OPTIMIZATION OF A TWO-PHASE ANAEROBIC DIGESTION SYSTEM

by

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ABSTRACT

Fundamental aspects of the biochemistry and microbiology of the numerous stages involved in the anaerobic biological conversion of organic matter to methane, as well as recent developments in the field of anaerobic digestion technology, have indicated that the physical separation and individual optimization of the two major steps of the process, that is acidogenesis and methanogenesis, may have advantageous results in the performance and stability of the system.

In order to optimize the two-phase anaerobic digestion system treating a complex meat based wastewater, the operating conditions for the optimum performance of the individual phases, as well as the overall process were determined.

Initially, the acidogenic phase was studied separately using a continuous-flow stirred tank (CSTR) reactor. The influence of the operational parameters, such as hydraulic retention time, organic loading rate, influent substrate concentration, pH and temperature on the first phase of anaerobic digestion has been investigated. It has been shown that the fermentation pattern of the acidogenic phase is generally not affected by the operational variables, the main fermentation products invariably being acetic and propionic acids. The concentration of acetic acid increased with the hydraulic retention time, influent substrate concentration, pH in the range of 5 to 8, and temperature in the range of 25 to 40 °C. In contrast, the concentration of propionic acid depended mainly on the substrate availability with a consistent proportion of the order of 7% of the influent COD being converted to it.

The kinetics of biomass growth and product formation were also studied. It has been demonstrated that the Monod equation completely failed to describe the growth of the acidogenic bacteria, while there was evidence of product inhibition. Two of the most commonly used inhibition models were applied, the non-competitive and the Haldane-type models, the former giving superior results.

The performance of the two-phase system was assessed using a CSTR reactor for the acidogenic phase and a fluidised bed for the methanogenic phase. Different ratios of the hydraulic retention time of the acidogenic to that of the methanogenic reactor were examined, while the overall hydraulic retention time of the system was kept constant. Furthermore, different influent substrate concentrations and different overall hydraulic retention times were applied. The results have shown that the two-phase system is capable of COD removals superior or similar to the single-stage system, when the volume of the methanogenic reactor is reduced by up to 25% and an equal volume is introduced as an acidogenic reactor. The effect of phase separation on the effluent characteristics, biogas production and biomass hold-up was assessed. Finally, the principles of fluidisation were employed to calculate the biofilm thickness of the fluidised bed carrier particles, and the kinetics of substrate removal in the fluidised bed reactors were discussed.

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NOMENCLATURE

AFBR	Anaerobic Fluidised Bed Reactor
COD	Chemical Oxygen Demand
CSTR	Continuous-flow Stirred Tank Reactor
HRT	Hydraulic Retention Time (T)
HOA	Hydrogen Oxidising Acetotrophs
HOM	Hydrogen Oxidising Methanogens
OHPA	Obligate Hydrogen Producing Acetogens
OLR	Organic Loading Rate ($M L^{-3} T^{-1}$)
NHOA	Non-Hydrogen Oxidising Acetotrophs
NRB	Nitrate Reducing Bacteria
SRB	Sulphate Reducing Bacteria
SRT	Solids Retention Time (T)
TSS	Total Suspended Solids
VFA	Volatile Fatty Acids
VS	Volatile Solids
VSS	Volatile Suspended Solids
D	Dilution Rate (T^{-1})
d_m	Medium (Sand) Diameter (L)
d_p	Particle (Bioparticle) Diameter (L)
I	Inhibitor Concentration ($M L^{-3}$)
k_d	Specific Decay Rate of Microorganisms (T^{-1})
K_i	Inhibition Constant of μ ($M L^{-3}$)
K_i'	Inhibition Constant of q_p ($M L^{-3}$)
K_s	Substrate Saturation Constant of μ ($M L^{-3}$)
K_s'	Substrate Saturation Constant of q_p ($M L^{-3}$)
N_{Re}	Particle Reynolds Number
P	Moisture Content
P_o	Influent VFA Concentration ($M L^{-3}$)
P_e	Effluent Product (VFA) Concentration ($M L^{-3}$)
r_p	Rate of Product Formation ($M L^{-3} T^{-1}$)
Q	Flowrate ($L^3 T^{-1}$)
q_p	Specific Rate of Product Formation (T^{-1})
q_{max}	Maximum Specific Rate of Product Formation (T^{-1})

R_s	Specific Rate of Substrate Removal (T^{-1})
R_{max}	Maximum Specific Rate of Substrate Removal (T^{-1})
S	Substrate (COD) Concentration (ML^{-3})
S_o	Influent Substrate Concentration (ML^{-3})
S_e	Effluent Substrate Concentration (ML^{-3})
$S_{e,c}$	Effluent Substrate Concentration available to Acidogens (ML^{-3})
S_p	Equivalent COD Concentration of the Products (ML^{3})
U	Liquid Upflow Velocity (LT^{-1})
U_t	Bioparticle Terminal Settling Velocity (LT^{-1})
V	Reactor Volume (L^3)
V_s	Sand Volume (L^3)
X_{exp}	Biomass Hold-up of Expanded Bed (ML^{-3})
x	Biomass (Total Suspended Solids) Concentration (ML^{3})
x_e	Effluent Biomass (Total Suspended Solids) Concentration (ML^{-3})
x_o	Influent Biomass (Total Suspended Solids) Concentration (ML^{-3})
Y	Biomass Yield Coefficient
δ	Biofilm Thickness (L)
ϵ	Bed Porosity
η_l	Liquid Viscosity ($ML^{-1}T^{-1}$)
θ	Hydraulic Retention Time (T)
μ	Specific Rate of Biomass Growth (T^{-1})
μ_{max}	Maximum Specific Rate of Biomass Growth (T^{-1})
ρ	Biomass Dry Density (ML^{-3})
ρ_l	Liquid Density (ML^{-3})
ρ_m	Medium (Sand) Density (ML^{-3})
ρ_p	Particle (Bioparticle) Density (ML^{-3})

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1. Anaerobic Wastewater Treatment

The anaerobic digestion process is a bacterial fermentation by which organic matter is converted to carbon dioxide and methane. As a treatment process for organic wastes its most notable features are that it destroys organic pollutants without requiring any additional oxygen and that it produces a useful by-product, a gaseous fuel containing about 60 % methane.

In spite of their present significance and future potential, anaerobic waste treatment processes have not always enjoyed a favourable reputation (McCarty, 1964). Anaerobic digestion was perceived as a sensitive biological process that required careful pH and temperature control, and protection from toxic substances. Its use was restricted to the treatment of secondary sludge and high-strength organic wastes (above 4 g COD L^{-1}); the aerobic treatment of the latter was uneconomic due to oxygen transfer limitations which resulted in increased hydraulic retention times (Cillie *et al.*, 1969).

In recent years, however, the search for sources of renewable energy has given a new boost to anaerobic digestion. The latter is now seen as an elegant process to dispose of an organic waste through its bioconversion to energy. The recent increase in understanding of the microbiological and biochemical aspects of the process has led to improved stability of the existing commercially operated digesters, while the development of new types of reactors such as the fixed-film reactors (Bull *et al.*, 1983a) and the two-phase anaerobic digestion systems (Bull *et al.*, 1984a) have further increased process stability. On the other hand, the development of new types of reactors which has enabled higher volumetric loading capacities to be achieved has increased the interest in treating dilute, as well as concentrated, wastes in anaerobic digesters (Forster, 1984).

The advantages of anaerobic digestion over aerobic can further be summarised by the following:

1) the anaerobic process requires considerably less energy, while at the same time useful energy can be recovered in the form of biogas;
2) relatively little waste sludge is produced as bacterial growth yield is low: hence, the costs of disposal of the organic residues are lower than those of aerobic systems;
3) almost any waste that can be treated aerobically, can also be treated anaerobically (Speece, 1983). The only known substances that require oxygen for their bacterial degradation are lignin, n-paraffins, some plastics and compounds with ether linkages (Sahm, 1984). There are, however, some substances which are not degradable under aerobic conditions that can be decomposed anaerobically (e.g. pectin) (Mudrack and Kunst, 1986);
4) anaerobic biomass has the ability to lie dormant for several months and then be fully operational within 2-3 days (Anderson *et al.*, 1984);
5) the nutrient requirements are very low.

In an economic context, many industries cannot now tolerate the increased costs of the aerobic treatment of their wastestreams. Anaerobic techniques, offering the prospect of a reduction in total costs of at least 50 % (Verstraete, 1983), will more frequently be applied, especially in those industries emitting warm and concentrated effluents.

1.2. Biochemistry and Microbiology of Anaerobic Digestion

The anaerobic biological conversion of organic matter to methane is a complex process involving a number of microbial populations linked by their individual substrate and product specificities. However, due to the complexity of the process and in order to simplify the mathematical representation of the actual

system, the overall process is often represented by three stages occurring simultaneously within the anaerobic digester (McCarty, 1964; Marsili-Libelli and Nardini, 1985):

1) hydrolysis of high molecular weight, often insoluble biodegradable polymers;

2) production of fatty acids from the soluble organic monomers;

3) methane generation.

In reality, however, there are at least six recognisable steps (Gujer and Zehnder, 1983; Harper and Pohland, 1986), each mediated by a specific group of microorganisms. As shown in Figure 1, these steps comprise:

1) hydrolysis of high molecular often insoluble materials (polymers), which are converted by enzymes to soluble fragments (monomers) such as sugars, amino acids, organic acids and alcohols;

2) conversion of organic monomers to acetic, propionic and butyric acids, hydrogen, carbon dioxide, and other organic products such as ethanol and lactic acid;

3) anaerobic oxidation of the intermediate products to acetic acid, hydrogen and carbon dioxide by the obligate hydrogen producing acetogenic (OHPA) bacteria;

4) acetogenic respiration of homoacetogens;

5) acetoclastic methane fermentation;

6) conversion of hydrogen to methane.

Furthermore, other bacteria, namely the nitrate-reducing bacteria (NRB) and the sulphate-reducing bacteria (SRB) which are also found in anaerobic digesters compete with the methanogenic bacteria for the substrate. These bacteria are known to be involved in the following steps: oxidation of the intermediate products to acetic acid and carbon dioxide; oxidation of acetic acid to carbon dioxide; oxidation of hydrogen (Harper and Pohland, 1986).

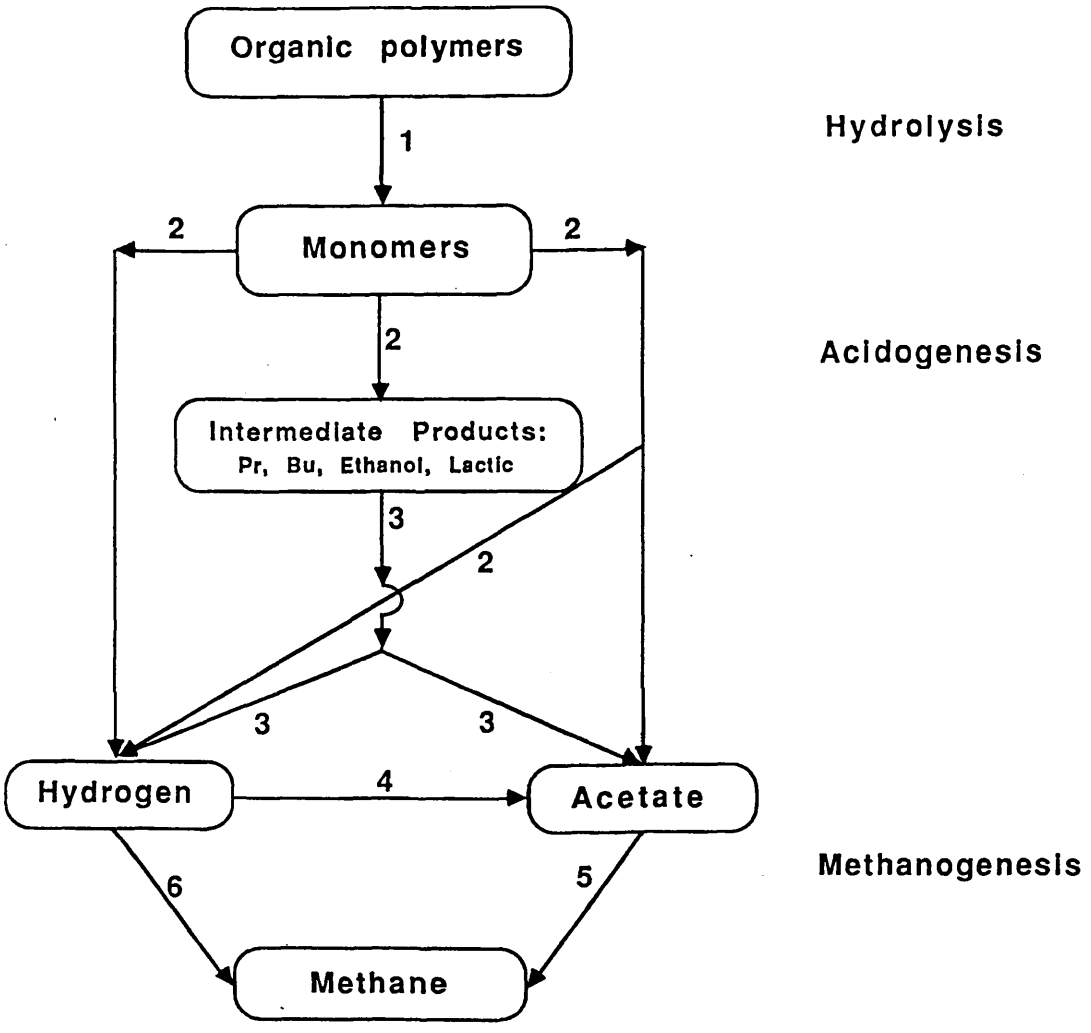


Figure 1: Schematic representation of processes operative during the biological conversion of organic wastes to methane

In order for the overall process to proceed efficiently, a dynamic balance between the various steps is necessary. The overall reaction rate is then equal to the slowest reaction rate (rate-limiting reaction), provided of course that a healthy population of all bacteria required is present in the digester. The hydrolytic step (Fig. 1, step 1) is often the rate-limiting step of the anaerobic degradation of particulate matter (Eastman and Ferguson, 1981). It has been suggested that this is mainly due to steric hindrance of the activity of hydrolysing enzymes by non-degradable polymers (e.g. waxes and lignins) (Mudrack and Kunst, 1986).

The microbial products of the organic monomer breakdown are various and include volatile fatty acids, ethanol, lactic acid and hydrogen (Fig. 1, step 2). The distribution of the individual volatile fatty acids and other organic products during the anaerobic degradation of a given substrate has been demonstrated to be variable as a result of the alternative pathways of catabolism that can be utilised by the bacterial population (Cohen *et al.*, 1984). The several possible metabolic routes for the anaerobic degradation of carbohydrates are presented in Figure 2; the individual biochemical conversions and pathways have not been included. Although any of these reactions can take place theoretically, whether they actually occur is dependent on the condition of the medium created by the nature of the substrate, and the presence of the appropriate microorganisms. In an anaerobic digester, acetate is generally the principal intermediate product, although other volatile fatty acids such as propionate, butyrate, iso-butyrate, valerate and iso-valerate may also be present in smaller amounts. Large amounts of propionic acid in the effluent of the anaerobic digester is the first sign of stress and/or overloading of the reactor, while large amounts of butyric acid usually suggest failure or "souring" of the digester (Asinari di San Marzano *et al.*, 1981). Products such as lactate and ethanol usually appear shortly after overloading and/or when some environmental stress has been applied to the reactor. The diverse reactions involved in this stage require

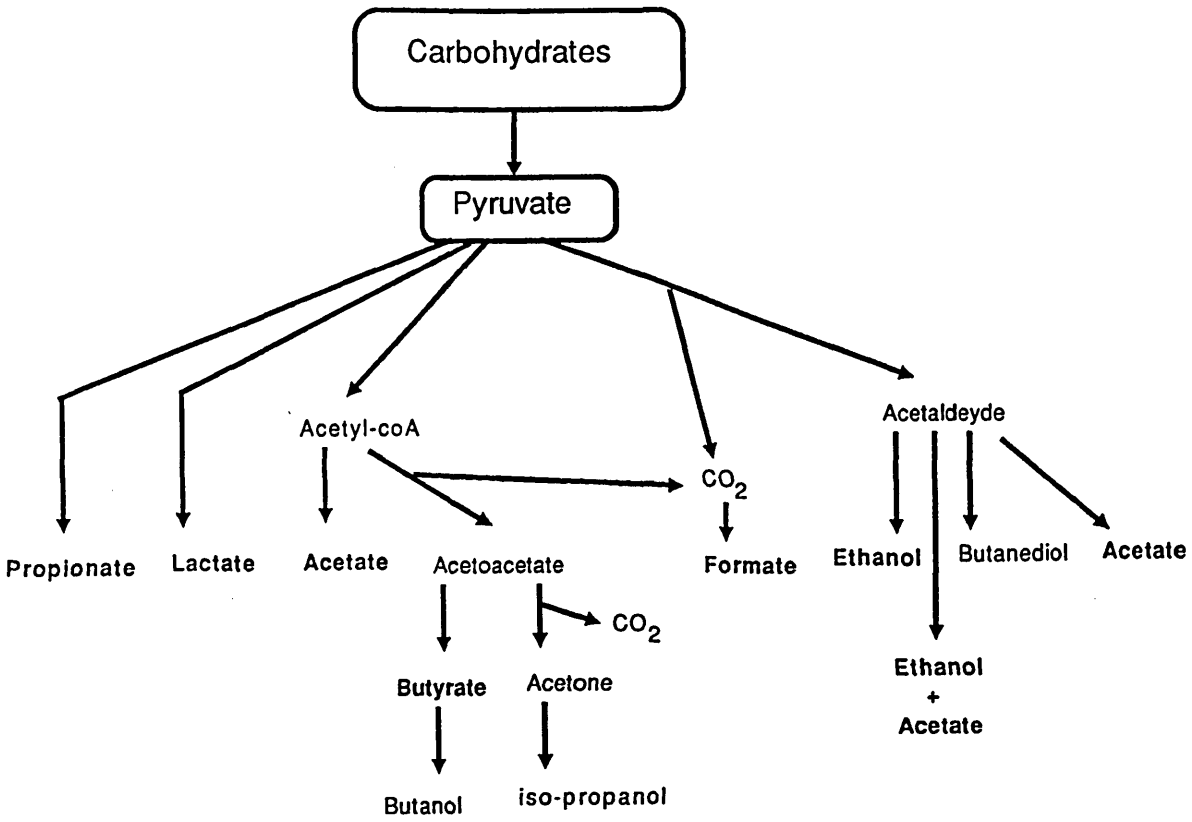


Figure 2 : Possible metabolic routes for the anaerobic degradation of carbohydrates (after Mudrack and Kunst, 1986)

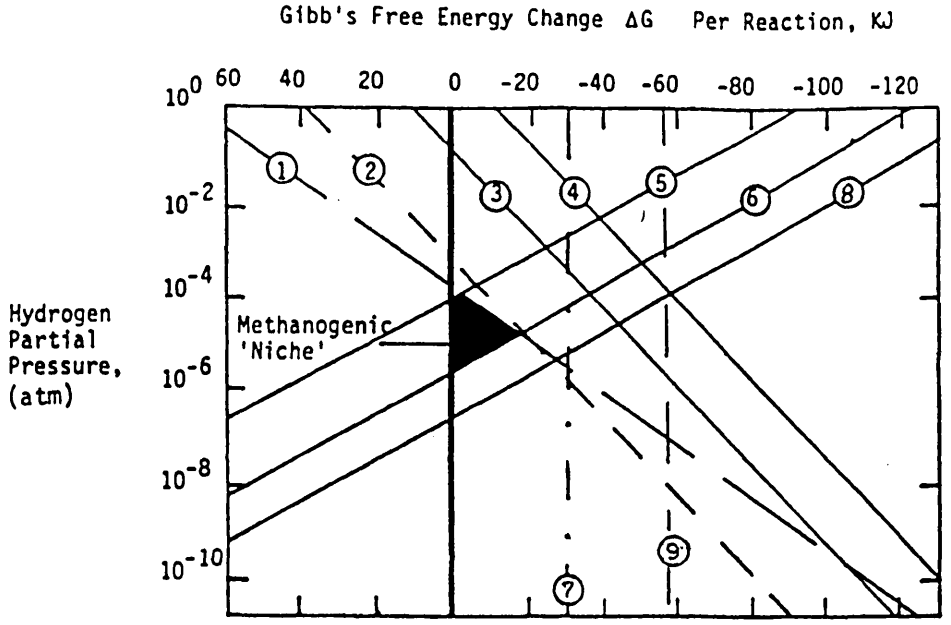
many types of bacteria which can utilise various complex organic substrates. The presence of different bacterial groups has been demonstrated by many authors (Toerien and Hattingh, 1969). These are mainly obligate anaerobes, although facultative bacteria are found which typically account for less than 10 % of the population (Toerien *et al.*, 1967). Furthermore, they are generally fast growing bacteria with doubling times in the range of 1.5 to 12 h (Henze and Harremöes, 1983).

The anaerobic degradation of the intermediate organic compounds to acetic acid is mediated by a group of bacteria known as the obligate hydrogen producing acetogenic (OHPA) bacteria (Fig. 1, step 3). A healthy population of these bacteria ensures the fast assimilation of the intermediate products. However, as noted above, during periods of overloading and/or other process stress in anaerobic digesters, accumulation of the intermediate products, mainly propionic and butyric acids, are observed (Andrews and Pearson, 1965). These imbalances have often been correlated with increased hydrogen concentrations. Kaspar and Wuhrmann (1978) reported a linear increase in the propionic acid concentration when the hydrogen partial pressure was increased from 10^{-4} to 1.5×10^{-2} atm. Novak and Carlson (1970) observed that long chain fatty acid degradation was inhibited by hydrogen. More recently, Barnes *et al.* (1984) observed an increase in propionic and other volatile acids immediately following an increase of the hydrogen partial pressure from 2×10^{-4} to 1.5×10^{-3} atm, as induced by a shock loading applied to a fluidised bed anaerobic digester treating molasses waste-water. However, it was only lately that the importance of hydrogen sensitivities, moderated by syntrophic associations between hydrogen-producing bacteria and hydrogen-consuming bacteria, was recognised. It is now known that the former can only survive when their products of metabolism are continuously removed from the medium. This can be achieved in their symbiosis with hydrogen-consuming microorganisms i.e. methanogenic

bacteria. The importance of the OHPA bacteria can also be demonstrated by the fact that the hydrogen and the acetate synthesised by their metabolism in an anaerobic digester treating a complex waste, have been estimated to provide the substrate for 54 % of the total methane produced (Kaspar and Wuhrmann, 1978). These bacteria are intolerant of pH fluctuations and must be maintained at neutral pH for maximum efficiency. Finally, their doubling times are of the order of 2-6 d (McInerney *et al.*, 1979; Boone and Bryant, 1980), i.e. longer in general than those of the methanogens, which are in the range of 0.2-2 d when hydrogen, formate or high levels of acetate are used as substrate (Ghosh and Klass, 1978).

To quantify the effects of the variation in hydrogen partial pressure, thermodynamic models and equilibrium assumptions have been used by several authors (McCarty, 1981; Gujer and Zehnder, 1983). The hydrogen-dependent reactions occuring during anaerobic digestion, as well as the graphical representation of the free energy change per reaction ΔG as a function of hydrogen partial pressure, are illustrated in Figure 3. The free energy change of a reaction is the measure of the amount of energy released. Part of this energy is used by the cell in form of ATP; the amount of ATP formed per unit of free energy released is dependent upon the metabolic pathway employed, each one of which has its own efficiency of ATP production. As can be seen from Figure 3, propionic acid oxidation to acetate (line 1) becomes favourable only at hydrogen partial pressures below 10^{-4} atm, while butyric acid oxidation (line 2) becomes favourable at 10^{-3} atm. Similarly, ethanol and lactate oxidations (lines 3 and 4) are inhibited by hydrogen partial pressures close to 1 atm. Also evident from the same diagram is the favourability of hydrogen oxidation by SRB (line 8) over conversion of hydrogen to methane (line 6) at all hydrogen partial pressures, and the favourability of acetate cleavage by SRB (line 9) over methanogens (line 7).

The methanogenic bacteria examined to date can utilise directly as substrate



(1) Propionate → Acetate: $CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + H^+ + HCO_3^-$

(2) Butyrate → Acetate: $CH_3CH_2CH_2COO^- + H_2O \rightarrow 2CH_3COO^- + H^+$

(3) Ethanol → Acetate: $CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + H^+ + 2H_2$

(4) Lactate → Acetate: $CH_3CHOHCOO^- + H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 2H_2$

(5) Hydrogen → Acetate: $2HCO_3^- + 4H_2 + H^+ \rightarrow CH_3COO^- + 4H_2O$

(6) Hydrogen → Methane: $HCO_3^- + 4H_2 + H^+ \rightarrow CH_4 + 3H_2O$

(7) Acetate: → Methane: $CH_3COO^- + H_2O \rightarrow HCO_3^- + CH_4$

(8) Hydrog. Oxid. by SBR: $SO_4^{2-} + 4H_2 + H^+ \rightarrow HS^- + 4H_2O$

(9) Acetate Oxid. by SBR: $SO_4^{2-} + CH_3COO^- + H^+ \rightarrow 2HCO_3^- + H_2S$

Figure 3 : The hydrogen dependent reactions occuring during anaerobic digestion, and the graphical representation of the standard free energy change per reaction ΔG as a function of hydrogen partial pressure, when the concentration of other products is: acetic acid, 25 mM; propionic, butyric, lactic acids, and ethanol, 10 mM; sulphate and sulphite, 5 mM; bicarbonate, 20 mM; methane, 0.7 atm (after Harper and Pohland, 1986)

only one or more of the following compounds: hydrogen, single-carbon compounds such as carbon dioxide, carbon monoxide, methanol, and formate, and the two-carbon compound, acetic acid. The reactions involved are presented in Table 1. It has been reported that when a mixture of primary and secondary sludges is digested, 70 % of the methane produced originates from acetate, while 30 % is formed through hydrogen oxidation (Kaspar and Wuhrmann, 1978).

Table 1 : Standard free energies associated with methanogenic conversion of different substrates (after Harper and Pohland, 1986)

Substrate	Reaction	$\Delta G_o, kJ\,mol^{-1}\,CH_4$
Hydrogen	$4H_2+HCO_3^-+H^+ \rightarrow CH_4+3H_2O$	-135.6
Acetic Acid	$CH_3COO^-+H_2O \rightarrow CH_4+HCO_3^-$	-31.0
Methanol	$CH_3OH+H_2 \rightarrow CH_4+H_2O$	-121.1
Methanol	$4CH_3OH \rightarrow 3CH_4+HCO_3^-+H_2O+H^+$	-102.5

All isolated methanogenic bacteria can oxidise hydrogen and reduce carbon dioxide to methane, with the one exception, *Methanothrix sp.*, which can only utilise acetate. Some species can utilise formic acid, while *Methanosarcina* was found to utilise methanol and methylamine also. Acetic acid can only be utilised by two genera: *Methanosarcina* and *Methanothrix*. According to their ability to utilise the various substrates, the methanogenic bacteria can be classified as follows:

1) the hydrogen oxidising acetotrophs (HOA) which can utilise both H_2/CO_2 and acetate, i.e. *Methanosarcina*;
2) the non-hydrogen oxidising acetotrophs (NHOA) which can utilise only acetate and not H_2/CO_2, i.e. *Methanothrix*;
3) the hydrogen oxidising methanogens (HOM) which do not cleave acetate, but use H_2/CO_2 as substrates.

The free energy change during H_2/CO_2 methanogenesis is very negative (Table 1); this results in the high affinity of HOA and HOM bacteria for H_2. It has been reported that the hydrogen removal rate in a methanogenic reactor is actually only 1% of the maximum possible (Kaspar and Wuhrmann, 1978). During the methanogenic conversion of acetate the change in free energy is much smaller. As a result the removal of H_2/CO_2 proceeds preferentially over the acetate cleavage. This can result in inhibition of acetate cleavage by the methanogens when the level of hydrogen is high (Van den Berg *et al.*, 1976). The same conclusions can also be drawn from Figure 3; a hydrogen concentration in the gas phase above 10^{-4} atm will favour the methanogenic conversion of hydrogen (line 6) over the acetoclastic reaction (line 7).

Comparison of the kinetic parameters of hydrogen oxidising acetotrophs (HOA) such as *Methanosarcina sp.*, and non-hydrogen oxidising acetotrophs (NHOA) such as *Methanothrix sp.*, has shown that the latter have much lower substrate utilisation rates and their maximum growth rate is only one-fourth of that of HOA bacteria, although their affinity for acetate is much higher. The values of the biokinetic parameters of these bacteria are shown in Table 2.

Table 2 : Kinetics of acetate cleavage to methane

Reference	Culture	μ_{max} (d^{-1})	K_s ($\frac{mgCOD}{L}$)	Y ($\frac{gVSS}{gCOD}$)	R_s ($\frac{gCOD}{gVSS\,d}$)
Smith and Mah, (1978)	*Methanosarcina barkeri*	0.60	320	0.04	15.0
Zehnder *et al.*, (1980)	*Methanothrix soehngenii*	0.11	30	0.03	3.7

The above observations agree with the finding that *Methanosarcina sp.* developed throughout or in the inlet of reactors, wherever the acetate concentration

was equal to or higher than 350 mg L^{-1} of acetic acid. Only when the acetate concentration was less than 350 mg L^{-1} was *Methanothrix sp.* the prevailing species in the reactor, outcompeting the growth of *Methanosarcina sp.* (Ehlinger *et al.*, 1987a).

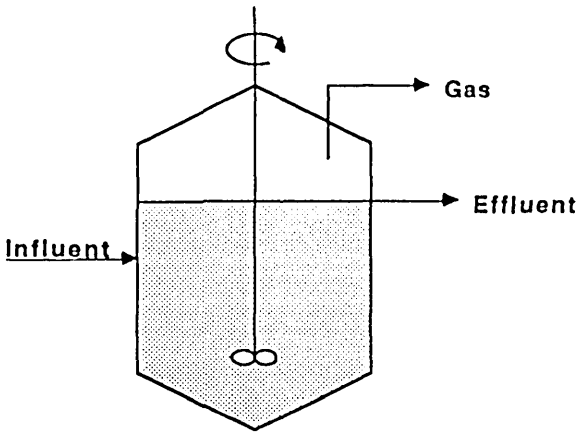
The rapid colonisation of the carrier particles of an anaerobic fluidised bed has been achieved by substituting part of the methanogenic substrate with methanol (Bull *et al.*, 1983b); methanogenesis through methanol has a high free energy change (Table 1), which suggests that more energy is available for growth. The resulting population is expected to be rich in *Methanosarcina sp.*, which is the only known methanogenic bacterium to utilise methanol (Harper and Pohland, 1986).

1.3. Anaerobic Digesters

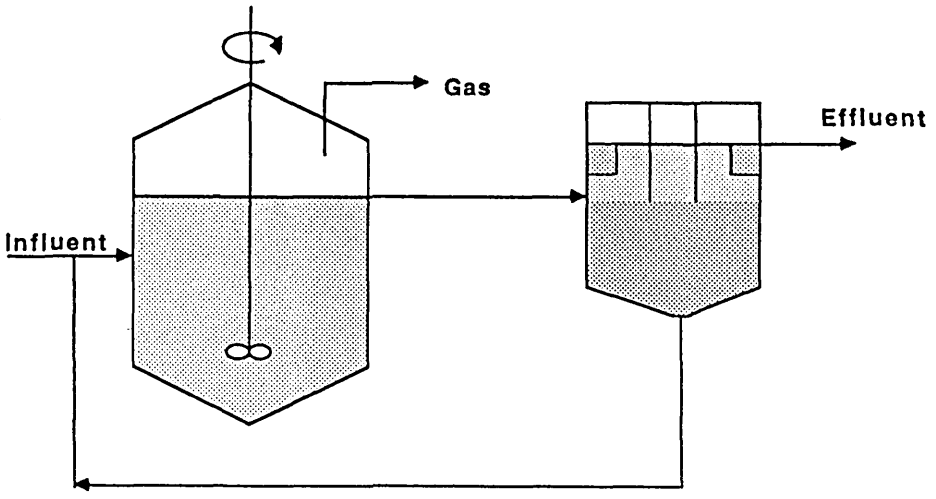
1.3.1. General Aspects

The types of reactor proposed in the literature for the anaerobic treatment of wastes are several. The initial designs were simple stirred reactors without any solids (biomass) recycle (Fig. 4). The slow growth rates of methanogenic bacteria necessitated the maintenance of reactor solids retention times as long as 30 d (Sahm, 1984). These reactors were quickly superseded by others which incorporated the separation and recycle of biomass (anaerobic contact process), which allowed the hydraulic retention time to be reduced to less than 15 d. However, even with systems designed to minimise the loss of solids, the maximum organic loading rate achieved was less than 4 kg COD $m^{-3} d^{-1}$ (Forster, 1984).

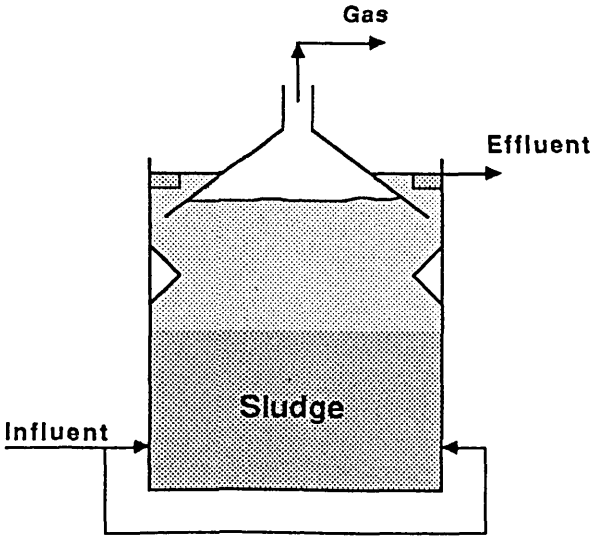
In order to overcome the difficulty of settling and recycling biomass, other reactor types were later introduced. These can be broadly divided into two groups, namely the non-attached growth systems (Fig. 4) and the fixed-film ones (Fig. 5). The former reactors depend for their performance on the ability of the microorganisms to form flocs or granules that can freely settle within the reactor.



Conventional Digester

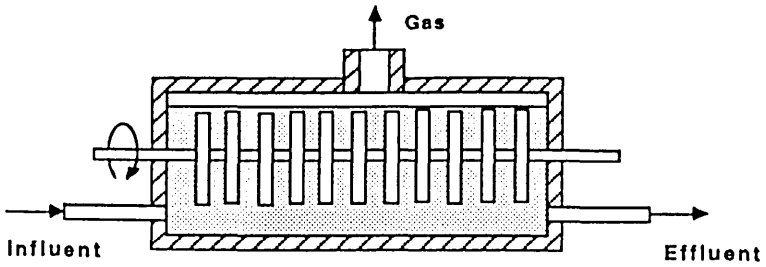


Anaerobic Contact Digester

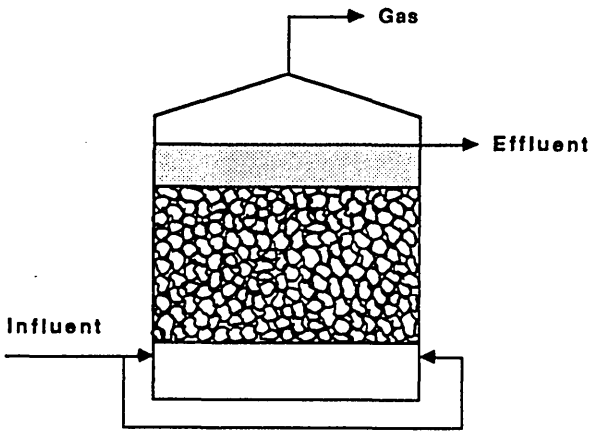


Upflow Anaerobic Sludge Blanket Reactor

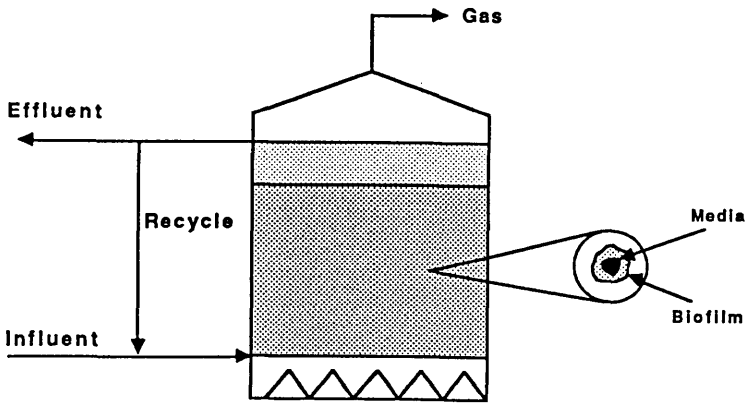
Figure 4 : Non-attached growth anaerobic digesters



Anaerobic Rotating Biological Contactor



Anaerobic Filter



Anaerobic Expanded/Fluidised Bed Reactor

Figure 5 : Fixed-film anaerobic digesters

Besides variations of the conventional reactors with or without recycle which are also included in this group, a new type of reactor has been introduced, the upflow anaerobic sludge blanket reactor (UASB) (Lettinga *et al.*, 1980). In this type of reactor the biomass forms large granules and flocs. These are kept in suspension by the gas bubbles produced and the upwards flowing wastestream; the latter should be controlled in order to avoid loss of biomass with the effluent (Fig. 4). The biomass concentration in the blanket is high (10-30 $g VSS L^{-1}$), resulting in good performance and high organic loading rates (40 $kg COD m^{-3} d^{-1}$). However, only certain wastes develop granular sludge quite readily (sugar-processing waste and wastes containing mainly volatile fatty acids), while for other wastes the granulation process is very slow or does not occur at all (Hulsoff-Pol *et al.*, 1983). Another problem with this type of reactor is that the low upflow velocity results in accumulation of any sediment found in the influent stream, which in turn produces decreased activity of the sludge blanket (Christensen *et al.*, 1984).

The anaerobic fixed-film reactors are characterised by the fact that microbial growth is promoted on an inert support medium. Almost any surface submerged in a nutrient-containing medium can be colonised by bacterial cells. The immobilised cells produce extracellular substances such as polysaccharides that can extend from the cell to form a structural matrix; the entire assemblage is commonly termed a biofilm (Characklis and Cooksey, 1983). The fixed-film anaerobic reactors can conveniently be classified into two distinct types, stationary- or fixed-medium systems and moving-medium ones. The anaerobic filters and the anaerobic rotating biological contactors (RBC) are included in the first group, while the anaerobic expanded and fluidised bed reactors are found in the second. Other types of reactor have also been presented in the literature, but they are mainly combinations of the above-mentioned ones.

The rotating biological contactor (RBC) became widespread in aerobic

processes of wastewater treatment, although its anaerobic application is less documented. Microorganisms attach to the inert plastic medium, which is usually a disc-array configuration partly or fully immersed and rotating horizontally in a vessel containing the wastewater (Fig. 5). The velocity of the revolution of the discs provides some control on the thickness of the biofilm (Borchardt, 1971).

The anaerobic filters, first investigated by Young and McCarty (1969), consist of a stationary inert medium, often non-porous (Fig. 5). Once an active microbial population is established, these reactors demonstrate a remarkable resilience to variable loading rates and to moderate environmental changes such as pH or temperature. According to the direction of movement of the wastestream, they can further be divided into upflow and downflow reactor types. In the upflow reactors the biological solids are held by attachment and entrapment within the component parts of the medium; accumulation of suspended matter is observed, although an equilibrium is generally established when the material is biodegradable. However, accumulation of refractory substances is undesirable and that is why the downflow configuration was introduced. Downflow filters may suffer blockages if an excessively small medium is employed; therefore, relatively large diameters ($> 2\,\mathrm{cm}$) are used which limit the specific surface area ($< 200\ \mathrm{m^2 m^{-3}}$) (Stronach *et al.*, 1986a). Due to the restricted area the biomass grows in thick layers (1-4 mm) around the packing particles and that can introduce diffusional limitations to the process. Another problem arising in the downflow reactors is channelling, which is less pronounced in the upflow systems.

The fixed-film concept was further extended with the development of expanded and fluidised beds (Fig. 5). These processes consist of passing the wastestream upwards through a bed of almost uniformly-sized fine particles such as sand (diameter < 1 mm). In the case of fluidised beds, the velocity of the liquid is sufficient to expand the bed beyond the point at which the frictional drag is equal

to the net downward force exerted by gravity. In this way, the particles can move freely, while the entire surface of each grain becomes available for biological colonization. The application of the fluidised bed principle to biological systems was originally proposed by Atkinson and Davies (1972). Initially, it was applied to denitrification processes by Jeris and Owens (1975), while expanded beds were first applied to the anaerobic digestion of wastewater by Jewell *et al.* (1981). The factors that contribute to the effectiveness of the expanded/fluidised bed process include (Cooper and Wheeldon, 1980; Stronach *et al.*, 1986a):

1) maximum contact occurs between the liquid and the biocoated particles;
2) liquid-film diffusional resistances are minimal due to particle motion and liquid velocities;
3) channelling, plugging and gas hold-up problems are generally circumvented;
4) small particles provide a large specific surface area (2000 m^2m^{-3}), which results in high biomass concentration (up to 40 $g\,VSS\,L^{-1}$) and reduced reactor size;
5) fast-settling particles permit high liquid velocities (10-30 $m\,h^{-1}$); this offers the possibility of treating dilute wastewaters (0.1-1.0 $g\,COD\,L^{-1}$) and allows the use of tall and thin reactors (small area requirements).

The expanded beds are known to result in a final effluent containing very low suspended solids (less than 5 $mg\,L^{-1}$): this is due to the trapping and filtering of the fine inert particles. Fluidised bed produce an effluent higher in suspended solids since all inert particles are removed from the reactor; therefore, they are more suitable for the treatment of wastes high in refractory solids. Potential disadvantages of the expanded and fluidised bed processes are the long periods of start-up required for the development of a balanced biofilm, the high energy requirements due to the high recirculation ratio, and the costly and not always fully

functioning liquid distribution systems which are required for uniform fluidisation in large scale systems.

1.3.2. Fundamentals of Anaerobic Fluidised Bed Reactors

Because all the claimed advantages of anaerobic fluidised bed reactors (AFBR) are derived from their ability to retain high biomass concentrations as biofilms within the reactor, the understanding of the phenomenon of biofilm formation is essential to the successful application of the AFBR technology.

Biofilm has been defined as the gelatinous matrix formed on media surfaces and is primarily composed of carbohydrates which are polymerised to produce linear or branched polysaccharides. The polysaccharide materials are considered to be microbial non-viable excretions or secretions (extracellular polymers). The biofilm acts as a binder which holds large colonies of microorganisms together (Characklis, 1973). The biofilm surface is highly adsorptive, partially due to its polyelectrolyte nature, and can collect significant quantities of silt, clay and other detritus (Characklis and Cooksey, 1983), while it allows the biofilm microorganisms to grow in an extremely dilute nutrient medium (Shieh and Keenan, 1986).

The nature of the nutrient-containing substrate was shown to affect the amount of biomass attached. It has been demonstrated that when glucose and other sugars are used, the growth of the biofilm on the media surface is stimulated because these substrates provide "building blocks" for polysaccharide production (Characklis, 1973). Ehlinger *et al.* (1987b) reported that the polysaccharide concentration of a biofilm was higher when glucose instead of a mixture of volatile fatty acids was used (7 $g L^{-1}$ as compared to 0.5 $g L^{-1}$), although the protein concentration was less affected (3.6 $g L^{-1}$ as compared to 1.8 $g L^{-1}$). This observation also suggests that polysaccharides are mainly secreted by acidogenic bacteria. The presence of certain nutrients such as nitrogen, phosphorus and sulfur in growth-

limiting concentrations also affect the polysaccharide production; Wilkinson (1958) reported that the polysaccharide production was maximised when the concentration of nitrogen became growth limiting. As a result, abundant biofilms which readily slough are typical of growth in nutrient solutions high in oxidizable materials, whereas dense, readily adhering biofilms are more common in low concentration nutrient solutions (Heukelekian, 1956).

The carrier medium most frequently utilised in AFBRs is sand, while other carriers such as synthetic resin, Al_2O_3, and active carbon have also been used (Jewell *et al.*, 1981, Switzenbaum and Danskin, 1982; Chen *et al.*, 1985b). The surface characteristics of the carrier medium used are shown to affect the biomass attachment (Characklis and Cooksey, 1983). Stimulation of the biofilm attachment by precoating the carrier particles with synthetic and naturally produced polymers was found partially successful (LaMotta *et al.*, 1982; Stronach *et al.*, 1987).

The total mass of biofilm is a direct function of the biofilm dry density ρ and the biofilm thickness δ. Consequently, both parameters affect the substrate conversion rates (Grady, 1983). It is, however, important to emphasize that a thick biofilm does not necessarily have a greater substrate conversion rate than a thin one. When the biofilm thickness is less than the critical value, the whole biofilm is active: the critical value of biofilm thickness is determined by the mass transfer limitations imposed by the extracellular polymers (Mulcahy *et al.*, 1981).

The biofilm thickness has been reported to be a direct function of the substrate loading rate (Characklis, 1981), unless the excess biomass is wasted or the height of the fluidised bed is controlled (Shieh *et al.*, 1981a). In the AFBRs the accumulation of the biofilm on the carrier medium is significantly affected by attrition among the bioparticles. This is in agreement with the observation that start-up using sand of 0.35 mm is more rapid than using sand of 0.75 mm diameter, mainly due to the lower shear forces present with smaller particles (Heijnen *et al.*,

1986). It also agrees with the suggestion of Shieh and Keenan (1986) that during the start-up of an AFBR, the superficial upflow velocity should be maintained above the minimum fluidisation velocity; in this way, a lower shear environment is provided and thus the biofilm formation is enhanced. In addition, the observed biomass concentrations in the lower region of the reactor are usually less than those observed in the middle and upper part, since the shear forces there are maximal (Bull *et al.*, 1984c). This points out the significance of adequate wastewater distribution at the inlet of the reactor, where much of the kinetic energy should be dissipated.

The above observations suggest that the optimal AFBR substrate conversion does not occur at maximum total biomass concentration or at maximum effectiveness of biomass, but rather at maximum effective biomass concentration throughout the reactor; in a NO_3^--N^- removal system, the biofilm thickness for the maximisation of the above parameters were found equal to 100, 50, and 80 μm, respectively (Shieh and Keenan, 1986).

The biofilm dry density is higher when the bulk-liquid concentration is high and/or the biofilm is thin, since the substrate availability is not affected. When the bulk-liquid concentration is low and/or the biofilm is thick, however, then the inner part of the biofilm enters the endogeneous respiration phase, which causes a decrease in biofilm dry density (Hoehn and Ray, 1973). The decrease in biofilm dry density with increasing biofilm thickness is very significant for the operation of AFBRs; less dense bioparticles tend to concentrate at the top of the reactor, which can lead to bioparticle wash-out and bed height control problems (Shieh and Keenan, 1986). Furthermore, the viable cell numbers are relatively low compared to the total biofilm volume, occupping 1-10% in the case of dilute nutrient solutions (Characklis, 1981). LaMotta (1976) has shown that beyond a certain biofilm thickness, no viable cells are found as monitored by the ATP concentration.

The temperature has also been shown to be an important factor for biofilm attachment. Although the optimal temperature for biomass growth is usually in the range of 35 to 37 °C, experiments have shown that polysaccharide production is greater at lower temperatures (Shieh and Keenan, 1986). This explains the observation that the optimum temperature for biofilm development lies between 20 and 30 °C (Shieh *et al.*, 1981b). Similarly, Boening and Larsen (1982) reported an increase of the attached biomass with temperature decreasing from 35 to 12.5 °C.

The kinetics of biological fluidised reactors have been studied by various researchers (LaMotta, 1976; Rittmann and McCarty, 1980; Mulcahy *et al.*, 1981; Shieh *et al.*, 1981b). The complexity of the mathematical modelling arises mainly from the fact that the combined effects of microbial processes and mass transfer phenomena have to be considered. Moreover, the fluidisation mechanism of biological bed reactors is more complicated than that of non-biological ones due to biofilm growth; this affects the overall density of the bioparticles and therefore, the expansion of the fluidised bed. In the following a summary of the basic equations describing the operation of AFBRs is given.

The bed porosity ϵ is a function of the liquid upflow velocity U ($L T^{-1}$) and the bioparticle terminal settling velocity U_t (LT^{-1}) according to the Richardson-Zaki correlation:

$$\frac{U}{U_t} = \epsilon^n \tag{1}$$

where n is a constant known as the expansion index. The latter can be expressed as an empirical function of the particle Reynolds number N_{Re}:

$$n = 10.35\, N_{Re}^{-0.18} \tag{2}$$

where $N_{Re} = \frac{d_p\, \rho_l\, U_t}{\eta_l}$, d_p is the bioparticle diameter (L), and ρ_l and η_l the density ($M L^{-3}$) and the viscosity ($M L^{-1} T^{-1}$) of the liquid respectively (La Motta,

1976).

The bioparticle terminal settling velocity can be calculated using Newton's law:

$$U_t = \left[\frac{0.75\ (\rho_p - \rho_l)\ g\ d_p}{C_D\ \rho_l} \right]^{1/2} \qquad (3)$$

where ρ_p is the density of the bioparticle (ML^{-3}), g is the gravitational acceleration (LT^{-2}) and C_D is Newton's drag coefficient which can be calculated by empirical equations as a function of Reynolds number N_{Re} and the shape of the particle. It is obvious that the lighter the bioparticle density, the lower the terminal settling velocity U_t and therefore, the more susceptible the particle to get washed out of the reactor. The density of the bioparticle ρ_p can be calculated from equation (4), when the medium (carrier) density ρ_m, the dry biofilm density ρ, the moisture of the biofilm P, the diameter of the medium particles d_m and that of the biocoated particles d_p are known:

$$\rho_p = \rho_m \left[\frac{d_m}{d_p} \right]^3 + \frac{\rho}{(1-P)} \left[1 - \left(\frac{d_m}{d_p}\right)^3 \right] \qquad (4)$$

The moisture content has been found experimentally to be almost constant and approximately 92.5 % for most applications (Shieh *et al.*, 1981a).

The substrate removal in a heterogeneous bioreactor such as a fluidised bed reactor can be described by the following steps:

1) transport of substrate from the bulk liquid to the liquid-biofilm interface (external mass transfer);

2) transport of substrate within the biofilm (internal mass transfer);

3) substrate conversion reactions within the biofilm.

The mathematical models of fluidised beds presented in the literature differ, since some of them account for both external and internal mass transfer resistances

(Rittmann and McCarty, 1980; Rodrigues *et al.*, 1983), while others take into consideration only the resistance within the biofilm and neglect the external mass transfer limitations (Shieh, 1980; Andrews, 1982). It has actually been found that, under the hydraulic conditions prevailing in common biological fluidised bed applications, the external mass transfer is negligible (Kissel, 1986). The internal mass transfer has been assumed to follow Fick's first law and thus the bioparticle continuity equation results in the following expression for the substrate conversion rate per unit biofilm mass R (T^{-1}) (Shieh and Keenan, 1986):

$$R = \frac{D_e}{r^2} \frac{d}{dr} \left(r^2 \frac{dS_r}{dr}\right) \tag{5}$$

where D_e is the effective diffusivity in the biofilm (L^2T^{-1}), r is the radial distance measured from the bioparticle centre (L), and S_r is the intra-biofilm substrate concentration in the radial distance r (ML^{-3}). The intrinsic kinetics are usually assumed to follow the Monod equation:

$$R = \rho\, k\, \frac{S_r}{K_r + S_r} \tag{6}$$

where ρ is the biofilm dry density, and k and K_r are constants. To simplify the computational work, the last equation can be reduced to zero or first order for $S_r \gg K_r$ and $S_r \ll K_r$ respectively (Shieh and Keenan, 1986).

Assuming that the diffusional limitations within the biofilm are also negligible, the equation used for the overall reaction coincides with that of the intrinsic reaction. Furthermore, when a recycle ratio greater than 5 is used, the reactor behaves as a completely mixed system, and the mass balance of substrate removal across the reactor results in the following expression for the observed specific rate of substrate consumption R_s (T^{-1}) (Shieh and Keenan, 1986):

$$R_s = \frac{S_o - S_e}{\theta\, X_{exp}} = \rho\, k\, \frac{S_e}{S_e + K_r} = R_{max} \frac{S_e}{S_e + K_r} \tag{7}$$

where X_{exp} is the biomass concentration of the reactor, expressed per unit volume of expanded bed (ML^{-3}), θ is the hydraulic retention time of the liquid calculated on the expanded bed volume (T), S_o and S_e are the influent and effluent substrate concentrations respectively (ML^{-3}), and R_{max} the maximum specific rate of substrate consumption (T^{-1}).

The mathematical simulation of the performance of anaerobic fluidised beds has shown that improved removal efficiencies can be obtained when a plug-flow rather than a completely-mixed regime is employed (Rittmann and McCarty, 1980); the effluent quality is superior due to stratification of the microbial population along the reactor. Harper and Pohland (1986) recommended the use of plug flow reactors, arguing further that the growth of *Methanosarcina sp.* will be encouraged in the inlet of the reactor where the highest concentration of acetate is likely to be found. However, in cases where highly acidic substrates are treated, an increased recirculation ratio ensures a better mixing between the influent and the effluent streams. As a result, sufficient pH adjustment can be achieved without the need of external pH control, which often requires the addition of costly buffers (De Walle and Chian, 1976; Koster, 1986).

Anaerobic fluidised bed reactors have been reported to treat various wastewaters such as meat and dairy wastes (Bull *et al.*, 1982; Stephenson and Lester, 1986), yeast water (Heijnen, 1983), phenol (Cheng and Chian, 1982), heat treat liquor (Hickey and Owens, 1981), whey permeate (Boening and Larsen, 1982) and molasses (Barnes *et al.*, 1984), efficiently. In Table 3, the values of the main operational characteristics of some of these processes are presented.

The amount and composition of the biogas produced by complete digestion varies with the substrate used. This becomes obvious when the overall equation describing the breakdown of a carbohydrate is applied (Mosey, 1981):

$$C_nH_aO_b + (n - \frac{a}{4} - \frac{b}{2})\,H_2O \rightarrow (\frac{n}{2} - \frac{a}{8} + \frac{b}{4})\,CO_2 + (\frac{n}{2} + \frac{a}{8} - \frac{b}{4})\,CH_4 \qquad (8)$$

Table 3 : Operational parameters of anaerobic fluidised bed reactors

Reference	Substrate	Carrier type	Carrier diameter (mm)	T (°C)	S_o ($g L^{-1}$)	OLR ($kg m^{-3} d^{-1}$)	θ (h)	%COD Removal
Switzenbaum and Jewell (1980)	Glucose	Al_2O_3	0.5	10-30	0.05-0.6	0.8-43	0.3-6.0	40-80
Jewell *et al.* (1981)	Municipal Sludge	Resin	1.0	20		0.6-35	0.2-5.0	5-85
Hickey and Owens (1981)	Heat Treatment liq.			30-35	10		4-21	52-75
	Acid Whey				53		4-19	66-89
	Soft Drink				6		36-120	72-84
Bull *et al.* (1982)	Miik Waste	Sand	0.22	37	5	4.5	15	90
Boening and Larsen (1982)	Whey Permeate	Coal	0.18	35	3-10	8-27	2.4-14	70-95
				20	3.5-4	8-21	5-10	40-75
				15	2.2-3	5-12	2-14	25-55
Frostell (1982)	Molasses	Sand	0.15	30	8.7	24	8	43
Li *et al.* (1982)	Whey Permeate	Sand	0.5	30-35	35		43.6	76
Switzenbaum and Danskin (1982)	Sweet Whey	Al_2O_3	0.5	25-31	10	9-60	4.1-27	37-93
Heijnen (1983)	Yeast Water	Sand	0.2	37	2.5	50	1	>90
Biver (1984)	Glycerin	Sand	0.4	20	1.4	7	4	95
Bull *et al.* (1983c)	Glucose/Oxoid	Sand	0.22	22	1.25	2.5	12	90
Schraa and Jewell (1984)	Sucrose	Diat. earth	0.45	55	1.5-16	7-154	0.5-5.2	30-90
Chen *et al.* (1985a)	Glucose	Carbon	0.6	35	0.5-90	<50	0.5-8	90

The methane content, as well as the percentage of methane in the gas, as calculated by Mosey (1981), are presented in Table 4.

Table 4: Theoretical gas yield coefficient for the anaerobic digestion process

Type of organic matter	Analysis	Methane Yield Coefficient (m^3 kg^{-1})	% CH_4 in Gas
All Types	COD	0.35	—
Carbohydrates	VS	0.39	50
Fats	VS	1.03	72
Proteins	VS	0.51	53
Acetate	direct	0.37	50
Propionate	direct	0.47	58
Methanol	direct	0.84	75

1.3.3. Two-Phase Anaerobic Digestion

The anaerobic digesters described so far can be considered as single-stage systems, since all biochemical and microbiological processes take place in the same reactor. Over the last few years, however, a new configuration, the so-called two-phase anaerobic digestion system, was introduced and investigated (Ghosh and Klass, 1978; Cohen *et al.*, 1979; Bull *et al.*, 1984a). Two-phase anaerobic digestion involves two biologically active digesters in series and allows for spatial separation of the acidogenic and methanogenic populations: in the first digester, complex organic substances (carbohydrates, proteins, fats) are hydrolysed and fermented mainly to volatile fatty acids by a number of bacteria collectively known as acidogenic bacteria, while in the second digester, the organic acids are converted to biogas by the combined activity of acetogenic (OHPA) and methanogenic bacteria.

The advantages of phase separation are claimed to be several (Ghosh *et al.*, 1975; Massey and Pohland, 1978; Breure and Van Andel, 1984; Sahm, 1984). Phase separation allows the maintenance of appropriate densities of acidogenic and methanogenic bacteria in separate reactors. It enables the maximisation of the rates of acidification and methanogenesis by application of optimal operational conditions, which accommodate the nutritional requirements, physiology, pH optima, growth and nutrient uptake kinetics of both groups of bacteria.

The physical separation of the acidogenic and methanogenic phases also results in better process control and thus increased stability of the anaerobic digestion system. Possible overloading of the methanogenic reactor can be detected at earlier stages and prevented by adapting the supply of the acidified effluent of the first reactor to an optimal employment of the methanogenic activity present in the methane reactor. The acidification reactor can also serve as a buffer system when the composition of the wastewater is variable and can provide a constant substrate for methanogens; the latter are known to adapt slowly to varying substrate content and composition (Cohen *et al.*, 1982). Moreover, Cohen *et al.* (1981) have shown that the time required for a two-phase system to return to its initial performance after a loading shock is only half the time required for the single-phase system. Phase separation can also help the elimination of compounds that are hazardous or toxic to methanogenic bacteria (e.g. oxygen, sulphate, formaldehyde). The importance of the removal of these compounds in separate acidogenic reactors has been emphasized by Lettinga *et al.* (1983).

Another argument put forward in support of phase separation is that the growth of acidogens in a single-stage process may exert a detrimental effect on the sludge settling characteristics, especially when acidogenic degradation of proteinaceous materials occurs (Cohen, 1983). The two-phase configuration enables the disposal of the faster growing acidogenic bacteria without loss of the

methanogenic bacteria; it has been estimated that the yield of the former is approximately 8%, while that of the latter is only 1.2% (Breure and Van Andel, 1984). The activity of methanogenic bacteria in the methanogenic reactor of a two-phase system has been found to be 3 times higher than that of a single-stage system, using either continuous-flow stirred tank reactors (Cohen *et al.*, 1980) or upflow sludge blanket reactors (Lettinga *et al.*, 1983). The implication of this observation is that higher organic loadings can be applied to the methanogenic reactor, which allows smaller methanogenic reactors to be used. When a fluidised bed reactor is used for methanogenesis, smaller sand particle size and consequently lower pumping costs are required, because of the low biomass yield and the biofilm characteristics of methanogens (Sutton and Li, 1983). Finally, it has been claimed that phase separation leads to the production of biogas of a higher methane content and therefore a higher caloric value as compared to one-phase processes; a possible explanation for this is given by Lettinga *et al.* (1983) who suggest that this is due to the reduction of NO_3^-, NO_2^- occurring in a single-stage system.

The two-phase anaerobic process has been suggested to be most efficient and therefore to be preferred when dealing with a liquid contaminated primarily with carbohydrates (starch, sugar, pectin). In these cases, excessive acidification of the influent, resulting in reduced stability of the single-phase system, is highly likely (Mudrack and Kunst, 1986). When, however, fats or proteins are the principal constituents, a single-stage system may appear more appropriate. However, experimental evidence suggests that the application of the two-phase process is exceptionally effective when wastes containing fats are treated in this way, since the inhibitory effect of the produced long-chain fatty acids upon the methanogenic bacteria is reduced (Hanaki *et al.*, 1987). Although the two-phase process seems best suited for the treatment of soluble-type wastewaters which present a high potential for volatile acid accumulation, superior performance has also been demonstrated for

particulate-type substrates such as sewage sludge and agricultural wastes (Keenan, 1976; Norrman and Frostell, 1977; Rijkens, 1981; Colleran *et al.*, 1983).

Phase separation can be achieved by membrane separation (Hammer and Borchardt, 1969) and by kinetic and pH control (Ghosh *et al.*, 1975; Massey and Pohland, 1978; Cohen *et al.*, 1979; Bull *et al.*, 1984a). The types of reactor used in two-phase systems cover the full range of anaerobic digesters. It should be noted, however, that when the first reactor is a fixed-film one, no real phase separation can be obtained since methanogenic bacteria can grow within the biofilm. As a result, the system operates as a two-stage rather than a two-phase one. The main operational parameters for some of the two-phase systems presented in the literature are shown in Table 5. The percentage of the initial COD converted to volatile fatty acids (degree of acidification) varied between 10 % and 96 %, depending on the substrate used and the hydraulic retention time applied in the first reactor. However, in all cases the performance of the two-phase system was comparable or superior to that of the single stage one. This agrees with the observation of Cohen *et al.* (1985) that even partial acidification (as low as 10 %) can actually stimulate the methanogenic activity of the bacterial population in the second reactor.

The effluent of an acidogenic reactor contains mainly acetic, propionic and butyric acids, although higher fatty acids are found at lower concentrations. Products such as lactate and ethanol, which are produced through less favourable pathways, are generally not present during steady-state operations (Cohen *et al.*, 1984). According to recent findings (Cohen *et al.*, 1984), there are two important fermentation types occurring complementary to each other, namely the butyric acid and the propionic acid types. Butyric acid fermentation is characterised by the production of butyrate, acetate, hydrogen and carbon dioxide as the main fermentation products, while propionic acid type fermentation results mainly in the formation of propionate, acetate and some valerate, with no significant gas

Table 5 : Operational parameters of two-phase anaerobic digestion systems

Reference	Substrate	Influent COD Conc. ($gCOD\,L^{-1}$)	ACID REACTOR Type	θ (h)	pH	%acidif.	%remov	METHANE REACTOR Type	θ	pH	OVERALL *OLR*	%remov.
Massey and Pohland (1978)	Glucose based	3.9	CSTR	11-23	6.4	54-73		CSTR	68-97	6.6	0.8-1.2	54-60
Ghosh *et al.* (1975)	Sewage sludge	5.3	CSTR	13	5.8	62		CSTR	155	7.2	3.2	40
Ghosh and Henry (1981)	Soft drink	45	CSTR	52	4.7	20	1	CSTR	125	7.5	6.1	95
Cohen *et al.* (1979)	Glucose	10 Gluc.	CSTR	10	6.0	96	12	CSTR	100	7.8		98
Tanaka and Matsuo (1986)	Milk waste	1.5	CSTR	24	6.1	37	10	Upflow	82	6.5	0.3	92
Pipyn and Verstraete (1979)	Distillery waste	10	CSTR	16-72				Upflow	14		14	78
Bull *et al.* (1984a)	Glucose based	6-12	CSTR	1.7-10	3.5-5.5	18-22		AFBR	17-100	6.9	1.8-15	50-92
Stephenson and Lester (1986)	Synthetic meat waste	2.5-5	CSTR	3.5	6.4	34	6-23	AFBR	12	7.5	3.6-7.6	75-81
Roy and Jones (1982)	Glucose	0.73 TOC	CSTR	4.5	4.3			Upflow	2.4	7.4	7.3	83
Zepeng *et al.* (1985)	Molasses Alcohol waste	30	Upflow	3.6	5.1		8-15	Upflow	2.6	7.0	5.4	89
		34	Upflow	14	5.0			Upflow	2.4	7.4	7.3	83
	Monosod. Glutamate waste	25	Upflow	24	5.0			Upflow	2.6	7.0	5.4	89
		17	Upflow	13	4.5							

production.

In light of the recent understanding of the importance of hydrogen in anaerobic digestion processes, some researchers have maintained that the physical separation of acidogenic and methanogenic bacteria, as practiced in the two-phase anaerobic systems, cannot enhance anaerobic rates, since it disturbs the interspecies transfer of hydrogen. It is now recognised that, due to the suppression of growth of methanogenic bacteria in the acidogenic reactor, accumulation of hydrogen can occur. This may lead to an alternation of the fermentation pattern of the acidogenic phase towards increased utilization of metabolic routes that do not employ H^+ reduction as a means of disposing of electrons, but instead employ organic electron acceptors. As a result more propionic and butyric acids are produced (Cohen, 1983). However, the constant production of propionic and/or butyric acids in the first reactor can be beneficial for the methanogenic reactor, since it enables the development of a healthy population of obligate hydrogen producing acetogenic (OHPA) bacteria and the associated hydrogen-oxidising methanogens, which ensures the rapid assimilation of these acids in the second reactor (Asinari di San Marzano *et al.*, 1981; Harper and Pohland, 1986).

In order to optimise the two phases of anaerobic digestion, it would be useful to engineer the operation of the first reactor towards those acids which are preferable substrates for methanogenesis. The rate of butyric acid removal has been found to be higher than that of the rest of the volatile fatty acids (Verstraete *et al.*, 1981; Cohen *et al.*, 1982; Mudrack and Kunst, 1982; Dohányos *et al.*, 1985). As a result, the rate-limiting step of butyrate degradation in methanogenic digesters is the conversion of the acetate produced to methane (Kaspar and Wuhrmann, 1978). The rate-limiting step of propionate degradation is the splitting of propionate to acetate, which is slower than the conversion of the acetate produced to methane (Lin *et al.*, 1986). A potential problem, however, for large scale operations

producing butyrate, might be odour control (Verstraete *et al.*, 1981).

The inhibitory effect of increased hydrogen concentration on the anaerobic degradation of propionate, as well as on the thermodynamics of the reaction, have been described in a previous paragraph. It is of interest to note that propionate degradation is largely inhibited during periods of high activity of the butyrate converting bacteria, because of the elevated hydrogen concentrations the latter produce (Wiegant *et al.*, 1986). Acetate exerts a less strong influence on the thermodynamics of the conversion of propionate, although it is also known to be inhibitory (Kaspar and Wuhrmann, 1978; Wiegant *et al.*, 1986). The above observations agree with the finding that the degradation of propionate proceeds more rapidly when individual acids rather than a mixture of volatile acids are used (Verstraete *et al.*, 1981) and it is therefore suggested that controlled acidification leading to one or only a few intermediates would be desirable.

Separate acidogenesis as part of a two-phase process has been studied in reactors treating glucose, sucrose, starch, pectin and gelatin as substrates (Cohen *et al.*, 1979; De la Torre and Goma, 1981; Breure and Van Andel, 1984; Dohányos *et al.*, 1985). These studies have shown that the products of the first phase, the conversion of substrate to volatile fatty acids, as well as the optimum conditions of the first phase, depend greatly on the substrate used. The operational parameters such as hydraulic retention time, organic loading rate, temperature and pH also affect the content and composition of the acidification reactor effluent. It is important to know the effect of these parameters, firstly because it can help in the engineering of the first phase towards more desirable products, and secondly because it can indicate those parameters to which the system is more sensitive. According to some researchers, there is little dependence of the product distribution of the first phase on the hydraulic retention time (Zoetemeyer *et al.*, 1982a; Breure and Van Andel, 1984), while others have found that it has a considerable effect on it

(Joergensen, 1978; Cohen *et al.*, 1984). Similarly, contradictory results have been reported for the effect of pH on the effluent composition, which was shown to be negligible in the range of 5 to 7 (Joergensen, 1978; Zoetemeyer *et al.*, 1982a), although other researchers found a more pronounced influence (Verstraete *et al.*, 1981; Breure and Van Andel, 1984; Dohányos *et al.*, 1985). Elevated temperatures were found to result in higher product concentrations and biomass growth rates, because of less pronounced product inhibition (De la Torre-Lozano *et al.*, 1980). Finally, increasing organic loading rates has been variously reported to result in the production of more propionic acid (Bull *et al.*, 1984a; Stoppok and Buchholz, 1985), and conversely more butyric acid and other products (Mudrack and Kunst, 1982).

1.4. Bacterial Growth and Biokinetics

1.4.1. Fundamentals of Continuous Culture Kinetics

The basis for the description of microbial growth was laid by Monod (1949): he assumed that the change in the biomass concentration x (M L^{-3}) with time is a linear function of x:

$$\frac{dx}{dt} = \mu x \tag{9}$$

where μ is the specific growth rate of the microorganisms (T^{-1}) and indicates the rate of biomass production per unit of biomass present in the system. Furthermore, Monod observed the value of μ to change with the substrate concentration S (ML^{-3}) according to:

$$\mu = \mu_{max} \frac{S}{K_s + S} \tag{10}$$

where μ_{max} is the maximum specific growth rate when there are no restrictions to the substrate availability (T^{-1}) and K_s is a constant (ML^{-3}). The latter indicates the substrate concentration for which the observed specific growth rate μ is half the

maximum value μ_{max}, a large value of K_s suggesting that a high substrate concentration is required for maximum growth. Therefore, K_s is commonly referred to as the half velocity coefficient or the substrate saturation constant.

A constant relationship was also found to exist between the mass of bacteria produced and the amount of substrate utilised (Monod, 1949):

$$Y = \frac{dx}{dS} \tag{11}$$

where Y is a constant usually termed the biomass yield coefficient.

When the kinetics of a continuous culture are studied, and assuming that the biomass concentration of the influent x_o equals zero, the mass balance of the biomass in the chemostat can be expressed as:

$$\text{Net Accumulation} = \text{Growth} - \text{Loss} - \text{Decay}$$

$$\frac{dx_e}{dt} = \mu\, x_e - D\, x_e - k_d\, x_e \tag{12}$$

where x_e is the biomass concentration in the chemostat and in the effluent ($M L^{-3}$), D is the dilution rate (T^{-1}), calculated as the ratio of the influent flowrate over the volume of the chemostat, and k_d is the specific decay rate of the microorganisms (T^{-1}). At steady state $\frac{dx_e}{dt} = 0$, so equation (12) reduces to:

$$\mu = D + k_d \tag{13}$$

A mass balance on the substrate concentration S_e in the chemostat gives:

$$\text{Net Accumulation} = \text{In} - \text{Out} - \text{Consumption for Growth}$$

$$\frac{dS_e}{dt} = (S_o - S_e)\, D - \frac{\mu\, x_e}{Y} \tag{14}$$

where S_o is the influent substrate concentration and S_e is the substrate concentration in the chemostat and in the effluent (ML^{-3}). At steady state $\frac{dS_e}{dt} = 0$, which combined with equations (13) and (14) produces the following expression:

$$\frac{D\,(S_o - S_e)}{x_e} = \frac{k_d}{Y} + \frac{D}{Y} \tag{15}$$

Similarly, a mass balance on the product concentration P_e in the chemostat gives:

Net Accumulation = Production + Input − Output

$$\frac{dP_e}{dt} = r_p - D\,(P_e - P_o) \tag{16}$$

where r_p is the rate of product formation per unit of reactor volume ($ML^{-3}T^{-1}$), P_o is the product concentration in the influent, and P_e is the product concentration in the chemostat and in the effluent (ML^{-3}). At steady state $\frac{dP_e}{dt} = 0$, which combined with equation (16) gives:

$$q_p = \frac{r_p}{x_e} = \frac{D\,(P_e - P_o)}{x_e} \tag{17}$$

where q_p is the specific rate of product formation (T^{-1}) and indicates the amount of products formed per unit biomass present in the reactor per unit time.

In order to describe the rate of product formation r_p as a function of the biomass of the system, Luedeking (1967) proposed the following equation:

$$r_p = \alpha\left[\frac{dx}{dt}\right] + \beta\, x \tag{18}$$

where $\left[\frac{dx}{dt}\right]$ is the rate of biomass production ($ML^{-3}T^{-1}$) and α and β are constants. When the product formation is only growth-associated, the constant β is equal to zero, and the rate of product formation depends only on the biomass growth rate (Blakebrough, 1967; Bailey and Ollis, 1977). By a combination of equations (9) and (18) the following expression can be derived:

$$q_p = \alpha\,\mu + \beta \tag{19}$$

The above description of the kinetics of a continuous culture is based upon the assumption that the biomass yield coefficient Y is constant [eq. (11)]. This

assumption is employed in practically all microbial growth models presented in the literature. However, there is evidence that the yield coefficient Y is not constant. According to Stover *et al.* (1983) only k_d is a true biokinetic constant, while Y is a variable one. The concept of the specific decay rate k_d is based on the assumption that some of the synthesized materials undergo endogeneous oxidation to produce energy not only for growth, but also to maintain the viability of the cells that have already been synthesized (Gaudy and Gaudy, 1980). Alternatively, the maintenance coefficient m has been used by other researchers: the maintenance requirement of a population of microorganisms is the amount of substrate required to provide energy for movement, internal repairs, preservation of concentration gradients etc. (Ghosh *et al.*, 1975). However, the assumption of constant substrate utilisation was again the basis for all kinetic calculations: the fractional substrate assimilations for synthesis and growth energy were assumed to be constant.

Biomass growth kinetics during anaerobic digestion has been studied by various researchers (Lawrence and McCarty, 1969; Ghosh and Klass, 1978; Anderson and Duarte, 1980). In Table 6, a comparison of the kinetic constants of the

Table 6 : Growth parameters of anaerobic bacteria

	μ_{max} (d^{-1})	K_s (gCOD L^{-1})	Y	Reference
Acidogenic Fermentation				
Glucose	7.2	0.4	0.14	Ghosh & Klass (1978)
Sewage Sludge	3.8	18.3	0.28	Ghosh & Klass (1978)
Cellulose	1.7	30.8	0.13	Ghosh & Klass (1978)
Methanogenic Fermentation				
Acetate	0.49	4.2	0.28	Ghosh & Klass (1978)
Overall Process				
Glucose	0.16	0.2	0.21	Anderson & Duarte(1980)
Glucose/Peptone	0.27	3.0	0.19	Anderson & Duarte(1980)

acidogenic and the methanogenic fermentation of various substrates is presented. It is now obvious that the specific growth rate of acidogenic bacteria is higher than that of the methanogenic bacteria, indicating that greater dilution rates can be applied to acidogenic reactors without wash-out of their bacterial populations [eq. (13)]. Furthermore, the biomass yield coefficient of acidogenic bacteria is 5 to 10 times higher than that of the methanogenic bacteria. Finally, the easily biodegradable substrates (e.g. glucose) have lower substrate saturation constants K_s than those that are not easily biodegradable (e.g. cellulose).

1.4.2. Inhibition Models

The Monod kinetics as described in the previous section have been extensively used over the last decades in the modelling of the anaerobic digestion processes (Ghosh and Pohland, 1974; Oi *et al.*, 1984; Ng *et al.*, 1985). The implication of the basic equation [eq. (10)] is that no other restrictions are imposed on the specific growth rate of the biomass, other than the substrate availability. However, during the culturing of microorganisms, growth may be inhibited. This may be caused by:

1) the substrate, if it is present in very high concentration,
2) a product formed by the microorganisms or another compound of the medium.

During the acidogenic phase of anaerobic digestion, there is evidence that product inhibition occurs, which results in decreased observed specific growth rates (Ierusalimsky, 1967; De la Torre-Lozano *et al.*, 1980; Zoetemeyer *et al.*, 1982c). The Monod equation [eq. (10)] no longer holds true. The proposed inhibitors, as well as the type of inhibition function, presented in the literature are very diverse. Hill and Barth (1977) introduced a Haldane-type inhibition model, which originates from enzyme kinetics:

$$\mu = \mu_{max} \frac{1}{1 + \frac{K_s}{S} + \frac{I}{K_i}} \qquad (20)$$

where I is the inhibitor concentration (ML^{-3}) and K_i is the inhibition constant. The inhibition constant has the same dimensions as the concentration of the inhibitor and a low value of the constant signifies strong inhibition. In this model, the un-ionised volatile fatty acids were assumed to act as inhibitors. The same inhibition function has been used by other researchers: they assumed that the inhibitor was either the un-ionised volatile fatty acids (Marsili-Libelli and Nardini, 1985; Moletta *et al.*, 1986), or the total concentration of propionic and butyric acids (Sinechal *et al.*, 1979). However, although successful for the prediction of the performance of an anaerobic digester, this inhibition function was only validated with data taken from single-stage anaerobic digesters and not from the first stage of a two-phase anaerobic digestion process. It is, therefore, essential that this model is applied to experimental data of an acidogenic reactor, in order to ensure that it can be applied for the prediction of the performance of the acidogenic phase.

Another inhibition expression reported in the literature and applied to the acidogenic phase is the non-competitive one. This is also derived from enzyme kinetics, and was first introduced by Ierusalimsky (1967):

$$\mu = \mu_{max} \frac{S}{S + K_s} \frac{K_i}{K_i + I} \tag{21}$$

The inhibiting effect of propionic acid was shown to be more pronounced than that of acetic acid. The same inhibition model was also applied during the propionic acid conversion of glucose by a *Propionibacterium sp.*. The specific growth rate of the organism was then found to be inhibited only by the propionic acid concentration (Hendricks *et al.*, 1985). However, it appears that other volatile fatty acids, such as butyric acid (Zoetemeyer *et al.*, 1982c), as well as long chain fatty acids (Hanaki *et al.*, 1981), can also have inhibitory effects on acidogenesis.

Substrate inhibition of the acidogenic phase has also been reported for a system treating glucose (Van Heuvel, 1986). In a combined model, the substrate

inhibition was assumed to follow the Haldane-type equation, while the product inhibition followed the non-competitive type with the un-ionised butyric acid acting as the sole product inhibitor:

$$\mu = \mu_{max} \frac{1}{1 + \frac{K_s}{S} + \frac{S}{K_{si}}} \frac{K_i}{K_i + I} \tag{22}$$

where K_{si} is the substrate inhibition constant (ML^{-3}).

Finally, De la Torre-Lozano *et al.* (1980) have studied the acidogenic phase of anaerobic digestion separately and have proposed the following inhibition model, assuming that the total volatile fatty acid concentration acts as inhibitor:

$$\mu = \mu_{max} \frac{K_i}{K_i + I} (1 - \lambda S_o) \tag{23}$$

where S_o is the influent substrate concentration (ML^{-3}), and λ is a constant (L^3M^{-1}).

The specific rate of product formation q_p can also be described by using models similar to the ones describing the specific biomass growth rate μ. This becomes obvious from equation (19): when product formation is only growth associated, the constant β is equal to zero and q_p is proportional to the value of μ. The equations describing q_p according to the non-competitive and the Haldane-type inhibitions models respectively are expressed as follows:

$$q_p = q_{max} \frac{S}{S + K_s'} \frac{K_i'}{K_i' + I} \tag{24}$$

and

$$q_p = q_{max} \frac{1}{1 + \frac{K_s'}{S} + \frac{I}{K_i'}} \tag{25}$$

The values of the biokinetic constants K_s' and K_i' would be expected to be the same as K_s and K_i respectively, obtained from the specific biomass growth rate models. However, analysis of experimental data has shown that discrepancies may

exist between the values determined by the two methods (Aiba *et al.*, 1973).

The modelling of the biomass growth and product formation in the acidification phase of anaerobic digestion can be particularly useful for the prediction of the performance of conventional single-stage anaerobic digesters, as well as the performance of the acidification reactor of a two-phase anaerobic digestion system. This application can lead to the better understanding and process control of the various anaerobic digestion systems.

CHAPTER 2

OBJECTIVES

The numerous advantages of anaerobic digestion over conventional aerobic methods make the former an ideal alternative over the latter for treating industrial wastes. There are, however, certain drawbacks to its applications, such as the prolonged periods required for start-up and the process instability which results in the failure of operating digesters.

In order to increase the stability of anaerobic digestion, two-phase anaerobic systems have been introduced and investigated. The physical separation of the acidogenic and methanogenic phases can increase stability because overloading of the methane reactor can be prevented by proper control of the acidification step. Furthermore, it enables the maximisation of the rates of acidification and methanogenesis by applying the optimum operational conditions for each group of bacteria, as determined by their biokinetic and metabolic properties.

In order to optimize the two phases of anaerobic digestion, it is necessary to engineer the operation of the first reactor towards those acids which are preferable substrates for methanogenesis. It has been shown that butyric acid is such an intermediate. Furthermore, the production of one or only a few major intermediates was also found to be more advantageous to methanogenesis than the production of a variety of intermediate products .

Separate acidogenesis as a part of a two-phase process has been studied in reactors treating various synthetic substrates. These studies have shown that the products of the first phase may vary. Furthermore, the operational parameters, such as hydraulic retention time, organic loading rate, influent substrate concentration, temperature and pH also affect the content and composition of the effluent of the acidification reactor. Unfortunately, contradictory results have been reported for the effect of these parameters; the latter is important to know firstly because it can help

to engineer the first phase towards more desirable products, and secondly because it can indicate those parameters to which the system is most sensitive.

A further understanding of the acidogenic phase requires the knowledge of the growth patterns of acidogenic bacteria. The Monod equation has been used extensively to describe their growth, although there is evidence that inhibition occurs in the first phase of anaerobic digestion. Several attempts have been made to model the acidogenic phase in the presence of inhibition: the proposed inhibitors, as well as the form of the inhibition function, vary.

Recently, new types of fixed-film reactors, namely the expanded and fluidised bed reactors have been introduced and used successfully for the treatment of wastewaters. In these reactors the biofilm is attached around small carrier particles which are kept in suspension by the upflow movement of the wastestream. The advantages of such systems are numerous: high biomass concentration, the possibility of low hydraulic retention times without wash-out of methanogenic bacteria, high tolerance of rapid temperature and organic loading rate changes, controlled bacterial film thickness, and reduced channelling, plugging and gas hold-up problems.

The objectives of this study were thus defined as follows:

1) To assess the influence of operational parameters such as hydraulic retention time, influent substrate concentration, organic loading rate, pH and temperature on the performance of acidogenic reactors.
2) To model the growth rate of the acidogenic bacteria and the rate of product formation during acidogenesis.
3) To assess the influence of phase separation in the anaerobic degradation of a complex waste, using a continuous-flow stirred tank reactor for the acidogenic phase and a fluidised bed for the methanogenic phase.
5) To determine the optimum ratio between the hydraulic retention time of the acidogenic reactor and that of the methanogenic reactor.

CHAPTER 3

MATERIALS AND METHODS

3.1. Laboratory Scale Acidification Reactors

3.1.1. Description of the Acidification Reactor Used in the Studies of the First Phase of Anaerobic Digestion

The schematic diagram of the apparatus utilised for the studies of the first phase of anaerobic digestion (acidogenesis) is presented in Figure 6.

The synthetic wastewater was pumped into the reactor vessel via a sterile silicone tubing line using an LKB Multi-perpex pump (LKB Ltd., Croydon, U.K.). The reactor vessel was a commercial Ultroferm laboratory fermenter (LKB-Biotec 1602-012, LKB Ltd., Croydon, U.K.) with a working volume of 2-4 L. The fermenter was also supplied with a control unit (LKB-Biotec 1601-023), with modules, actuators and transducers for stirrer speed, temperature, pH control, mechanical foam-breaking, and dissolved-oxygen monitoring (Fig. 7). The mechanical stirrer could be operated at a maximum speed of 1500 ± 10 r.p.m., while the temperature could be set between 4 and 70 °C with an accuracy of ± 1 °C. The pH was adjusted as required with an accuracy of ± 0.05 units, using solutions of $2M$ $NaOH$ and $2M$ HCl.

The effluent of the reactor was removed through a submerged overflow system, which was also used for the adjustment of the reactor volume. The gases produced were collected into a bottle filled with acidified distilled water (0.5 M H_2SO_4) through one of the ports provided at the top of the reactor. The top of the reactor vessel was supplied with a number of ports, the unused ones being sealed with silicone rubber septa. The sampling of the reactor liquid was performed through one of the ports by means of a plastic syringe supplied with a specially constructed long wide-bore stainless steel needle.

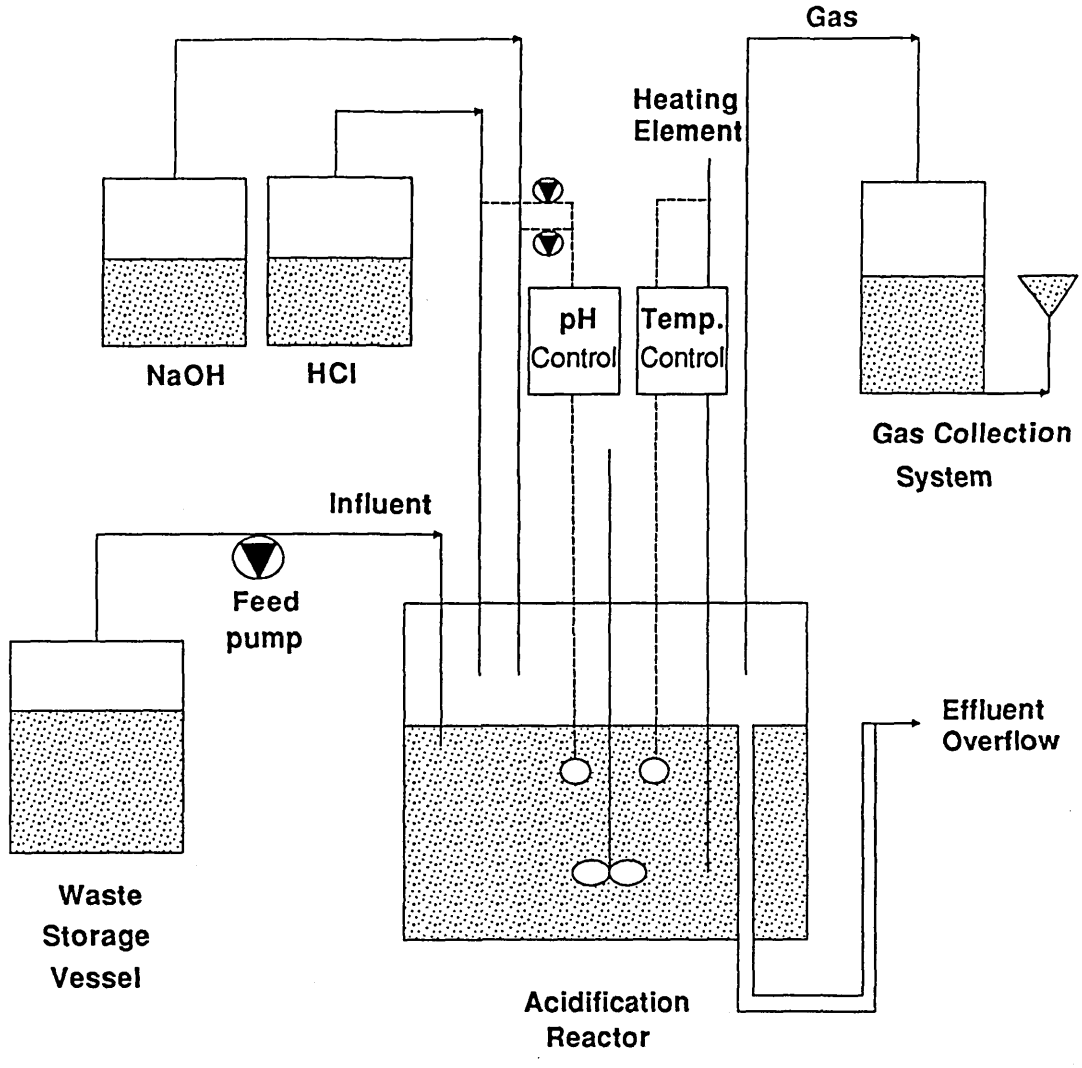


Figure 6 : Schematic diagram of the reactor used in the first phase (acidification) studies

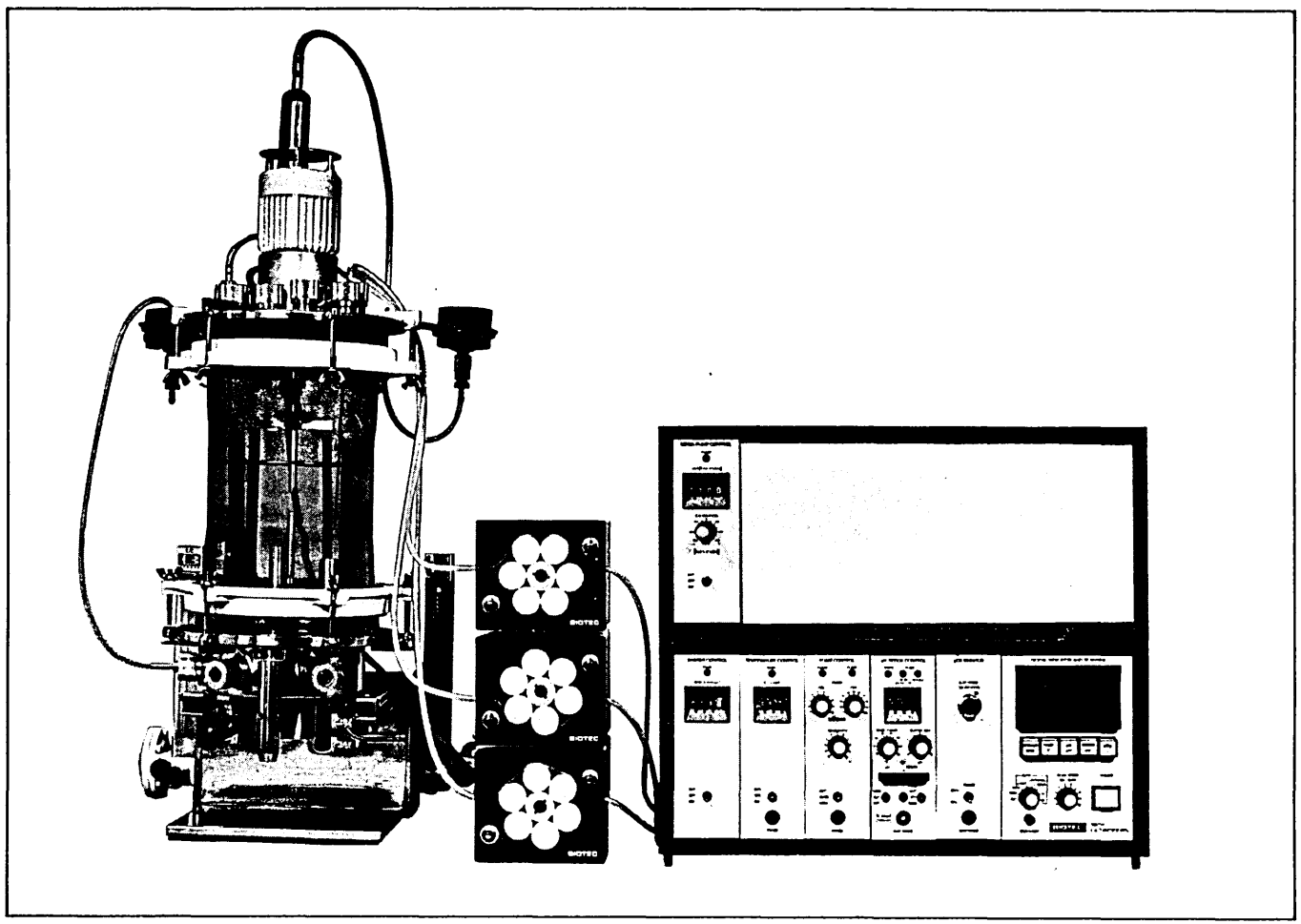


Figure 7 : Ultroferm fermentation system (Biotec 1601)

3.1.2. Description of the Acidification Reactors Used in Two-Phase Studies

In the two-phase studies, three different acidification reactors were utilised. These reactors were constructed of borosilicate glass, using components of glassware commercially supplied as Quickfit (Corning, U.K.). In the following description of the apparatus, the reference number for each component is appended in brackets.

A flat bottom fermentation vessel (FV500), which was adapted by a side arm to maintain a constant volume of 245 mL was used as the acidification reactor of system 1 (Fig. 8). The top was fitted with a five socket flange lid (MAF1/75), silicone grease being applied to the ground glass joint, prior to clamping it securely to the vessel. For the construction of the acidification reactors of the other systems (systems 2 and 3), larger vessels (FV1000) coupled with the corresponding flange lids (MAF1/100) were used, the side arms being adapted to maintain the required volumes of 500 mL and 750 mL, respectively.

Influent was pumped into the vessel by means of a LKB Multi-perpex pump via a straight tubing connector (MF10/2). A thermometer pocket (SH4A) facilitated temperature measurement, while another thermometer pocket was sealed with a silicone rubber septum and used as the sampling port. The remaining sockets were sealed with closed-end stoppers (SB19). The gases produced were not collected separately, but left the reactor together with the effluent liquid.

Each reactor was supported above a magnetic stirrer (Stuart Ltd., Camlab, Cambridge, U.K.), which provided adequate stirring of the reactor contents. The maintenance of the appropriate temperature in all reactors was achieved as follows: a length of silicone tubing was wrapped around each reactor and heated water was circulated through the tubing via a thermostatic control unit (model SU5, Grant Instruments Ltd., Camlab, Cambridge, U.K.). The temperature of the circulating water was adjusted to ensure that the required temperature of 37 ± 1 °C was

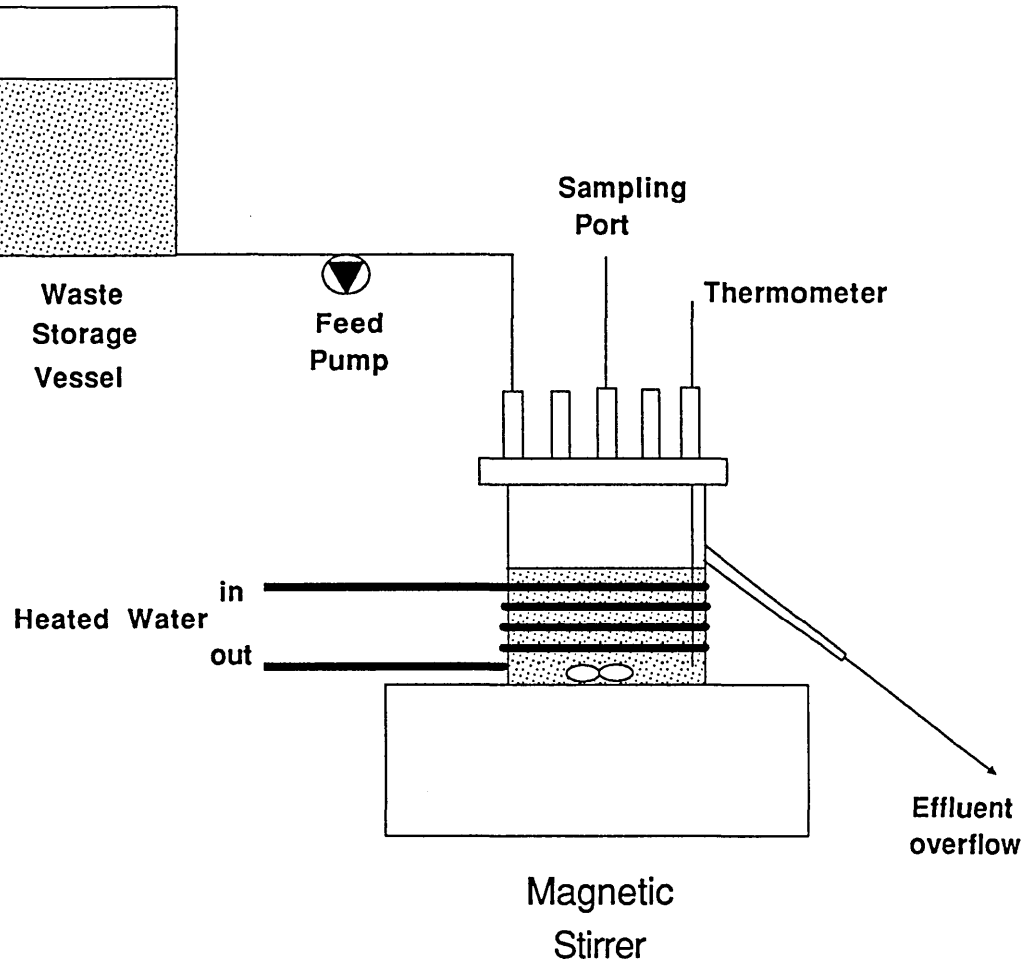


Figure 8 : Schematic diagram of the acidification reactors used in the two-phase anaerobic digestion systems

maintained within the acidification reactors.

A reactor of similar construction and of a useful volume of 500 mL was also utilised during the studies of the first phase to supply a constant quality inoculum throughout the experiments. This reactor was operated for a two months period under constant conditions before a constant quality of well-acclimated inoculum could be obtained.

3.1.3. Operation of the Acidogenic Reactors

During the operation of the acidogenic reactors in this study, the operational parameters utilised were the hydraulic retention time θ and the organic (COD) loading rate (OLR). The hydraulic retention time θ (h) of a completely-mixed, continuous-flow stirred tank reactor (CSTR) is given by the following equation:

$$\theta = \frac{V}{Q} \tag{26}$$

where V is the volume of the reactor (L) and Q is the influent flowrate ($L\,h^{-1}$). The value of θ is the inverse of the dilution rate D as defined for equation (12).

The organic loading rate OLR ($kg\,COD\,m^{-3}\,d^{-1}$) can be calculated from the following equation:

$$OLR = \frac{Q \times S}{V} \times 24 \tag{27}$$

where S is the influent substrate concentration ($g\,COD\,L^{-1}$). The multiplication factor is a result of the choice of units, which are the ones most frequently encountered in the literature.

3.2. Laboratory Scale Fluidised Bed Reactors

3.2.1. Description of Anaerobic Fluidised Bed Reactors

Four anaerobic fluidised beds were employed during the two-phase studies of anaerobic digestion. Each reactor had an empty volume of 2 L and was used in conjunction with the following: recycle and overflow chambers, influent and recycle

lines, a heating system, and a gas collection system. A schematic representation of the assembly is shown in Figure 9.

The fluidised bed column was constructed of extruded acrylic tubing (Visijar-Tuckers Plastics, Croydon, U.K.) of 50 mm I.D. and 5 mm wall thickness and was 1 m in height (Bull *et al.*, 1982). The base of the column was connected by a 12 TPI screw thread joint to an acrylic boss; the latter was internally machined to a conical shape (Fig. 10). A stainless steel inlet pipe with a 5 mm I.D. was directed into the apex of the cone, constituting the liquid distribution system and enabling the uniform fluidisation of the fluidised bed carrier particles. The top of the reactor was connected to the column in a similar manner to that of the basal connection through a screw thread joint (Fig. 10). Although a gas draw-off outlet was provided, it was not used: the outlet was gas sealed, so that the produced gases were forced to escape together with the effluent liquid.

The effluent liquid left the top of the column through a 5 mm I.D. steel port, situated 2 cm below the screw thread of the acrylic boss at the top of the column (Fig. 10), and flowing through 5 mm I.D. flexible PVC tubing passed into the overflow chamber (Fig. 9). A sampling port was incorporated on the PVC line, which consisted of a 3-way, 4 mm polypropylene stopcock (Nalgene, BDH Apparatus Div., Dagenham, U.K.).

Two chambers were used to hold the liquid waste-water, namely the recycle and the overflow. The recycle chamber was designed to receive the influent waste-stream, thus ensuring that the influent passed through the reactor rather than flow directly out with the overflow effluent. These chambers were connected to each other by a length of 5 mm I.D. flexible PVC tubing, allowing liquid flow from one to the other. The pressure was equalised between the chambers by interconnecting their gas phases (Fig. 9).

Both chambers were constructed of extruded acrylic tubing of 30 cm height,

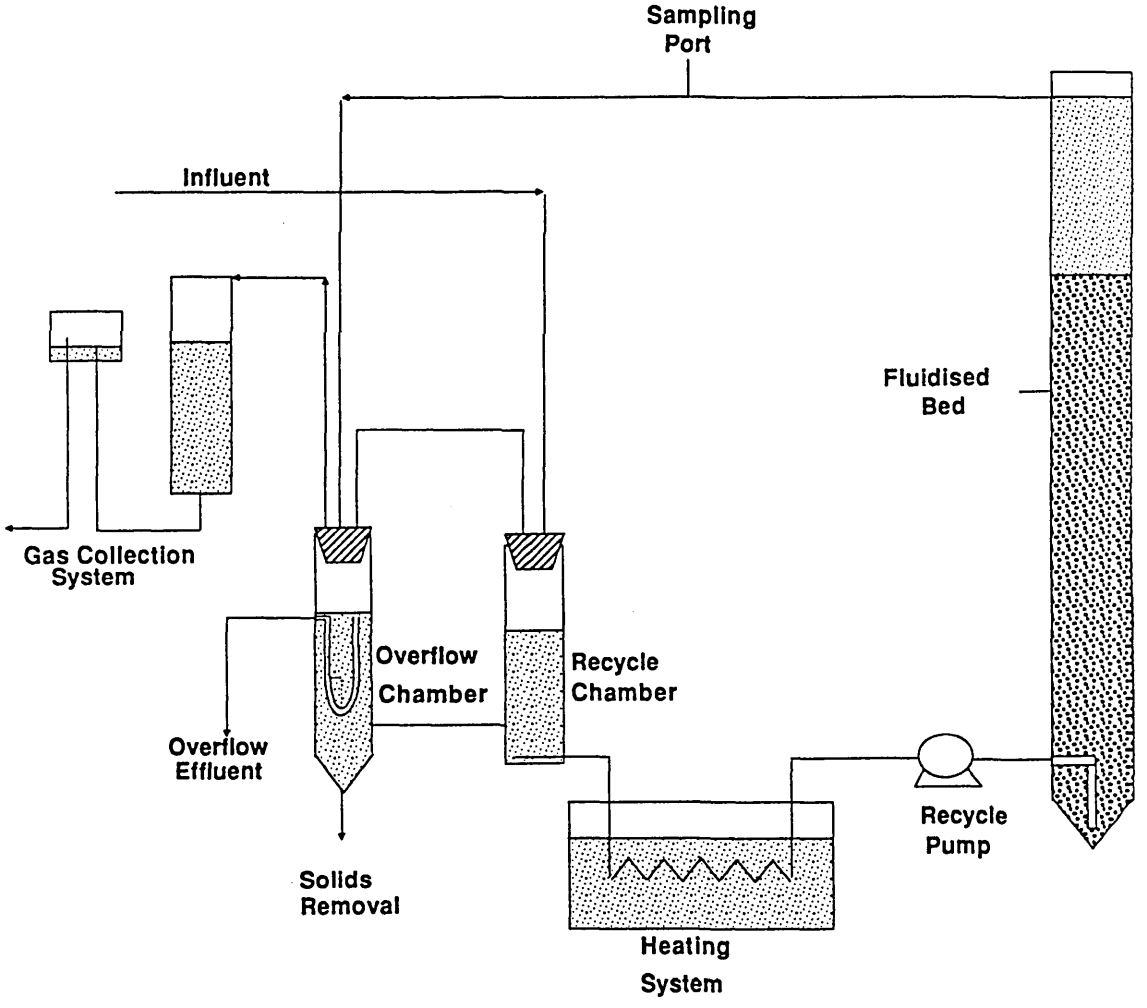


Figure 9 : Schematic diagram of the anaerobic fluidised bed reactor system

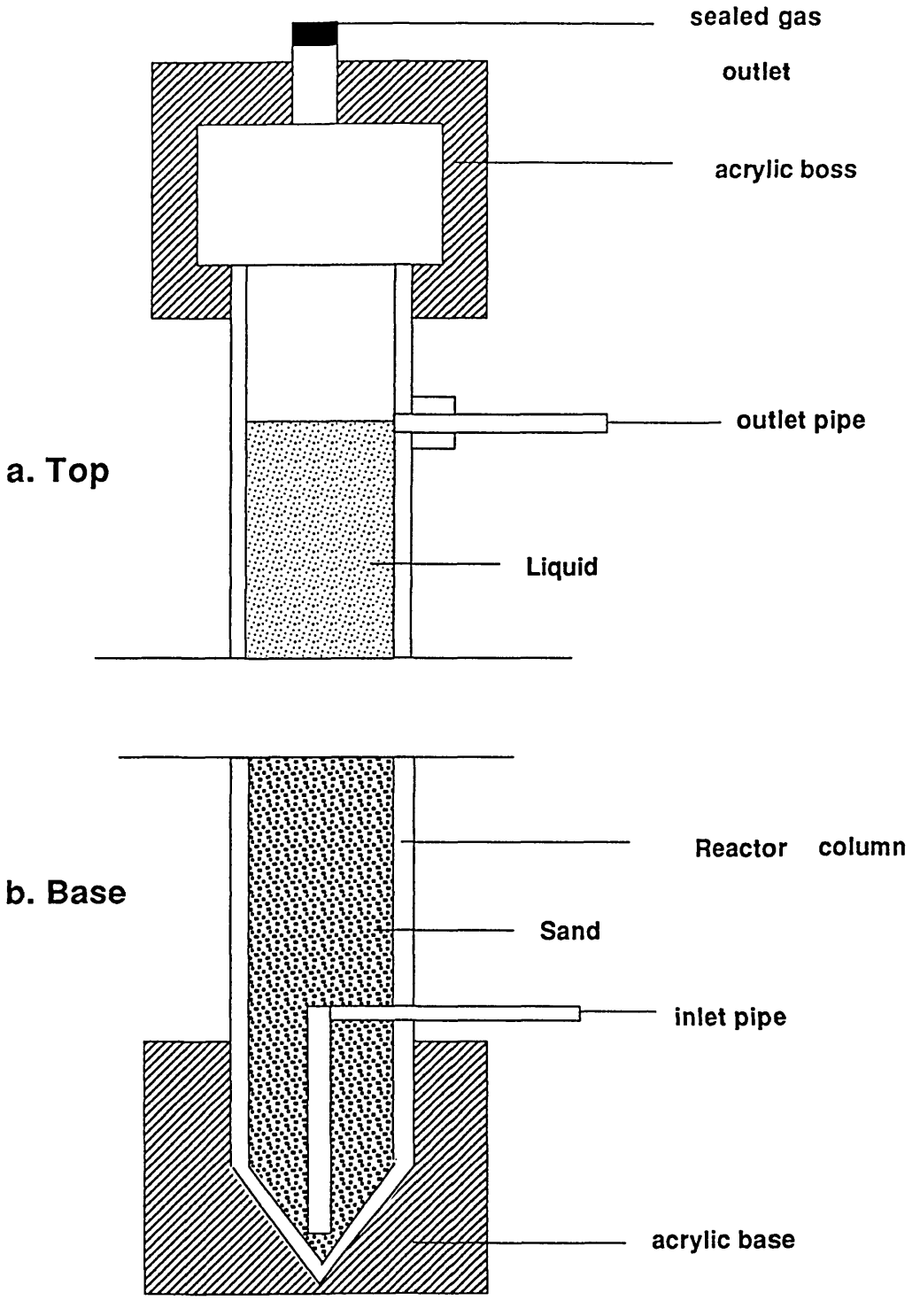


Figure 10 : Representation of the construction of the top and base of the fluidised bed column

60 mm I.D., and a wall thickness of 5 mm. Their tops were sealed with rubber bungs, through which holes had been pierced and short lengths of 6 mm O.D. pyrex tubing inserted to receive the influent or recycled liquid (as applied for the recycle and the overflow chamber respectively). Although the recycle chamber utilised a flat base, a conical base was constructed for the overflow chamber; this allowed the collection and removal of the particles that had been carried out of the fluidised bed column. The base consisted of an acrylic boss, internally machined to conical shape and provided with a 1 cm I.D. stainless steel outlet, which was fitted to the apex of the cone (Fig. 11). The overflow chamber also included a U-shaped glass outlet, which was fully submerged into the liquid, thus preventing gas escape. The overflow outlet was finally connected to the drainage system via a length of silicone tubing.

As mentioned above, the recycle chamber was designed to receive the influent waste-water (Fig. 9). In the case of the single-stage system, a LKB Multi-perpex pump (LKB Ltd., Croydon, U.K.) was used which pumped the influent liquid from the storage vessel through a silicone tubing line into the recycle chamber of the fluidised bed. In the two-phase anaerobic digestion system, the overflowing effluent of the acidogenic reactor flowed freely into the recycle chamber of the anaerobic fluidised bed, the latter serving as the methanogenic reactor.

The mixed recycled and influent waste-water left the recycle chamber by 10 mm I.D. flexible PVC tubing and was pumped into the fluidised bed via a Watson Marlow HRSV 214 Flow inducer (Watson Marlow, Falmouth, Cornwall, U.K.). The pump was fitted with 6.3 mm I.D. silicone rubber tubing and was operated at a sufficient rate to maintain the required expansion of the fluidised bed reactor. The tubing and the pump head rollers were lubricated with glycerol to increase the tubing life. The PVC line that contained the liquid was also passed through a heated water bath (Conair Churchill Ltd., Uxbridge, Middlesex, U.K.). In this way, the temperature of the fluidised bed could be maintained at the required

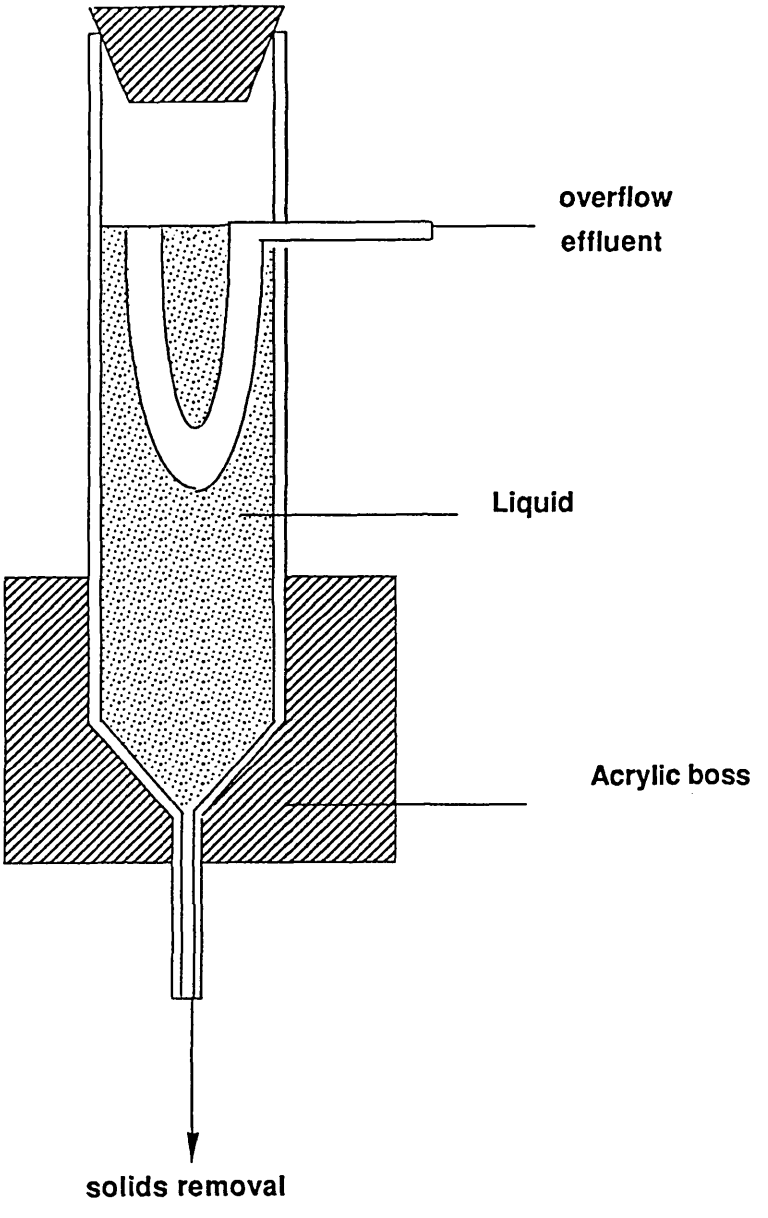


Figure 11 : Diagrammatic representation of the construction of the overflow chamber

level of $37 \pm 1\,^{\circ}C$. Furthermore, the water bath was equipped with a stirring Chircotherm unit (Tecam, Cambridge, U.K.) which permitted efficient heat transfer.

The gas production was measured by displacement of acidified water (0.5 M H_2SO_4). The gas collector connected to each anaerobic fluidised bed consisted of a glass cylinder of 18 cm I.D. and of 60 cm height (Fig. 9). A silicone rubber septum was provided, which served as the sampling port for gas analysis.

The tubing connections in the various parts of the apparatus were made by polypropylene push-fit connectors (Gallenkamp Ltd., Loughborough, U.K.). Polypropylene non-return valves (Gallenkamp Ltd., Loughborough, U.K.) were inserted in the inlet of the fluidised bed column to prevent backflow and subsequent loss of the reactor contents in the event of failure or rupture of a connecting line. All other interconnecting pipework comprised 5 mm I.D. PVC tubing.

3.2.2. Operation of Anaerobic Fluidised Bed Reactors

3.2.2.1. Biomass Support Material. The carrier particles of the fluidised bed reactors were silica sand B15 Redhill 65 (British Industrial Sands Ltd., Redhill, Surrey, U.K.). An amount of sand, adequate for the operation of the four fluidised bed reactors, was sieved and the fraction retained between 200 and 600 μm sieves (American Standard Sieves) was used. The physical properties of the sieved sand are given in Table 7.

Table 7: Physical properties of the carrier sand particles

Mean Particle size	0.40 mm
Particle density	2.65 g cm^{-3}
Bulk density	1.43 g cm^{-3}
Porosity	46 %

3.2.2.2. Operational Parameters of the Fluidised Bed Reactors. The hydraulic retention time θ and the organic loading rate OLR in a fluidised bed reactor can be defined in a way similar to that used in the case of the completely mixed acidification reactors [eq. (26) and (27), respectively]. The volume V employed in the calculations is the expanded volume of the fluidised bed. The influent flowrate Q in the fluidised bed reactor of a two-phase system is equal to the influent flowrate of the corresponding acidogenic reactor, since the two reactors are connected in series and the total effluent of the first reactor is passed into the second one.

The solids retention time SRT in the application of fluidised bed reactors is the time that the attached biomass remains within the reactor. In a biological system, the SRT (d) can be calculated as the ratio of the total biomass present inside the reactor over the rate at which biomass is removed from the reactor, either in the form of the suspended solids of the effluent, or as part of the system biomass control. When biomass is removed from the reactor solely as suspended solids in the effluent stream, the following expression can be used:

$$SRT = \frac{X_{exp}\ V}{x_e\ Q}\ \frac{1}{24} \tag{28}$$

where X_{exp} is the attached biomass concentration, expressed as volatile solids per unit volume of expanded bed ($g\,VSS\,L^{-1}$), V is the volume of the expanded bed (L), x_e is the biomass concentration in the effluent expressed as total suspended solids concentration ($g\,TSS\,L^{-1}$), and Q is the effluent flowrate which is equal to the influent flowrate ($L\,h^{-1}$). The multiplication factor is used to achieve consistency of units, when the SRT is required in days.

3.3. Synthetic Waste-Water

The waste-water used in this study was based on meat extract and glycerol together with essential inorganic nutrients. The composition producing an influent COD concentration of 2.5 gL^{-1} is given in the following table (Table 8). For influent of a different COD concentration, the amounts of all organic and inorganic constituents were adjusted *pro rata*. The composition of the synthetic waste-water is similar to the one used by previous researchers (Bull *et al.*, 1982; Stephenson and Lester, 1986; Stronach *et al.*, 1987). However, the amount of ammonium chloride was reduced in order to avoid chloride inhibition when the highest COD concentrations were applied. Furthermore, Bull *et al.* (1983b) suggested that enhanced methanogenic growth could be achieved in a fluidised bed reactor when the influent contained a reduced concentration of ammonium chloride.

Table 8 : Composition of the synthetic waste-water

Composition	Grade	Concentration ($mg L^{-1}$)
Oxoid 'Lab Lemco' Powder (L29)		1950
Glycerol	GPR	200
Ammonium Chloride	Analar	50
Sodium Chloride	Analar	50
Potassium Dihydrogen Phosphate	Analar	30
Calcium Chloride (hydrated)	Analar	24
Magnesium Sulphate (hydrated)	Analar	7.5

A modification of the above mentioned waste-water composition was utilised during the studies of the two-phase anaerobic digestion system. A trace element solution was added to the influent substrate; this is known to promote

methanogenic activity (Wiegant *et al.*, 1983). The stock trace element solution was added at a concentration of 0.05 $mL\,L^{-1}$ for 2.5 $g\,COD\,L^{-1}$ and this amount was adjusted proportionately for different COD concentrations. The composition of the stock trace element solution was proposed by Wiegant *et al.* (1983) and is presented in Table 9. All chemicals used were of Analar grade (BDH Chemicals Ltd., Dagenham, U.K.).

Table 9 : Composition of the stock trace element solution

Trace element	Concentration ($g\,L^{-1}$)
Cobalt nitrate	3.180
Ferrous chloride	3.136
Ethylene diamine tetraacetic acid (EDTA)	1.107
Manganous chloride	1.067
Sodium selenate	0.195
Ammonium molybdate	0.096
Boric acid	0.050
Zinc chloride	0.050
Nickel chloride	0.092
Cupric nitrate	0.039
HCl concentrated	1.0

The waste-water was prepared in 10 or 20 L quantities with distilled water and autoclaved at 121 °C for one hour to prevent bacterial growth in the influent holding vessel and thus ensure a consistent influent quality.

3.4. Sampling Procedures

Effluent samples of the acidogenic reactors were removed by immersing a specially constructed wide-bore long stainless steel needle into the contents of the

reactor through the silicone rubber seal provided. The liquid was then pulled slowly into a plastic syringe. The sample size was in the range of 20 to 30 mL.

The effluent samples of the fluidised bed reactors were collected at the sampling port (a 3-way stopcock inserted on the effluent line, as described in section 3.2.1.). The sample size was in the range of 40 to 60 mL.

Immediately after collection, the pH and turbidity of the sample were measured and then the residue of the sample was filtered through a Whatman GF/C fibreglass filter. Two aliquots of the filtered sample were then preserved separately for COD and volatile fatty acid analysis, as described in the corresponding sections (sections 3.5.1. and 3.5.4. respectively). Whenever analysis of unfiltered sample was required, part of the unfiltered sample was preserved separately.

The influent waste-water was also analysed daily for COD and volatile fatty acids, although no pH or TSS determination were required. The influent samples were preserved similarly to the effluent samples.

All glassware used was cleaned by soaking in a 5% (v/v) solution of 'Decon 90' detergent (BDH Chemicals Ltd., Dagenham, U.K.) for 24 hours and thoroughly rinsed with distilled water.

3.5. Analytical Methods

3.5.1. Determination of Chemical Oxygen Demand

The method adopted was based on the standard methods for the examination of waters and associated materials issued by the Standing Committee of Analysts (1977), and included minor modifications previously employed by Bull *et al.* (1982) and Stronach *et al.* (1987).

Prior to the analysis, the sample of reactor effluent was filtered and diluted with acidified distilled water to a final concentration of 1% (v/v) of nitric acid

(HNO_3) in the sample to be analysed. This method allowed the preservation of the COD value, because bacterial growth was inhibited. Furthermore, due to the low pH value, the removal of the dissolved H_2S was achieved by purging the diluted sample with nitrogen for 0.5 min (Wang *et al.*, 1985).

The dilution of the sample was such as to provide an expected COD concentration in the range of 250 to 450 $mg\,L^{-1}$, whenever this was possible. In this way, the precision of the analysis was improved; the error incorporated with the pipetting of the sample into the COD flasks was standardised, since the size of sample was kept constant and equal to 5 mL. However, the precision of the analysis was never better than ± 5%.

The sample was boiled under reflux for two hours in a 250 mL round bottom flask (Quickfit, Corning, U.K.) with 9.5 mL of oxidising reagent and 0.5 mL of mercuric sulphate solution (200 $g\,L^{-1}$ in 10 % (v/v) sulphuric acid). The oxidising reagent was prepared by mixing 1500 mL of silver sulphate solution (10 $g\,L^{-1}$ in concentrated sulphuric acid) with 400 mL potassium dichromate solution (7.6618 $g\,L^{-1}$ in distilled water). A distilled water 5 mL blank was also prepared and analysed together with the samples.

After cooling, 40 mL of distilled water were added to the flask through the condenser and the sample was titrated against 0.0625 M ferrous ammonium sulphate in 2% (v/v) sulphuric acid using ferrous phenanthroline as an indicator. The titre of the ferrous ammonium sulphate solution was tested regularly over a standard solution of potassium dichromate. The correction factor of the titrant (*C.F.*) was then calculated as the theoretical consumption over the measured one.

The COD of the sample ($g\,L^{-1}$) was estimated using the following equation:

$$\mathrm{COD} = \frac{(T_b - T_d)\,0.5}{V_d} \times (D.F.) \times (C.F.) \qquad (29)$$

where T_b is the titrant consumption of the blank (mL), T_d is the titrant

consumption of the diluted sample (mL), V_d is the volume of the diluted sample (mL), and ($D.F.$) is the dilution factor equal to the final volume of the sample over the volume of the sample before the dilution.

3.5.2. Measurement of pH

The sample pH was determined electrometrically using an Orion Research Model 701 pH meter, with compensation for temperature. The pH-meter was standardised prior to each measurement using a pH 7.0 buffer solution (Gallenkamp, Loughborough, U.K.). The latter was renewed weekly.

3.5.3. Determination of Total Suspended Solids

The total suspended solids (TSS) of the effluent samples were determined using a turbidimeter (Hach, model 2100A, Camlab, Cambridge, U.K.). This was calibrated over a range of concentrations and the response (optical density) was found to be linear for total suspended solids concentrations up to 120 $mg L^{-1}$ (Fig. 12). Appropriate dilutions with distilled water were made whenever the TSS concentration of the sample was higher.

For the calibration of the instrument, the standard method for the examination of waters and associated materials issued by the Standing Committee of Analysts (1980) was used. The samples were filtered through Whatman GF/C fibreglass filters. Prior to their use, the filters were rinsed with distilled water and dried at 105 ^{o}C to constant weight. The same procedure was also employed for the used filters. The difference between the two weights constituted the total suspended solids of the sample. This procedure was followed for three different effluents, one taken from an acidogenic reactor, the second one being the effluent of a methanogenic fluidised bed reactor, and the third one being the supernatant of digested sludge. The three calibration lines were very close. The derived cumulative calibration line for the calculation of biomass concentration expressed as total

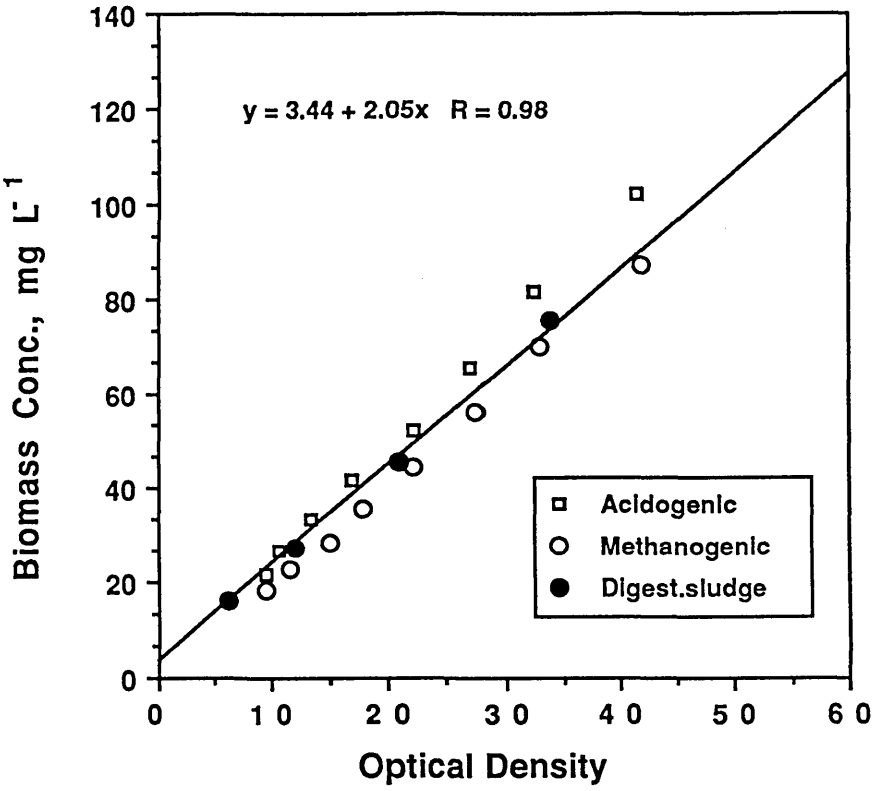


Figure 12 : Calibration graph for the total suspended solids concentration as a function of the optical density

suspended solids concentration ($g\,L^{-1}$) is as follows:

$$x = 2.05\,(O.D.) + 3.44 \qquad (30)$$

where $(O.D.)$ is the optical density of the sample, taking values between 0 and 50. Whenever dilution of the sample was applied, appropriate corrections were made for the calculation of the biomass concentration of the initial sample.

3.5.4. Determination of Volatile Fatty Acid Concentration

The concentration of the individual volatile fatty acids, namely acetic, propionic, butyric, iso-butyric, valeric and iso-valeric acids, in the samples was determined by gas chromatography (GC) using a flame ionisation detector (FID).

Initially, a Hewlett-Packard 5700A Gas Chromatograph (Hewlett-Packard, Winersh, Berks, U.K.) with an on-column injector was used for the analysis. A glass column of 1 m x 2 mm I.D. was packed with packing consisting of 0.3% Carbowax with 0.1% phosphoric acid on 60/80 Carbopack (Supelco Inc., Supelchem, Sawbridgeworth, Herts, U.K.). The oven temperature was maintained at 120 °C, while the injector and the detector temperatures were 180 and 250 °C respectively. The carrier gas was oxygen-free nitrogen, supplied at a flow rate of 40 $mL\,min^{-1}$. The integrator used was a Hewlett Packard Model 3380A (Hewlett-Packard, Winersh, Berks, U.K.), which could be calibrated for the direct reading of the sample concentration.

The analysis of individual volatile fatty acids was at a later stage performed on a capillary column. A Perkin Elmer 8420 Capillary gas chromatograph with a split/splitless injector, and an incorporated integrator was used, coupled with a Perkin-Elmer GP-100 Graphics Printer (Perkin-Elmer, Beaconsfield, Bucks, U.K.). A fairly polar bonded phase fused silica column was used, which was 12 m x 0.3 mm with a film thickness of 0.5 μm (12QC3/BP21 0.5, SGE, Milton Keynes, U.K.). The carrier gas was helium at a pressure of approximately 7 psig. The temperature of

the oven was programmed to change during the analysis, and the exact settings are presented in Table 10.

Table 10 : Capillary column gas chromatograph settings for the volatile fatty acid analysis

Injection temperature	190 °C
Detector temperature	250 °C
Initial temperature	80 °C
Initial time	1 min
Rate of temperature rise	30 °Cmin^{-1}
Final temperature	135 °C

Prior to the analysis, the instrument was calibrated using a standard solution at a concentration of 100 mgL^{-1} of each acid (WSFA-2 free acid standard, Supelco Inc., Supelchem, Sawbridgeworth, Herts, U.K.). For the calibration of the instrument, the method of external standard, based on the comparison of the peak areas, was employed. The sample size injected when the packed column was used, was 1μL. However, smaller amounts were injected when the capillary column was used (0.25 μL). Small size samples are generally preferable, since they minimise the effect of the "water peak" (hydrolytic decomposition products of the stationary phase) which may interfere with free acid peaks.

Before the analysis each sample was acidified to pH 2 using formic acid or meta-phosphoric acid (Standing Committee of Analysts, 1979). The required amount was 2% formic or 4% meta-phosphoric acid in the final solution. Since the available formic acid was contaminated with acetic acid, blank samples, containing an equal concentration of formic acid in distilled water, were also analysed. No blank was required when the sample was acidified with phosphoric acid.

Formic and phosphoric acids are incorporated into the solution to ensure that

the column inlet is acidic: in this way, the active sites are inactivated and thus the adsorption of the volatile fatty acids on the column, a phenomenon known as ghosting, is prevented. When a packed column is used, improved performance is ensured by injecting 5% formic acid between consecutive sample injections. The same treatment can also be repeated whenever peak tailing occurs (GC Bulletin 751F, Supelco Inc.).

3.5.5. Determination of Lactic Acid and Ethanol

The lactic acid and ethanol concentrations were determined using a High Performance Liquid Chromatograph (HPLC) (Series IV, Perkin-Elmer, Beaconsfield, Bucks, U.K.) coupled to a refractometer detector (model LC-25, Perkin-Elmer, Beaconsfield, U.K.). An Aminex HPX-87H column (Bio-Rad, Watford, U.K.), 3 m x 7.8 mm I.D. was used in conjuction with 0.01 N H_2SO_4 as eluent at a flow rate of 0.8 $mL\,min^{-1}$. The sample preparation was the same as that used for the volatile fatty acid analysis. A large amount of the sample was injected into an internal loop injector, the latter having a volume of 10 μL. A standard solution of 250 $mg\,L^{-1}$ of each compound was used for the calibration of the instrument.

During this analysis, the concentration of the individual volatile fatty acids could also be determined. By comparison of these results with those obtained using the standard method, the accuracy of the latter was assessed. Furthermore, increased precision of the volatile fatty acids analysis was achieved by using the standard method.

3.5.6. Determination of Volatile Solids

The volatile solids concentration X in the anaerobic fluidised bed reactors was estimated using the following method. A nickel crucible was washed and heated at 500 °C for 30 minutes. A known volume of sand support media was then removed from the bed, transferred to the nickel crucible and dried at 105 °C to a

constant weight. The crucible and contents were then transferred to a muffle furnace and heated at 500 °C for 30 min. After cooling, volatile solids ($gVS\,L^{-1}$) were determined from the following equation:

$$X = \frac{W_{105} - W_{500}}{V_{sample}} \times 1000 \qquad (31)$$

where W_{105} and W_{500} are the weights (g) of the crucible and contents after heating at 105 and 500 °C respectively and V_{sample} is the volume of the sand sample (mL). The volatile solids concentration has been calculated on the non-expanded (settled) bed volume. To calculate the volatile solids concentration of the expanded bed volume the following equation can be applied:

$$X_{exp} = X \frac{100}{100 + \%\ expansion} \qquad (32)$$

The proportion of attached biomass compared to total biomass (attached and suspended) was also tested. To this end, two sand samples were analysed for volatile solids, the first one analysed upon removal from the fluidised bed and the second one washed with distilled water and then introduced to the nickel crucible.

3.5.7. Gas Composition

The method used was that of Kirk *et al.* (1982) and employed a modified gas chromatograph (Gallenkamp, Loughborough, U.K.). The instrument was adapted to contain two parallel packed columns containing Chromosorb 102 and molecular sieve 5A (Phasesep Ltd., Queensferry, Clwyd), each passing through opposite cells of a thermal conductivity detector. The chromatograph was calibrated using 100% methane (BOC Ltd., Special Gases). Methane was determined by injection of a 500 μL gas sample into the Chromosorb 102 column. The gas samples were collected with a gas-tight syringe through the silicone-sealed sampling port of the gas collection columns. Traces were recorded on a Servogor 120 chart recorder (Servoscript, Bracknell, Berks, U.K.) and comparison of peak heights made.

3.6. Preparation of Constant Quality Inoculum

For the kinetic studies of the first phase of anaerobic digestion, a constant quality well-acclimated acidogenic population was required. This was obtained from a continuous-flow stirred acidogenic reactor with a working volume of 500 mL, initially inoculated with anaerobically digested sewage sludge (Hogsmill Valley Water Pollution Control Works, Thames Water Authority). The description of this reactor is given in a previous section (3.1.2.). The influent substrate of this reactor had a composition similar to the one used during the experimental studies of the acidogenic phase, with a COD concentration of 2.5 gL^{-1}. The hydraulic retention time θ of the reactor was 32 h, which was equal to the longest hydraulic retention time used in the studies. In this way, only the bacterial species with shorter doubling times were allowed to grow.

3.7. Pasteurisation of Sludge

In order to assess the effect of the inoculum on the product distribution of the first phase of anaerobic digestion, digested and activated sludge inocula were used, as well as corresponding pasteurised inocula. For the pasteurisation of sludge, a method proposed by Cohen *et al.* (1984) was employed, which consisted of the following steps: the activated sludge was firstly thickened by centrifugation, a step which was not necessary for the digested sludge; a 25 mL thickened sample was subsequently added to a 100 mL Ehrlenmeyer flask; the latter was closed with a cotton wool plug and heated for 20 minutes in a shaking water bath at 80 °C. Subsequently, the flasks were cooled quickly by cold water, and the procedure was repeated for a second time. The pasteurised sludge was used for inoculation immediately after its preparation.

3.8. Batch Studies of Acidogenic Bacteria

The growth of acidogenic bacteria was initially studied in batch mode. A constant amount (30 mL) of substrate was introduced into 50 mL test tubes, which were specifically designed for use with the turbidimeter (Hach, 2100A, Camlab, Cambridge, U.K.). The test tubes were autoclaved at 121 °C for 1 h and sealed with sterile suba-seals. After cooling, the tubes were inoculated with approximately 1 mL of acidogenic inoculum and placed in a constant temperature shaking water bath. The optical density of the test tube content was measured every 1 to 2 h. The substrate concentrations employed varied between 0.4 and 10 gL^{-1} of COD, while the temperatures chosen were 25, 37 and 45 °C. For increased accuracy, duplicates were prepared for all experimental conditions. The calculations for the determination of the specific growth rate of acidogenic bacteria is given in a following section (3.9.1.).

3.9. Kinetic Calculations

3.9.1. Estimation of Biokinetic Constants of the Monod Equation

When the Monod equation [eq. (10)] is used to describe the growth of bacterial biomass, linear transformations can be used for the estimation of the biokinetic constants μ_{max} (d^{-1}), and K_s (gL^{-1}). The transformations most commonly used are the following:

$$\frac{1}{\mu} = \frac{1}{\mu_{max}} + \frac{K_s}{\mu_{max}} \frac{1}{S} \tag{33}$$

and

$$\frac{S}{\mu} = \frac{K_s}{\mu_{max}} + \frac{S}{\mu_{max}} \tag{34}$$

where μ is the observed specific growth rate of the microorganisms (d^{-1}), and S is the substrate concentration within the reactor (gL^{-1}). In the first case, $\frac{1}{\mu}$ is plotted versus $\frac{1}{S}$, the intercept and the slope (regression coefficient) being equal to $\frac{1}{\mu_{max}}$ and $\frac{K_s}{\mu_{max}}$ respectively. In the second case, $\frac{S}{\mu}$ is plotted versus S, the intercept and

the slope being equal to $\frac{K_s}{\mu_{max}}$ and $\frac{1}{\mu_{max}}$ respectively.

When the kinetics of a microbial population are measured in batch systems, μ (d^{-1}) can be calculated from the following equation, which is derived by integration of equation (9):

$$ln\ x_t = ln\ x_o + \mu\ t \qquad (35)$$

where x_t and x_o are the biomass concentration at time t and zero, respectively ($g\,L^{-1}$), and t is the time (d^{-1}). The starting time of the logarithmic increase of the biomass is considered as time zero (Gaudy and Gaudy, 1980).

During bacterial growth in batch systems, more than one phase can be recognised (Fig. 13). Initially, there is a lag period with no observable increase in the biomass concentration. This is followed by the period of exponential growth, which in a semi-logarithmic plot is presented as linear. Finally, a period of declining growth, associated with limiting substrate concentration, is observed. The last phase can further be separated into the logarithmically decreasing phase, the stationary phase, the accelerating auto-digestion and finally, the decelerating auto-digestion.

When a continuous culture is studied, the value of μ can be calculated from equation (13), provided that the values of the dilution rate D and the specific decay rate k_d are known. In addition, k_d can be calculated from the yield equation [eq. (15)].

The substrate concentration S in a completely mixed stirred tank reactor (CSTR) is equal to the effluent substrate concentration S_e. When a complex substrate is used, the substrate concentration is most frequently expressed as $g\,COD\,L^{-1}$. During the growth studies of acidogenesis, however, not all the COD present constitutes a substrate for the acidogenic bacteria: part of it originates from the volatile fatty acids, which are produced during acidogenesis and which cannot be utilised by acidogens. Therefore, the COD concentration S used in the kinetic

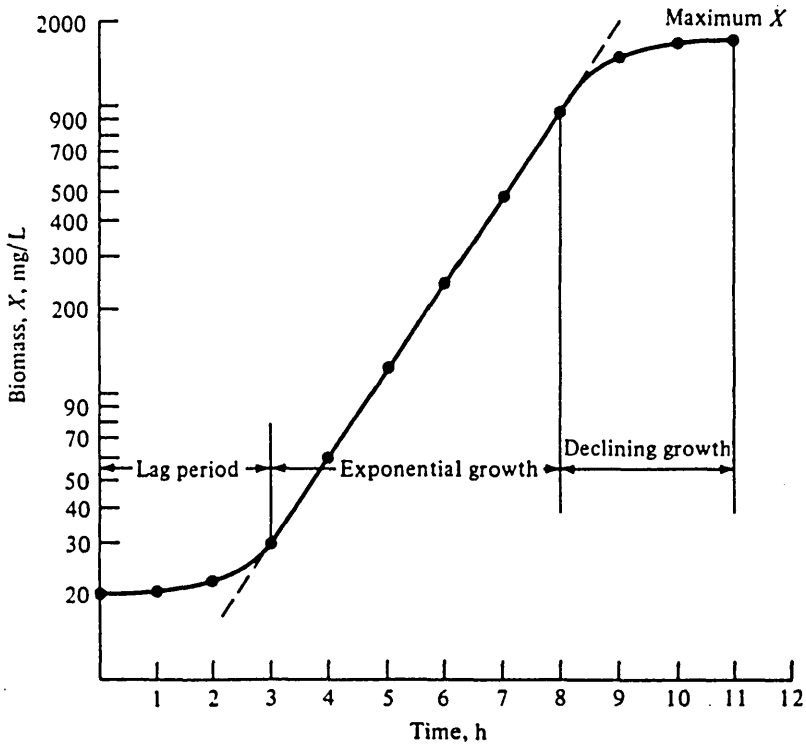


Figure 13 : Biomass concentration as a function of time in a batch culture (after Gaudy and Gaudy, 1980)

equations of acidogenic bacteria should be corrected by subtracting from the measured value the COD exerted by the mixture of the volatile fatty acids. This method has been used by other researchers (Massey and Pohland, 1978; Eastman and Ferguson, 1981), and the theoretical COD conversion factors for the individual volatile acids are given in Table 11. During subsequent studies of the acidogenic bacteria, the corrected COD concentration is represented by $S_{e,c}$ and it is always applied whenever the substrate concentration is required.

Table 11 : COD conversion factors for the volatile fatty acids

Acid	g COD g^{-1} acid
Acetic acid	1.066
Propionic acid	1.512
Butyric acid	1.816
Valeric acid	2.037

3.9.2. Estimation of Biokinetic Constants in the Case of Non-Linear Equations

When a growth rate equation other than the Monod equation is used, the need for a non-linear technique arises, since no linear transformation of the equation can be derived. To solve the regression problem, a ready optimization subroutine of the NAG library (Subroutine E04JAF) was employed. This subroutine used a quasi-Newton algorithm for finding the constrained minimum of the error function.

The non-linear curve-fitting has been described by other researchers as not entirely satisfactory, because it is sensitive to initial guesses for the numerical values of the biokinetic constants, and because 'best-fit' values with little physical meaning can be obtained (Gaudy *et al.*, 1986).

To circumvent some of these difficulties, the search for the values of the biokinetic constants was maintained within a bounded range. The bounds for the

biokinetic constants estimated in this study are summarised in Table 12.

Table 12 : Range of search for the biokinetic constants

Constants		Allowable range
μ_{max}	(d^{-1})	$12 \leq \mu_{max} \leq 85$
K_s	(g COD L^{-1})	$0.01 \leq K_s \leq 10$
K_i	(g inhibitor L^{-1})	$0.01 \leq K_i \leq 5$
k_d	(d^{-1})	$0.01 \leq k_d \leq 6.6$
q_{max}	(g VFA L^{-1})	$55 \leq q_{max} \leq 385$

The lower bound of μ_{max} was 12 d^{-1}, which was the highest specific growth rate experimentally observed during this study and corresponded to a hydraulic retention time of 2 h and a doubling time of the acidogenic bacteria equal to 1.4 h. The upper bound of μ_{max} is 7 times the lower bound, and it corresponds to a doubling time of 0.2 h. A larger value of μ_{max} would not have a physical meaning, since all values found in the literature for the biomass growth rate during acidogenesis of even the most easily degradable substrates are smaller.

The range of search for the maximum specific rate of product formation q_{max} has similarly been defined: the lower bound was the highest specific rate of product formation experimentally observed during the present study, while the upper bound was 7 times this value, consistent with the selection of the upper bound of μ_{max}.

The value of K_s is a measure of the degree of biodegradability of the substrate (waste-water): the lower its value, the more easily degradable the substrate. An increase of K_s has been reported when the acidogenic bacteria are adapted to very concentrated solutions (Ng *et al.*, 1985). However, during the present studies, the highest COD concentration used was 24 g L^{-1} and therefore, an upper bound of K_s equal to 10 g L^{-1} should be considered reasonable.

The upper limit of the inhibition constant K_i was set to 5 $g L^{-1}$. This is in agreement with the values reported in the literature, when the inhibitor was the total volatile fatty acid (VFA) concentration (De la Torre-Lozano *et al.*, 1980), one or more of the individual VFA (Hendricks *et al.*, 1985; Zoetemeyer *et al.*, 1982c), or the un-ionised VFA concentration (Hill and Barth, 1977). Finally, the range of search for the k_d value was defined according to the minimum and maximum values found in the literature.

In order to find the optimum value of the above mentioned biokinetic constants, a statistical technique known as weighted linear regression was used (Edwards and Wilkie, 1968; Davies and Goldsmith, 1984). This method is used when the variance (s^2) is not the same for all values of y_i, which happens particularly when y_i takes values over a wide range. The objective function (*O.F.*) to be minimised is then the weighted sum of squares of the differences between the observed $y_{i,obs}$ and predicted $y_{i,pred}$ values:

$$O.F. = \sum_{1}^{n} \left[w_i \, (y_{i,obs} - y_{i,pred})^2 \right] \tag{36}$$

where n is the number of observations, $w_i = 1/s_i^2$ and s_i^2 is the variance of y_i. The goodness-of-fit of each model was estimated by calculating the average relative error (*R.E.*) according to the following formula (Davies and Goldsmith, 1984):

$$R.E. = \frac{\sum_{1}^{n} \left| \frac{y_{i,obs} - y_{i,pred}}{y_{i,obs}} \right|}{n} \times 100\% \tag{37}$$

The variable y_i introduced in the error function (objective function) was the inhibitor concentration, whatever the inhibitor was in each case. When a regression technique is employed, the dependent variable should be regressed upon the independent one, so that the error function of the variable with the highest variation is minimised (Davies and Goldsmith, 1984). The inhibitor (volatile fatty acid)

concentration was chosen as such a variable, since it was measured with the highest standard deviation.

3.10. Calculation of the Biofilm Thickness of Fluidised Bed Particles

For the calculation of the biofilm thickness around the sand particles of the fluidised bed reactors, a computer program was developed which calculates the biofilm thickness δ when the initial sand volume V_s, the superficial velocity U and the biomass hold-up of the expanded bed X_{exp} are given. The computer solution is based on an iterative procedure schematically illustrated in Figure 14; the equations, as well as the symbols used in this figure, have been described in section 1.3.2. This procedure is similar to the fluidisation algorithm proposed by Shieh and Keenan (1986). To solve the problem a number of assumptions were made:

1) the mean diameter of the sand particles r_m was assumed to be equal to 400 μm, since the sand fraction used in the present study was sieved between 300 and 500 μm;
2) the dry density of the biomass ρ was assumed to be 0.04 $g cm^{-3}$, according to the existing literature on similar systems (Shieh *et al.*, 1981a). Furthermore, this value of ρ gave a better prediction for both the biomass hold-up of the expanded bed and the volume of the expanded bed;
3) the moisture P of the biofilm was assumed to be 92.5 %, according to the literature (Shieh *et al.*, 1981a);
4) the density of the sand ρ_m was 2.65 $g cm^{-3}$, as measured using standard methods (British Standards Institution, 1975);
5) the density ρ_l and viscosity η_l of the wastewater were assumed to be equal to those of distilled water, that is 1 $g cm^{-3}$ and 1 cp, respectively.

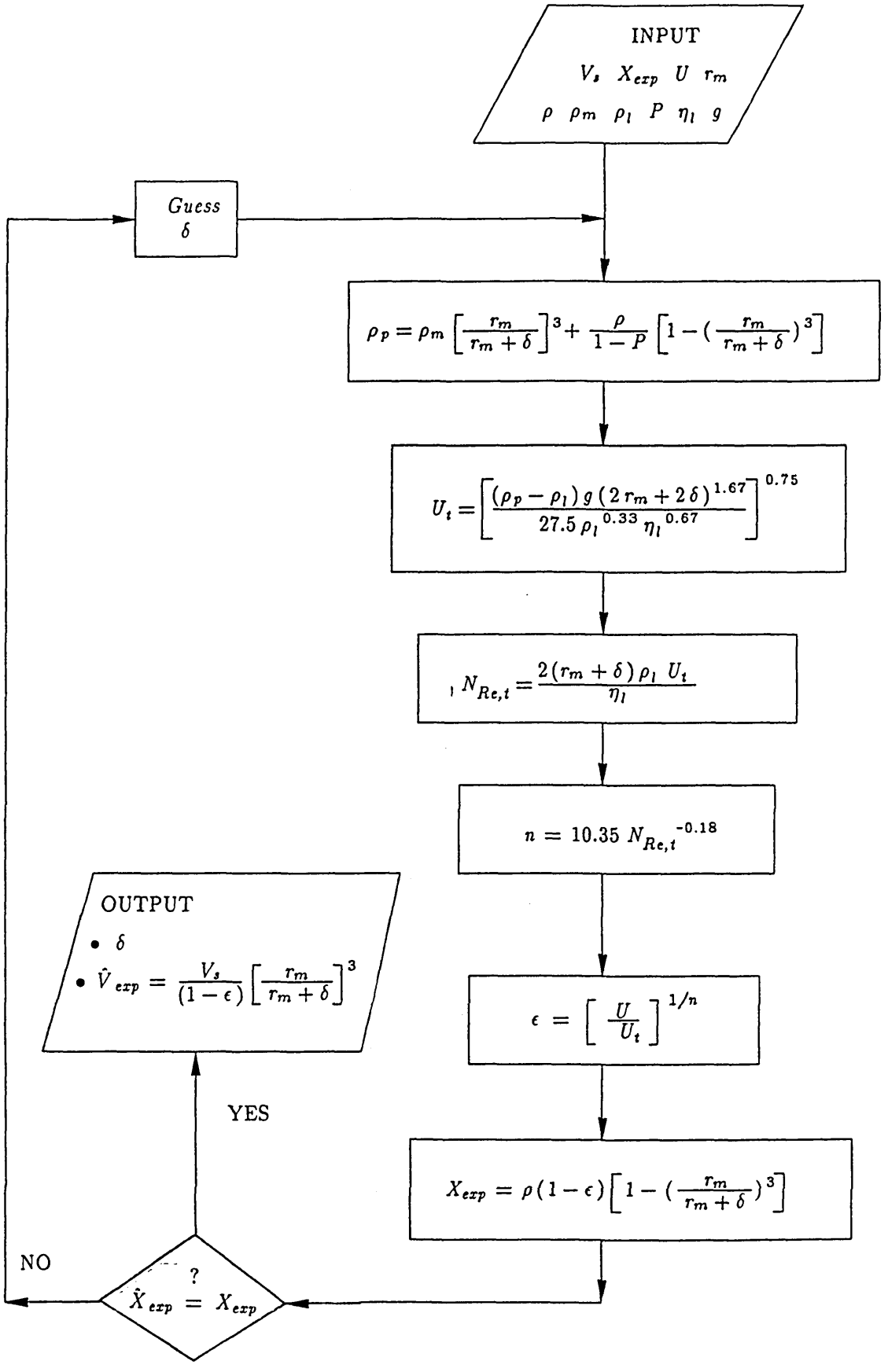


Figure 14 : Fluidisation Algorithm for the Calculation of the Biofilm Thickness δ in a Fluidised Bed Reactor

The biofilm thickness δ was calculated so that the predicted biomass hold-up of the expanded bed $\hat{X}_{exp}$ was equal to the measured one X_{exp}. A further comparison was also made between the measured value of the expanded bed volume V_{exp} and that predicted by the model $\hat{V}_{exp}$.

3.11. Statistical Analysis

3.11.1. Calculation of the Variance of a Function

To calculate the variance $VAR\,(f)$ of a function $f(x_1, x_2)$ when the variances of x_1 and x_2 are known, the following equation can be used:

$$VAR\,(f) = \left[\frac{\partial f}{\partial x_1}\right]^2 VAR\,(x_1) + \left[\frac{\partial f}{\partial x_2}\right]^2 VAR\,(x_2) \qquad (38)$$

where $VAR\,(x_i)$ is the variance of x_i. Using this equation, two useful expressions can be derived, when the function f is given:

$$VAR\,(x_1) = (x_1)^4\, VAR\,(\tfrac{1}{x_1}) \qquad (39)$$

and

$$VAR\,(x_2) = (x_1)^2 \left[VAR\,(\tfrac{x_2}{x_1}) + (x_2)^2\, VAR\,(\tfrac{1}{x_1})\right] \qquad (40)$$

These equations can be used for the determination of the variance of μ_{max} and K_s, as a function of $\frac{1}{\mu_{max}}$ and $\frac{K_s}{\mu_{max}}$ and their variances, calculated from the linear transformations of the Monod equation [eq. (33) and (34)]. The same equations can also be applied for the determination of the variance of k_d and Y, as a function of $\frac{1}{Y}$ and $\frac{k_d}{Y}$ and their variances calculated from equation (15).

3.11.2. Analysis of Variance

The analysis of variance permits the variance between samples (such as different treatments or reactors) and the variance within samples (which represents the inherent variation or the experimental error) to be distinguished. Furthermore, using the F-test of homogeneity of variance, one can assess whether the variance between samples is greater than that within samples (Davies and Goldsmith, 1984).

CHAPTER 4

RESULTS

4.1. *Kinetic Study of Acidogenic Bacteria in Batch Culture*

The kinetics of acidogenic bacteria were initially studied in batch culture. During the exponential growth period of a batch culture (Fig. 13), the specific growth rate μ can be calculated from equation (35).

The biomass concentration was determined by directly measuring the turbidity of the culture; the latter was held in a special test tube appropriate for use with the turbidimeter. This procedure was repeated for a number of different substrate concentrations in the range of 0.4 to 10 gCOD L^{-1}. Two to three replicates were measured for each substrate concentration to increase accuracy. For the inoculation, a well acclimated acidogenic population was used, as described in section 3.6. The amount of the inoculum introduced in each test tube was adjusted so that the measured turbidity was always in the linear range.

The calculated specific growth rates μ at 37 °C for the different substrate concentrations are presented in Table 13. The calculations were based on the data of the exponential growth period observed between 0 and 6.5 hours after inoculation at 37 and 45 °C and between 0 and 18 hours after inoculation at 25 °C.

Assuming the validity of the Monod equation [eq. (10)], the biokinetic constants μ_{max} and K_s can be calculated using the linear transformations presented in section 3.9.1. [eq. (33) and (34)]. The substrate concentration, S, utilised with these equations is equal to the initial substrate concentration in the test tube. Although this is an approximation of the actual substrate concentration (the substrate concentration changes with time, because of the bacterial growth), it is commonly used for batch cultures. The graphical representations and linear

Table 13 : Values of specific growth rate μ of acidogenic bacteria at 37 °C

Substrate Concentration (gCOD L^{-1})	$\frac{1}{\mu}$ (d) Range			Average	STD
0.4	1.216	0.834	1.034	1.027	0.156
0.6	0.790	0.808	0.795	0.796	0.009
0.8	0.612	0.582	0.482	0.558	0.056
1.0	0.447	0.434	0.486	0.455	0.022
1.4	0.465	0.417	0.404	0.428	0.026
2.0	0.308	0.373	0.295	0.324	0.035
3.0	0.274	0.365	0.252	0.296	0.048
6.0	0.191	0.208	0.187	0.195	0.009
10.0	0.169	0.178	0.168	0.174	0.004

Note: STD is the standard deviation.

regression coefficients of the two equations are presented in Figures 15 and 16, respectively. The calculated values of μ_{max} and K_s as derived from the two linear transformations are presented in Table 14. The calculation of the standard deviation of μ_{max} and K_s was carried out according to equations (39) and (40) respectively, and is presented in the same table in brackets.

Table 14 : Estimation of biokinetic constants in batch at 37 °C

	$\frac{1}{\mu_{max}}$	$\frac{K_s}{\mu_{max}}$	μ_{max} (d^{-1})	K_s (gCOD L^{-1})
$\frac{1}{\mu} = \frac{1}{\mu_{max}} + \frac{K_s}{\mu_{max}} \frac{1}{S_o}$ (33)	0.146 (0.035)	0.357 (0.016)	6.835 (1.626)	2.439 (0.590)
$\frac{S_o}{\mu} = \frac{K_s}{\mu_{max}} + \frac{S_o}{\mu_{max}}$ (34)	0.137 (0.006)	0.376 (0.050)	7.297 (0.293)	2.740 (0.382)

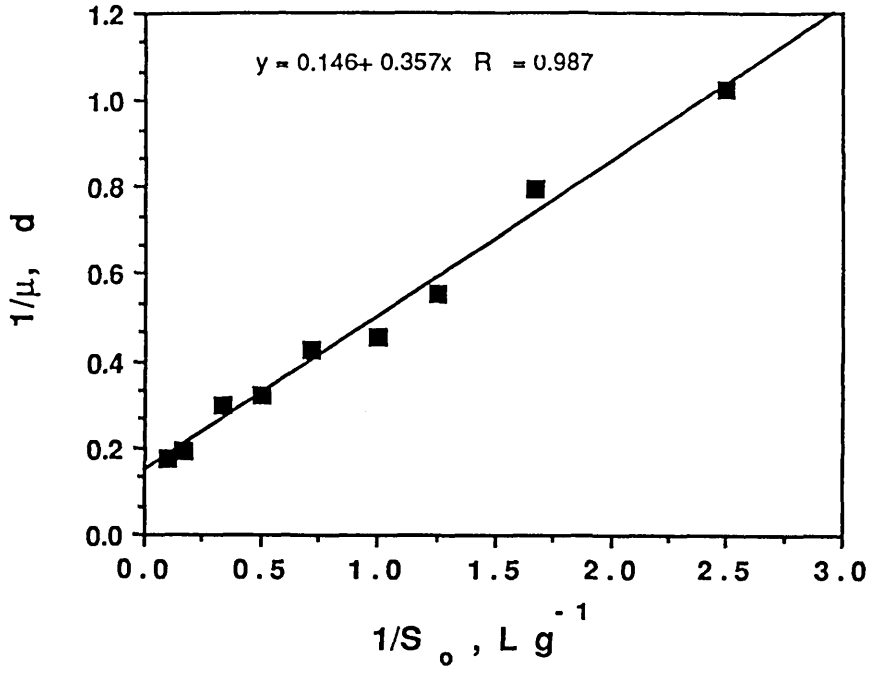


Figure 15 : Plot of $\frac{1}{\mu}$ versus $\frac{1}{S_o}$ for the calculation of the biokinetic constants μ_{max} and K_s, according to the Monod equation

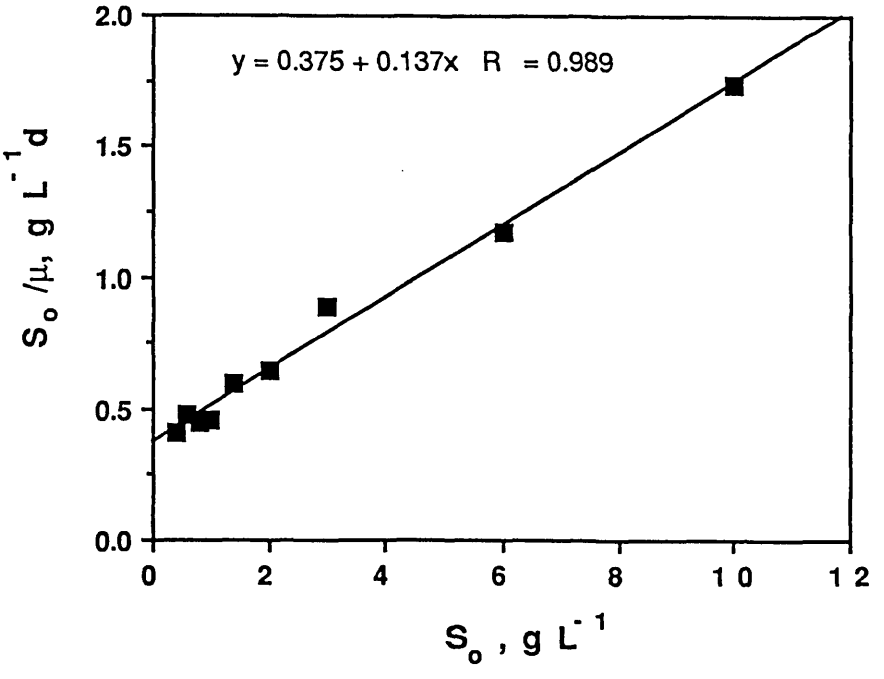


Figure 16 : Plot of $\frac{S_o}{\mu}$ versus S_o for the calculation of the biokinetic constants μ_{max} and K_s, according to the Monod equation

The estimates of the biokinetic constants μ_{max} and K_s, obtained from the two linear transformations, are very close (Table 14). The same applies for the correlation coefficient of the two lines (Fig. 15 and 16). However, the variance of the estimates was much lower when the second equation [eq.(34)] was used. This suggests the superiority of equation (34), at least for the given experimental data.

The effect of temperature on the values of the Monod constants was also studied. Three different temperatures, namely 25, 37 and 45 °C, were applied. The application of the linear transformation of equation (34) was again proven more successful. In Table 15, the estimates of μ_{max} and K_s, as well as the minimum doubling time of the bacteria, are presented for the different temperatures. The minimum doubling time t_d (h) can be calculated from the following equation:

$$t_d = \frac{ln\,2}{\mu_{max}} \times 24 \qquad (41)$$

where μ_{max} is the specific growth rate (d^{-1}). The standard deviation of each parameter is indicated in the same table in brackets. As can be seen from Table 15, the temperature mainly affects the value of μ_{max} and consequently that of t_d, while K_s is not drastically changed.

Table 15 : Comparison of the biokinetic constants in batch at different temperatures

Temperature (°C)	μ_{max} (d^{-1})	K_s (gCOD L^{-1})	t_d (h)
25	1.911 (0.094)	2.159 (0.460)	8.71
37	7.297 (0.293)	2.740 (0.382)	2.28
45	5.025 (0.821)	2.404 (1.536)	3.31

4.2. Evaluation of the Effect of Operational Parameters on the Performance of an Acidification Reactor

4.2.1. Experimental Design and Reproducibility

In order to assess the influence of operational parameters on the performance of an acidogenic reactor, a set of experiments were designed. This was intended to show the effect of each one of the operational variables (i.e. hydraulic retention time θ, influent substrate concentration S_o, organic loading rate OLR, pH and temperature), while the others were kept constant. However, because of the interdependence of θ, S_o and OLR, only one of these parameters could be kept constant, while the other two changed with respect to one another. It should also be noted that no pH control was applied, unless the effect of pH was to be examined.

Six series of experiments were conducted, for which the corresponding values of the operational variables are given in Table 16. Each series of experiments consisted of 4 to 7 individual experiments, each one representing the steady-state obtained for the chosen conditions. In order to achieve a steady-state the reactor was left to equilibrate over 4 hydraulic retention times or two days, whichever was longer, after which the effluent characteristics were measured over another period of 4 hydraulic retention times (Bull *et al.*, 1984a). Reinoculation had to take place at the beginning of each series of experiments, the inoculum being taken from a continuous-flow stirred tank acidogenic reactor as described in section 3.6.

Experimental reproducibility was tested by comparing the effluent characteristics of experimental runs conducted under similar operational conditions. Four such experimental runs were selected, for which the operational parameters and the effluent characteristics are given in Table 17, where S_e is the filtered

Table 16 : Operational conditions applied to the acidogenic reactor during the studies of the first phase of anaerobic digestion

Series	Run	Operational Conditions	θ (h)	S_o (gCOD L^{-1})	OLR (kg $m^{-3}d^{-1}$)	T (°C)	pH
I	I.1.	constant OLR	16.9	6.60	9.4	35	6.16
	I.2.		8.2	3.20	9.4	35	6.00
	I.3.		4.0	1.73	8.6	35	5.80
	I.4.		2.0	0.74	8.9	35	5.75
II	II.1.	constant θ	8.0	0.75	2.2	33	6.15
	II.2.		8.0	1.56	4.7	33	6.01
	II.3.		7.6	2.96	9.4	33	5.82
	II.4.		7.6	6.07	19.2	33	5.85
	II.5.		7.6	12.39	39.1	33	6.04
	II.6.		8.0	18.53	58.5	33	6.17
	II.7.		8.0	23.98	75.7	33	6.29
III	III.1.	constant S_o (low)	16.0	3.02	4.5	36	6.05
	III.2.		8.3	3.01	8.7	36	6.00
	III.3.		5.9	2.94	12.0	36	5.93
	III.4.		3.9	3.00	18.5	36	5.90
	III.5.		3.0	3.00	24.0	36	5.79
	III.6.		2.0	3.00	36.0	36	5.71
IV	IV.1.	constant S_o (high)	33.8	11.90	8.7	36	6.35
	IV.2.		16.3	11.90	17.5	36	6.12
	IV.3.		8.1	12.28	36.4	36	6.04
	IV.4.		6.0	12.28	48.8	36	5.52
	IV.5.		4.1	12.33	72.7	36	5.55
	IV.6.		3.0	12.00	96.0	36	5.55
	IV.7.		2.1	11.94	138.5	36	5.59
V	V.1.	Temperature variation	8.0	2.92	8.8	40	5.76
	V.2.		8.0	2.99	9.0	35	5.54
	V.3.		8.0	2.75	8.3	30	5.40
	V.4.		8.0	3.03	9.1	25	5.20
VI	VI.1.	pH variation	8.0	2.92	8.8	35	8
	VI.2.		8.0	2.92	8.8	35	7
	VI.3.		8.0	2.92	8.8	35	6
	VI.4.		8.0	2.92	8.8	35	5

Table 17 : Operational parameters and effluent characteristics of experimental runs compared for repeatability

Run	θ (h)	S_o ($gCOD\,L^{-1}$)	T (°C)	TSS ($mg\,L^{-1}$)	S_e ($gCOD\,L^{-1}$)	VFA ($g\,L^{-1}$)
I.2.	8.2	3.20	35	149	2.51	0.995
II.3.	7.6	2.96	33	144	2.58	0.857
III.2.	8.3	3.01	36	164	2.37	1.176
V.2.	8.0	2.99	35	152	2.46	0.829
Mean	8.0	3.04	35	152	2.48	0.964
STD	0.3	0.11	1.3	8.5	0.09	0.159
Relative STD	4%	4%	—	6%	4%	16%

effluent COD concentration, VFA is the total volatile fatty acid concentration, and TSS is the total suspended solids concentration.

The biomass concentrations and the filtered effluent COD concentrations were very similar in all runs, with a relative standard deviation of less than 6%. This variation was less than those associated with the analytical methods used to determine these parameters, which were 8% and 7%, respectively. The standard deviation of the measured volatile fatty acid concentrations was 0.16 $g\,L^{-1}$ (relative standard deviation 16%), which was higher than the variation of the analytical method (approximately 10%). This may be partly explained by the fact that the operational parameters applied to the reactor during the experimental runs, although similar, were not identical. Nevertheless, the deviation observed was small compared to the differences in volatile acid concentrations obtained within an experimental series (e.g. 1.14 to 3.85 $g\,L^{-1}$) and it was therefore considered that the performance of the acidogenic reactor could be regarded as consistent under constant operating conditions.

4.2.2. Influence of Hydraulic Retention Time, Organic Loading Rate and Initial Substrate Concentration on the Degree and Rate of Acidification

In order to examine the influence of hydraulic retention time θ, organic loading rate OLR, and initial substrate concentration S_o on the performance of the first stage of anaerobic digestion, the experimental data of series I, II, III and IV were used and analysed. In Table 18 and Table 19, the effluent characteristics of series I and II, and series III and IV are presented, respectively. The percentages of the major individual volatile fatty acids are given, while the numbers in brackets denote the standard deviation corresponding to each value.

To quantify the influence of the operational variables on the performance of the acidogenic reactor, the degree of acidification and the rate of product formation were calculated.

The degree of acidification was expressed as the percentage of the initial substrate concentration converted to volatile fatty acids and other fermentation products (e.g. ethanol, lactate). The initial substrate concentration S_o was measured in gCOD L^{-1} and the quantity of fermentation products was converted, according to Table 11, to the theoretical equivalent in gCOD L^{-1} S_p i.e. the nominal COD exerted by the mixture of the products:

$$\% \text{ acidification} = \frac{S_p}{S_o} \times 100 \qquad (42)$$

The rate of product formation per unit of reactor volume r_p (g L^{-1} d^{1-}) was another important parameter for the evaluation of the performance of the acidogenic reactor:

$$r_p = \frac{(P_e - P_o)}{\theta} \times 24 \qquad (43)$$

where P_o and P_e are the influent and effluent product concentrations respectively (g L^{-1}) and θ is the hydraulic retention time (h). For the waste-water utilised in this study, P_o was very low and constituted approximately 0.8% of the influent COD.

Table 18 : Effluent characteristics of the acidogenic reactor at steady state during experimental series I and II

Series	Run	*TSS* mg L^{-1}	S_e gCOD L^{-1}	*VFA* mg L^{-1}	*%Ac*	*%Pr*	*%Bu*	*%Val*
I	I.1.	310 (25)	5.19 (0.30)	2.351 (0.168)	56.8	17.9	10.0	9.0
	I.2.	149 (12)	2.52 (0.29)	0.994 (0.036)	50.7	12.6	14.1	16.6
	I.3.	53 (5)	1.15 (0.10)	0.213 (0.025)	24.9	53.5	7.0	10.3
	I.4.	26 (5)	0.59 (0.10)	0.111 (0.020)	36.0	43.2	7.2	6.3
II	II.1.	63 (4)	0.66 (0.02)	0.178 (0.016)	42.9	40.4	7.3	3.9
	II.2.	107 (7)	1.36 (0.05)	0.368 (0.018)	46.2	34.0	9.5	2.7
	II.3.	144 (9)	2.58 (0.10)	0.857 (0.025)	41.8	33.3	4.0	15.6
	II.4.	238 (10)	5.45 (0.16)	1.536 (0.111)	34.7	35.9	8.3	15.3
	II.5.	556 (26)	11.26 (0.65)	3.040 (0.305)	35.1	29.5	8.2	21.8
	II.6.	829 (43)	15.65 (1.07)	4.509 (0.359)	51.0	25.9	7.6	11.6
	II.7.	1182 (79)	21.97 (0.66)	5.434 (0.386)	45.9	32.8	12.6	3.4

Note: standard deviation is shown in brackets

Table 19 : Effluent characteristics of the acidogenic reactor at steady state during experimental series III and IV

Series	Run	*TSS* $mg\,L^{-1}$	S_e $gCOD\,L^{-1}$	*VFA* $mg\,L^{-1}$	*%Ac*	*%Pr*	*%Bu*	*%Val*
III	III.1.	190 (9)	2.35 (0.10)	1.273 (0.054)	51.6	26.5	4.9	10.9
	III.2.	164 (10)	2.37 (0.04)	1.176 (0.054)	45.2	36.3	5.4	7.1
	III.3.	166 (12)	2.48 (0.09)	1.103 (0.024)	44.2	34.5	4.6	11.3
	III.4.	144 (27)	2.49 (0.06)	0.867 (0.021)	45.4	32.2	4.7	12.3
	III.5.	118 (11)	2.54 (0.03)	0.690 (0.024)	43.7	32.3	4.3	14.2
	III.6.	111 (12)	2.58 (0.05)	0.540 (0.045)	46.2	34.1	3.6	11.0
IV	IV.1.	792 (17)	10.04 (0.45)	3.853 (0.473)	38.2	28.8	21.5	2.9
	IV.2.	599 (26)	10.63 (0.47)	2.604 (0.325)	48.8	27.9	21.4	—
	IV.3.	556 (34)	10.97 (0.36)	2.447 (0.189)	45.6	35.0	17.4	—
	IV.4.	479 (6)	10.32 (0.36)	1.724 (0.162)	24.7	54.6	14.8	—
	IV.5.	411 (36)	11.22 (0.06)	1.576 (0.074)	27.9	49.6	16.1	—
	IV.6.	404 (42)	11.04 (0.39)	1.483 (0.071)	25.5	56.2	14.0	—
	IV.7.	264 (45)	10.95 (0.16)	1.137 (0.072)	32.0	47.5	15.7	—

Note: standard deviation is shown in brackets

In Figures 17 and 18, the degree of acidification is plotted versus the hydraulic retention time, and organic loading rate, respectively. The influent COD concentration can be indirectly evaluated from these figures. The degree of acidification exhibited a tendency to vary proportionally with the hydraulic retention time and inversely with the organic loading rate and COD concentration.

More observations can be made with reference to the degree of acidification obtained during the individual experimental series. The degree of acidification achieved during experimental series II, produced under the regime of constant hydraulic retention time, did not change markedly with the organic loading rate (Fig. 18) or the influent substrate concentration. The maximum acidification was achieved for a COD loading rate equal to 9.4 $kg\,m^{-3}d^{-1}$ and an influent COD equal to 3 $g\,L^{-1}$. Further increases in the organic loading rate and influent concentration did not significantly affect the degree of acidification, which remained almost constant even for COD loadings as high as 72 $kg\,m^{-3}d^{-1}$ and influent COD concentrations of 24 $g\,L^{-1}$. This implies that the hydraulic retention time is the operational parameter with the greatest impact on the degree of acidification.

A comparison of the data from experimental series III and IV, generated using constant influent COD concentrations equal to 3.15 and 12 $g\,L^{-1}$ respectively, showed that the degree of acidification achieved during the former series was invariably higher than that obtained during the latter, at similar hydraulic retention times (Fig. 17). The negative effect of the higher initial substrate concentration is thus indicated. Another observation was the existence of a critical hydraulic retention time, below which the degree of acidification dropped abruptly. This changed slightly with the influent substrate concentration and was found to be equal to 6 and 8 h for experimental series III and IV, respectively (Fig. 17).

The degree of acidification obtained during experimental series I, produced under constant organic loading rate, increased significantly with hydraulic retention

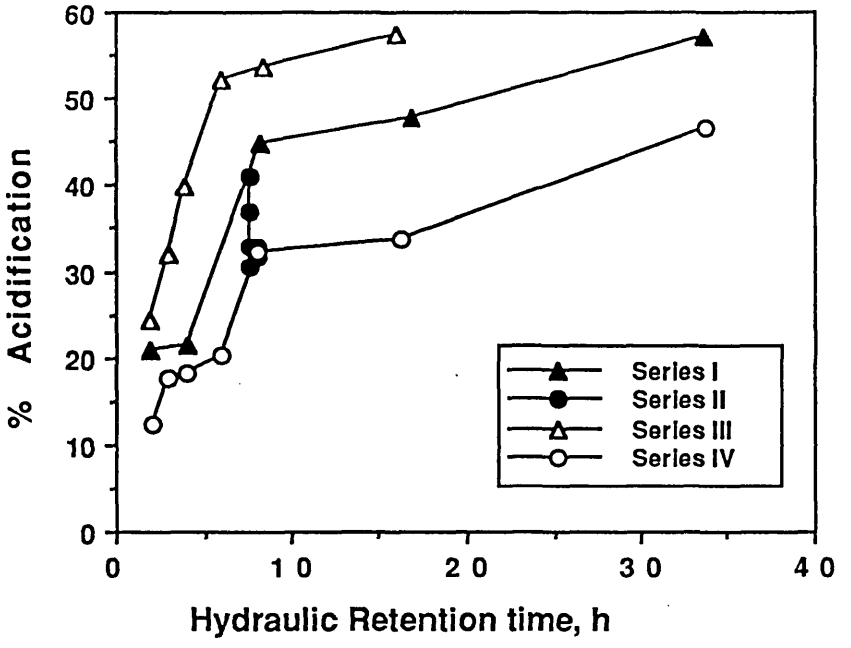


Figure 17 : Effect of hydraulic retention time on the degree of acidification during experimental series I, II, III, and IV

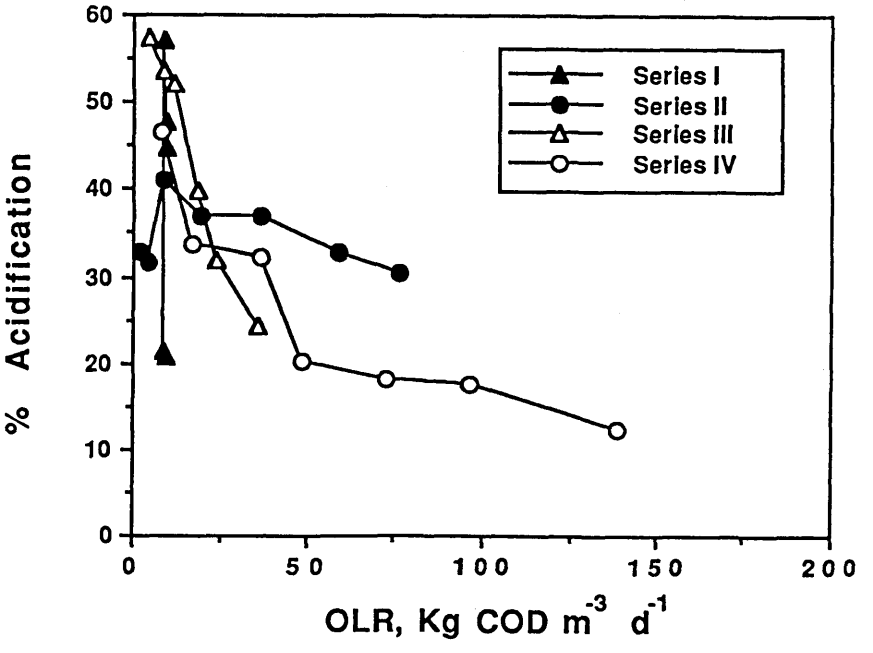


Figure 18 : Effect of organic loading rate on the degree of acidification during experimental series I, II, III, and IV

time (Fig. 17), although this coincided with increasing substrate concentration, which has been shown to repress the acidification. This suggested that the effect of hydraulic retention time on acidification was much more pronounced than that of substrate concentration. The same conclusion can be reached by comparing series II and series I (Fig. 17): the degree of acidification obtained during series II, using constant hydraulic retention time, did not change markedly when the influent COD changed from 7.5 to 24 $g^{-1}L$. In contrast, the degree of acidification obtained during series I, using constant loading, and varying hydraulic retention time, changed significantly for influent COD between 0.74 and 13 gL^{-1}. The existence of a step increase in the degree of acidification was also observed during series I for hydraulic retention times greater than 8 h or initial COD concentrations in excess of 3 gL^{-1}.

In order to verify the above observations and quantify the effect of each operational parameter on the degree of acidification, stepwise multiple regression was used (Draper and Smith, 1981). This technique selects those variables that are most significant for the regression and discards the least useful ones. In this way, the best model is obtained which maximises the correlation coefficient R while minimising the number of input terms.

The regression line obtained was the following:

$$\% \text{ acidification} = 34.2 + 0.681\ (\theta) - 0.181\ (OLR), \quad R = 0.767 \qquad (44)$$

where θ is the hydraulic retention time (h), and OLR is the organic loading rate ($kg\,COD\,m^{-3}\,d^{-1}$). The above two variables were found to be the most important for the prediction of the degree of acidification, while the calculation of the t-statistics of the multiple regression coefficients further indicated that θ was the most important of the two. Thus, to increase the degree of acidification, long hydraulic retention times are required, even for the less concentrated substrates.

The rate of product formation was found to increase with increasing influent

substrate concentration (Fig. 19) and to decrease when the hydraulic retention time was increased (Fig. 20). The effect of the influent substrate concentration on the rate of product formation was more pronounced than that of the hydraulic retention time (Fig. 19 and 20). To verify the above observation, the stepwise multiple regression technique was employed. The regression line obtained was the following:

$$r_p = 2.87 - 0.244\ (\theta) + 0.652\ (S_o), \quad R = 0.956 \tag{45}$$

where r_p is the rate of product formation ($g\,L^{-1}d^{-1}$) and S_o is the influent COD concentration ($g\,L^{-1}$). The application of the t-test to the multiple regression coefficients showed that the influent substrate concentration was the operational variable that most affected the rate of product formation. Thus, the more concentrated the influent substrate, the higher the rate of product formation, even for great hydraulic retention times.

4.2.3. Influence of Temperature on the Degree and Rate of Acidification

The experimental data obtained from series V, produced under constant influent substrate concentration, constant hydraulic retention time, no pH control and variable temperature, were analysed to assess the influence of temperature on the first phase of anaerobic digestion (Table 20).

Table 20 : Effluent characteristics of the acidogenic reactor at steady state during experimental series V

Series	Run	*TSS* $mg\,L^{-1}$	S_e $gCOD\,L^{-1}$	*VFA* $mg\,L^{-1}$	*%Ac*	*%Pr*	*%Bu*	*%Val*
V	V.1.	154 (8)	2.44 (0.18)	0.864 (0.031)	35.2	35.0	6.7	16.2
	V.2.	152 (4)	2.46 (0.09)	0.829 (0.024)	33.3	36.1	8.9	14.7
	V.3.	157 (2)	2.53 (0.06)	0.627 (0.056)	34.8	38.0	7.0	13.2
	V.4.	141 (8)	2.61 (0.06)	0.619 (0.042)	31.5	43.9	7.8	11.3

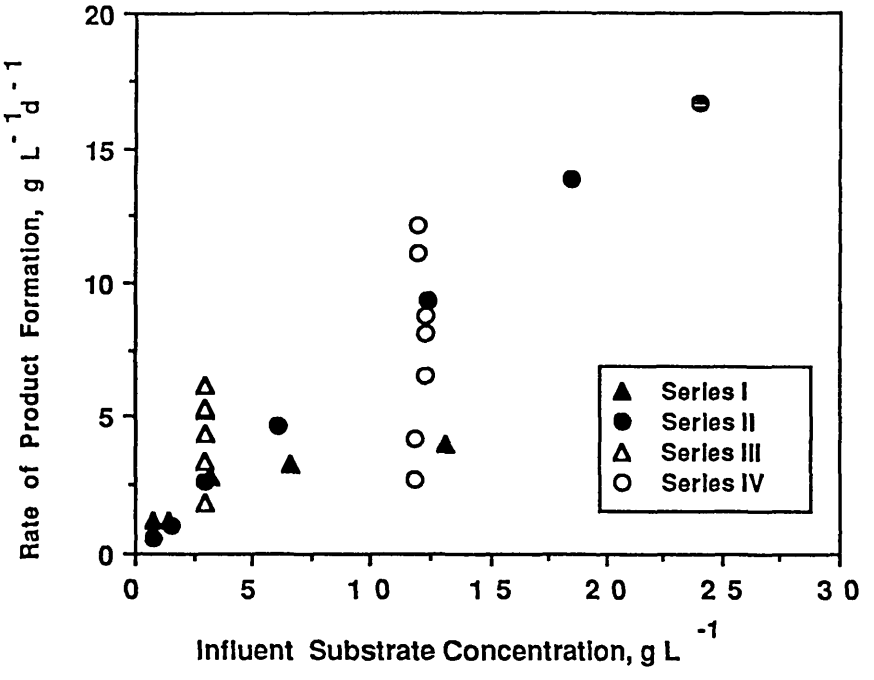


Figure 19 : Effect of influent substrate concentration on the rate of product formation during experimental series I, II, III, and IV

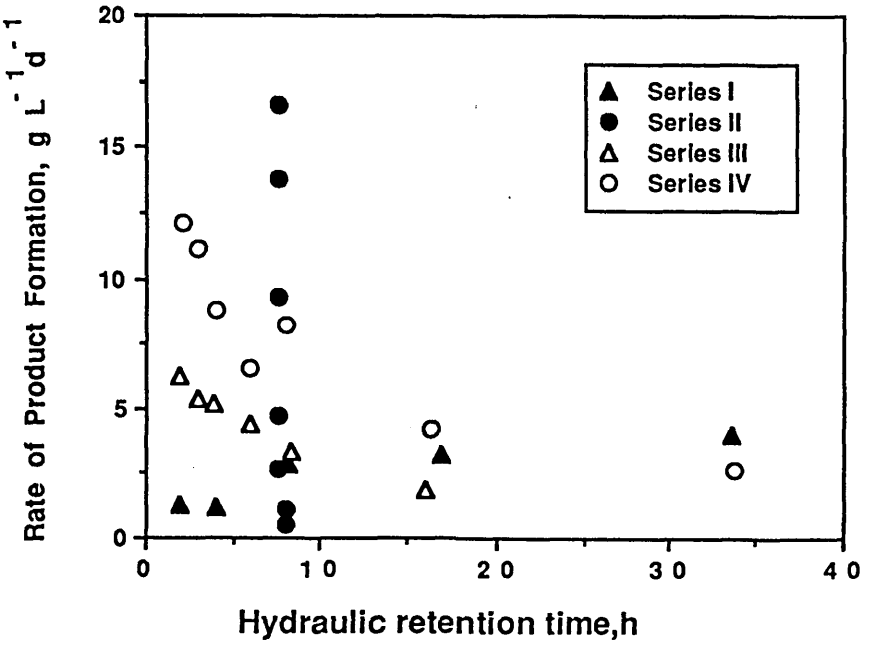


Figure 20 : Effect of hydraulic retention time on the rate of product formation during experimental series I, II, III, and IV

In the following figure, the degree of acidification and the rate of product formation are presented for the temperatures studied (Fig. 21). Both parameters were found to decrease with decreasing temperature over the whole range of temperatures studied (25 to 40 °C).

In order to quantify the effect of temperature on the rate of product formation, a standard Arrhenius-type equation was used:

$$ln\ k = ln\ k_o - \frac{E}{R'\ T} \tag{46}$$

where k is the reaction velocity, k_o is the frequency factor, E is the activation energy (J mol^{-1}), R' is the gas constant (8.314 J K^{-1} mol^{-1}) and T is the absolute temperature (°K).

During the present study, the reaction velocity was equal to the rate of product formation r_p (g acids produced L^{-1} d^{-1}). The natural logarithm of r_p was plotted versus $\frac{1}{T}$, and by using linear regression, the coefficients of the equation were calculated as follows:

$$ln\ (r_p) = 8.568 - \frac{19840}{R'\ T}\ ,\ R = 0.929 \tag{47}$$

Hence the activation energy of the reaction was found to be 19.84 kJ mol^{-1}.

The temperature correction factor Q_{10} could then be calculated. This indicated how many times the overall reaction rate would increase if the temperature is increased by 10 °C.

$$Q_{10} = \frac{rate\ at\ (\ T°C + 10\ °C)}{rate\ at\ (\ T°C)} \tag{48}$$

For this system the value of Q_{10} at 30 °C was found to be 1.29.

It should be noted here that the Arrhenius equation is only valid within one range of temperatures (e.g. mesophilic, thermophilic, or psychrophilic), which was the case during the present study.

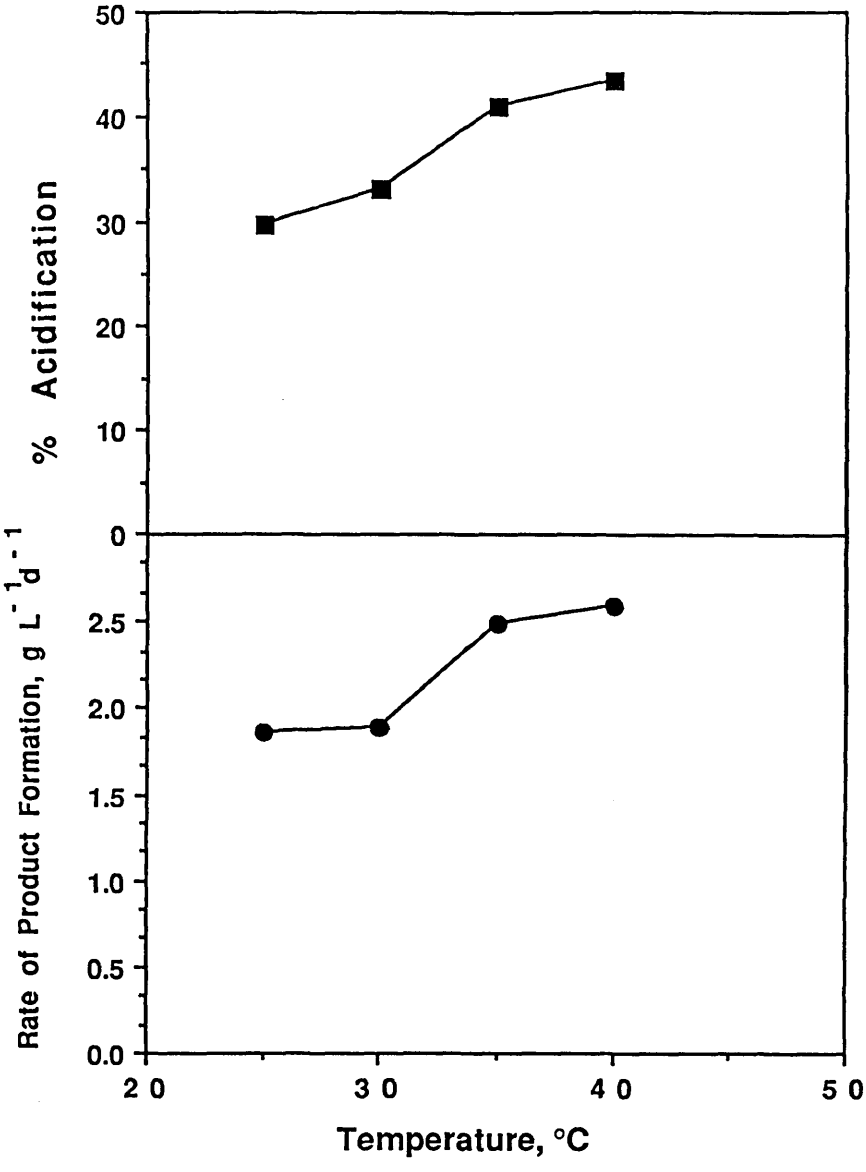


Figure 21 : Effect of temperature on the degree of acidification and rate of product formation during experimental series V

4.2.4. Influence of pH on the Degree and Rate of Acidification

The experimental data obtained from series VI, produced under constant influent substrate concentration, constant hydraulic retention time, constant temperature, and variable pH (Table 16), were analysed in order to assess the influence of pH on the first phase of anaerobic digestion. The effluent characteristics of the acidogenic reactor are presented in Table 21.

Table 21 : Effluent characteristics of the acidogenic reactor at steady state during experimental series VI

Series	Run	*TSS* $mg\,L^{-1}$	S_e $gCOD\,L^{-1}$	*VFA* $mg\,L^{-1}$	*%Ac*	*%Pr*	*%Bu*	*%Val*
VI	VI.1.	136 (10)	2.54 (0.10)	0.659 (0.027)	38.4	33.7	9.1	10.6
	VI.2.	155 (4)	2.45 (0.04)	0.841 (0.031)	39.7	35.2	9.3	8.6
	VI.3.	155 (5)	2.48 (0.04)	0.597 (0.042)	36.0	39.2	10.2	11.7
	VI.4.	125 (3)	2.64 (0.07)	0.500 (0.023)	26.0	42.2	10.8	16.4

The effect of pH on the degree of acidification and the rate of product formation is shown in Figure 22. It can clearly be seen that the optimum pH for both parameters was 7 or close to it.

4.2.5. The Effect of Operational Parameters on Product Distribution

The effluent of the acidogenic reactor was analysed and the concentration of individual volatile fatty acids, lactic acid and ethanol was measured for all experimental series. It was found that acetic and propionic acids were always the main products (Tables 18, 19, 20, and 21). Expressed as a proportion of the total products, the percentage of acetic acid in the effluent ranged from 25 to 57%,

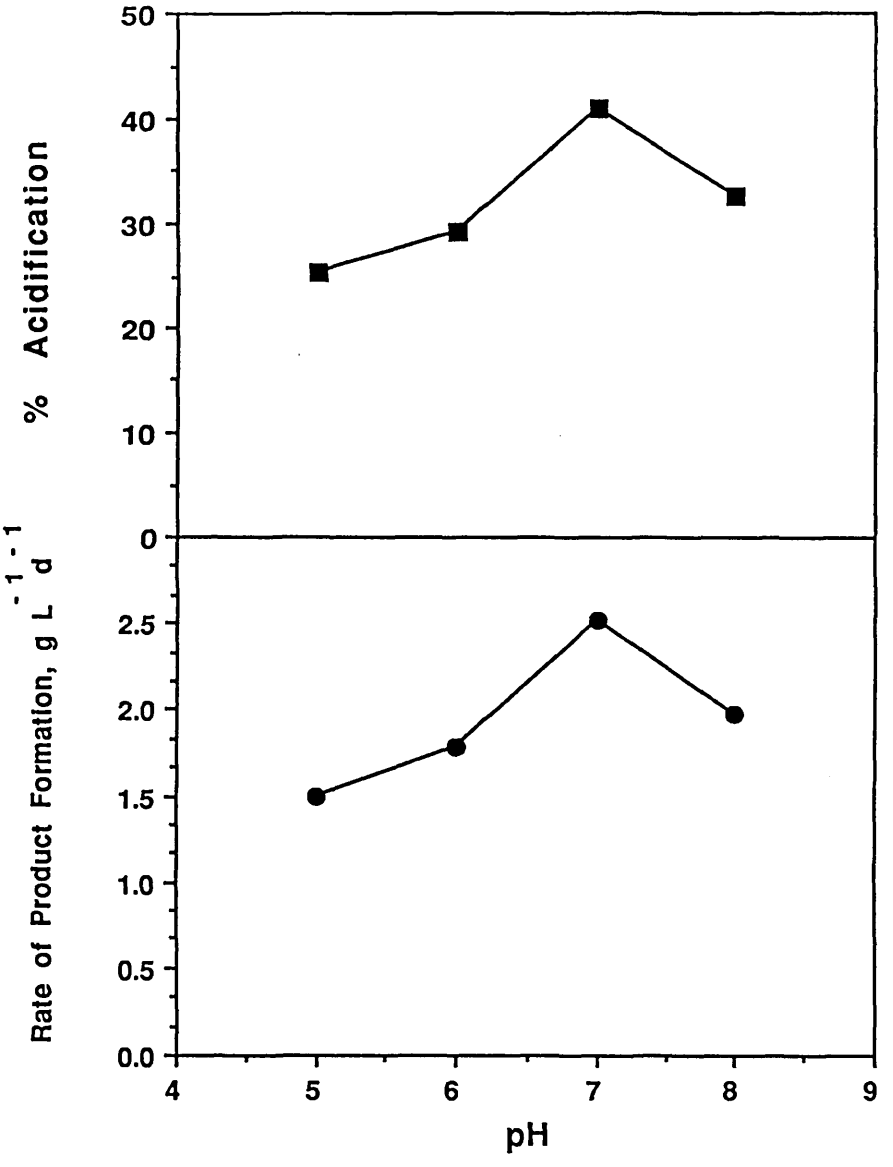


Figure 22 : Effect of pH on the degree of acidification and the rate of product formation during experimental series VI

with a mean value of 40 % , while that of propionic acid ranged from 13 to 55 %, with a mean value of 35 %. The next most important products were butyric and valeric acids, both found in the range of 4 to 17 %. Other volatile fatty acids, such as iso-butyric, 2-methyl butyric and iso-valeric acids, were found at even lower concentrations. Lactic acid was present at certain experiments at concentrations of less than 30 mg L^{-1}, while ethanol was not detected in any experimental run.

In order to investigate the influence of the various operational parameters on the distribution of the volatile acids produced, the concentrations of the individual acids were correlated to the hydraulic retention time, organic loading rate and initial substrate concentration by means of multiple regression.

A very high correlation coefficient was found to exist between the propionic acid concentration and the influent substrate concentration (Fig. 23), while no correlation was found between the propionic acid concentration and the organic loading rate or the hydraulic retention time:

$$(Pr) = 0.052 + 0.069\ (S_o), \qquad R = 0.958 \qquad (49)$$

where (Pr) is the propionic acid concentration (g L^{-1}) and (S_o) is the influent COD concentration (g L^{-1}).

This correlation implied that the amount of propionic acid produced was almost entirely determined by the initial substrate concentration, with the other parameters such as hydraulic retention time and organic loading rate affecting propionate production very little. The regression line does not change when the propionic acid concentrations produced during experimental series V and VI are used. This indicated that neither the temperature nor the pH affected the propionic acid concentration significantly. The proportion of substrate transformed to propionic acid was consistently of the order of 7 %.

The concentration of acetic acid was similarly correlated to the influent substrate concentration (Fig. 24). The correlation coefficient was relatively low,

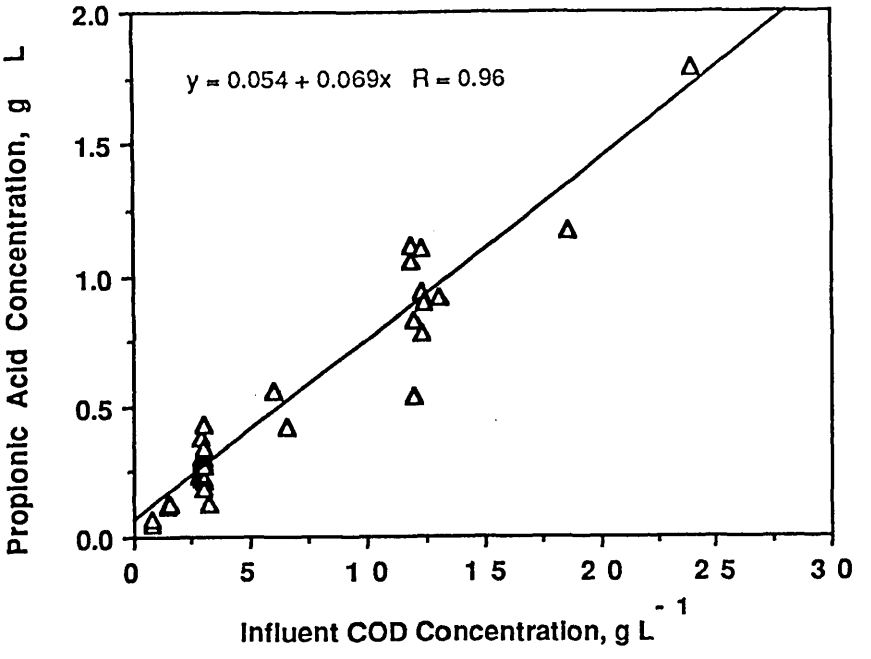


Figure 23 : Effect of influent substrate concentration on propionic acid concentration

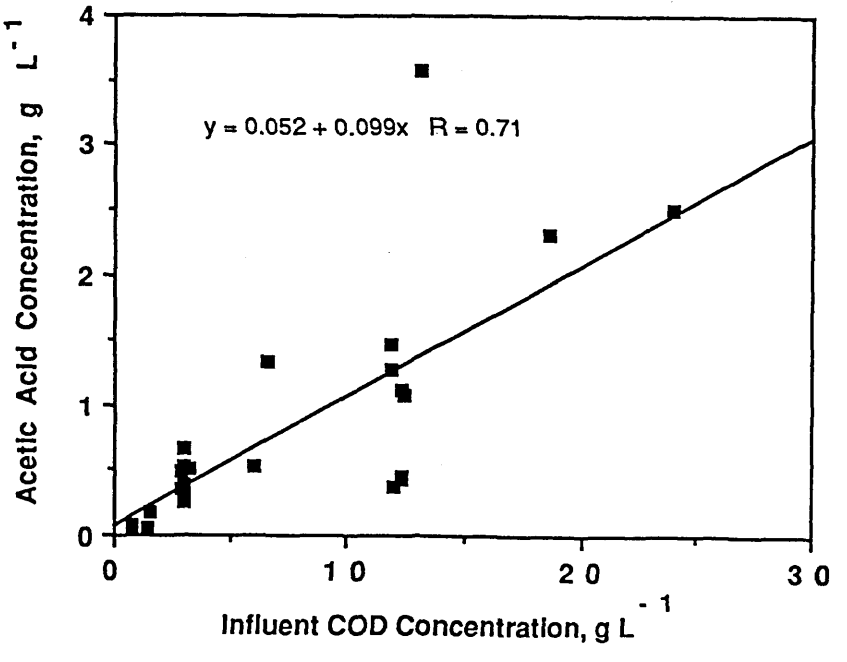


Figure 24 : Effect of influent substrate concentration on acetic acid concentration

which indicated that acetic acid production was possibly influenced by other parameters. Using multiple regression to study the influence of the influent substrate concentration and the hydraulic retention time simultaneously, a better correlation was obtained. The equation derived for this regression was the following:

$$(Ac) = -0.313 + 0.083\,(\theta) + 0.054\,(S_o), \qquad R = 0.881 \qquad (50)$$

where (Ac) is the acetic acid concentration ($g\,L^{-1}$), and θ is the hydraulic retention time (h). As can be seen from this equation, the concentration of acetic acid increased proportionally with both the hydraulic retention time and the influent COD concentration.

The percentage of acetic and propionic acids, as a proportion of the total volatile fatty acids, was also correlated to the operational parameters. There was a certain tendency for the percentage of acetic acid to increase with the hydraulic retention time and the influent substrate concentration. Also, the percentage of propionic acid decreased with increasing hydraulic retention time. However, in neither case could a quantitative relationship be statistically derived.

The effect of pH on the product distribution was studied using the data of experimental series VI. The concentration of acetic and propionic acids ($g\,L^{-1}$), as well as the proportions of the main four acids expressed as percentages of the total are shown in Figure 25. The concentration of propionic acid did not appear to be significantly affected by the decrease in pH. Conversely, the concentration of acetic acid decreased consistently with the pH. The distribution of the individual acids was not severely affected by the pH, except for a slight decline in the percentage of acetic acid and a corresponding increase in that of propionic acid at pH 5.

The effect of temperature on the product distribution was studied using the data of experimental series V. The concentrations of acetic and propionic acids, as well as the proportions of the four most important acids, expressed as percentages of the total, are shown in Figure 26. The concentration of propionic acid was

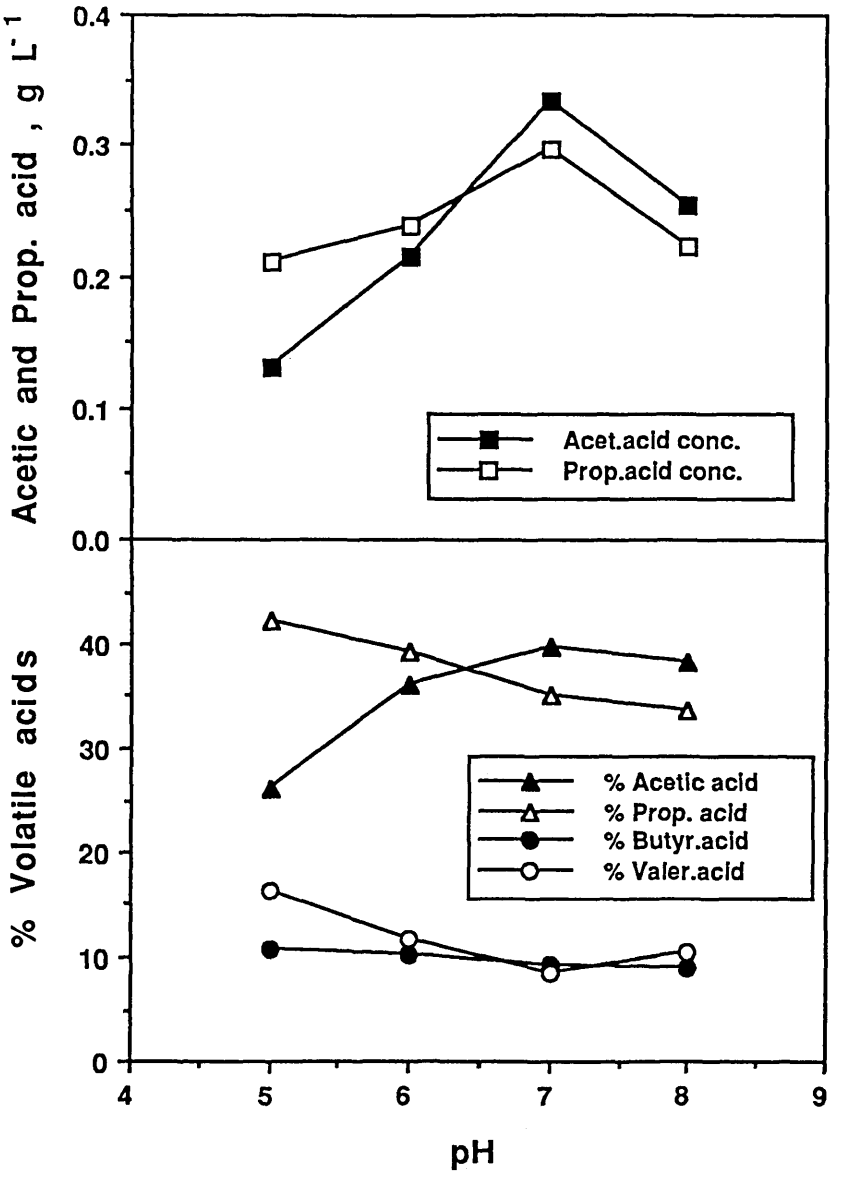


Figure 25 : Effect of pH on acetic and propionic acids concentration and volatile fatty acid distribution during experimental series VI

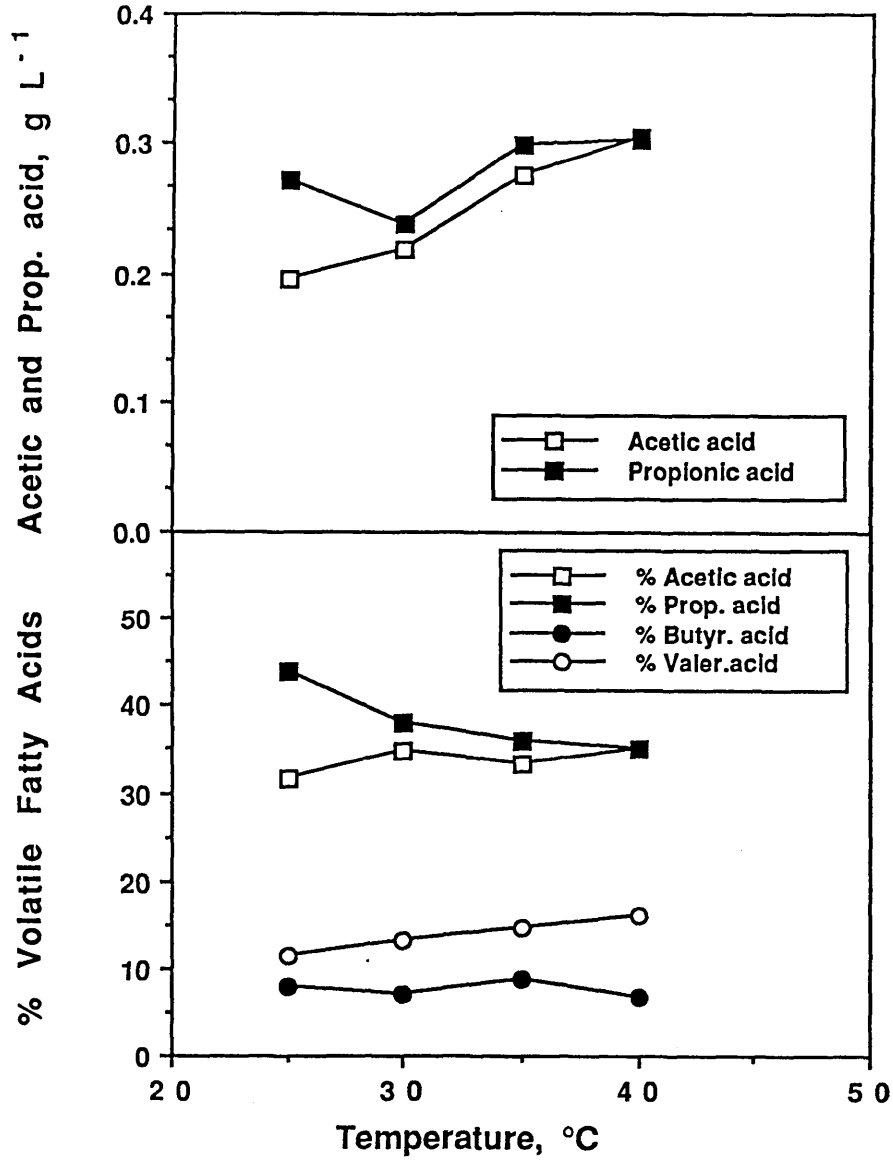


Figure 26 : Effect of temperature on acetic and propionic acids concentration and volatile fatty acid distribution during experimental series V

relatively constant for temperatures between 25 and 40 °C, although the concentration of acetic acid increased consistently with increasing temperature. The percentage of propionic acid decreased with increasing temperature, while that of acetic acid slightly increased. These results were in agreement with previous observations that the production of propionic acid was only slightly affected by operational parameters, and was mainly limited by substrate availability.

4.3. Kinetic Study of Acidogenic Bacteria in Continuous Culture

4.3.1. Estimation of the Biomass Yield and the Specific Decay Rate of Acidogenic Bacteria

In order to study the growth of acidogenic bacteria, it is necessary to have an estimate of their specific decay rate k_d (d^{-1}). The latter, as well as the biomass yield Y, can easily be calculated from equation (15):

$$\frac{D\,(S_o - S_{e,c})}{x_e} = \frac{k_d}{Y} + \frac{D}{Y} \qquad (15)$$

where D is the dilution rate (d^{-1}), S_o is the influent substrate concentration ($gCOD\,L^{-1}$), $S_{e,c}$ is the corrected effluent substrate concentration and equal to the effluent COD concentration S_e minus the COD exerted by the mixture of the products S_p, and x_e is the biomass concentration ($gTSS\,L^{-1}$). By plotting the specific rate of substrate consumption $\frac{D\,(S_o - S_{e,c})}{x_e}$ versus the dilution rate D, and using linear regression, the slope $\frac{1}{Y}$ and the intercept $\frac{k_d}{Y}$ were estimated (Fig. 27). To calculate the variance of Y and k_d, equations (39) and (40) were used.

The value obtained for k_d was 0.052 d^{-1} with a standard deviation of 0.379, while that of Y was 0.099 g biomass g^{-1} COD utilised with a standard deviation of 0.007. The correlation coefficient of the regression line was 0.95. Using a t-test, the specific decay rate was shown not to be statistically different to zero at $p = 0.05$.

The precision of the determination of k_d was very low. To overcome this problem, the estimation of k_d was also incorporated into the inhibition model and another value of k_d was calculated.

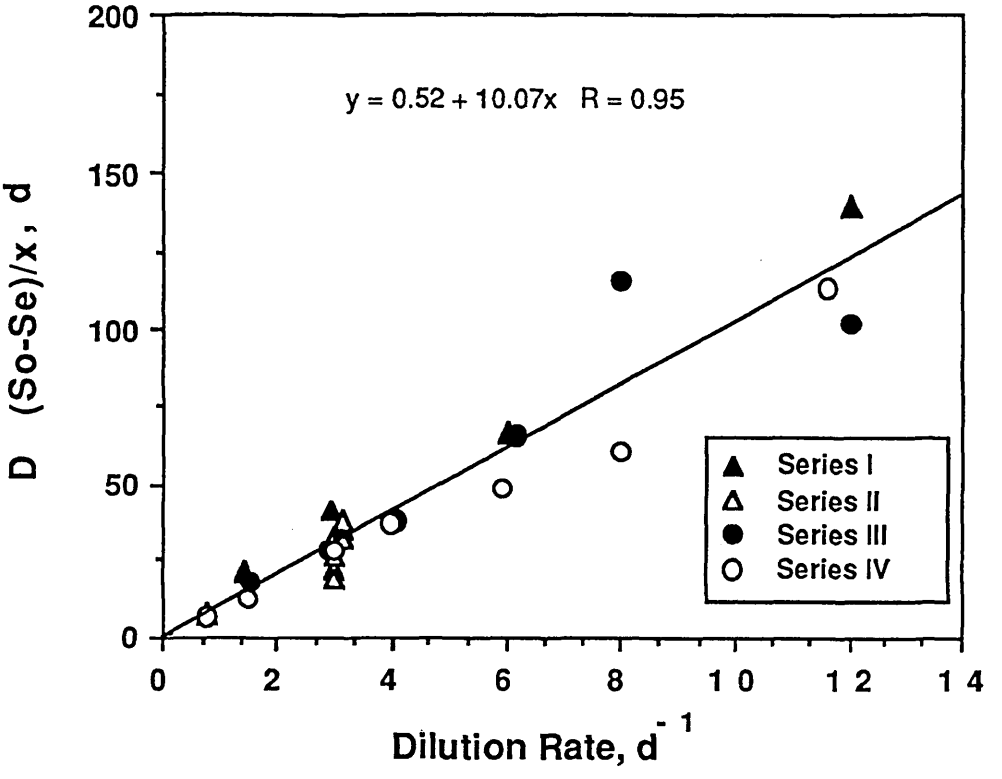


Figure 27 : Specific rate of substrate consumption $\left[\frac{D\,(S_o - S_e)}{x_e}\right]$ versus dilution rate D

4.3.2. Application of Inhibition Models for the Calculation of the Maximum Specific Growth Rate, μ_{max}, of Acidogenic Bacteria

Before proceeding to the evaluation of the inhibition models proposed for the growth of the acidogenic bacteria, it must be noted that the Monod equation completely failed to represent the system, although it is still used in modelling and has been used successfully for the description of batch culture kinetics (section 4.1.). In Figure 28, the inverse of the specific growth rate ($\frac{1}{\mu}$) is plotted against the inverse of the effluent substrate concentration ($\frac{1}{S_{e,c}}$) according to equation (33). Although the expected slope would be positive, the one obtained was far from that. The growth rate obtained was lower than expected when the substrate concentration, and consequently the product concentration, were high.

To determine the model that best fitted the experimental data, the model for non-competitive inhibition [eq. (21)] was initially evaluated with inhibition exerted by the total volatile fatty acids (VFA). The experimental data used in this evaluation were derived from experimental series I, II, III and IV. In Table 22, the calculated values of the biokinetic constants μ_{max}, K_s, K_i and k_d are given, while the observed and the predicted values of the total volatile fatty acid concentration (VFA) are shown in Figure 29.

To investigate the sensitivity of the parameter estimation to the value of the specific decay rate k_d, the model was re-evaluated using the experimentally calculated value of k_d, which was equal to 0.052 d^{-1}; the new values calculated for the biokinetic constants are also given in Table 22. The average relative error as defined in equation (37) was 28.7%, when the k_d value was calculated simultaneously with the rest of the biokinetic constants, and 36.7% when k_d was assumed equal to 0.052 d^{-1} as calculated from the yield data [eq. (15)]: it is obvious that a better fit of the experimental data to the inhibition model could be achieved by using the former method.

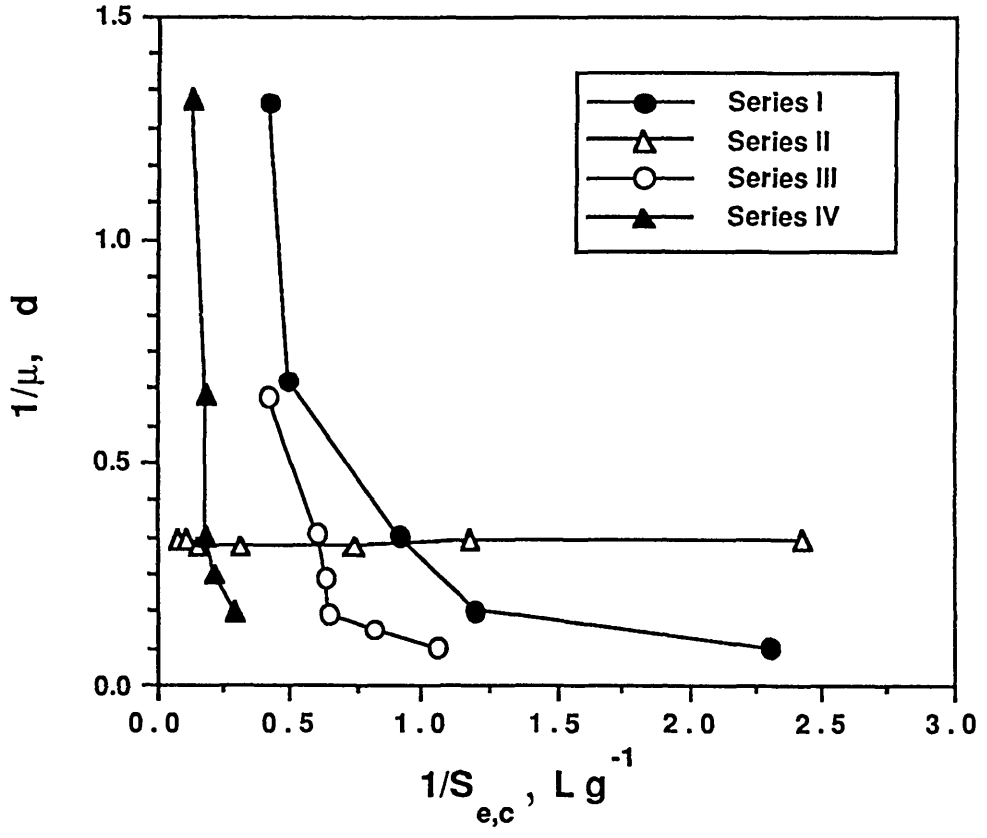


Figure 28 : Plot of $\frac{1}{\mu}$ versus the inverse of the corrected effluent substrate concentration $S_{e,c}$

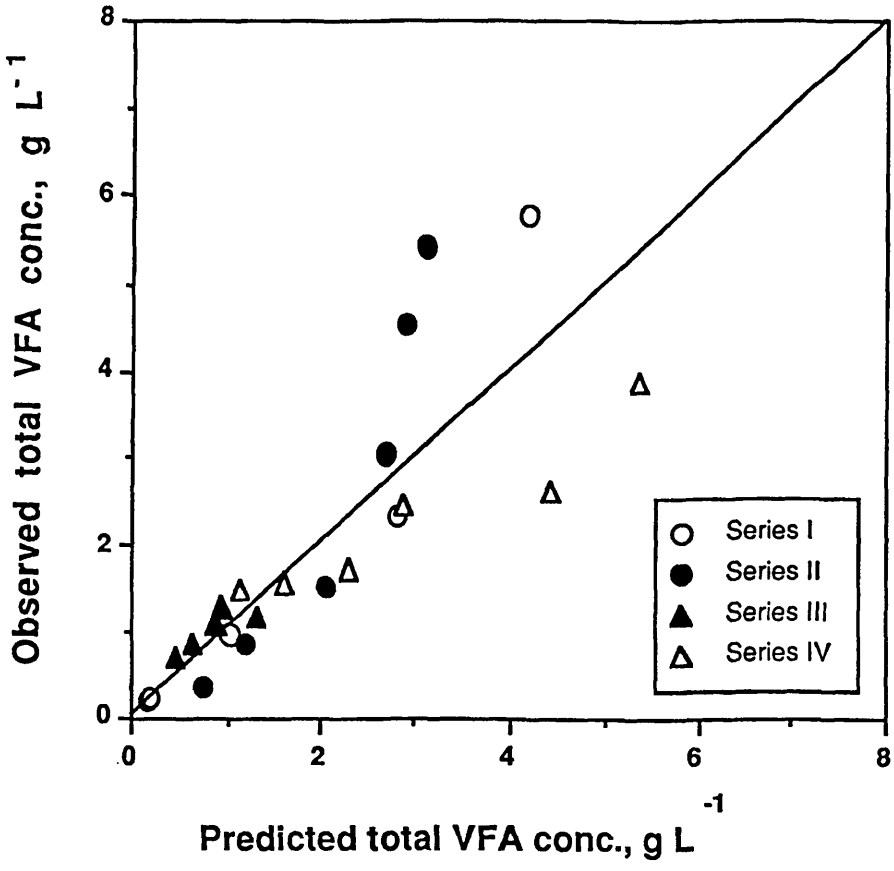


Figure 29 : Plot of the observed values of the total volatile fatty acid (VFA) concentrations versus those predicted by the non-competitive inhibition model, with simultaneous calculation of k_d

Table 22 : Values of the biokinetic constants, as predicted by the non-competitive inhibition model, and regression lines between the observed and the predicted values of the total volatile fatty acid concentrations

	k_d calculated simultaneously	k_d calculated from yield data
μ_{max} (d^{-1})	32.6	39.2
K_s (gL^{-1})	1.768	1.996
K_i (gL^{-1})	0.633	0.212
k_d (d^{-1})	1.747	0.052
Average Relative Error	28.73 %	36.74 %
Regression coefficient (standard deviation)	0.927 (0.142)	0.651 (0.137)
Intercept (standard deviation)	0.184 (0.343)	0.892 (0.335)
Correlation coefficient R	0.824	0.729
Standard deviation about regression (gL^{-1})	0.936	1.131

In order to test the goodness-of-fit further, the observed values of total VFA were regressed upon the predicted ones; ideally the regression line should pass through zero and have a regression coefficient equal to 1. The values of the regression coefficient and the intercept obtained for the two evaluations are given in Table 22. In the case where k_d was calculated by the model, the regression coefficient and the intercept of the regression line were statistically equal to unity and zero respectively, at $p = 95\%$. However, for k_d equal to 0.052 d^{-1}, the regression line had a regression coefficient and an intercept statistically different to unity and zero respectively, showing that the model gave biased predictions.

The second inhibition model evaluated was the Haldane-type [eq. (20)] using the total volatile fatty acids as inhibitors. The values of the biokinetic constants were initially estimated simultaneously with the specific decay rate k_d, and then separately by using the experimentally calculated value of k_d equal to 0.052 d^{-1}. The results of the two evaluations are given in Table 23, together with the regression lines between the observed and the predicted values of the total volatile fatty acid concentrations (VFA). The average relative error was lower when the value of k_d was calculated by this model, and a better agreement between the observed and the predicted values of the total VFA concentration was found; these values are presented in Figure 30.

Table 23 : Values of the biokinetic constants, as predicted by the Haldane-type inhibition model, and regression lines between the observed and the predicted values of the total volatile fatty acid concentrations

	k_d calculated simultaneously	k_d calculated from yield data
μ_{max} (d^{-1})	26.4	39.6
K_s ($g L^{-1}$)	2.349	2.192
K_i ($g L^{-1}$)	0.282	0.107
k_d (d^{-1})	0.536	0.052
Average Relative Error	47.92 %	58.62 %
Regression coefficient (standard deviation)	0.752 (0.173)	0.722 (0.198)
Intercept (standard deviation)	0.718 (0.388)	0.921 (0.405)
Correlation coefficient R	0.698	0.633
Standard deviation about regression ($g L^{-1}$)	1.184	1.281

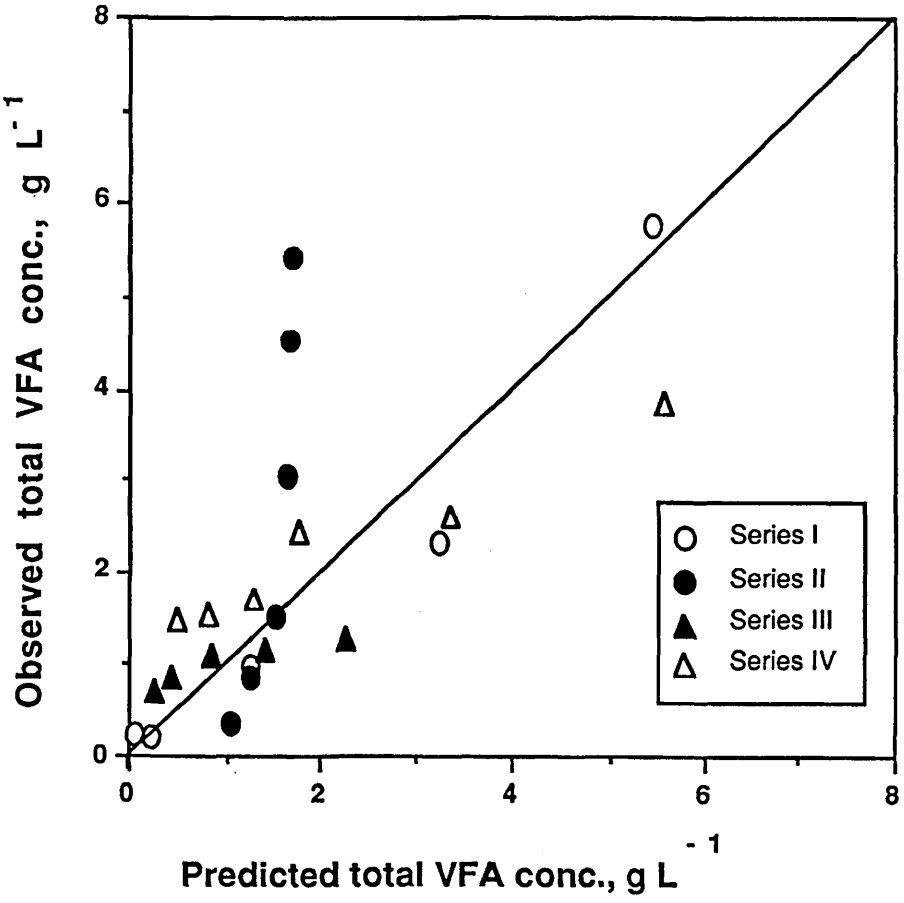


Figure 30 : Plot of the observed values of the total volatile fatty acid (VFA) concentrations versus those predicted by the Haldane-type inhibition model, with simultaneous calculation of k_d

The application of the Haldane-type inhibition model appeared to give inferior results to the non-competitive inhibition model. To test this observation, the F-test for equality of variance was applied. This test indicated that the variance between the observed and the predicted values of the product concentration was statistically higher at $p = 0.05$ when the Haldane model instead of the non-competitive model was employed. This probability level is not highly significant and may indicate that the difference in variance was the result of chance. However, the application of the Haldane equation led to substantial under-estimation of the product concentration at low concentrations, and this was verified by the fact that the intercept of the regression line between the observed and the predicted values was statistically different to zero. Therefore, it could be concluded that the non-competitive model was the most applicable.

Both inhibition models were also evaluated using un-ionised instead of total volatile fatty acids as inhibitors. The concentrations of the un-ionised VFA were calculated indirectly from the determinations of the total concentration and the effluent pH values, using the appropriate ionisation constants. Similarly, the inhibiting effect of propionic and butyric acids was evaluated. In both cases, however, the regression of the observed values on the predicted ones was very poor, and hence no data for the biokinetic constants are presented here.

4.3.3. Application of Inhibition Models for the Calculation of the Maximum Rate of Product Formation, q_{max}, of Acidogenic Bacteria

The relationship between the specific rate of product formation q_p [eq. (17)] and the specific biomass growth rate μ was investigated using equation (19):

$$q_p = \frac{(P_e - P_o)}{x_e} = \alpha \mu + \beta \qquad (19)$$

For the calculation of μ according to equation (13), the estimates of k_d derived from the previous analyses were used, that is 0.052, 0.536 and 1.747 d^{-1}. In Table 24, the regression coefficient α and the intercept β are given, when q_p is

expressed in g VFA g^{-1} TSS d^{-1} and μ in d^{-1}. The values obtained for the regression coefficient α and the correlation coefficient R were not affected by the value of k_d. The latter affected only the value of the intercept β. However, the application of a t-test showed that for all regression lines, the intercept was not statistically different to zero at $p = 0.05$.

Table 24 : Linear regression of the specific rate of product formation q_p versus the specific biomass growth rate μ

k_d (d^{-1})	Regression coefficient (stand. deviation)	Intercept (stand. deviation)	Correlation coefficient R
0.052	4.02 (0.29)	3.08 (1.65)	0.945
0.536	4.02 (0.29)	1.13 (1.76)	0.945
1.747	4.02 (0.29)	-3.73 (2.07)	0.945

Since the specific rate of product formation q_p is proportional to the specific growth rate μ, it becomes obvious that a model similar to the one used for the prediction of μ could be used for the prediction of q_p. The values of the biokinetic constants K_s and K_i would be expected to be the same as those obtained from the growth rate equations, although this has not always been found to be the case (Aiba *et al.*, 1973).

The inhibition models employed previously for the description of μ were also applied to describe the specific rate of product formation q_p. The equations used for the non-competitive type and the Haldane-type of inhibition were equations (24) and (25), respectively:

$$q_p = q_{max} \frac{S}{S + K_s'} \frac{K_i'}{K_i' + I} \tag{24}$$

and

$$q_p = q_{max} \frac{1}{1 + \frac{K_s'}{S} + \frac{I}{K_i'}} \tag{25}$$

The variable introduced into the error function was the total volatile fatty

acid concentration. The calculated values of the biokinetic constants q_{max}, K_s' and K_i' are given in Table 25, while the observed and predicted values of the total volatile fatty acid concentrations are shown in Figures 31 and 32 respectively for the two models. The average relative error between the observed and the predicted values of the total VFA was lower when the non-competitive inhibition model was applied. However, the variation for both models was too large and therefore, no conclusions can be derived for the relative effectiveness of the two models.

Table 25 : Values of the biokinetic constants, as calculated by the non-competitive and the Haldane-type inhibition models, applied for the description of the specific rate of product formation q_p

	Non-competitive Model	Haldane-type Model
q_{max} ($gVFA\,g^{-1}TSS\,d^{-1}$)	125.75	148.71
K_s' ($g\,L^{-1}$)	2.713	2.161
K_i' ($g\,L^{-1}$)	0.146	0.150
Average Relative Error	54.33 %	76.22 %
Regression coefficient (standard deviation)	0.532 (0.117)	0.689 (0.187)
Intercept (standard deviation)	1.038 (0.326)	0.960 (0.392)
Correlation coefficient R	0.712	0.960
Standard deviation about regression ($g\,L^{-1}$)	1.160	1.275

4.4. Effect of Inoculum on the Product Distribution of the Acidogenic Reactor

The effect of the inoculum on the product distribution of the first phase of anaerobic digestion was studied in batch. The method followed was similar to that employed to study the kinetics of acidogenesis in batch: test tubes that could be

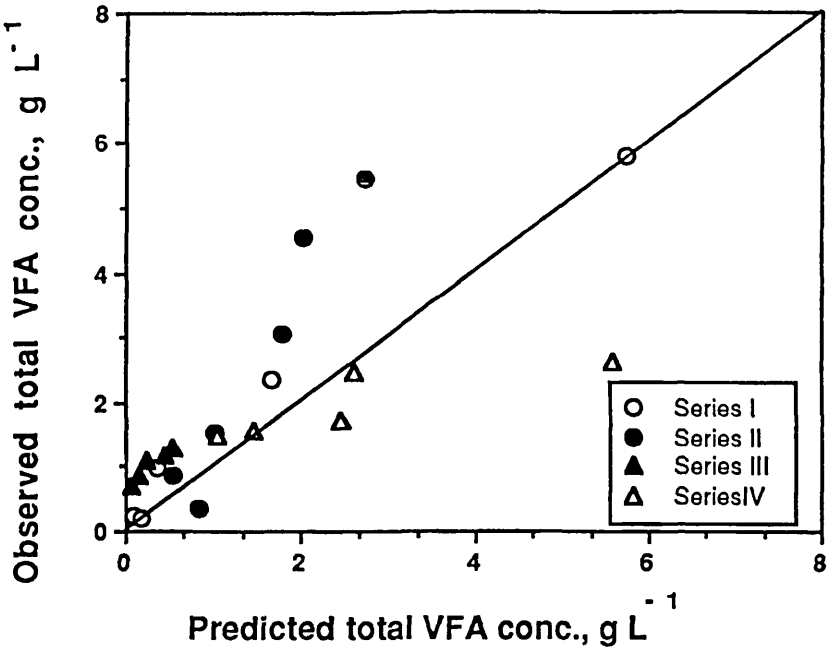


Figure 31 : Plot of the observed values of the total volatile fatty acid (VFA) concentrations versus those predicted by the non-competitive inhibition model, when applied for the description of the specific rate of product formation q_p

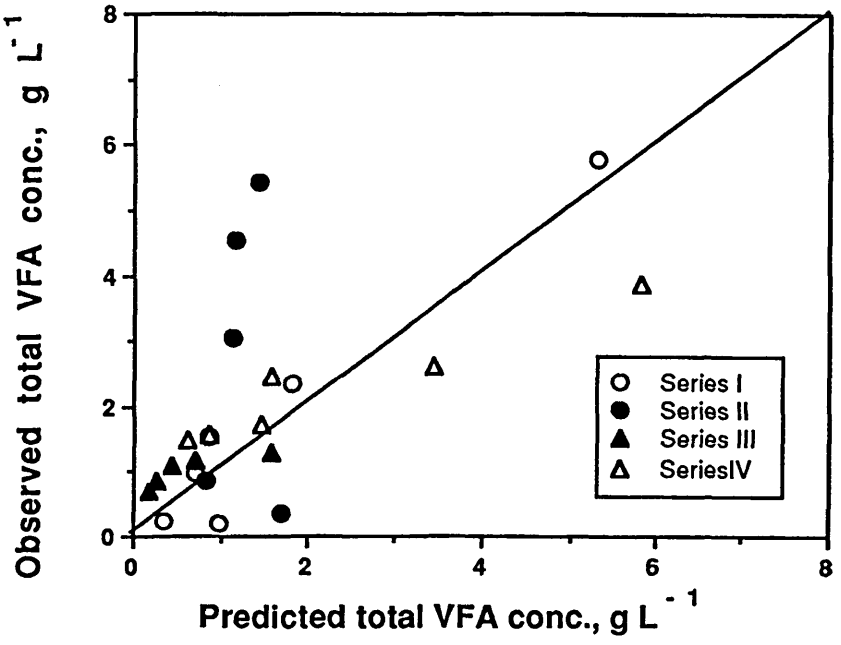


Figure 32 : Plot of the observed values of the total volatile fatty acid (VFA) concentrations versus those predicted by the Haldane-type inhibition model, when applied for the description of the specific rate of product formation q_p

used with the turbidimeter were filled with substrate at a concentration of 3 g COD L^{-1} and autoclaved. They were then inoculated with four different inocula: activated sludge, pasteurised activated sludge, anaerobic digested sludge and pasteurised anaerobic digested sludge. The pasteurisation technique was described in section 3.7. The percentages of the four major fatty acids produced are given in Table 26, while the standard deviation of the four replicates are presented in brackets. Using the analysis of variance technique, it was shown that the distribution of the products was not affected by the initial inoculum.

Table 26 : Percentages of the volatile fatty acids produced when different inocula are used

Inoculum	Acetic acid	Propionic acid	Butyric acid	Valeric acid
Activated sludge	69.0 (3.1)	18.7 (5.1)	7.7 (1.8)	4.7 (0.5)
Pasteurised activated sludge	66.3 (6.6)	12.6 (2.4)	13.5 (5.0)	7.6 (1.9)
Digested sludge	67.8 (2.4)	14.3 (1.5)	13.1 (3.4)	4.8 (1.7)
Pasteurised digested sludge	67.1 (5.1)	14.3 (0.8)	12.5 (4.8)	6.0 (1.1)

4.5. Characterisation of Start-up Performance of the Two-Phase Anaerobic Digestion Systems

4.5.1. Start-up Procedures

In order to assess the effect of phase separation on the performance of an anaerobic digester, four anaerobic digestion systems were tested simultaneously: systems 1, 2 and 3 consisted of two stages, a continuous-flow stirred tank acidogenic reactor and a fluidised bed methanogenic reactor. System 4 was a single-stage anaerobic fluidised bed reactor used as control. Details of the experimental

apparatus used, as well as the construction of each reactor, are given in Chapter 3. The volume of the individual reactors of each system are shown in Table 27, the volume of the methanogenic reactors corresponding to the expanded bed volume.

Table 27 : Volume of the individual reactors used in the four anaerobic digestion systems

System	Acidogenic Reactor (L)	Methanogenic Reactor (L)	Total Volume (L)
1	0.235	1.645	1.880
2	0.500	1.500	2.000
3	0.745	0.745	1.450
4 (Control)	—	1.500	1.500

Prior to inoculation, all reactors were filled with wastewater at a COD concentration equal to 3 gL^{-1}: only 50% of the COD was derived from the meat waste, while the other 50% was replaced by its COD equivalent of methanol. All reactors were left to equilibrate at the operating temperature of $37 \pm 1\,^{\circ}C$. The recycle pumps of the fluidised beds were adjusted to give a 30% bed expansion to provide for the expanded bed volumes presented in Table 27. Due to variations in the pressure drop throughout the fluidised bed systems, as well as wearing of the tubing of the recycle pumps, the upflow velocity required to maintain the chosen expansion varied between 6 to 14 $m\,h^{-1}$, corresponding to an upflow flowrate in the range of 12 to 28 $L\,h^{-1}$. Taking into account that the influent flowrate during the whole range of the two-phase anaerobic digestion experiments varied between 0.05 to 0.15 $L\,h^{-1}$, the recycle ratio of the fluidised beds can be calculated as being not less than 1:80. This relatively high recycle ratio can be attributed to the large height to width ratio of the fluidised bed construction, as well as the high strength of the wastewater which dictated low influent flowrate for the range of organic loading rates studied.

The acidogenic reactors were then inoculated with freshly collected, screened ($< 1\,mm$), digested sludge at a concentration of 6%(v/v), while the methanogenic reactors were inoculated with similar sludge at a concentration of 10%(v/v). Following the inoculation, the reactors were operated in batch mode for 48 hours, after which continuous feed flow was started.

The regime followed during the start-up period has been previously employed successfully by other researchers, for the rapid start-up of anaerobic fluidised bed reactors (Bull *et al.*, 1983b; Stronach *et al.*, 1986; Stephenson and Lester, 1986). It consists of a stepped increase of the organic loading rate in conjuction with a stepped decrease of the hydraulic retention time and stepped increase of the influent COD concentration. This regime is employed together with the gradual substitution of the methanol of the influent with waste. In Figure 33, the hydraulic retention time HRT, influent COD concentration S_o, organic loading rate OLR and % methanol of the influent are presented. These values of the operational parameters represent the overall values applied to each system, and were identical for the four anaerobic systems. Because of differences in the total volume of the four systems, different influent flowrates had to be applied to ensure that the overall hydraulic retention times were the same. The hydraulic retention time of each component reactor was a fraction of the overall hydraulic retention time equal to the reactor volume over the total volume of the system. Similarly, the organic loading rates of the individual reactors were different to the overall rates and could be calculated as already described in section 3.2.2.2. [eq. (27)].

4.5.2. Performance of the Acidogenic Reactors during Start-up

During the start-up period the values of the major operational parameters applied to the acidogenic reactors of the two-phase systems were measured daily: these parameters were the temperature, the influent flowrate conducive of the

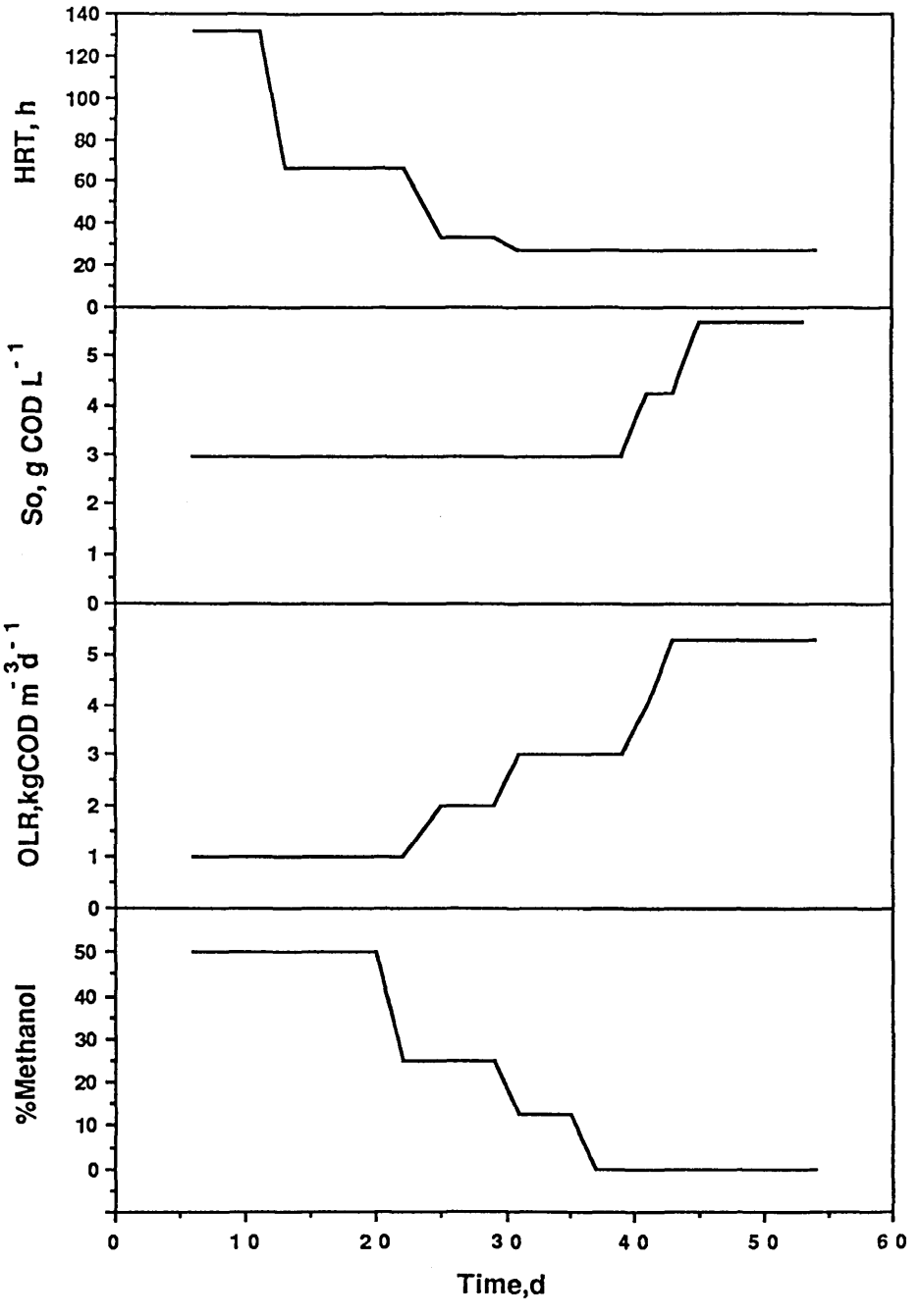


Figure 33 : Start-up regime for the anaerobic digestion systems

hydraulic retention time, and the influent COD concentration. The effluent characteristics of the acidogenic reactors, such as filtered COD concentration, total suspended solids, pH and volatile fatty acid concentration were monitored every two days. The performances of the acidogenic reactors during the start-up period are presented in Figures 34, 35 and 36, using the notation A1, A2 and A3 for the acidogenic reactors of systems 1, 2 and 3, respectively.

The pH of the effluent was always in the range of 5.2 to 6.6, although after day 40 it attained a value of approximately 6 in all reactors (Fig. 34). No pH control was ever applied to any of the acidogenic reactors. The total suspended solids (TSS), which are generally expected to be higher the longer the hydraulic retention time of the acidogenic reactor, fluctuated significantly until day 30 (Fig. 34). This fluctuation was attributed to operational problems associated with the separate collection of the gases produced in the acidogenic phase from those produced in the methanogenic phase: the effluent of the acidogenic reactor could not flow freely into the recycle chamber of the fluidised bed system, resulting in hydraulic retention times longer than the nominal ones. The gas collection system of the acidogenic reactors was removed at day 30, after which no major fluctuations of the total suspended solids were observed.

The filtered effluent COD concentrations of the acidogenic reactors are presented in Figure 35. The solid line represents the nominal influent COD concentration. The % filtered COD removals are also presented in the same figure. At the beginning of the start-up period, the observed COD removals were relatively large, being in the range of 20 to 40 %. These were mainly due to the long hydraulic retention times, which allowed some methanogenic bacteria to grow. The latter were further encouraged by the methanol present in the feed. The % COD removals were gradually reduced to between 10 and 20 %.

In Figure 36 the total concentration of the volatile fatty acids, as well as the

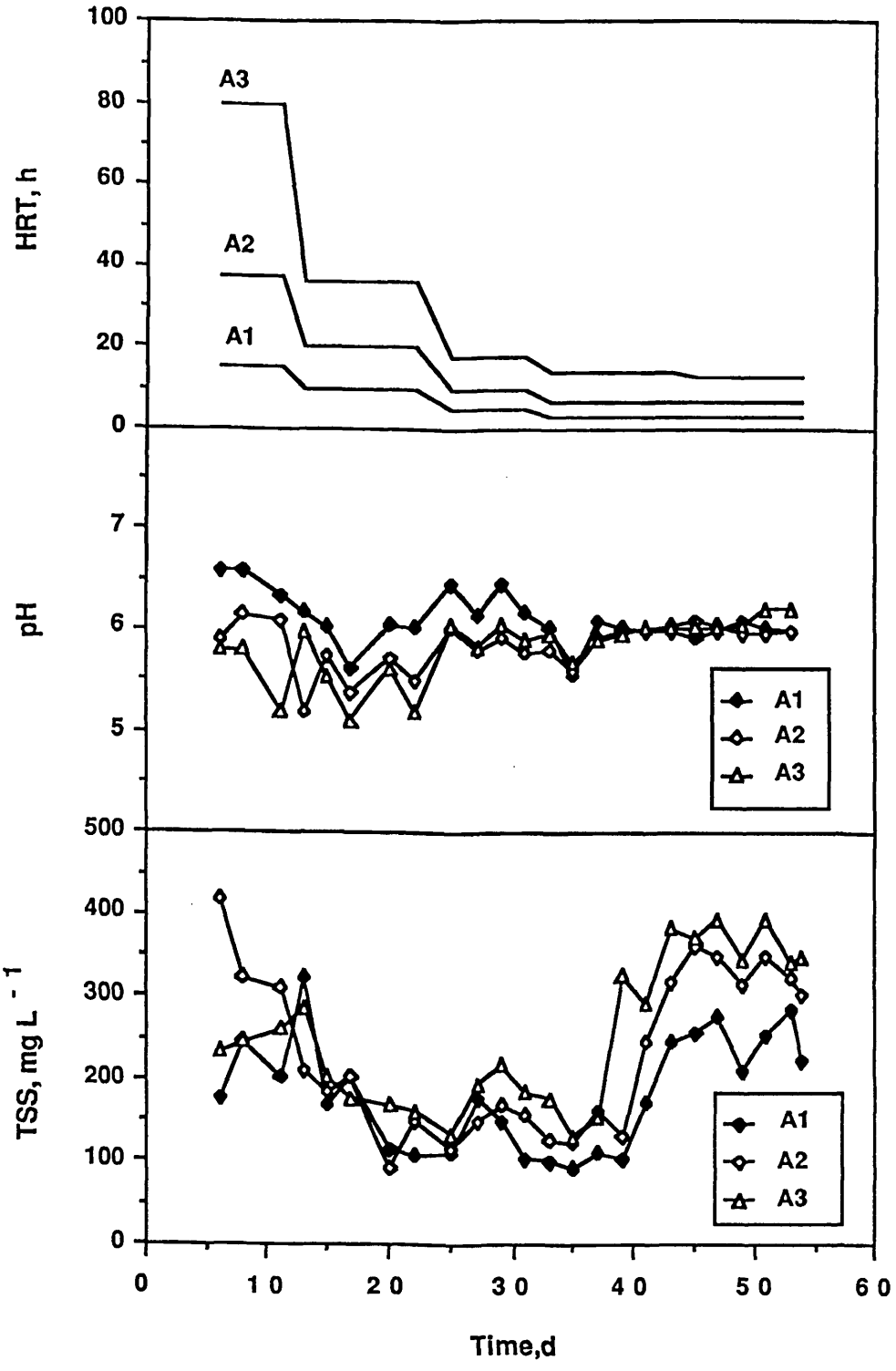


Figure 34 : Hydraulic retention time (HRT), pH and total suspended solids (TSS) of the acidogenic reactors of the two-phase systems during start-up (A1, A2, and A3 represent the acidogenic reactors of systems 1, 2 and 3, respectively).

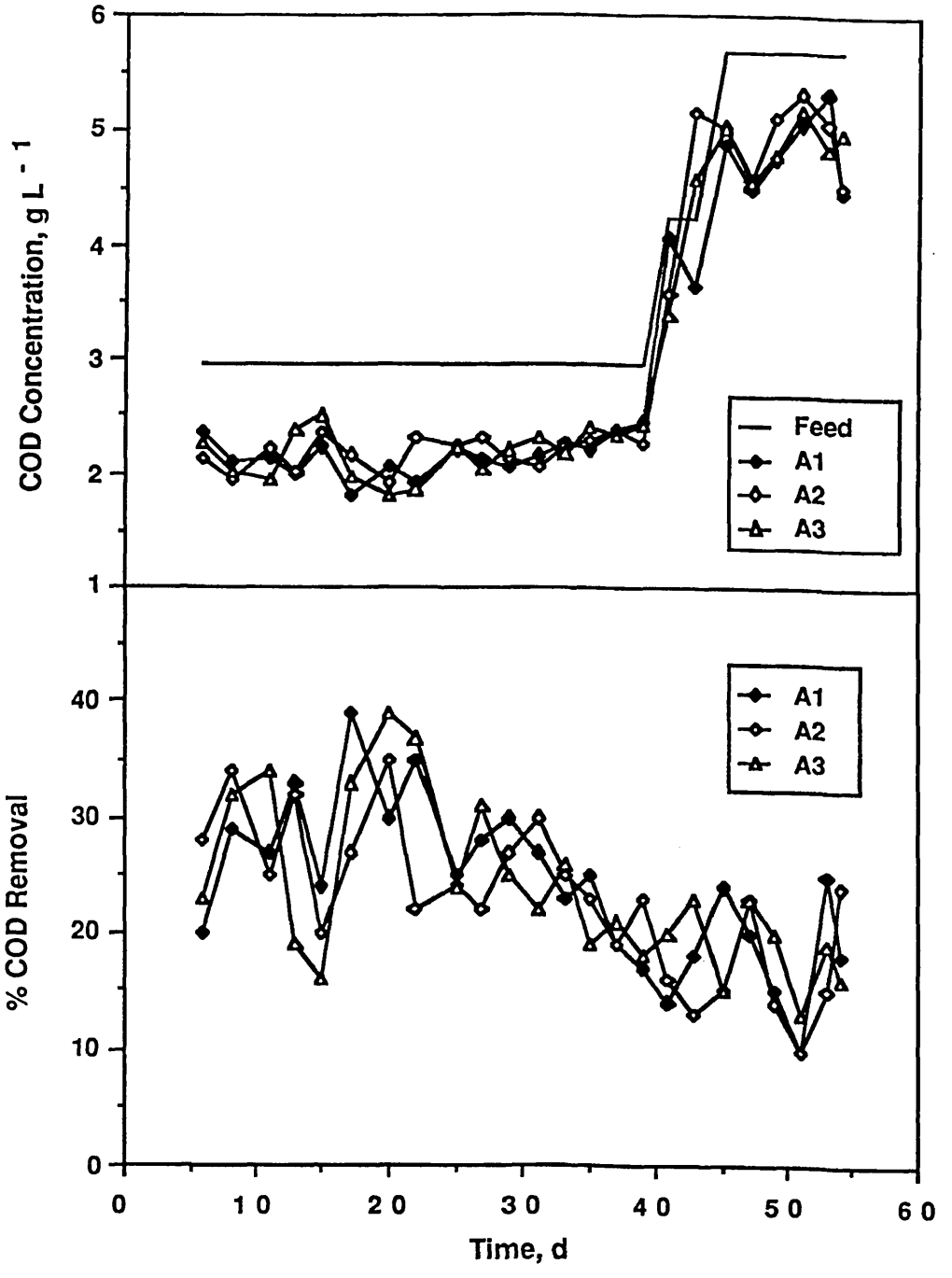


Figure 35 : Influent and filtered effluent COD concentration (S) and % filtered COD removal of the acidogenic reactors of the two-phase systems during start-up (A1, A2, and A3 represent the acidogenic reactors of systems 1, 2 and 3, respectively).

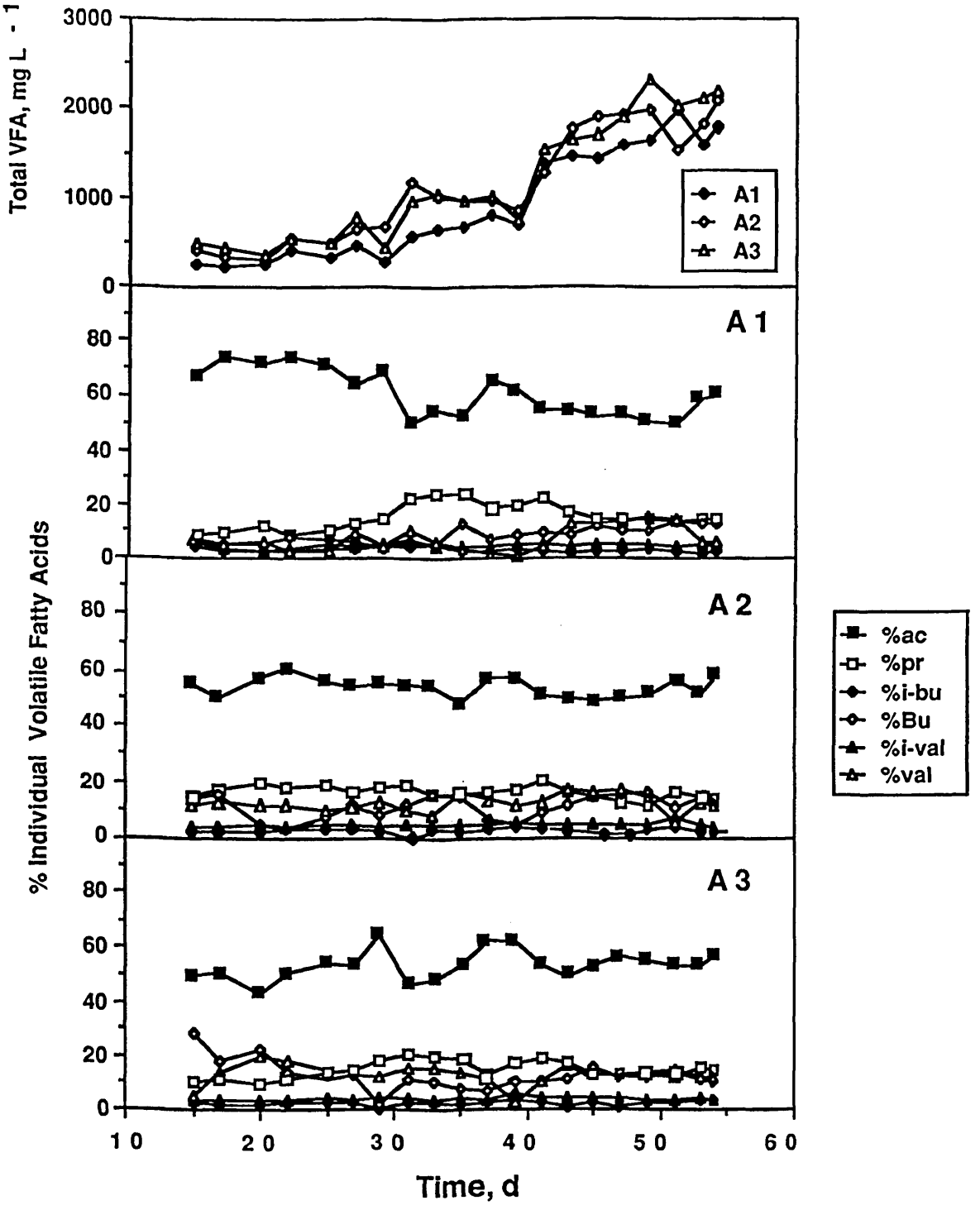


Figure 36 : Total volatile fatty acid concentration and distribution of the individual acids in the effluent of the acidogenic reactors of the two-phase systems during start-up (A1, A2, and A3 represent the acidogenic reactors of systems 1, 2 and 3, respectively).

percentage of each individual acid are presented. Acetic acid was always the predominant fatty acid, its proportion varying between 45 and 70% for all acidogenic reactors. During the start-up period, the degree of acidification previously defined as the percentage of the initial COD concentration converted to volatile fatty acids [eq. (42)], varied between 15 to 30 % until day 30, after which it assumed values between 35 to 55%.

4.5.3. Performance of the Methanogenic Reactors during Start-up

During the start-up period of the two-phase anaerobic digestion systems, the values of the major operational parameters applied to the methanogenic fluidised bed reactors were measured daily: these parameters were the temperature, the % bed expansion determining the volume of the beds, and the influent flowrate and COD concentration (also applying to the acidogenic reactors). The effluent characteristics such as filtered and unfiltered COD concentration, total suspended solids, pH and volatile fatty acids were monitored every two days. The rate of gas production was also measured daily, since it was most sensitive to system disturbances and could therefore be used for the early detection of various operational problems such as temperature or feed pump failures, leakages etc. The composition of the evolved gas was measured regularly once a week. The performances of the methanogenic reactors during the start-up period are presented in Figures 37, 38, 39 and 40 using the notation M1, M2, M3 and M4 for systems 1, 2, 3 and 4 (control), respectively.

During the first week of operation, the pH values of the methanogenic fluidised beds were controlled to approximately 7 by addition of 0.1 *M* NaOH. The pH control was operated once a day or whenever necessary, the amount of alkali to be added being determined by titration of a known volume of the effluent and calculation of the amount needed to neutralise the whole volume. After day 10, no pH control was required, as the pH was in the range of 6.6 to 6.9 and it gradually

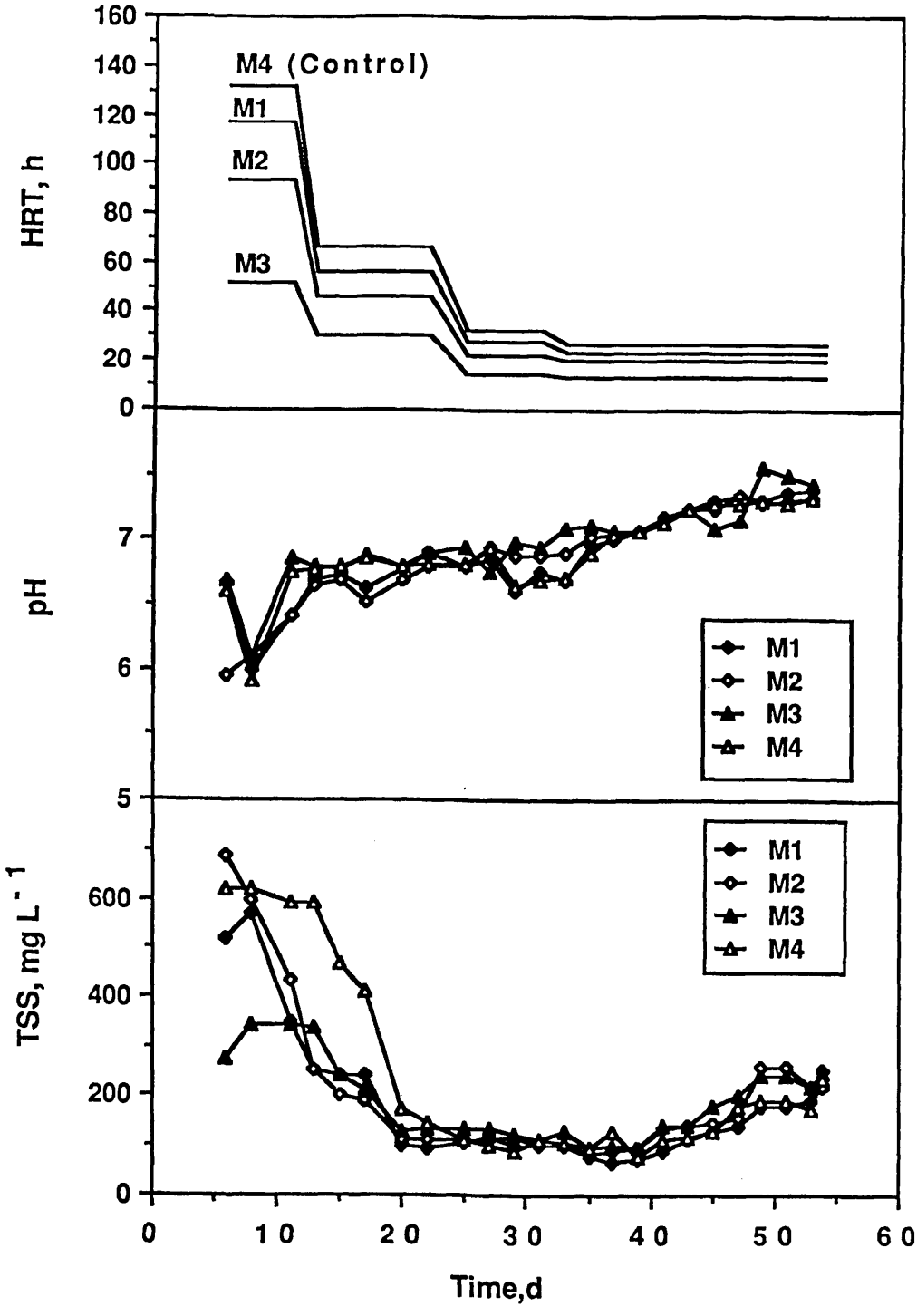


Figure 37 : Hydraulic retention time (*HRT*), pH and total suspended solids (TSS) of the methanogenic reactors of the anaerobic digestion systems during start-up (M1, M2, M3 and M4 represent the methanogenic reactors of systems 1, 2, 3 and 4, respectively).

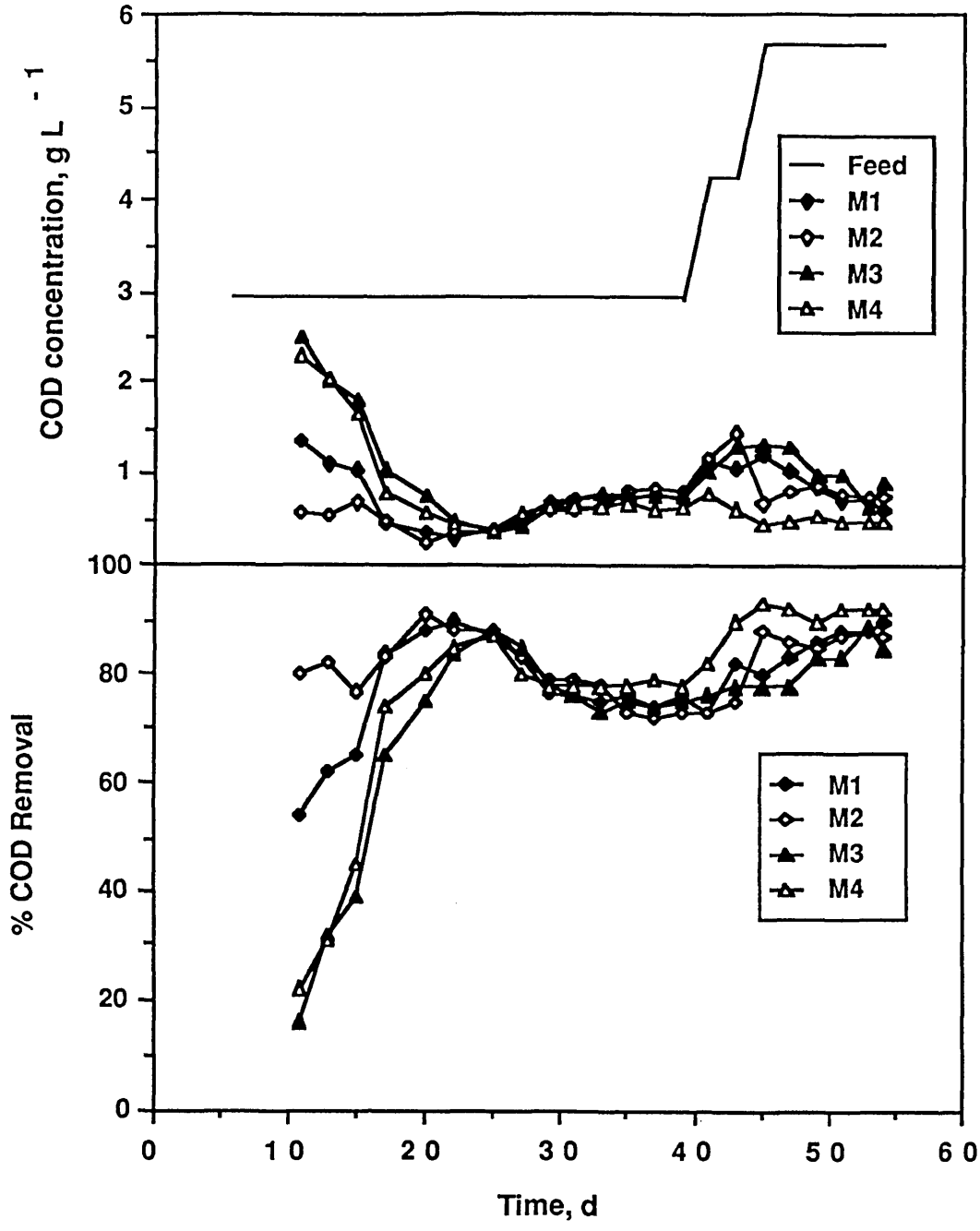


Figure 38 : Influent and filtered effluent COD concentration (S) and % filtered COD removal of the anaerobic systems during start-up (M1, M2, M3 and M4 represent the methanogenic reactors of systems 1, 2, 3 and 4, respectively).

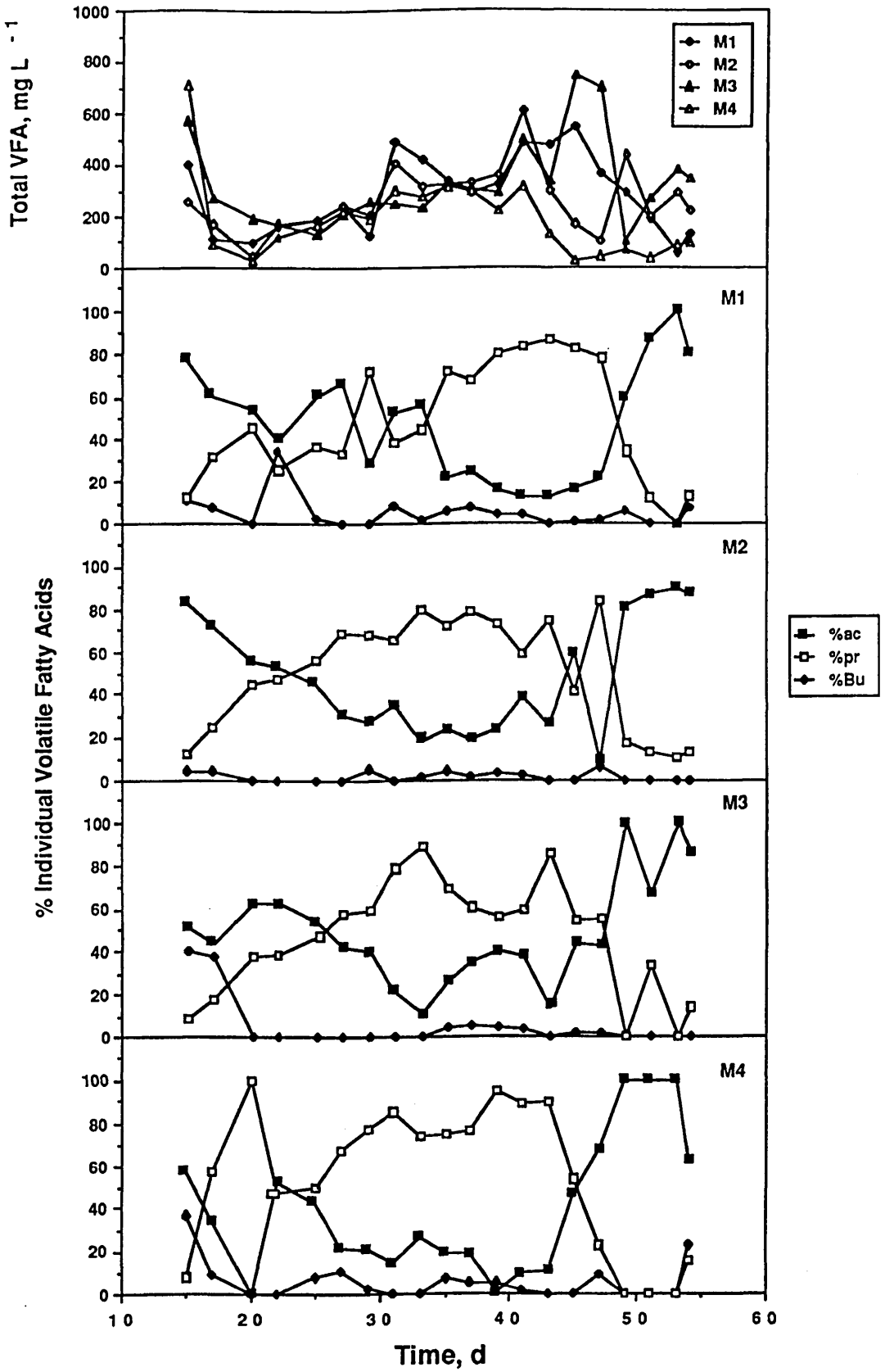


Figure 39 : Total volatile fatty acid concentration and distribution of the individual acids in the effluent of the anaerobic systems during start-up (M1, M2, M3 and M4 represent the methanogenic reactors of systems 1, 2, 3 and 4, respectively).

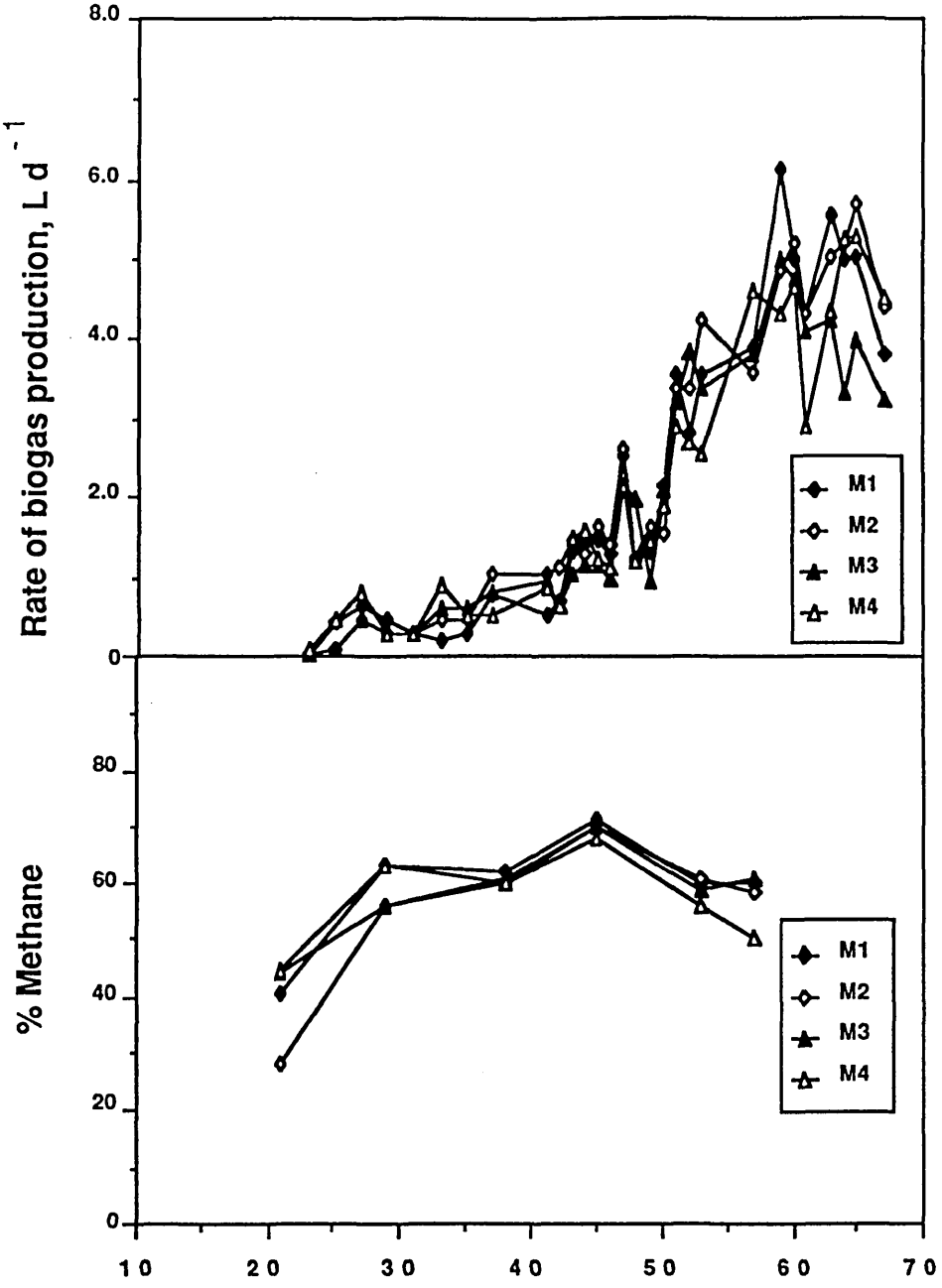


Figure 40 : Rate of biogas production and % CH_4 in the biogas produced from the anaerobic digestion systems during start-up (M1, M2, M3 and M4 represent the methanogenic reactors of systems 1, 2, 3 and 4, respectively).

increased to values between 7.1 and 7.5 for all methanogenic reactors (Fig. 37). The total suspended solids of the effluent after day 20 were in the range of 50 to 150 mgTSS L^{-1}, while after day 40 they increased from 150 to 300 mg TSS L^{-1} due to the increased influent COD concentration and organic loading rate (Fig. 37). It is of interest to note that during the first two weeks, the single-stage anaerobic digester (M4) retained a considerably higher concentration of total suspended solids compared to those of the two-phase systems (M1, M2 and M3).

After day 20, the % COD removal of all the systems was in the range of 75 to 92% (Fig. 38). However, during the beginning of the start-up period the removals of systems 1 and 2 were considerably higher than those of systems 3 and 4: at day 12, systems 1 and 2 were removing 80 and 55% of the influent COD respectively, while systems 3 and 4 were removing only 16 and 22% respectively. These % COD removals represent the overall values and not those corresponding to the methanogenic fluidised bed of each system (although the two coincide in the case of the single-stage system 4).

The total concentration of the volatile fatty acids in the effluent was always less than 800 mg L^{-1} and occasionally was less than 100 mg L^{-1} (Fig. 39). The distribution of the volatile fatty acids varied noticeably throughout the start-up period, although the main acids were acetic and propionic, while butyric acid appeared in concentrations of less than 10% of the total volatile fatty acid concentration (Fig. 39). After day 20 the concentration of propionic acid tended to increase, constituting as much as 80% of the total VFA concentration, while towards the end of the start-up period acetic acid was again the prevailing acid in all methanogenic reactors.

Finally, in Figure 40 the rate of gas production, as well as the % CH_4 in the gas evolved, are presented. No gas production could be detected in any system until day 13. After that, the rate of gas production increased steadily, as did the applied

organic loading rate. The percentage of CH_4 in the evolved gas was in the range of 28 to 45% at day 22, although it gradually increased reaching a maximal 70% in some reactors. At the end of the start-up period, the % CH_4 was considerably different between the two-phase and the single-stage system, the biogas of the former containing 58 to 62% methane, while that of the latter only 50% methane.

4.6. Steady-state Operation of the Two-Phase Anaerobic Digestion Systems

4.6.1. Experimental Design

After a period of fifty five days had elapsed since the initial start-up of the reactors, the performances of the two-phase anaerobic digestion systems (systems 1, 2 and 3), as well as that of the control single-stage digester (system 4) were determined at steady-state. The influent substrate concentration was identical for the four anaerobic digestion systems and the same was the case for the overall hydraulic retention times and organic loading rates of all systems. However, the hydraulic retention time of the acidogenic and the methanogenic reactors varied proportionately for each system with respect to the individual volume of each reactor (Table 27): the hydraulic retention times of the methanogenic reactors were 87.4, 74.8, 50 and 100% of the overall hydraulic retention times for systems 1, 2, 3 and 4, respectively. This configuration allowed the direct comparison of the four systems in order to determine the optimum ratio of the volumes of the individual (acidogenic and methanogenic) reactors in the two-phase systems.

To ensure the validity of the derived conclusions, three different experiments were conducted, the two-phase anaerobic systems being compared at three different steady-states. The values of the operational parameters applied to all systems during each experiment are presented in Table 28. The experimental design was such as to allow a further comparison between the different steady-states to be made. Experiments I and II, conducted under similar organic loading rates, could be compared to assess the effect of influent COD and/or hydraulic retention time.

Table 28 : Operational parameters applied to the two-phase anaerobic digestion systems during the steady-state experiments.

Experiment	overall θ (h)	S_o (g COD L^{-1})	OLR (kg COD $m^{-3}d^{-1}$)
I	27.0	5.9	5.28
II	13.5	3.0	5.26
III	13.5	5.9	10.56

Similarly, experiments II and III, conducted under similar hydraulic retention times, could be compared to assess the effect of organic loading rate and/or influent COD concentration. Finally, experiments I and III, using the same influent COD concentration, could be compared to give evidence on the effect of the organic loading rate and/or the hydraulic retention time.

Following the start-up period as described in section 4.5., the two-phase anaerobic digestion systems were left to equilibrate over 18 to 21 days. This period has successfully been used by other researchers to provide adequate time for fixed-film anaerobic digestion systems to reach equilibrium (Bull *et al.*, 1984a). Considering that the solids retention time (biomass retention time) of an anaerobic fluidised bed reactor lies in the range of 4 to 8 days (Stephenson and Lester, 1986; Stronach *et al.*, 1987), the equilibration period adopted was long enough to allow a new bacterial distribution to be established. After steady-state was reached, the effluent characteristics were measured daily or twice a day over a period of 6 days. In this way the steady-state performance of the reactor was ensured, while obtaining enough replicates to allow statistical comparison of the four systems.

4.6.2. Steady-state Performance of the Two-Phase Anaerobic Digestion Systems

After the equilibration period, as described in the previous section, had elapsed, the effluent characteristics of all reactors were measured at steady-state. The mean values of the measured parameters are presented in Tables 29, 30 and 31

Table 29 : Steady-state performance of the two-phase anaerobic digestion systems during experiment I

	System 1	System 2	System 3	System 4 (Control)
Influent COD conc. ($g L^{-1}$)	5.94	5.94	5.94	5.94
Overall *OLR* ($Kg m^{-3} d^{-1}$)	5.28	5.28	5.28	5.28
Temperature (°C)	37	37	37	37
Configuration	two-phase	two-phase	two-phase	single-stage
Acid Reactor				
Hydraulic retent. time (h)	3.4	6.8	13.5	—
Effluent : pH	6.15 (0.03)	6.13 (0.03)	6.27 (0.03)	—
TSS ($mg L^{-1}$)	246 (24)	306 (8)	345 (16)	—
filtered COD ($g L^{-1}$)	4.765 (0.122)	4.692 (0.109)	4.480 (0.178)	—
unfiltered COD ($g L^{-1}$)	5.115 (0.142)	5.126 (0.117)	4.970 (0.185)	—
total VFA ($mg L^{-1}$)	1751 (123)	1996 (130)	2070 (104)	—
Acetic acid ($mg L^{-1}$)	960 (68)	1085 (52)	1135 (56)	—
Propionic ac. ($mg L^{-1}$)	301 (16)	319 (13)	335 (19)	—
i-Butyric ac. ($mg L^{-1}$)	58 (11)	53 (7)	56 (5)	—
Butyric acid ($mg L^{-1}$)	153 (54)	215 (48)	180 (35)	—
i-Valeric ac. ($mg L^{-1}$)	90 (21)	81 (12)	90 (7)	—
Valeric acid ($mg L^{-1}$)	190 (45)	243 (36)	274 (21)	—
Methane Reactor				
Hydraulic retent. time (h)	23.6	20.2	13.5	27.0
Effluent : pH	7.37 (0.02)	7.34 (0.02)	7.44 (0.04)	7.30 (0.03)
TSS ($mg L^{-1}$)	214 (14)	230 (15)	221 (19)	199 (20)
filtered COD ($g L^{-1}$)	0.409 (0.102)	0.529 (0.056)	0.580 (0.075)	0.488 (0.041)
unfiltered COD ($g L^{-1}$)	0.712 (0.118)	0.856 (0.043)	0.894 (0.089)	0.770 (0.031)
total VFA ($mg L^{-1}$)	95 (22)	193 (38)	201 (69)	31 (9)
Acetic acid ($mg L^{-1}$)	67 (10)	167 (29)	161 (56)	26 (9)
Propionic ac. ($mg L^{-1}$)	9 (4)	22 (6)	35 (11)	—
Butyric acid ($mg L^{-1}$)	9 (4)	4 (3)	6 (4)	6 (3)
Biogas Production				
Production rate ($L d^{-1}$)	5.05	5.67	3.98	5.30
Biogas % CH_4	60	58	61	50
m^3 CH_4 Kg^{-1} COD utilised	0.347	0.364	0.373	0.385

Table 30 : Steady-state performance of the two-phase anaerobic digestion systems during experiment II

	System 1	System 2	System 3	System 4 (Control)
Influent COD conc. ($g L^{-1}$)	2.96	2.96	2.96	2.96
Overall *OLR* ($Kg m^{-3} d^{-1}$)	5.26	5.26	5.26	5.26
Temperature (°C)	37	37	37	37
Configuration	two-phase	two-phase	two-phase	single-stage
Acid Reactor				
Hydraulic retent. time (h)	1.7	3.4	6.8	—
Effluent : pH	6.11 (0.03)	6.07 (0.03)	6.13 (0.03)	—
TSS ($mg L^{-1}$)	108 (11)	123 (12)	162 (21)	—
filtered COD ($g L^{-1}$)	2.480 (0.103)	2.467 (0.112)	2.335 (0.161)	—
unfiltered COD ($g L^{-1}$)	2.634 (0.095)	2.642 (0.112)	2.564 (0.145)	—
total VFA ($mg L^{-1}$)	884 (62)	955 (137)	889 (152)	—
Acetic acid ($mg L^{-1}$)	545 (47)	538 (51)	486 (81)	—
Propionic ac. ($mg L^{-1}$)	132 (8)	181 (27)	175 (26)	—
i-Butyric ac. ($mg L^{-1}$)	24 (2)	30 (9)	20 (3)	—
Butyric acid ($mg L^{-1}$)	39 (4)	73 (17)	52 (15)	—
i-Valeric ac. ($mg L^{-1}$)	41 (2)	50 (12)	38 (7)	—
Valeric acid ($mg L^{-1}$)	102 (6)	83 (41)	118 (27)	—
Methane Reactor				
Hydraulic retent. time (h)	11.8	10.1	6.8	13.5
Effluent : pH	7.21 (0.02)	7.18 (0.02)	7.26 (0.04)	7.23 (0.03)
TSS ($mg L^{-1}$)	150 (17)	142 (7)	158 (13)	138 (12)
filtered COD ($g L^{-1}$)	0.167 (0.017)	0.175 (0.027)	0.216 (0.041)	0.196 (0.030)
unfiltered COD ($g L^{-1}$)	0.381 (0.037)	0.377 (0.026)	0.441 (0.040)	0.392 (0.038)
total VFA ($mg L^{-1}$)	48 (24)	35 (10)	51 (11)	19 (8)
Acetic acid ($mg L^{-1}$)	32 (15)	26 (8)	32 (10)	15 (5)
Propionic ac. ($mg L^{-1}$)	4 (5)	3 (4)	7 (5)	—
Butyric acid ($mg L^{-1}$)	5 (3)	4 (3)	3 (2)	4 (2)
Biogas Production				
Production rate ($L d^{-1}$)	5.24	5.04	4.39	3.66
Biogas % CH_4	65	62	68	56
m^3 CH_4 Kg^{-1} COD utilised	0.395	0.340	0.447	0.299

Table 31 : Steady-state performance of the two-phase anaerobic digestion systems during experiment III

	System 1	System 2	System 3	System 4 (Control)
Influent COD conc. ($g L^{-1}$)	5.94	5.94	5.94	5.94
Overall *OLR* ($Kg\ m^{-3}\ d^{-1}$)	10.56	10.56	10.56	10.56
Temperature (°C)	37	37	37	37
Configuration	two-phase	two-phase	two-phase	single-stage
Acid Reactor				
Hydraulic retent. time (h)	1.7	3.4	6.8	—
Effluent : pH	6.13 (0.03)	6.09 (0.03)	6.14 (0.03)	—
TSS ($mg L^{-1}$)	181 (23)	232 (47)	276 (47)	—
filtered COD ($g L^{-1}$)	4.986 (0.289)	4.841 (0.217)	4.637 (0.161)	—
unfiltered COD ($g L^{-1}$)	5.244 (0.300)	5.170 (0.206)	5.029 (0.186)	—
total VFA ($mg L^{-1}$)	1572 (8)	1607 (110)	1663 (125)	—
Acetic acid ($mg L^{-1}$)	880 (56)	903 (83)	944 (63)	—
Propionic ac. ($mg L^{-1}$)	253 (29)	277 (11)	257 (15)	—
i-Butyric ac. ($mg L^{-1}$)	54 (14)	49 (7)	49 (9)	—
Butyric acid ($mg L^{-1}$)	135 (30)	188 (40)	198 (37)	—
i-Valeric ac. ($mg L^{-1}$)	97 (31)	85 (9)	85 (10)	—
Valeric acid ($mg L^{-1}$)	153 (40)	106 (46)	130 (76)	—
Methane Reactor				
Hydraulic retent. time (h)	11.8	10.1	6.8	13.5
Effluent : pH	7.30 (0.02)	7.26 (0.02)	7.29 (0.04)	7.27 (0.03)
TSS ($mg L^{-1}$)	152 (14)	157 (11)	213 (20)	160 (11)
filtered COD ($g L^{-1}$)	0.415 (0.061)	0.446 (0.038)	0.550 (0.034)	0.437 (0.054)
unfiltered COD ($g L^{-1}$)	0.631 (0.062)	0.669 (0.035)	0.852 (0.035)	0.665 (0.058)
total VFA ($mg L^{-1}$)	59 (45)	120 (44)	127 (24)	34 (23)
Acetic acid ($mg L^{-1}$)	45 (34)	80 (28)	94 (18)	27 (18)
Propionic ac. ($mg L^{-1}$)	5 (8)	36 (16)	31 (8)	3 (5)
Butyric acid ($mg L^{-1}$)	4 (2)	—	3 (2)	4 (3)
Biogas Production				
Production rate ($L d^{-1}$)	10.38	10.90	8.30	9.42
Biogas % CH_4	68	64	67	55
$m^3\ CH_4\ Kg^{-1}$ COD utilised	0.398	0.372	0.424	0.368

for experiments I, II and III, respectively, while their corresponding standard deviations are shown in the same tables in brackets.

During all experiments the performances of the four systems were entirely satisfactory (Tables 29, 30 and 31): more than 90% of the initial COD concentration was removed from the filtered effluents, the pH of the effluents were always in the range of 7.18 to 7.44 and no accumulation of volatile fatty acids was observed. The total volatile fatty acid concentrations ranged between 19 and 200 $mg\,L^{-1}$, the main acid produced being acetic. The total suspended solids of the effluents were moderate, having values between 138 and 230 $mg\,L^{-1}$. Finally, methane production was close to the theoretical value at 25 °C (0.38 $m^3\,CH_4\,kg^{-1}$ COD utilised), varying between 0.30 and 0.42 $m^3\,CH_4\,kg^{-1}$ COD utilised: this variation was mainly attributed to the limited accuracy of the gas measurement in the gas collection system used in this study.

During experiment I (Table 29), the acidification reactors of systems 1, 2 and 3 have generally performed as expected, according to the previous experiments on the first phase of anaerobic digestion (section 4.2.): the main volatile fatty acid was acetic acid, whilst significant concentrations of propionic acid and lower concentrations of butyric acid were also observed. As a consequence of the equal influent COD concentration, the longer the hydraulic retention time was, the higher were the total volatile fatty acid and total suspended solid concentrations. The degrees of acidification were 40.9, 46.9, and 48.6%, while the %COD removals calculated on the unfiltered effluents as they entered each methanogenic reactor were 13.9, 13.7 and 16.3% for systems 1, 2 and 3, respectively.

It should be noted here that the performances of the acidogenic reactors of the two-phase systems were not directly comparable to the performance of the acidogenic reactor described in section 4.2.. This was partly attributed to the fact that during the two-phase experiments the reactor contents were gently stirred by

means of a magnetic stirrer, while during the acidogenic phase experiments the reactor was supplied with a mechanical stirrer operated at 400 rpm for vigorous mixing without any visible wall growth. Additionally, the inoculum used in the two-phase experiments was not the one used for the acidogenic phase experiments, the latter being acclimated to the particular wastewater by operating under constant conditions for a period of two months (section 3.6.). Finally, the wastewater used in the two-phase study was slightly different; it contained a trace element solution, while during the first thirty days of the start-up period it also contained considerable amounts of methanol.

The quality of the effluents of the methanogenic reactors of the four systems were very similar. Due to the small differences observed between the four systems, as well as the relatively large standard deviations of the measurements, statistical analysis was required to assess whether the observed differences were significant. The four systems were initially compared using the F-test of the one-way analysis of variance. Once it was established that the effluent characteristics of the four systems were statistically different ($p = 0.01$), a t-test analysis was conducted between the effluent characteristics of the control reactor and those of each of the two-phase systems: this enabled the determination of the systems that were statistically better or worse than the control. The method followed was similar to the least significant differences method, varying only in that the comparison was always made against the control reactor. The standard deviation, s, of the mean values was calculated as the square root of the residual mean squares and the degrees of freedom were those of the residuals as given by the analysis of variance table of the whole set of data. The standard deviation of the difference of the mean values was then calculated from equation (51):

$$\mathrm{STD} = s\sqrt{\frac{2}{n}} \tag{51}$$

where n is the number of observations of each of the mean values.

During experiment I (Table 29), the acidification reactors removed 13.9, 13.7, and 16.3 % of the unfiltered COD, while the degrees of acidification were 40.9, 46.9, and 48.6 % for systems 1, 2, and 3, respectively. The main volatile fatty acid was acetic acid, although considerable amounts of propionic acid were produced together with small amounts of butyric acid. The filtered COD concentration of effluent of the methanogenic reactor of system 1 was lower than those of the other systems, indicating the superiority of system 1. The difference was shown to be statistically significant at $p = 0.05$. The filtered COD concentration of system 2 was not statistically higher than that of the control system, although that was the case for the COD concentration of system 3. The unfiltered COD concentrations of systems 1 and 2 were not significantly different to that of the control system ($p = 0.05$), while system 3 produced effluent with unfiltered COD values statistically higher than those of the control system. The % COD removals of all systems followed similar patterns to those obtained for the effluent COD concentrations; the exact values are presented in Table 32. The total suspended solids concentration of the

Table 32 : The % COD removal of the filtered and the unfiltered effluent of the two-phase anaerobic systems during steady-state operation

	% filtered COD removal			% unfiltered COD removal		
	Exp. I	Exp. II	Exp. III	Exp. I	Exp. II	Exp. III
System 1	93.1 (1.7)	94.3$^{\alpha}$ (0.6)	93.0$^{\alpha}$ (1.0)	88.0$^{\alpha}$ (2.0)	87.1$^{\alpha}$ (1.3)	89.4$^{\alpha}$ (1.0)
System 2	91.1$^{\alpha}$ (0.9)	94.1$^{\alpha}$ (0.9)	92.5$^{\alpha}$ (0.6)	85.6$^{\alpha}$ (0.7)	87.3$^{\alpha}$ (0.9)	88.7$^{\alpha}$ (0.6)
System 3	90.2 (1.3)	92.7$^{\alpha}$ (1.4)	90.7 (0.6)	85.0 (1.5)	85.1 (1.4)	85.7 (0.6)
System 4 (control)	91.8 (0.7)	93.4 (1.0)	92.6 (0.9)	87.0 (0.5)	86.8 (1.3)	88.8 (1.0)

Note: $^{\alpha}$ indicates which measurements are not significantly different from the control at $p = 0.05$

control system (system 4) was the lowest observed, its difference from system 1 not being significant, while those from systems 2 and 3 were significant at $p = 0.05$. Finally, the control system produced effluent with significantly ($p = 0.05$) lower total volatile fatty acid concentrations, although the volatile fatty acid concentrations of all the reactors were consistently low. The methane production of all systems was very close to the theoretical value at 25°C of 0.38 $m^3 CH_4 kg^{-1}$COD utilised, although the $\% CH_4$ differed significantly, taking values between 58 and 61% for the two-phase systems and 50% for the single-stage system (Table 29).

During experiment II (Table 30), the acidification reactors removed 11.0, 11.8 and 13.4% of the unfiltered COD, while the degrees of acidification were 40.1, 44.1 and 41.6% for systems 1, 2 and 3, respectively. The total volatile fatty acid concentration of the acidification reactor of system 3 was lower than those of systems 1 and 2, although it would be expected to be higher on the grounds of the longer hydraulic retention time. The F-test of the analysis of variance showed that the effluent characteristics of the four systems were statistically different at $p = 0.05$. The filtered COD concentration of system 1 was the lowest observed during experiment II, while the COD concentrations of systems 2 and 3 were respectively slightly lower and higher than that of the control system. However, none of the observed values were significantly different from the control system (system 4) at $p = 0.05$. Similarly, the unfiltered COD concentration of system 1 was the lowest observed, its difference from the control not being statistically significant ($p = 0.05$). The unfiltered COD of system 2 was also not significantly lower than the control, while that of system 3 was significantly higher than the control. The % COD removals of the filtered and the unfiltered effluent of the four systems are presented in Table 32. The control system displayed the lowest total suspended solids concentration, although the observed differences were not statistically significant except for system 3. Finally, the total volatile fatty acid concentration of

the control system was significantly lower than those of systems 1 and 3, while the effluent volatile acids of system 2 were not statistically different from those of the control. The methane production was not always close to the theoretical value of 0.38 m^3 CH_4 kg^{-1} COD utilised, which could possibly be attributed to erratic gas production measurements. The biogas produced by the two-phase systems contained a higher percentage of methane compared to that of the single-stage system (65, 62, 66 and 56 % CH_4 for systems 1, 2, 3, and 4, respectively).

After the completion of experiment II, the influent COD concentration and consequently the organic loading rate were gradually doubled for experiment III. The increase of the organic loading was well tolerated by all reactors without any volatile fatty acid accumulation and/or drop of the pH. However, the operation of the single-stage reactor was disturbed due to considerable sand losses ($\sim$10 %). The sand was collected and was reintroduced into the fluidised bed reactor, but without success since it was soon washed out of the reactor. To counteract the sand losses of the control reactor, the new volume was recorded and the influent flowrate was adjusted so that the hydraulic retention time of system 4 was again 13.5 h and equal to the overall hydraulic retention time of systems 1, 2 and 3.

During experiment III (Table 31), the acidification reactors removed 11.7, 13.0 and 15.3 % of the unfiltered COD concentration, while the degrees of acidification were 36.6, 37.0, and 38.4 % for systems 1, 2 and 3, respectively. The F-test, in conjuction with the analysis of variance, indicated that the effluent characteristics of the fluidised beds of the four systems were statistically different at $p = 0.01$. System 1 displayed the lowest filtered COD concentration, as well as the lowest total suspended solid concentration. However, both of these parameters, as well as the total volatile fatty acid concentration, did not differ significantly from the control system. System 2 was also statistically the same as the control, although the volatile fatty acid concentration of its effluent was higher than that of the

control. Finally, the performance of system 3 was the least satisfactory, its differences from the control being statistically significant. The %COD removals of the four systems are presented in Table 32. The methane production of the four systems was close to the theoretical, while the percentage of methane in the biogas varied significantly between the two-phase systems and the single-stage system (68, 64, 67 and 55% for systems 1, 2, 3, and 4, respectively).

The above analysis of data has shown that the reductions in the volumes of the fluidised bed reactors by 12.6 and 25.2% (system 1 and 2, respectively) and the replacement of these volumes by completely mixed acidogenic reactors, resulted in comparable or improved performances of the anaerobic systems: during all experiments, systems 1 and 2 displayed similar or improved % filtered and unfiltered COD removals (Table 32). Furthermore, the two-phase systems demonstrated a consistently higher percentage of methane in the biogas produced. However, the total effluent volatile fatty acids concentrations of the two-phase systems were consistently higher than the acids concentrations of the single-stage system, constituting approximately 17 to 41% of the effluent COD as compared to 8 to 16% of the COD of the single-stage system.

By comparing the three experiments, some interesting observations can be made. During experiment II, conducted under the same organic loading rate as experiment I but with a shorter hydraulic retention time, improved % filtered COD removals were observed for all reactors, while the % unfiltered COD removals were not statistically different. This is an indication that the hydraulic retention time was sufficiently long for the anaerobic system to operate satisfactorily. During experiment III, conducted under the same hydraulic retention time as experiment II but with twice the influent COD concentration and consequently twice the organic loading rate, the % filtered and unfiltered COD removals were not statistically inferior to those observed during experiment II. This implies that the anaerobic

digestion systems can tolerate the increased COD concentrations well and can operate satisfactorily at organic loading rates as high as 10.56 kg COD $m^{-3} d^{-1}$. Similarly, the % removals observed during experiments I and III were not statistically different, although the hydraulic retention time during experiment III was reduced by 50%, the influent COD concentration remaining the same.

The biomass hold-up of the methanogenic fluidised bed reactors was measured at the end of each steady-state. The sand samples of all reactors were taken from outlets placed at 35 cm distance from the bottom of the columns. Due to the fact that the volume and consequently the height of the fluidised beds were not the same, the outlet was located in approximately the middle of the beds of systems 1, 2 and 4, while it was very close to the top of the bed of system 3. This resulted in erratic readings for system 3, due to accumulation of non-attached or sloughed biomass at the top of the fluidised bed column. Two measurements were made for each reactor at each steady-state and the mean values are presented in Table 33. The accuracy of the measurements was approximately 8%. The biomass hold-up presented in Table 33 is expressed in grams of volatile solids per litre of expanded bed, assuming a 30% bed expansion. This assumption is not totally valid, since the porosity of the settled bed changes due to biomass accumulation. However,

Table 33 : Biomass hold-up of the anaerobic fluidised bed reactors during steady-state operation

	Biomass hold-up (g VS L^{-1} expand. bed)		
	Exp. I	Exp. II	Exp. III
System 1	2.00	1.91	2.61
System 2	2.09	1.93	2.57
System 3	1.71	(10.32)	(10.71)
System 4 (Control)	4.78	6.23	6.91

the error introduced is estimated to be less than 5% and it can therefore be neglected. No correction was made for the suspended biomass concentration due to the low TSS concentration (< 230 mg L^{-1}), as well as the small volume of liquid introduced into the crucibles together with the sand samples.

An attempt was made to calculate the solids retention time of the biomass in the fluidised bed reactors according to equation (28). However, this equation is not applicable when the influent total suspended solids concentration is different to zero. Furthermore, the fact that the influent to the fluidised bed reactors contained a higher concentration of total suspended solids than the corresponding effluent (at least during experiments I and III) suggested that part of the influent biomass concentration was either attached or underwent lysis. These phenomena could not be quantified due to the several assumptions incorporated. Therefore, the solids retention time SRT of only the single-stage anaerobic digester was calculated and it was found to be 27.0, 25.4 and 24.3 d for experiments I, II and III, respectively.

It is of interest to note that the attached biomass of the control system was significantly higher than that of the two-phase systems (Table 33). This was attributed to the substantial growth of acidogenic, as well as methanogenic bacteria on the fluidised bed carrier particles, as the former are known to have higher biomass yields than the latter. The increased biomass hold-up of system 4 was probably responsible for the operational problems observed between experiments II and III: due to the increased biomass hold-up the bioparticles become lighter with lower settling velocities [eq. (4) and (5)] and they were therefore easily carried out of the reactor.

In order to elucidate the phenomena associated with fluidisation and biofilm thickness, a computer program was developed which calculates the biofilm thickness around the fluidised bed particles, when the biomass hold-up, the initial volume of the sand and the upflow velocity of the liquid are given. Details of the calculations,

as well as the basic assumptions, are given in section 3.10. (Fig. 14). The biofilm thicknesses as calculated by the proposed model for all fluidised bed reactors at steady-state, are presented in Table 34. The biomass dry density ρ was assumed equal to 0.04 $g\,cm^{-3}$ according to the existing literature. Furthermore, for this value of ρ, the biomass hold-up of the expanded bed X_{exp} (g VS per L of expanded volume), as well as the volume of the expanded bed, were predicted within 1 and 5 % of the observed values, respectively. A different value of the biomass dry density ρ resulted in proportionally different biofilm thickness. However, the patterns presented in Table 34 did not change: the two-phase systems demonstrated a consistently thinner biofilm in comparison to the single-stage system. More importantly, the biofilms of all anaerobic fluidised beds during all studied steady-states are characterised as thin and fully penetratable: according to Atkinson and Fowler (1974) a biofilm thickness of up to 100 to 200 μm is active, which means that it is fully penetrated by substrate and diffusional limitations are negligible.

Table 34 : Biofilm thickness δ of the fluidised bed particles, as calculated by the fluidisation model during steady-state operation

	Biofilm thickness δ (μm)		
	Exp. I	Exp. II	Exp. III
System 1	14.0	14.5	18.2
System 2	14.4	14.7	16.3
System 3	11.9	—	—
System 4 (Control)	38.9	57.4	61.9

This final observation is very significant, because it simplifies the kinetic study of methanogenesis in the fluidised bed reactor: most of the kinetic models proposed in the literature for the description of attached biomass systems incorporate mass transfer limitations due to the resistance of the boundary layer

surrounding the biofilm and/or resistance within the biofilm. In the present study, the biofilm thickness is very small and therefore the diffusional limitations within the biofilm are negligible. Furthermore, there is considerable evidence that the external mass transfer (liquid boundary layer) resistance in a fluidised bed reactor is also neglible, due to the high superficial velocities (Shieh, 1980; Andrews, 1982; Toldrá *et al.*, 1986). Under these conditions the order of the overall reaction is equal to the biofilm intrinsic reaction. Taking into account that the fluidised beds behave as completely mixed reactors when the recycle ratio is greater than 5, as is the case in the present study, a simple equation can be used to describe the overall specific rate of substrate removal R_s ($g\,COD\,g^{-1}VS\,d^{-1}$):

$$R_s = \frac{(S_o - S_e)}{X_{exp}\,\theta} = R_{max}\,\frac{S_e}{K_r + S_e} \qquad (7)$$

where R_{max} is the maximum specific rate of substrate removal ($g\,COD\,g^{-1}VS\,d^{-1}$) and K_r is the substrate saturation constant ($g\,COD\,L^{-1}$). When $S_e << K_r$ the reaction becomes first order, while for $S_e >> K_r$ the reaction becomes zero order.

By plotting the specific rate of substrate removal R_s versus the effluent substrate concentration S_e an important observation can be made: the single-stage anaerobic digester (system 4) demonstrated an invariably lower specific rate of substrate removal than the two-phase anaerobic systems (Fig. 41). More specifically, R_s was found to be in the range of 0.75 to 1.32 $g\,COD\,g^{-1}VS\,d^{-1}$ for the single stage reactor, while it was in the range of 2.15 to 4.10 $g\,COD\,g^{-1}VS\,d^{-1}$ for the two-phase systems. Moreover, the specific rate of substrate removal is almost constant for different effluent concentrations S_e. Although the experimental points are few and therefore no definite conclusions can be derived, this observation suggests that the reaction order is zero, at least for the range of effluent concentrations studied.

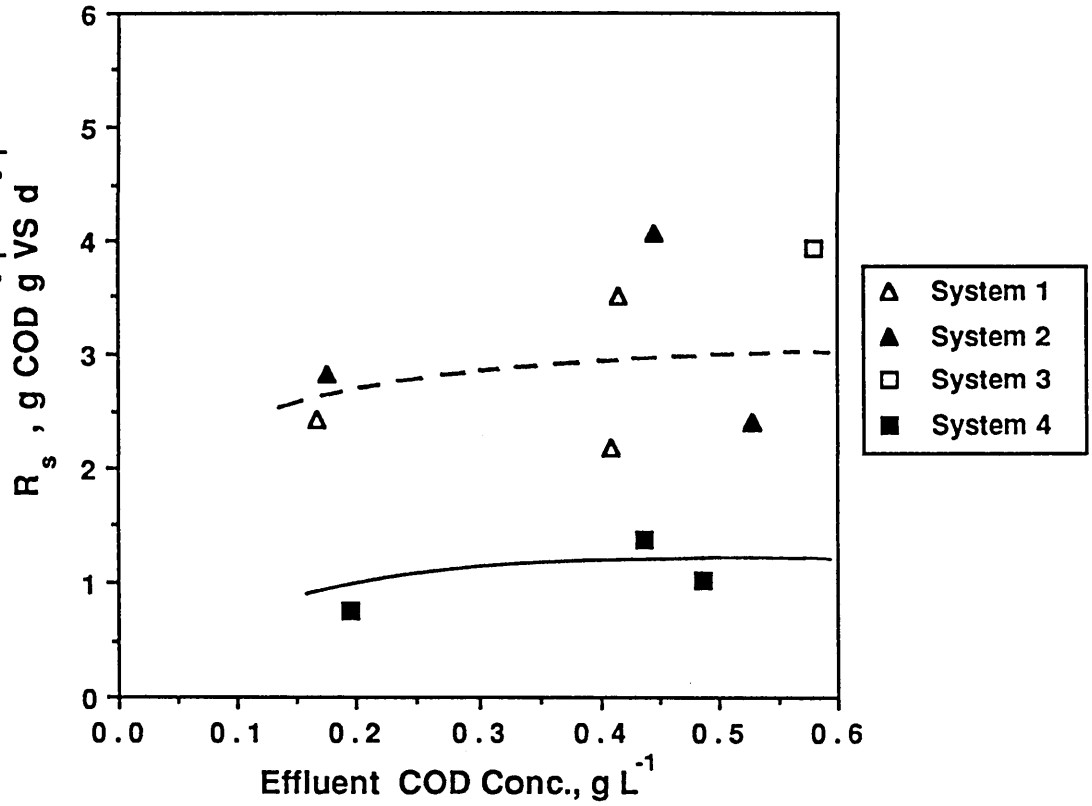


Figure 41 : Specific rate of substrate removal R_s versus the effluent substrate concentration S_e

CHAPTER 5

DISCUSSION

The Monod equation has been successfully applied to the description of the kinetics of acidogenic bacteria in batch culture in this study. However, it was demonstrated that it is not suitable for the description of continuous culture kinetics of acidogenic bacteria due to pronounced product inhibition (section 4.3.2.). A possible explanation for the observed discrepancies between the batch and the continuous culture kinetics could lie in the nature of the batch culture experiments, which made product inhibition rather unlikely. At the beginning of the exponential growth period, the product concentration was zero and it gradually increased. However, due to the low biomass concentration (which allowed the direct measurement of the culture turbidity, the biomass concentration throughout the experiment not exceeding 80 $mg L^{-1}$of total suspended solids), only a small percentage of the initial substrate concentration was used up and consequently, the product concentration was low. As time progressed, the observed decline in the biomass growth rate did not necessarily coincide with substrate depletion, but it could be attributed to increasing product inhibition. The biomass growth rate at this later stage would possibly be more representative of the biomass growth rate in a continuous culture in presence of product inhibition.

Other researchers have also observed differences between the values of the biokinetic constants as calculated for batch and continuous cultures; Ghosh and Klass (1978) reported that the kinetics of continuous fermentation were generally faster than those of batch fermentations. They attributed this partially to the inferior mixing characteristics of the batch culture. Atkinson (1983) stated that the continuous culture kinetics represent steady conditions whereas the batch culture kinetics reflect a range of conditions. Finally, Gomez and Goma (1986) reported the

pronounced effect of the inoculum size in the kinetics of acidogenic bacteria in batch culture.

The values of the biokinetic constants μ_{max} and K_s at 37 °C as calculated from the batch culture experiments were 7.30 d^{-1} and 2.74 gCOD L^{-1}, respectively. The value of the saturation constant K_s is relatively close to the substrate concentration identified as critical for the degree of acidification in continuous culture (section 4.2.2.), as well as the K_s value as calculated from the inhibition models (section 4.3.2).

Given the maximum specific growth rate μ_{max}, the minimum doubling time, t_d, of the acidogenic bacteria was calculated according to equation (41) and found to be 2.28 h. This value compares well with the minimum doubling times of acidogenic bacteria growing in batch cultures, which were calculated to be 2.3, 4.3, and 9.8 h for glucose, sewage sludge, and cellulose, respectively (Ghosh and Klass, 1978). However, there is ample experimental evidence that acidogenic bacteria can grow much faster in continuous cultures; the minimum doubling time of acidogenic bacteria growing on glucose was calculated to be 0.55 h (Ghosh and Klass, 1978), while other researchers have reported values as low as 1.15 h (Blakebrough, 1967) and 1.35 h (Zoetemeyer *et al.*, 1982c) under the influence of substrate and/or product inhibition. Furthermore, according to the results of the present study, the acidogenic bacteria can survive at hydraulic retention times as low as 2 h, which corresponds to a doubling time of 1.39 h. It would appear that the biokinetic constants of the acidogenic bacteria as calculated in batch culture during the present study differ significantly from the continuous culture constants and therefore they should be used for continuous culture applications with caution.

According to the present understanding of the processes taking place in anaerobic digestion, the production of acetic and butyric acids as the main intermediates produced in the acidogenic phase would be beneficial to the overall

system performance, since they can easily be assimilated by the bacterial population present in the digester: the rate of butyric acid degradation to acetic acid has been found to be higher than the rest of the volatile fatty acids (Kaspar and Wuhrmann, 1978; Cohen *et al.*, 1982; Mudrack and Kunst, 1982; Dohányos *et al.*, 1985), while propionate degradation has been reported to be the rate limiting step of the overall process, being even slower than the conversion of acetate to methane (Lin *et al.*, 1986). During the first phase of anaerobic digestion (acidogenesis) the production of certain volatile fatty acids has been reported to be encouraged by appropriate manipulation of the operational variables (Joergensen, 1978; Verstraete *et al.*, 1981; Mudrack and Kunst, 1982; Cohen *et al.*, 1984). The variables hydraulic retention time θ, organic loading rate OLR, influent substrate concentration S_o, pH and temperature were examined during the present study.

The predominant fermentation intermediates produced from the complex meat-extract waste during the acidogenic continuous culture experiments of the present study were always acetic and propionic acids, independent of the values of the operational parameters. The concentration of butyric acid was generally less than 10 % of the total volatile fatty acid concentration, although the values of the operational parameters covered the whole range of those found in practical applications. These results suggest that the nature of the intermediate products at steady-state are largely determined by the type of the substrate used. This is also supported by the experimental results of other researchers: the products of the first phase of anaerobic digestion were mainly acetic and butyric acids when easily degradable carbohydrates (e.g. glucose, sucrose) were used as substrate (Cohen *et al.*, 1979; De la Torre and Goma, 1981). However, when more complex wastes were studied, increased amounts of propionic acid were produced: during the acidification of a yeast waste, propionic acid was the main product (Dohányos *et al.*, 1985), while the fermentation products of gelatin were acetic, propionic and valeric acids

(Breure and Van Andel, 1984).

The variation in the type of volatile fatty acids produced during the acidogenic phase of anaerobic digestion is not only affected by the substrate used, but has also been associated with the initial inoculum and the bacterial population present in the reactor: during the acidogenic fermentation of glucose and in the presence of a spore-forming population, the main products were acetic and butyric acids (Van Andel *et al.*, 1986). However, a different fermentation pattern with acetic and propionic acids as the main fermentation products was observed when a pasteurised inoculum was used or when the spore-forming bacteria were washed out of the reactor, after sporulation was induced by irregular or low rate feed supply (Van Andel *et al.*, 1986). A potential change in the fermentation pattern of the acidogenic reactor of a two-phase system after the application of some environmental stress could be detrimental to the overall process, since methanogenic bacteria are known to adapt slowly to changing substrate type and concentration. Therefore, the manipulation of the fermentation towards more propionic rather than butyric acid seems to be advisable (Van Andel *et al.*, 1986).

Although the above results indicate the possibility of engineering the anaerobic fermentation of glucose alternatively towards butyric or propionic acids, they do not necessarily suggest that the same result could be achieved with a more complex waste. To test the effect of the initial inoculum on the product formation during the acidogenic fermentation of the complex waste of the present study, a series of batch experiments was conducted (section 4.4.). The results showed that the product distribution does not change with the inoculum. These results suggest that the type of substrate may dictate the fermentation pattern and consequently a population selection could occur. This observation is in agreement with the results recorded by one group of researchers who, working under similar conditions, have reported that the biomass obtained during high rate glucose acidification was of a

granular nature, while that obtained during gelatin acidification formed a slime easily attachable to all types of surfaces (Breure and Van Andel, 1984).

After establishing that acetic and propionic acids are the main volatile fatty acids produced during the acidogenic fermentation of the present complex waste, it is of interest to make some comments concerning their degradation. It has already been stated that propionate degradation is slower than the acetate conversion to methane (Lin *et al.*, 1986). Furthermore, propionate degradation is thermodynamically favourable only when the partial pressure of hydrogen is sufficiently low (Harper and Pohland, 1986) and it therefore is suppressed during periods of high activity of the butyrate-degrading bacteria (Wiegant *et al.*, 1986). Nevertheless, the presence of concentrations of propionate higher than those routinely observed in the influent of methanogenic reactors has been suggested to be advantageous to reactor performance (Henzon *et al.*, 1986), and Gourdon and Vermande (1987) have reported that propionate concentrations as high as 4 to 6 $g L^{-1}$ do not affect methanogenesis or propionate degradation. The latter is believed to be the case, because the bacterial system is forced to organise itself around the compulsory syntrophy between the obligate hydrogen producing and the methanogenic bacteria, and consequently appropriate amounts of obligate hydrogen producing (OHPA) bacteria are maintained at all times (Asinari Di San Marzano *et al.*, 1981).

The degree of acidification achieved during the current study varied between 15 and 60% of the initial COD concentration. For initial COD concentrations and hydraulic retention times above the critical values identified as 3 $g L^{-1}$ and 6 h respectively, the acidification achieved was between 30 and 60%. This compares well with the conversion achieved by Breure and Van Andel (1984) with gelatin, which was as low as 40%. However, when glucose or other easily degradable substrates are used, an almost complete acidification (99%) can be obtained (Cohen

et al., 1979).

Acidification was found to increase with hydraulic retention time θ and decrease with increasing substrate concentration S_o and organic loading rate *OLR*. The opposite held, however, for the rate of product formation r_p. As a result, the optimum conditions for the first phase have to be established as a compromise between the two. According to Cohen *et al.* (1985), even partial acidification of the substrate can stimulate significantly the methanogenic activity of the population of a second reactor: these workers reported that the maximum specific COD conversion rates of sludge increased two to four times when the degree of acidification was only 3 to 10%, as compared with a three fold increase for complete acidification of the influent to the second reactors. This suggests that optimum conditions of the first stage should not aim for maximisation of the degree of acidification, but for the maximum rate of acid production. Since the latter for a given substrate concentration is maximised for short hydraulic retention times, the minimum possible hydraulic retention time should be used. Alternatively, the optimum hydraulic retention time could be the critical value determined in the current study, because then the degree of acidification would also be relatively high. According to the results presented, the critical hydraulic retention times were 6 and 8 h for initial substrate concentrations equal to 3 and 12 g COD L^{-1}, respectively. This shows that the higher the initial substrate concentration, the longer the optimal hydraulic retention time will be.

In the present study the effect of the operational parameters was quantified and their relative importance demonstrated in determining the degree of acidification and the rate of product formation: the degree of acidification was mainly determined by the hydraulic retention time, with the influent substrate concentration and the organic loading rate moderately affecting it. In contrast, the rate of product formation was determined mainly by the influent substrate

concentration, and for the feasible hydraulic retention times, a high rate of product formation cannot be achieved unless a concentrated influent substrate is used.

The temperature appeared to affect the acidogenic phase according to the Arrhenius equation. The activation energy was calculated to be 19.84 kJ mol^{-1}. This is at the lower end of the range of activation energies reported in the literature for mesophilic anaerobic microorganisms grown in a completely mixed single-stage reactor (Muck and Grady, 1974). The low value of the activation energy implies that the acidogenic phase tolerates temperature variations better than the single-stage anaerobic digestion system. The optimum temperature was determined to be 40 °C. However, according to Zoetemeyer *et al.* (1982b), a suboptimal temperature is preferable, firstly in order to avoid disturbances of the system by the high death rate which occurs at high temperatures, and secondly because it will lower the energy requirements of the system.

The optimum pH required in order to achieve both a high degree of acidification and a high rate of product formation was found to be 7. This is in general agreement with the optimum pH reported for different substrates: pH 7 for gelatin (Breure and Van Andel, 1984), pH 7 for glucose (Bull *et al.*, 1984a), and pH 6.3 for amino acids (Breure and Van Andel, 1984). However, lower values of the optimum pH have been determined previously, such as pH 5 for starch (Mudrack and Kunst, 1982) and pH 6 for glucose (Cohen *et al.*, 1979). Since the main products of the former are acetic and propionic acids and of the latter are acetic and butyric acids, it could be speculated that the optimum pH is different for the two types of acidogenic fermentation, that is, the propionic acid type and the butyric acid type.

The product distribution, and more specifically, the concentration of acetic acid produced, was found to depend on the operational parameters. Longer hydraulic retention times resulted in increased acetic acid concentration. In contrast,

the concentration of propionic acid did not change with the hydraulic retention time. It was found to be a function of the initial substrate concentration only. Furthermore, for the waste used in the present study a consistent 7% of the influent COD was converted to propionic acid. These observations are in agreement with the data presented by Stoppok and Buchholz (1985), where the amount of propionic acid produced consisted of approximately 7% of the influent substrate concentration, while the concentration of acetic acid varied according to the hydraulic retention time θ and the influent substrate concentration S_o. Joubert and Britz (1986) also found that the propionic acid concentration was constant for a range of pH values and a temperature of 27 °C. The engineering implication of the above observation is that low production of propionic acid might only be achieved when less dilute substrate concentrations are used.

The effect of pH on the composition of the volatile fatty acids produced was assessed and the concentration of acetic acid was found to be affected by pH to a greater extent than the concentration of propionic acid, although both acids reached a maximum concentration at pH 7. The percentage of acetic acid as a proportion of the total volatile fatty acids seemed to increase with increasing pH, while the percentage of propionic acid slightly decreased. At pH 7, acetic and propionic acids were 39 and 35% of the total volatile acids, respectively. However, at pH 5, acetic and propionic acids were 25 and 42% , respectively. This agrees with the results reported by Breure and Van Andel (1984) on gelatin, who found that the percentage of acetic acid decreased while the percentage of propionic acid increased, when the pH changed from 6.0 to 5.3.

The effect of temperature on the composition of the effluent of the acidogenic reactor was also determined. The effect of temperature on the concentration of acetic acid was much more pronounced than its effect on the concentration of propionic acid, which was almost constant for the range of 25 to 40 °C. The

propionic acid produced as a proportion of the total volatile fatty acids increased when the temperature decreased. This is in agreement with the results reported by De la Torre and Goma (1981), who also found a change in composition with temperature, and more specifically, an increased percentage of propionic acid at lower temperatures.

The experiments conducted at 35 ± 1.5 °C without pH control were further used for the calculation of the biomass yield Y and of the specific decay rate k_d of the acidogenic bacteria according to equation (15). The values obtained for Y and k_d were 0.099 ± 0.007 g biomass g^{-1} COD utilized and 0.052 ± 0.379 d^{-1}, respectively (section 4.3.1.). These values lie at the low end of the ranges reported in the literature (Table 35). As can be seen from Table 35, large variations are observed in the experimentally determined values of the specific decay rate and the yield coefficient of the acidogenic bacteria, which could probably justify the large standard error observed during the determination of k_d in the present study. A possible explanation is the one suggested by Stover *et al.* (1983) that only k_d is a true biokinetic constant, while the yield coefficient is a variable one. It has also been

Table 35 : Values of the specific decay rate k_d and the yield coefficient Y of the acidogenic bacteria

Specific Decay Rate k_d (d^{-1})	Yield Coefficient (g VSS g^{-1} COD)	Substrate	Reference
0.34	0.48	sludge	Eastman and Ferguson (1981)
0.08	0.12	—	Young and McCarty (1967)
6.14	0.17	dextrose	Ghosh and Pohland (1974)
—	0.08 - 0.016	glucose	Zoetemeyer *et al.* (1982c)
0.16	—	palm oil	Ng *et al.* (1985)
0.87	0.54	dextrose and beef extract	Andrews and Pearson (1965)

reported that the molar yield for the growth of even a pure culture is affected by the pathway of substrate breakdown, the specific growth rate, the possibility of anaerobic electron transport during fermentation and the availability and use of external hydrogen acceptors (Atkinson, 1983). This is in agreement with the finding of De la Torre and Goma (1981) that the acidogenic biomass yield increased when a selected (acclimated) population was used, although other conditions were kept constant. These observations suggest that an accurate determination of k_d is not possible if equation (15) is to be used. A more reliable value for k_d is expected to be obtained when equation (13) and the inhibition model for the description of the specific growth rate are solved simultaneously.

The application of the two inhibition models demonstrated that the non-competitive model could fit the experimental data better than the Haldane-type model. When the former was applied and k_d was calculated simultaneously with the other biokinetic constants, the minimum relative standard error of 28.7% was obtained. This deviation, although large, can partially be explained by the fact that the precision of the analytical methods was limited and never less than 8% for the individual variables. In addition it should be noted that reinoculation had to take place between the different runs. Although an effort was made to keep a standard quality for the inoculum, variation may have resulted due to acclimation of the micro-organisms or other environmental conditions. Even for the same inoculum, variation in the performance of a chemostat can be observed, due to the instability of a continuous culture in presence of inhibition: this arises from the fact that the same biomass growth rate can correspond to substrate limitation or product inhibition (Edwards, 1970; Philbrook and Grady, 1983). This implies that the observed steady-states can easily be upset by environmental disturbances and give non-representative information. Another problem involved when the kinetics of a mixed population are studied in a chemostat is the population selection, which

occurs in response to changing hydraulic retention time. This is usually followed by changes in the biokinetic constants of the biomass present (Philbrook and Grady, 1983). Finally, wall growth may cause variation of the performance of an acidogenic reactor, especially when continuous cultures are studied and therefore long equilibration times are required (Duarte and Anderson, 1982). Nevertheless, continuous culture experiments are indispensable, especially when the dynamics of the system are to be studied.

By solving equation (13) and the non-competitive inhibition model for the biomass growth rate simultaneously, a new value was calculated for the specific decay rate k_d, which was found to be equal to 1.747 d^{-1}. This value lies well within the range reported in the literature (Table 35).

The application of the non-competitive inhibition model to the experimental data of the present study has also yielded values for the biokinetic constants μ_{max} and K_s. The maximum growth rate μ_{max} in the absence of inhibition was found to be 32.6 d^{-1}, which corresponds to a minimum hydraulic retention time of the acidogenic reactor of 0.74 h and a doubling time of the acidogenic bacteria of 0.51 h. This compares well with the reported doubling times t_d of acidogenic bacteria growing in continuous culture, which were equal to 0.49 h, 0.61 h and 0.56 h for glucose, galactose and dextrose, respectively (Ghosh and Pohland, 1972; 1974). According to the experimental results of other researchers, the doubling time of acidogenic bacteria in the presence of substrate and/or product inhibition, is at least as low as 1.15 h (Blakebrough, 1967) and 1.35 h (Zoetemeyer *et al.*, 1982c). It should be noted here that the calculated value of μ_{max} was not very sensitive to the inhibition model used or the k_d value, the values obtained always lying in the range of 26.4 to 39.2 d^{-1}.

The value of the substrate saturation constant K_s was calculated to be 1.768 g COD L^{-1}. The substrate saturation constant of a biological system depends largely

on the concentration (Ng *et al.*, 1985), as well as the type of the substrate used (Ghosh and Klass, 1978): bacteria growing on cellulose or other poorly degradable substrates normally have high saturation constants (Ghosh and Klass, 1978). The calculated value of K_s was larger than those reported in the literature for glucose, indicating that the wastewater used in the present study was less easily biodegradable. The value of K_s was found to be insensitive to the inhibition model or the k_d value, the values obtained being in the range of 1.8 to 2.3 g COD L^{-1}. However, when the non-competitive model was applied for the description of the specific rate of product formation, a higher value of 2.71 g COD L^{-1} was obtained for the substrate saturation constant K_s'. This is very close to the concentration of 3 g COD L^{-1} identified as critical for the system reported here, since the proportion of the substrate converted to volatile fatty acids (degree of acidification) sharply declined at lower substrate concentrations. Although it cannot be established statistically that the values of K_s and K_s' are significantly different, this observation suggests that the limiting substrate concentration could be different for the biomass growth rate and the product formation rate; this phenomenon has also been observed by Aiba *et al.* (1973).

The inhibition constant K_i of the non-competitive inhibition model was calculated to be 0.633 g L^{-1} of the total volatile fatty acids. This compared well with the values found previously, such as 0.740 g L^{-1} of acetic acid (Ierusalimsky, 1967), 0.810 g L^{-1} propionic acid (Hendricks *et al.*, 1985) and 0.990 g L^{-1} of total volatile fatty acids (De la Torre-Lozano *et al.*, 1980). However, K_i was very sensitive to the k_d value, as well as the inhibition model used. Lower values were predicted for the K_i when the Haldane-type model was used or the k_d value was assumed to be equal to 0.052 d^{-1}.

The application of equation (19) in conjuction with equations (13) and (17) to the experimental data has shown that the intercept β was statistically equal to

zero at a probability level $p = 0.05$ for all calculated values of k_d. This result also indicates that only the cells found in the exponential growth phase produced acids and not those found in the stationary phase [eq. (18)]. This is in agreement with the results of another study, which have demonstrated that the activity of the non-growing cells during acidogenesis was not significant, unless a pH controlled culture was used (De la Torre and Goma, 1981). In contrast to these results, it has been suggested that in the presence of product inhibition, uncoupling of biomass growth and product formation may occur, which would lead to product formation by the resting cells (Herrero, 1983). Moletta *et al.* (1986) have similarly used a complicated model with different expressions for biomass growth and metabolite production, and for product formation by resting cells. However, the present study has shown that this phenomenon does not occur, especially when low hydraulic retention times and consequently large μ values are used [eq. (17)].

The calculated value for the regression coefficient α of equation (19) was found to be equal to 4.02. This compares well with the values reported in the literature: in the absence of pH regulation, the value of α ranged between 3.0 and 3.16, although considerably higher values have been reported for cultures with pH regulation (De la Torre and Goma, 1981).

When the two inhibition models were applied to the description of the specific rate of product formation, the estimated values for the maximum specific rate of acid production q_{max} were found to be 125.7 and 148.7 g VFA per gTSS per day for the non-competitive [eq. (24)] and the Haldane-type [eq. (25)] models, respectively. These values are larger than those usually observed, although the maximum volatile fatty acid productivity measured in practice with sucrose as substrate was 180 g VFA per gTSS per day (De la Torre and Goma, 1981). An interesting observation is that the value of q_{max} as calculated for the two inhibition models is very close to the maximum specific rate of acid production as calculated

from equation (19) and for μ equal to μ_{max}: by substituting the values of μ_{max} calculated from the inhibition models, q_{max} was found to be in the range of 110 to 160 g VFA per g TSS per day. The consistency of the q_{max} value calculated by the two methods suggests that q_{max} has a physical meaning and that it could be achieved under appropriate conditions.

Anaerobic digestion as a process for waste-water treatment has been traditionally hampered because of the time requirements of the start-up periods. During the two-phase experiments of the present study, this problem has been successfully tackled by employing the start-up regime introduced by Bull *et al.* (1983b). This method consists of a stepped increase of the organic loading rate in conjuction with a stepped decrease in the hydraulic retention time θ and a stepped increase of the influent substrate concentration S_o. Furthermore, at the beginning of the start-up period as much as 50 % of the influent COD concentration S_o is substituted with methanol, which is gradually replaced with waste. This method has also been successfully employed by other researchers (Stronach *et al.*, 1986b; Stephenson and Lester, 1986). Methanol is among the few substrates that can readily be utilized by methanogenic bacteria: it has been shown that at least one acetoclastic methanogen, that is *Methanosarcina sp.*, is capable of consuming methanol, while *Methanothrix sp.*, which is the other important acetoclastic methanogen, cannot utilise it (Harper and Pohland, 1986). The free energy change of the reaction of methanol conversion to methane is highly negative, which makes more energy available for bacterial growth and accelerates the start-up process (Harper and Pohland, 1986). In addition, methanol has been suggested to be beneficient during the start-up of anaerobic digesters, since it cannot be utilised by the sulphate reducing bacteria and therefore it prevents them out-competing the methanogenic bacteria on the fluidised bed carrier particles (Oremland and Polcin, 1982).

The significance of the start-up procedures have previously been emphasized not only for the rapid start-up of the methanogenic reactors, but also for the good performance of the systems at later stages. Stronach *et al.* (1987) have observed that variable conditions during start-up have resulted in reduced % COD removals at steady-state. Cánovas-Dias and Howell (1987) have similarly indicated that a synthetic feed media was preferable for the rapid start-up of an anaerobic digester which would later be used for a real treatment application: the main intermediate products of the real waste should be incorporated in the synthetic media in order to encourage the development of a balanced methanogenic population within the reactor. This last observation is consistent with the findings of other researchers who have reported that a rapid start-up is encouraged by feeding the anaerobic digester with pre-acidified wastewater: Tanaka and Matsuo (1986) found that the start-up period of a single-stage digester was 7 times longer than that of a two-phase system.

During the present study, the performances of the two-phase systems (systems 1 and 2) were considerably better than that of the single-stage system at the beginning of the start-up period: at day 15, the %COD removals of systems 1 and 2 were 65 and 80% respectively as compared with 30% of the single-stage system. Nevertheless, the differences were less pronounced in later stages.

The steady-state experiments of the present study were designed so that the optimum size of the acidogenic and the methanogenic reactors could be assessed. The hydraulic retention times of the methanogenic reactors were 87.4, 74.8, 50.0 and 100.0 % of the overall hydraulic retention time for systems 1, 2, 3 and 4, respectively. The validity of the derived conclusions was further tested by examining three different steady states as presented in Table 28. The experimental design allowed the determination of not only the optimum reactor sizes, but also the effect of phase separation on the performance, the effluent characteristics and the quality of the biogas evolved in anaerobic fluidised bed systems.

During all experiments the performances of the four systems (the two-phase systems and the single-stage control system) were very stable and satisfactory. More than 90 % of the initial COD concentration was removed from the filtered effluent at organic loading rates as high as 10.5 kg COD $m^{-3}d^{-1}$. Furthermore, no accumulation of volatile fatty acids was observed, while the pH of the effluent was always in the range of 7.18 to 7.44: a slightly alkaline pH value has been found to be optimum for acetate and propionate cleavage and methanogenesis (Boone and Xun, 1987). The effluent characteristics did not always vary significantly between the two-phase systems and the single-stage reactor, and taking into account the relatively large standard deviation of the measurements, statistical analysis had to be incorporated into the evaluation of the data in order to determine the significance of the observed differences.

The percentage of COD removal of the filtered, as well as the unfiltered effluent, of the four systems were presented in Table 32. It can be noted from this table that the filtered and unfiltered COD removals of the two-phase systems 1 and 2 were superior or statistically the same as those of the single-stage control reactor for all three steady-states (experiments I, II and III). On the other hand, the filtered and unfiltered COD removals of system 3 were always lower than that of the control system; the observed differences were statistically significant ($p = 0.05$) in all cases, the only exception being the filtered COD removals obtained during experiment II. These observations suggest that the reduction of the volume of the methanogenic reactor by as much as 25 % and the simultaneous introduction of an acidogenic reactor of equal volume, have either improved the filtered and unfiltered COD removals of the system or have left them unchanged. However, further reduction in the volume of the methanogenic reactor (system 3) resulted in reduced COD removals, suggesting that the increased acidification occurring in the first reactor cannot adequately stimulate methanogenesis in the fluidised bed

methanogenic reactor.

Another parameter significant for the evaluation of the relative effectiveness of the single- and two-phase systems, is the total suspended solids concentration. Statistical analysis demonstrated that the total suspended solids concentrations of the single-stage digester (system 4) were not statistically different to those of system 1 and 2, while the observed differences were statistically significant for system 3.

The effect of phase separation had a very pronounced effect on the total volatile fatty acid concentration in the effluent: the two-phase anaerobic digestion systems had consistently higher volatile fatty acid concentrations than the single-stage system. This observation is consistent with the finding of other researchers studying two-phase anaerobic digestion systems (Lettinga *et al.*, 1983). An important implication of this observation is that the breakdown of the influent substrate is more complete when phase separation is applied. More importantly, a low volatile fatty acid concentration can be limiting for the growth of methanogenic bacteria: according to Table 2, the substrate saturation coefficients K_s for the growth of the main acetotrophic methanogens e.g. *Methanosarcina sp.* and *Methanothrix sp.* are 300 $mg\,L^{-1}$ and 30 $mg\,L^{-1}$ of acetic acid, respectively. During steady-state operation in the present study, the total concentrations of volatile fatty acids in the effluent of the single-stage reactor were approximately 30 $mg\,L^{-1}$ and 19 $mg\,L^{-1}$ for influent substrate concentrations of 6 $g\,COD\,L^{-1}$ and 3 $g\,COD\,L^{-1}$, respectively and consisted mainly of acetic acid. These VFA values are very low, suggesting that the growth of both species of acetotrophic bacteria could be limited. Conversely, the two-phase anaerobic digestion systems demonstrated total volatile fatty acid concentrations in the range of 59 to 200 $mg\,L^{-1}$ and 35 to 51 $mg\,L^{-1}$ for influent substrate concentrations equal to approximately 5 and 2.5 $gCOD\,L^{-1}$, respectively. These values were considerably higher than those of the single-stage system, indicating that the application of phase-separation ensures that more

substrate is available for the slow growing methanogenic bacteria. The importance of the acetic acid concentration has also been emphasised by Van den Berg *et al.* (1976) who reported that the rate of acetic acid consumption in a methanogenic reactor is almost constant for a wide range of acetic acid concentrations and it drops abruptly when its concentration is below 13 $mg\,L^{-1}$. Another potential problem of the operation of the anaerobic reactor at low volatile fatty acid concentrations is the possibility that the sulphate reducing bacteria (SRB) may grow to out-compete the methanogenic bacteria: the former were found to have a K_s value of approximately 12 $mg\,VFA\,L^{-1}$, which suggests that they can grow at concentrations even lower than those required for the growth of *Methanothrix* (Yoda *et al.*, 1987a). Had this happened, the percentage of CH_4 in the biogas evolved would decrease (Anderson *et al.*, 1984). It should be noted here that the volatile fatty acid concentration decreases with decreasing influent substrate concentration S_o, and as a result the application of a more dilute influent substrate concentration would lead to even lower concentrations of the volatile fatty acids and exacerbation of the problems associated with this.

The biomass hold-up of the fluidised bed reactors during the present study varied between 1.9 to 2.6 grams of volatile solids per litre of expanded bed ($g\,VS\,L^{-1}$ exp. bed) for the two-phase systems and 4.8 to 6.9 $g\,VS\,L^{-1}$ exp. bed for the single-stage system.

These biomass concentrations are considerably lower than the greatest ones reported in the literature for anaerobic expanded and fluidised bed reactors: Schraa and Jewell (1984) reported a maximum biomass hold-up of 60 $g\,L^{-1}$ for small diatomaceous earth carrier particles, Heijnen (1983) observed 40 $gVS\,L^{-1}$ using 0.2 mm diameter sand, while Enger *et al.* (1986) achieved 50 g VS L^{-1} in a full-scale two-phase fluidised bed reactor using similar sand. A number of factors are responsible for the high biomass concentrations reported: the reduced particle size

greatly affects the biomass hold-up, since it increases the surface area available for bacterial colonisation (Jewell, 1983). In addition, increased organic loading rates have been reported to increase significantly the concentration of the attached biomass (Schraa and Jewell, 1984; Shapiro and Switzenbaum, 1984; Chen *et al.*, 1985a; Rudd *et al.*, 1985). Finally, the biomass hold-up greatly depends on the upflow velocity, which determines the shear stress on the biofilm surface (Trulear and Characklis, 1982). Shieh *et al.* (1981a) have demonstrated the effect of the upflow velocity using a fluidisation model similar to the one used in the present study (section 3.10.).

The concentration of the attached biomass obtained during the present study is in good agreement with that of other anaerobic fluidised bed reactors using sand particles of similar size (0.4 mm diameter) and operated at similar organic loading rates: Stephenson and Lester (1986) reported that the biomass concentrations at an organic loading rate of 10 $kg\,COD\,m^{-3}\,L^{-1}$ were equal to 5.7 and 3.0 $g\,VS\,L^{-1}$ exp. bed for a single-stage and a two-phase methanogenic fluidised bed, respectively. Toldrá *et al.* (1986) reported biomass hold-up of less than 5 $g\,VS\,L^{-1}$ exp. bed for organic loading rates as high as 36 $kg\,COD\,m^{-3}\,d^{-1}$ using acetic acid as substrate and sand particles with an average diameter of 0.5 mm.

The biomass concentrations observed in the single-stage fluidised bed reactor were significantly different and approximately 2.5 times higher than those observed in the two-phase systems when operated under similar conditions. This behaviour can be expected, since in the single-stage reactor more acidogenic bacteria are allowed to grow on the carrier particles: the latter bacteria are known to have a high biomass yield of approximately 0.10 $g\,VS\,g^{-1}\,COD$ utilised as compared with 0.03 $g\,VS\,g^{-1}\,COD$ utilised for the methanogenic bacteria (Henze and Harremöes, 1983). The growth of acidogenic bacteria on the carrier particles of the single-stage fluidised bed reactor was largely encouraged by the composition of the effluent: in

the single-stage system the volatile fatty acids constituted only 8, 16 and 9% of the effluent COD concentration as compared with 35, 32 and 25% in the two-phase system during experiments I, II and III, respectively. Thus, in the single-stage system more complex substrate was available for the growth of acidogenic bacteria. The biomass concentration of attached film systems has previously been reported to be greatly dependent on the type of the substrate used: Ehlinger *et al.* (1987b) reported that the biomass hold-up was higher when the substrate consisted of carbohydrates rather than volatile fatty acids or pre-acidified waste.

The increased biomass concentration of the single-stage reactor was not accompanied by increased methanogenic activity; the specific rate of substrate utilisation R_s of the single-stage system varied between 0.8 and 1.4 $g\,COD\,g^{-1}\,VS\,d^{-1}$, while that of the two-phase system ranged between 2.2 and 4.1 $g\,COD\,g^{-1}\,VS\,d^{-1}$. Similarly, other researchers have reported that phase separation significantly increased the methanogenic activity of the bacterial population: Cohen *et al.* (1985) measured a two- to four-fold increase in the methanogenic activity, when the influent substrate was completely or partially acidified. A possible explanation of the reduced methanogenic activity of the single-stage system is the one suggested by Ehlinger *et al.* (1987b) concerning the chemical composition of the biofilm: the polysaccharide concentration of a biofilm was higher when glucose instead of a mixture of volatile fatty acids was used (7 $g\,L^{-1}$ as compared with 0.5 $g\,L^{-1}$), although the protein and consequently the microbial concentration was less affected (3.6 $g\,L^{-1}$ as compared to 1.8 $g\,L^{-1}$). The observed differences in the methanogenic activity of the two-phase systems compared with the single-stage one, could also be attributed to differences in the methanogenic bacteria prevailing in each system: due to the higher acetate concentration in the effluent of the two-phase systems more *Methanosarcina sp.* are expected to grow, while the methanogenic population of the single-stage reactor would be expected to be enriched in

Methanothrix sp.. The former has been found to have in suspended culture a higher specific rate of substrate utilisation, R_s, equal to 15 $g COD g^{-1} VS d^{-1}$ as compared with 4 $g COD g^{-1} VS d^{-1}$ of the latter (Smith and Mah, 1978; Zehnder *et al.*, 1980). Unfortunately, the assumption of different microbial populations was not verified by microscopic observations. It should be noted here that the observed R_s values of the two-phase systems were considerably higher than most values reported in the literature for single-stage fluidised bed reactors: Shieh *et al.* (1985) measured a maximum specific rate of substrate removal equal to 2 $g COD g^{-1} VS d^{-1}$, while in other systems values as low as 0.1 $g COD L^{-1} VS d^{-1}$ have been reported (Yoda *et al.*, 1987b).

The increased biomass hold-up of the single stage reactor is a drawback rather than an advantage of such a system, since it is not accompanied by increased methanogenic activity. Furthermore, it appears to be the cause of the operational problems observed in the single-stage reactor between experiments II and III: when the organic loading rate was increased from 5 to 10 $kg COD m^{-3} d^{-1}$, a considerable proportion of the sand particles ($\sim 10\%$) was washed out of the reactor. Although this sand was returned to the reactor, it was shortly washed out again. This behaviour was attributed to a rapid increase of the biofilm thickness: the upflow velocity was higher than the settling velocity of certain bioparticles, which were consequently carried away from the fluidised bed. Furthermore, Timmermans and Van Haute (1984) reported the existence of a critical biofilm thickness beyond which gas bubbles adhere to the biofilm surface, resulting in a sudden biomass wash-out. To avoid the wash-out of these particles, the upflow velocity should be adjusted and/or a biomass control system should be incorporated in the fluidised bed design. However, in the two-phase system, the low biomass production rate enables its undisrupted operation for much longer periods: Tanaka and Matsuo (1986) have shown that in a two-phase system the biomass yield in the second reactor was as

low as 0.043 g VS g^{-1} COD utilised, as compared to 0.257 g VS g^{-1} COD utilised in the first reactor, which enabled the stable operation of the methanogenic reactor without any sludge-wasting.

The fluidisation model used in the present study (section 3.10.) has allowed the calculation of the biofilm thickness δ around the sand carrier particles of the fluidised bed reactors. The biofilm thickness δ of the two-phase systems was considerably lower than that of the single-stage system, ranging between 14 to 18 μm as compared to 39 to 62 μm for the single-stage system, the higher values observed for higher organic loading rates. These results agree well with the values reported by other researchers working on fluidised bed reactors with sand for carrier particles of 0.2 to 0.5 mm diameter: Switzenbaum and Eimstad (1987) have measured a biofilm thickness of 50 μm when the influent substrate was glucose and the organic loading rate 12 kg COD m^{-3} d^{-1}, while Toldrá *et al.* (1986) have reported a biofilm thickness in the range of 15 to 25 μm for an influent substrate consisting mainly of acetic acid and for organic loading rates in the range of 1.5 to 36 kg COD m^{-3} d^{-1}. Similarly, other researchers have reported biofilm thicknesses of less than 20 μm (Jewell, 1987) or close to 40 μm (Wang *et al.*, 1986).

The biomass dry density ρ used in the above calculations was equal to 0.04 g cm^{-3}, while the moisture content of the biofilm P was assumed equal to 92.5 %. These values of ρ and P gave the best agreement between the predicted and the observed values of the biomass hold-up of the expanded bed X_{exp}, as well as of the volume of the expanded bed V_{exp}. The value of ρ has been reported to depend on the operational conditions applied to the system: Characklis and Cooksey (1983) have found ρ to increase with turbulence and organic loading rate, taking values between 0.01 and 0.05 g cm^{-3}, while Shieh *et al.* (1981a) reported ρ to be in the range of 0.04 to 0.06 g cm^{-3} in anaerobic fluidised bed systems. Therefore, the chosen value of 0.04 g cm^{-3} can be considered reasonable. Furthermore, a sensitivity

analysis has shown that by assuming ρ equal to 0.02 $g cm^{-3}$, that is, lower by 50 %, the biofilm thickness δ is calculated in the range of 30 to 40 μm for the two-phase systems and in the range of 80 to 120 μm for the single-stage system: these values are lower than 100 to 200 μm, which is reported to be the maximum thickness for fully penetrated biofilms (Atkinson and Fowler, 1974). Therefore, the biofilms obtained during the present study can be considered thin and the diffusional limitations within them assumed negligible. This conclusion has also been reached by other researchers studying anaerobic fluidised bed reactors (Toldrá *et al.*, 1986; Wang *et al.*, 1986; Denac *et al.*, 1988).

The assumption of negligible diffusional limitations within the biofilm simplifies significantly the kinetic analysis of the fluidised bed reactors. The mathematical modelling of substrate utilisation by a biofilm becomes complicated when diffusional limitations within the biofilm, as well as in the boundary liquid film, are incorporated (Rittmann and McCarty, 1980). However, there is sufficient evidence that the external mass transfer (boundary liquid film) limitations are also negligible: Mulcahy *et al.* (1981) measured the external mass transfer resistance and found that, if incorporated, it affected the overall results by a maximal 5 %. Trulear and Characklis (1982) reported that the external mass transfer resistance decreased with increasing upflow velocity, while Kissel (1986) concluded that the external mass transfer resistance is negligible in fluidised bed reactors. The assumption of negligible external mass transfer resistance was also supported by the experimental work of other researchers on fluidised bed reactors (Toldrá *et al.*, 1986; Wang *et al.*, 1986). Finally, Andrews and Trapasso (1985) indicated that liquid-phase mass transfer resistance is a less severe problem in anaerobic than aerobic beds, since the gas bubbles agitate the liquid film and thus increase the mass transfer coefficient. Furthermore, anaerobic treatment is usually applied to high-strength wastes, resulting in higher effluent concentrations and thus reduced external mass transfer

limitations.

Under the above mentioned assumptions the Monod equation can be used to describe the overall kinetics according to equation (7). By plotting the specific rate of substrate removal R_s versus the effluent substrate concentration S_e, it became apparent that the single-stage system demonstrated an invariably lower specific rate of substrate removal than the the two-phase systems: R_s was found in the range of 0.75 to 1.32 $g\,COD\,g^{-1}\,VS\,d^{-1}$ as compared with 2.15 to 4.10 $g\,COD\,g^{-1}\,VS\,d^{-1}$ of the two-phase systems. The causes for the observed differences between the single-stage and the two-phase configurations were discussed in a previous paragraph of the present section and were attributed to the microbial population present in the digesters. It should be noted here that R_s is almost constant for increasing S_e. This pattern suggests that the reaction order is zero or close to it. A comparison with the literature indicates that the order of the reaction could actually be zero; Rodrigues *et al.* (1983) have calculated zero order kinetics, Toldrá *et al.* (1986) reported that the order of the reaction was close to zero and equal to 0.27, while the application of the Monod equation [eq. (7)] by Shieh *et al.* (1985) resulted in a K_s value equal to 154 $mg\,COD\,L^{-1}$, thus suggesting zero order kinetics for effluent concentrations higher than K_s.

The number of the experimental points obtained during the present study was too low to allow a more detailed kinetic analysis of the fluidised bed reactors. However, it would be interesting for future research to incorporate kinetic models other than the Monod equation: Hill and Barth (1977) have proposed an inhibition model for the methanogenic reactor with the un-ionised volatile fatty acids acting as inhibitors. The kinetic models applicable to fixed-film systems could, however, be markedly different than those applied to suspended growth systems: Denac *et al.* (1988) have shown that no hydrogen inhibition occurred within a biofilm, although hydrogen has been shown to be inhibitory for methanogenesis in suspended growth

cultures (Kaspar and Wuhrmann, 1978; Wiegant *et al.*, 1986). Similarly, the effect of the un-ionised volatile fatty acids on attached biomass growth could be less pronounced.

Another parameter that can be used as a criterion for the efficient operation of an anaerobic digestion system is the concentration of methane in the biogas: the proportion of CH_4 in the evolved biogas of the two-phase systems was consistently higher by approximately 10% than that of the single-stage system. Other researchers have also observed increased methane in two-phase systems: Sutton and Li (1983) observed an increase in methane concentration from 60 to 85%, when the two-phase instead of the single-stage configuration was used. A possible explanation could be that in a single-stage system the metabolic activity of the attached acidogenic bacteria results in increased CO_2 production without any increase in the production of CH_4. Moreover, according to the previous analysis, the methanogenic population of the two-phase systems is expected to be enriched in *Methanosarcina sp.* rather than *Methanothrix sp.*: the former has the ability to produce CH_4 by utilising H_2/CO_2 which the latter lacks. Poels *et al.* (1985) have shown that the proportion of CH_4 in the biogas can increase by as much as 10 %, when the H_2 produced is utilised by hydrogen-utilising methanogens in an auxiliary reactor. Finally, due to the lower acetate concentration more sulphate reducing bacteria are allowed to grow in the single-stage reactor: although the latter do not affect the COD removal rates, they result in biogas with decreased CH_4 and increased H_2S which is not desirable because of its corrosive activity (Anderson *et al.*, 1984).

The comparison of experiments I, II and III have resulted in some interesting observations concerning the effect of influent substrate concentration S_o, hydraulic retention time θ and organic loading rate on the performance of the anaerobic digestion systems.

The influent substrate concentration S_o greatly affected the effluent

concentration S_e: when S_o changed from 5.94 to 2.96 $g L^{-1}$, S_e changed from approximately 0.50 to 0.20 $g L^{-1}$ during all steady-states observed in the present study. The same observation has also been made by other researchers and on this basis it was suggested that the Monod equation should not be used for the description of attached biofilm kinetics, since it predicts S_e independent of S_o. The fact that S_e depends on S_o does not necessarily compromise the validity of the Monod equation, the latter appearing to reproduce sufficiently well the patterns observed in the present study.

During experiment II, conducted under the same organic loading rate as experiment I but with shorter hydraulic retention time and lower influent substrate concentration S_o, improved COD removals were observed for all reactors. This observation suggests that the overall hydraulic retention time applied during experiment II (13.5 h) was sufficiently long for the anaerobic digestion system to operate satisfactorily at the chosen organic loading rate. Rudd *et al.* (1985) similarly demonstrated that lower S_o resulted in increased COD removal, although the organic loading rate was constant. The increased COD removals observed during the present study could also be attributed to hysteresis phenomena: during experiment I higher influent concentration (S_o) resulted in increased effluent concentration (S_e) and consequently increased biomass hold-up on the fluidised bed carrier particles. A reduction in S_o and consequently S_e was not accompanied by the corresponding reduction in the already attached biomass. The application of high S_o in order to ensure increased biomass hold-up and therefore better performance of the system has been used by certain researchers and is known as the maximum loading start-up (Heijnen, 1986). To improve performance, Rittmann and Brunner (1984) similarly proposed the cyclic operation of anaerobic fluidised beds using alternatively high and low influent substrate concentrations: this method is based on the observation by Rittmann and McCarty (1981) that an established biofilm is

capable of substrate removals below the minimum concentration S_e required for biofilm support, if the influent S_o is lower than the S_o used to establish the film.

A comparison between experiments II and III conducted under the same hydraulic retention time θ but double S_o and consequently double organic loading rate, demonstrated that the performance of the anaerobic systems was slightly affected. The observed decline in the efficiency of the systems was very small, although the organic loading rate was raised from 5 to 10 $kg\,COD\,m^{-3}\,d^{-1}$. This observation agrees with the results presented by Switzenbaum and Eimstad (1987) who have observed a slight increase in the COD removal when the S_o was doubled but the hydraulic retention time θ was left unchanged. Oakley *et al.* (1984) also found that the increase of the organic loading up to 11.9 $kg\,COD\,m^{-3}\,L^{-1}$ under constant θ was very well tolerated by an anaerobic system without a major decline in its performance ($\sim$ 80 %); this behaviour could possibly be attributed to the increased S_e which allowed more methanogenic bacteria to grow on the carrier particles.

A comparison between experiments I and III conducted using equal S_o but half the hydraulic retention time θ, has shown that the COD removal during the latter was slightly improved although the organic loading rate was double. A possible explanation could be that the quality of the methanogenic population has improved with time. It is also possible that due to the low S_e concentrations imposed during experiment II, more *Methanothrix sp.* were allowed to grow, thus enabling the operation of the reactor at lower S_e. Finally, the effect of recycle ratio could be of importance; during experiment I the recycle ratio was approximately 150:1 as compared to 75:1 during experiments II and III. The increased recycle ratio has been shown to adversely affect the performance of a fluidised bed reactor (Rittmann, 1982), while lower recycle ratio allows better stratification of the microbial population along the reactor (Andrews and Trapasso, 1985).

It is of interest to note that the overall system efficiency, defined as the percentage of COD removal of the filtered or the unfiltered effluent, was not significantly affected by phase separation, the differences always being less than 4 %. This is in agreement with the findings of Bull *et al.* (1984a) that the percentage of COD removal of the two-phase system was not considerably higher than the single-stage system, unless a high organic loading rate ($> 8\,kg\,COD\,m^{-3}d^{-1}$) was applied. Similarly, Therkelsen and Carlson (1979) found almost identical performances of one- and two-phase digestion of a complex solid waste. In contrast, other researchers reported significantly improved performances of two-phase systems treating soft-drink waste (Ghosh and Henry, 1981) and sewage sludge (Ghosh, 1987). The two-phase configuration was also reported to result in increased COD removals when the waste contained high concentrations of fats, since inhibition of the methanogens by the produced long-chain fatty acids was prevented (Tanaka and Matsuo, 1986; Hanaki *et al.*, 1987). It should be noted here that most of the existing literature in favour of phase separation does not provide direct comparison between the single-stage and the two-phase configurations (Massey and Pohland, 1978; Roy and Jones, 1982; Morris and Burgess, 1984; Stoppok and Buchholz, 1985, Zepeng *et al.*, 1985). Furthermore, many of the so called two-phase systems are actually two-stage systems, which allow the growth of methanogenic bacteria in the first reactor (Sutton and Li, 1983; Wechs, 1984).

The application of phase separation in anaerobic digestion has been shown to enjoy several advantages over the single-stage configuration. Besides its superior steady-state characteristics, analysed in the present study, the two-phase configuration has also been reported to have the ability to tolerate organic, temperature and pH shocks more easily than the single-stage system (Cohen *et al.*, 1982; Bull *et al.*, 1984a; Tanaka and Matsuo, 1986).

Given the various advantages of the two-phase systems, the optimum size of

the acidogenic reactor was defined as the one which allowed a COD removal superior or statistically the same as that of the single-system. The optimum volume of acidogenic reactor was thus found to be in the range of 12 to 25% of the overall active volume, which during the present study allowed for a hydraulic retention time in the range of 1.7 to 6.8 h.

Despite the operational advantages of the two-phase systems, the incorporation of a second reactor has often been criticized as uneconomical, since a more complex design and more extensive measuring and control devices are required (Sahm, 1984). However, the reduction of the methanogenic reactor by as much as 25 % in a two-phase system results in considerable reduction of the investment cost of the most expensive pieces of equipment (the fluidised bed reactor and the recycle pumps) and decreased operating costs. Further savings are also achieved due to the improved quality of the biogas evolved. Thus, a more detailed analysis is necessary to facilitate the economic evaluation which should take into account all the above-mentioned factors to determine the economic viability of the phase separation.

CHAPTER 6

CONCLUSIONS

1) The operational parameters such as hydraulic retention time, organic loading rate, influent substrate concentration, pH and temperature affect the degree and rate of acidification in the acidogenic reactor. However, the fermentation pattern is not affected, the main products always being acetic and propionic acids.

2) The degree of acidification is primarily determined by and is an increasing function of the hydraulic retention time, while the organic loading rate adversely affects acidification to a smaller degree.

3) The rate of acid production is primarily determined by and is an increasing function of the influent substrate concentration, while the hydraulic retention time adversely affects acid production rate to a smaller degree.

4) The optimum temperature for maximum degree and rate of acidification is 40 ^{o}C. The temperature effect on the rate of acidification follows an Arrhenius type equation with an activation energy of 19.84 kJ mol^{-1}.

5) The optimum pH for maximum degree and rate of acidification is 7.

6) The amount of propionic acid produced during the first phase of anaerobic digestion is not significantly affected by the operational parameters. Propionate is mainly determined by substrate availability with a consistent 7 % of the influent substrate converted to this acid. In contrast, the amount of acetic acid produced is significantly affected by the operational parameters.

7) The inoculum of the acidogenic reactor does not affect the type of volatile fatty acids produced, the main intermediates always being acetic and propionic acids.

8) The yield coefficient Y and the specific decay rate k_d of the acidogenic biomass were calculated from the yield data and were found equal to 0.099 g biomass per g COD utilised and 0.052 d^{-1}, respectively.

9) The Monod equation failed to describe the growth of acidogenic bacteria in continuous culture, while there was evidence of product inhibition. Two of the most commonly used inhibition models were applied, the non-competitive and the Haldane-type models, using the total volatile fatty acids concentration as inhibitor. The former model gave a better prediction of the acidogenic biomass growth rate than the latter model.

10) The maximum specific growth rate μ_{max}, the substrate saturation constant K_s, the inhibition constant K_i and the specific decay rate k_d as calculated by the non-competitive inhibition model for the acidogenic bacteria growing on the specific wastewater were found to be equal to 32.6 d^{-1}, 1.768 g COD L^{-1}, 0.633 g VFA L^{-1}, and 1.747 d^{-1}, respectively.

11) The specific rate of product formation q_{max} in the acidogenic phase was found to be proportional to the specific growth rate μ, suggesting that only cells found in the exponential growth phase produced acids and not those found in the stationary phase.

12) The application of the non-competitive inhibition model for the description of the specific rate of product formation gave superior results to the Haldane-type model. The maximum specific rate of product formation q_{max} was found to be 125.8 g VFA g^{-1} TSS d^{-1}, while the substrate saturation constant K_s' and the inhibition constant K_i' were found to be equal to 2.71 g COD L^{-1} and 0.146 g VFA L^{-1}, respectively.

13) A rapid start-up of the anaerobic fluidised bed reactors was accomplished by following the particular start-up regime (Bull *et al.*, 1983b): at day 55, more than 85% of the influent COD concentration was removed at an organic loading rate of 5.3 kg COD m^{-3} d^{-1}. During start-up, no pH control was required after day 10, while the total volatile fatty acid concentration never exceeded 750 mg L^{-1}.

14) During steady-state operation, the two-phase anaerobic digestion systems

demonstrated COD removals superior or statistically the same as that of the single-stage system whenever the volume of the acidification reactor constituted up to 25% of the overall volume. However, reduced COD removals were observed, when the volume of the acidification reactor was 50 % of the total volume.

15) The volatile fatty acid concentration of the effluent of the two-phase systems was higher than that of the single-stage system, constituting 17 to 41 % of the effluent COD concentration as compared to 8 to 16 % of the single-stage system.

16) The biogas evolved from the two-phase anaerobic digestion systems was of superior quality in comparison to that of the single-stage system, since its methane content was 6 to 13 % higher than that of the latter.

17) The biomass hold-up of the single-stage fluidised bed reactor was significantly higher than that of the methanogenic fluidised bed reactors of the two-phase systems. However, the methanogenic activity of the biomass of latter reactors was 2 to 5 times higher than that of the single-stage system.

18) The biofilm thickness around the fluidised bed carrier particles was simulated using a mathematical model and it was found to be in the range of 12 to 18 μm and 39 to 62 μm for the two-phase and the single-stage fluidised bed reactors, respectively.

19) The optimum ratio of the volume of the acidogenic reactor to that of the methanogenic reactor was defined as the one which allowed COD removals superior or statistically the same as the single-stage system. The optimum volume of the acidogenic reactor was thus found to be in the range of 12 to 25 % of the overall volume, which during the present study allowed for a hydraulic retention time in the range of 1.7 to 6.8 h.

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