slurry (Kiely, 1984), and is not therefore included here. The fate of each of the polymers will now be considered.

LIGNIN

Lignin is a highly complex aromatic polymer and plays a role in cementing the polysaccharide components in cell walls both physically and chemically, thereby increasing the mechanical strength of wood as a composite material and its decay resistance toward micro-organisms. It represents an important fraction of the solids of dairy cattle slurry, and values ranging from 3-19% of the total solids (mean 12%) have been reported from eight studies (Table 1.2). The large variation in the composition of the slurries is attributed to the factors considered in section 1.1. The lignin content of beef cattle slurry is frequently lower than that of dairy cattle slurry (Table 1.2) as a consequence of the higher energy ration fed to beef cattle. There are three basic types of lignin polymer which are formed via a random free-radical condensation mechanism, and composed of structural units consisting of different proportions of coniferyl, sinapyl and p-coumaryl alcohol polymers (Higuchi, 1980). In addition to the complex structure of lignin, three properties are particularly important with respect to microbial degradation (Higuchi, 1980):

(i) lignin has a compact stucture that is insoluble in water and is difficult to wet and penetrate by

micro-organisms.

(ii) the intermonomer linkages that account for the structural rigidity are a variety of carbon-carbon and carbon-ether bonds.

(iii) the intermonomer linkages in lignin are not directly hydrolyzable.

Lignin is therefore a formidable substrate for microbial catabolism, although it has been successfully degraded under aerobic but apparently not to any great extent under anaerobic conditions (Zeikus, 1980b; Zeikus et al., 1982; Sleat and Robinson, 1984). The aerobic degradation of lignin is a slow process and is brought about by a powerful oxidation of the complex polymer (Higuchi, 1980; Zeikus, 1980b; Higuchi, 1982; Sleat and Robinson, 1984; Schoemaker et al., 1985). Thus, as a consequence of the low Eh operative in anaerobic digesters (and other anaerobic environments) such powerful oxidation reactions are not feasible, and since alternative degradative mechanisms apparently do not exist (or are extremely slow), lignin is largely recalcitrant to anaerobic digestion.

A small apparent destruction of lignin has been observed in some studies with cattle slurry (Table 1.3) and pig slurry (Iannotti et al., 1979), and may be attributed either to a deficiency in the analytical procedure or to the removal of peripheral aromatic rings (Prins, 1977; Zeikus, 1980b; Sleat and Robinson, 1984). Soluble lignin-related aromatic compounds such as phenol or benzoate are degraded under anaerobic conditions (Zeikus, 1980b; Zeikus et

al., 1982; Boyd et al., 1983; Sleat and Robinson, 1983, 1984; Fedorak and Hrudey, 1984; Mountfort et al., 1984; Colberg and Young, 1985). Furthermore lignin is known to shield some of the ∝-cellulose and hemicellulose from degradation, and it has been proposed that the ultimate degradability of a particulate waste is inversely proportional to its lignin content (Chandler et al., 1980). However, other studies have indicated that the degradability of cattle slurry may be determined by more than its lignin content alone (Hashimoto et al., 1981; Hills and Kemmerle, 1981).

In an attempt to improve the digestibility of cellulosic materials, a number of pretreatments (eg. heat, chemical, enzymatical) have been successfully applied. Perhaps the most common of these is a mild alkaline treatment which has increased the digestibility of wastes upto four-fold, apparently by opening up the cellulosic stucture and decreasing its lignin content (eg. Lindberg et al., 1984; Kolankaya et al., 1985; Pavlostathis and Gossett, 1985).

ALPHA-CELLULOSE

Holocellulose, that is total cellulose, is composed of ∝-cellulose and hemicellulose. ∝-cellulose represents a significant proportion of the solids of dairy cattle slurry and a mean value of 20% total solids has been obtained from

eight studies (Table 1.2). The degradation of ∝-cellulose, plus other carbohydrates, accounts for approximately 40% of the methane produced from domestic sludge (Fig. 1.4). ∝-cellulose is a linear homogeneous polymer of D-anhydroglucopyranose units linked by β 1-4 glucosidic bonds. The number of glucose units per molecule of ∝-cellulose may be as high as 14,000 (Cowling, 1975), and these glucose chains stack together to give cellulose fibrils of plant cell walls. Within each microfibril the flat ribbon-like molecules of ∝-cellulose are bound laterally by hydrogen bonds and associated in various degrees of order. Some regions contain highly orientated parallel molecules and are termed crystalline, whilst those of lesser order are called paracrystalline or amorphous regions.

∝-cellulose and the other major constituents of natural fibres are insoluble molecules and are deposited within the cell walls in an intimate physical mixture of considerable structural complexity. Micro-organisms that degrade natural fibres live on the exterior fibre surface or in the lumina. Here they secrete extracellular cellulases that induce the dissolution of the highly-polymeric constituents of the fibre to soluble products which may be assimilated and metabolized by the organisms. The necessary physical contact can only be achieved by diffusion of the enzyme to susceptible sites on the gross surface of the fibre, or the microfibrillar and molecular surfaces within the fibre wall. Thus, any structural feature of the fibre

or its constituents that limits the accessibility or diffusion of cellulolytic enzymes will exert a profound effect on the susceptibility of the fibre to hydrolysis. A number of such factors have been identified, and will now be considered (see Cowling (1975) for a consideration of further features):

(1) The crystallinity of ∝-cellulose is an important factor influencing its hydrolysis, for the cellulase enzymes more readily degrade the amorphous regions of the ∝-cellulose structure than the less accessible crystalline areas (Cowling, 1975; Fan et al., 1980). Indeed, significant increases in the crystallinity of ∝-cellulose have been observed during hydrolysis. Pretreatments which reduce the degree of crystalline perfection (eg. ball milling) have increased the rate of hydrolysis of cellulosic materials, but apparently not the ultimate degradability (Millet et al., 1975; Fan et al., 1980; Lee and Fan, 1982; Hashimoto, 1983; Jerger et al., 1983). An alternative pretreatment (mild acid hydrolysis) has, however, increased the rate of ∝-cellulose hydrolysis without affecting the crystallinity of the polymer (Grethlein, 1985).

(2) Perhaps the most important factor influencing the degradation of ∝-cellulose fibres is the shape, size and surface properties of the fibre capillaries in relation to the shape, size and diffusion of the cellulase enzymes. The capillary voids in fibres include gross capillaries (20nm-10μm) and cell-wall capillaries which are substantially smaller, even when fully expanded (after

moisture has been absorbed - Cowling, 1975). If the enzymes penetrate the cell-wall capillaries, the greater surface area exposed ensures that the rate of hydrolysis is greater than in the gross capillaries. The cellulase enzymes have been estimated to have (if spherical) a diameter of approximately 5-6nm (Cowling, 1975; Grethlein, 1985) and would therefore be expected to diffuse readily within the gross capillaries, but only in a small proportion of the cell wall capillaries. Indeed, a linear relationship has been established between the initial rate of α-cellulose hydrolysis and the surface area accessible to a molecule with a nominal diameter of 4-5nm (Fan et al., 1980; Grethlein, 1985; Grous et al., 1986) but not the overall surface area (Fan et al., 1980).

(3) The hydrolysis of α-cellulose fibres is also affected by their moisture content. Moisture hydrates the α-cellulose molecule and opens up the cell-wall capillaries, this makes them more accessible to the cellulases and increases the rate of hydrolysis (Cowling, 1975).

(4) The degradability of α-cellulose is also influenced by the nature of associated substances. α-cellulose is frequently closely linked with lignin which restricts its degradation. This is particularly important in anaerobic environments where lignin is itself virtually recalcitrant to degradation, and also shields some of the α-cellulose from attack (see previous section on lignin).

The slow degradation of cellulosic materials is believed to be the rate limiting step in the anaerobic

digestion of particulate wastes such as cattle slurry. The slowness of this initial step is not a consequence of the primary structure of α-cellulose, but the second and tertiary structure discussed previously. The enzyme cellulase brings about the hydrolysis of α-cellulose, and is not a single enzyme but a complex of several (Tsao, 1984). The cellulolytic enzymes of Clostridium thermocellum (a bacterium commonly found in thermophilic digesters) have been described by Zeikus (1980a) and consist of:

(i) Extracellular; Endoglucanase cellulase:

cellulose + H_2O ⟶ cellodextrins + cellobiose

(ii) Cell bound; Cellodextrin phosphorylase:

cellodextrins + Pi ⟶ glucose-1-phosphate + cellobiose

(iii) Cell bound; Cellobiose phosphorylase:

cellobiose + Pi ⟶ glucose-1-phosphate + glucose

When grown in monoculture, Clostridium thermocellum ferments cellulose to ethanol, acetate, carbon dioxide and hydrogen (Zeikus, 1980a). However, C.thermocellum forms stable associations with methanogenic and non-methanogenic bacteria in digesters, and this has a profound effect on the fermentation end products. For example when associated with a methanogen, acetate, methane and carbon dioxide are the major end products as a result of inter-species hydrogen transfer (Zeikus, 1980a). A considerable amount of research

has been dedicated to an examination of cellulolytic bacteria in co-cultures (eg. Latham and Wolin, 1977; Laube and Martin, 1981; LeRuyet et al., 1984) and in tri-cultures (eg. Laube and Martin, 1981, 1983). A number of bacteria have been implicated in cellulolysis in digesters, including species of Clostridium, Bacteroides, Butyrivibrio and Ruminococcus.

HEMICELLULOSE

Hemicelluloses are defined as the polysaccharide fraction that may be extracted from delignified plant tissue by alkali, but not by water or ammonium oxalate solution (Prins, 1977). Hemicellulose is commonly the first or second most abundant of the proximate constituents in dairy cattle slurry and a mean value of 24% total solids has been obtained from eight studies, although there was a considerable range of values (Table 1.2).

Hemicellulose, unlike α-cellulose, is a complex polysaccharide that is not well defined in terms of organic structure. It consists of a heterogeneous mixture of monomeric units including D-glucose, D-mannose, D-galactose, D-xylose, L-arabinose, D-gluconic acid and 4-O-methyl-D-gluconic acid, which form a variety of linear and highly branched polymers (Ghosh et al., 1985). Although the enzymatic hydrolysis of hemicellulose is relatively easy, the complexity of hemicellulolytic enzyme systems exceeds that of the cellulases. Hemicellulose is first

hydrolyzed by enzymes such as xylonases, xylobiase and other hemicellulases, to xylans, arabans, mannans, glucans and galactans. These polysaccharides are then hydrolyzed to soluble oligosaccharides and sugar monomers (Prins, 1977), and finally fermented to substrates suitable for the second stage of digestion (Fig. 1.4).

Hemicellulose is located in the lignocellulosic fraction of dairy cattle slurry, and provides the link between lignin and α-cellulose. The degradation of hemicellulose is therefore restricted to some extent by the physical factors discussed previously for α-cellulose. Up to 40% hemicellulose and 41% α-cellulose were destroyed during the anaerobic digestion of dairy cattle slurry (Table 1.3), although the actual proportion destroyed varied considerably with such factors as the source of the cattle slurry and the digester operating conditions employed. It is anticipated that if α-cellulose and hemicellulose (of general formula $C_n H_{2n} O_n$) were completely degraded to gaseous end products, a gas yield of $0.8 m^3$ kgVS destroyed^{-1} would be achieved, and the biogas produced would contain 50% methane (Table 1.4). The small variation in the theoretical biogas yields estimated in five studies (Table 1.4) is due to the range of compounds taken as standards. A thorough determination of the composition of cellulosic materials in dairy cattle slurry (although likely to vary from source to source) would permit more accurate values to be obtained. A number of bacterial species have

been implicated in hemicellulose degradation and include members of the genus Bacteroides and Ruminococcus (Tsao, 1984).

ASH

Ash is taken to be that material not volatilized in a furnace at 550°C, and represents 13-24% of the total solids in dairy cattle slurry, with a mean value of 18% from eight studies (Table 1.2). It is an estimate of the inorganic or the total mineral content of the slurry. A significant proportion (17-69%) of the total ash has been found to be silica ash (Hills and Kemmerle, 1981; Hills and Roberts, 1981) and is therefore likely to have originated from the scraping of concrete floors, or the bedding of cattle on sand. The complete composition of dairy cattle slurry ash has been determined by Anthony (1971) and Martin et al. (1983). Ash is taken to be completely recalcitrant to digestion and the apparent destruction sometimes observed is believed to be due to settling out in the digester (see section 3.5.3).

PROTEIN

The term crude protein is extensively used in determinations of the proximate constituents of animal wastes. It is obtained by multiplying the total organic nitrogen concentration of the waste by 6.25, and therefore

assumes that all organic nitrogenous compounds are proteins. This involves some degree of error since, for example, purine and pyrimidine bases and urea may also be present. Crude protein estimates also include free amino acids, and the amino acid composition of a sample of dairy cattle slurry has been determined after hydrolysis (Anthony, 1971; Martin et al., 1983). A majority of the protein in cattle slurry is believed to be of bacterial origin (Hobson, 1981), and represents 13-24% total solids (mean value 17%) in the slurry (Table 1.2).

The overall destruction of protein in an anaerobic digester is rather less than might have been anticipated, as a fair amount of protein synthesis also takes place. The hydrolysis of protein leads to the formation of peptides and eventually individual amino acids. It has been estimated that approximately 21% of the methane liberated from the anaerobic digestion of domestic sludge originates from protein degradation (Fig. 1.4). The amino acids are then fermented to hydrogen, carbon dioxide, acetate and longer VFA (Fig. 1.4); some of these reactions are discussed in section 1.2.2. The proteolytic enzymes from two rumen bacteria have been recently described (Tsao, 1984).

The complete degradation of proteins to gaseous end products is likely to give a gas yield of 1.0 m^3 kgVS $destroyed^{-1}$, and a biogas of approximately 68% methane (Table 1.4). The large variation in these predicted values (Table 1.4) is due to the standards taken as a starting point, in some cases individual amino acids have been

adopted, whilst in others a more general formula has been used. To obtain an accurate gas yield per VS destroyed for dairy cattle slurry (from a particular source) it is necessary to determine the amino acid composition. Previously, alanine, aspartic acid, glutamic acid and leucine have been found to be the most abundant amino acids in dairy cattle slurry, although there was some variation (Anthony, 1971; Martin et al., 1983).

LIPIDS

Lipids (fats) are a heterogenous group of organic compounds which are soluble in organic solvents but insoluble in water. The lipid content of digester samples is determined by procedures in which the lipids are extracted into organic solvents such as ether or chloroform. Short chain volatile fatty acids (VFA) are not generally considered as lipids (see section 3.3.1). Lipids represent only a small fraction of the total solids of dairy cattle slurry, an average value of 3% having been obtained from four studies (Table 1.2). An examination of the lipid content of pig slurry after hydrolysis revealed that long chain fatty acids represented 74% total lipids and primarily consisted of palmitic, stearic, oleic and linoleic acids (Iannotti et al., 1979).

The initial step in the degradation of triglycerides and membrane lipids such as phosphoglycerides is a hydrolysis reaction carried out by lipases to liberate long

chain fatty acids. In some circumstances these long chain fatty acids have reached high concentrations in the digester and proved to be inhibitory (Hanaki et al., 1981; Roy et al., 1985). However, this is not generally the case and long chain fatty acids are degraded by the β-oxidation pathway to yield acetate, hydrogen and short chain VFA (Jeris and McCarty, 1965; Chynoweth and Mah, 1971; Weng and Jeris, 1976; Roy et al., 1985); the fate of these products is considered in section 1.2.2. It has been suggested (Roy et al., 1985) that the β-oxidizing organisms may form a syntrophic association with H_2-scavenging bacteria, in a similar manner to that described for bacteria β-oxidizing VFA (sections 1.2.2 - 1.2.4). The degradation of lipids is sensitive to the retention time employed and lipolysis is severely restricted at retention times of less than eight days (at 35°C - O'Rourke, 1968). Approximately 39% of the methane liberated from the anaerobic digestion of domestic sludge is believed to have originated from lipid destruction (Fig. 1.4). Some lipids are not degraded in the cow's rumen and are probably also not degraded in an anaerobic digester, examples are waxes and esters of long chain fatty acids with primary alcohols (Prins, 1977).

It is anticipated that the complete degradation of lipids will give the highest gas yield (1.4 m^3 kgVS destroyed^{-1}) and the biogas richest in methane (70% methane) recorded from any of the polymers (Table 1.4). The heterogeneous mixture of compounds incorporated as lipids

has ensured that there is some variation in the theoretical gas yields. A complete analysis of the lipid composition would enable more accurate values to be determined.

1.2.2 The Intermediary Steps

In the intermediate stage of anaerobic digestion (Fig. 1.4 - stage 2), the hydrolysis and fermentation products from stage one are degraded into substrates suitable for methanogenesis. These intermediate products primarily consist of VFA (but not acetate) and originate from; the addition of such acids in the feed, the fermentation of amino acids and sugars, and a small proportion from the β-oxidation of long chain fatty acids. The intermediate stage of anaerobic digestion involves the activities of two distinct groups of bacteria, the obligate proton-reducing (H_2 forming) acetogenic bacteria, and the homoacetogenic bacteria (Fig. 1.4 - reactions 4 & 5). It has been estimated that there are 10^6 obligate proton-reducing bacteria, and 10^5-10^6 homoacetogenic bacteria per cm^3 in anaerobic sludge digesters (Zeikus, 1980a).

The obligate proton-reducing acetogenic bacteria are a complex of species involved in at least four types of reactions:

(1) The oxidation of alcohols, such as ethanol, to acetate and hydrogen (Bryant *et al.*, 1967).

(ii) The β-oxidation of VFA (McInerney et al., 1979, 1981; Shelton and Tiedje, 1984; Henson and Smith, 1985; Roy et al., 1985; Stieb and Schink, 1985; Tomei et al.,1985).

(iii) The decarboxylation of propionate to acetate, carbon dioxide and hydrogen (Boone and Bryant, 1980).

(iv) The degradation of aromatic compounds (eg. Mountfort et al., 1984).

These bacteria are all extremely sensitive to molecular hydrogen, and few have been studied in pure culture. Indeed it is less than twenty years since Bryant et al. (1967) demonstrated Methanobacillus omelianskii to be a co-culture of a methanogenic and obligate proton-reducing bacterium, and not a single organism.

The homoacetogenic bacteria have only recently been implicated in anaerobic digestion, and are responsible for the oxidation of hydrogen with concomitant reduction of carbon dioxide to acetate (Balch et al., 1977; Adamse, 1980; Braun et al., 1981; Leigh et al., 1981; Braun and Gottschalk, 1982). The extent of their contribution to the digestion process (Fig. 1.4 - reaction 5), however remains largely unknown.

The sulphate-reducing bacteria represent only a small fraction of the bacterial flora of an anaerobic digester (10^4-10^5 organisms cm^{-3}; Zeikus, 1980a), and trace amounts of H_2S are commonly detected in the digester headspace. The sulphate-reducing bacteria may play a small but important role in digestion, as H_2S is required by several

methanogens, and will precipitate potentially toxic metal ions. It is anticipated that the sulphate-reducing bacteria will co-exist with the methanogenic bacteria in digesters operated on animal slurries, as their thermodynamic advantage is diminished at the low sulphate concentrations typically found in animal slurries (Archer, 1983).

FATE OF STRAIGHT CHAIN FATTY ACIDS

The β-oxidation of fatty acids under anaerobic conditions is thermodynamically unfavourable, and bacteria carrying out these reactions have only been cultivated in co-culture with hydrogen scavenging partners (sulphate-reducing bacteria or methanogenic bacteria) that maintain a low partial pressure of hydrogen. However, the range of acids degraded by a single organism was rather limited in each case, and none could degrade propionate in addition to other fatty acids, suggesting some degree of specialisation (Table 1.5). Even numbered VFA (eg. butyrate, caproate - Table 1.6) were converted almost entirely to acetate and hydrogen, although breakdown products (eg. butyrate from caproate) were released and subsequently degraded (Stieb and Schink, 1985). Odd numbered VFA (eg. valerate, heptanoate - Table 1.6) were β-oxidized to acetate, hydrogen and propionate. Butyrate is generally the most abundant of these acids, and it has been estimated that approximately 8% of methane produced from cattle slurry originates through its degradation (Mackie and

Bryant, 1981).

In addition to the β-oxidation of VFA, it has been suggested that small quantities of valerate may be requred by cellulolytic and methanogenic bacteria for growth (Prins, 1977; Baldwin and Allison, 1983).

FATE OF PROPIONATE

With the exception of acetate, propionate is often quantitatively the most important of the VFA, and it has been estimated that 13-16% total methane produced during the anaerobic digestion of dairy cattle slurry is formed through propionate (Mackie and Bryant, 1981), although higher values have been anticipated by Boone (1984a). A single bacterium (Syntrophobacter wolinii) has been described which degrades propionate in a syntrophic co-culture with a hydrogen scavenging organism (Boone and Bryant, 1980):

Equation 1 ($\Delta G^{0'}$ = + 76.1 kJ reaction^{-1}):

$$CH_3CH_2COO^- + 3H_2O \longrightarrow CH_3COO^- + HCO_3^- + H^+ + 3H_2$$

Equation 2 (In co-culture with a sulphate reducing bacterium $\Delta G^{0'}$ = -151.3 kJ reaction^{-1}):

$$4CH_3CH_2COO^- + 3SO_4^{2-} \longrightarrow 4CH_3COO^- + 4HCO_3^- + H^+ + 3HS^-$$

Equation 3 (In co-culture with a methanogenic bacterium $\Delta G^{0'}$ = -102.4 kJ reaction^{-1}):

$$4CH_3CH_2COO^- + 3H_2O \longrightarrow 4CH_3COO^- + HCO_3^- + H^+ + 3CH_4$$

Acetate, carbon dioxide and hydrogen were the products of this reaction (equation 1), and S.wolinii was incapable of degrading other VFA (Boone and Bryant, 1980). Recent studies of propionate degradation have indicated that the actual mechanism of degradation may be very different to that of other larger VFA, and initially involve conversion to succinate (Koch et al., 1983; Boone, 1984b; Schink, 1985).

Three molecules of hydrogen are produced from the degradation of a single propionate molecule (reaction 1), compared with only two from the oxidation of butyrate (Table 1.6). The degradation of propionate is therefore thermodynamically more sensitive to high partial pressures of hydrogen than butyrate degradation. Under standard conditions, propionate and butyrate catabolism are both thermodynamically unfavourable reactions ($\Delta G^{0'}$ = +76.1kJ mol^{-1} and +48.1kJ mol^{-1} respectively), however in the presence of hydrogen-utilising organisms the overall reactions may become exergonic (equations 2 and 3), although the growth of such co-cultures is extremely slow. The generation time of a dixenic-culture of S.wolinii, methanogen and sulphate reducer (monoxenic cultures with the methanogen were not obtained) was about half as fast (161 hours) as the monoxenic culture of S.wolinii and a sulphate reducer alone (87 hours) (Boone and Bryant, 1980). Propionate may also be assimilated by methanogenic bacteria as a carbon-source (Eikmanns et al., 1983).

FATE OF BRANCHED VOLATILE FATTY ACIDS

Very little is known of the sources and fate of branched VFA compared with straight chain acids, but present information is summarised in Table 1.7. Branched VFA are present in small quantities in cattle slurry feed, and also originate from the β-oxidation of larger fatty acids and the oxidative deamination of valine, leucine and isoleucine (Table 1.7). These two reactions are conducted by bacteria that form syntrophic associations with hydrogen scavenging organisms in both anaerobic digesters and sediments. Further to this it has been suggested that i-butyrate may be formed directly from n-butyrate by an isomerization reaction (eg. Cohen *et al.*, 1982a,b), and that branched VFA may be produced from the degradation of benzoic compounds (Colberg and Young, 1985) and 1,2-propanediol (Czerkawski *et al.*, 1984).

Three mechanisms by which branched VFA may be degraded have been described (Table 1.7). Bacteria have been identified which are capable of β-oxidizing iso-heptanoic acid (McInerney *et al.*, 1979, 1981) and 2-methyl butyric acid (Shelton and Tiedje, 1984; Stieb and Schink, 1985), although apparently not i-butyrate and i-valerate (reactions which are not considered feasible (Lehninger, 1975)). However, evidence has been presented to suggest that i-butyrate may be isomerized to n-butyrate and then β-oxidized to acetate (Shelton and Tiedje, 1984; Zinder

et al., 1984). Branched VFA are also important growth factors for many methanogenic and cellulolytic bacteria (Prins, 1977; Whitman et al., 1982; Baldwin and Allison, 1983; Eikmanns et al., 1983), for they may be readily converted to the amino acids valine, leucine and isoleucine.

HOMOACETOGENIC BACTERIA

Several bacteria have been described which bring about the oxidation of hydrogen and the reduction of carbon dioxide to acetate according to the equation:

$$4H_2 + 2HCO_3^- + H^+ \longrightarrow CH_3COO^- + 4H_2O$$

These include Acetobacterium woodii (Balch et al., 1977), Clostridium aceticum (Adamse, 1980; Braun et al., 1981), Acetogenium kivui (Leigh et al., 1981) and Acetobacterium wieringae (Braun and Gottschalk, 1982). The role of these bacteria in anaerobic digestion is poorly understood, and Braun et al. (1979) estimated the viable counts of homoacetogenic bacteria to be approximately 1% of that of H_2-utilising methanogenic bacteria in sludge and lake sediments. Furthermore it has been reported that acetate synthesis from hydrogen and carbon dioxide is a small but significant reaction in the anaerobic digestion of cattle slurry (Mackie and Bryant, 1981; Boone, 1982) and in anaerobic sediments (Lovley and Klug, 1983; Jones and Simon, 1985).

1.2.3 Methanogenesis

Methane biosynthesis is limited to a specialized group of obligately anaerobic bacteria, the methanogens (Fig. 1.4, stage three). It has been estimated that there are approximately 10^6-10^8 methanogens cm^{-3} digester (Zeikus, 1980a). They are perhaps the most strictly anaerobic bacteria known and detailed studies require stringent procedures to maintain a low Eh and ensure the exclusion of oxygen from cultures (Zeikus, 1977; Kirsop *et al.*, 1984).

The methanogenic bacteria are a diverse group of organisms quite different to most other bacteria and have been assigned to the kingdom Archaebacteria. Although they share many features in common with eubacteria (eg. size, absence of cellular organelles, ribosomal sub-unit size), there are a number of important distinct archaebacterial features. These include: ribosomal RNA sequence homology; absence of thymidine in the 'common arm' of transfer RNA; ether-linked poly-isoprenoid glycerol lipids; the absence of peptidoglycan in cell walls; absence of dihydrouracil and distinct transcription series. Furthermore, methanogenic bacteria contain a series of co-enzymes (eg. coenzyme F420, coenzyme M and factor F430) which are absent from most other bacteria. The genetic analysis of methanogens is still in its infancy, although recent developments have considerably increased our knowledge of the bioenergetics of these

organisms. A number of reviews have been published which deal with the biology, general physiology and classification of methanogenic bacteria: Zeikus, 1977; Anthony, 1982; Mah, 1982; Zehnder et al., 1982; Daniels, 1984; Daniels et al., 1984; Kirsop, 1984; Zeikus et al., 1985; Archer and Harris, 1986.

Substrates that may be used as both carbon and energy sources include H_2/CO_2, formate, methanol, carbon monoxide, methylamine and acetate. A majority of methanogens are capable of growth on H_2/CO_2, approximately half can utilise formate, whilst members of only two species (Methanothrix and Methanosarcina) can utilise methanol, methylamine and acetate. The most important substrates for methanogenesis in anaerobic digestion are believed to be acetate, from where approximately 70% of the methane is derived, and H_2/CO_2 from where the remainder originates (Smith and Mah, 1966; Mountfort and Asher, 1978; Mackie and Bryant, 1981).

Formate is produced as a fermentation end-product during digestion and is a suitable substrate for methanogenesis. The addition of formate to mixed cultures stimulates methanogenesis (Hungate et al., 1970; Archer, 1983). However, very low concentrations of formate have been found in rumen fluid (5-20μM; Hungate et al., 1970) and sludge digesters (17-130μM; Schauer et al., 1982), although a high turnover has been proposed (Hungate et al., 1970). Methanogens have been described that are capable of degrading formate at concentrations as low as

15μM (Schauer *et al.*, 1982), although it appears likely that it may be converted to H_2/CO_2 rather than directly to methane (Archer, 1983). Further evidence for this is that acetate and H_2/CO_2 accounted for 99-105% of the methane produced from the anaerobic digestion of dairy cattle slurry (Mackie and Bryant, 1981).

METHANE FROM ACETATE

The acetate pool within an anaerobic digester is formed from the degradation of larger molecules (Fig. 1.4 - reactions 2,3,4), the reduction of carbon dioxide (Fig. 1.4 - reaction 5) and the addition of acetate in the feed. Two mechanisms of methane production from acetate have been identified and are summarised in Table 1.8. The first mechanism, termed the aceticlastic reaction is undertaken only by methanogenic bacteria, and involves the decarboxylation or 'splitting' of acetate in such a manner that the methyl group is primarily converted to methane, whilst the carboxyl group is converted to carbon dioxide. That this is the pattern observed with labelled acetate in cattle slurry digesters suggests that this is the major route of acetate degradation (Mountfort and Asher, 1978; Mackie and Bryant, 1981). The decarboxylation of acetate (Table 1.8, mechanism 1) is the least thermodynamically favourable of the methanogenic substrates with only 31 kJ mol^{-1} released. Models accounting for carbon and electron flow permitting energy conservation have been proposed for

aceticlastic methanogens, and are believed to involve electron transport phosphorylation rather than substrate level phosphorylation (Daniels et al., 1984; Zeikus et al., 1985). Two genera of methanogenic bacteria are capable of splitting acetate, and it has been proposed that at low acetate concentrations Methanothrix species (km 0.7mM, $\hat{\mu}$ 0.1 day^{-1}) will outcompete Methanosarcina species (km 3-5mM, $\hat{\mu}$ 0.3 day^{-1}), but that at high substrate concentrations the Methanosarcina type will predominate (Gujer and Zehnder, 1983; Archer, 1984).

A second mechanism of acetate degradation has been recently identified and is a two stage process (Zinder and Koch, 1984). The first step (Table 1.8, equation 2a) involves the 'oxidation' of acetate to hydrogen and carbon dioxide by non-methanogens, and the second step (Table 1.8, equation 2b) is the reduction of carbon dioxide to methane by methanogenic bacteria. The net reaction is the same as the first mechanism, but one can differentiate between the two by the use of ^{14}C-labelled substrates. Non-aceticlastic production of methane from acetate is carried out by a syntrophic co-culture, and the acetate oxidizing organism has not been isolated in pure culture (Zinder and Koch, 1984). This is likely to be a consequence of an extreme sensitivity to molecular hydrogen. Acetate oxidation is even more thermodynamically unfavourable (see Table 1.8, equation 2a) than propionate oxidation (see section 1.2), therefore exceptionally low hydrogen partial pressures must be maintained to permit acetate oxidation,

and this represents an example of inter-species hydrogen transfer.

METHANE FROM HYDROGEN AND CARBON DIOXIDE

The reduction of carbon dioxide to methane with concomitant hydrogen oxidation (Fig. 1.4, reaction 7) is carried out uniquely by methanogens and accounts for approximately 30% of the methane formed during anaerobic digestion (Mountfort and Asher, 1978). Almost all methanogenic bacteria are able to utilise H_2/CO_2 mixtures as the sole carbon and energy sources. The reduction of carbon dioxide is carried out according to equation 2b (Table 1.8), although the enzymology of the reaction between H_2/CO_2 is presently still uncertain (but see Daniels et al., 1984; Zeikus et al., 1985).

The hydrogen-utilising methanogenic bacteria are the main hydrogen consuming organisms in anaerobic digesters (Mackie and Bryant, 1981; Boone, 1982) and have an extremely low affinity for molecular hydrogen (Table 1.9). This permits extremely low partial pressures of hydrogen to be maintained (Table 1.10) and ensures that the degradation of propionate and longer VFA is thermodynamically feasible (see section 1.2.4 for further details).

1.2.4 Interactions in an Anaerobic Digester

In a multistage process such as anaerobic digestion, the conditions must be such that each individual reaction is exergonic (ie. $\Delta G < 0$). Under standard conditions (pH 7.0, all reactants 1 M or 1 atm.) this is clearly not the case and many reactions are, infact, endergonic ($\Delta G^{0'} > 0$), eg. the β-oxidation of VFA (section 1.2.2). Thus for successful digestion, the concentration of free intermediates (eg. acetate, propionate, hydrogen) must be such that VFA oxidation, acetate decarboxylation and hydrogen oxidation are all thermodynamically feasible. This is achieved by the maintenance of low concentrations of the two major intermediates, acetate and hydrogen, chiefly by methanogenic bacteria. Hydrogen is particularly important in this respect, and its concentration in digesters is so low (Table 1.10), that it has proved difficult to measure. Archer (1983) has calculated that for all the reactions in anaerobic digestion to be thermodynamically feasible (under typical digester conditions), the hydrogen concentration should be in the range of $0.25\text{-}8\times10^{-5}$ atmospheres. At higher concentrations, hydrogen yielding reactions (eg. β-oxidation of VFA) will not be tenable, and at lower concentrations hydrogen consuming reactions (eg. CO_2 reduction) will not be possible. However, it is likely that even in the best circumstances less than one molecule of ATP will be formed per substrate molecule and that growth will therefore be slow.

The maintenance of a low hydrogen partial pressure is of fundamental importance to the digestion process, and is achieved by inter-species hydrogen transfer. The dependence of the obligate proton-reducing bacteria on the methanogenic bacteria (or other hydrogen scavenging organisms) to maintain low hydrogen partial pressures is so great that up to now the VFA β-oxidizing bacteria have only been isolated as syntrophic co-cultures. It has been proposed that the hydrogen producing and consuming reactions are closely associated in microenvironments within the digester (Schoberth, 1983; Boone, 1984a) and it has been calculated that the mean distance between the site of hydrogen production and consumption is only 76μm (Gujer and Zehnder, 1983).

Low hydrogen concentrations are maintained in anaerobic digesters (Table 1.10) by an extremely rapid turnover of hydrogen (10^4-10^5 hr^{-1}; Kaspar and Wuhrmann, 1978a; Robinson and Tiedje, 1982). On the other hand VFA are turned over approximately once per hour (Kaspar and Wuhrmann, 1978a; Mackie and Bryant, 1981) and steady-state concentrations in mesophilic digesters are generally in the range of 0.1-5.0mM for acetate, 0.01-1.00mM for propionate, with the longer acids present at lower concentrations. However, despite the extremely rapid turnover of hydrogen it has been calculated that its utilisation proceeds at 1% maximum rate, whilst the acetate

degrading systems operate at approximately half the maximal rate, and propionate catabolism occurs at 10-15% maximal rate (Kaspar and Wuhrmann, 1978a). This is of fundamental importance to anaerobic digestion as it ensures that low hydrogen partial pressures are maintained in all except the most adverse circumstances, and that the β-oxidation of VFA proceeds unrestricted. It is anticipated that, as a consequence of the rapid hydrogen turnover, the hydrogen concentration would rise virtually instantaneously during periods of severe digester instability (Zehnder and Koch, 1983). Indeed a sudden rise in the hydrogen partial pressure either artificially (Kaspar and Wuhrmann, 1978a,b; Boone, 1982) or by the addition of detrimental feed loads (Asinari di San Marzano et al., 1981; Mackie and Bryant, 1981; Barnes et al., 1983) has caused a rise in the concentration of the longer chain VFA, particularly propionate. Apparent Ks values for hydrogen have been determined for several methanogens (Table 1.9), and it has been suggested that methanogens have a minimum threshold of approximately $2x10^{-5}$ atmospheres (0.02μM) for hydrogen (Lovley, 1985). The concentrations found in natural environments are compatible with these values (Table 1.10).

In addition to hydrogen, it is likely that propionate degradation will be sensitive to a rise in the acetate concentration (and that butyrate degradation will be even more sensitive). The addition of acetate at 80mM severely restricted propionate degradation (Kaspar and Wuhrmann, 1978b), whilst smaller additions had a limited but

discernable effect (Boone, 1982; Zehnder and Koch, 1983), and the addition of 8mM acetate was without effect (Kaspar and Wuhrmann, 1978b). It has been suggested that some of the digester reactions may also be sensitive to carbon dioxide (Finney and Evans, 1975; Hansson, 1982), although in view of the fact that the partial pressure remains relatively constant during digestion, it is doubted that this will be of much consequence.

The close harmony amongst the terminal reactions of anaerobic digestion was noted by Boone (1982) when acetate or carbon dioxide/hydrogen were added to a steady-state digester operated on cattle slurry, and brought about a rise in methane production. The hydrolysis of particulate components of the cattle slurry was uneffected by these additions and this strongly suggests that the digestion of soluble substrates was not a limiting factor (Boone, 1982). It is widely considered that the rate-limiting step in the digestion of a particulate waste such, as cattle slurry, is the initial hydrolysis and fermentation reactions (Fig. 1.4, reaction 1), and not the degradation of the intermediates (Fig. 1.4, reactions 2-7) (Hobson, 1981; Archer, 1983; Gujer and Zehnder, 1983). Two slow steps have been identified in the digestion of soluble wastes, acetogenesis from longer VFA and methanogenesis from acetate (Archer, 1983; Gujer and Zehnder, 1983; Archer, 1984; Kirsop, 1984; Kirsop *et al.*, 1984).

1.3 THE STABILITY OF ANAEROBIC DIGESTION

1.3.1 Introduction

When operating under steady-state conditions, the activities of the different metabolic groups of bacteria (see Fig. 1.4) are compatible, and there is no accumulation of catabolic end products within the digester. However, when the fermentation process is subjected to a sudden change in operating parameters, digestion becomes unbalanced as the various metabolic groups of bacteria respond in a different manner. This has been observed, for example; in digesters subjected to toxic compound addition (Kruize, 1983; Speece and Parkin, 1983; Hilpert *et al.*, 1984), to sudden temperature fluctuations (van Velsen and Lettinga, 1980; Temper *et al.*, 1983), to organic overloading (Pohland and Bloodgood, 1963; Barnes *et al.*, 1983; Temper *et al.*, 1983; Kennedy *et al.*, 1985), and to a change in substrate (van den Berg and Lentz, 1981). Indicators of digester instability have been frequently taken to be a rise in the acetic or VFA concentration and a fall in pH and methane production (Mueller *et al.*, 1959; Graef and Andrews, 1974; Hill and Barth, 1977). In cases of severe digester instability the VFA have reached toxic concentrations and brought about souring of digestion and a complete failure of the process. VFA toxicity has been found to increase with diminished pH (eg. Kroeker *et al.*, 1979); this is attributed

to the increased preponderance of the unionised form of the fatty acids, for it has been demonstrated that the bacterial cytoplasmic membrane is freely permeable to such acids in their unionised, but not their ionised form (Kell et al., 1981).

1.3.2 Effect of Temperature Shock Treatments

Several studies have been dedicated to an examination of the optimal operating temperature for the mesophilic anaerobic digestion of cattle slurry (eg. Zeeman et al., 1983a; Hawkes et al., 1984). However, the effect of temperature shock treatments was not considered. Working with pig slurry, van Velsen and Lettinga (1980) found that digesters operated on a 15 day retention time (RT) were not greatly effected by daily temperature changes from 30°C to 20°C and back to 25°C. However, when daily fluctuations from 20°C to 40°C were applied for five successive days, digestion did become unstable and 17 days were required for complete recovery. Experiments conducted with sewage sludge digesters at a 4 - 11 day RT (Temper et al., 1983) also found that small temperature changes (eg. 3°C fall over three days, 10°C drop for two hours) had only a transient effect on digester stability. On the other hand, four days were required for the rate of biogas production to return to normal after the temperature had been lowered from 35°C to 18°C for 10 hours. None of these studies have, however,

considered the likely position with a farm-scale digester where a failure in heating devices might be expected to lead to daily temperature falls of the order of 8°C (Skilton et al., 1985).

1.3.3 Causes and Symptoms of Digester Instability

In many studies of anaerobic digestion under stressed conditions a rise in the acetic acid or total VFA concentration has been taken to be one of the most important indicators of instability (Mueller et al., 1959; Graef and Andrews, 1974; Hill and Barth, 1977). Often this rise has been accompanied by a fall in methane production and consequently it has been frequently assumed that the acetate-utilising methanogens represent the group of bacteria most sensitive to a sudden change in operating conditions. Indeed, several studies have restricted themselves to an examination of digester stability with acetate-enriched cultures (Parkin et al., 1983; Speece and Parkin, 1983). However, some authors have differentiated between individual VFA and in these circumstances it has been suggested that propionic acid may be the most sensitive of the fatty acids to shock treatments (eg. Pohland and Bloodgood, 1963; Asinari di San Marzano et al., 1981).

Although digester bacteria clearly respond in a different manner to shock treatments (with some groups very

sensitive to sudden changes in operating conditions and others relatively uneffected), our present knowledge of the anaerobic digestion process is not sufficient to determine which groups of micro-organisms cause the instability and which are effected. The different response of the individual VFA to shock treatments has been predicted in a mathematical model presented by Mosey (1983). This model proposed that a rise in the hydrogen partial pressure will bring about a larger rise in the concentration of propionate than acetate or butyrate (as a consequence of increased formation and a greater thermodynamic sensitivity of propionate degradation to molecular hydrogen). Experimental evidence has been produced to support this hypothesis, and high hydrogen partial pressures have been measured during periods of digester instability (Barnes et al., 1983; Whitmore et al., 1985; Archer et al., 1986; Whitmore and Lloyd, 1986; Mosey, Personal communication). In these circumstances the hydrogen-utilising methanogenic bacteria are likely to be the most sensitive group of organisms to the shock treatment (for they are chiefly responsible for hydrogen removal), and the obligate proton-reducing bacteria the next most sensitive. Other interpretations of the different responses of the VFA are possible however, for the obligate proton-reducing bacteria are also sensitive to a rise in the acetate concentration (see section 1.2.4), and a small rise in the acetate concentration may cause digester instability.

It is likely that the response of the different

bacterial groups to various shock treatments will vary from waste to waste, with the actual digester conditions employed and the shock treatment applied, particularly, for example, when compounds that specifically inhibit one group of organisms enter the digester. The careful monitoring of each step in the digestion process will considerably improve our understanding of the digestion process.

1.4 ADVANCED REACTORS

1.4.1 Introduction

Anaerobic digestion has been chiefly conducted in conventional digesters such as continuously stirred tank reactors (CSTR) and unstirred tank reactors. In these digesters the solids, hydraulic and bacterial retention time are equivalent. In an attempt to improve the economics of the process, new and innovative digestion schemes have been developed. These are designed to maximise methane yields and production rates, increase process stability and decrease the energy requirements and costs. The advanced digester designs considered here all act by retaining active biomass within the digester (in a variety of ways) so that the bacterial retention time is longer than the hydraulic retention time, and the digestion process independent of the bacterial growth rate. In addition, some digesters are

designed to retain slowly degraded solids, so that the solids retention time is greater than the hydraulic retention time.

Six types of advanced reactors will be briefly considered; the anaerobic contact process, the upflow anaerobic filter, the downflow stationary fixed-film reactor, the fluidized/expanded bed, the upflow anaerobic sludge blanket, and two stage systems. A general description of advanced reactors, and a comparison of their performance using different substrates may be found in the following references: van den Berg et al., 1981; Hall et al., 1982; Henze, 1983; Switzenbaum, 1983; van den Berg and Kennedy, 1983; Kirsop, 1984.

1.4.2 The Anaerobic Contact Process

The anaerobic contact process may be considered as a CSTR with solids and bacterial recycle. Micro-organisms and partially degraded solids are returned to the reactor via a sedimentation tank (Fig. 1.5a), so that the solids and bacterial retention times are greater than the hydraulic retention time. This type of digester has been studied in detail at the National Research Council in Ottawa, and wastes treated include food processing wastes and simulated sewage sludge (van den Berg and Lentz, 1981; van den Berg et al., 1981).

1.4.3 The Upflow Anaerobic Filter

A number of different types of fixed film reactor have been developed for anaerobic waste treatment, and they have been classified into eight basic configurations (Henze and Harremoes, 1983). One of these is the upflow anaerobic filter, which is packed with an inert support material ideally with a high void volume and specific area (eg. gravel, rocks, ceramic, fired clay, plastic media) that acts as a stationary support surface for microbial film attachment (Fig. 1.5b). The upflow anaerobic filter has a very high volume loading capacity and consequently very short retention times may be employed. Wastes treated generally contain soluble organics or organics that are readily converted into soluble components, and feeds with a high particulate solids content are considered unsuitable as blockages are frequently encountered. The upward flow of the waste materials may also be impeded by excessive bacterial growth on the support material, in the digester void spaces, and in clumps at the bottom of the digester. In these circumstances it has proved necessary to backwash the reactor.

The upflow anaerobic filter is not, therefore, particularly suited to the digestion of particulate animal slurries (Henze and Harremoes, 1983; Young, 1983), although after pretreatment pig slurry has been successfully treated (Smith *et al.*, 1977; Chavadej, 1981; Brumm and Nye, 1982;

Colleran et al., 1983; Hasheider and Sievers, 1984). A variety of pretreatments have been employed such as pre-digestion, dilution and sieving to produce a 'soluble' waste of <1% total solids, with a very high VFA concentration. When pig slurry of a higher solids concentration has been applied blockages have been encountered. Cattle slurry does not appear to have been treated in an anaerobic filter, which may be a consequence of the lower concentration of easily degradable soluble components (especially VFA) compared with pig slurry.

A novel reactor has been recently described in which an upflow anaerobic filter and upflow anaerobic sludge blanket (section 1.4.6) are combined in a single reactor (Guiot and van den Berg, 1984, 1985). This reactor is suitable for the treatment of soluble wastes, which firstly pass through the sludge blanket and then the anaerobic filter.

1.4.4 Downflow Stationary Fixed-Film Reactor

The downflow stationary fixed-film (DSFF) reactor is also a fixed-film digester, and is in many respects similar to the upflow anaerobic filter. Feed is introduced into the DSFF reactor from the top and flows down distinct vertical channels to which a bacteria film is attached (Fig. 1.5c). The downward flow ensures that little suspended growth or particulate material is retained in the digester. A range

of support materials such as needle punched polyester, glass, polyvinyl chloride, potter's clay and red drain tile have been found to be suitable for use (Kennedy and van den Berg, 1982a,b,c).

Particulate animal wastes have been successfully treated and the reactor is less prone to blockages than the upflow anaerobic filter, consequently stringent pretreatments of the slurry have been unnecessary. However, the slurry has been sieved and diluted in all cases. Pig slurry of 1% TS (Harvey et al., 1984) and 2-3% TS (Kennedy and van den Berg, 1982a,b,c) and cattle slurry of 4% TS (Liao and Lo, 1985) have been successfully treated. Higher gas yields were produced from the anaerobic digestion of separated cattle slurry (SCS) in the DSFF reactor than in conventional digesters at all retention times tested (Liao and Lo, 1985). At the longer retention times employed (12-16 days), the biogas yield from a DSFF reactor was approximately double that from a conventional digester, whilst at retention times of less one day biogas was produced from a DSFF reactor but not a conventional digester (Liao and Lo, 1985). The enhanced performance of the DSFF reactor compared with the conventional digester may allow a large reduction in digester volume required to treat a fixed volume of waste, and hence significantly improve the economics of the process.

1.4.5 The Fluidized/Expanded Bed

The anaerobic fluidized/expanded bed (Fig. 1.5d) differs from the DSFF reactor and the upflow anaerobic filter in that most of the biomass is attached as a thin biofilm on small support particles. Inert support particles commonly used include sand, granular activated carbon, anthracite and glass, and they are maintained in a fluidized state by the upward velocity of the waste. Thus the effluent must normally be recycled. Fluidized/expanded beds have been reported to operate at lower retention times and/or higher organic loadings than anaerobic filters (Switzenbaum, 1983; Kirsop, 1984), although the waste should still be soluble. Pretreated cow slurry has been successfully digested in both fluidized and expanded bed reactors, although food processing and other soluble wastes have been more frequently applied (Henze and Harremoes, 1983).

1.4.6 The Upflow Anaerobic Sludge Blanket Reactor

The upflow anaerobic sludge blanket (USAB) reactor has no packing material and takes advantage of the fact that, with the correct physical and chemical conditions, anaerobic sludge can be flocculated and formed into granules with excellant settling properties. The waste is passed upwards through a relatively dense zone of granular sludge

and then through a less dense zone of flocculated sludge particles (Fig. 1.5e). For the successful operation of the UASB reactor, it is essential that the sludge is retained within the system. This is accomplished with an internal gas-solids separator and by minimizing mixing and sludge recirculation. Active biomass and solids are therefore maintained independently of the hydraulic retention time.

Complex liquid wastes of <1% total solids (eg. diluted domestic sewage, diluted fattening calf slurry and slaughterhouse waste) have been successfully treated in a UASB, although it is anticipated that the entrapment of non or poorly biodegradable particulate matter in the sludge is likely to appreciably lower the efficiency of the system. The upflow anaerobic sludge blanket process has been reviewed by Lettinga *et al*. (1983), and a novel reactor has been recently described in which an upflow anaerobic filter and sludge blanket are combined in a single reactor (Guiot and van den Berg, 1984, 1985).

1.4.7 Two-Stage Digesters

It has been proposed that anaerobic digestion may be enhanced if carried out in two or more stages, each occurring in a separate reactor, rather than a single digester. The rationale is that the conditions required for optimal functioning of the various stages of digestion are different, and that by separating the stages each may be

optimized individually and more easily controlled. However, it is anticipated that the economics of the digestion process may be adversely effected when more than a single vessel is used.

The anaerobic digestion of glucose in a two-stage system has been widely studied by workers in The Netherlands (eg. Cohen et al., 1979, 1980, 1982a,b; Zoetemeyer et al., 1982a,b,c). In the first reactor VFA are formed from glucose, and in the second reactor they are converted to methane. A number of particulate animal wastes have been successfully treated in a two-stage system (Kirsop, 1984), including pig slurry (Smith et al., 1977; Colleran et al., 1983), dairy waste water (Nieuwenhof, 1982) and crop residues (Colleran et al., 1983). The anaerobic digestion of particulate agricultural wastes in a two-stage digester involves hydrolysis of the particulate matter in the first reactor to yield high VFA concentrations, and then methanogenesis when this is applied to an upflow anaerobic filter (Smith et al., 1977; Colleran et al., 1983) or an UASB (Nieuwenhof, 1982).

Table 1.1 Summary of Comparisons of the Mesophilic Anaerobic Digestion of Whole Cattle Slurry and Separated Cattle Slurry

WHOLE CATTLE SLURRY				SEPARATED CATTLE SLURRY				RT (days)	REF
%VS dest	GY added	GY dest	CH_4 prod	%VS dest	GY added	GY dest	CH_4 prod		
36	0.27	0.75	0.67	44	0.37	0.84	0.90	10	a
26	0.20	0.77	0.99	29	0.23	0.79	1.14	5	a
12	0.09	0.69	0.20	15	0.13	0.90	0.18	16	b
12	0.07	0.60	0.20	19	0.16	0.89	0.30	12	b
10	0.07	0.63	0.24	25	0.20	0.77	0.38	10	b
11	0.06	0.49	0.26	25	0.17	0.65	0.40	8	b
7	0.04	0.63	0.25	20	0.16	0.79	0.50	6	b
-	0.07 -0.35	-	-	-	-	-	-	20 -30	c
-	0.03 -0.25	-	-	-	-	-	-	10 -12	c
-	-	-	-	-	0.31	-	0.28	15 -17	d
-	-	-	-	-	0.26	-	0.51	9 -10	d
-	-	-	-	-	0.18	-	0.68	5 -6	d
-	0.32	-	0.31	-	0.32	-	0.19	inf.	e
25	0.08	0.31	0.24	30	0.26	0.84	0.50	10	f
21	0.07	0.35	0.26	28	0.26	0.90	0.68	8	f
---------failed----------				23	0.20	0.89	0.61	6	f
29	0.26	0.88	0.42	37	0.35	0.94	0.34	20	g
-	-	-	-	37	0.31	0.85	0.40	15	g
-	-	-	-	16	0.23	1.44	0.32	16	h
-	-	-	-	19	0.25	1.30	0.38	12	h
25	0.08	0.31	0.24	26	0.23	0.89	0.47	10	h
21	0.06	0.29	0.26	25	0.20	0.80	0.49	8	h
7	0.03	0.45	0.15	24	0.19	0.80	0.63	6	h
---------failed----------				15	0.13	1.00	0.40	5	h
---------failed----------				20	0.10	0.51	0.35	4	h
---------failed----------				23	0.06	0.28	0.32	3	h
---------failed----------				10	0.05	0.51	0.42	2	h
---------failed----------				3	0.03	1.23	0.37	1.5	h
---------failed----------				3	0.03	0.93	0.40	1	h

CONTINUED OVER

Table 1.1 Continued

Abbreviations

%VS dest	percentage volatile solids destroyed
GY added	gas yield (m^3 biogas kg VS added^{-1})
GY dest	gas yield (m^3 biogas kg VS destroyed^{-1})
CH_4 prod	methane production (m^3 methane m^3 digester^{-1} day^{-1})
RT (d)	retention time (days)

References

a	Rorick et al. (1980)
b	Lo et al. (1983)
c	Zeeman et al. (1983a)
d	Hawkes et al. (1984)
e	Kiely (1984)
f	Liao et al. (1984)
g	Pain et al. (1984)
h	Liao and Lo (1985)

Note

The results obtained by the original authors have been converted to the above units with the information provided. However, the following details should be noted:

1. All experiments were conducted at 35°C except Rorick et al. (40°C) and Lo et al. (30°C).

2. The solids content was approximately 7 - 8% for whole cattle slurry and 4% for separated cattle slurry, except in the study of Rorick et al. where both slurries were added at 4.1% VS.

3. The results for separated cattle slurry reported by Lo et al. (b), Kiely (e) and Liao et al. (f) are the mean of two degrees of separation.

Table 1.2 The Proximate Constituents of Dairy and Beef Cattle Slurry

Ash	Lipd	∝cell	Hcell	Lign	CP	VFA	Reference
Dairy Cattle Slurry							
15	n/d	----41----		7	16	n/d	Chandler et al. 1980
22	3	15	11	9	24	8	Rorick et al. 1980
17	n/d	27	48	19	16	n/d	Hills & Kemmerle 1981
18	n/d	25	54	18	n/d	n/d	Hills & Roberts 1981
13	3	----31----		14	15	n/d	Martin et al. 1983
17	3	31	12	12	13	<1	Robbins et al. 1983
24	2	12	15	3	15	3	Kiely 1984
16	n/d	----26----		17	n/d	1	Singh et al. 1984
18	3	20	24	12	17	2	OVERALL MEAN
Beef Cattle Slurry							
28	4	17	19	7	15	1	Varel et al. 1977
9-18	n/d	9-25	13-25	3-6	n/d	3-6	Hashimoto et al. 1981
14	n/d	16	9	4	17	8	Hashimoto 1983
29	3	----40----		9	17	n/d	Martin et al. 1983
21	3	17	16	6	16	4	OVERALL MEAN

Abbreviations

lipd	lipid
∝cell	∝-cellulose
hcell	hemicellulose
lign	lignin
CP	crude protein
VFA	volatile fatty acids
n/d	not determined

Note

1. No other proximate constituents have been reported
2. All values are expressed as a percentage of the total solids from the original publication.
3. Where ∝-cellulose and hemicellulose have not been determined individually the sum is given.
4. The results of Hashimoto et al. (1981) are from a number of feed rations

Table 1.3 Comparison of the Destruction of Volatile Solids, Lignin, ∝-cellulose and Hemicellulose During the Anaerobic Digestion of Dairy Cattle Slurry

% Proximate Constituent Destroyed				Reference
VS	Lignin	∝-cell	hcell	
35	12	35	33	Hills & Kemmerle (1981)
13-34	*	7-41	24-33	Hills & Roberts (1981)
20	n/d	35-40	35-40	Kiely (1984)

Abbreviations

VS	Volatile Solids
∝-cell	∝-cellulose
hcell	hemicellulose
n/d	not determined

Note

* Hills & Roberts found -15 to 11% lignin was destroyed

Table 1.4 The Theoretical Biogas Yield (m^3kgVS destroyed^{-1}) and Methane Content of Biogas Produced from the Three Major Degradable Components of Dairy Cattle Slurry

Theoretical Biogas Yield (m^3 kg VS dest^{-1})			Theoretical Methane (as % in biogas)			Reference
Carb	Prot	Lip	Carb	Prot	Lip	
0.9	0.6	1.5	50	84	70	Badger et al. 1979
0.9	1.2	1.2	50	54	70	Iannotti et al. 1979
0.7	1.0	1.4	50	>50	72	Hawkes 1983
0.8	1.2	1.3	50	65	70	Schoberth 1983
0.8	0.9	1.5	(no values given)			Hawkes & Hawkes 1986
0.8	1.0	1.4	50	68	70	MEAN

Abbreviations

Carb Carbohydrate
Prot Protein
Lip Lipid

Note

The gas composition values do not take into account the relative solubility of carbon dioxide or methane

Table 1.5 Summary of Bacteria Capable of Degrading Straight Chain Volatile Fatty Acids

Name of Isolate	VFA degraded	Reference
Syntrophomonas wolfei	butyrate(c4) - caprylate(c8)	McInerney et al. (1979,1981)
Syntrophobacter wolinii	propionate(c3)	Boone and Bryant (1980)
spore forming and non-spore forming organism	butyrate(c4) - caproate(c6)	Shelton and Tiedje (1984)
non-spore forming organism	butyrate(c4)	Henson and Smith (1985)
Syntrophomonas sp.	butyrate(c4) - caproate(c6), plus even no. long chain acids upto c18	Roy et al. (1985)
Clostridium bryantii	butyrate(c4) - undecanoate(c11)	Stieb and Schink (1985)
spore forming organism	butyrate(c4)	Tomei et al. (1985)

Table 1.6 Proposed Reactions Involved in the Catabolism of Volatile Fatty Acids by Syntrophomonas wolfei (McInerney et al., 1979)

Butyrate:

$$CH_3(CH_2)_2COO^- + 2H_2O \longrightarrow 2CH_3COO^- + 2H_2 + H^+$$

Valerate:

$$CH_3(CH_2)_3COO^- + 2H_2O \longrightarrow CH_3CH_2COO^- + CH_3COO^- + 2H_2 + H^+$$

Caproate:

$$CH_3(CH_2)_4COO^- + 4H_2O \longrightarrow 3CH_3COO^- + 4H_2 + 2H^+$$

Heptanoate:

$$CH_3(CH_2)_5COO^- + 4H_2O \longrightarrow CH_3CH_2COO^- + 2CH_3COO^- + 4H_2 + 2H^+$$

Table 1.7 Summary of Reactions Involving Branched Volatile Fatty Acids in Anaerobic Environments

REACTIONS	Organism	Reference
β-oxidation:		
iso-heptanoic acid ⟶ i-valerate + acetate + H_2	*Syntrophomonas wolfei*	McInerney *et al.*, 1979, 1981
2-methylbutyric acid ⟶ propionate + acetate + H_2	*Clostridium bryantii*	Stieb & Schink, 1985
2-methylbutyric acid ⟶ propionate + acetate + H_2	one spore forming and one non-spore forming bacteria	Shelton & Tiedje, 1984
Oxidative Deamination:		
valine ⟶ i-butyric	*Bacteroides ruminicola*	Allison, 1978
leucine ⟶ i-valerate	*Megasphaera elsdenii*	Allison, 1978
isoleucine ⟶ 2-methylbutyric acid	-	Nagase & Matsuo, 1982
	Acidaminobacter hydrogenoformans	Stams & Hansen, 1984
	Desulfovibrio sp.	Stams *et al.*, 1985
Isomerization:		
i-butyrate ⟶ n-butyrate	Spore forming bacterium	Shelton & Tiedje, 1984
	-	Zinder *et al.*, 1984
	-	Cohen *et al.*, 1982a,b
Incorporation into cellular carbon:		
i-butyrate ⟶ valine	*Methanococcus voltae*	Whitman *et al.*, 1982
i-valerate ⟶ leucine	Three species of methanogenic bacteria	Eikmanns *et al.*, 1983
2-methylbutyric acid ⟶ isoleucine	many rumen bacteria	Prins, 1977
		Baldwin & Allison, 1983

Table 1.8 Mechanisms for Methanogenesis from Acetate and Their Energy Yield under Standard Conditions ($\Delta G^{0'}$ from Thauer et al., 1977)

Mechanism 1. (acetate splitting)	$\Delta G^{0'}$ (kJ mol^{-1})
$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$	- 31

Mechanism 2. (acetate oxidation)

(a)	$CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 4H_2 + H^+$	+105
(b)	$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$	-136
sum	$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$	- 31

Table 1.9 Apparent Ks values for Hydrogen of Methanogenic and other Bacteria

Isolate	Apparent Ks (μM)	Ref
Methanobacterium ruminantium	1	a
Methanobacterium thermoautotrophicum	8	b
Methanosarcina barkeri	15 - 25	b
Methanobrevibacter arboriphilus	6	b
Methanospirillum hungatei	5 - 12	b
Methanobacterium formicicum	6	b
Sulphate reducing bacteria	1 - 3	b
Rumen fluid & digester sludge	6	c

References

a Hungate *et al*. (1970)
b Kristjansson & Schonheit (1983)
c Robinson & Tiedje (1982)

Table 1.10 Measured Hydrogen Concentrations in Different Habitats (Steady-state conditions)

Hydrogen Concentration		Reference	Habitat
atmospheres	µM		
$<10^{-4}$	<0.1	Kaspar & Wuhrmann (1978a)	Sludge digesters
0.5×10^{-4}	0.05	Robinson & Tiedje (1982)	Sludge digesters
$<10^{-5}$	<0.01	Robinson & Tiedje (1982)	Sediments
$10^{-5} - 10^{-3}$	0.01 - 1	Zehnder et al., (1982)	Sediments
2.5×10^{-4}	0.25	Barnes et al., (1983)	Soluble Waste digesters
$4 - 8 \times 10^{-5}$	0.04 - 0.08	Mosey (1983)	Sludge digesters
$0.5 - 1 \times 10^{-4}$	0.05 - 0.1	Mosey & Fernandes (1984)	Sludge digesters

Fig. 1.1 Effect of the Solids Concentration on the Gas Yield (per VS added) from Cattle Slurry at a 20 day RT and 30°C (Results of Singh et al. (1984)).

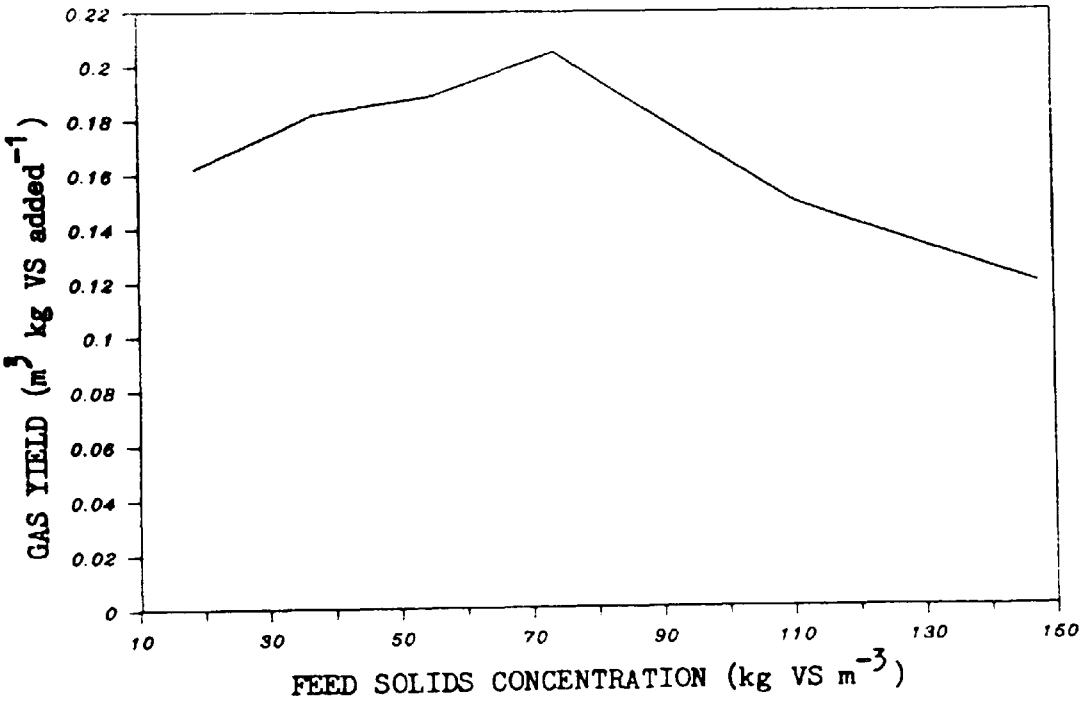


Fig. 1.2 Effect of the Solids Concentration on the Methane Yield (per VS added) from Cattle Slurry at 35°C and four Retention Times (Results of Hashimoto (1982)).

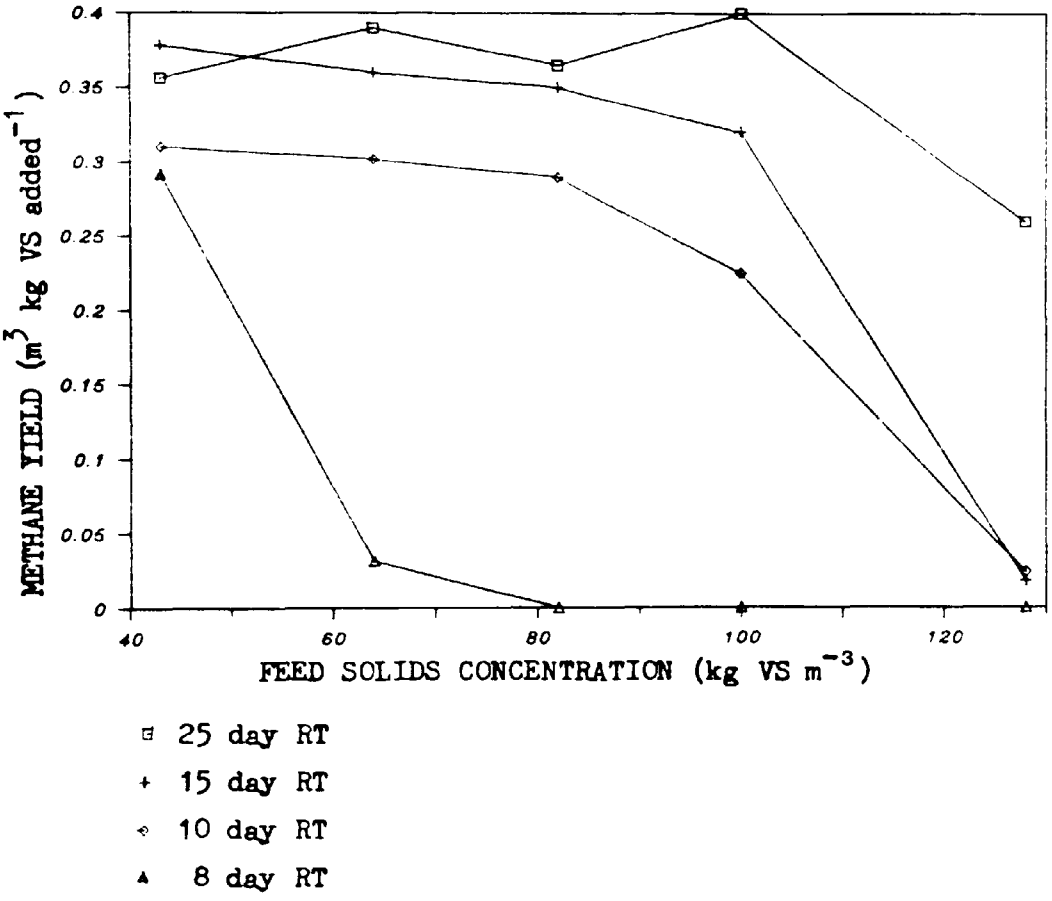


Fig. 1.3 Effect of the Solids Concentration on the Gas Yield (per VS added) from Dairy Cattle Slurry.

Figure 1.3a Results of Lo et al. (1983)

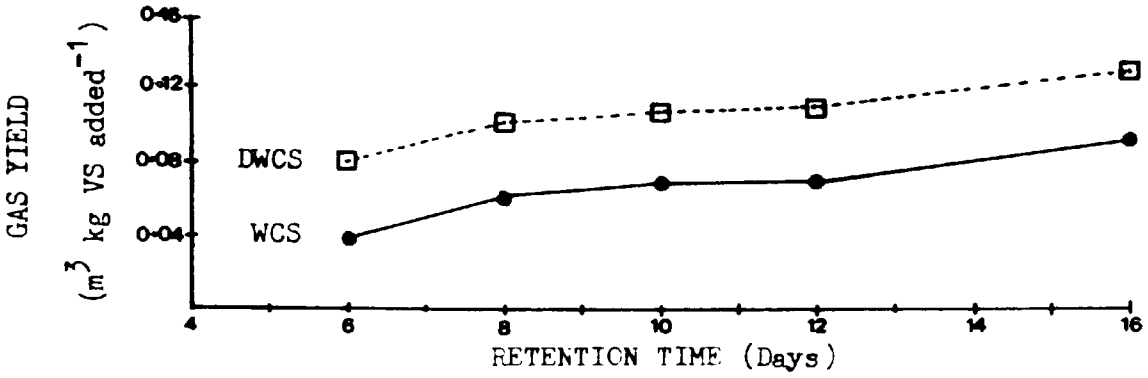


Figure 1.3b Results of Liao et al. (1984)

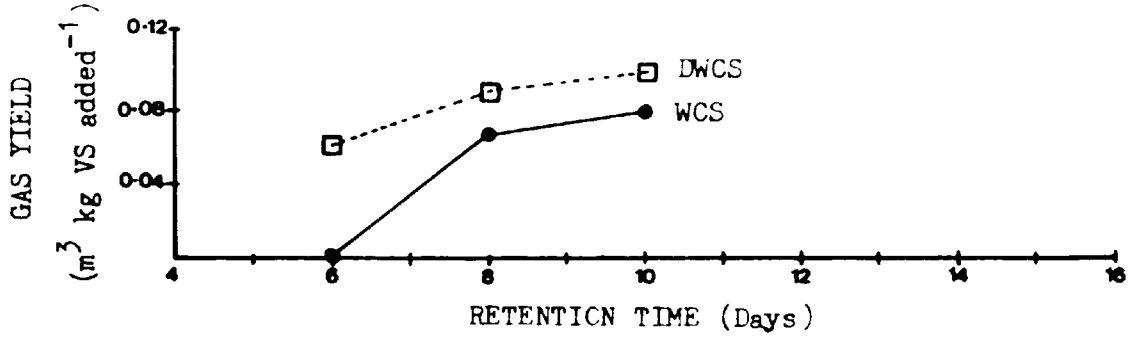
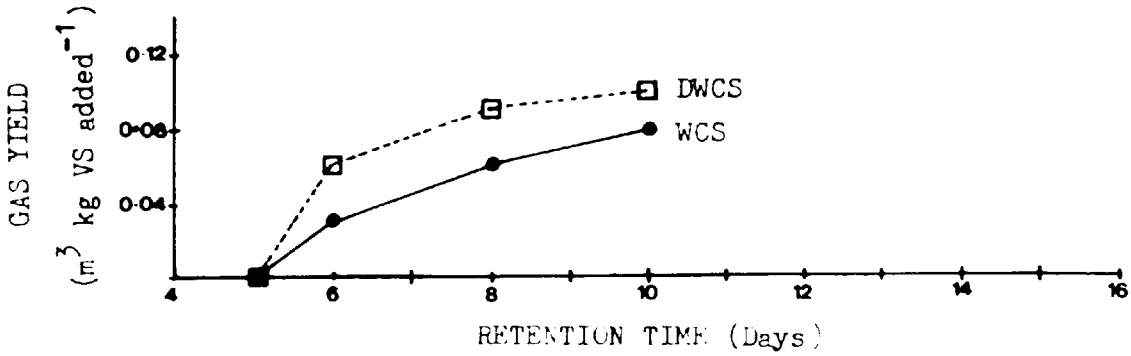


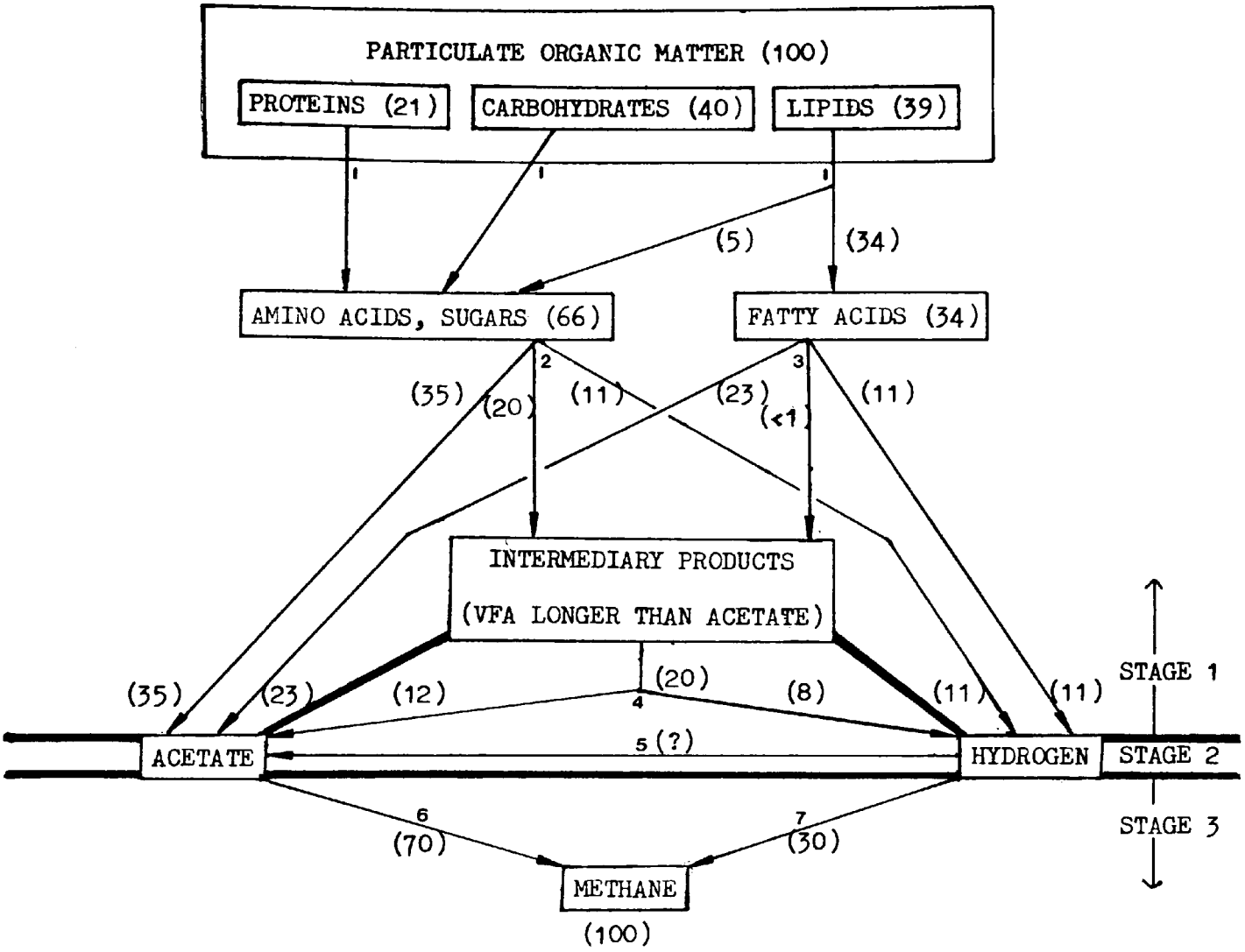
Figure 1.3c Results of Liao and Lo (1985)



DWCS - Diluted Whole Cattle Slurry

WCS - Whole Cattle Slurry

Fig. 1.4 Proposed Reaction Scheme for the Anaerobic Digestion of Domestic Sludge (Adapted from Gujer and Zehnder, 1983).



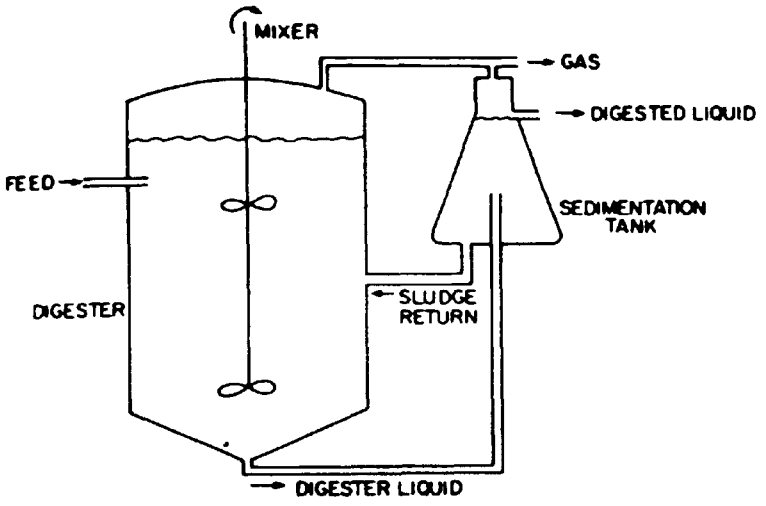
Stage 1 Reactions:
1 Initial hydrolysis of particulate organic material
2 Fermentation of amino acids and sugars
3 Anaerobic β-oxidation of long chain fatty acids

Stage 2 Reactions:
4 β-oxidation of short chain volatile fatty acids
5 Oxidation of H_2 with reduction of CO_2 to acetate

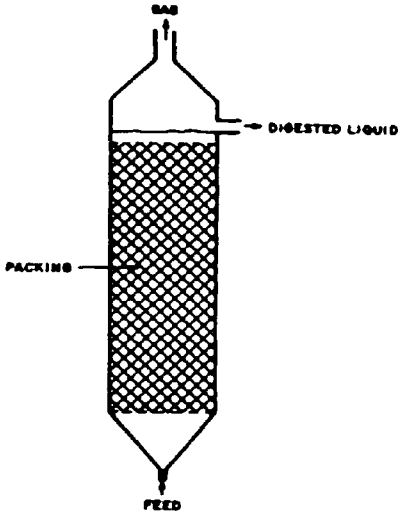
Stage 3 Reactions:
6 Formation of methane by aceticlastic methanogens
7 Formation of methane by CO_2 reducing methanogens

Values in brackets indicate substrate flow (stoichiometrically) in form of percentage of COD (chemical oxygen demand) or methane equivalents.

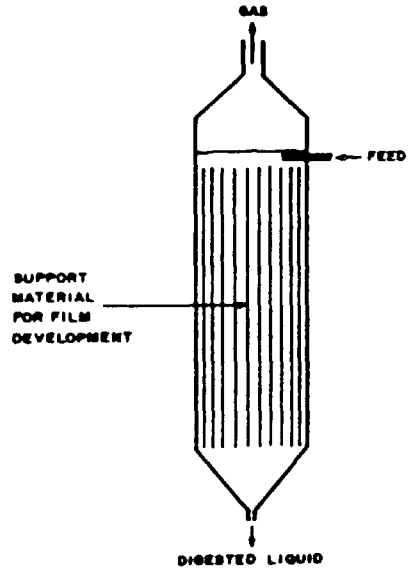
Fig. 1.5 Sketches of Advanced Reactors. (A) Anaerobic Contact Reactor; (B) Upflow Anaerobic Filter; (C) Downflow Stationary Fixed-film Reactor; (D) Anaerobic Fluidized/ Expanded Bed; (E) Upflow Anaerobic Sludge Blanket.



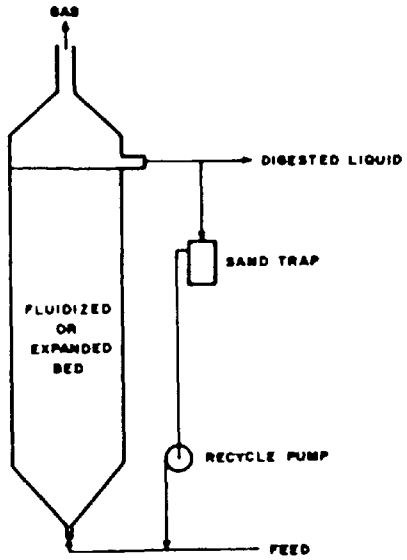
(A)



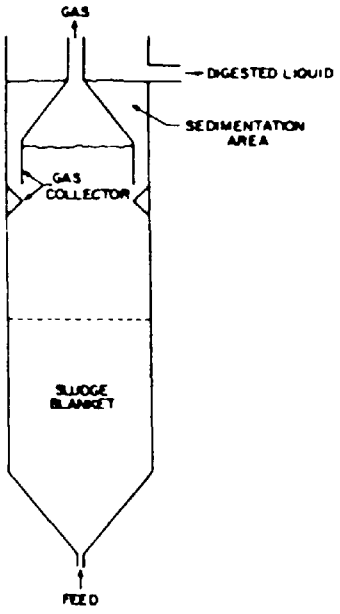
(B)



(C)



(D)



(E)

CHAPTER TWO

MATERIALS AND METHODS

2.1 SOURCE OF CATTLE SLURRY

Slurry was obtained from the herd of lactating British Friesian dairy cows of The Animal and Grassland Research Institute, Reading, UK. Details of the cows' diet are given in Table 2.1. The cattle were housed throughout the year in cubicle sheds with sawdust bedding. The cubicle shed passage ways were tractor-scraped twice daily into an underground cross-channel which also received some parlour and collecting yard washings. Mechanical scrapers in the cross-channel conveyed slurry to a reception pit outside the building where it was mixed and pumped, either to a mechanical separator or to the whole cattle slurry (WCS) feed tank. Slurry was separated with a commercial two stage roller press machine (Farrow Irrigation Ltd.) mounted on a 4m high gantry to allow collection of the solid fraction in a trailer and gravity transfer of the separated liquid to a feed tank. The separator employed a conventional brushed screen as a primary stage, followed by a roller pressing stage consisting of a semicircular screen with 3mm diameter perforations, over which passed a series of brushes and rollers. Fresh batches of separated and whole cattle slurry were obtained from the two feed tanks and stored at -20°C (within three days of arriving at the Polytechnic) until required. Where necessary the slurries were each mixed to homogeneity prior to storage at -20°C. Immediately prior to use the slurry was thawed

and kept at 4°C until required.

2.2 DIGESTER OPERATION

2.2.1 Five-Litre Daily Fed Digesters

Five-litre laboratory-scale digesters (Fig. 2.1) were constructed out of standard quickfit glassware. A stirrer shaft of 3mm stainless steel was fitted with two propellers separated by approximately 100mm, and manufactured from 60mm diameter stainless steel discs. The shaft entered the digester through a stirrer guide, and the sleeve gland contained water as a gas seal. Stirring was achieved with a Citenco stirrer set to approximately 300 rpm. A polythene tube was sealed into the largest port (diameter 30mm) of the lid; this dipped well below the level of the liquid in the digester and served as a feeding port. Effluent was also removed from this port with a 100cm^3 plastic syringe with modified end. Of the remaining three ports, one was used to take gas samples for compositional determinations, the second was connected to a gas meter and the third connected to a water manometer.

The digesters were maintained at 35°C by immersion in an aquarium tank heated by a Grant FH15 flowheater (Grant Instruments Ltd, Cambridge, UK). Temperature fluctuations were applied to the digesters

(where appropriate) by lowering the surrounding water temperature. On these occasions a flowcooler (Grant FC15) was also present. Initially the digester temperature was lowered from 35°C by approximately 8°C per day. Once at 3-6°C, the digester temperature was raised back to 35°C either rapidly (in a single day) or slowly (over several days). Thermocouples situated in the digesters revealed that approximately eight hours were required for an 8°C temperature drop to be effected, whilst only four hours were necessary to bring about an 8°C rise in digester temperature.

The digesters forming part of the temperature shock experiment (Chapter 5) were fed once daily at noon, seven days per week (except where indicated at a 10 day retention time (RT), when they were not fed at all), whilst the digester used in comparison with the anaerobic filter (Chapter 6) was fed only five days a week (once a day, Monday to Friday). Immediately prior to feeding the intra-digester pH, daily gas volume and composition were determined and an amount of digester slurry equal to the feed volume, removed for analysis. The digesters were stirred for ten minutes before and after feeding; otherwise the digesters were unstirred. The experiments in which these digesters were used are indicated in Table 2.2.

2.2.2 Ten-Litre Daily Fed Digesters

Two identical, 10 l digesters were constructed of perspex (Fig. 2.2). A stirrer shaft of 3mm stainless steel was fitted with two propellers separated by approximately 210 mm, and manufactured from 130 mm diameter stainless steel discs. The shaft entered the digester through a stirrer guide, and the sleeve gland contained water as a gas seal. The digesters were stirred continuously at 120 rpm with a Heidolph R2R50 stirrer (Heidolph - Elektro KG, Kelheim, West Germany). Feeding was achieved by a displacement method, where the required volume was poured into the digester and the effluent collected from the side-arm (Fig. 2.2). Biogas exited from a third port, from where samples for compositional determinations were taken. The digesters were maintained at 35+/-0.5°C in a constant temperature cabinet constructed of polystyrene. The temperature was controlled by an electric fan heater with adjustable thermostat.

Immediately prior to feeding, the intra-digester pH, daily biogas volume and composition were determined. Samples of the effluent (taken directly from the digester rather than via the exit side-arm) and feed were analyzed on Mondays (total solids only at a 25 day RT, complete analysis at a 10 day RT) and Thursdays (complete analysis at both retention times). The experiments in which these digesters were used are indicated in Table 2.2).

2.2.3 Batch Digester Operation

Batch digestion tests were conducted in both 2 l and 5 l unmixed reactors. The initial reactor contents are described in Table 2.3. Once a small volume had been removed for analysis (as indicated in Table 2.3), the digester head space (approximately 2 l in the 5 l vessels, and 0.5 l in the 2 l reactors) was gassed with oxygen-free nitrogen, and the digester incubated at 35°C until biogas production ceased. Results from control digesters operated on seed alone were subtracted in all cases. (The experiments in which these batch digesters were used are indicated in Table 2.2).

2.2.4 Operation of the Anaerobic Filter

A glass vessel (Fig. 2.3) was randomly packed with Filterpak CR50 (Mass Transfer International, Kirkby Stephen, Cumbria, UK) and had a working volume of 20 l (including recirculation tubing). The packing material and inside surface of the digester had a surface area : volume ratio of 230 m^2/m^3. Slurry was continuously recirculated by pumping (Valisi peristaltic pump RE2, Valisi SNC, Milan, Italy) from an overflow arm back into the bottom of the digester and then upwards at 3.5 l $hour^{-1}$. The minimum diameter of the tubing was 13 mm. Biogas exited from a third port, where samples for compositional determinations were taken (Fig.

2.3). The digester was maintained at 35°C by a heated water jacket in an insulating cabinet, and the effluent temperature was checked frequently and varied from 34-35°C, (except during periods of heater failure).

Immediately prior to feeding, the biogas volume and composition were determined. Feeding was achieved by draining the required volume of effluent from the bottom of the digester, and then pumping feed into the top of the digester (see Fig. 2.3), until the initial digester volume was restored. The slurry was then recirculated as previously described. The digester was fed five times a week (once a day, Monday to Friday). The experiments in which this reactor was used are indicated in Table 2.2.

2.3 ANALYTICAL TECHNIQUES

2.3.1 Total and Volatile Solids

The total solids concentrations of slurries was determined by drying samples of 25 (+/- 1.5)ml in glass beakers using a commercial microwave oven (Hitachi or National) set on 'defrost'. After two hours a constant weight was achieved.

The volatile solids content of samples dried using a microwave oven was determined by incineration in a 'Eurotherm' electric furnace (Carbolite Ltd, Sheffield) at

500°C for 30 minutes. The samples were placed in preweighed porcelain crucibles, and then reweighed after incineration, when cool.

2.3.2 Holocellulose, Hemicellulose and Alpha-cellulose

Holocellulose (total cellulose) was determined by the method of Allen *et al.* (1974) with modifications necessitated by the physical properties of cattle slurry. This is a delignification procedure that preserves hemicellulose as well as α-cellulose as an estimate of the entire polysaccharide fracton.

Wet samples of 30ml (diluted with water so that the total solids content was approximately 0.5g) were incubated in a water bath at 75°C for four hours, with 0.3g of sodium chlorite and 1ml of 10% v/v acetic acid added initially and after one, two, and three hours. After exactly four hours, samples were cooled in ice cold water. The remaining solids were separated using a bench centrifuge (MSE Centaur 1, MSE Ltd, UK) at full speed for ten minutes, and washed in deionised water (twice), acetone (twice) and diethyl ether (once) by resuspending the pellet, mixing and re-centrifuging. The final pellets were washed into weighed beakers with diethyl ether to ensure complete transfer. The ether was allowed to evaporate overnight, and the extracts further dried for 30 minutes in the microwave oven (at 'defrost' setting). The volatile solid content of the

extracts was then determined, and the weight of non-volatile matter subtracted.

Breakdown of the holocellulose was carried out with potassium hydroxide, this dissolved away the hemicellulose to leave α-cellulose in a reasonably pure form (Allen et al., 1974). The derived holocellulose (as above except that double the quantities were used) was incubated at 20°C with 20ml of 24% potassium hydroxide. After two hours the solids were removed by centrifugation, and the pellet washed (by resuspension and centrifugation) with deionised water (twice), acetone (twice) and finally with diethyl ether (once). The final pellets were washed into weighed beakers with diethyl ether to ensure complete transfer. The ether was allowed to evaporate overnight, and the extracts dried for 30 minutes in the microwave oven (at defrost setting). The volatile solid content of the extracts was then determined, and the weight of non-volatile matter subtracted. The white product was taken to be α-cellulose, hemicellulose was derived by subtracting α-cellulose from holocellulose.

2.3.3 Lignin

Lignin was estimated by a procedure in which all other organic compounds were destroyed. Sample pre-treatment removed lipids (with ether), soluble carbohydrates (with water) and proteins (with dilute acid).

The holocellulose complex was then hydrolysed with 72% acid followed by 3% acid, to leave lignin (Allen et al., 1974).

To a pre-weighed extraction thimble was added approximately 1g of dried slurry (as total solids, dried in a microwave oven), the thimble was then reweighed and glass wool added to prevent sample loss. This was extracted with ether in a Soxhlet apparatus for approximately six hours. The thimbles were then removed and the ether allowed to evaporate overnight. The following morning the entire thimble contents (except the glass wool) were transferred to a 1 l tall beaker and boiled gently for three hours in 400 ml deionised water. A watchglass ensured that the volume remained constant. 22 ml 10% H_2SO_4 was then added and the sample boiled for a further hour. After settling, the supernatant was removed with a sintered filter stick (No. 3), and the solids left to dry overnight. To the residue was then added 15 ml 72% H_2SO_4 (at 12-15°C), this was stirred with the filter stick for one minute and incubated at 18-20°C with occasional stirring. After two hours the acid concentration was reduced to 3% by the addition of 560 ml deionised water, the filter stick was washed and removed, and the sample boiled gently for four hours (a watchglass ensured that the volume remained constant). The contents were then allowed to settle, and filtered immediately through a preweighed No. 3 sintered glass crucible. Hot water was washed through to remove the acid. The following morning the sintered crucible was dried for one hour in the microwave oven (at

'defrost' setting). The volatile solid content of the extract was then determined and the weight of non-volatile matter subtracted. Although the pore size of the No. 3 sintered crucible is 20-30 μm, it was evident that smaller lignin particles were retained (see Table 4.1). This is attributed to the pores becoming blocked during filtration, so that the actual pore size was considerable smaller than the initial pore size. Further evidence of the blockage of the filtration pores was that the filtration process frequently took at least one hour.

2.3.4 Total Lipid Assay

The assay used was based on that of Bligh and Dyer (1959), with modifications necessitated by the physical properties of the slurry and using an antioxidant instead of N_2 and cooling to prevent extract oxidation.

8 ml wet samples were homogenised for 60 seconds with 10 ml chloroform and 20 ml methanol. A further 10 ml chloroform and then 10 ml deionised water were added and the samples homogenised for a further 60 seconds after each addition. The chloroform contained $5mgl^{-1}$ BHQ (Butyrated Hydroxy Quinoline) as an antioxidant. The samples were then centrifuged on a bench centrifuge for 15 minutes at top speed. This facilitated the separation of the (lower) chloroform layer containing the lipids from the water/methanol layer. Solid impurities collected in a

compact layer at the phase boundary and were discarded when the samples were transferred into a separated funnel. The entire chloroform layer was then transferred into a weighed beaker and the chloroform allowed to evaporate overnight. The beaker was then dried for 15 minutes in the microwave oven (set to 'defrost') and the weight of lipids present determined.

2.3.5 Crude Protein

Crude protein was estimated by multiplying the organic nitrogen concentration by a factor of 6.25. The organic nitrogen concentration was calculated by subtracting the ammonium concentration (see below) from the total Kjeldahl nitrogen concentration. The total nitrogen concentration was determined by Kjeldahl digestion followed by steam distillation. 0.5 g cattle slurry (wet weight) was added to 2ml acidic selenium dioxide solution ($20gl^{-1}$ selenium dioxide in 50% v/v H_2SO_4) and heated over a low flame until colourless. This was followed by steam distillation into boric acid plus indicator (methyl red and methylene blue) and back titration with 0.01 M HCl.

2.3.6 Ammonium Concentration

Samples of cattle slurry were acidified by the

addition of an equal volume of 0.1 M HCl. After centrifugation for ten minutes at full speed on the bench centrifuge (MSE Centaur 1, MSE Ltd, UK), 1.0 ml supernatant was transferred to the still along with 2 ml of 10 M NaOH. The distillate was collected in boric acid plus indicator (methyl red and methylene blue) and back titrated with 0.01M HCl.

2.3.7 pH

pH was determined using a Russell combination pH electrode (Auchtermuchty, Scotland) attached to a Philips PW9049 pH meter. All measurements of digester pH were make by inserting the electrode into the digester, to avoid changes as a consequence of CO_2 release (see section 5.2). The electrode was calibrated daily before use with a pH 7.0 buffer held at 35°C or the appropriate temperature.

2.3.8 Volatile Fatty Acid - The Spectrophotometric Method

Prior to the successful operation of the gas-chromatograph, the total volatile fatty acid (VFA) concentration was determined by a spectrophotometric method. This method (Montgomery et al., 1962) is more rapid than the gas-chromatograph technique but is considerably less accurate (particularly at concentrations below 2mM) and

fails to differentiate between the acids. The results are expressed as equivalents of acetate, as acetate was used for calibration purposes.

Cattle slurry samples were filtered through Whatman No. 1 filter paper; and 0.5 ml filtrate was added to 1.5ml ethylene glycol and 0.2 ml 50% v/v sulphuric acid in a test tube. The tubes were mixed and heated for three minutes in boiling water before cooling in cold water. 0.5 ml 100gl^{-1} hydroxylamine chloride and 2.0 ml 4.5M NaOH were then added and the tubes mixed again. After one minute, samples were added to 10 ml ferric chloride solution (20gl^{-1} ferric chloride in 2% v/v sulphuric acid) in 25 ml volumetric flasks, which were then filled to 25 ml with deionised water and shaken. After five minutes optical densities were measured at 500 nm. A sample containing 0.5 ml deionised water was also processed and used as a reference in the spectrophotometer (Varian Techtron 635, Varian Assoc., UK). Calibration curves were obtained using acetic acid standards, and calibration was found to be linear upto at least 83 mM (5000 ppm).

2.3.9 Volatile Fatty Acid - Gas Chromatography

The individual volatile fatty acid (VFA) concentrations (C-2 to C-6) were determined by a method in which samples were acidified and then extracted into diethyl

ether. The separation of acids achieved was superior to that achieved with aqueous methods (Fig. 2.4), and the problems of column fouling were also avoided.

At the end of each experiment slurry samples that had been stored at -20°C were thawed and acidified by the addition of formic acid (Aristar grade, BDH, Poole, UK) to 10% v/v, and then clarified by centrifugation (one minute at full speed in a Micro-Centaur centrifuge (MSE Ltd, UK)). An equal volume of diethyl ether was then added to the supernatant and the sample shaken 20 times; the diethyl ether fraction was then transferred to an automatic sampler vial. The VFA concentration was measured using a 6ft x 4mm glass column of 15% SP1220/1%H_3PO_4 on 100/120 Chromosorb W/AW (Supelco Inc., USA) in a Vista 6000 gas chromatograph (Varian Assoc., UK) at 145°C with nitrogen the carrier gas at 30cm^3 min^{-1}. The injector was set to 130°C and the flame ionisation detector to 155°C. 1.5µl ether extracted samples were injected onto the column by an automatic sampler (Varian Assoc., UK). The machine was calibrated after each 20 samples with a standard, and gave a standard error of less than 2% on all occasions for each acid. Each acid responded in a linear fashion at least within a range of: 0.05-500 mM (3-30000 ppm) for acetate; 0.01-215mM (1-16000 ppm) for propionate; 0.01-64 mM (1-5600 ppm) for n-butyrate and i-butyrate; 0.01-28 mM (1-2800 ppm) for n-valerate and i-valerate; 0.01-24 mM (1-2800 ppm) for n-caproate and i-caproate. This method did not distinguish between 2-methyl butyric acid and 3-methyl

butyric acid (i-valeric acid), and both have been recorded as i-valerate. A typical separation of the VFA is given in Fig. 2.4. Sample injections were alternated with 10% formic acid (in ether) injections to avoid ghosting from VFA and long chain fatty acids (which were also partially extracted by this procedure). The results were analyzed by a 401 Chromatography Data Station (Varian Assosc., UK).

2.3.10 Gas Composition

The methane and carbon dioxide content of the biogas was determined using a 2m x 0.125" Poropak T (80-100 mesh) metal column in a Vista 6500 gas chromatograph (Varian Assoc., UK) at 60°C with helium as the carrier gas at 30 cm^3 min^{-1} and a thermal conductivity detector. The machine was calibrated daily with a standard mixture comprising 63.6% methane and 36.4% carbon dioxide (Hyline standard, BOC, London) and gave a standard error of less 0.5% on all occasions. The results were analyzed by a 401 Chromatography Data Station (Varian Assoc., UK), and are expressed throughout as a percentage of the total biogas.

The hydrogen sulphide and ammonia concentration in the digester headspace were determined using gas analysis tubes (Draeger Ltd., London).

2.3.11 Gas Production

The volume of biogas produced was measured daily before feeding using a 0.25 litre gas meter (Alexander Wright and Co Ltd., London) filled with light transformer oil.

2.3.12 Sieving Procedure

250 ml WCS or 500ml SCS (each containing approximately 20g total solids) were applied to a series of sieves and washed through with 10.5 l of tap water. The solids were then removed and the process repeated, at least 6 l of each slurry was sieved on each occasion to ensure reproducability. Brass mesh sieves with a nominal aperture of 1700, 1000, 710, 300, and 63 μm (A. Gallenkamp Co Ltd., London) were used along with a 15 μm plastic mesh (Nytal HD15, Polak Ltd., London). When the sieving was complete each size fraction was dried in the microwave oven (defrost setting) to a constant weight.

2.3.13 Sample Preparation for the Scanning Electron Microscope

Samples to be viewed with the SEM (scanning electron microscope) were prepared by critical point drying. Small

sections of the anaerobic filter packing material were first fixed for 30 minutes in 3% glutaraldehyde, and then sequentially immersed for 15 minutes in 30%, 50%, 80%, 100% ethanol, ethanol:amyl acetate (50:50 v/v) and finally amyl acetate. Transfers between these solutions were carried out radidly to avoid drying. The samples were then placed in a critical point drying pressure vessel (Polarson Ltd., Watford), and the amyl acetate removed by flushing the apparatus with the drying liquid, carbon dioxide, in which the sample was immersed for a further hour. The carbon dioxide was taken past its critical point of 31.5°C and 1100 psi by heating in a sealed vessel to 35-40°C, then opening a valve to release the pressure. Dried samples were coated with gold in a Nanotech Sputter-coater before viewing with a SEM (Cambridge Instuments, model no. 105).

2.3.14 ATP Determinations

ATP (adenosine 5'-triphosphate) has been successfully used as a monitor of viable microbial biomass in both anaerobic digesters (Callander and Barford, 1980) and the rumen (Forsberg and Lam, 1977; Wallace and West, 1982). The firefly-luciferase assay of ATP is the most commonly adopted assay procedure, as the method is both rapid and extemely sensitive. Although other ATP extraction procedures have been described (Fosberg and Lam, 1977; Callander and Barford, 1980), more consistent results were

obtained in this study with an extraction procedure based on that used by Wallace and West (1982). ATP extracts were prepared by rapidly removing pairs of rings from the anaerobic filter (so as to minimise any effect from oxygen) and submerging each in 100ml ice-cold 0.25M sulphuric acid. The rings were stirred occasionally, removed after five minutes, and a small portion of the acidified extracts centrifuged at full speed for ten minutes at 4°C in a Micro Centaur (MSE Ltd., UK). 0.4 ml portions of the supernatant were then neutralised with the addition of 0.2ml 1M NaOH, 0.4ml 1M Tris-HCl pH 7.8, 0.2ml buffer (100mM Tris-acetate pH 7.8, 30mM $MgCl_2$, 2mM EDTA (Ethylenediaminetetra-acetic acid)) and 0.8ml deionised water. The final pH was 7.5-8.0. The ATP concentration of the extracts was determined immediately. 200µl of tested extract was added to a luminometer assay tube, and placed in a Picolite Luminometer (Packard Instruments Ltd., Caversham, UK) which was maintained at 20°C. 40µl ice-cold ATP monitoring reagent (luciferase - LKB Instruments, Crawley, UK) was then added and after a five second delay, the light emitted was counted for 30 seconds. Calibrations were made with a standard of 10^{-6} M ATP (in the buffer described above) which was serially diluted in the same buffer (minus $MgCl_2$). A background containing the buffer alone (minus $MgCl_2$) was subtracted in each case.

Table 2.1 Summary of Cows' Diet

Date of Slurry Arrival	Batch	Composition of Diet		
		%Maize silage	%Straw	%Conc+
December 1982	A	62	4	34
October 1983	B	73	5	22
June 1984	C*	67	5.5	27.5

* for 62 cows only; this was supplied mixed with slurry from a further 36 cows fed grass silage and concentrates ad lib.

+ the concentrates comprised (per 1016.5 kg): Barley 700kg, Wheat 80kg, Soya 210kg, High phosphorus minerals 9kg, Beta 103 1kg, Beta 101 0.5kg, Beta 107 5kg and Limestone 11kg.

Table 2.2 Summary of Digesters Applications

(1) 5-litre Daily Fed Digesters

Temperature shock experiments (Chapter 5)

Anaerobic filter study (Chapter 6)

(2) 10-litre Daily Fed Digesters

Effect of separation on the digestion process (Chapter 3)

(3) Batch Digester Operation

(a) 5-litre reactors

- comparison of ultimate degradability of WCS and SCS (Chapter 3)
- comparison of ultimate degradability of sieved WCS fractions (Chapter 4)

(b) 2-litre reactors

- comparison of ultimate degradability of WCS & SCS (Chapter 3)

(4) Anaerobic Filter

Anaerobic filter study (Chapter 6)

Table 2.3 Summary of Contents of Batch Digesters Prior to Digestion

Chapter 3.4.1 Comparison of Ultimate Digestion of WCS and SCS from batch A Slurry

Digester Designation	SCS	WCS	Control
WCS added (l)	0	1.0	0
SCS added (l)	1.0	0	0
Seed added (l)	0.5	0.5	1.5
Total volume (l)	1.5	1.5	1.5
Volume removed for analysis (l)	0.1	0.1	0.1
Final volume (l)	1.4	1.4	1.4

NB seed was from SCS digester at 25 day RT and 35°C

Chapter 3.4.2 Comparison of Ultimate Digestion of WCS at Three Solids Concentrations

Digester Designation	1	2	3	Control
WCS added (l)	1.5	1.0	0.5	0
Seed added (l)	0.2	0.2	0.2	0.2
<15μm sieved fraction added (l)	0	0.5	1.0	1.5
Total volume (l)	1.7	1.7	1.7	1.7
Volume Removed for Analysis (l)	0.2	0.2	0.2	0.2
Final volume (l)	1.5	1.5	1.5	1.5

NB seed was from SCS digester at 25 day RT and 35°C

Chapter 3.4.3 Comparison of Ultimate Digestion of WCS and SCS at Similar Solids Concentration

Digester Designation	SCS	WCS	Control
WCS added (l)	0	0.87	0
SCS added (l)	1.39	0	0
Seed added (l)	0.5	0.5	0.5
Deionised water added (l)	2.11	2.63	3.5
Total volume (l)	4.0	4.0	4.0
Volume removed for analysis (l)	0.1	0.1	0.1
Final volume (l)	3.9	3.9	3.9

NB seed was from WCS and SCS digesters (equal volumes) at 25 day RT and 35°C

CONTINUED OVER

Table 2.3 continued

Chapter 4.3 Comparison of Small Variations in Solid Addition on the Ultimate Anaerobic Digestion of Sieved WCS Solids

Digester Designation	A	B	C	D	E	Control
Solids added (g)	300	300	600	900	900	0
Seed added (l)	0.5	0.5	0.5	0.5	0.5	0.5
Deionised water added(l)	0.6	0.6	0.3	0	0	0.9
<15μm size fraction (l)	2.7	2.7	2.7	2.7	2.7	2.7
Total volume (l)	4.1	4.1	4.1	4.1	4.1	4.1
Removed for analysis (l)	0.1	0.1	0.1	0.1	0.1	0.1
Final volume (l)	4.0	4.0	4.0	4.0	4.0	4.0

NB solids were mixed sieved fractions >15μm (as wet weight) seed was a mixture of WCS/SCS (equal volumes) from 25 day RT and 35°C

CONTINUED OVER

Table 2.3 (continued)

Chapter 4.4 Comparison of the Ultimate Degradability of Sieved Fractions of WCS

Digester Designation / ADDITION	>1700 µm	1000-1700µm	710-1000µm	300-710µm	15-300µm	<15µm	RCS	Control
Solids >1700µm (g)	1076	0	0	0	0	0	221	0
Solids 1000-1700µm (g)	0	817	0	0	0	0	133	0
Solids 710-1000µm (g)	0	0	827	0	0	0	79	0
Solids 300-710µm (g)	0	0	0	877	0	0	236	0
Solids 63-300µm (g)	0	0	0	0	482	0	131	0
Solids 15-63µm (g)	0	0	0	0	191	0	50	0
<15µm size fraction (l)	2.52	2.78	2.77	2.72	3.42	3.60	2.75	0
Seed added (l)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	4.1
Total Volume (l)	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1
Removed for analysis (l)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Final volume (l)	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0

NB Sieved solids were added as wet weight.
Seed was from SCS digester at 25 day RT and 35°C.
RCS - reconstituted whole cattle slurry.

Fig. 2.1 Sketch of Five-Litre Conventional Digester

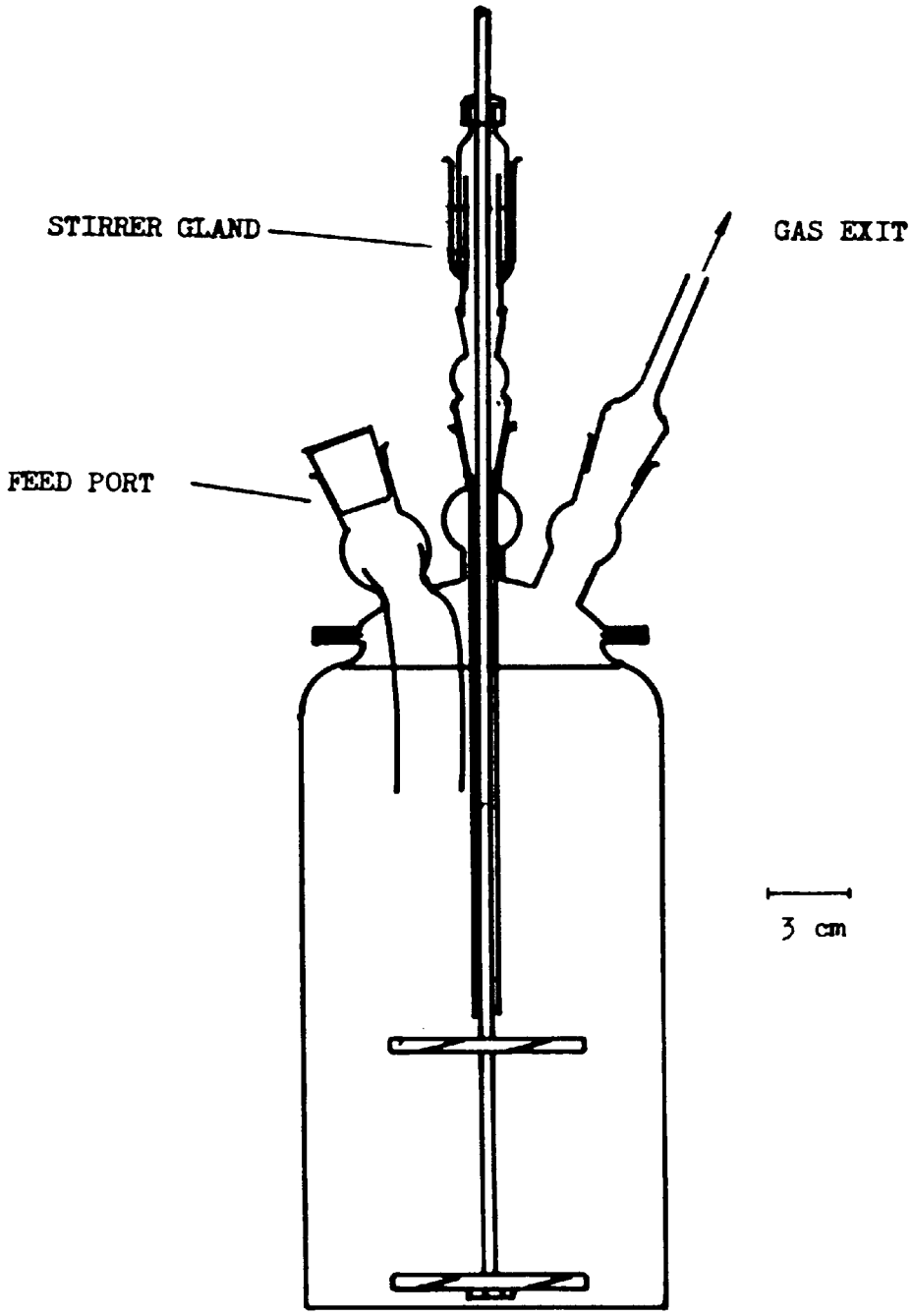


Fig. 2.2 Sketch of Ten-Litre Continuously Stirred Digester

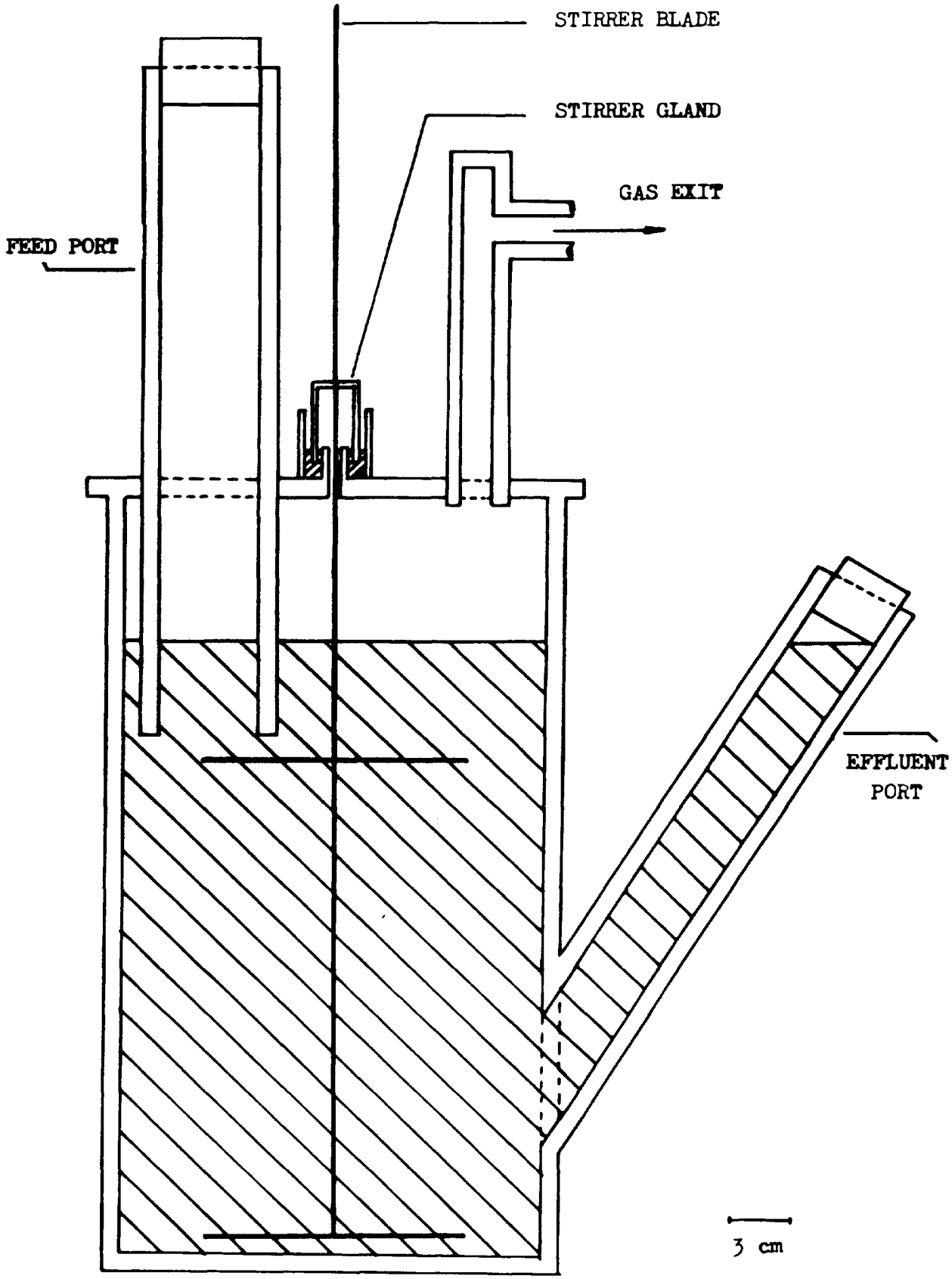


Fig. 2.3 Sketch of the Upflow Anaerobic Filter

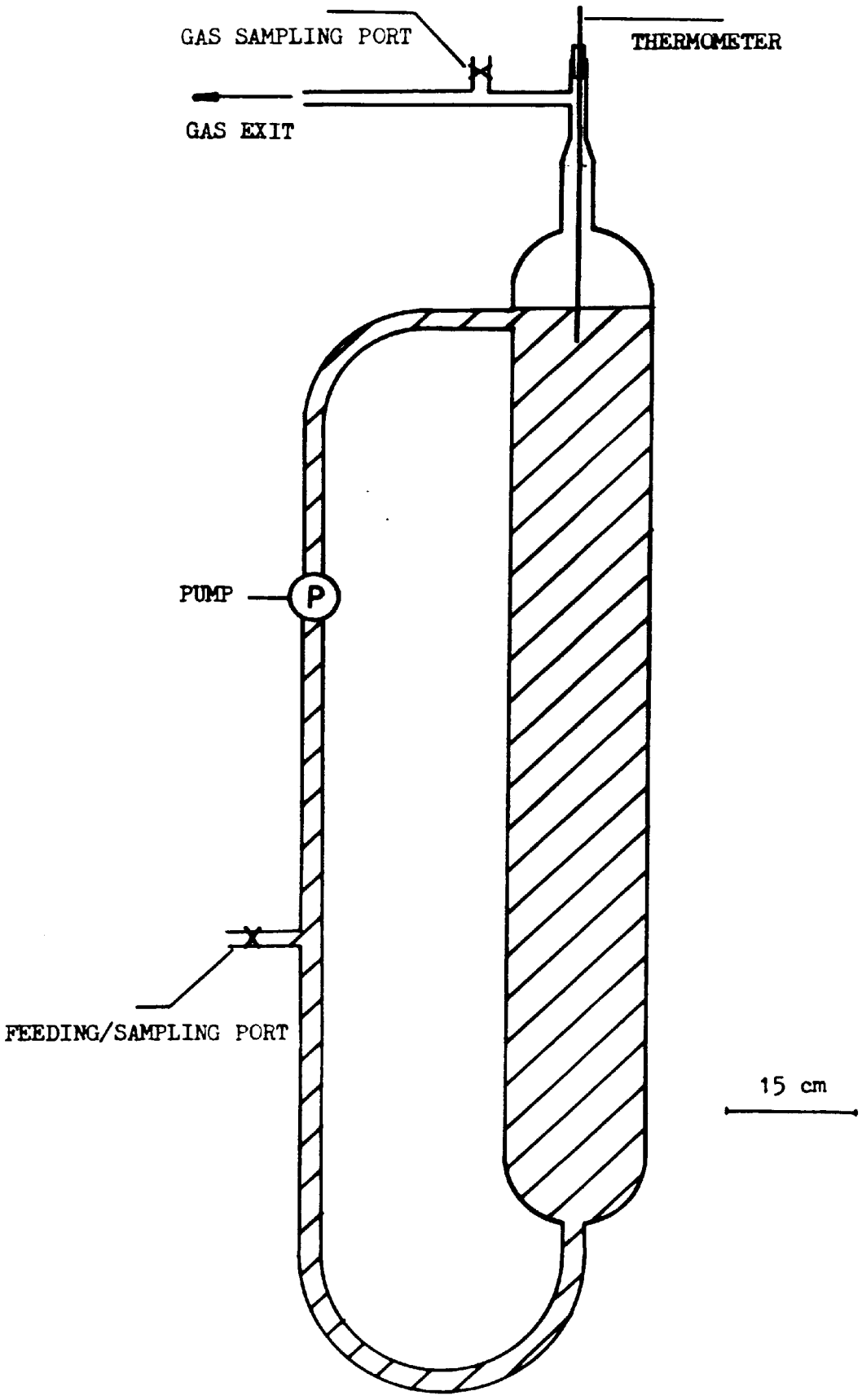
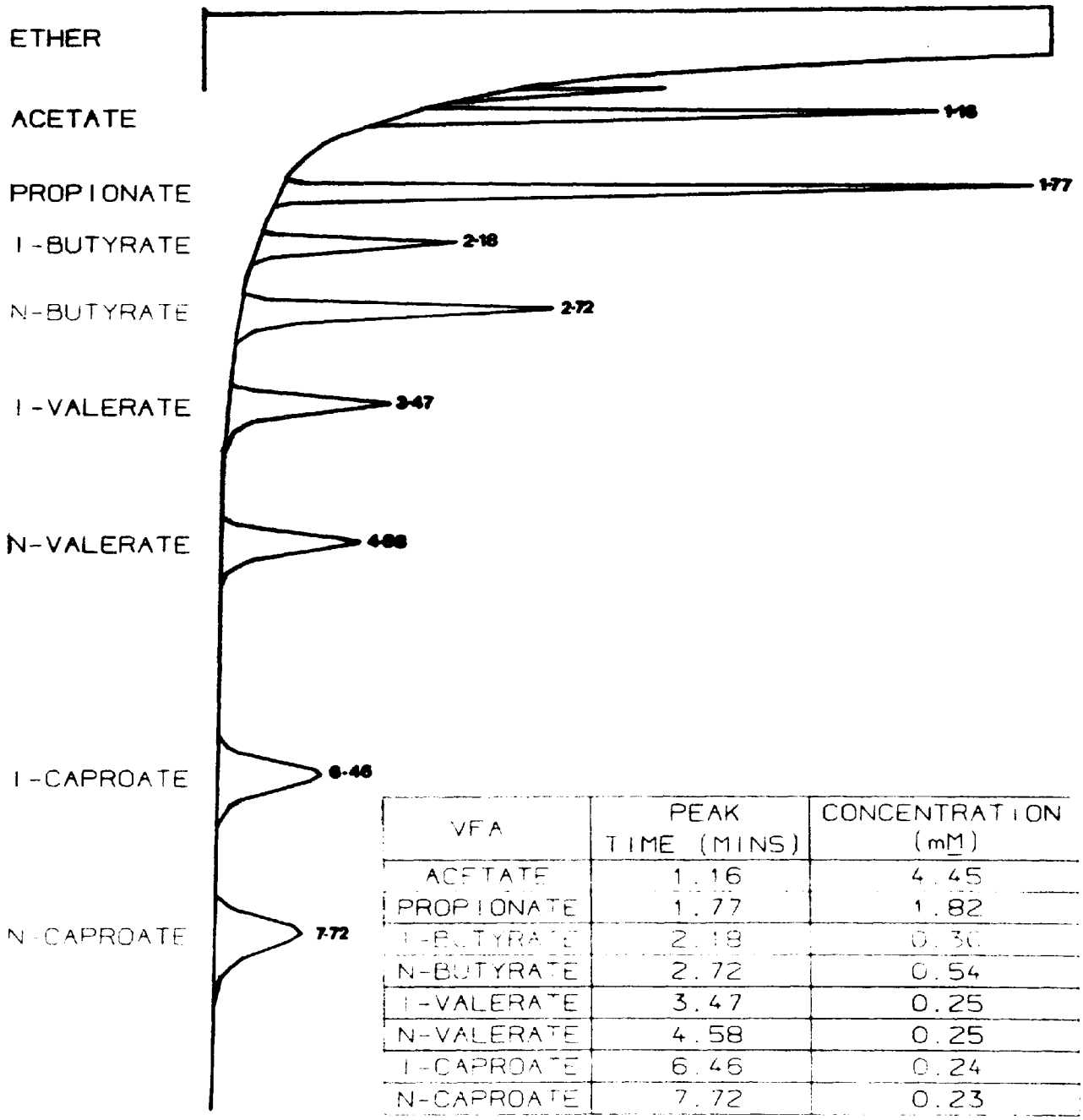


Fig. 2.4 Typical Separation of Volatile Fatty Acids by Gas Chromatography



VFA	PEAK TIME (MINS)	CONCENTRATION (mM)
ACETATE	1.16	4.45
PROPIONATE	1.77	1.82
I-BUTYRATE	2.18	0.30
N-BUTYRATE	2.72	0.54
I-VALERATE	3.47	0.25
N-VALERATE	4.58	0.25
I-CAPROATE	6.46	0.24
N-CAPROATE	7.72	0.23

CHAPTER THREE

COMPARISON OF THE ANAEROBIC DIGESTION OF WHOLE AND SEPARATED CATTLE SLURRY

3.1 INTRODUCTION

Previous studies have indicated that higher gas yields (per VS added) and a higher volume of methane (per m^3 digester) may be obtained from the mesophilic anaerobic digestion of SCS than WCS (see Table 1.1). This would allow smaller digesters and shorter retention times to be employed, and hence make anaerobic digestion more economically viable. However, such studies have, to a large extent, failed to provide an explanation for this enhancement of digestion.

In this study an attempt has been made to characterize WCS and SCS, and examine the mesophilic anaerobic digestion of the slurries. Characteristics of WCS and SCS examined include the solids concentration, particle size distribution and composition (Chapters 3.2 and 3.3). Studies of the anaerobic digestion of WCS and SCS (Chapter 3.4 and 3.5) have been conducted at an infinite retention time (in batch reactors) and at a 25 day and 10 day RT. In addition to confirming that higher methane and gas yields were obtained from SCS than WCS, an explanation of the enhancement in the efficiency of digestion was also sought.

3.2 THE PARTICLE SIZE DISTRIBUTION OF WCS AND SCS

The particle size distribution of WCS and SCS (from batch B - section 2.1) was determined by applying

approximately 20g dry weight of slurry to a series of standard sieves according to the procedure outlined in section 2.3.12. Only 14% total solids of WCS were retained by the largest sieve (nominal aperture 1700μm), but it is anticipated that a larger proportion might have been retained if the cattle had been bedded on straw rather than sawdust. The largest proportion of WCS solids were found in the <15μm size fraction which passed through all the sieves (Fig. 3.1). A significant proportion of the solids from WCS have been found to pass through a sieve of this magnitude in previous studies (Chang and Rible, 1975; Pain et al., 1978). The solids retained by the 63μm and larger sieves were chiefly composed of partially degraded plant material, whilst the solids passing through this sieve were of a mud-like consistancy. The 63-300μm and 300-710μm size fractions included significant quantities of sand, which presumably originated from the effects of scraping concrete floors.

The separator removed 39% of the solids from WCS, and solids were removed from all size fractions, although a greater proportion were lost from the larger size fractions (indeed a third of solids lost were from the largest size fraction). Eighty-five percent of the solids retained by the 1700μm sieve in WCS were removed by the separator, compared with 34-52% of the solids of particles sized between 63-1700μm, and only 9-14% solids less than 63μm in size (Fig. 3.1). Thus, separation brought about a significant reduction in the size of particles within the

slurry. For example, the geometric mean diameter of SCS solids was only 80μm, compared with 316μm for the solids of WCS.

SUMMARY

1. The particle size distribution of WCS and SCS from batch B was determined, and the largest percentage of solids were located in the <15 μm size fraction for both slurries.
2. 39% total solids in WCS were removed by separation, chiefly from the larger size fractions. Thus, separation brought about a reduction in the mean particle size.

3.3 THE COMPOSITION OF WHOLE CATTLE SLURRY AND SEPARATED CATTLE SLURRY

3.3.1 The Proximate Constituents

The initial composition of WCS and SCS have been determined as gross or proximate constituents. Seven proximate constituents have been assayed for in this study (lipids, crude protein, VFA, α-cellulose, hemicellulose, lignin and ash) according to procedures outlined in section 2.3.

VFA have been included as a proximate constituent, as it is believed that they may be retained when slurry samples are dried in the microwave oven. Evidence for this

will now be considered.

VOLATILE FATTY ACID VOLATILISATION DURING TOTAL SOLID DETERMINATIONS

In a majority of previous studies, total solids (TS) determinations have been carried out by drying samples overnight at 105°C. However, an alternative procedure has been adopted in this study with samples being dried in a microwave oven. Previously, approximately 90% VFA have been reported to be lost during conventional total solids determinations (van Velsen, 1977). To determine whether VFA were also volatilised in the microwave oven, additions of glacial acetic acid (the predominant acid in most anaerobic digesters) were made to 25 ml SCS (mean %TS = 3.20, mean original VFA concentration = 48.5m<u>M</u> (2,910 ppm)). Each sample was directly compared with a control to which no addition of acetate was made, and the results were disregarded if the control solids concentration varied by more than 3% from the mean.

The proportion of acetate retained as total solids increased as the acetate addition fell. For example, when included at 1.59<u>M</u> only 7% of acetate added initially was retained upon drying, compared with 17% when added at 0.44<u>M</u>, and 80% at 33m<u>M</u> (Fig. 3.2a). However, the weight of acetate retained did increase with addition (Fig. 3.2b), when acetate was added at 33m<u>M</u> approximately 27m<u>M</u> was retained as total solids, compared with 45m<u>M</u> when added at

200mM, and 111mM when 1.59M acetate was added. With acetate additions of less than 0.67M, an equation of:

$$y = 0.115x + 0.021$$

where: y = acetate retained (M)

x = acetate added (M)

may be used to represent this relationship (Fig. 3.2b). The volatilisation of acetate was uneffected by allowing samples (to which acetate had been added) to stand for one hour at room temperature prior to TS determinations, or by neutralising the acidified sample with sodium hydroxide.

When 1.09g and 9.31g glacial acetic acid were diluted into a final volume of 25ml with deionised water (final concentrations 0.73M and 6.2M respectively), all was volatilized after two hours in the microwave oven. However, acetate was never completely volatilized when added to SCS and dried in a similar manner. It is probable therefore that some of the added acetate became bound to, or associated with SCS in some manner and not released upon drying. The initial VFA concentration of SCS was 48.5mM, so with an addition of 30.8mM acetate (the lowest addition made), the final concentration was 79.3mM. In this case 80% of the added acetate was retained, and it is likely that a majority of the original 48.5mM may also have been retained. The VFA concentrations encountered in these studies were frequently below 79.3mM, and so it may be assumed that a majority of the VFA were retained as TS upon drying.

The retention of VFA (at concentrations <80mM) in the TS assay, when conducted in the microwave oven rather than the conventional oven therefore represents an important difference between the two procedures. Furthermore, it may account for the apparently higher values obtained in the microwave oven when identical samples have been compared by the two procedures (Webb, 1984).

To ascertain whether VFA were extracted by the total lipid assay, glacial acetic acid was added at 203mM to four samples of SCS and compared with four samples to which no addition was made. The mean lipid content of SCS alone was $4.36gl^{-1}$ and $4.34gl^{-1}$ with acetate addition. Thus none of the acetate was retained in the total lipid assay (an increase of $12.2gl^{-1}$ would have been anticipated if all of the added acetate had been retained).

VFA are therefore likely to be retained in the total solids assay when conducted in the microwave oven and since they were not extracted by the total lipid assay, have been included as a separate proximate constituent in this study.

3.3.2 The Composition of WCS and SCS from Batch A

The holocellulose, ash and VFA content of the slurries were determined by the procedures described in section 2.3, and the sum of the other proximate constituents obtained by subtraction (Table 3.1). Over half of the WCS solids were composed of holocellulose (α-cellulose and

hemicellulose). The separator removed 51% TS from WCS, and 75% of this was holocellulose (Table 3.1). Holocellulose therefore represented a smaller proportion of the solids in SCS than in WCS. A more thorough examination of the proximate constituents was carried out with slurry from batch B.

3.3.3 The Composition of WCS and SCS from Batch B

Lipids, VFA and crude protein were primarily located in the <15μm size fraction (see Chapter 4), and were not removed to any great extent by the separator (Table 3.2). WCS and SCS therefore contained approximately equal quantities (in terms of gl^{-1}) of these three constituents. However, in WCS they represented 17% TS compared with 27% TS in SCS (Table 3.2). The values obtained for WCS compare relatively well with those obtained in previous studies (Table 1.2), in which lipids have been found to represent 2-4% TS of beef and dairy cattle slurry, VFA 1-8% TS, and crude protein 13-24%. The discrepancy between the crude protein concentration in this and previous studies may be attributed to the inclusion of ammonia as protein in previous studies. In both slurries it was found that hemicellulose and α-cellulose represented a substantial proportion of the total solids and hemicellulose was slightly more abundant than α-cellulose (Table 3.2). WCS contained 35.8gl^{-1} holocellulose (which represented 44% total solids) and SCS 16.5gl^{-1} (33% total solids). These

values agree well with those obtained in previous studies of the composition of beef and dairy cattle slurry (Table 1.2). Mechanical separation removed a significant quantity of holocellulose from WCS and it accounted for 60% total solids lost during separation. The large removal of holocellulose is attributed to the separation process chiefly removing material from the larger particle size fractions and the material in these size fractions primarily consisting of α-cellulose and hemicellulose (Chapter 4).

37% of the lignin and 40% of the ash were removed by the separator, compared with 39% of the total solids. The closeness of these values ensured that lignin and ash represented a similar proportion of the solids in both slurries, 20-21% total solids were lignin and 19% ash. The diminished removal of lignin and ash compared to holocellulose is attributed to them both being relatively evenly distributed throughout all the size fractions and not chiefly located in the larger size fractions (see Chapter 4). Lignin and ash have been found to represent a similar proportion of the total solids in previous determinations of the proximate constituents of beef and dairy cattle slurry (Table 1.2).

The C:N ratio of the waste was lowered from 16:1 to 10:1 by separation and the available (non-lignin) C:N ratio fell from 11:1 to 7:1 (Table 3.2). Thus, separation may be thought to have a deleterious effect on the digestion process, since the ratio was further reduced from the optimal range of 25-35:1 reported for dairy cattle slurry

(Hills, 1979; Hills and Roberts, 1981; Robbins et al., 1983). However, it is doubted that the excess of nitrogen will have a harmful effect on digestion in this study. Instead, it represents an under-utilisation of the potential of the process, in that this C:N ratio will support a much greater solids destruction.

Differences were apparent in the composition of the two batches of WCS and SCS (Tables 3.1 and 3.2), despite both coming from the same source (indeed even greater variations have been observed previously (Hawkes et al., 1984)). This may be partly attributed to variations in the cattle diet (see Table 2.1), the manner of slurry collection and a number of other factors (see section 1.2.1).

SUMMARY

1. Glacial acetic acid was not volatilised when included at typical digester concentrations in slurry samples which were dried in the microwave oven, nor was it extracted by the total lipid assay. The VFA have therefore been included as a separate proximate constituent.

2. Seven proximate constituents were assayed for: lipids, crude protein, VFA, α-cellulose, hemicellulose, lignin and ash. Together they accounted for 100-105% total solids.

3. Holocellulose (α-cellulose and hemicellulose) was the most abundant of the proximate constituents (33-57% total solids) in all the slurries tested.

4. The separator chiefly removed holocellulose from WCS;

ash and lignin were lost to a lesser extent; and lipids, crude protein and VFA were virtually uneffected.

5. Compositional differences were found between the two batches of slurry. Slurry from batch A contained more holocellulose and less ash than batch B.

3.4 COMPARISON OF THE ANAEROBIC DIGESTION OF WCS AND SCS IN BATCH REACTORS

Batch reactors have been used to determine the maximum or ultimate degradability of dairy cattle slurry. Comparisons of the ultimate anaerobic digestion of WCS and SCS have been carried out at 35°C with two consignments of slurry received from The Animal and Grassland Research Institute.

3.4.1 Comparison of the Anaerobic Digestion of WCS and SCS from Batch A

The anaerobic digestion of SCS and WCS from batch A of slurry (composition, see Table 3.1), has been examined in paired two-litre batch reactors at 35°C. The initial composition and operation of the reactors has been described in section 2.2.3. Biogas production ceased after 46 days, and an average of 13.1 litres of biogas was yielded from SCS and 23.7 l from WCS (Table 3.3). The larger volume

of biogas produced from WCS is attributed to the larger solids addition. However, the rate of biogas production was proportionately slower from WCS than SCS. By day six, 50% total biogas from SCS had been liberated, whilst nine days were required for a similar proportion to be yielded from WCS (Fig. 3.3). Possible explanations for the slower rate of biogas production from WCS are considered in the following sections. The biogas liberated from SCS was slightly richer in methane than that from WCS (Table 3.3) and this may be attributed to the different composition of the two slurries (Table 3.1).

Despite the greater volume of biogas produced from the ultimate digestion of one litre of WCS compared with one litre of SCS, a higher gas yield was obtained from SCS (0.41 m^3 kgVS added^{-1}; methane yield 0.26 m^3 kgVS added^{-1}), than WCS (0.34 m^3 kgVS added^{-1}; methane yield 0.21 m^3 kgVS added^{-1}). Thus separation improved the efficiency of digestion. 42% of the volatile solids from the SCS were destroyed compared with only 38% volatile solids of WCS (Table 3.3). A significant proportion of the volatile solids were therefore recalcitrant to degradation and approximately half of this was holocellulose.

3.4.2 Comparison of the Anaerobic Digestion of WCS at Three Solids Concentrations

The variations in the ultimate anaerobic digestion of WCS and SCS observed above may be due to the different solids concentration of the two slurries. To determine the effect of solids concentration on the ultimate gas and methane yields and the rate and composition of biogas produced, the anaerobic digestion of WCS (from batch B - composition given in Table 3.2) was investigated at three solids concentrations in two litre batch reactors. The operation of these reactors has been described in section 2.2.3.

Biogas was produced at different rates from the three digesters despite each containing the same substrate. Biogas liberation from digester one (86.4g VS added) peaked on days 22 and 37, whilst production from digester two (54.4g VS added) peaked on days 17, 22 and 30 and digester three (29.3g VS added) peaked on day 10 (Fig. 3.4). Although the gas meter attached to digester one stopped on day 40, it can be calculated that (providing the gas yield was the same as for digesters two and three) only 57% total biogas had been produced from digester one by day 40, compared with 91% from digester two, and 99% total biogas from digester three. Thus, the period required for ultimate anaerobic digestion increased with solids addition. It is likely that when larger solid additions were employed, digestion became inhibited, perhaps as a consequence of a

raised VFA concentration. This may be due either to VFA formation in the digester, or to the initially higher VFA concentrations (Table 3.4).

The biogas composition in the digester headspace varied during digestion (Fig. 3.4), but similar mean values (64.9 - 65.3% methane, see Table 3.4) were obtained from the three digesters. The periods of greatest biogas production were frequently accompanied by an increase in the methane content of the biogas (Fig. 3.4), and are due to an increase in activity of the methanogenic bacteria.

The gas yields and solids destruction obtained from WCS were uneffected by the solids addition despite the different rates of biogas production (Table 3.4). Mean gas yields of 0.50 m^3 kgVS added^{-1} (methane yield 0.33 m^3 kgVS added^{-1}) and 1.02 m^3 kgVS destroyed^{-1} were obtained, and 50% of the volatile solids were destroyed (Table 3.4). The different solids concentration of WCS and SCS tested in the previous experiment is therefore unlikely to have effected the ultimate degradability and ultimate gas yields. However, the slower liberation of biogas from WCS (Fig. 3.3) may well be at least partly attributed to the higher solids concentration of WCS.

3.4.3 Comparison of the Anaerobic Digestion of WCS and SCS at Similar Solids Concentrations

To negate the effect of the solids concentration on

the digestion process observed in sections 3.4.1 and 3.4.2, the ultimate digestion of a similar weight of WCS and SCS solids (approximately 60g VS per 4l) was examined in paired batch reactors. Variations in the rate of biogas production must therefore now be attributed to differences in composition, particle size or other factors. Batch reactors of 4l final volume were set up and operated as described in section 2.2.3.

Biogas production from the pair of digesters operated on SCS was initially a little more rapid than from the pair of digesters in which WCS was the provided substrate (Fig. 3.5), and by day 10, 54% of the total biogas had been liberated from SCS, and 50% of the total biogas from WCS. The large differences previously observed in the rate of biogas production between WCS and SCS (Fig. 3.3), are therefore chiefly attributed to variations in the concentration of solid material tested. The biogas in the digester headspace was richer in methane when SCS was employed as the substrate compared with WCS (Fig. 3.5), and the highest methane concentration recorded from SCS was 67.4% (day 21) and 62.0% from WCS (day 19).

A slightly larger proportion of the volatile solids of WCS were destroyed compared with SCS, although approximately half of the solids were recalcitrant in each case (Table 3.5). However, as a consequence of the slightly greater biogas yield per solids destroyed for SCS, identical gas yields of 0.46 m^3 kgVS $added^{-1}$ were obtained from the

two slurries (Table 3.5). It should be remembered that the higher solids content of WCS will ensure that more biogas and methane will be liberated from WCS per volume of slurry.

3.4.4 Discussion of Batch Reactor Tests

The ultimate methane yield, total biogas yield and solids destruction obtained from the anaerobic digestion of WCS and SCS were uneffected by the concentration of solids added. However, larger solid additions brought about a slower rate of biogas production. The proportionately faster rate of biogas production from one litre of SCS than one litre of WCS (Fig. 3.3) was chiefly attributed to differences in the concentration of solids tested, since a comparison at similar solids concentrations (ie. same weight of slurry rather than the same volume) showed biogas to be liberated only slightly more rapidly from SCS than WCS (Fig. 3.5). In these circumstances, therefore the more rapid degradation of SCS must be due to other factors, such as particle size or composition. The compositional changes brought about by mechanical separation also effected an increase in the quality of biogas. WCS contained more holocellulose than SCS (Tables 3.1 and 3.2) and when degraded this yielded a gas poorer in methane than did the destruction of other proximate constituents (see Table 1.4).

Small differences in composition between the two batches of dairy cattle slurry (Tables 3.1 and 3.2) may be

responsible for the ~~three~~ slightly higher ultimate methane and gas yields (per VS added) produced from batch B of slurry than batch A. Particle size and the solids concentration were considered to have no effect on the ultimate degradability of dairy cattle slurry (section 1.2.1). A further consequence of the different composition of the two batches of slurry was that separation had a different effect on their digestion. Higher methane and gas yields (per VS added) were produced from the ultimate anaerobic digestion of SCS than WCS, from batch A of slurry. Thus, mechanical separation improved the efficiency of digestion. However, with slurry from batch B, similar ultimate gas yields (per VS added) were produced from SCS and WCS, although a slightly higher ultimate methane yield (per VS added) was obtained from SCS.

SUMMARY

1. The ultimate digestibility of dairy cattle slurry was independent of the concentration of slurry tested. However, large additions of solids led to a reduction in the rate of biogas production.

2. The proportionately faster rate of biogas production from SCS than WCS was chiefly attributed to its lower solids concentration. However, other factors (eg. composition, particle size) play a lesser role.

3. The biogas liberated from SCS contained 2-5% more methane than that evolved from WCS.

4. Higher ultimate methane and biogas yields (per VS added) were produced with slurry from batch B than from batch A.

5. Higher ultimate methane and gas yields (per VS added) were obtained from SCS than WCS from batch A of slurry. Thus, mechanical separation improved the efficiency of digestion. However, although a slightly higher ultimate methane yield (per VS added) was produced from SCS than WCS from batch B of slurry, similar ultimate gas yields (per VS added) were obtained.

6. Approximately half of the volatile solids were recalcitrant to digestion in all circumstances. Mechanical separation did not increase solids degradation from batch B of slurry, although a small enhancement was noted with batch A.

3.5 COMPARISON OF THE ANAEROBIC DIGESTION OF WHOLE CATTLE SLURRY AND SEPARATED CATTLE SLURRY AT A 10 DAY AND 25 DAY RT

At an infinite retention time, more methane and total biogas were liberated from WCS than an equal volume of SCS (with batch B of slurry), although a similar total biogas yield (per VS added) and a slightly lower methane yield (per VS added) were obtained. At finite retention times however, the proportionately slower rate of biogas production from WCS (due to inhibition (Figs. 3.3 and 3.4) and other effects (Fig. 3.5)) became an increasingly important factor. Higher

gas yields (per VS added) were now produced from SCS, and as the retention time was shortened more methane and total biogas were eventually liberated from SCS than from an equal volume of WCS (see Table 1.1).

The semicontinuous anaerobic digestion of WCS and SCS (from batch B of slurry) was studied in two identical ten-litre reactors (Fig. 2.2), operated at 35°C. Each digester was stirred continuously at 120 rpm to alleviate the problems of scum formation, and operated according to the procedure described in section 2.2.2. Approximately two retention times were allowed in all cases before results were taken, and this proved to be sufficient to enable steady-state conditions to be established. Results were taken over a period of at least two retention times, during which there was no significant rise or fall in biogas production, VFA concentration or any other measured parameter. The digesters were initially operated at a 25 day RT for 43 days and results taken over a further 91 days. A 10 day RT was then employed and, after allowing 17 days for the digester to stabilize, results were taken during a further 24 days of operation.

3.5.1 Gas Yields/Solids Destruction

In the periods during which the digester was taken to be at steady state, there was little weekly variation and no gradual increase or decrease in biogas production. Gas

yields of 0.14 m^3 kgVS added^{-1} (methane yield 0.08 m^3 kgVS added^{-1}) and 0.27 m^3 kgVS added^{-1} (methane yield 0.16 m^3 kgVS added^{-1}) were obtained with WCS at retention times of 10 days and 25 days respectively (Table 3.6). These gas yields fall within the range of those reported previously for whole dairy cattle slurry (Table 1.1).

Higher gas yields were obtained from SCS than WCS at both retention times employed (Table 3.6). A total biogas yield of 0.23 m^3 kgVS added^{-1} (methane yield 0.15 m^3 kgVS added^{-1}) was produced at a 10 day RT, and 0.33 m^3kgVSadded^{-1} (methane yield 0.21m^3 kgVS added^{-1}) at a 25 day RT (Table 3.6). These yields are comparable with others obtained in this (Chapter 5) and previous studies (Table 1.1). Shortening the retention time led to a greater fall in the methane and total biogas yield (per VS added) from WCS than SCS (Table 3.6). The gas yield (per VS added) from WCS fell to 59% ultimate yield (see Table 3.5) at a 25 day RT and to only 30% ultimate yield at a 10 day RT, whereas with SCS the total biogas yield fell to 72% ultimate yield (see Table 3.5) at a 25 day RT and 50% at a 10 day RT. A similar pattern was also observed for the methane yields (Table 3.6).

At an infinite retention time (Fig. 3.3) and a 25 day RT, more methane and total biogas were liberated (per litre of digester) from WCS than SCS (Table 3.6). However, the fall in gas production from WCS as the retention time was shortened, was so great that at a 10 day RT 9% more methane and 1% more total biogas were liberated from each litre of

SCS compared with WCS (Table 3.6). More methane was also liberated per litre of SCS digester than WCS digester at retention times of less than 16 days in previous studies (Table 1.1). The biogas evolved from SCS was richer in methane than that produced from WCS at both retention times employed (Table 3.6). This confirms observations made in batch studies (Table 3.3 and Fig. 3.5).

The gas yield per VS added is dependent upon the biodegradability of the waste (that is the percentage of VS destroyed) and the amount of biogas yielded when the solids are destroyed (m^3 kgVS destroyed^{-1}). In this study the volatile solids of WCS were more degradable than those of SCS, for example at a 25 day RT, 37% VS of WCS were degraded compared with only 29% VS of SCS (Table 3.6). Therefore, although separation has increased the efficiency of digestion, the digestibility of the slurry (ie. %VS destroyed) has not been improved. However, the volume of biogas yielded per mass of substrate destroyed was much greater for SCS than WCS. Yields of 1.10-1.12 m^3 kgVS destroyed^{-1} were recorded for SCS and 0.61-0.70 m^3 kgVS destroyed^{-1} for WCS (Table 3.6). The greater efficiency of digestion after separation was therefore largely due to the fact that the solids that were degraded yielded more biogas. The possible effect of three differences in slurry characteristics on the digestion process will now be considered.

3.5.2 Effect of Total Solids Concentration on the Digestion Process

It is anticipated that an overloading of digestion may lead to the accumulation of toxic products (eg. ammonia, VFA), and hence to the inhibition of digestion and perhaps failure. However, although the daily destruction of solids was greater when WCS was provided as the substrate rather than SCS (Table 3.7 - presumably as a consequence of the initially higher solids content of WCS), this did not result in digester failure but only in unbalanced digestion. It is doubted that the accumulation of ammonium ions proved toxic in this study, as the concentration only reached 1842 ppm (Table 3.8); although such problems have been encountered previously when cattle have been fed a high protein diet (Zeeman et al., 1983a,b). When operated at a 25 day RT low VFA concentrations were found in both the WCS (1.29mM) and SCS (0.66mM) digesters (Table 3.9). Acetate was the predominant acid (0.58-0.90mM), the concentration of propionate was low (0.05-0.36mM), and the larger acids were only present at trace concentrations. These values are typical of those found in well balanced digesters (see Chapter 5). Once steady-state conditions were established at a 10 day RT, it was apparent that the total VFA concentrations were higher than those found previously at a 25 day RT (Table 3.9), propionate was now the predominant acid (3.47-7.63mM) rather than acetate (1.90-4.18mM), and this, along with the rise in the concentration of

branched acids may be taken as evidence of overloaded digestion (see Chapter 5). The greatly elevated concentration of propionate (7.63mM) at a 10 day RT (where it exceeded the concentration in the feed - 6.56mM) is attributed to a rise in its rate of formation (as a consequence of increased polymer hydrolysis) exceeding its rate of degradation. The failure of the obligate proton-reducing bacteria to degrade propionate and longer straight chain VFA may be attributed to inhibition by molecular hydrogen, although other possibilites are considered in Chapters 5 and 7. The sensitivity of the branched acids to the drop in retention time (Table 3.9) is probably due to their increased formation and recalcitrance to digestion. The different response of the individual fatty acids to digester stress has also been observed in temperature shocked (Chapter 5) and overloaded digesters (Chapter 6) and is considered further in Chapter 7.2.

The raised VFA concentration may have two effects on the digestion process. Firstly, very high concentrations may inhibit the digester bacteria and bring about a souring of digestion. Indeed, that digesters have been successfully operated on SCS but not WCS at very short retention times (eg. Liao et al., 1984) may, in some circumstances, be attributed to an overloading of digestion. Although digestion did not fail in this study, it is likely that the high VFA concentations reached in the WCS digester at a 10 day RT exerted an inhibitory effect on the digestion process, and that this contributed to the lower production

of methane and total biogas (per m^3 digester) from WCS than SCS. It is, however, difficult to quantify the extent of VFA inhibition on the digestion process. A second effect of the raised VFA concentration is that the VFA lost in the effluent represent the loss of a potential source of methane. Approximately 1.3 litres more biogas per day (15% rise) would have been produced from the WCS digester at a 10 day RT if the VFA had been completely degraded, whilst a further 0.5 litres per day (6% rise) would have been evolved from SCS. The problems of digester overloading have been relieved by slurry dilution, and significant increases in the gas yield (per VS added) have been reported (Lo et al., 1983; Liao et al., 1984; Liao & Lo, 1985).

3.5.3 Effect of Compositional Differences on the Digestion Process

The composition of solid material destroyed during digestion is likely to effect the digestion process (see Table 3.10). Two of the proximate constituents, ash and lignin, were virtually recalcitrant to degradation. The small apparent destruction of ash (Table 3.10) was chiefly due to the settling out of sand in the digester. At the end of the experiment the SCS digester was dismantled and 94% of the ash that had been apparently destroyed during operation at a 10 day RT and 25 day RT located. The final weight of ash in the SCS digester was 55g, and it is doubted that this

will have formed a significant dead space. There was no evidence that any of the other fractions of the waste had settled out in the digester. Up to 6% of the lignin polymer was apparently degraded in this study (Table 3.10). This may be due to a limited degradation of the lignin polymer (Zeikus, 1980b; Sleat & Robinson, 1984) or simply to experimental error. The fate of ash and lignin will not be considered further.

For convenience the remaining solids will be considered as either holocellulose (α-cellulose and hemicellulose) or 'soluble' components. Lipids, VFA and crude protein are grouped together as 'soluble' components since they were primarily located in the <15μm size fraction (Chapter 4). The use of this term is not intended to imply that they were truly soluble, for example a majority of the protein is believed to be of bacterial origin (Hobson, 1981). At both retention times employed differences were apparent in the composition of solids destroyed for WCS and SCS (Tables 3.7 and 3.10). With WCS a larger proportion of destroyed solids were attributed to holocellulose degradation (49-59%), than to the destruction of the 'soluble' components (34-39%); whereas with SCS, only 30-46% total solids destroyed was attributed to holocellulose breakdown and 51-59% to the removal of the 'soluble' components (Table 3.10). The different composition of the solids destroyed during the anaerobic digestion of WCS and SCS had a profound effect on the digestion process and will be considered later.

The variation in the composition of the solid material degraded was chiefly attributed to a larger destruction of holocellulose in the WCS digester. For, 6.5-7.9g holocellulose was destroyed per day in the WCS digester compared with only 2.3-3.0g per day in the SCS digester (Table 3.7). The difference in the destruction of the 'soluble' components was considerably smaller, in the WCS digester 3.4-6.4g per day were destroyed compared with 2.3-5.7g per day in the SCS digester (Table 3.7). This variation is ascribed to the initial composition of the two slurries; both contained similar quantities of the 'soluble' components, whilst WCS contained approximately twice the amount of holocellulose (Table 3.2). Three effects of the differences in the composition of material destroyed have been identified and are now considered:

EFFECT ON GAS YIELD (PER VS ADDED) AS THE RETENTION TIME IS DROPPED

The difference in composition of the solids destroyed partly accounted for the greater reduction in the gas yield (per VS added) from WCS compared with SCS when the retention time was shortened (Table 3.6). The percentage of lipids and crude protein degraded in both slurries did not fall substantially when the retention time was shortened (Table 3.10), whereas the destruction of holocellulose fell by almost half as the retention time was dropped from 25 days (33-42% destroyed) to 10 days (18-22% destroyed). Thus, the

greater fall in the gas yield (per VS added) from WCS compared with SCS as the retention time was reduced is, to some extent at least, attributed to holocellulose representing a larger proportion of the destroyed solids in WCS than SCS (Table 3.10).

Studies conducted with sewage sludge at 35°C (O'Rourke, 1968) revealed significant reductions in lipid hydrolysis when the retention time was dropped below eight days and it is possible that a similar effect may be found with dairy cattle slurry at such a retention time. It should be remembered that the greater reduction in the efficiency of the digestion of WCS may also be partially attributed to the loss of VFA in the effluent, discussed previously (section 3.5.2).

EFFECT ON BIOGAS YIELD (PER VS DESTROYED)

From the results obtained in this study (Table 3.6) and previous studies (Table 1.1), it is apparent that the biogas yield per solids destroyed was significantly higher for SCS than for WCS. These differences are partially attributed to variations in the composition of the wastes destroyed. Several authors have calculated the biogas volume expected per kg solids destroyed for carbohydrates, lipids and proteins, the three major degradable components of dairy cattle slurry (Table 1.4). Thus, since the proximate constituents that have been destroyed are known,

the theoretical biogas yield per solids destroyed may be calculated. Using the mean values from Table 1.4, slightly higher gas yields were predicted for SCS (1.05-1.12 m^3 kgVS destroyed^{-1}) than for WCS (0.97-1.00 m^3 kgVS destroyed^{-1}). The predicted and experimental (Table 3.6) yields were similar for SCS, whilst the predicted yields for WCS were much higher than those actually obtained (Table 3.6). From the above considerations, the lowest theoretical gas yield would be 0.8 m^3 kgVS destroyed^{-1} if carbohydrates (essentially holocellulose) were the sole substrate (Table 1.4). However, a number of gas yields (per solids destroyed) have been obtained for WCS and SCS (Tables 1.1 and 3.6) below this theoretically minimum value. It therefore seems likely that other factors may also account for the apparently low biogas yield per solids destroyed:

(1) Formation of VFA (or other volatile substances) that will not be included in volatile solids determinations. This will give an erroneously high destruction of volatile solids and hence a low biogas yield per VS destroyed. It is doubted, however, that this will have been of much consequence in this study since VFA were included in the volatile solids assay.

(2) Formation of dissolved carbon dioxide. This will not be included in the volatile solids determination, and therefore also give an erroneously high biogas yield per solids destroyed.

(3) These calculations assume that polymers have been wholly converted to carbon dioxide and methane. The partial

degradation of polymers, the formation of intermediary products in the digester or the incorporation of carbon into cell biomass may therefore all bring about changes in the C/H/O ratio of the waste, and lead to inaccuracies in the estimation of the theoretical biogas yield. The release of hydrogen or oxygen (although not necessarily in the gaseous form) will give an apparent destruction of volatile solids but no biogas will be formed.

(4) A range of compounds have been taken as standards for the calculation of the theoretical biogas yield and account for the variations in Table 1.4. The determination of the exact composition of cattle slurry would permit more suitable standards to be chosen, and produce more accurate theoretical biogas yields per VS destroyed.

EFFECT ON BIOGAS COMPOSITION

The greater destruction of holocellulose in the WCS digester accounted for the poorer quality of biogas released compared with SCS (Table 3.6). From theoretical considerations it has been proposed that the biogas liberated from carbohydrate degradation will contain only 50% methane, whilst that from the digestion of lipids or crude protein would contain approximately 70% methane (Table 1.4).

3.5.4 Effect of Particle Size on The Digestion Process

The selective removal of some of the larger particles from WCS by the separator brought about a reduction in particle size (Fig. 3.1), and it is anticipated that this reduction in particle size may itself increase the rate of biogas production. The anaerobic digestion of differently sized particles from WCS is examined in Chapter 4, and discussed further in Chapter 7.1.

SUMMARY

1. Gas yields of 0.14 and 0.27 m^3 kgVS added^{-1} (methane yield 0.08 and 0.16 m^3 kgVS added^{-1}) were obtained from WCS at retention times of 10 days and 25 days respectively.

2. Gas yields of 0.23 and 0.33 m^3 kgVS added^{-1} (methane yield 0.15 and 0.21 m^3 kgVS added^{-1}) were obtained from SCS at retention times of 10 days and 25 days respectively. Thus separation improved the efficiency of digestion.

3. The improved efficiency of digestion was not due to a raised degradability of the waste (ie. VS destruction) but to the fact that the solids that were destroyed yielded more biogas per unit weight.

4. The greater destruction of solids at a 10 day RT (particularly in the WCS digester) led to unbalanced digestion, VFA toxicity and also the loss of VFA - a rich source of methane.

5. Ash and lignin were virtually recalcitrant to degradation.

6. Differences were identified in the composition of solids destroyed in the two slurries. With WCS, holocellulose represented over half of the solids destroyed. Whereas with SCS, lipids, crude protein and VFA made the major contribution to solids destruction. This is attributed to the initial composition of the two wastes.

7. The digestion of WCS was restricted to a greater extent than that of SCS when the retention time was dropped. This is partly attributed to holocellulose representing a larger proportion of WCS solids, and its degradation being particularly sensitive to a fall in retention time. The greater accumulation of VFA in the WCS digester at a 10 day RT also ensured that the gas yield (per VS added) was reduced more than for SCS.

8. The higher gas yield (per VS destroyed) and higher methane content of the biogas liberated from the digestion of SCS than WCS was due to the initial composition of the two wastes.

Table 3.1 Comparison of the Proximate Constituents of Whole Cattle Slurry and Separated Cattle Slurry from Batch A

Proximate Constnt.	WCS		SCS		Loss on Separation		%PC Lost
	gl^{-1}	%TS	gl^{-1}	%TS	gl^{-1}	%TS	
Holocell	49.1/0.4/2	57	16.2/0.0/2	38	32.9	75	67
Ash	8.1/0.1/2	9	7.1/0.0/2	17	1.0	3	12
VFA	3.1/0.1/2	4	3.0/0.1/2	7	0.1	0	3
Lig. lip. & protn	25.9/---/-	30	16.2/---/-	38	9.7	22	38
T.Solids	86.2/0.9/2	100	42.5/1.7/2	100	43.7	100	51

Values represent : mean/standard deviation/no. of samples

Abbreviations

Holocell - holocellulose

Lig - lignin

Lip - lipid

Protn - protein

PC - proximate constituent

Table 3.2 Comparison of the Proximate Constituents of WCS and SCS from Batch B.

Prox. Con.	WCS		SCS		Loss on Separation		%PC Loss
	gl^{-1}	%TS	gl^{-1}	%TS	gl^{-1}	%TS	
Lipids	5.8/1.9/14	7	6.0/2.4/14	12	0.2	gain	gain
VFA	2.4/0.4/24	3	2.1/0.3/23	4	0.3	1	12
Cr. Prot	5.4/1.1/12	7	5.2/1.6/11	11	0.2	1	4
Hemicell	21.8/3.1/8	27	8.9/2.9/10	18	12.9	40	59
∝-cell	14.0/3.1/8	17	7.6/1.4/10	15	6.4	20	46
Lignin	16.5/1.7/12	20	10.4/1.0/16	21	6.1	19	37
Ash	15.8/1.0/12	19	9.5/1.4/12	19	6.3	20	40
T. Solids	81.7/2.4/13	100	49.7/1.9/14	100	32.0	100	39
NH_4^+ (ppm)	1329/225/10		1345/233/10				
C:N ratio	16:1		10:1				
Available C:N ratio	11:1		7:1				

Notes

Values represent : mean/standard deviation/no. of samples
The constituents added up to 100-105% total solids and have been converted to 100% for ease of comparison.

Abbreviations

Cr. Prot - Crude Protein
Hemicell - Hemicellulose
∝-cell - ∝-cellulose
PC - Proximate Constituent
T. Solids - Total Solids

Table 3.3 Comparison of the Anaerobic Digestion of Separated Cattle Slurry and Whole Cattle Slurry in Batch Reactors.

	Separated Cattle Slurry			Whole Cattle Slurry		
	Before Digestion	After Digestion	Destroyed	Before Digestion	After Digestion	Destroyed
VS in digester (g)	32.1/0.0	18.5/0.9	13.6/0.9	70.2/0.4	43.3/0.9	26.9/0.9
Holocellulose in digester (g)	14.7/0.1	9.9/1.4	4.8/1.5	44.1/2.0	22.4/0.6	21.7/2.6
VFA (as mM acetate equiv.)	51.7/1.7	<1.0/0.0	-	50.0/1.7	2.1/0.0	-
pH	7.56/0.01	7.93/0.04	-	7.38/0.10	7.67/0.05	-
%CH_4 in headspace		64.2/3.2			62.6/5.2	
Total gas volume (l)		13.1/0.1			23.7/0.0	
Total methane volume (l)		8.4/0.6			14.8/0.2	
Gas Yield ($m^3 kgVS\ added^{-1}$)		0.41/0.00			0.34/0.00	
Methane Yield ($m^3 kgVS\ added^{-1}$)		0.26/0.02			0.21/0.00	
Gas Yield ($m^3 kgVS\ destroyed^{-1}$)		0.97/0.06			0.88/0.05	
%VS destroyed		42/3			38/2	

The seed has been subtracted from the above values. The values above represent the mean/standard deviation of a pair of digesters, except for the gas composition for which four samples were taken.

Table 3.4 Comparison of the Anaerobic Digestion of Whole Cattle Slurry at Three Solids Concentrations in Batch Reactors.

	Digester One			Digester Two			Digester Three		
	BD	AD	D	BD	AD	D	BD	AD	D
gVS in digester	86.4	42.9	43.5	54.4	30.4	24.0	29.3	13.2	16.1
VFA (as acetate equiv. (mM))	64.6	5.0	-	38.3	1.2	-	24.2	1.7	-
pH	7.03	7.82	-	7.05	7.62	-	7.48	7.74	-
Mean % methane in headspace		65.2			65.3			64.9	
Total gas volume (l)		*			25.81			15.73	
%VS destroyed		50			44			55	
Gas yield ($m^3 kgVS\ added^{-1}$)		*			0.47			0.54	
Methane yield ($m^3 kgVS\ added^{-1}$)		*			0.31			0.35	
Gas yield ($m^3 kgVS\ destroyed^{-1}$)		*			1.07			0.97	

* Total gas volume was not available as the gas meter stopped on day 40. The value for the seed has been subtracted in each case.

Abbreviations

BD - Before Digestion
AD - After Digestion
D - Destroyed

Table 3.5 Comparison of the Anaerobic Digestion of Whole Cattle Slurry and Separated Cattle Slurry at Similar Solids Concentrations

	Separated Cattle Slurry			Whole Cattle Slurry		
	Before Digestion	After Digestion	Destroyed	Before Digestion	After Digestion	Destroyed
gVS in digester	60.1/3.9	32.9/0.1	27.2/4.2	54.0/3.1	26.5/1.6	27.5/3.3
Total gas volume (l)		27.5/0.8			24.9/0.0	
% Methane in headspace		64.7/1.7			60.7/1.0	
Gas yield (m^3kgVS added^{-1})		0.46/0.04			0.46/0.03	
Methane yield (m^3kgVS added^{-1})		0.30/0.03			0.28/0.02	
Gas yield (m^3kgVS destroyed^{-1})		1.01/0.18			0.90/0.11	
% VS destroyed		45/4			51/3	

The seed has been subtracted from the above values.
The values represent : mean/standard deviation of a pair of digesters, except for the gas composition which is calculated from days 5 to 42 (n = 46; see Fig. 3.5) for a pair of digesters.

Table 3.6 Comparison of Ultimate Methane Yields, Gas Yields, Solids Destruction and Biogas Composition of Whole Cattle Slurry and Separated Cattle Slurry at a 25 day RT and a 10 day RT.

	Separated Cattle Slurry		Whole Cattle Slurry	
	25 day RT	10 day RT	25 day RT	10 day RT
Gas yield (m^3kgVS added^{-1})	0.33/0.04/13	0.23/0.01/3	0.27/0.02/13	0.14/0.00/3
Gas yield (m^3kgVS added^{-1}) as a % of ultimate yield	72	50	59	30
Methane yield (m^3kgVS added^{-1})	0.21/0.03/13	0.15/0.01/3	0.16/0.01/13	0.08/0.00/3
Methane yield (m^3kgVS added^{-1}) as a % of ultimate yield	70	50	57	29
%VS destroyed	29/6/13	21/1/3	37/9/13	23/2/3
Gas yield (m^3kgVS destroyed^{-1})	1.12/0.13/13	1.10/0.21/3	0.7? /0.05/13	0.61/0.06/3
Volume of biogas (l biogas l digester^{-1} day^{-1})	0.53/0.06/13	0.93/0.01/3	0.71/0.05/13	0.92/0.02/3
Methane volume (l methane l digester^{-1} day^{-1})	0.34/0.04/13	0.59/0.01/3	0.43/0.03/13	0.54/0.01/3
% methane in headspace	63.9/1.1/42	63.3/1.5/20	60.3/1.4/42	58.6/0.9/16

Notes

Values represent : mean/standard deviation/no. of samples
All (except gas composition) have been calculated on a weekly basis. See section 2.2.2 for frequency of sampling.

Table 3.7 Comparison of the Proximate Constituents Destroyed per day in Digesters Operated on Whole Cattle Slurry and Separated Cattle Slurry

	g day^{-1} destroyed in WCS digester		g day^{-1} destroyed in SCS digester	
	25-d RT	10-d RT	25-d RT	10-d RT
Holocellulose (α-cellulose & hemicellulose)	6.5	7.9	2.3	3.0
'Soluble' components (lipids, VFA, & crude protein)	3.4	6.4	2.3	5.7
Others (lignin, ash)	0.3	2.1	0.1	1.0
TOTAL	10.2	16.4	4.7	9.7

Values determined from feed and digester effluent concentrations - see Table 3.10

Table 3.8 Comparison of Measured Parameters During the Digestion of Whole Cattle Slurry and Separated Cattle Slurry

	WCS		SCS	
	25day RT	10day RT	25day RT	10day RT
NH_4^+ (ppm)				
in feed	1130/104/6	1529/127/4	1260/238/6	1511/84/4
in effluent	1572/187/14	1842/171/5	1605/137/11	1775/110/5
pH				
of feed	7.57/0.50/7	-	8.01/0.24/7	-
of effluent	7.53/0.25/8	-	7.51/0.24/8	-
H_2S (ppm)				
in biogas	446/119/7	1750/674/6	1520/175/7	1621/666/6
NH_3 (ppm)				
in biogas	<1/ - /7	<1/ - /6	<1/ - /7	<1/ - /6

Notes

No pH readings were taken at a 10 day RT

Values represent : mean/standard deviation/ no. samples

Table 3.9 Volatile Fatty Acid Concentrations (mM) in Whole Cattle Slurry and Separated Cattle Slurry Digesters

	Feed		Effluent			
			WCS		SCS	
	WCS	SCS	10-d RT	25-d RT	10-d RT	25-d RT
Acid:						
acetate	25.67/2.84	22.49/4.88	4.18/1.33	0.90/0.42	1.90/0.63	0.58/0.37
propionate	6.56/0.78	5.96/1.31	7.63/0.91	0.36/0.19	3.47/0.59	0.05/0.05
i-butyrate	0.54/0.13	0.63/0.18	0.46/0.05	0.01/0.00	0.05/0.01	0.01/0.00
n-butyrate	1.80/0.25	1.60/0.34	0.22/0.05	0.01/0.00	0.03/0.01	0.01/0.00
i-valerate	0.75/0.15	0.81/0.24	0.64/0.08	0.01/0.00	0.08/0.03	0.01/0.00
n-valerate	0.41/0.10	0.40/0.13	0.23/0.05	<0.01	0.02/0.01	<0.01
i-caproate	0.21/0.07	0.20/0.05	0.19/0.08	<0.01	0.03/0.01	<0.01
n-caproate	0.05/0.02	0.03/0.01	0.04/0.02	<0.01	0.01/0.00	<0.01
TOTAL	35.99/3.45	32.12/6.37	13.59/1.43	1.29/0.56	5.59/1.03	0.66/0.45
No. of samples analysed	22	23	16	11	16	9

Values represent : mean/standard deviation.

Table 3.10(a) Comparison of Proximate Constituent Destruction of Whole Cattle Slurry

Whole Cattle Slurry at a 25 day RT

Proximate Constituent	Feed (gl^{-1})	Effluent (gl^{-1})	Destrn. (gl^{-1})	% contrib to TS Destrn	%Destrn of P.C.
Lipid	5.9/1.5/8	2.4/1.0/13	3.5	14	59
VFA	2.5/0.2/6	0.1/0.0/11	2.4	9	96
Crude Protein	6.7/1.3/8	4.0/1.4/13	2.7	11	40
Hemicellulose	21.3/4.8/6	12.0/2.1/14	9.3	36	44
α-cellulose	14.5/3.7/6	8.6/1.7/13	5.9	23	41
Lignin	14.7/1.6/6	13.8/2.0/8	0.9	4	6
Ash	13.2/1.0/7	12.4/3.0/14	0.8	3	6
TOTAL SOLIDS	78.8/3.2/8	53.3/7.3/14	25.5	100	32

Whole Cattle Slurry at a 10 day RT

Lipid	5.6/2.6/6	3.0/1.6/6	2.6	16	46
VFA	2.4/0.3/16	1.0/0.1/16	1.4	8	58
Crude Protein	4.1/0.8/4	1.7/0.4/5	2.4	15	59
Hemicellulose	22.2/2.7/2	17.1/0.9/4	5.1	31	23
α-cellulose	13.6/1.4/2	10.7/0.9/4	2.9	18	21
Lignin	18.2/1.9/6	17.7/3.1/3	0.5	3	3
Ash	18.4/1.4/5	16.9/1.0/7	1.5	9	8
TOTAL SOLIDS	84.5/0.1/5	68.1/0.3/7	16.4	100	19

Continued over

Table 3.10(b) Comparison of Proximate Constituent Destruction of Separated Cattle Slurry

Proximate Constituent	Feed (gl^{-1})	Effluent (gl^{-1})	Destrn (gl^{-1})	% Contrbn to TS	%Destrn of P.C.
Separated Cattle Slurry at a 25 day RT					
Lipid	5.3/1.9/8	3.1/1.8/8	2.2	19	42
VFA	2.2/0.3/7	0.0/0.0/9	2.2	19	100
Crude Protein	6.1/0.4/7	4.6/1.1/14	1.5	13	25
Hemicellulose	8.6/3.4/8	6.3/1.1/13	2.3	19	27
α-cellulose	8.0/1.7/8	4.8/1.0/13	3.2	27	40
Lignin	9.4/0.9/8	9.2/0.5/6	0.2	2	2
Ash	7.8/1.3/8	7.7/1.4/12	0.1	1	1
TOTAL SOLIDS	47.4/1.5/9	35.7/3.0/14	11.7	100	25
Separated Cattle Slurry at a 10 day RT					
Lipid	6.5/3.2/6	4.2/2.3/6	2.3	24	35
VFA	2.0/0.5/16	0.4/0.1/16	1.6	17	80
Crude Protein	4.4/1.0/4	2.7/0.6/5	1.7	18	39
Hemicellulose	9.2/1.3/2	7.6/0.4/4	1.6	16	17
α-cellulose	7.2/0.3/2	5.8/0.6/4	1.4	14	19
Lignin	11.4/1.0/8	11.2/1.3/5	0.2	2	2
Ash	11.2/1.5/5	10.3/0.3/7	0.9	9	8
TOTAL SOLIDS	51.9/2.6/5	42.4/0.6/7	9.7	100	19

Note

Values represent Mean/Standard Deviation/Number of Samples for feed and effluent. Single values are means.

Fig. 3.1 Particle Size Distribution of Whole Cattle Slurry and Separated Cattle Slurry Solids

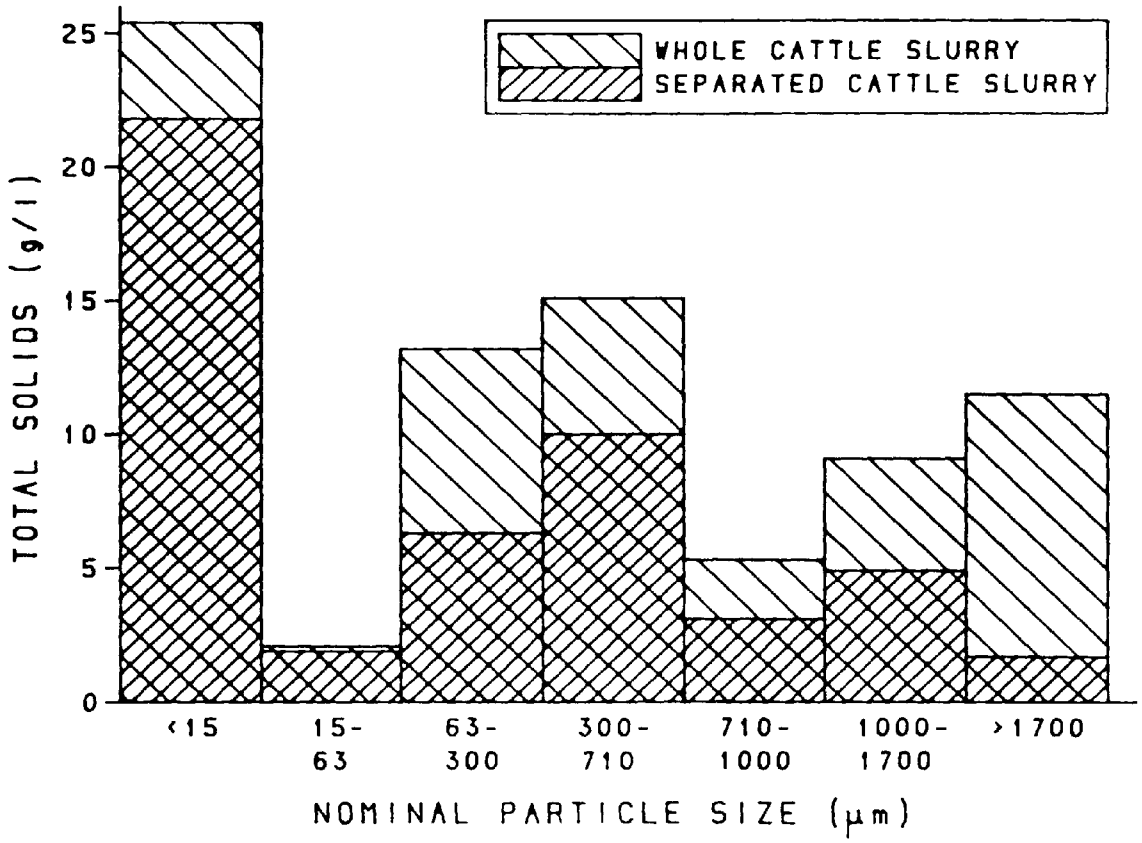
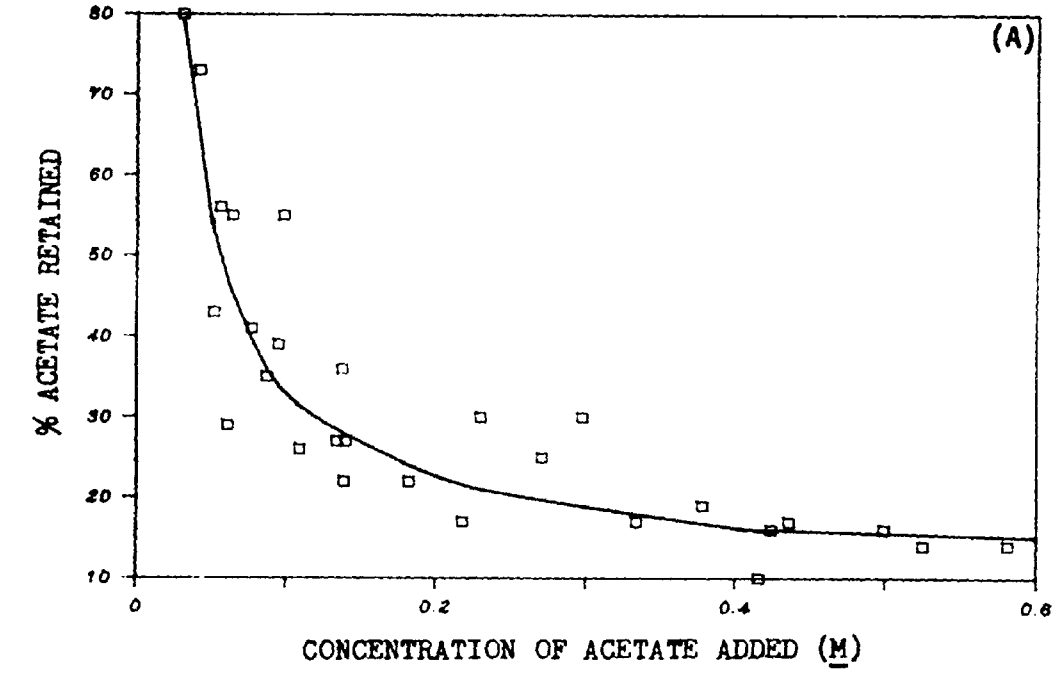


Fig. 3.2 Effect of Concentration of Acetate Added to Separated Cattle Slurry on its Retention in Total Solid Determinations



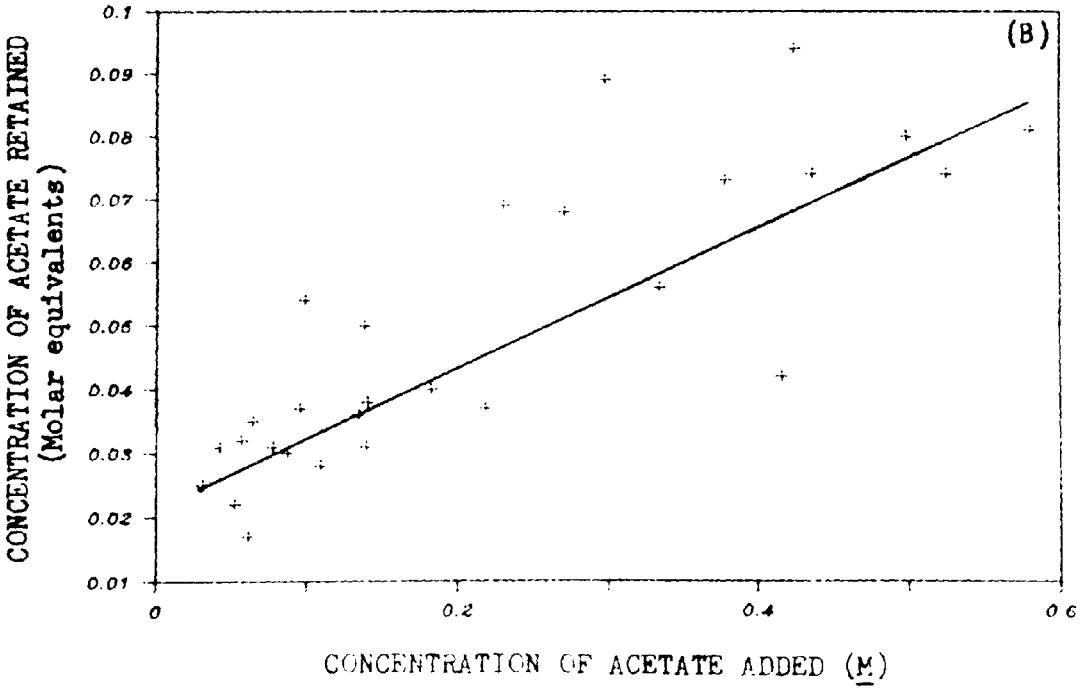


Fig. 3.3 Comparison of Gas Production (litres per day) from the Batch Digestion of Whole and Separated Cattle Slurry

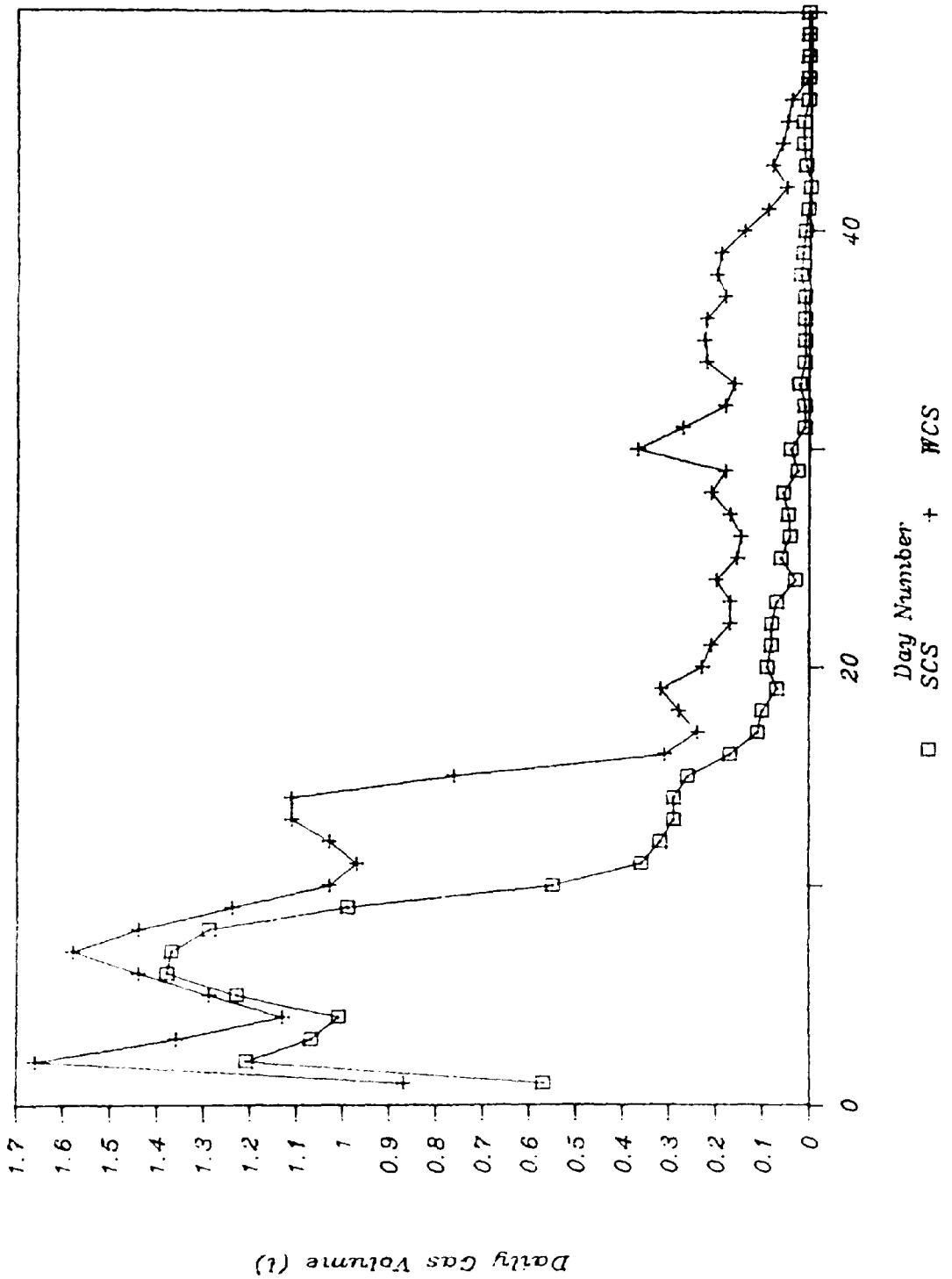


Fig. 3.4 Comparison of the Daily Gas Volume and Composition of Biogas Produced from the Anaerobic Digestion of Whole Cattle Slurry at Three Solids Concentrations in Batch Reactors

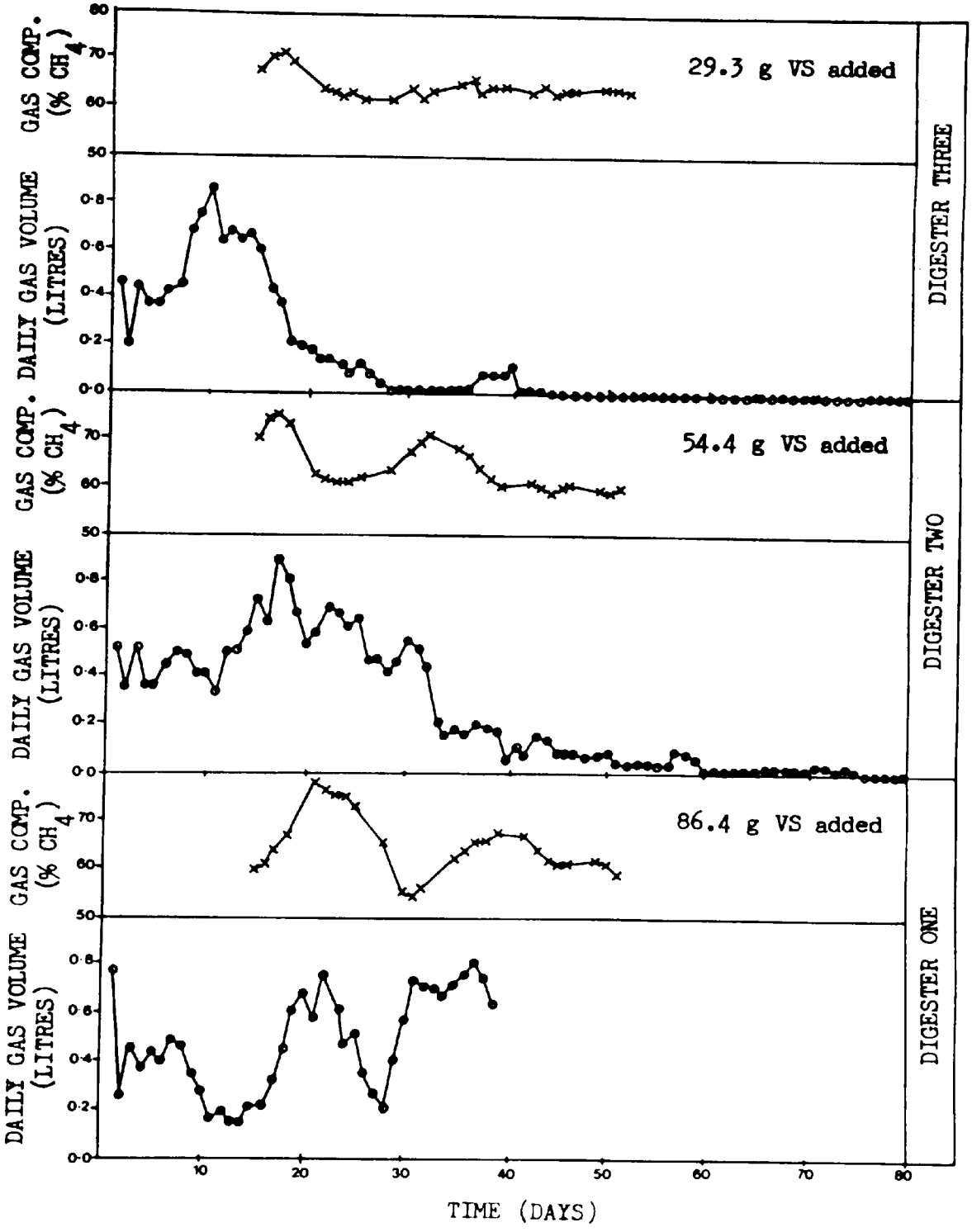
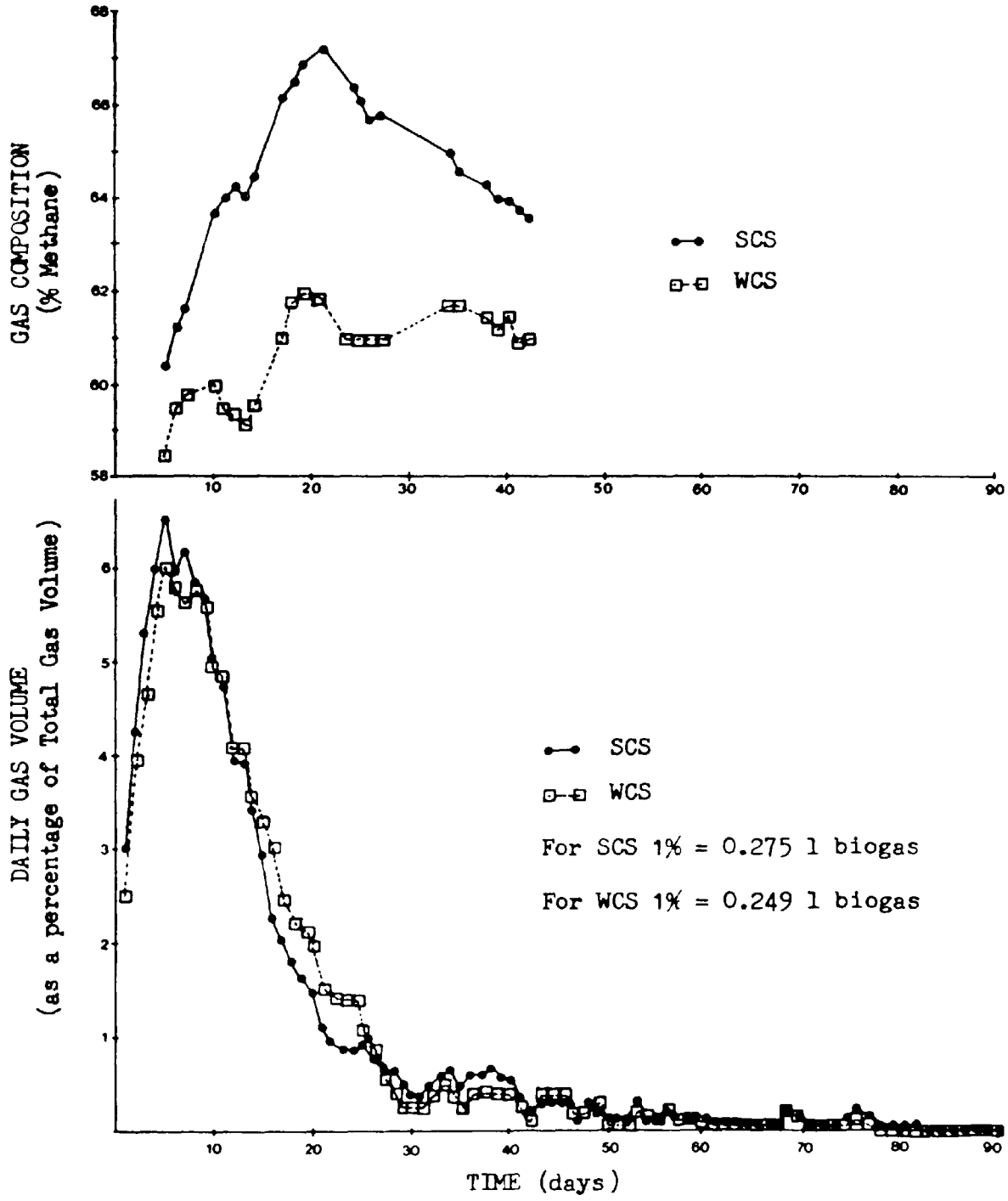


Fig. 3.5 Comparison of the Anaerobic Digestion of Whole and Separated Cattle Slurry at Similar Solids Concentrations in Batch Reactors



CHAPTER FOUR

THE ANAEROBIC DIGESTION OF SIEVED PARTICLES OF WHOLE CATTLE SLURRY

4.1 INTRODUCTION

Studies of the anaerobic digestion of SCS and WCS have revealed that higher gas yields (per VS added) were produced from SCS than WCS, and that at retention times of less than 16 days more methane was liberated per volume of digester (Tables 1.1 and 3.6). This enhancement of digestion after separation has been, at least partly attributed to the different solids concentration, composition and particle size distribution of the two slurries. A further characterisation of WCS was now carried out by sieving WCS into seven particle size fractions and determining the composition and ultimate digestibility of these fractions. It was envisaged that information derived from this study would be of assistance in interpreting the differences observed previously in the characteristics and degradability of WCS and SCS.

4.2 THE COMPOSITIONAL DISTRIBUTION OF WHOLE CATTLE SLURRY

The composition of the seven particle size fractions of WCS (see Fig. 3.1) have been determined according to the procedures outlined in section 2.3. Seven proximate constituents were assayed for and accounted for 92-106% of the total solids. The sum of the concentrations of proximate constituents have been adjusted to 100% for

convenience, with a proportional change in the concentration of each constituent (Table 4.1). The three largest size fractions (>710μm) consisted almost entirely of hemicellulose (21-35% total solids), α-cellulose (32-40% TS) and lignin (17-22%). This is attributed to the fact that these three size fractions were primarily composed of partially degraded plant material. In addition to a large quantity of partially degraded plant material, the 63-300μm and 300-710μm size fractions also contained significant amounts of sand. The sand is believed to originate from the scraping of the animal shed floors. Hemicellulose, α-cellulose and lignin together represented 56-58% total solids of these two size fractions, ash 25-36% and lipids, VFA and crude protein only 8-14% (Table 4.1). The two smallest particle size fractions were of a mud-like consistancy and contained larger amounts of lipids, VFA and crude protein (Table 4.1). The solids passing through the 15μm sieve contained smaller amounts of holocellulose (14% TS), but significant quantities of lignin (23% TS), ash (25% TS) and lipids, VFA and crude protein (38% TS). The low holocellulose:lignin ratio of the smaller particle size fractions, compared with the larger particle size fractions (Table 4.1) is attributed to the partial degradation of smaller cellulosic particles in the cow's rumen. Lower C:N and available (non-lignin) C:N ratios were also recorded for the smaller size fractions. One consequence of the different composition of the particle size fractions of WCS was that the removal of the larger size fractions brought

about a change in slurry composition. Large amounts of hemicellulose and α-cellulose were removed by the separator, compared to lesser quantities of lignin and ash, and virtually no lipids, crude protein or VFA.

By combining the particle size distribution of WCS (see Fig. 3.1) with the composition of the different particle size fractions (Table 4.1), the compositional distribution of WCS has been determined (Fig. 4.1 and Table 4.2). The sum of each proximate constituent accounted for 89-114% of the total solids in the original WCS (Table 4.2). To simplify matters, a summary of Table 4.2 has been provided as Table 4.3. Here, the seven size fractions have been combined to give three ranges of approximately equal total solids content. Although a similar proportion of WCS solids were located in each size range, a majority of lipids (52%), VFA (100%) and crude protein (70%) were found in particles of <63μm. Ash was chiefly located in the <63μm and 63-710μm size ranges and lignin was evenly distributed throughout all the size ranges. Hemicellulose and α-cellulose were primarily found in the >710μm size range (Table 4.3).

SUMMARY

1. The 710-1000μm, 1000-1700μm and >1700μm size fractions of WCS were chiefly composed of lignin, hemicellulose and α-cellulose. These size fractions contained only small amounts of lipid, crude protein and ash.

2. The 63-300μm and 300-710μm size fractions contained large amounts of ash, hemicellulose, α-cellulose and lignin and small amounts of lipid and crude protein.

3. The <15μm and 15-63μm size fractions contained significant amounts of lipids, crude protein and VFA. Ash and lignin were still present in large amounts, but there was little α-cellulose or hemicellulose.

4. Over half of the lipids, crude protein and VFA present in WCS were located in the <63μm size fractions. Hemicellulose and α-cellulose were chiefly found in the >710μm size fraction. Lignin was relatively evenly distributed throughout all the size fractions, and ash was primarily located in the <710μm size fractions.

4.3 EFFECT OF SMALL VARIATIONS IN THE VOLATILE SOLID ADDITION ON THE ULTIMATE ANAEROBIC DIGESTION OF SIEVED WHOLE CATTLE SLURRY SOLIDS

Previously, large differences in the quantity of WCS solids added to batch reactors (19-57g VS per litre), had no effect on the ultimate digestibility of the waste, but did influence the rate of biogas production (see section 3.4.2). The effect of smaller variations in the concentration of solids added to batch reactors (6-17g VS per litre) was now considered. The purpose of this experiment was to act as a control for the batch tests described in section 4.4, where the ultimate anaerobic digestion of particle size fractions

of WCS was determined. (A control was required as different quantities of tested solids were inadvertantly added to each reactor). The design of this experiment was the same as that used in section 4.4 (and described in section 2.2.3), except that a homogeneous mixture of solids from all the particle size fractions of WCS >15μm was used (see section 4.2).

One hundred and eight days were required for the ultimate anaerobic digestion of the homogeneous sieved solids. The destruction of volatile solids and gas and methane yields were independent of the initial solids concentration (Table 4.4), as found previously (section 3.3.2). A mean methane yield of 0.29 m^3 kgVS $added^{-1}$ was obtained, and the mean gas yield was 0.48 m^3 kgVS $added^{-1}$ (Table 4.4). The rate of biogas production and the composition of biogas liberated were, however, effected by the initial concentration of solids added (Figs. 4.2 and 4.3). A longer time period was required for biogas production with increasing solids addition (Fig. 4.2); for example by day 10, 57-59% of the total biogas had been liberated from digesters A and B (22-24g VS added), but only 44% from digester C (46g VS added), and 33-34% from D and E (66-68g VS added). This delay in biogas production is due to the inhibition of digestion, and has been observed previously (section 3.3.2). Despite each reactor containing the same substrate, the methane content of the biogas decreased as the concentration of solids added increased (Fig. 4.3 and Table 4.4). This is most likely attributed to

a proportionately smaller contribution of the seed to total biogas production with increasing solids addition, for the biogas liberated from the control reactor was richer in methane (mean 74.5%) than that from the test digesters (Fig. 4.3 and Table 4.4). The failure to observe a similar effect on biogas composition when WCS was examined at three solids concentrations (see Table 3.4) is presumably attributed to a larger production of biogas from the test substrate in these circumstances. Thus the rate of biogas production and its composition were dependent upon the concentration of solids added, and this point will need to be borne in mind when the batch tests conducted in section 4.4 are considered.

SUMMARY

1. The ultimate destruction of volatile solids, and ultimate methane and gas yields were independent of solids addition within the range of 5.6-17.0g VS per litre.
2. The rate of biogas production and methane content of the biogas both decreased with increasing solids addition in the range of 5.6-17.0g VS per litre.

4.4 THE ULTIMATE ANAEROBIC DIGESTION OF SIEVED FRACTIONS OF WHOLE CATTLE SLURRY

WCS was sieved into six particle size fractions, and the ultimate anaerobic digestion of the fractions determined in batch reactors. A further reactor contained

reconstituted whole cattle slurry (RCS). RCS was obtained by mixing the sieved fractions back together in the original proportions in which they were found in WCS. An eighth digester acted as a control. Additions of sieved solids (5-24g VS per litre) to the batch reactors were made upto a final volume of 4.1 litres with seed (0.5 l added) and the <15μm size fraction (ie. the proportion of WCS passing through a 15μm sieve). The <15μm size fraction was added to ensure a reasonable C:N ratio and that no soluble factor was limiting. The full experimental protocol has been described in section 2.2.3.

4.4.1 Gas Yields Obtained From the Particle Size Fractions

Two hundred and twenty-six days were required for the ultimate anaerobic digestion of the solids from the >1700μm size fraction, and 126 days were allowed for the remaining size fractions. Only small differences were found in the ultimate digestibility of the six size fractions (from 47-57% VS were destroyed), and a slightly greater proportion of the larger size particles were degraded than the smaller particles (Fig. 4.4a and Table 4.5). This may provide an explanation for the reduction in the ultimate degradability of cattle slurry solids sometimes observed after separation, for it was these large, more degradable particles that were removed by the separator. The composition of solid material destroyed in each size fraction is considered in section

4.4.2.

Gas yields ranging from 0.86-1.11 m^3 kgVS destroyed^{-1} were obtained for the tested samples, and there was no apparent relationship between this and particle size (Fig. 4.4b and Table 4.5). From observations made with WCS and SCS (Tables 3.3, 3.5 and 3.6) and theoretical considerations (Table 1.4), it was anticipated that lower gas yields (per VS destroyed) would have been produced from the larger particle size fractions of WCS. Using the theoretical gas yields (from Table 1.4, and assuming that VFA, lipids and protein give a gas yield of 1.2 m^3 kgVS destroyed^{-1}) and taking account of the composition of solids destroyed (Table 4.7), theoretical yields of 0.80-0.82 m^3 kgVS destroyed^{-1} were predicted for the four largest particle size fractions, compared with 1.01-1.11 m^3 kgVS destroyed^{-1} actually obtained; whilst the theoretical gas yields predicted for the 15-300μm particle size fraction (0.88 m^3 kgVS destroyed^{-1}), the <15μm fraction (1.13 m^3 kgVS destroyed^{-1}) and RCS (0.91 m^3 kgVS destroyed^{-1}) compare well with those obtained. The apparently high gas yields (per VS destroyed) obtained from the four largest size fractions is most likely attributed to an overestimation of biogas production, perhaps as a consequence of gas meter malfunctions.

Total gas yields of 0.43-0.63 m^3 kgVS added^{-1} were recorded, and higher gas yields were produced from the larger size fractions (Fig. 4.4c and Table 4.5). Indeed, total biogas yields higher than, or equal to, that from RCS were produced from the three largest size fractions, whilst

the yields from the three smaller size fractions were lower. Since similar ultimate gas yields (per VS added) were obtained for WCS and SCS, it was anticipated that similar ultimate gas yields (per VS added) would also be obtained for the sieved particle size fractions. The larger yields obtained from the larger size fractions may be due to an overestimation of biogas production, as discussed previously. However, no clear pattern emerged between the ultimate methane yield and particle size (Fig. 4.4d and Table 4.5).

It is doubted that the ultimate degradability of the particle size fractions was influenced by the concentration of solids tested (see sections 3.3.2 and 4.3), or by the actual size of the particles (see section 1.2.1). Thus, the ultimate degradability was solely dependent upon the composition of tested samples. The composition of solids destroyed is now considered.

4.4.2 The Components of the Volatile Solids Destroyed

The composition of the volatile solids degraded has been calculated for RCS (reconstituted whole cattle slurry) and each of the particle size fractions (Tables 4.6 and 4.7). From 47-57% volatile solids were destroyed, 49-80% holocellulose and 0-73% lipids, protein and VFA (Table 4.7). A greater proportion of holocellulose was destroyed in particles larger than 15μm (72-80% destroyed) than in the

<15μm size fraction (49% destroyed). This is attributed to the more easily degraded holocellulose having been previously removed from the smaller particles during passage through the cow's rumen (see also holocellulose : lignin ratio of these particles - Table 4.1). Undegraded holocellulose is presumably complexed with lignin, and as a result, recalcitrant to digestion. The slightly higher degradability of the larger size fractions may also be related to their lower lignin content, although the degradability of each fraction was much greater than anticipated from Chandler's observations (Chandler et al., 1980 - see section 1.2.1). Furthermore, the large contribution of lignin and ash to the <15μm size fraction of WCS may provide an explanation of the poor degradation of the 'soluble' portion of dairy cattle slurry observed previously (Zeeman et al., 1983a). The variable degradation of lipids, VFA and protein (0-25% destroyed) in particles >300μm (Table 4.7) is attributed to their low concentration leading to a high degree of error in their measurement. A larger proportion (50-73%) of the lipids, VFA and protein were degraded in the three samples (<15μm and 15-300μm particle size fractions and RCS) in which they were present in significant amounts (Table 4.7).

Holocellulose represented 96-100% volatile solids destroyed in the four largest size fractions, with the degradation of protein, lipids and VFA accounting for only 0-4% (Table 4.7). However, lipids, proteins and VFA represented 20% of the VS destroyed in particles from

15-300μm, and 82% of the solids in particles <15μm (Table 4.7).

4.4.3 Rate and Composition of Biogas Production

The smaller particle size fractions of WCS were degraded proportionately more rapidly than the larger particle size fractions (Fig. 4.5 and Table 4.8) and the differences in the rates of biogas production were much greater than accounted for by variations in the solids concentration alone (see section 4.3). The more rapid degradation of the <15μm particle size fraction and (to a lesser extent) the 15-300μm size fraction, compared with the size fractions >300μm may be due to particle size itself, although in view of the fact that all particles from 300-1700μm were degraded at approximately the same rate, it is more likely that differences relate to variations in composition. The 300-1700μm particle size fraction and the >1700μm particle size fraction (whose degradation was particularly slow since a large proportion of the solids floated and were out of contact with the aqueous phase) were composed almost entirely of α-cellulose, hemicellulose and lignin (88-95% VS) and contained very little protein, lipid and VFA (5-12% VS), whilst the two smaller size fractions contained significantly more protein, lipid and VFA (20-52% VS) and less α-cellulose, hemicellulose and lignin (48-80% VS). The degradation of cellulosic particles has also been

reported to be the slowest step in the digestion of particulate wastes in previous studies (eg. Hobson, 1981).

The composition of the biogas in the headspace of the digesters varied little between days 22 and 58 (Fig. 4.6) as a majority of the biogas had been liberated by day 22 (Fig. 4.5). Biogas in the headspace of digesters containing the <15μm and 15-300μm size fractions was richer in methane (mean 73.9% and 67.9% respectively (Table 4.5)) than that in the control digester (mean 64.7% methane), whilst the biogas released from the particle size fractions larger than 300μm was poorer in methane (mean 56.0-60.4%; Table 4.5). The difference in the quality of biogas liberated from the particle size fractions is attributed to the different composition of solids destroyed (Table 4.7); for it is anticipated that the biogas liberated from the digestion of lipids, protein and VFA will contain approximately 70% methane, whilst that from holocellulose will contain only 50% methane (Table 1.4).

4.4.4 Effect of Digestion on Particle Size

After digestion the solids were sieved again and some reduction in particle size identified (Tables 4.9 and 4.10). The digestion process effected a reduction in the particle size range in which 22-49% of the solids were retained, whilst no change was detected with 45-78% of the solids, and a small proportion apparently increased in size (5-9%). The

apparent increase in size is attributed to errors in the sieving procedure (including the presence of seed which was subtracted on each occasion). A smaller proportion (22%) of particles larger than 1700μm were reduced in size by digestion, compared with particles initially sized from 15-1700μm (31-49% reduced in size). This was presumably due to small changes in particle size having a lesser effect upon particles so large. A reduction in particle size has also been observed after the passage of material through the rumen (Kennedy, 1985).

SUMMARY

1. Slightly higher destructions of volatile solids were obtained for the larger size sieved particles, compared with the smaller sized particles.
2. Biogas production from the smaller particle size fractions of WCS was more rapid and richer in methane than that from the larger size fractions. This is attributed to the different composition of the size fractions.
3. 72-80% holocellulose in particles >15μm was degraded, but only 49% holocellulose in the <15μm size fraction.
4. 96-100% volatile solids destroyed in particles >300μm was attributed to holocellulose destruction, but only 80% in particles sized from 15-300μm and 18% in particles <15μm. The remainder is due to VFA, protein and lipid degradation.
5. 22-49% particles apparently decreased in size during digestion and moved from a higher to a lower particle range.

Table 4.1 Percentage (by weight) of Each Proximate Constituent in the Particle Size Fractions of Whole Cattle Slurry

Proximate Constituent	Size Fraction (μm)							% in WCS
	<15	15-63	63-300	300-710	710-1000	1000-1700	>1700	
Lipids	11	10	9	4	6	2	4	6
VFA	9	0	0	0	0	0	0	3
Crude Protein	18	10	5	4	4	3	4	9
Hemicellulose	9	11	21	24	21	35	32	22
α-cellulose	5	21	17	20	40	37	32	20
Lignin	23	28	20	12	20	17	22	18
Ash	25	20	28	36	9	6	6	22
TOTAL SOLIDS	100	100	100	100	100	100	100	100
C:N Ratio	7:1	20:1	38:1	49:1	55:1	79:1	52:1	18:1
Available C:N Ratio	5:1	11:1	26:1	37:1	39:1	60:1	37:1	13:1
Holocellulose: Lignin Ratio	0.6:1	1:1	2:1	4:1	3:1	4:1	3:1	2:1

Table 4.2 Compositional Distribution of WCS. The Weight of Each Proximate Constituent in Each Particle Size Fraction in one litre of WCS

Proximate Constituent	Size Fraction (μm)							Sum of P.C. (gl^{-1})	Quantity in WCS * (gl^{-1})	% accounted for
	<15	15-63	63-300	300-710	710-1000	1000-1700	>1700			
Lipid	2.7	0.2	1.2	0.6	0.3	0.2	0.4	5.6	4.9	114
VFA	2.3	0	0	0	0	0	0	2.3	2.1	109
Crude Protein	4.5	0.2	0.6	0.5	0.2	0.3	0.4	6.7	7.5	89
Hemicellulose	2.1	0.2	2.9	3.6	1.1	3.1	3.6	16.6	18.0	92
α-cellulose	1.3	0.4	2.1	3.1	2.1	3.3	3.9	16.2	16.0	101
Lignin	5.7	0.6	2.5	1.8	1.1	1.5	2.4	15.6	14.1	110
Ash	6.3	0.4	3.6	5.2	0.4	0.5	0.6	17.0	17.4	98
TOTAL SOLIDS	24.9	2.0	12.9	14.8	5.2	8.9	11.3	80.0	80.0	-

* Quantities actually found in WCS (see Table 4.1)

Abbreviation: P.C. - Proximate Constituent

Table 4.3 A Summary of the Distribution of the Proximate Constituents in Three Particle Size Ranges of Whole Cattle Slurry

	Particle Size Range (μm)		
	<63	63-710	>710
Lipids	52	32	16
VFA	100	0	0
Crude Protein	70	16	14
Hemicellulose	14	39	47
α-cellulose	10	32	58
Lignin	40	28	32
Ash	39	52	9
TOTAL SOLIDS	34	34	32

The percentage of each proximate constituent (and total solids) in the three size ranges are given (ie. 52% lipids was located in the <63μm range, 32% in the 63-710μm range and 16% in the range >710μm).

Table 4.4 Comparison of Solids Destruction and Gas Yields from the Ultimate Anaerobic Digestion of Sieved Homogeneous Whole Cattle Slurry Solids

Digester	A	B	C	D	E
gVS added	22.2	24.5	45.7	66.4	68.1
gVS undegraded	9.5	11.1	24.9	34.2	31.2
gVS destroyed	12.7	13.4	20.8	32.2	36.9
Total gas volume (l)	11.01	10.53	21.35	33.09	34.32
Mean % CH_4 in biogas	62.3	62.5	59.4	57.1	57.2
Total CH_4 volume (l)	6.86	6.58	12.68	18.89	19.63
%VS destroyed	57	55	46	48	54
GY ($m^3 kgVS\ added^{-1}$)	0.50	0.43	0.47	0.50	0.50
MY ($m^3 kgVS\ added^{-1}$)	0.31	0.27	0.28	0.29	0.29
GY ($m^3 kgVS\ destroyed^{-1}$)	0.87	0.79	1.03	1.03	0.93

Abbreviations

GY - Gas Yield

MY - Methane Yield

Table 4.5 Summary of Gas Yields and Solids Destructions From the Ultimate Anaerobic Digestion of Sieved Size Fractions of Whole Cattle Slurry

Particle Size (μm)	gVS added	gVS destryd	T.G.V. (l)	Mean % methane	% VS destryd	GY added	MY added	GY dest.
>1700	98.5	51.6	52.44	56.0	52	0.53	0.30	1.02
1000-1700	47.4	27.1	30.00	60.2	57	0.63	0.38	1.11
710-1000	53.1	26.7	27.09	60.4	50	0.51	0.31	1.01
300- 710	55.7	26.2	26.42	60.4	47	0.47	0.28	1.01
15- 300	29.4	14.7	12.58	67.9	50	0.43	0.29	0.86
<15	21.3	10.0	10.52	73.9	47	0.49	0.36	1.06
RCS	60.7	32.5	30.99	59.2	54	0.51	0.30	0.96

Abbreviations
RCS - Reconstituted Whole Cattle Slurry
T.G.V. - Total gas volume (litres)
GY added - Gas Yield ($m^3 kgVS\ added^{-1}$)
MY added - Methane Yield ($m^3 kgVS\ added^{-1}$)
GY dest. - Gas Yield ($m^3 kgVS\ destroyed^{-1}$)

Table 4.6 Composition of Sized Particle Size Fractions of WCS Before and After Ultimate Anaerobic Digestion

Particle Size (μm)	Composition Before Digestion				Composition After Digestion			
	holocell (g)	lignin (g)	VFA,CP &lipids (g)	Total VS (g)	holocell (g)	lignin (g)	VFA CP &lipids (g)	Total VS (g)
>1700	67.0	23.1	8.4	98.5	17.5	23.1	6.3	46.9
1000-1700	36.6	8.6	2.5	47.4	7.3	8.6	4.4	20.3
710-1000	35.5	11.7	5.9	53.1	10.0	11.7	4.7	26.4
300- 710	38.4	10.5	6.8	55.7	11.8	10.5	7.2	29.5
15- 300	14.9	8.5	6.0	29.4	3.2	8.5	3.0	14.7
<15	3.7	6.4	11.2	21.3	1.9	6.4	3.0	11.3
RCS	42.8	18.7	15.8	77.3	14.0	18.7	4.4	37.1

The composition of the size fractions before digestion was ascertained by the procedures described in Chapter 2. The composition of the volatile solids after digestion was determined in the following manner; holocellulose and total volatile solids - direct measurement; Lignin - it has been assumed that none was degraded (see Chapter 3); lipids, VFA and crude protein - by subtraction.

Abbreviations
RCS - Reconstituted Whole Cattle Slurry
holocell - holocellulose
CP - Crude Protein

Table 4.7 Composition of Size Fractions Destroyed During Ultimate Anaerobic Digestion, the Percentage of Each Constituent Destroyed and Their Contribution to Volatile Solid Destruction

Particle Size (μm)	Weight of Proximate Constituent Destroyed			Percentage Destruction			Percentage Contribution to Volatile Solid Destruction	
	holocell (g)	VFA, lipid, protein (g)	VS (g)	holocell	VFA, lipid protein	VS	holocell	VFA, lipid & protein
>1700	49.5	2.1	51.6	74	25	52	96	4
1000-1700	29.0	-1.9	27.1	80	0	57	100	0
710-1000	25.5	1.2	26.7	72	20	50	96	4
300- 710	26.6	-0.4	26.2	76	0	47	100	0
15- 300	11.7	3.0	14.7	79	50	50	80	20
<15	1.8	8.2	10.0	49	73	47	18	82
RCS	28.8	11.4	40.2	67	72	52	72	28

Abbreviations

RCS - Reconstituted Whole Cattle Slurry
holocell - Holocellulose

Note

See Table 4.6 for digester contents before and after digestion.

Table 4.8 Comparison of the Rate of Biogas Production From the Six Sieved Fractions of Whole Cattle Slurry

Particle size (μm)	VS added (gl^{-1})	% final gas volume liberated by day 10
>1700	24.6	5
1000 - 1700	11.9	25
710 - 1000	13.3	24
300 - 710	13.9	16
15 - 300	7.4	44
<15	5.3	88
RCS	15.2	18

RCS - Reconstituted whole cattle slurry

Table 4.9 Effect of Anaerobic Digestion on the Particle Size Distribution of Sieved Fractions of Whole Cattle Slurry

Particle size After Dig.(μm)	Particle Size Before Digestion (μm)				
	>1700 (dig. 1)	1000-1700 (dig. 2)	710-1000 (dig. 3)	300-710 (dig. 4)	15-300 (dig. 5)
>1700	78	5	0	0	0
1000 - 1700	3	46	9	0	0
710 - 1000	3	14	51	7	3
300 - 710	9	22	26	62	4
63 - 300	6	2	4	14	31
15 - 63	(1)	(11)	(10)	(17)	22
<15	(*)	(*)	(*)	(*)	40
TOTAL	100	100	100	100	100

Note

The above values represent the percentage in each particle size fraction after digestion.

* The 15-63 μm and <15 μm size fractions are combined for digesters 1-4. In digester 5, 86% of the solids were initially sized between 63-300 μm, and 14% between 15-63 μm.

Table 4.10 The Proportion of Particle Size Fractions of Whole Cattle Slurry Increasing in Size, Remaining Unchanged, and Decreasing in Size After Ultimate Anaerobic Digestion.

Digester	Particle Size (μm) Before Digestion	Change In Size After Digestion		
		% Larger	% Same	% Smaller
1	>1700	--	78	22
2	1000 - 1700	5	46	49
3	710 - 1000	9	51	40
4	300 - 710	7	62	31
5	15 - 300	7	45	48

Fig. 4.1 Size and Composition Distribution of Total Solids in One Litre of Whole Cattle Slurry

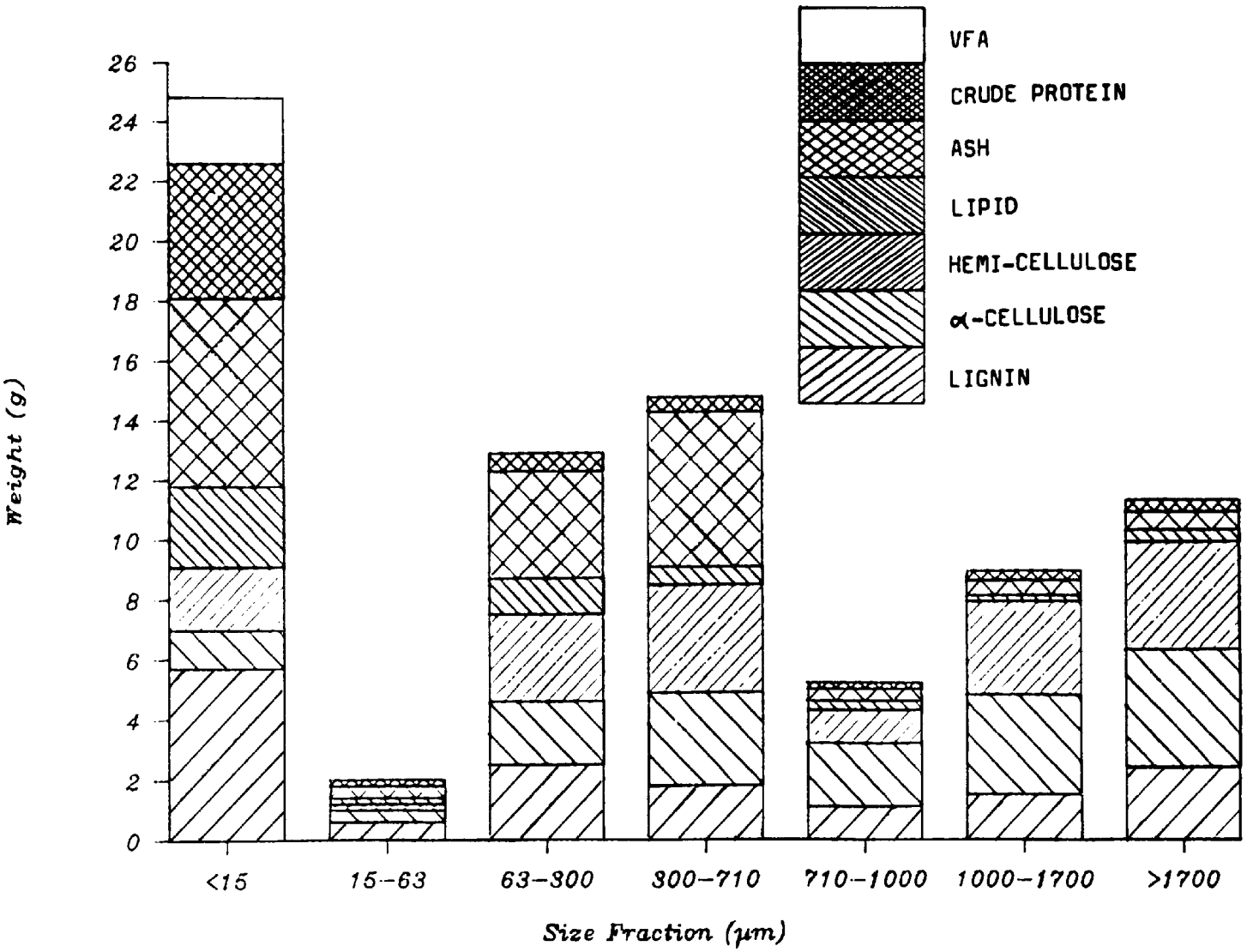


Fig. 4.2 Plot of Daily Gas Volume Produced During the Batch Digestion of Sieved Homogeneous Cattle Slurry Solids

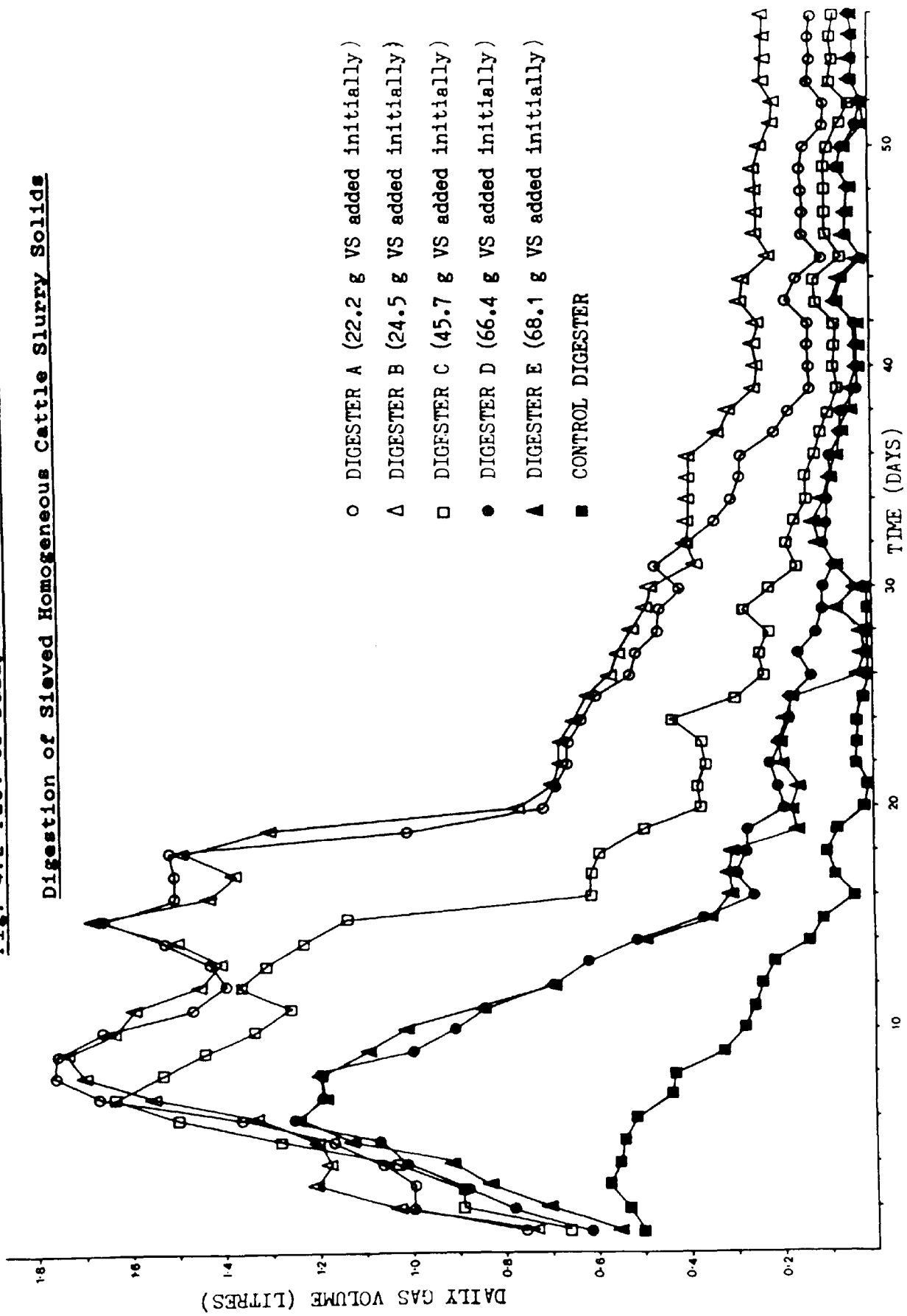


Fig. 4.3 Plot of Composition of Biogas in the Digester Headspace During the Batch Digestion of Homogeneous Cattle Slurry Solids

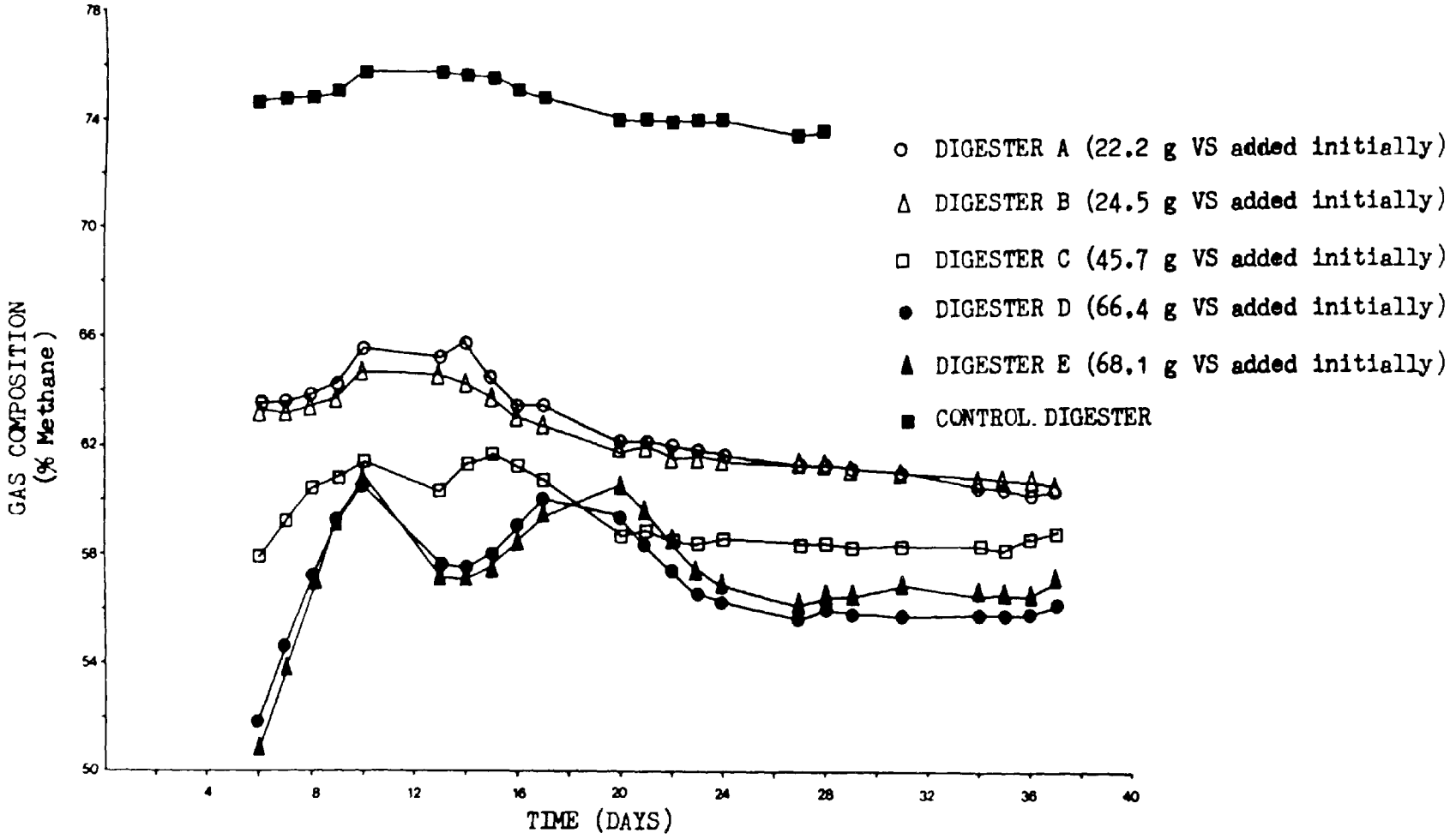


Fig. 4.4 The Ultimate Anaerobic Digestion of Sieved Fractions of Whole Cattle Slurry

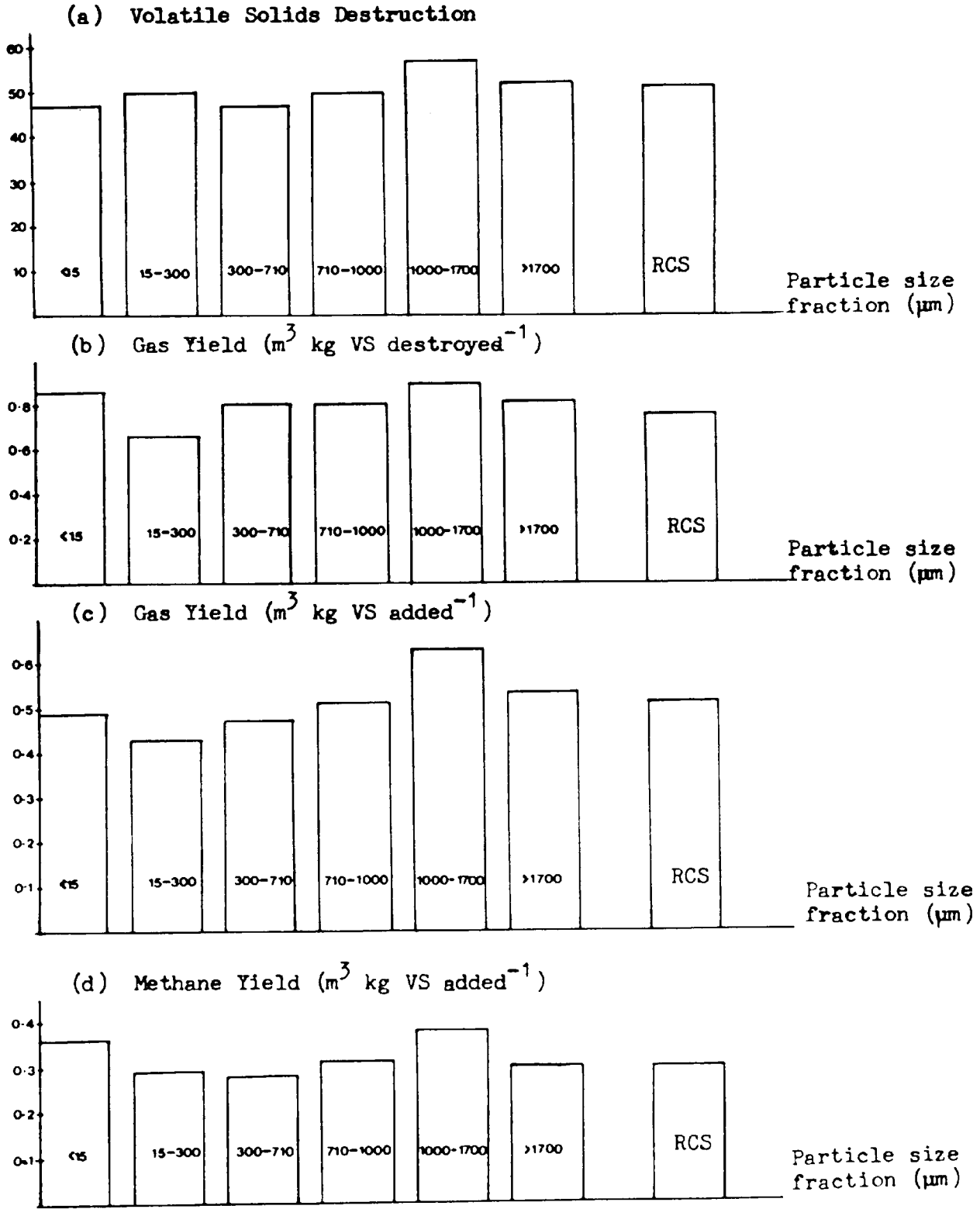
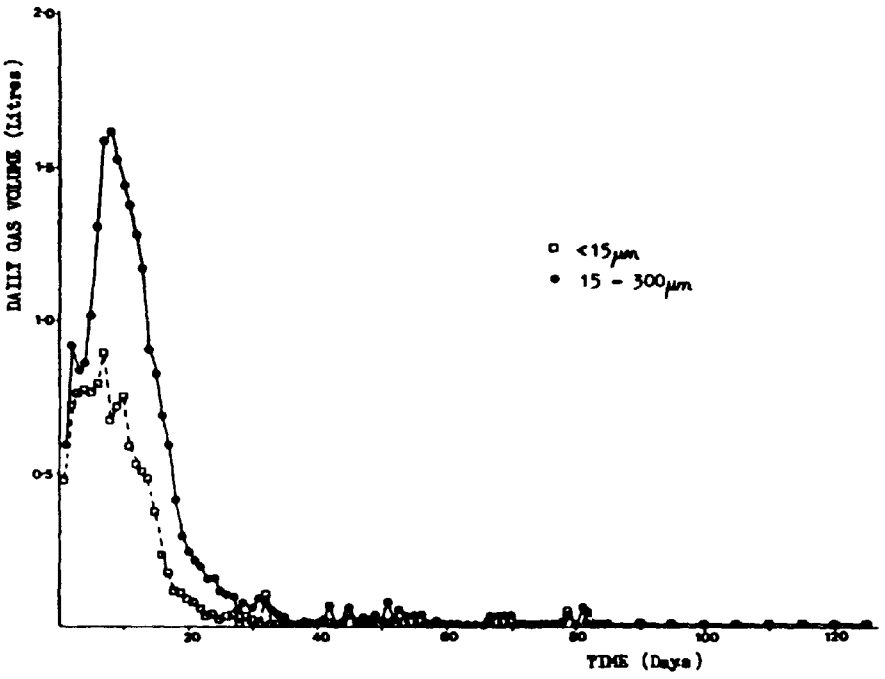


Fig. 4.5a Plot of Daily Gas Production from Sieved Fractions of Whole Cattle Slurry (see Fig. 4.5b)



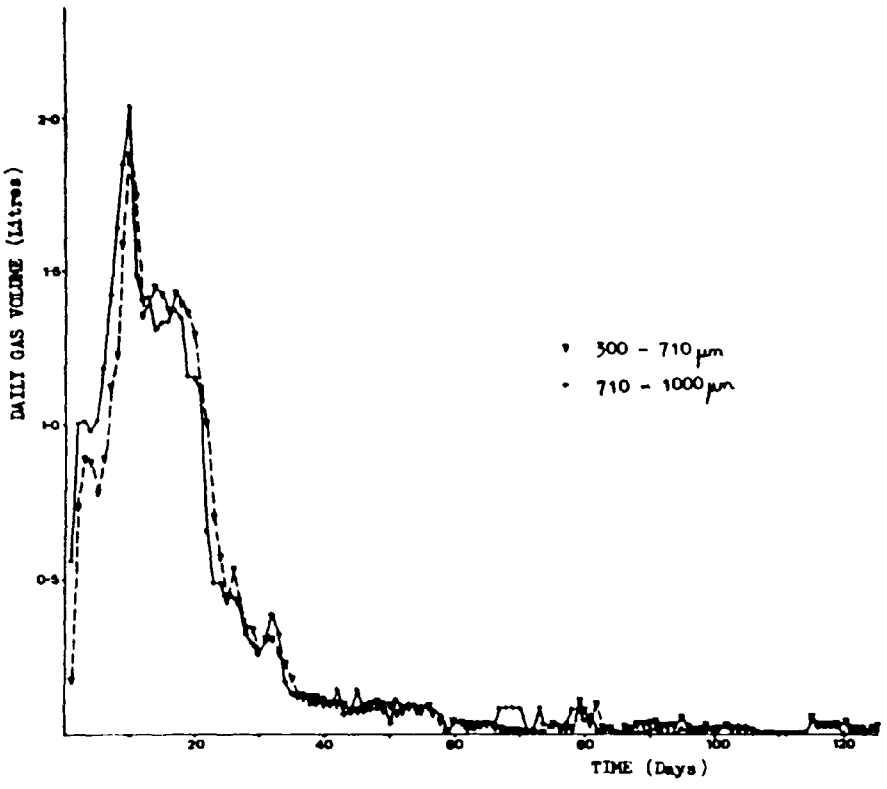
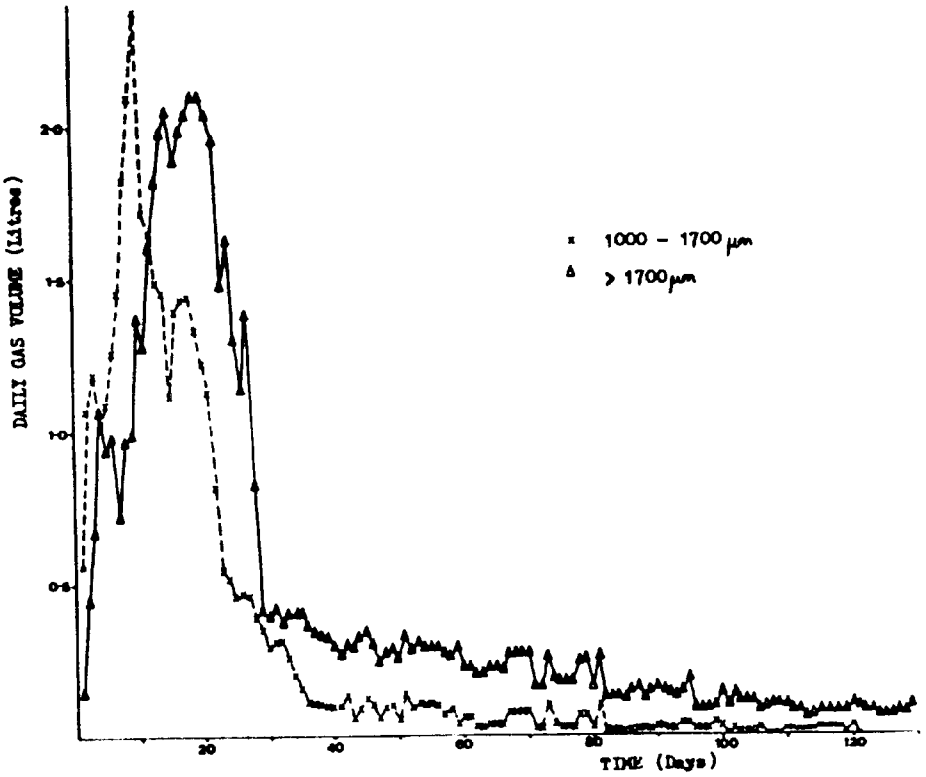


Fig. 4.5b Plot of Daily Gas Production from Sieved Fractions of Whole Cattle Slurry (see Fig. 4.5a)



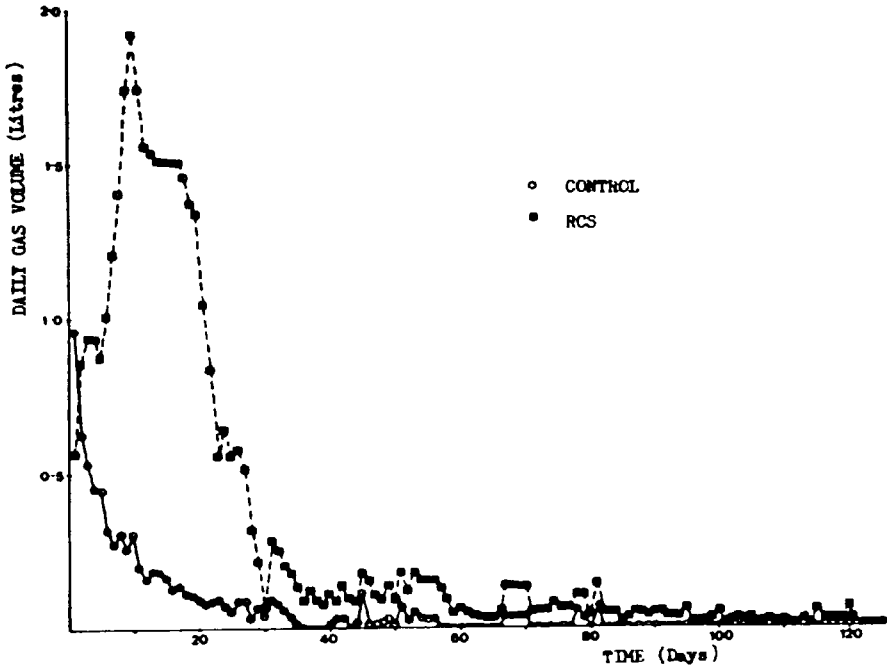
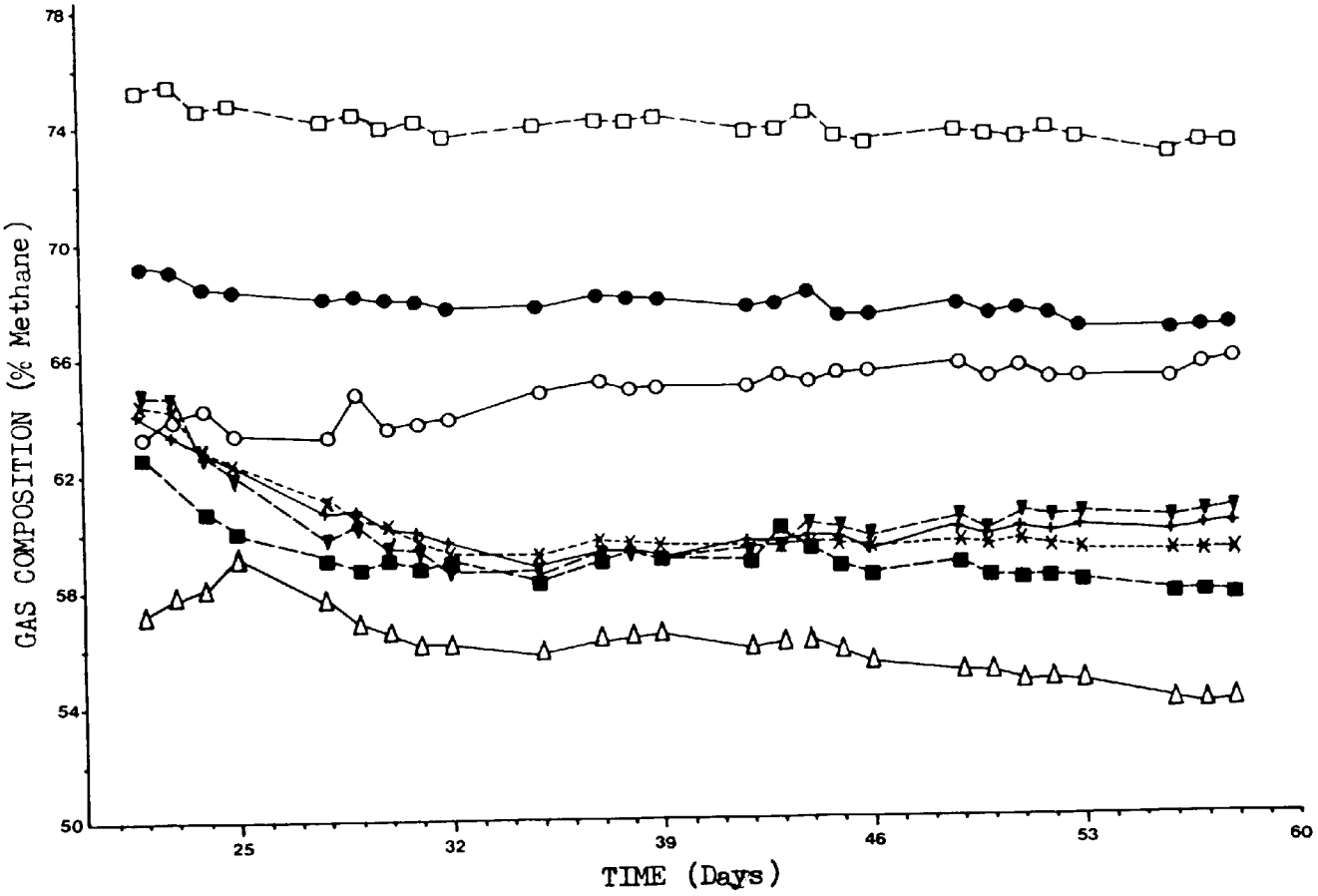


Fig. 4.6 Plot of Composition of Biogas in the Digester Headspace During the Batch Digestion of Sieved Fractions of Whole Cattle Slurry



Δ Size fraction >1700 μm; 98.5 g VS added

x Size fraction 1000 - 1700 μm; 47.4 g VS added

+ Size fraction 710 - 1000 μm; 53.1 g VS added

▼ Size fraction 300 - 710 μm; 55.7 g VS added

● Size fraction 15 - 300 μm; 29.4 g VS added

□ Size fraction <15 μm; 21.3 g VS added

○ Control reactor

■ RCS; 60.7 g VS added

CHAPTER FIVE

EFFECT OF TEMPERATURE SHOCK TREATMENTS ON THE STABILITY OF ANAEROBIC DIGESTERS OPERATED ON SEPARATED CATTLE SLURRY

5.1 INTRODUCTION

The sensitivity of anaerobic digesters operated on pig slurry and sewage sludge to temperature shock treatments has been recently examined (van Velsen and Lettinga, 1980; Temper et al., 1983). However, similar investigations have not been conducted with cattle slurry, nor have these studies considered the likely position in a full-scale digester where a failure in heating devices might be expected to lead to daily temperature falls of the order of 8°C (Skilton et al., 1985). It is doubted that excessive temperature rises will be a problem with full scale digesters, as a safety device is generally present to prevent such an occurrence.

The aim of this study was to monitor digester stability during falls and then rises in the operating temperature, and also to recommend a recovery procedure that may be adopted with full-scale digesters when temperature failure occurs. Experiments were conducted at a 10 and 25 day RT with five litre laboratory-scale digesters operated on SCS (batch A, Table 3.1). Once the digesters had been maintained at 35°C for two retention times, temperature fluctuations were applied to two pairs of digesters, a third pair acting as a control. Initially, the digesters were subjected to temperature drops of approximately 8°C per day, down to 3-6°C. The digester temperature was then increased back to 35°C either rapidly (in a single day) or slowly (over several days). The effect of starving the

digesters during the recovery period was also examined at a 10 day RT. To monitor digester stability, daily measurements were made of the biogas volume and composition, digester pH, and the individual VFA concentrations (C-2 to C-6).

However, as all the digesters used in these studies were fed semi-continuously (that is once per day, seven days a week), an initial study was carried out to determine how representative a sample taken immediately prior to feeding was of steady-state conditions within a digester. In previous studies, methane production and the VFA concentration cycled over a 24 hour period in daily fed digesters (Mountfort and Asher, 1978; Mackie and Bryant, 1981).

5.2 DAILY CYCLING WITHIN AN ANAEROBIC DIGESTER

To determine the accuracy of samples taken immediately prior to feeding as a reflection of the real steady-state in dairy cattle slurry digesters, a digester was operated at 35°C and a 25 day RT, fed once per day and monitored continuously. Immediately prior to feeding (0-4.5 hours) biogas was produced at a rate of 60cm^3 hr^{-1}, but increased dramatically after feeding (Fig. 5.1). Stirring the digester prior to feeding liberated approximately 100cm^3 of trapped biogas, and after feeding biogas production continued at approximately 160cm^3 hr^{-1} for seven hours (Fig.

5.1). This then decreased over an eight hour period to give a rate of 60cm^3 hr^{-1} until the digester was stirred and fed again. In these circumstances, therefore, biogas was not produced at a steady rate but cycled over a 24 hour period, with half of the total biogas produced in the first seven and a half hours. A similar pattern of biogas production has been observed previously (Mountfort and Asher, 1978; Mackie and Bryant, 1981), and may be attributed to the removal of rapidly degraded fractions of the feed.

Variations in the quality of biogas produced were also observed (Fig. 5.2), for example the amount of gas not attributed to carbon dioxide or methane in the digester headspace increased from 5% to 13% during feeding (Fig. 5.2). In a total of 17 similar studies, significant increases in the concentration of gas not attributed to carbon dioxide or methane were noted on twelve occasions, with an average concentration of 4% before feeding and 8% after feeding. This is presumably attributed to digester design and the feeding procedure adopted permitting the entry of air. Furthermore the concentration of methane and carbon dioxide also varied relative to each other. In the three hours after feeding, the methane content of biogas in the digester headspace fell from 66.4% to 65.0% (Fig. 5.2), but recovered overnight to the original value. This was primarily due to an increased production of carbon dioxide in the three hour period immediately after feeding (when 0.33 l methane and 0.21 l carbon dioxide were produced), and may be ascribed to the activity of

facultatively anaerobic bacteria utilising the small amount of oxygen present, and to inhibition of the methanogenic bacteria by traces of oxygen and a raised redox potential. A similar initial increase in carbon dioxide production compared with methane evolution has been observed previously (Mountfort and Asher, 1978; Mackie and Bryant, 1981). Thus it would seem likely that the methane concentration immediately prior to feeding may be a slight overestimate of that actually produced during digestion, as half of the total biogas was liberated during the first seven and a half hours of digestion, and during the first half of this period (at least), the methane content of the biogas was lower than that found immediately prior to feeding.

The intra-digester pH was monitored continuously over a 67 hour period by placing a pH electrode permanently in the digester (Fig. 5.3). This revealed only small changes in the digester pH, with the pH falling slightly (mean of 0.03 pH units) when the digester was stirred prior to feeding (Fig. 5.3). Monitoring the digester pH immediately prior to feeding would therefore seem to represent a reasonable indication of the steady-state pH. However, during the temperature shock experiment it was not practicable to have an electrode permanently located in each digester, and an alternative was sought by comparing two further methods (Table 5.1). The measurement of six replicate samples revealed that placing a washed electrode directly in the digester led to a mean overestimate of pH by 0.02 pH units (maximum 0.06 pH units), whilst transferring

the sample to a beaker before measurement led to an overestimate of 0.07 pH units (maximum 0.11 pH units - Table 5.1). The former procedure was therefore adopted in this study. The increase in pH values is presumably attributed to the escape of dissolved carbon dioxide.

It was anticipated that the VFA concentration might also cycle during a 24 hour period. For example if the feed total VFA concentration is 50mM and the concentration in the digester prior to feeding is 2mM, then at a 10 day RT the total VFA concentration immediately after feeding will be approximately 7mM. Thus, it is likely that the VFA concentration will decrease during the 24 hour period. Indeed such changes have been observed in previous studies with cow slurry, where the VFA concentration reached a peak two hours after feeding (and not immediately after feeding), and then declined to basal levels over the remainder of the 24 hour period (Mountfort and Asher, 1978; Mackie and Bryant, 1981). The VFA concentrations recorded immediately prior to feeding therefore represents the lowest found in the digester during a 24 hour period.

SUMMARY

1. The semi-continuous feeding (once a day, seven days a week) of an anaerobic digester operated on SCS caused a 24 hour cycling of biogas production, biogas composition and the VFA concentration. Strict steady-state conditions do not therefore exist.

2. Sampling immediately prior to feeding represented a

reasonable indicator of the digester pH, but may lead to a small overestimation of methane production. The VFA concentration will represent the lowest found during the 24 hour cycle.

5.3 TEMPERATURE SHOCK EXPERIMENTS CONDUCTED AT A 25 DAY RT

5.3.1 Characteristics of the Control Digesters

Once stabilized, there was little daily variation in digester performance (Fig. 5.4 and Table 5.2) and no significant difference between individual digesters prior to the shock treatments (Figs. 5.4 - 5.6). The steady-state characteristics of the digester effluent and feed are summarised in Table 5.2. A gas yield of 0.38m^3 kgVS added^{-1} (methane yield 0.24m^3 kgVS added^{-1}) was obtained and agrees with those obtained in previous studies of the anaerobic digestion of dairy cattle slurry after passage through a mechanical separator (Tables 1.1 and 3.6).

5.3.2 Effect of a Drop in Operating Temperature

The temperature drops of approximately 8°C per day, applied from day seven, had no discernable effect on digester pH (Figs. 5.5 and 5.6), confirming it to be only a

crude indicator of digester instability (Mueller et al., 1959; Pohland and Bloodgood, 1963; Asinari di San Marzano et al., 1981). However, increases in the total VFA concentration (Figs. 5.5 and 5.6) and individual VFA concentrations (Fig. 5.7) verified these to be more sensitive parameters.

Lowering the digester temperature led to successive reductions in the volume of biogas liberated, which was negligible below 10°C (Figs. 5.5 and 5.6). The quality of biogas in the digester headspace increased from 63% methane at 35°C to 79% at 3°C (Fig. 5.6), and was attributed to a raised solubility of carbon dioxide at lower temperatures.

5.3.3 Effect of a Rapid Rise in Digester Operating Temperature

Increasing the digester temperature from 6°C to normal mesophilic conditions in a single step had no discernable effect on digester pH, but the total VFA concentration diminished to the initial steady-state level within two days (Fig. 5.5). The sudden temperature increase was accompanied by a dramatic rise in biogas production and its methane content, and eight days were required before steady-state conditions were re-established (Fig. 5.5). The delay in digester recovery (when the total VFA concentration had diminished within two days) is presumably attributed to the slow degradation of relatively low methane-yielding

cellulosic materials. The cellulolytic bacteria have therefore responded more slowly to the rapid temperature rise than some of the other digester bacteria (eg. those responsible for VFA degradation). Fortunately, however, this does not drastically destabilize the digestion process as holocellulose is non-toxic, and the only effect is a delayed return to steady-state conditions.

5.3.4 Effect of a Slow Rise in the Digester Operating Temperature

A total of ten days elapsed before the digester restabilized when the operating temperature was raised up to 35°C in six daily increments (Fig. 5.6), compared with only eight days when the temperature was raised in a single step. Initial increases in the operating temperature had little effect on the digestion process due to the low operating temperature (over the range 3-21°C); there was only limited biogas production (primarily attributed to expansion and the physical release of carbon dioxide, although a small amount may have been of microbial origin), the intra-digester pH dropped from 7.44 to 7.23 and the total VFA concentration reached a peak of 13.04mM (day 15). This comprised 10.51mM acetate, 1.93mM propionate, 0.12mM i-butyrate, 0.13mM n-butyrate, 0.25mM i-valerate, 0.03mM n-valerate, 0.05mM i-caproate and 0.02mM n-caproate (Fig. 5.7). Digester recovery began once the temperature

was raised above 21°C; previously accumulated substrates were degraded, biogas production and pH increased, and the VFA concentration fell. However, the VFA were not all removed at the same rate, for example the concentration of propionic acid diminished proportionately more slowly than did the concentration of acetic acid (Fig. 5.7). Seven days after initially raising the digester temperature from 3°C (on the second day of operation at 35°C - day 19) the concentration of each acid had fallen to the previous steady-state level.

The small rises in digester temperature brought about successive increases in biogas production, but a fall in its methane content (Fig. 5.6). The largest daily gas volume was produced on the first day of operation at 35°C (day 18), and after four further days the rate of biogas production was equivalent to that in the control digesters.

SUMMARY

1. Lowering the digester temperature from 35°C by approximately 8°C per day brought about a fall in biogas production, but a rise in the quality of biogas in the digester headspace, and the digester VFA concentration.
2. Raising the operating temperature from 3-6°C up to normal mesophilic conditions in one day restored steady-state conditions within eight days; whilst ten days were required when the temperature was raised by approximately 8°C per day.

3. Signs of unbalanced digestion were observed when the operating temperature was raised slowly to 35°C, for example the concentration of propionate fell less rapidly than did the concentration of acetate.

5.4 TEMPERATURE SHOCK EXPERIMENTS CONDUCTED AT A 10 DAY RETENTION TIME

5.4.1 Characteristics of the Control Digesters

After the acclimation period there was again very little variation in the performance of digesters not subjected to shock treatments (Fig. 5.8), and no significant difference between such digesters (Figs. 5.8-5.10). The steady-state characteristics of the digester feed and effluent are summarised in Table 5.2. A gas yield of 0.34 m^3 kgVS added^{-1} (methane yield 0.22 m^3 kgVS added^{-1}) was obtained which falls within the range of yields obtained previously (Tables 1.1 and 3.6).

When no further additions of slurry were made to the digesters maintained at 35°C, the total VFA concentration fell from 1.64mM to an undetectable level (<0.01mM) in a single day, and there was a more gradual decrease in digester pH (Fig. 5.8). Starving the digester also brought about a steady decrease in the quality and quantity of biogas produced (Fig. 5.8).

5.4.2 Effect of a Temperature Drop

Dropping the digester temperature from 35°C to 6°C over four days effected an increase in the VFA concentration from 1.64mM to 14.20 mM (Fig. 5.10), with each acid increasing in concentration (Fig. 5.11). These increases may be attributed to the addition of acids in the feed, and the formation of VFA within the digester. The temperature drops also brought about a decrease in digester pH (Figs. 5.9 and 5.10). This may be at least partly attributed to a rise in the VFA concentration and an increase in dissolved carbon dioxide.

5.4.3 Effect of a Rapid Temperature Rise with Continued Feeding

Raising the digester operating temperature from 6°C to 35°C in a single day re-established steady-state conditions within six days when a 10 day RT was employed. The total VFA concentration fell steadily and the pH increased, so that by day 24 both had reached the value found prior to the shock treatment (Fig. 5.9). The largest daily production of biogas was obtained after two days at 35°C and remained slightly above that found prior to the shock treatment for a further four days (Fig. 5.9). The

methane content of the biogas fell to 56% when the digester reached 35°C, but then improved slowly and within four days was equivalent to that found prior to the temperature shock treatment (Fig. 5.9).

5.4.4 Effect of a Rapid Temperature Rise without Feeding After Day 16

Rapidly raising the operating temperature of a digester to which fresh slurry was not added after day 16 brought about a rapid decrease in the total VFA concentration and a small rise in digester pH (Fig. 5.9). The daily fed and starved digesters initially produced similar quantities of biogas, however after four days at 35°C biogas production from the starved digester represented only half of that evolved from the daily fed digester (Fig. 5.9). The advantages of not feeding a stressed digester were more clearly seen when the operating temperature was raised slowly (section 5.4.6).

5.4.5 Effect of a Slow Temperature Rise with Continued Feeding

Slowly increasing the digester operating temperature from 6°C brought about further rises in the VFA

concentration and further falls in pH (Fig. 5.10). However, once restored to 35°C the VFA concentration began to fall and the pH rise (Fig. 5.10). That digester recovery only commenced when the operating temperature reached 35°C, rather than 21°C (as in the previous cases), may be attributed to the extremely high VFA concentration (46.61mM on day 21) proving inhibitory (Mueller et al., 1959; Pohland and Bloodgood, 1963; Kroeker et al., 1979). Complete digester recovery required at least eight days at 35°C (12 days since the temperature was first raised).

The large increase in the total VFA concentration (Fig. 5.10) was not solely attributed to the addition of acids in the feed. The total VFA concentration and that of a majority of the individual acids on day 21 (when the highest VFA concentration was reached) was larger than that anticipated from feed addition alone, and must therefore be due to VFA formation within the digester (Fig. 5.11 and Table 5.3). Furthermore, individual acids did not respond in the same manner (Fig. 5.11 and Table 5.3). More details of the imbalance between the individual VFA concentrations was noted when the total VFA concentration began to drop (Fig. 5.10), and the individual acids were removed at different rates (Fig. 5.11). The largest concentrations of acetate, n-butyrate and n-caproate were found on day 21, whilst propionate, i-butyrate and n-valerate all peaked on day 23, and i-valerate and i-caproate peaked three days after this (Fig. 5.11). Furthermore, on day 29 the digester

had been at 35°C for eight days but the total VFA concentration (10.59 mM) was still well above that found prior to the shock treatment (Fig. 5.11 and Table 5.4). The total VFA concentration now represented 23% of that found on day 21, acetate 17%, propionate 36%, n-butyrate 1% and i-valerate 96% (Fig. 5.11 and Table 5.4). The temperature shock treatment therefore elicited large changes in the total acid concentration, but all the VFA did not respond in the same manner.

From these observations an order of response to digester instability has been determined for the individual VFA. Although acetate remained the predominant acid in all circumstances, the concentrations of the branched acids were proportionately more susceptible to the temperature shock treatment (in respect of their original concentration in steady-state reactors) than acetate or n-butyrate. The order of response is: i-butyrate ~ i-valerate ~ i-caproate > propionate > n-valerate ~ n-caproate > n-butyrate ~ acetate. Those to the left accumulated proportionately more rapidly and were degraded proportionately less quickly during the temperature shock treatments. Thus the total acid concentration or acetic acid concentration would appear not to be the most sensitive indicator of digester instability, and it would be more appropriate to monitor changes in the concentration of branched VFA or propionic acid.

Raising the digester temperature by approximately 8°C per day brought about successive increases in biogas production (Fig. 5.10). It is likely that the initial

increases were at least partly attributed to expansion and the physical release of carbon dioxide, for similar quantities of biogas were released from the daily fed and starved digesters (Fig. 5.10). After two days at 35°C the rate of biogas production exceeded that found prior to the shock treatment, and remained slightly elevated for at least a further seven days. The methane content of the biogas initially fell as the digester operating temperature was raised, but once restored to 35°C began to increase again (Fig. 5.10).

5.4.6 Effect of a Slow Temperature Rise Without Feeding After Day 16

Slowly raising the operating temperature of a digester to which fresh slurry was not added after day 16, brought about a much more rapid drop in the VFA concentration than found in the daily fed digester (Fig. 5.10). Once the temperature was raised above 20°C, the VFA concentration began to fall and reached previous steady-state levels within three days. This fall was accompanied by a fall and then a rise in the pH. That the VFA concentration and pH both fell initially may be taken as evidence that the VFA concentration is not the only factor influencing the digester pH.

The increase in biogas production was similar to that found in the daily fed digester for the first four

temperature increases (Fig. 5.10). However, the rate of biogas production then fell away rapidly in the starved digester, and 12 days after the temperature increase it was only one-tenth of that liberated from the daily fed digester (Fig. 5.10). The continuing production of biogas is attributed to the degradation of holocellulose and other accumulated material. The cessation of feeding therefore helped prevent digester overloading and may also have enhanced digester recovery, as when feeding was resumed on day 29 (after a break of 12 days) normal biogas production commenced within four days (results not shown). However, further work remains to be carried out, to determine the starvation period required to bring about the most rapid digester recovery.

SUMMARY

1. Reducing the digester operating temperature brought about a large increase in the VFA concentration. This was attributed to the non-degradation of acids added in the feed and to VFA formation in the digesters. The individual VFA did not all respond in the same manner to the shock treatment.

2. Raising the operating temperature from 6°C to 35°C restored biogas production, and effected a decrease in the VFA concentration. When the temperature was increased in a single day steady-state conditions were re-established within six days, whilst at least twelve days were required

when the operating temperature was raised slowly.

3. Starving the digesters whilst the temperature was restored to 35°C prevented severe digester overloading and may bring about a more rapid recovery from the temperature shock treatment.

5.5 DISCUSSION

The operating temperature of laboratory-scale digester was lowered from 35°C to 3-6°C in a similar manner to that anticipated on a full-scale digester due to heating failure, and two different recovery procedures were employed. Raising the temperature back up to 35°C in a single day restored steady-state conditions within six days when a 10 day RT was employed, and within eight days at a 25 day RT. However, when the temperature was raised over several (five to six) days, at least twelve days were required at a 10 day RT, and ten days at a 25 day RT before steady-state conditions were re-established. It is therefore proposed that the original digester operating temperature should be restored as soon as possible after a heating failure. The more rapid recovery when the operating temperature was raised to 35°C in a single step is attributed to the immediate removal of potentially toxic VFA, and to the prevention of the further build-up of undegraded feed and recalcitrant intermediates. Starving the digester during the recovery period also prevented the

further accumulation of toxic VFA and may enhance digester recovery. However, further work is required to determine the period of digester starvation that may bring about the most rapid recovery following temperature shock.

The different response of the individual VFA to the temperature shock may provide an insight into the interactions in an anaerobic digester. An order of sensitivity of VFA to the shock treatment has been established: i-butyrate ~ i-valerate ~ i-caproate > propionate > n-valerate ~ n-caproate > acetate ~ n-butyrate. Those to the left accumulated proportionately more rapidly in the temperature stressed digester and were removed proportionately less quickly during the recovery period, although acetate remained the predominant acid. The response of the straight-chain VFA is, to a large extent, consistent with the sensitivity of their degradation to molecular hydrogen (Boone and Bryant, 1980; Hanaki et al., 1981). The effect of hydrogen on acetate degradation is less clearly understood, no effect would be anticipated on thermodynamic grounds, although it has been reported to be inhibitory in some, but not all, cases (Harper and Pohland, 1986). Although no monitoring of the hydrogen partial pressure was possible in this study, it has been found to rise during periods of instability in previous studies (Barnes et al., 1983; Whitmore et al., 1985; Archer et al., 1986; Whitmore and Lloyd, 1986; Mosey, Personal Communication). Such an increase would most likely be attributed to a fall in activity of the

H_2-utilising methanogens (the chief hydrogen consuming bacteria - see section 1.2), perhaps as a consequence of a greater sensitivity to the temperature changes employed.

However, although the proportional increase in the acetate concentration was not as large as for other VFA, it did rise during periods of instability, and always remained the most abundant acid. Thus, the activity of the acetate-utilising methanogens was also restricted during the temperature shock treatment. Indeed, it is likely that the turnover of acetate may fall during periods of instability (since higher VFA were not degraded), and that the acetate concentration may not, therefore, have increased as much as might otherwise be anticipated. Furthermore, these studies do not preclude the possibility that the acetate-utilising methanogens were the group of organisms most susceptible to the temperature shocks applied, and that a rise in the acetate concentration inhibited the obligate proton-reducing bacteria (see section 1.3). The larger increase in the concentration of propionate than n-butyrate, would however render this possibility doubtful, since the oxidation of n-butyrate is more thermodynamically sensitive to acetate than the oxidation of propionate. Only by the careful monitoring of each of the individual steps of digestion can the above assertions be verified or refuted, and the complex interactions in anaerobic digestion fully understood.

The routes of formation and degradation of branched VFA are not as well understood as those of straight chain

acids, and have been briefly considered in section 1.2.2. The concentrations of branched VFA were all proportionately more sensitive to the temperature shock treatment than the concentration of the straight chain acids. This is likely to be due to an increase in formation (due perhaps to a rise in protein degradation, the isomerization of straight chain acids, or alternative routes of formation (see Table 1.7)), and their recalcitrance to degradation. In other studies where glucose was the substrate provided, a rise in the concentration of branched VFA was not observed (eg. Cohen et al., 1980), perhaps as a result of negligible protein degradation.

Table 5.1 Comparison of Methods of Determining the pH of Separated Cattle Slurry Digesters Operated at 35°C and a 25 day RT.

Replicate	pH in digester (after stirring) (a)	pH with washed electrode (b)	pH determined in beaker (c)
1	7.22	7.24	7.28
2	7.22	7.22	7.29
3	7.22	7.23	7.29
4	7.15	7.21	7.26
5	7.18	7.23	7.25
6	7.16	7.16	7.22
mean	7.19 (0.03)	7.21 (0.03)	7.26 (0.03)

Notes

(a) pH in digester after stirring. The pH was continuously recorded with an electrode permanently in the digester.

(b) pH with a washed electrode. After determination (a) the electrode was removed, washed and returned to the digester for determination (b).

(c) pH determined in a beaker. A sample was removed from the digester, transferred to a beaker and measured immediately.

(d) The values in brackets represent the standard deviation.

Table 5.2 Summary of Steady-state Characteristics of Digesters Operated at 35°C and a 25 and 10 day RT.

	Undigested Slurry 25 day RT	Digested Slurry 25 day RT	Undigested Slurry 10 day RT	Digested Slurry 10 day RT
pH	7.14/0.07/17	7.44/0.04/37	7.05/0.11/21	7.46/0.05/16
Total VFA Conc.(mM)	68.49/7.13/16	1.06/0.14/16	57.63/10.21/22	1.64/0.37/16
Acetate (mM)	51.91/5.87/16	0.80/0.13/16	43.24/7.92/22	1.38/0.27/16
Propionate (mM)	10.27/1.01/16	0.17/0.01/16	9.59/1.75/22	0.22/0.08/16
i-butyrate (mM)	1.02/0.13/16	0.02/0.00/16	0.65/0.06/22	0.01/0.00/16
n-butyrate (mM)	3.34/0.32/16	0.03/0.00/16	2.61/0.47/22	0.02/0.00/16
i-valerate (mM)	1.20/0.14/16	0.02/0.00/16	0.92/0.16/22	0.01/0.00/16
n-valerate (mM)	0.36/0.03/16	0.01/0.00/16	0.38/0.05/22	<0.01/16
i-caproate (mM)	0.38/0.03/16	0.01/0.00/16	0.19/0.02/22	<0.01/16
n-caproate (mM)	0.01/0.00/16	<0.01/16	0.05/0.01/22	<0.01/16
%Total Solids	4.31/0.36/30	2.74/0.20/ 8	4.14/0.34/21	2.83/0.10/15
%Volatile Solids	3.53/0.31/30	2.08/0.21/ 8	3.41/0.32/21	2.13/0.11/15
Daily Gas Volume (l)	-	2.69/0.28/42	-	5.80/0.26/52
%methane in biogas	-	62.8/0.9 /37	-	64.5/0.8 /40
Mean %VS destruction	-	41	-	38
GY (m^3kgVS added^{-1})	-	0.38	-	0.34
MY (m^3kgVS added^{-1})	-	0.24	-	0.22
GY (m^3kgVS destryd^{-1})	-	0.93	-	0.91

Notes

Values represent: mean/standard deviation/no. samples
GY - Gas Yield (mean value)
MY - Methane Yield (mean value)

Table 5.3 Comparison of Predicted (based on VFA additions) and Actual Volatile Fatty Acid Concentrations on Day 21 in the SCS digester at a 10 day RT.

VFA (mM)	Actual Conc. Day 13	Predicted Conc. * Day 21	Actual Conc. Day 21	Concn. Act-Pred	% diff. Act-Pred / Pred
Acetate	0.47	23.34	33.61	10.27	44
Propionate	0.04	5.14	9.40	4.26	83
i-butyrate	0	0.34	0.95	0.61	179
n-butyrate	0	1.40	0.85	-0.55	-39
i-valerate	0	0.49	1.15	0.66	135
n-valerate	0	0.21	0.35	0.14	67
i-caproate	0	0.10	0.20	0.10	100
n-caproate	0	0.03	0.10	0.07	233
TOTAL	0.51	31.05	46.61	15.56	50

* The predicted concentration on day 21 is calculated assuming that there has been no VFA degradation since the temperature was lowered on day 13.

Table 5.4 A Comparison of Individual Volatile Fatty Acid Concentrations on Day 21 and Day 29 in SCS Digester Operating at a 10 day RT.

VFA (mM)	Concentration on Day 21	Concentration on Day 29	% Remaining on Day 29
Acetate	33.61	5.82	17
Propionate	9.40	3.41	36
i-butyrate	0.95	0.04	4
n-butyrate	0.85	0.01	1
i-valerate	1.15	1.10	96
n-valerate	0.35	0.01	3
i-caproate	0.20	0.20	100
n-caproate	0.10	<0.01	0
TOTAL	46.61	10.59	23

Fig. 5.1 Plot of Hourly Gas Production from a 5-litre Laboratory Scale Digester Operated on Separated Cattle Slurry at 35°C and a 25 day RT

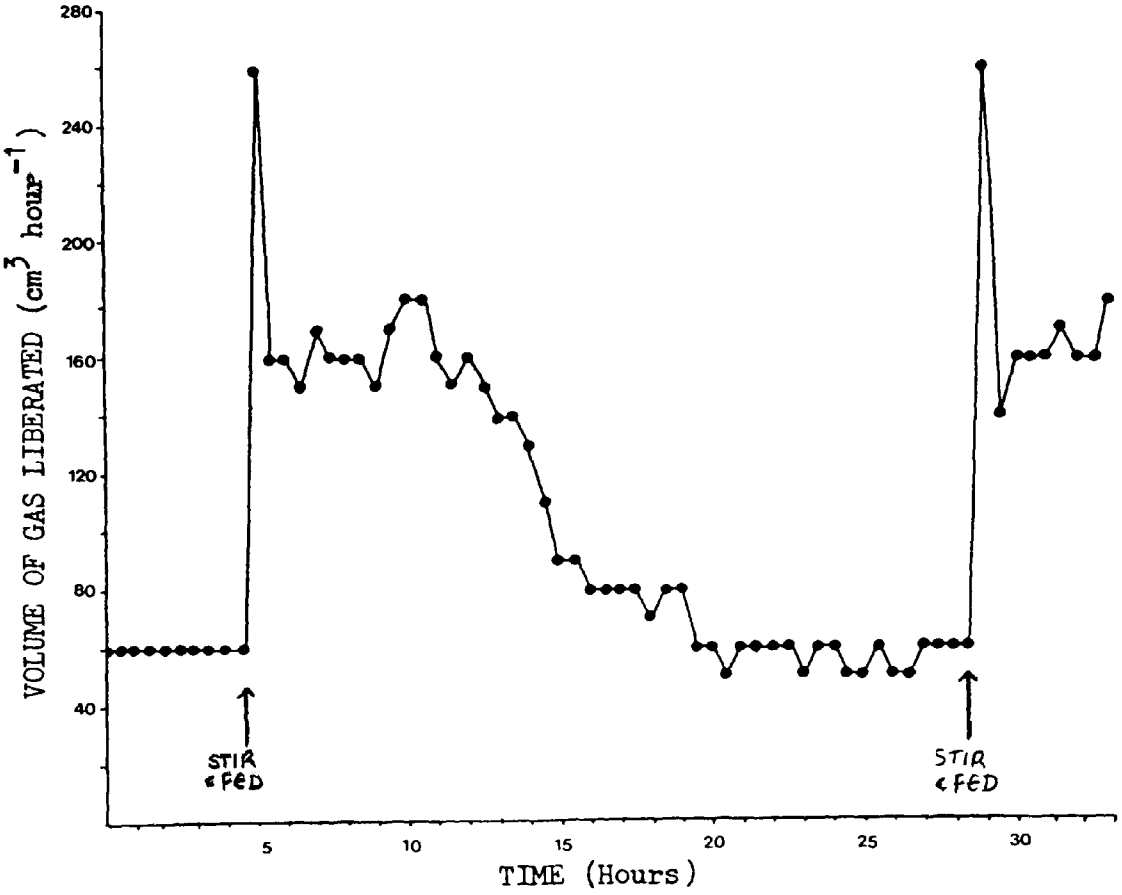


Fig. 5.2 Variations in the Composition of Biogas in the Headspace of a 5-litre Laboratory Scale Digester Operated on Separated Cattle Slurry at 35°C and a 25 dayRT.

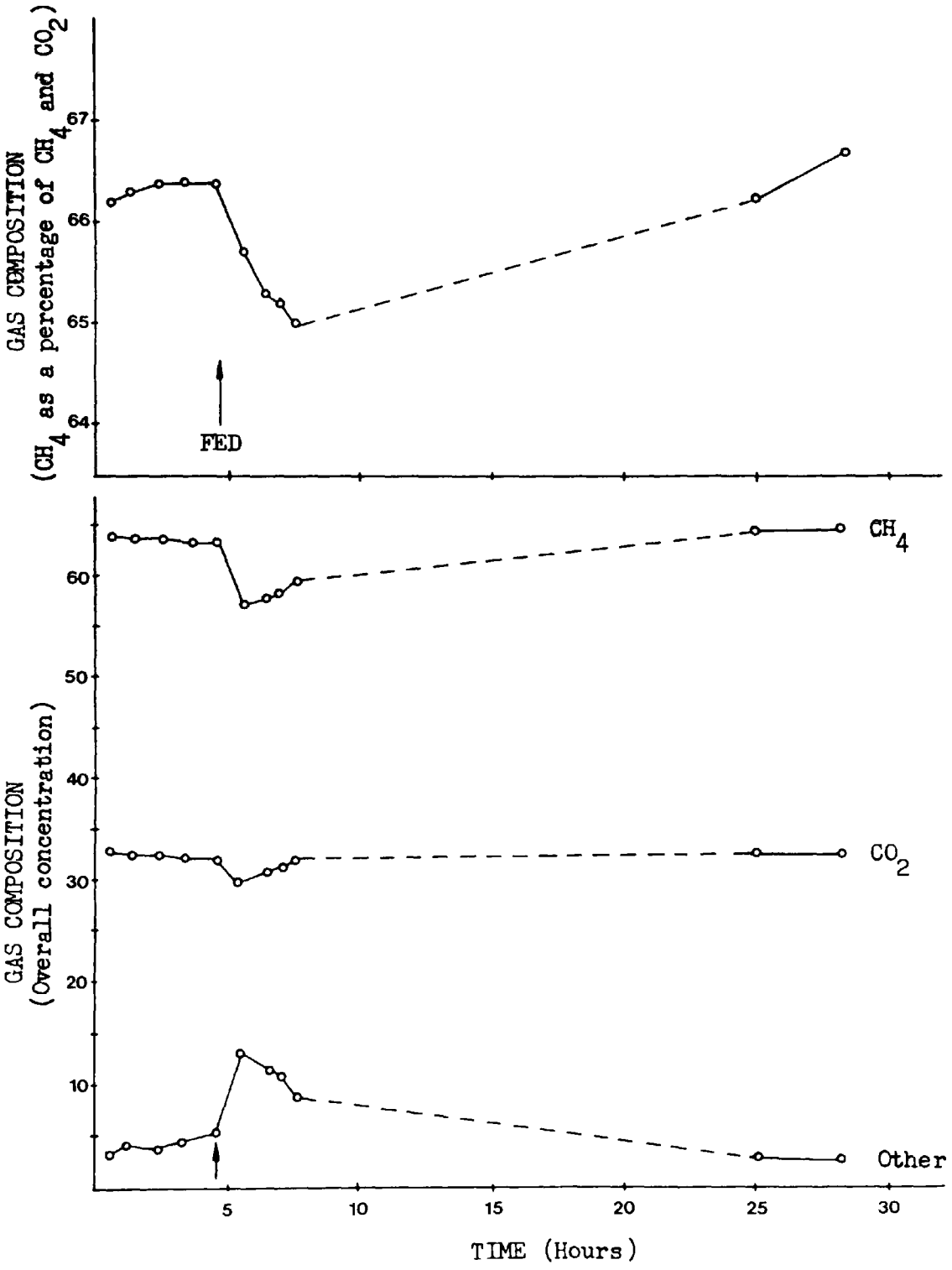


Fig. 5.3 Variations in Intra-digester pH of 5-litre Laboratory Scale Reactor Operated on Separated Cattle Slurry at 35°C and a 25 day RT

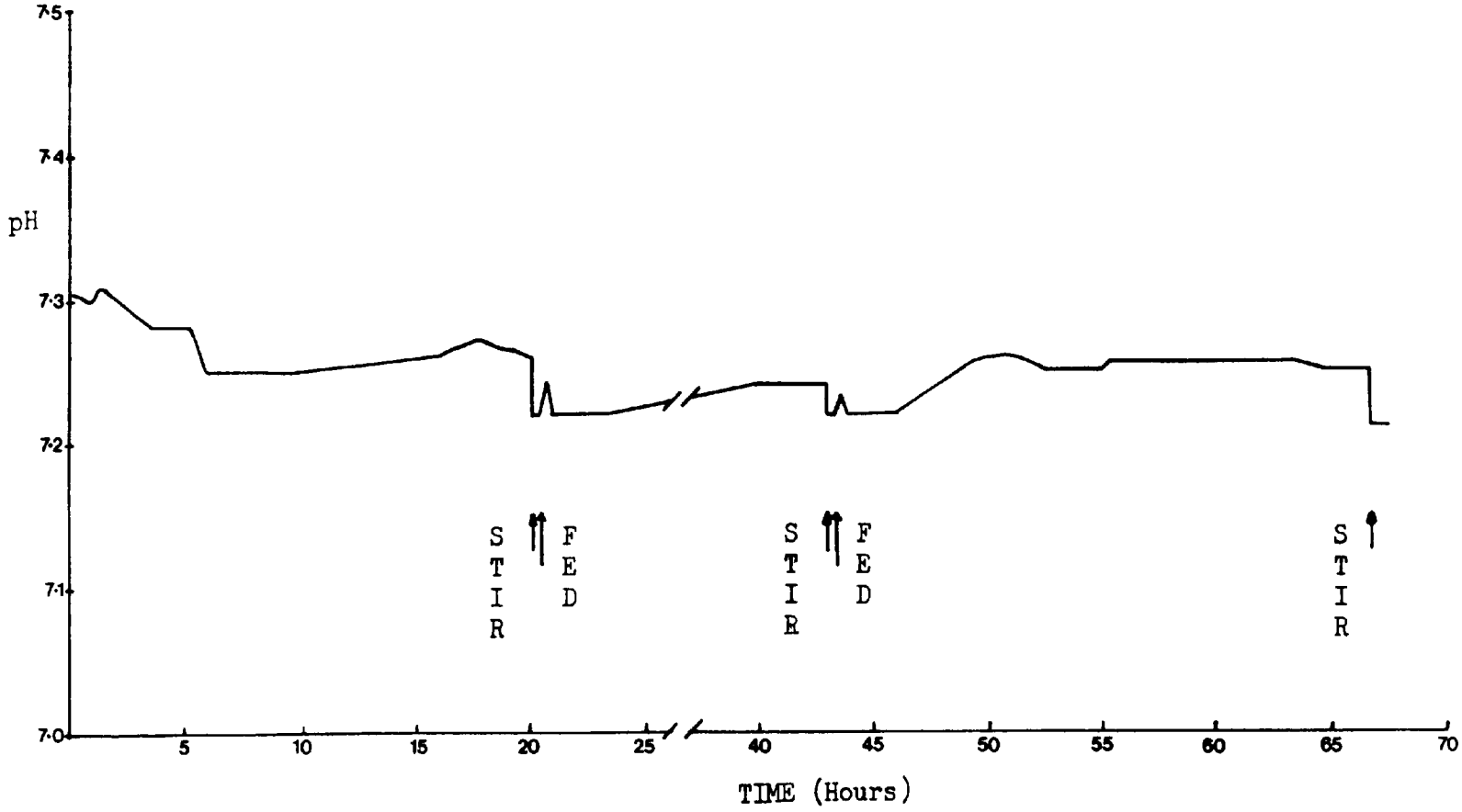


Fig. 5.4 Operation of Digesters on a 25 day RT and a Constant Temperature of 35°C

Fig. 5.5 Effect of Temperature Fall and then Rapid Temperature Rise on Digesters Operated on a 25 day RT

Fig. 5.6 Effect of Temperature Fall and then Slow Temperature Rise on Digesters Operated on a 25 day RT

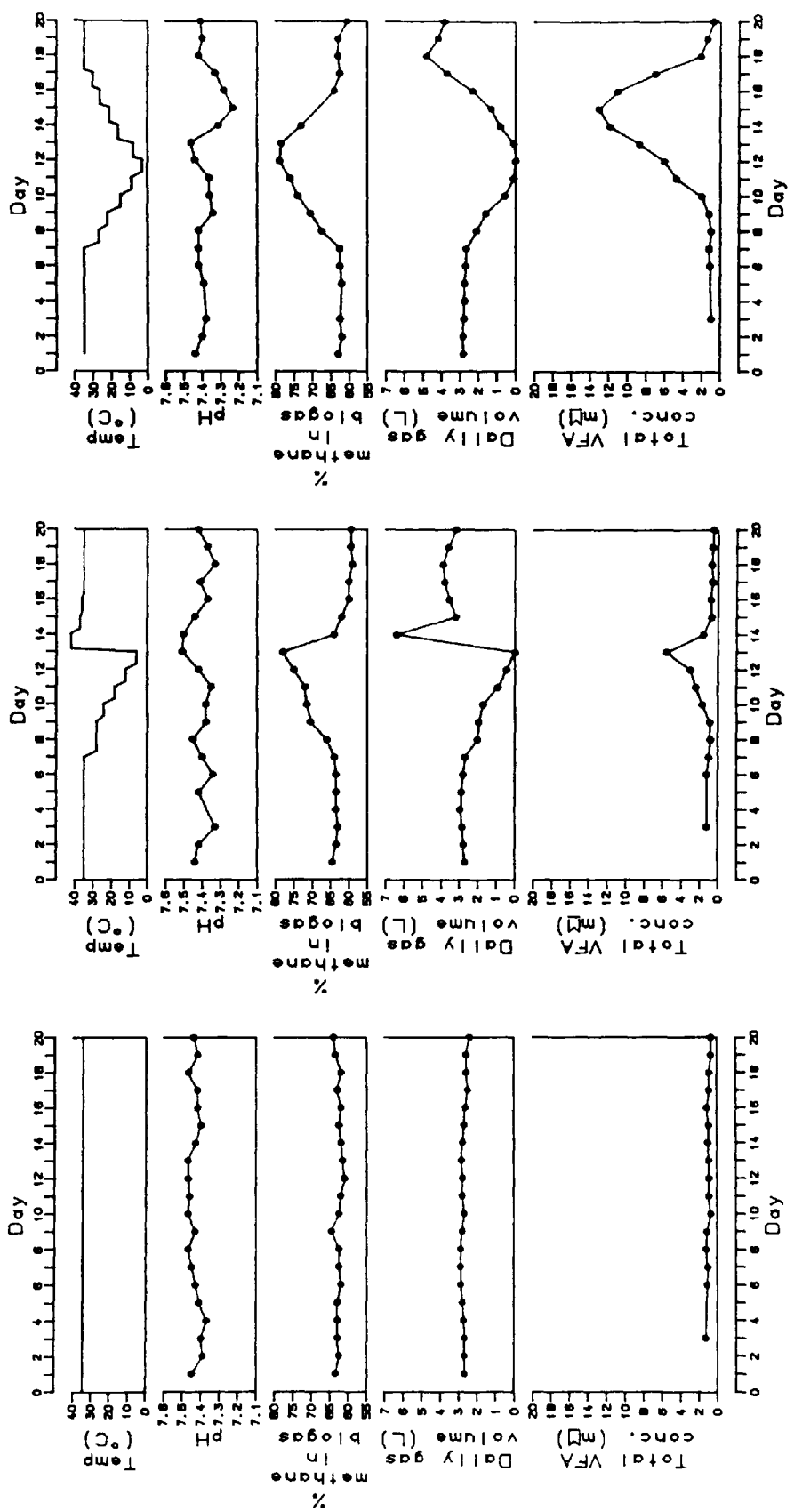


Fig. 5.4

Fig. 5.5

Fig. 5.6

Fig. 5.7 Effect of Temperature Fall and then Slow Temperature Rise on the Concentration of Individual VFA in Digesters Operated on a 25 day RT

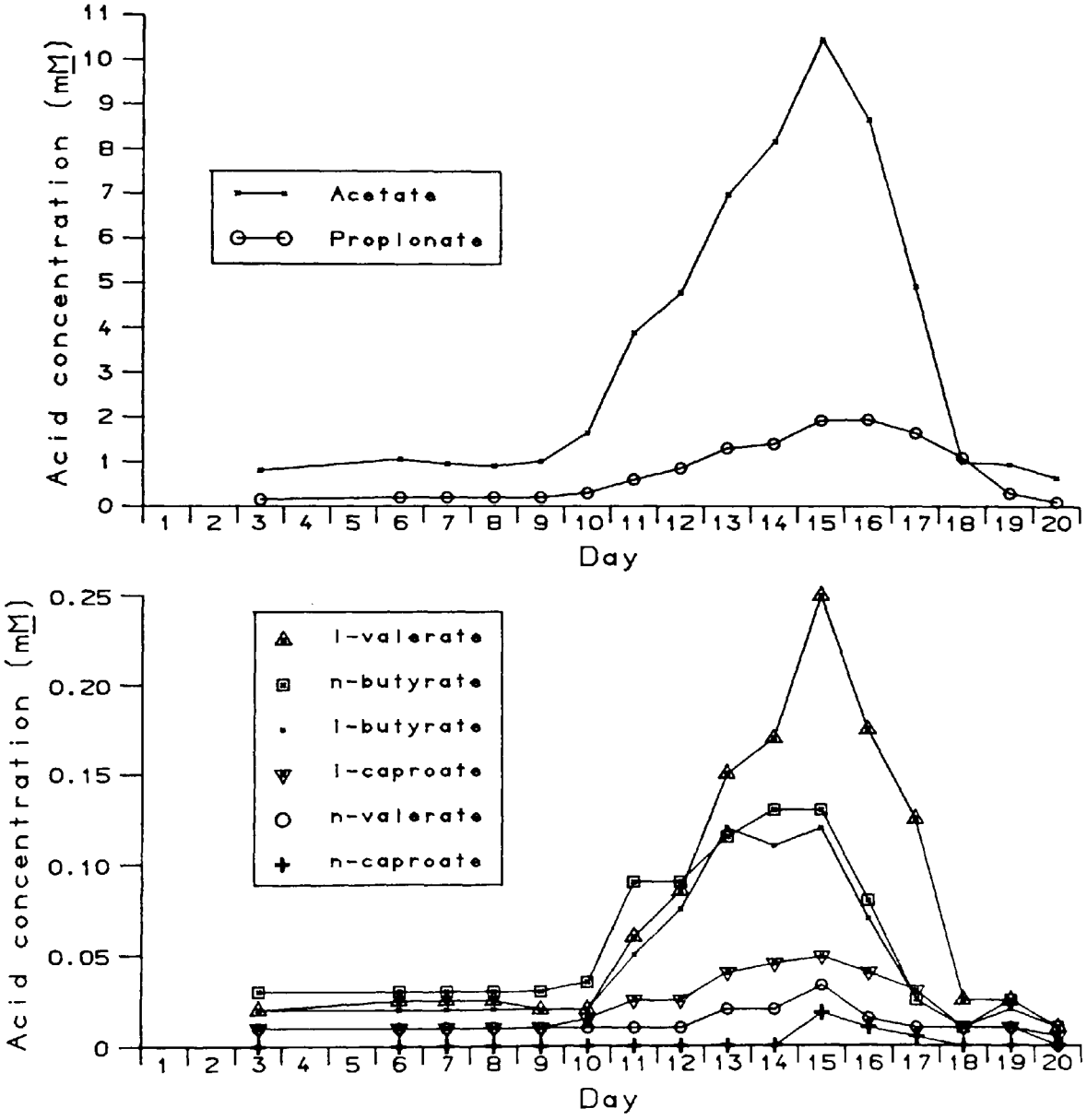


Fig. 5.8 Operation of Digesters on a 10 day RT and a Constant Temperature of 35°C (digester fed daily ●-●; digester not fed x-x)

Fig. 5.9 Effect of Temperature Fall and then Rapid Temperature Rise on Digesters Operated on 10 day RT (digester fed daily ●-●; digester not fed x-x)

Fig. 5.10 Effect of Temperature Fall and then Slow Temperature Rise on Digesters Operated on a 10 day RT (digester fed daily ●-●; digester not fed x-x)

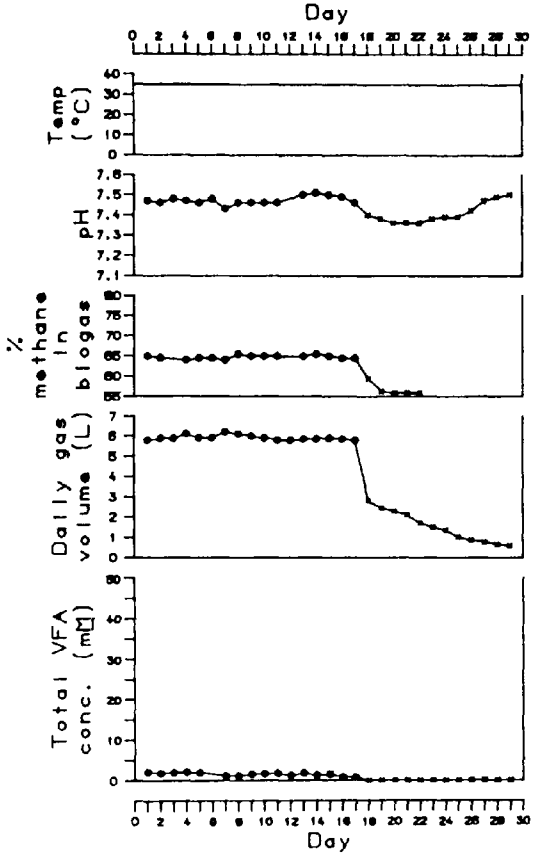


Fig. 5.8

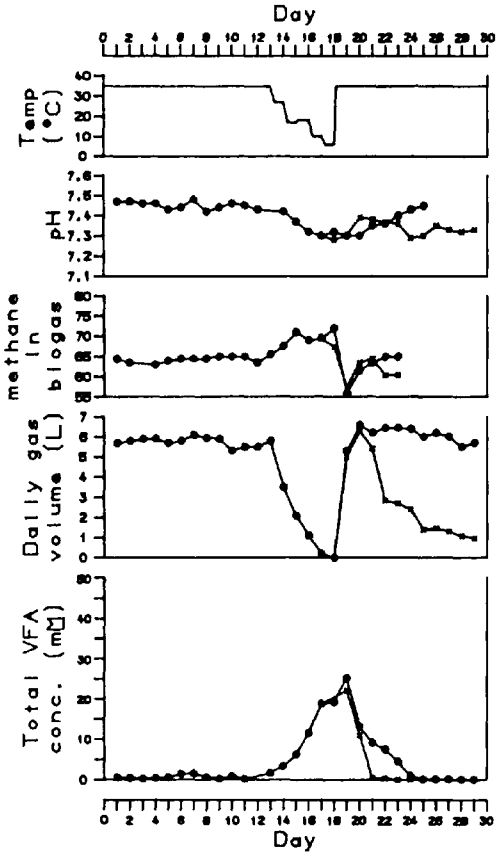


Fig. 5.9

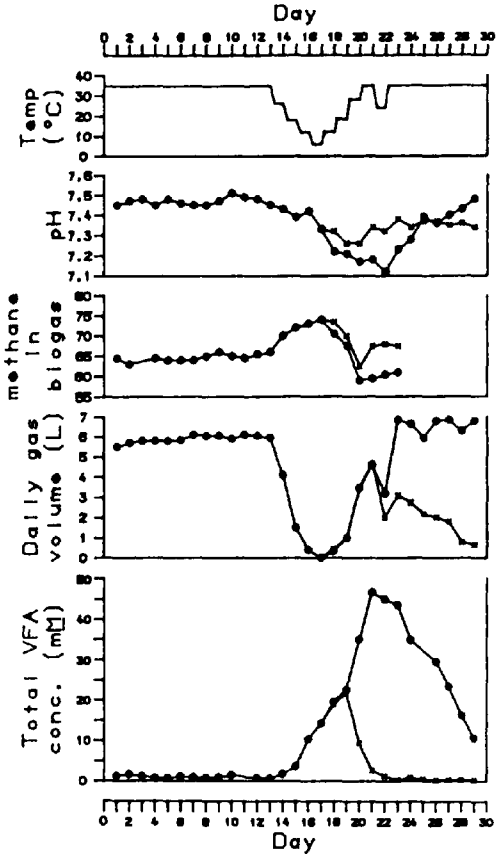
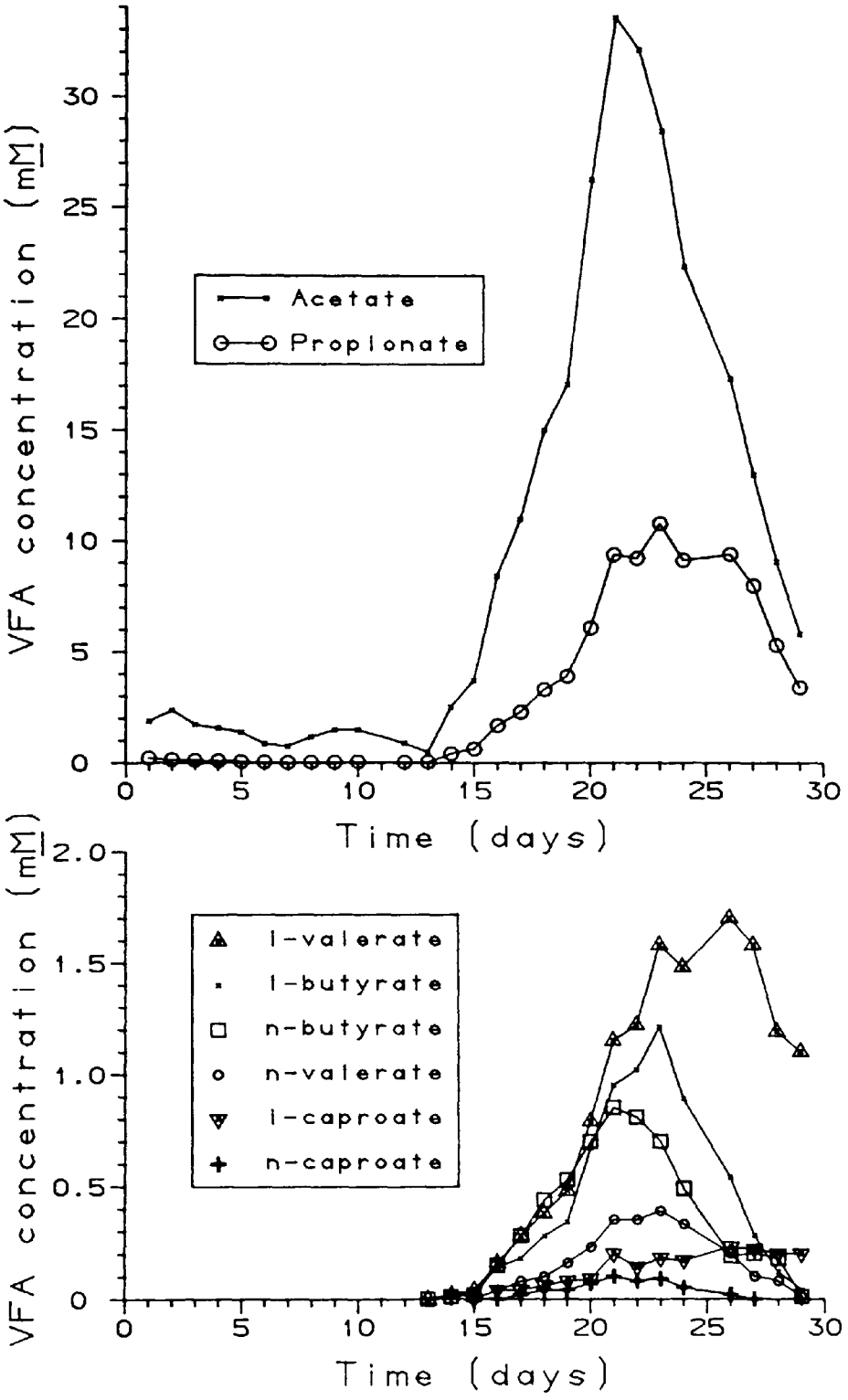


Fig. 5.10

Fig. 5.11 Effect of Temperature Fall and then Slow Temperature Rise on the Concentration of Individual VFA in Digesters Operated on a 10 day RT



CHAPTER SIX

THE ANAEROBIC DIGESTION OF SEPARATED CATTLE SLURRY IN AN UPFLOW ANAEROBIC FILTER

6.1 INTRODUCTION

Advanced reactors are primarily designed for use with rapidly degraded soluble wastes, and are not therefore particularly well suited to the anaerobic digestion of dairy cattle slurry, which is both slowly degraded and highly fibrous. However, after pretreatments that removed nearly all of the particulate material and lowered the solids concentration below 1% TS, cattle slurry has been treated in most advanced reactors (although apparently not the upflow anaerobic filter - see section 1.4); and SCS (4%TS) has been digested in a DSFF reactor (Liao and Lo, 1985). It is, however, anticipated that as a result of the greater surface area available for bacterial attachment in an upflow anaerobic filter, higher rates of biogas production may be achieved from SCS than found previously with a DSFF reactor.

The rate limiting step in the digestion of a particulate waste material such as SCS is believed to be the initial hydrolysis of particulate solids rather than one of the soluble steps (Hobson, 1981; Archer, 1983; Gujer and Zehnder, 1983). In these circumstances, therefore, it is envisaged that digestion may be only slightly enhanced by the attachment of bacteria to a support matrix, as the cellulolytic bacteria need to be closely associated with the substrate to effect hydrolysis. However, at short retention times where digestion may be overloaded and approaching failure, the presence of an attached biomass may lead to improved rates of biogas production. The retention of

methanogenic and obligate proton-reducing bacteria may prevent the accumulation of potentially toxic compounds such as VFA, and thereby reduce digester overloading.

Experiments were conducted to determine the feasibility of operating an upflow anaerobic filter on SCS, and ascertain whether the performance of such a reactor was superior to that of a conventional digester.

6.2 OPERATION OF THE UPFLOW ANAEROBIC FILTER

6.2.1 Start-Up

To start up the upflow anaerobic filter (design described in section 2.2.4), 10 litres of SCS (analysis - Table 6.1), 8 litres of deionised water and 2 litres of seed (from SCS digester operated at 35°C and a 25 day RT (see Chapter 3), were added to the reactor, the headspace was flushed with oxygen-free nitrogen and incubated at 35°C without slurry recirculation. Feeding of the digester commenced after 15 days, and a 10 day RT was employed. Despite frequent blockages (days 19-22, 26 and 29-31) the rate of biogas production and its methane content increased steadily over the next 18 days (Fig. 6.1). However, a heating failure (day 33) and blockages did not permit steady-state conditions to be established and the anaerobic filter was maintained in an unheated room (December 1984 - January 1985) from days 39 to 53 without feed addition or

slurry recirculation.

On day 53 the operating temperature was raised to 35°C, and on day 55 feeding and slurry recirculation resumed. The anaerobic filter was now operated on a 14.3 day RT (Fig. 6.1). After one retention time the weekly gas yields were calculated over a five week period (Table 6.2), until a heating failure again brought experimentation to an end on day 100. Despite operating difficulties which led to the digester not being fed on days 87 or 88, relatively consistent gas yields were obtained during these weekly periods (Table 6.2). A mean gas yield of 0.37 m^3 kgVS added^{-1} was obtained (methane yield 0.24 m^3 kgVS added^{-1}) and the values ranged from 0.35-0.41 m^3 kgVS added^{-1} (methane yield 0.21-0.26m^3 kgVS added^{-1}). These gas yields compare favourably with those obtained from SCS in conventional digesters (Tables 1.1, 3.6 and 5.2) operating at a similar retention time.

6.2.2 Comparison of Upflow Anaerobic Filter and Conventional Digester

Although the upflow anaerobic filter had been successfully operated at a 14.3 day RT it was essential to compare its performance directly with a conventional digester to determine whether digestion was indeed enhanced. A 5 day RT was chosen for this comparison, as this was the shortest retention time at which conventional SCS digesters

had been successfully operated at in our laboratory (Hawkes et al., 1984). A conventional 5 l digester was set up on day 111 with 4 l effluent from the filter and one litre of SCS (analysis - Table 6.1). The anaerobic filter was fed 4 l SCS on this day. To ensure that digestion proceeded smoothly, both digesters were initially operated on a 11.7 day RT for 10 days (from day 114). During this period the gas yield (and methane yield) from the anaerobic filter was not significantly greater than that from the conventional digester (Fig. 6.2 and Table 6.3). From day 125 both digesters were operated on a 5 day RT until feed stocks were exhausted. No blockage problems were encountered in the anaerobic filter during this period, although there was a heating failure (days 133 - 135). The filter was fed in the usual manner during this period and the results have not been corrected for any reduction in biogas production during operation at the lower temperature.

When the retention time was dropped from 11.7 days to 5 days (day 124), biogas production increased from both digesters (Fig. 6.2), and more biogas was liberated (per 20 l digester) from the anaerobic filter than the conventional digester on each day of operation at this retention time (Fig. 6.2 - except during temperature failure). Biogas production (and the weekly gas yield) increased slightly in the filter during operation at a 5 day RT (Fig. 6.2 and Table 6.3), suggesting that steady-state conditions had not been reached and that higher rates of biogas production (and gas yields) may be attained. However, biogas production

(and the weekly gas yield) decreased during operation of the conventional digester at a 5 day RT (Fig. 6.2 and Table 6.3), with the result that the anaerobic filter produced 14% more biogas (and methane) than the conventional digester during the first week of operation and 73% more biogas (82% more methane) during the third week (Table 6.3). Thus it is likely that the anaerobic filter is capable of operation at a 5 day RT, whilst the conventional digester may be failing.

The biogas produced from the two digesters was of a similar quality until day 136, when the methane content of biogas from the conventional digester fell away towards the end of each week (Fig. 6.2). The fall in biogas quality is a further symptom that the conventional digester was overloaded and approaching failure. The lower methane content of the biogas at the end of the week may be attributed to the feeding procedure adopted, whereby the digester was overloaded during the week but recovered to some extent over the weekend. Higher concentrations of hydrogen sulphide were recorded in the biogas liberated from the conventional digester (3000 ppm on day 143, 3100 ppm on day 144) than the anaerobic filter (1550 ppm on day 143, 1100 ppm on day 144).

During operation on a 11.7 day RT the highest total VFA concentration recorded in the anaerobic filter and the conventional digester were 5.0mM and 1.6mM respectively (Fig. 6.2). When the retention time was dropped to five days the VFA concentration increased in both digesters. The

total VFA concentration in the anaerobic filter reached 12.2mM (Fig. 6.2), whilst the concentration in the conventional digester rose to 40.7mM (approximately 2700ppm). In both digesters the VFA concentration increased gradually during the week and then fell back during the weekend (except during temperature failure in the anaerobic filter). This is again attributed to the feeding procedure adopted. The increase in the VFA concentration was not simply due to the non-degradation of acids added in the feed, but also to acid formation within the digester, for each acid increased in a different proportion to that found in the feed, and some were even found at a greater concentration than in the feed (Fig. 6.3 and Table 6.1). For example, between days 139 and 144 the concentration of acetate in the conventional digester was 52-80% of the feed value, whilst propionate was 100-110% of the feed concentration, i-butyrate 120-136% and n-butyrate 15-42%.

When feeding was stopped on day 144, biogas production from the conventional digester increased slightly, whilst production from the anaerobic filter fell rapidly (Fig. 6.2). The continuation of biogas production from the conventional digester is attributed to the degradation of accumulated VFA. However, after day 151 more biogas was produced from the filter (Fig. 6.2), this may be due to the greater retention of holocellulose in the filter. Possible consequences of this retention of holocellulose are considered later.

The enhanced performance of the upflow anaerobic

filter compared with the conventional digester at a 5 day RT is presumably attributed to the activity of the biomass associated with filter support rings preventing digester overloading. This permitted digestion in the anaerobic filter to approach steady-state conditions, whilst digestion in the conventional digester became unbalanced and failure appeared probable. The accumulation of VFA in the conventional digester had two main effects on the digestion process. Firstly, the higher VFA concentration in the effluent resulted in a greater loss of a potential source of methane than from the anaerobic filter. It can be calculated (for the highest VFA concentration on day 139) that an extra 9.2 l biogas would be produced per day (per 20 l digester volume) from the conventional digester if all the VFA had been degraded (a 43% increase) whilst only 2.7 l biogas day^{-1} (per 20 l digester volume) would have been produced from the anaerobic filter (a 7% rise). Secondly, and of greater importance, is the inhibitory effect of the high VFA concentrations reached in the conventional digester which, if allowed to rise much further, would have resulted in the souring and complete failure of digestion. The possibility that other compounds (eg. hydrogen) might also reach inhibitory concentrations should not be eliminated.

6.2.3 Dismantling of the Anaerobic Filter

On day 166 the anaerobic filter was dismantled. All

of the rings were covered in a slime layer (plates 1 and 2) and a considerable amount of solid material was retained within the rings, particularly in the lower half of the digester. Viable biomass determinations were carried out on support rings removed from six different heights within the digester. These revealed there to be a significant biomass attachment (Table 6.4). The ATP content of the slime associated with the rings ranged from $2.2\text{-}8.8\times10^{-8}$ moles $ring^{-1}$ (there were approximately 275 rings in the filter). In a previous study the mean ATP concentration of a conventional digester operated on SCS (25 day RT and 35°C) was 1×10^{-6} M. Therefore, the viable biomass associated with a single digester support ring ranged from 22 to 88 times that found in 1 ml conventional digester. The greater viable biomass attached to rings at the bottom of the digester is attributed to these rings having a greater slime attachment. Further evidence that micro-organisms were closely associated with the filter support rings was obtained when scanning electron microscopy (SEM) preparations of the rings were examined (plates 3 - 6). Groups of bacteria were identified on these preparations as being associated with the support matrix.

With such a large accumulation of solid material in the anaerobic filter (particularly in the lower half), it could be suggested that the enhancement of digestion was, to some extent at least, due to the trapping of solids within the support matrix. To determine how strongly the solids were attached to the support rings the reactor was filled

with water which was pumped upwards at 45 litres hour^{-1} (maximum pump speed) for several hours, and then pumped downwards at the same speed. This treatment removed some of the attached slime, but had no effect on the solids trapped within the matrix. The solids retention time was therefore probably greater than the hydraulic retention time in the anaerobic filter. Furthermore, it was also demonstrated that the flow of slurry within the digester had become channelled and that some of the solids were not mixing with the recirculating liquid. It is anticipated that the accumulation of solids within the filter rings may account for the frequent blockages encountered and eventually completely block the reactor. The enhancement of digestion might therefore also be attributed to the entrapment of solids and bacteria within the matrix, in addition to the looser association of bacteria with the filter support rings.

SUMMARY

1. An upflow anaerobic filter has been successfully operated at a 5-14 day RT with SCS as the substrate. However, solids did accumulate within the reactor and blockages were frequently encountered.

2. An upflow anaerobic filter and conventional digester have been compared directly at 35°C and a 5 day RT with SCS as the substrate. The conventional digester was overloaded, digestion was unbalanced and failure appeared

likely, whereas the anaerobic filter approached steady-state conditions and a mean gas yield of 0.26m^3 kgVS added^{-1} (mean methane yield 0.18m^3 kgVS added^{-1}) was obtained.

3. It is likely that the enhanced performance of the anaerobic filter was partly a consequence of the retained biomass maintaining low VFA concentrations and preventing digester imbalance, and partly due to the accumulation of solids giving a longer solids retention time than hydraulic retention time.

4. SEM observations and ATP assays have indicated that retained biomass may be present in the slime layer attached to the support rings and in solid material trapped within the matrix of the filter.

6.3 DISCUSSION

In this preliminary study SCS has been successfully treated in an upflow anaerobic filter at 35°C and a 5 - 14 day RT. This may represent the first example of the treatment of a particulate waste with a solids concentration of more than 1% TS in an upflow anaerobic filter. Previously, only the DSFF reactor of the advanced reactors had been used for the treatment of a fibrous waste with a solids concentration of more than 1% TS (see section 1.4). However, solids did accumulate within the matrix of the filter and blockages were a frequent problem. Indeed it is anticipated that the accumulation of solid material might

become so great that during a period of prolonged operation the digester may block completely. It is therefore recommended that the suitability of an upflow anaerobic filter for the treatment of SCS be further considered over a longer time period.

The gas and methane yields (per VS added) obtained at a 5 day RT from the upflow anaerobic filter were larger than those from the conventional digester, and those reported previously for a DSFF reactor (Liao and Lo, 1985). Furthermore, it also seemed likely that the conventional digester was approaching failure as VFA continued to accumulate and biogas production fall. The better performance of the upflow anaerobic filter was attributed to the activity of biomass attached to and trapped within the filter support matrix and solids retention within the support rings. The attached biomass was particularly important as it permitted digestion to proceed in a balanced fashion at a 5 day RT without bacterial washout. No attempt was made to identify the bacteria retained in the digester, although it is likely that it contained organisms responsible for the degradation of soluble substrates (eg. methanogenic bacteria, obligate proton-reducing bacteria - see Fig. 1.4, reactions 4 - 7). In view of the close interactions between these bacteria, and the present poor level of understanding of their interactions, it is difficult to be certain which group (or groups) of this community primarily acted to prevent the excessive

concentration of hydrolysis products (released from the first stage of digestion (see Fig. 1.4)) inhibiting digestion.

The present study has indicated that at short retention times it may be prudent to treat SCS in an upflow anaerobic filter rather than a conventional digester. Furthermore, the performance of the upflow anaerobic filter at a 5 day RT also compared most favourably with that of the DSFF reactor (Liao and Lo, 1985) at a similar retention time. However, further work remains to be carried out to determine whether this is matched by a similar response at longer retention times. In view of the problems of solids accumulation and blockages encountered during operation of the upflow anaerobic filter, it may be necessary to employ a DSFF reactor for the anaerobic digestion of dairy cattle slurry. An alternative approach to the problem of blockages would be to apply a finer mesh in the separator and thereby remove a greater proportion of the particulate solid material. This point is discussed further in section 7.1.3.

Table 6.1 Summary of Characteristics of SCS Applied to the Upflow Anaerobic Filter.

	mean	standard deviation	number of samples
Total Solids (%)	4.41	0.48	36
Volatile Solids (% of TS)	77.5	2.5	36
VFA concentration (mM)			
Total VFA	47.71	5.84	18
Acetate	34.76	4.17	18
Propionate	8.24	1.10	18
i-butyrate	0.69	0.13	18
n-butyrate	2.14	0.43	18
i-valerate	1.01	0.16	18
n-valerate	0.49	0.10	18
i-caproate	0.31	0.13	18
n-caproate	0.05	0.03	18

Table 6.2 Summary of Weekly Gas Yields From the Anaerobic Filter When Operated at a 14.3 day RT.

Days	Total gas Vol (l)	Mean $\%CH_4$ in biogas	g VS added	GY	MY
68-74	110.36	63.2	269.2	0.41	0.26
75-81	123.47	68.5	336.6	0.37	0.25
82-88	94.67	59.6	269.2	0.35	0.21
89-95	97.78	66.5	269.2	0.36	0.24
96-100	78.21	64.5	201.9	0.39	0.25
TOTAL (33 days)	504.47	64.5	1346.1	0.37	0.24

Abbreviations

GY - Gas Yield ($m^3 kgVS\ added^{-1}$)

MY - Methane Yield ($m^3 kgVS\ added^{-1}$)

Note

See Fig. 6.1 for daily biogas production, composition and periods of feeding.

Table 6.3 Summary of Weekly Gas Yields From the Conventional Digester and Anaerobic Filter When Operated at an 11.7 and 5 day Retention Time.

Digester	Period (days)	Total Gas Vol (l)	$\%CH_4$ in biogas	g VS added	MY	GY	$\frac{MY(F)-MY(CD)}{MY(F)}$ (%)	$\frac{GY(F)-GY(CD)}{GY(F)}$ (%)
Filter	114-124	218.71	68.7	656.6	0.23	0.33	1	2
CD	114-124	53.50	69.5	164.1	0.23	0.33	-	-
Filter	125-131	232.26	67.9	957.6	0.16	0.24	14	14
	132-138	234.90	68.5	957.6	0.17	0.25	31	19
	139-144	222.74	67.9	766.1	0.20	0.29	82	73
CD	125-131	51.10	67.9	239.4	0.14	0.21	-	-
	132-138	49.44	63.6	239.4	0.13	0.21	-	-
	139-144	32.21	64.0	191.5	0.11	0.17	-	-

Abbreviations
F - Filter (operating volume 20 l)
CD - Conventional Digester (operating volume 5 l)
GY - Gas Yield ($m^3 kgVS\ added^{-1}$)
MY - Methane Yield ($m^3 kgVS\ added^{-1}$)

Note
Days 114-124 : Retention Time was 11.7 days
Days 125-144 : Retention Time was 5 days

Table 6.4 Viable Biomass Determinations of Matter Associated With Anaerobic Filter Support Rings

Sample Site	Viable Biomass (Moles ATP per ring)		
	Replicates ($x10^{-8}$)		Mean ($x10^{-8}$)
	(1)	(2)	
1 (Top of digester)	1.5	4.9	3.2
2	1.6	2.9	2.2
3	3.5	1.7	2.6
4	2.4	3.8	3.2
5	8.0	7.0	7.5
6 (Bottom of digester)	9.5	8.0	8.8

Fig. 6.1 Plot of Biogas Production and Composition During Start-up of the Upflow Anaerobic Filter.

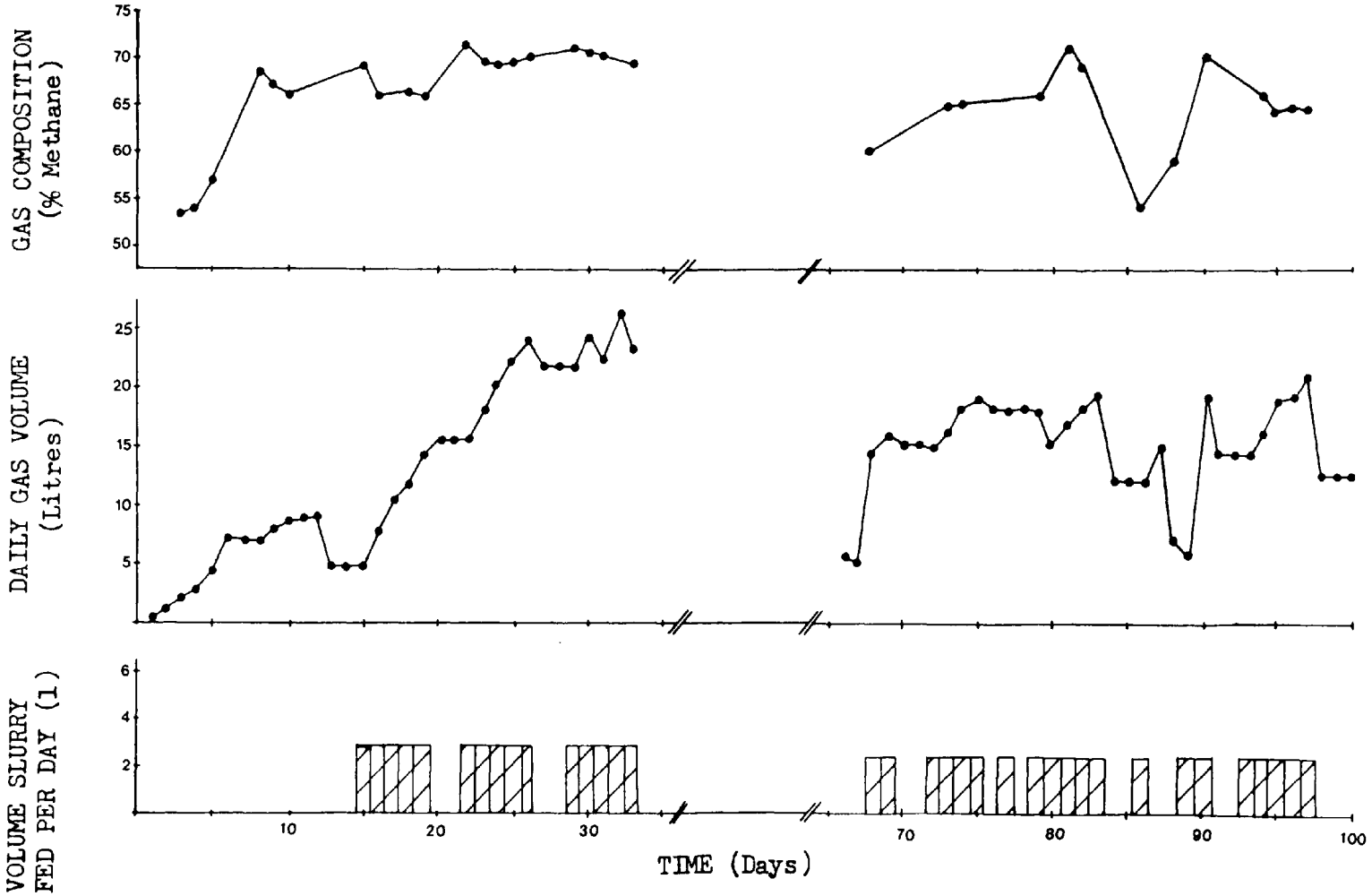


Fig. 6.2 The Operation of a Conventional Digester and Upflow Anaerobic Filter at an 11.7 day and 5 day RT. Plot of Volatile Fatty Acid Concentration, Biogas Composition and Daily Gas Volumes.

The gas volumes from the conventional digester have been multiplied by four, since the operating volume was one quarter of that of the anaerobic filter, and it has been assumed that equal volumes of biogas were produced on each day over the weekends.

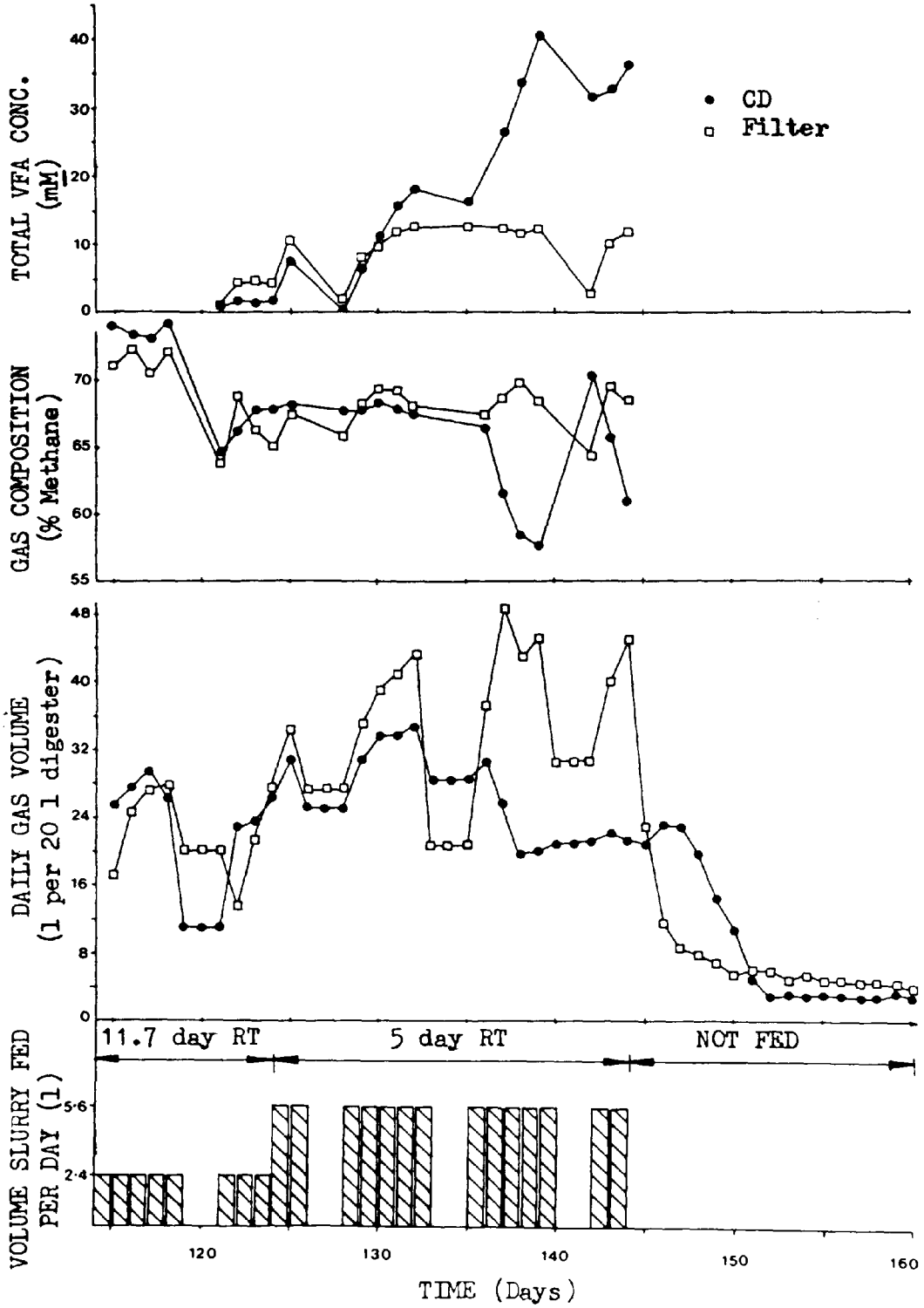
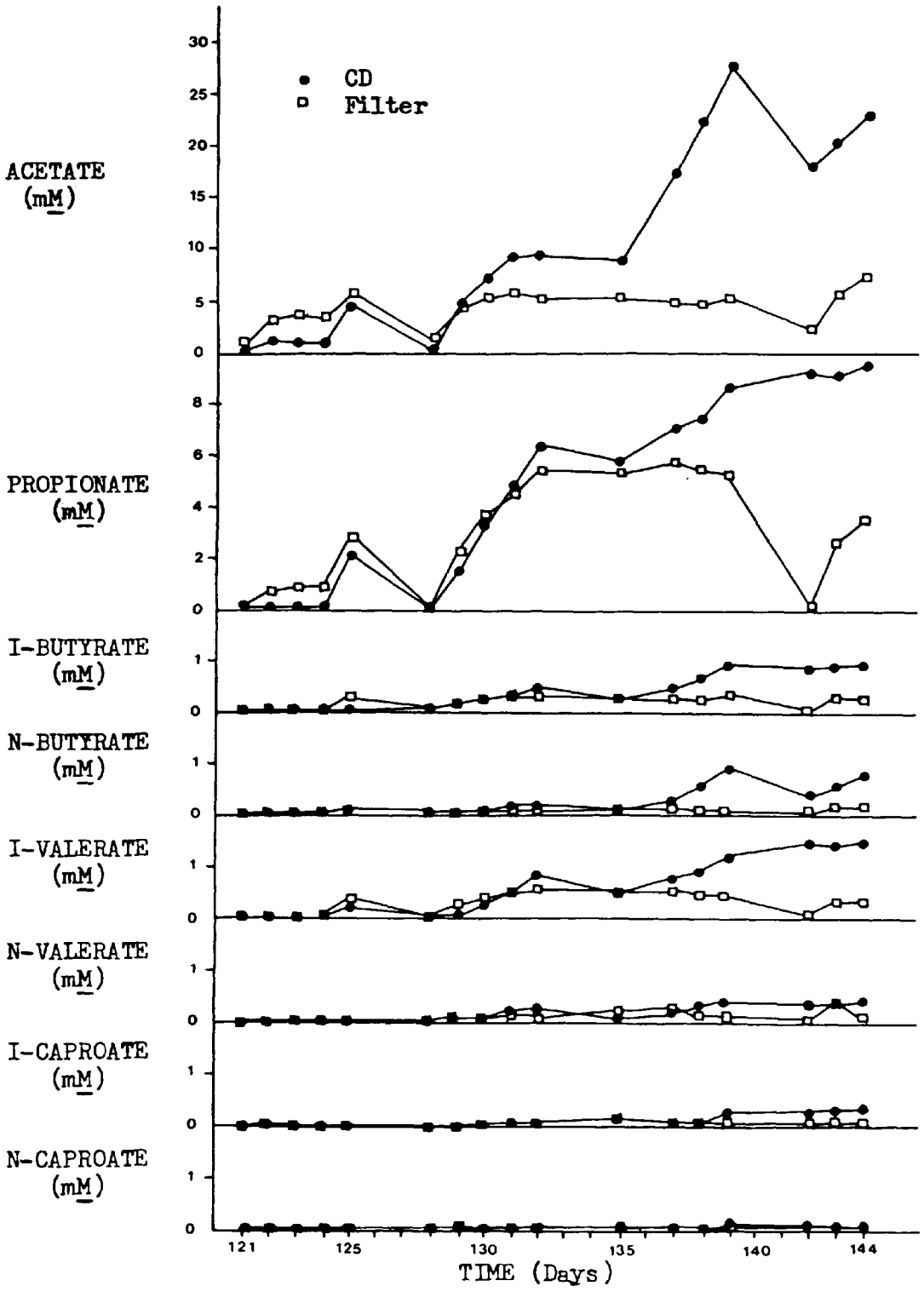


Fig. 6.3 Plot of Individual Volatile Fatty Acid Concentrations in an Upflow Anaerobic Filter and Conventional Digester operated at a 5 day RT

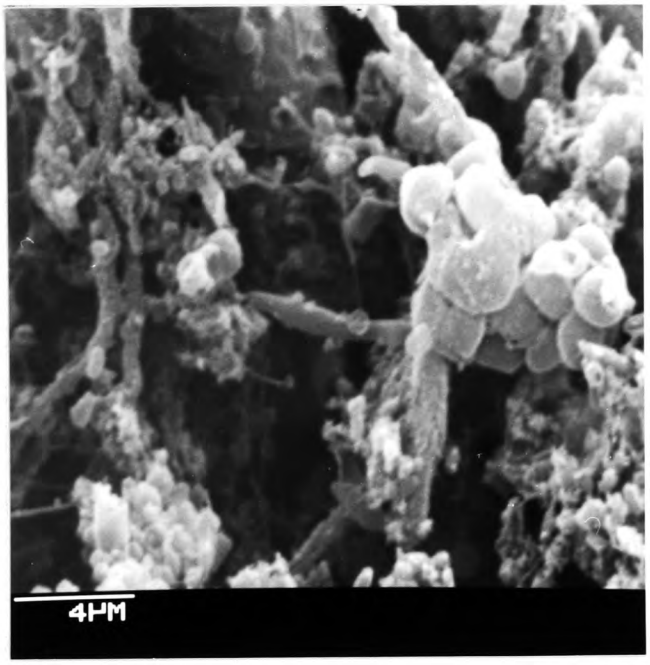


Plates 1 and 2. The Appearance of the Upflow Anaerobic Filter Support Rings on Day 166 (When the Filter was Dismantled). The associated slime layers may be distinguished

1

2

Plates 3-6. Scanning electron microscopy (SEM) preparations of the slime layer associated with the upflow anaerobic filter support rings. Groups of micro-organisms may be identified on these preparations



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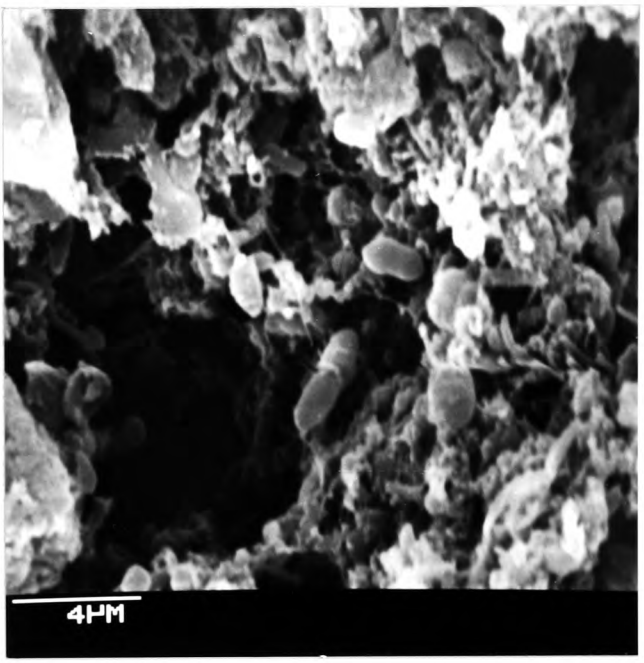

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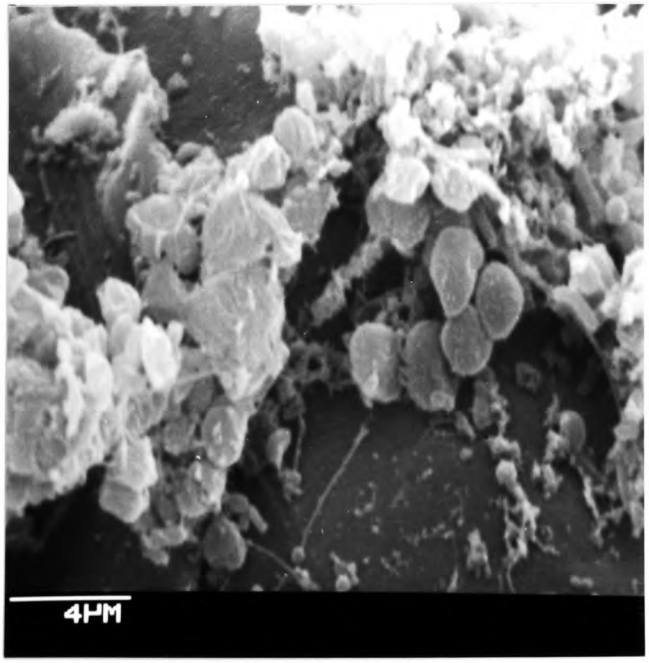

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6

CHAPTER SEVEN

DISCUSSION

7.1 EFFECT OF MECHANICAL SEPARATION ON THE ANAEROBIC DIGESTION OF DAIRY CATTLE SLURRY

7.1.1 The Enhancement of Digestion

Previous findings that passing WCS through a mechanical separator improved the efficiency of digestion have been confirmed. Higher methane and total biogas yields (per VS added), and a biogas richer in methane were produced from SCS than WCS at a 10 day and a 25 day RT, and in some instances also at an infinite retention time, (sections 3.4 and 3.5). Furthermore, more methane was liberated (per m^3 digester) from SCS than WCS at a 10 day RT. In a commercial situation it is anticipated that the slightly lower volume of methane liberated from SCS (per m^3 digester) at longer retention times may be offset by a fall in capital and operating costs, and an improvement in waste handling characteristics. Therefore, mechanical separation may permit both smaller digesters and shorter retention times to be employed, and hence make anaerobic digestion more economically viable. To ascertain the extent of any improvement in digestion economics is beyond the scope of this study, and is likely to be influenced by the characteristics of the waste, the degree of separation applied, retention time, operating temperature and the value of methane generated (but see section 7.1.3).

7.1.2 Factors Likely To Bring About The Enhancement Of Digestion

The higher methane and total biogas yield from SCS compared with WCS was due to the initial characteristics of the two slurries and three important differences were identified; the solids concentration, composition and particle size distribution. A simple explanation of the enhancement of digestion was not therefore possible as the three factors combined together, and their relative importance depended upon the operating conditions employed.

EFFECT OF SOLIDS CONCENTRATION

The solids concentration of dairy cattle slurry effected the digestion process in a similar manner to that anticipated (see section 1.1.2). It had no effect at an infinite retention time (in batch reactors - section 3.4), and exerted a negligible affect at a 25 day RT (section 3.5). In these circumstances digestion was not overloaded. However, the increased loading rate at a 10 day RT brought about a rise in the VFA concentration, in both the WCS and SCS digesters, and as a consequence of the different solids concentration VFA accumulated to a greater extent when WCS was the provided substrate (section 3.5). The removal of solids from WCS by the separator therefore reduced overloading and ensured that the digestion of SCS was

enhanced relative to WCS. It is envisaged that at shorter retention times, increasingly toxic VFA concentrations will be produced, and the gas yields (per VS added) from WCS will become further reduced relative to those from SCS. Indeed, in previous studies SCS has been successfully treated at retention times as low as one day, whilst digesters operated on WCS have been overloaded and failed when retention times of less than five days have been employed (Liao and Lo, 1985).

The adverse effect of the solids concentration on the digestion process was not, however limited to WCS, as a SCS digester was also overloaded when a 5 day RT was employed (section 6.2). Extremely high VFA concentrations were now recorded, and failure appeared likely. The problems of digester overloading may be overcome by employing a finer mesh in the separator, so as to remove a greater proportion of the solid material.

It should be added that the effect of the solids concentration on the digestion process was not strictly due to the actual concentration of solid material added, but to the amount destroyed. Therefore, any effect from overloading depended upon the degradability of the slurry under the operating conditions employed. In this study, 69 -117% more solids were destroyed per day in the WCS digester than the SCS digester (section 3.5). It is probable that the SCS used in this study was more digestible than that used previously, when digesters have been operated at 35°C and a 5 day RT (eg. Hawkes et al., 1984; Liao and Lo, 1985),

whereas failure appeared likely when such conditions were employed in this study (section 6.2).

The overloading of digestion occurred when the feed was added at such a rate that polymer degradation (see Fig. 1.4) was no longer the rate limiting stage of digestion. This resulted in VFA being formed more rapidly than they were degraded, and their consequent accumulation. The microbiological/biochemical basis for digester overloading is considered further in section 7.2.

EFFECT OF COMPOSITIONAL CHANGES

The passage of WCS through a mechanical separator removed 39% of the solids. This was chiefly from the larger particle size fractions and consisted of: 60% holocellulose, 19% lignin, 20% ash and 1% lipids, VFA and protein (the 'soluble' components - section 3.3). Ash and lignin were virtually recalcitrant to degradation and need not be considered further. The large removal of holocellulose from WCS compared to the negligible loss of the 'soluble' components (lipids, VFA and protein) brought about changes in the initial composition of the slurries and the composition of the solids destroyed. In studies conducted at a 10 and 25 day RT holocellulose represented 48-64% solids destroyed in WCS, and the 'soluble' components only 33-39%. In SCS, 31-49% solids destroyed was attributed to holocellulose degradation and 49-59% to the removal of the 'soluble' components (section 3.5).

At all retention times employed a slightly higher proportion of the volatile solids of WCS were destroyed compared with SCS (sections 3.4 and 3.5). Thus, solids degradability was not improved by separation (although it may be at shorter retention times). That previous studies frequently found solids digestibility to be slightly increased after passage through a mechanical separator (Table 1.1) may be attributed to variations in cattle slurry obtained from different sources. Instead of a rise in solids degradability, the higher methane and total biogas yields (per VS added) from SCS were chiefly attributed to a greater quantity of biogas liberated when the solids were destroyed (gas yield per VS destroyed). This was due to differences in the composition of material degraded, as the 'soluble' components yielded more biogas when destroyed than did holocellulose (Table 1.4). Higher gas yields per VS destroyed have also been recorded for SCS than WCS in previous studies (Table 1.1).

The different composition of WCS and SCS also ensured that the gas and methane yields from WCS fell more rapidly than those from SCS when the retention time was dropped. Lowering the retention time from 25 days to 10 days brought about a 45-49% fall in holocellulose degradation, but only a 1-8% fall in the removal of the 'soluble' components (percentages are means for WCS and SCS - see section 3.5). Thus, sufficient time was not available at a 10 day RT for complete holocellulose degradation, but it was

sufficient to permit an almost complete digestion of the 'soluble' components (that were ultimately degradable). The anaerobic digestion of lipids, VFA and protein became restricted when shorter retention times were employed with sewage sludge (O'Rourke, 1968), and it is likely that this might also be the case with dairy cattle slurry.

The larger contribution of holocellulose to solids destruction in WCS ensured that the biogas liberated was poorer in methane than that from SCS. It has been estimated that the biogas liberated from holocellulose degradation will contain 50% methane, and that from the degradation of 'soluble' components approximately 70% methane (Table 1.4). The biogas produced from WCS contained 58.6-60.3% methane and that from SCS 63.3-63.9% methane (sections 3.4 and 3.5); similar observations were also made in other studies (Liao et al., 1984; Pain et al., 1984; Liao and Lo, 1985).

EFFECT OF PARTICLE SIZE

A study of the ultimate anaerobic digestion of sieved WCS solids revealed that a slightly higher proportion of the solids in the larger particle size fractions were destroyed than in the smaller particle size fractions (section 4.4). This was attributed to the composition of the particles, particularly in that the smaller size fractions contained more lignin (section 4.2). The poor degradability of the liquid portion of dairy cattle slurry observed previously (Zeemen et al., 1983a) was presumably

attributed to its large ash and lignin content (which represented approximately half of the solids in particles <300μm in this study - section 4.2). It is probable that the removal of the more degradable large particles from WCS by the separator also accounted for the reduction in solids destruction observed after separation (section 3.5).

The more rapid production of biogas and its higher quality from the smaller particle size fractions of WCS compared with the larger particles, were not attributed to particle size itself, but to variations in the composition of the sieved fractions (section 4.4). Thus, the increase in gas and methane yields (per VS added) and biogas quality after separation at a 10 and 25 day RT was not attributed to the reduction in particle size brought about by the removal of larger sized particles, but to changes in composition this caused. Particle size is not therefore considered to play a large role in the digestion process.

INTERACTIONS BETWEEN THESE FACTORS

The relative effect of the solids concentration and substrate composition on the anaerobic digestion of WCS and SCS depended upon the operating conditions employed. The ultimate gas and methane yields (per VS added) obtained in batch reactors were not influenced by the slurry solids concentration or by differences in particle size. However, variations in slurry composition ensured that although a greater proportion of WCS solids were destroyed at an

infinite retention time, the solids from SCS yielded more biogas when destroyed, and similar ultimate methane and gas yields (per VS added) were attained for WCS and SCS (section 3.4). A further consequence of the different composition of the two slurries was that the biogas liberated from SCS was richer in methane (section 3.4).

The different solids concentration of WCS and SCS had little effect on the anaerobic digestion process at a 25 day RT, and no signs of overloading were observed. Insufficient time was available at a 25 day RT to permit complete solids degradation and the gas yields (per VS added) were less than those recorded in batch reactors. This effected the digestion of WCS (gas yield (per VS added) was 59% of the ultimate yield - section 3.5) to a greater extent than SCS (gas yield (per VS added) was 72% of the ultimate yield - section 3.5), and was presumably due to a fall in holocellulose degradation (see below).

Lowering the retention time from 25 days to 10 days brought about a further fall in the gas yield (per VS added) from both SCS (50% of the ultimate yield) and WCS (30% of the ultimate yield - section 3.5). The anaerobic digestion process was now slightly overloaded and VFA accumulated. This was more severe for WCS than SCS, as the initial solids concentration was higher. It has been calculated that an extra 6% biogas would have been produced from SCS and an extra 15% from WCS had the VFA been fully degraded (section 3.5). A second and more important consequence of the raised VFA concentration was the inhibitory effect that it

had on the digester bacteria. The effect is difficult to quantify, but was clearly sizeable since more methane and biogas were produced from SCS than WCS at a 10 day RT (section 3.5). A further factor influencing the digestion was the hydrolysis of holocellulose which fell by approximately half (in both slurries) as the retention time was dropped from 25 days to 10 days. It may be calculated that an extra 26% biogas would have been liberated from SCS and an extra 70% from WCS if holocellulose degradation had not fallen when the retention time was dropped. Thus, the greater fall in the gas yield (per VS added) from WCS than SCS was primarily due to holocellulose representing a larger proportion of the volatile solids in WCS (section 3.5). This fall in holocellulose degradation may be attributed to a greater sensitivity of the cellulolytic bacteria to the time available for substrate digestion, and/or the inhibitory effect of the raised VFA concentration. The accumulation of VFA and drop in holocellulose degradation accounted for 74% of the fall in the gas yield (per VS added) from SCS and 92% of the fall from WCS as the retention time was dropped from 25 to 10 days (section 3.5). Other factors that may have contributed to the reduced gas yield (per VS added) include particle size and the fall in the degradation of other proximate constituents, and further studies are required to evaluate their contribution. Compositional differences between the two slurries also ensured that, although a larger proportion of WCS solids were destroyed than SCS solids at a 10 day RT,

substantially more biogas was liberated from the destroyed solids of SCS, so that higher gas and methane yields (per VS added) were produced from SCS than WCS.

It is likely that the fall in the degradation of holocellulose and other components of the volatile solids will continue as the retention time is lowered further. However, it is envisaged that at shorter retention times the solids concentration of the two slurries will become an increasingly important factor, and that severe overloading and failure may occur.

7.1.3 The Optimal Degree of Separation

To bring about maximum methane production from dairy cattle slurry in a semi-continuously fed digester, the volume of methane liberated per day is optimized rather than the efficiency of digestion (gas yield per VS added). This is achieved by adding slurry at the optimal loading rate and depends upon the operating conditions employed (eg. temperature, retention time) and the digestability of the slurry under such conditions. The problem of adding WCS at too high a loading rate (and thereby overloading the digester) may be overcome by diluting the slurry or passing it through a mechanical separator. Of these two alternatives mechanical separation is preferred, primarily because it avoids the rise in costs associated with the treatment of an increased volume of material. At retention times of less than 16 days, the passage of WCS through a

separator that removed approximately 40% of the solids led to an increase in the volume of methane produced per m^3 of digester (section 1.1). Thus, in these circumstances the removal of at least 40% of the solids was necessary to achieve the optimal loading rate. However, it is likely that the removal of less than 40% of the solids may lead to an increased production of methane when retention times longer than 16 days are employed. Furthermore, it is anticipated that as the retention time is dropped it will be necessary to remove a successively larger proportion of WCS solids to achieve optimal methane production. To achieve this, it may for example be necessary to remove 20% of the solids at a 20 day RT, 40% at a 15 day RT, 60% at a 10 day RT and 75% at a 5 day RT. Different degrees of separation may be applied to the slurry by employing a number of mesh sizes in the separator. Furthermore, other factors (eg. improved waste handling characteristics) may make it expedient to employ a mechanical separator in circumstances where methane production is not increased. This and previous studies have been largely concerned with a separator that removed approximately 40% of the solids; this should now be expanded to include other degrees of separation, so that the optimal degree of separation at each retention time may be established.

During the anaerobic digestion of SCS in an upflow anaerobic filter, particulate matter accumulated in the reactor and blockages were frequently encountered. It

therefore seems likely that the successful treatment of dairy cattle slurry in an upflow filter requires the removal of more than the 40% of solids removed by the separator in this study. Thus, it is recommended that a smaller size mesh be employed in the separator. Results from this study indicate that if the <15μm particle size fraction was applied to the upflow anaerobic filter, the substrate would contain only 2.5%TS and would be rapidly degraded, and hence allow very short retention times to be employed.

7.2 STABILITY OF THE ANAEROBIC DIGESTION OF DAIRY CATTLE SLURRY

7.2.1 Indicators of Digester Instability

Digester instability in this study was caused by temperature shock treatments (Chapter 5), and overloading (in conventional digesters at a 5 and 10 day RT (Chapters 3 and 6) and in an upflow anaerobic filter at a 5 day RT (Chapter 6)). The temperature shock treatments may, however, be considered as a form of digester overloading, in that the lowered operating temperature prevented a complete degradation of the added substrate and brought about the accumulation of intermediates such as VFA. The causes of digester instability are considered further in section 7.2.2. Although the severity of overloading varied, a

similar pattern of response was observed in each case. Biogas production, the proportion of methane in the biogas and the digester pH all fell, whilst the total and individual VFA concentrations increased. Previous studies conducted with other wastes have revealed a similar response to digester instability (Pohland and Bloodgood, 1963; Barnes et al., 1983; Kennedy et al., 1985), although in many cases these parameters were not all measured together (eg. van Velsen and Lettinga, 1980; Kruize, 1983; Speece and Parkin, 1983; Temper et al., 1983; Hilpert et al., 1984).

The most severe overloading of digestion occurred in this study when the retention time of a conventional digester operated on SCS was dropped from 11.7 days to 5 days. This brought about a drop in the proportion of methane in the biogas (from 67% to 57%), a fall in biogas production and a rise in the total and individual VFA concentrations (section 6.2). The VFA concentration reached 40.7mM and chiefly consisted of acetate (28.0mM) and propionate (8.8mM), although the longer chained VFA (particularly branched VFA) also increased in concentration. Furthermore it appeared likely that the concentration of VFA would increase further, and eventually bring about a complete failure of digestion.

When the retention time of an upflow anaerobic filter operating on SCS was dropped in a similar manner (section 6.2) or temperature shock treatments applied to SCS digesters (sections 5.3 and 5.4) or a 10 day RT employed with SCS and WCS in conventional digesters, failure did not

appear likely, although symptoms of digester instability were observed on each occasion. In some cases small falls in the methane content of biogas were noted (sections 3.5, 5.3 and 5.4), whilst in other cases no effect was discernable (sections 3.5 and 6.2). A further indicator of overloaded digestion was a fall in digester pH, although this was noticed only during the more severe temperature shock treatments (sections 5.3 and 5.4). The most sensitive, and in some cases the only symptom of digester instability was a rise in the total and individual VFA concentrations (sections 3.5 and 6.2). Indeed, the total VFA concentration has been used successfully as a monitor of digester stability in previous studies (Mueller et al., 1959; Graef and Andrews, 1974; Hill and Barth, 1977). Results from this study have shown that a total VFA concentration exceeding 2mM indicates that the anaerobic digestion of dairy cattle slurry has become slightly unbalanced, whilst a concentration in excess of 20mM was considered serious, and appreciably higher concentrations may lead to failure (although the actual concentrations at which these effects are found is likely to be influenced by a number of factors such as the operating conditions employed and previous VFA concentrations).

In all cases of overloading the concentration of propionate and branched VFA were proportionately more sensitive than the concentration of acetate, butyrate or total acids. The greater sensitivity of propionate to shock treatments has been noted previously (eg. Pohland and

Bloodgood, 1963; Asinari di San Marzano et al., 1981), although the magnitude of the response of branched VFA (in that they were the most sensitive of the VFA) represents a novel finding. It is therefore recommended that changes in the concentration of propionate or branched VFA be used to monitor digester stability rather than the concentration of acetate or total VFA. Recent observations have, however, suggested that a rise in the partial pressure of hydrogen may be a further symptom of digester instability (Barnes et al., 1983; Whitmore et al., 1985; Archer et al., 1986; Whitmore and Lloyd, 1986). Many problems have been encountered in the measurement of hydrogen concentrations in digesters, and the results obtained in these studies indicate that it may represent an even more sensitive monitor than the concentration of individual VFA.

7.2.2 Causes of Digester Instability

Digester instability in this study was caused by the overloading of digestion, where the initial stages of digestion (degradation of lipids, protein and holocellulose - Fig. 1.4) proceeded more rapidly than the final stages. It was characterized by an accumulation of intermediates (eg. VFA) and a fall in methanogenesis (section 7.2.1). The dairy cattle slurry was not therefore toxic itself, although compounds released after partial degradation may exert an inhibitory effect on the digestion process. In the temperature shocked digesters, imbalance was caused by a

greater sensitivity of the obligate proton-reducing and methanogenic bacteria to the treatments employed (section 5.5), whilst in overloaded digesters (sections 3.5 and 6.2) the activity of bacteria responsible for the initial stages of digestion increased more rapidly than the activity of the obligate proton-reducing and methanogenic bacteria (Fig. 1.4). The greater sensitivity of the obligate proton-reducing and methanogenic bacteria to changes in the digester operating conditions may be attributed to the sensitivity of these organisms to the actual changes employed and their slow growth rate (eg. Archer, 1983; Mosey, 1983). The retention of obligate proton-reducing and methanogenic bacteria in the upflow anaerobic filter at a 5 day retention time ensured that these bacteria did not become washed out, and that digestion was better balanced at low retention times than in a conventional digester, where intermediate products accumulated and methane production fell (Chapter 6).

A difficult point to resolve is which of these groups of bacteria caused digester instability and which were affected. Evidence has been presented previously to suggest that a sudden change in operating parameters may bring about a rapid rise in the hydrogen partial pressure (Barnes et al., 1983; Whitmore et al., 1985; Archer et al., 1986; Whitmore and Lloyd, 1986), and that this may have a detrimental effect on digestion. The observations made in this study with overloaded (sections 3.5 and 6.2) and temperature shocked digesters (sections 5.3 and 5.4) are

consistent with this hypothesis, as the concentrations of VFA responded in a similar manner to that anticipated (on thermodynamic grounds) to a rise in the hydrogen partial pressure (in that the concentration of propionate increased proportionately more than did the concentrations of acetate and butyrate). In these circumstances it is anticipated that the hydrogen-utilising methanogenic bacteria (the chief hydrogen consumers - Braun et al., 1979; Mackie and Bryant, 1981; Boone, 1982) may be the group of organisms most sensitive to the changes applied. However, the activity of the acetate-utilising methanogens also fell during periods of instability and acetate accumulated. At the present time there is conflicting evidence as to whether a raised hydrogen partial pressure will inhibit acetate-utilising methanogenic bacteria (see Harper and Pohland, 1986), although these organisms may themselves be sensitive to changes in operating conditions or perhaps a change in one of the other parameters.

The concentrations of branched VFA were proportionately more sensitive to digester overloading (sections 3.5 and 6.2) and temperature shocks (sections 5.3 and 5.4) than the concentrations of straight chain VFA. This was due to an increase in their formation and a fall degradation. A poor level of understanding of the routes of formation and degradation of branched VFA makes these observations difficult to interpret (section 1.2); although it is possible that the increased formation may be linked to a rise in protein degradation, as leucine was one

of the most abundant amino acids in dairy cattle slurry (Anthony, 1971; Martin et al., 1983) and when deaminated produces i-valerate (section 1.2), the major branched VFA in stressed digesters (sections 3.5, 5.3, 5.4 and 6.2).

Digester instability in this study was caused by a failure of bacteria responsible for the final stages of digestion (the obligate proton-reducing and methanogenic bacteria) to maintain low concentrations of their substrates in temperature shocked (sections 5.3 and 5.4) and overloaded digesters (sections 3.5 and 6.2). This brought about a rise in the hydrogen partial pressure, the VFA concentrations and a fall in methane production. Prolonged periods of digester instability may lead to very large rises in the VFA concentrations, and if unchecked this may eventually cause souring and a complete failure of digestion.

7.2.3 Improving Digester Stability

Commercial digesters operating on livestock waste are prone to instability due to the addition of toxic compounds (eg. disinfectants or antibiotics) with the feed, failure to maintain a constant temperature or failure to maintain the optimum feed rate (Temper et al., 1983; Hilpert et al., 1984).

Results obtained in this study have demonstrated that overloading of the digestion process may occur before it is detected by measurements of pH and a fall in the methane of

the biogas. By monitoring the concentration of propionate and branched VFA (or perhaps hydrogen) it may be possible to identify this overloading, and therefore provide greater process control.

The study of temperature shocked digesters has shown that this type of failure is identical to overloading and that the best recovery method is to increase the temperature as rapidly as possible and to discontinue feeding in severe cases.

The attached biofilm in the upflow anaerobic filter helped to prevent digester overloading at a 5 day RT. This consortia of retained bacteria maintained low VFA concentrations and permitted high rates of methane production (section 6.2), and represents a further example of improving digester stability.

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