ERRATA

Page 6 section 4.08.1 "Biodegradation Tests" should read "Batch Tests".

Page 76, line 21 "for the start-up of the fluidised beds, filters and UASB reactors on pharmaceutical wastes" should be inserted after "fitted".

Page 134, lines 23,24,26; page 159, lines 7,8,10 : "$m^3kg^{-1}CODm^{-3}d^{-1}$" should read "$m^3CH_4$ kg^{-1}COD removed m^{-3}reactor volume d^{-1}".

Page 149, line 3 "Biodegradation" should read "Batch".

ANAEROBIC TREATMENT OF INDUSTRIAL WASTEWATERS IN HIGH RATE BIOREACTORS

by

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ABSTRACT

The literature describing the biochemical and microbiological aspects of anaerobic systems and the applications and limitations of the major anaerobic bioreactor types has been reviewed, with emphasis on the treatment of industrial wastewaters.

Laboratory scale anaerobic fluidised bed reactors were started up on a synthetic waste at 37°C under variable conditions and their performances evaluated. Start up was aided by stepped loading and continuous nitrogen purge; polymer addition promoted bacterial attachment but retarded the development of a balanced microbial population.

Reactor performance at bed expansions of 5-30% were examined. Moderate expansions had negligible effects on attachment and efficiency but the latter were impaired at the 5% level. Increasing trace element addition in fluidised beds at steady state did not affect COD removal or suspended solids concentrations but TVA levels decreased and methane production increased, indicating improved reactor stability.

The operational capabilities of fluidised beds restarted after downtime were determined. Full reactor performance was retained after 39 days downtime but restart after 89 days required manipulation as COD removal was poor and TVA levels high. Biomass activity was found to be greatest in the upper regions of the beds, with attached biomass more active than suspended.

The acclimation of fluidised beds to pharmaceutical wastes has been described. Acclimation was achieved although results indicated that one waste was nutrient limited and the second contained a non-degradable or toxic fraction which did not inhibit methanogenesis.

Laboratory scale anaerobic filter and upflow anaerobic sludge blanket reactors were designed and constructed and started up, with the fluidised beds, on high strength industrial wastes. Fluidised beds were found to be superior to filters and sludge blankets in COD removal although methane production was greatest in the sludge blankets. Volatile acids composition in fluidised beds was found to relate to substrate composition.

ACKNOWLEDGEMENTS

I would like to express my grateful appreciation to Dr. J. N. Lester and Dr. T. Rudd for their valuable guidance and encouragement during the course of this study and thesis preparation; to the Science and Engineering Research Council for financial support for this research; to my colleagues in the Public Health Engineering Section; and to Dr. Alan Cayless for constant support, good humour and invaluable assistance throughout.

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LIST OF ABBREVIATIONS

BOD	Biological oxygen demand
CASBER	Carrier associated sludge bed reactor
COD	Chemical oxygen demand
CSTR	Continuously stirred tank reactor
DOC	Dissolved organic carbon
HRT	Hydraulic retention time
OHPA	Obligate hydrogen producing acetogenic
PVC	Polyvinylchloride
RBC	Rotating biological contactor
SRT	Solids retention time
TOC	Total organic carbon
UASB	Upflow anaerobic sludge blanket
UVA	Undissociated volatile acid
VFA	Volatile fatty acid
VS	Volatile solids
VSS	Volatile suspended solids
TVA	Total volatile acids

CHAPTER 1
INTRODUCTION

1.1 INDUSTRIAL WASTE TREATMENT

The treatment of complex organic wastes such as primary and secondary wastewater sludges has long been accomplished by means of anaerobic biological conversion systems, but the protracted retention times and large volume units required precluded the use of the process for the treatment of high strength organic industrial wastewaters. In addition, anaerobic reactors were regarded as slow to start up, expensive to introduce and less stable under fluctuating environmental conditions than the aerobic systems (trickling filters, activated sludge) adopted for these wastestreams. Recently, however, restrictions caused by rising power costs have warranted increased interest in the economics of the operation of anaerobic treatment systems, in conjunction with a number of additional factors:

1. the energy costs of aeration can be eliminated, thus treatment can be achieved with an energy input considerably less than that required for some aerobic processes;
2. less sludge results from anaerobic metabolism as bacterial growth yield is low: removal costs of the sludge are therefore also low;
3. where by-product recovery is practicable, a net energy gain in the form of methane may be generated, especially when digester temperature can be maintained by waste heat from allied processes, or when the effluent is warm; and,

4. the development of systems such as expanded and fluidised beds, with high volumetric load capacities, short HRTs and long SRTs, has resulted in a reduction in reactor unit volumes and hence land area required and has also provided the possibility of treating less-concentrated wastewaters.

Almost any waste that can be treated aerobically can be treated anaerobically (Speece 1983) and the main considerations of the use of one system over the other must therefore be those of time, efficiency and cost. Table 1 enumerates various waste types which have been treated by anaerobic processes.

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

Anaerobic digestion processes are widely used in the treatment of sewage sludge although the biochemical reactions comprising various stages in the anaerobic degradation of organic materials have not yet been fully elucidated. The overall anaerobic conversion of biodegradable organic solids to the end products methane and carbon dioxide was initially believed to proceed in three stages which occurred simultaneously within the digester. These were:

TABLE 1 WASTES TREATED BY ANAEROBIC DIGESTERS

REACTOR TYPE	WASTE TYPE	REFERENCE
Anaerobic Filter	Leachate	Chian & DeWalle (1977)
	Starch production	Mosey (1978)
	Petrochemical	Lin Chou et al (1979)
	Bean blanching	van den Berg & Lentz (1979)
	Alcohol stillage	Dahab & Young (1981)
	Livestock	Newell (1981)
	Municipal	Genung et al (1982)
	Coal gasification	Cross et al (1982)
	Food & drink processing	Wheatley (1983)
Stirred Tank	Petrochemical	Lin Chou et al (1979)
	Sewage sludge	McConville & Maier (1979)
	Bean blanching	van den Berg et al (1980b)
	Agricultural	Lehmann & Wellinger (1981)
	Citrus peel press liquor	Lane (1983)
	Potato	Jewell & Cummings (1984)
	Chemical	Stephenson & Lester (1986)
UASB	Methanolic	Lettinga et al (1979a;1981)
	Bean blanching	van den Berg et al (1981)
	Sugar beet	Heertjes et al (1979)
	Citrus peel press liquor	Lane (1983)
	Potato	Christensen et al (1984)
	Chemical	van den Berg et al (1981)
Contact Reactor	Bean blanching/potato/pear peeling/rum stillage	van den Berg & Lentz (1978)
	Bean blanching	van den Berg et al (1980b)
	Synthetic	Anderson & Donnelly (1978)
	Fruit & vegetable processing	Lane (1984)
Expanded Bed	Synthetic	Switzenbaum & Jewell (1980)
	Synthetic	Morris & Jewell (1981)
	Municipal	Jewell et al (1981)
	Whey	Switzenbaum & Danskin (1981)
	Black liquor condensate	Norrman (1983)
	Synthetic	Schraa & Jewell (1984)
Fluidised Bed	Dairy/chemical/food/drink	Hickey & Owens (1981)
	Whey permeate	Sutton & Li (1981)
	Whey/food/chemical/bakery	Jeris (1983)
	Kraft bleach liquor	Salkinoja-Salonen et al (1983)
	Soy bean	Sutton et al (1982)
	Synthetic meat/dairy	Bull et al (1983a;1983b)
	Coke plant	Nutt et al (1984)
	Primary wastewater	Switzenbaum et al (1984)
	Synthetic meat	Stephenson & Lester (1986)
RBC	Synthetic	Tait & Friedman (1980)
Carrier Assisted	Molasses/sugar refining	Martensson & Frostell (1983)

1. the hydrolysis of insoluble biodegradable polymers;
2. the production of acid from smaller soluble organic molecules; and,
3. methane generation.

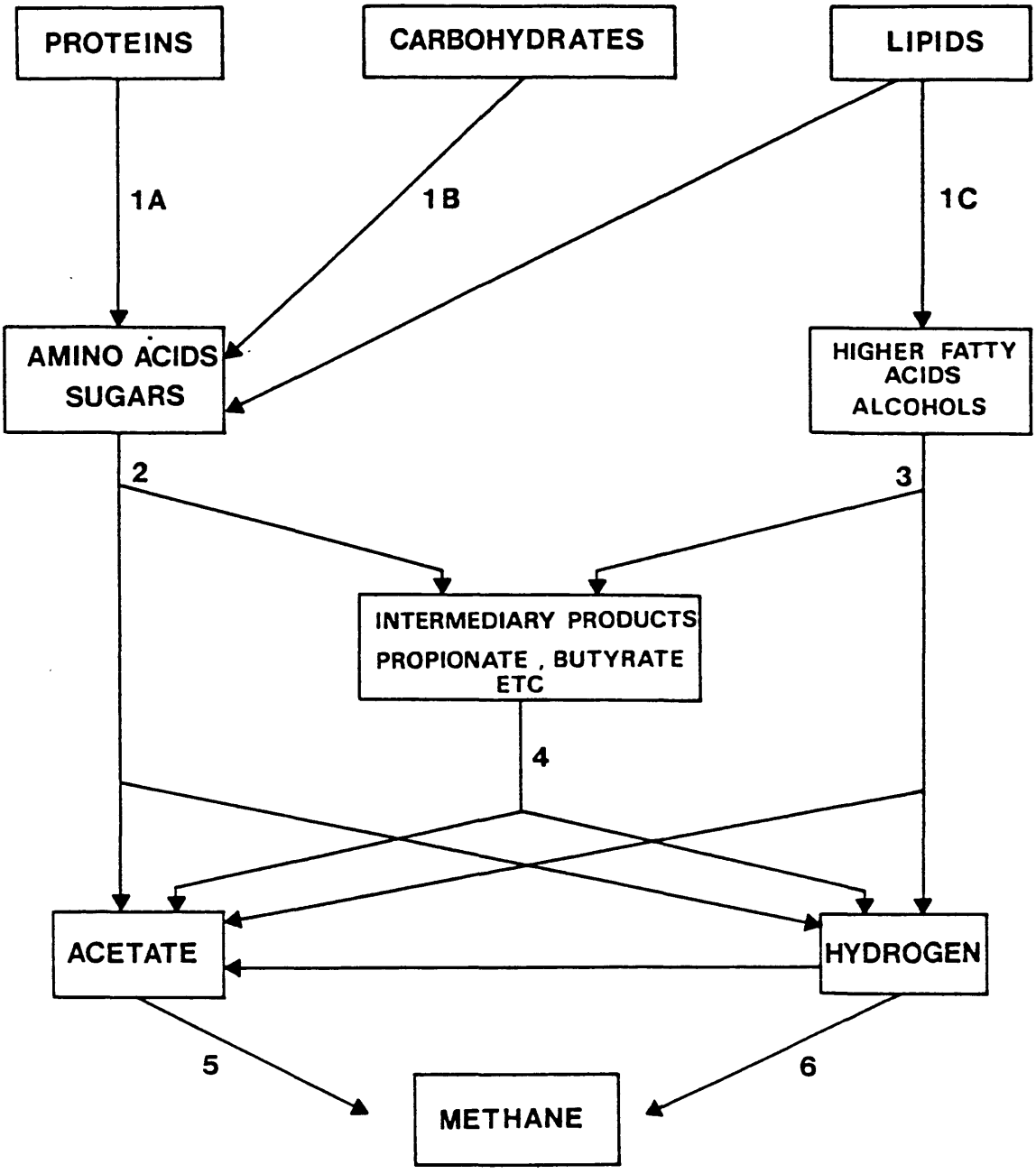
However, Gujer and Zehnder (1983) proposed a six step system in the anaerobic conversion of high molecular weight degradable organics to methane and carbon dioxide (Figure 1). These stages comprised:

1. hydrolysis of proteins, lipids and carbohydrates;
2. fermentation of sugars and amino acids;
3. anaerobic oxidation of long chain fatty acids and alcohols;
4. anaerobic oxidation of intermediates such as the volatile fatty acids (with the exception of acetate);
5. conversion of acetate to methane; and,
6. conversion of hydrogen to methane.

Determination of the rate-limiting step in the anaerobic digestion of complex, insoluble biodegradable molecules may be problematical: the rates of degradation depend not only upon their structures and substituent groups, but also upon the types of bacteria present in the digester and the efficiencies of these in substrate conversions under the prevailing conditions of influent flow rate, temperature and pH. Kaspar and Wuhrmann (1978) suggest that where soluble substrates are dominant, the rate-limiting reaction would tend to be the formation of methane from acetate rather than from fatty acids under steady state conditions. These authors conclude that a large surplus capacity of hydrogen-consumption in the digester buffers the partial pressure of dissolved hydrogen and stabilises

FIG 01

PATHWAY OF ANAEROBIC BIODEGRADATION (AFTER GUJER & ZEHNDER (1983) AND OTHERS)



1. Hydrolysis
2. Fermentation
3. Anaerobic (β) Oxidation
4. Anaerobic Oxidation
5. Decarboxylation of Acetate $CH_3COO^- + H_2O \longrightarrow CH_4 + HCO_3^-$
6. Hydrogen Oxidation $CO_2 + 4H^+ \longrightarrow CH_4 + 2H_2O$

the methanogenic ecosystem at values of pH low enough to allow the rapid oxidation of fatty acids.

In an anaerobic digester, the size of each group of bacteria will be in proportion to the amount of its particular substrate in the reactor system: the bacteria or microbial groups form the catalyst in each biologically mediated reaction. However, the quality of catalyst will not limit the rate of the reaction: substrate or nutrient concentration will be rate-limiting (Hobson 1983).

The major gases formed in the degradation process are methane and carbon dioxide and the fluxes of the diverse substrates or intermediary products are expressed as COD. If the main sink of electrons (or hydrogen) is carbon, as opposed to sulphur or nitrogen, then COD reduction results in the formation of methane (Gujer & Zehnder 1983).

The anaerobic conversion stages occurring in digesters have been analysed and expressed in terms of discrete reactions, as continuous cultures with one substrate that is limiting but with an excess of nitrogen (Hobson 1983; Hobson & McDonald 1980). Substrate utilisations can be described by simple Monod kinetics applicable to bacterial growth in pure culture chemostats. The growth rate of the biomass is assumed to be related to the biomass concentration:

$$\frac{dX_B}{dt} = \mu X_B \qquad (1)$$

where X_B = biomass concentration

μ = specific biomass growth rate.

The specific biomass growth rate is not a constant but

is related to the residual substrate concentration, S_2 in the system. The semi-empirical equation developed by Monod (1949) predicts that:

$$\mu = \frac{\mu_{max} S_2}{K_S + S_2} \qquad (2)$$

where μ_{max} = maximum specific biomass growth rate

K_S = half velocity coefficient

K_S describes the substrate concentration which supports a specific growth rate that is half the maximum rate, i.e. it provides an indication of the ease of biomass assimilation of the substrate.

Where one limiting dissolved substrate is involved, a basal model of degradation in digesters describes the residual substrate at different detention times in terms of the equation applied to completely mixed continuous cultures (Hobson 1983). At a dilution rate, D, at steady state, the residual limiting substrate concentration, S_2 is given by:

$$S_2 = \frac{D K_S}{\mu_{max} - D} \qquad (3)$$

Under steady state digester conditions, the bacterial growth rate is equal to the dilution rate, i.e.:

$$\frac{dX_B}{dt} = 0 \qquad (4)$$

and:

$$\mu = D = \frac{\mu_{max} S_2}{(K_S + S_2)} \qquad (5)$$

The concentration of substrate available for bacterial

assimilation governs, within limits, the growth rate of bacteria in the system; S_2, therefore, increases with the increase of growth rate imposed by the dilution rate, D. As the value of D approaches μ_{max}, the biomass begins to wash out of the vessel. The influent substrate concentration term, S_1, is not included in equations (3) and (5), and the substrate utilised by the microorganisms (S_1-S_2) governs only the amount of bacterial growth. The rate of substrate removal or utilisation can, however, be related to the influent substrate concentration by an equation similar to (5):

$$k = \frac{r_{x,max}\, S_2}{K_s + S_2} \qquad (6)$$

where k = substrate utilisation rate $[(S_1-S_2)/T]$

$r_{x,max}$ = maximum substrate utilisation rate

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

$$Y = \frac{\text{mass of bacteria formed}}{\text{mass of substrate removed}} \qquad (7)$$

where Y = biomass yield coefficient.

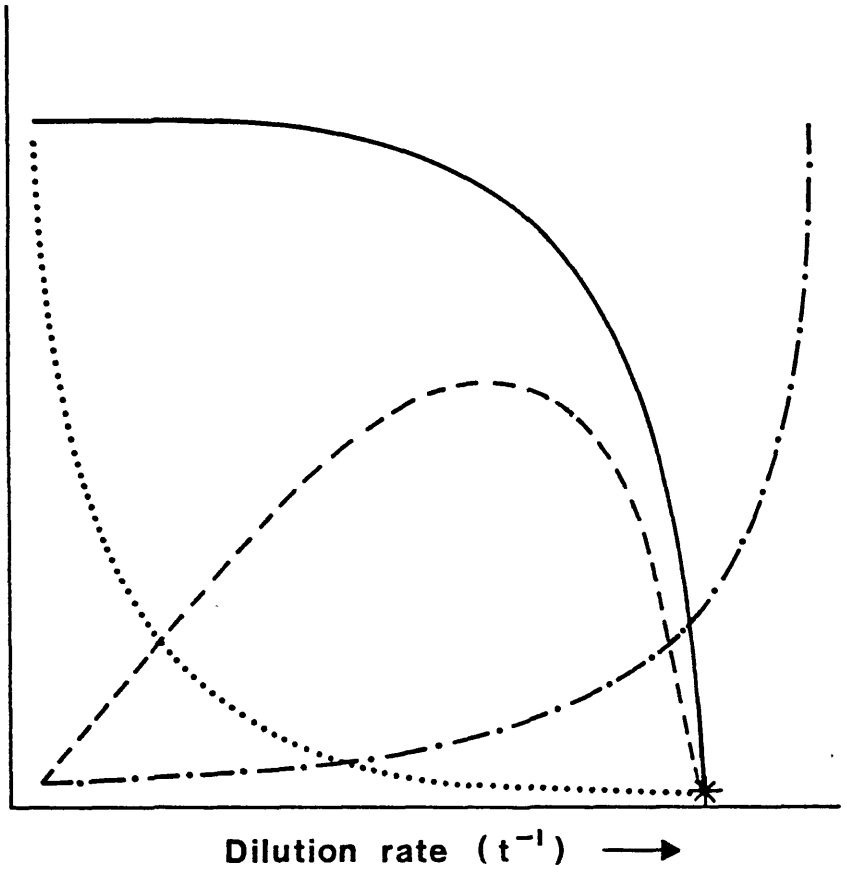
The biomass yield is of significance in the economic considerations of specific treatment processes, as it indicates the volume of excess sludge which will be produced and which will require treatment and disposal. A low yield coefficient is advantageous since it indicates that most of the influent carbon is being converted to methane and little resultant sludge accumulates. However, too low a biomass yield can also render the reactor sensitive to the effects of shock loading.

Substrate concentrations are expressed in grams or milligrams per litre, as these units are generally used in descriptions of experimental digesters (Hobson 1983). The rate of substrate utilisation (r_x) can thus be expressed as kg COD kg^{-1} VSS d^{-1} and the yield coefficient (Y) as kg VSS $(kg\ COD)^{-1}$. The dilution rate is the ratio of the flow rate of the pump, F, (l h^{-1}) and the volume of the digester, V, (l), being the flow rate per unit volume, or F/V; in comparisons of theoretical and experimental results, the reciprocal of this parameter (1/D), the retention time, is conveniently used. The variation of key digester features with dilution rate is shown in Figure 2 and the growth constants applicable to some anaerobic cultures are presented in Table 2.

Very few, if any, digester influent streams are completely homogeneous. Where complex substrates are present, bacterial degradation will not occur at the same rate for every specific substrate. The cumulative effect, nevertheless, of all the conversions occurring in the system will generally produce a smooth curve of degree of degradation against retention time (Hobson 1983).

As bacteria in general can only take up organic matter in soluble form, microbial assimilation of heterogeneous, particulate biopolymers requires breakdown or hydrolysis (Figure 1) as the first step. This process is mediated by extracellular enzymes, the reaction rates of which are influenced by pH, cell residence time and waste content of the digester. The pool of particulate material in the system must also be considered to include newly synthesised biomass (Gujer & Zehnder 1983).

FIG.02



Cell numbers are maintained over a range of dilution rates; at the point where substrate influent rate exceeds cell growth rate (D_{crit}), organisms wash out exponentially and S_2 increases rapidly.

VARIATION OF KEY DIGESTER FEATURES WITH DILUTION RATE.

TABLE 2 ANAEROBIC GROWTH CONSTANTS (after Henze & Harremoes 1983)

Culture	μ_{max} 35°C d^{-1}	Y_{max} maximum yield coefficient kgVSS(kgCOD)$^{-1}$	$r_{x, max}$, 35°C kgCOD(kgVSS.d^{-1})$^{-1}$		K_s kgCODm^{-3}
			100%active VSS	50%active VSS	
Acetogenic bacteria	2.0	0.15	13	7	0.2
Methanogenic bacteria	0.4	0.03	13	7	0.05
Combined culture	0.4	0.18	2	1	-

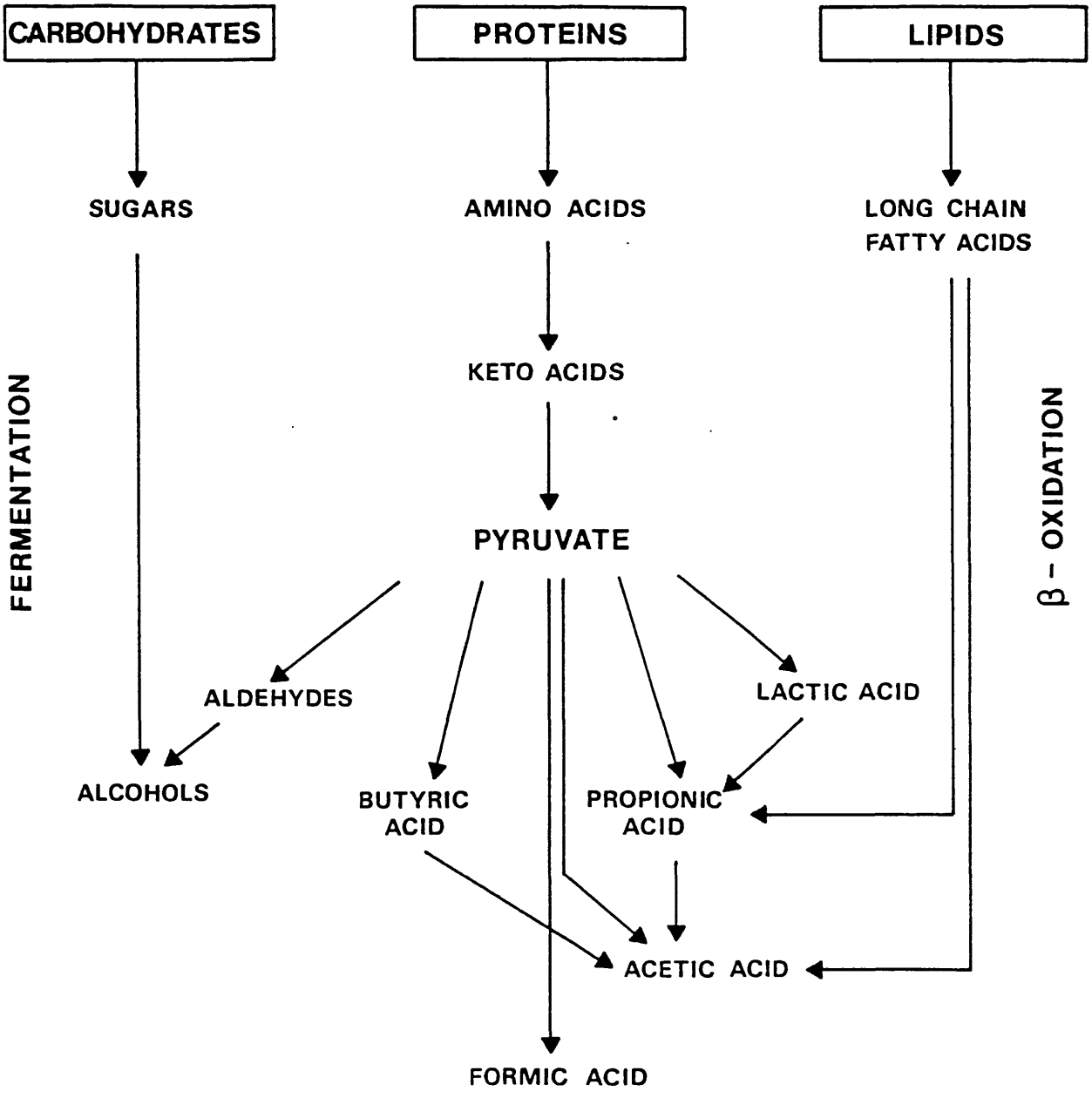
In a digester system stabilising domestic sludge under steady state conditions (i.e. HRT less than 12 days at 33°C), the rates of production of soluble organic materials are balanced by similar removal rates, thus ensuring that there is little accumulation of these dissolved organics (Cohen et al. 1982). The soluble fraction of the total organic carbon (TOC) in the system is generally less than 10% of the whole (i.e. present as dissolved organic carbon or DOC). In an anaerobic digester with a mean HRT of 40 days at 33°C, very short mean residence times of acetate (1 h) and propionate (0.7 h) were recorded (Kaspar & Wuhrmann 1978). The products of hydrolysis do not accumulate, and the distribution of the individual volatile fatty acids has been demonstrated to be variable, illustrating the differences in the pathways of catabolism utilised by the digester bacteria (Eastman &

Ferguson 1981).

The breakdown products of the hydrolysis phase form the substrates of the intermediary stages of the anaerobic digestion process (Figure 1). The acetogenic bacteria are the predominant microflora and acetate production is the principal result of their activity although higher volatile acids such as propionate, butyrate, iso-butyrate, valerate and iso-valerate may also be formed. The major routes of product formation by the acidogens are shown in Figure 3. Their conversions are either fermentations or β-oxidation processes.

Fermentation may be described as a microbiological reaction whereby organic compounds act as both electron donors and electron acceptors, whereas in anaerobic β-oxidation, the main sink for electrons is molecular hydrogen (Figure 1). It has been established in several instances that removal of the electrons generated in glycolysis during acid-forming metabolism can occur via the mechanism of interspecies hydrogen transfer, from acidogenic to methanogenic bacteria (Hungate 1967; Schiefinger et al. 1975; Winfrey et al. 1977).

During long chain fatty acid degradation, hydrogen production inhibits the reaction (Novak & Carlson 1970). Methanogenesis from hydrogen (stage 6 of Figure 1) is extremely dependent on pH and hence hydrogen accumulation at low pH inhibits anaerobic oxidation. Not all the intermediates present in sludge and wastewater treatment systems at steady state have been clearly identified and little is known of them as their concentrations are usually so low as to preclude extensive investigation. Around 70%



THE MAJOR ROUTES OF PRODUCT FORMATION BY THE ACID - PRODUCING BACTERIA (after Henze & Harremoes 1983).

FIG. 03

of the methane generated originates from acetate metabolism, but this figure includes the acetate formed by the degradation of higher fatty acids by the acetogenic bacteria (Kaspar & Wuhrmann 1978). The role of higher VFAs as digestion intermediates has been examined: it was estimated that 15% of the methane generated by anaerobic sludge digestion originated from propionate (Kaspar & Wuhrmann 1978); figures of 13% methane from propionate and 8% from butyrate during mesophilic digestion of cattle waste have also been reported (Mackie & Bryant 1981).

The reaction generating methane from acetate is a decarboxylation:

$$*CH_3COO^- + H_2O \longrightarrow *CH_4 + HCO_3^- \quad (8)$$

The conversion (stage 5 in Figure 1) has figured prominently in several investigations, not least because acetate is the dominant intermediary fermentation product in the decomposition of organic compounds under anaerobiosis in natural habitats (Kaspar & Wuhrmann 1978). The methanogenic breakdown of acetate has been kinetically analysed and four discrete phases distinguished (Powell et al. 1983):

1. a rapid methanogenic rate increase upon acetate addition;
2. an exponential rate increase with time, reflecting the exponential growth of methanogenic bacteria;
3. an approximately constant rate of methane production; and,
4. a rapid decline associated with acetate depletion.

The growth kinetics for acetate decarboxylating processes have been derived using enrichment cultures

(Lawrence & McCarty 1969), the organism involved probably being Methanosarcina spp. from which similar kinetic parameters have since been obtained (Smith & Mah 1978) (Table 3).

The principal acetate cleaver in the majority of anaerobic digesters is likely to be Methanothrix soehngenii, however, as it has a high affinity (and thus a small K_s) for acetate. This species has been reported in several reactors in the absence of Methanosarcina, and at HRTs greater than 15d at 35°C can successfully outcompete other bacterial systems (Gujer & Zehnder 1983). However, at high substrate concentrations, comparison of the typical growth kinetics of Methanosarcina and Methanothrix spp. shows that the former will predominate.

Methanogenesis from hydrogen is also a pH dependent conversion (Huber et al. 1982; Wildgruber et al. 1982). The following reaction occurs:

$$CO_2 + 4H_2 \longrightarrow CH_4 + 2H_2O \qquad (9)$$

All methanogens examined to date are capable of methane formation by the oxidation of hydrogen and the reduction of carbon dioxide, and this metabolic feature unites the diverse species of methanogenic bacteria (Balch et al. 1979).

The combined and coordinated metabolic activity of an anaerobic reactor population is required for the complete degradation of complex organic matter to carbon dioxide and methane. The intermediates necessary for certain microorganisms are produced as a consequence of the action of others and therefore consortia of bacteria are

TABLE 3 GROWTH KINETICS FOR METHANOGENESIS FROM ACETATE

T (^{o}C)	μ_{max}(d^{-1})	K_s (mgl^{-1}COD)	Source	Organism
25	0.24	930	Lawrence & McCarty (1969)	Methanosarcina*
30	0.24	356	Lawrence & McCarty (1969)	Methanosarcina*
35	0.34	165	Lawrence & McCarty (1969)	Methanosarcina*
35	0.44+	250+	Smith & Mah (1978)	Methanosarcina*
37	0.43	369	Massey & Pohland (1978)	Methanosarcina?
37	0.21+	241+	Wandrey & Aivasidis (1983)	M. barkeri

*Figures derived by Gujer & Zehnder (1983)

+Approximate values

frequently involved in these conversions.

A greater proportion of obligate, in comparison to facultative, anaerobes has been frequently isolated from anaerobic sludge digestion systems: obligate organisms have been reported to be present at 10 - 100 times the numbers of facultative bacteria, the results being attributed to the low Eh value of the environment (Mah & Sussman 1968). In digesters treating animal wastes however, facultative anaerobes comprised approximately half of the isolates obtained; these indicated the cellulosic nature of the influent and the hydrolytic functions of the digester flora (Hobson & Shaw 1974). The component microorganisms of a particular anaerobic regime are influenced by the reactor feedstock: the facultative population of a sewage digester was found to consist mainly of Escherichia coli, a dominant

coliform bacterium in the human digestive system, but upon acclimation to pig-waste as feedstock, the predominant facultative anaerobes subsequently isolated proved to be the streptococci of the porcine gut (Hobson & Shaw 1974).

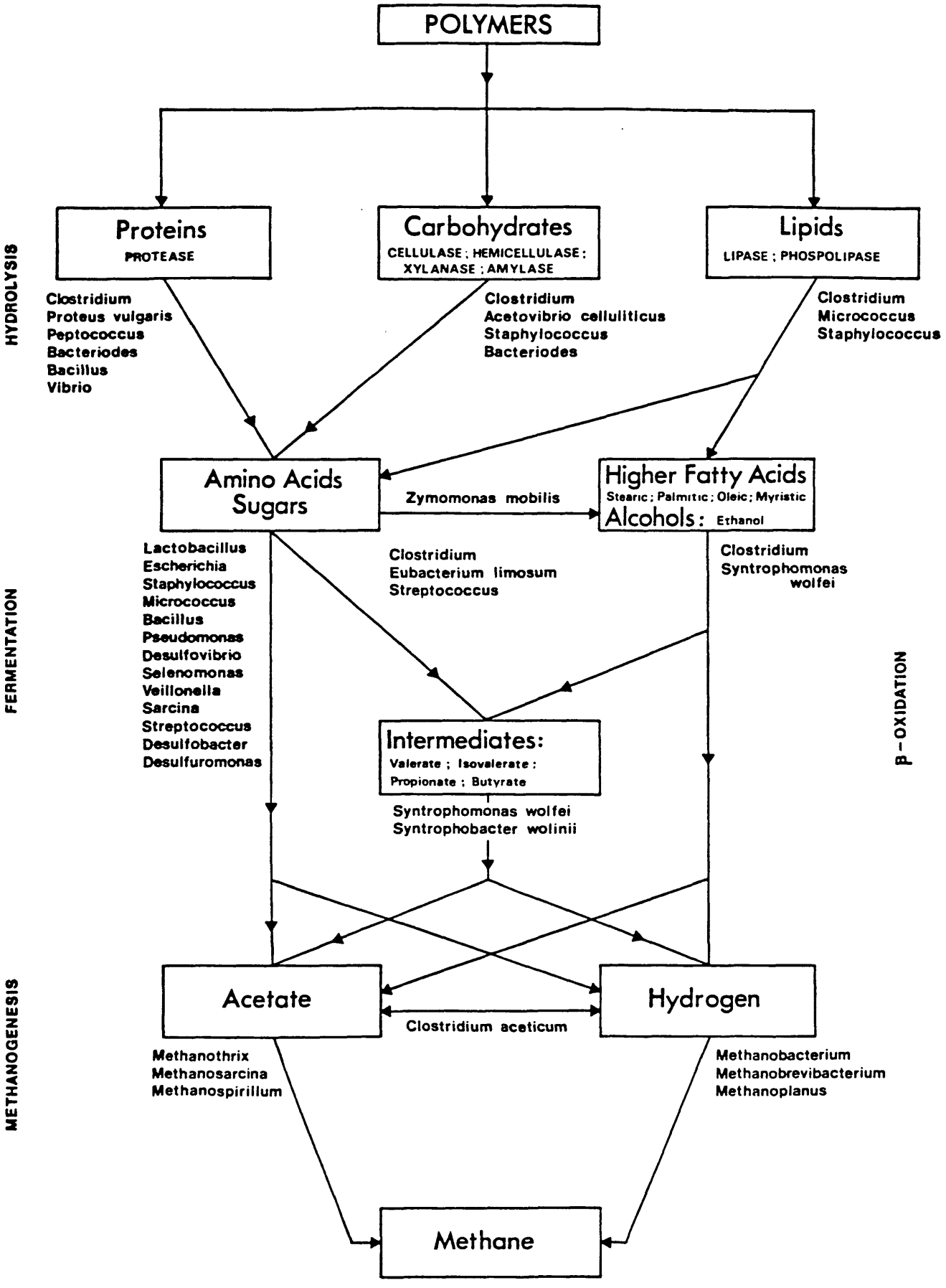
The majority of investigations into the microbiology of anaerobic digestions are based on mesophilic systems, and although the bacterial types may differ in thermophilic operations, similar reactions probably occur. Of the total population of a mesophilic digester, 9% were found to be thermophilic (55^{o}C) and 1% proved to be obligate thermophiles (60^{o}C) (Chin 1983).

Throughout the start-up phase of an anaerobic digestion process, the heterogeneous groups of microorganisms evolve in succession until the biocenosis reaches stabilisation and the various groups attain their final proportions (Hobson & Shaw 1974).

Hydrolysis of macromolecules such as lipids, proteins and carbohydrates under anaerobic reactor conditions is primarily an extracellular enzymic reaction and many microorganisms produce extracellular enzymes, chiefly hydrolases (Scott 1979). The function of hydrolases such as lipases, proteases and cellulases is the degradation of complex molecules into units which can be assimilated by the microbial cell. In an anaerobic digester where organic polymers form a substantial portion of the influent waste, the hydrolysing bacteria and their enzymes are of paramount importance because their activity produces the simpler substrates for the succeeding steps in the degradation sequence (Figure 4).

It has been suggested that high concentrations of

FIG.04



THE BREAKDOWN OF ORGANIC POLYMERS

hydrolysis products inhibit the hydrolytic enzymes of the relevant bacteria (Eastman & Ferguson 1981). Catabolite repression of enzyme synthesis is known; synthesis of the α-amylase of B. licheniformis and B. subtilis is repressed by glucose and by fructose in the case of B. stearothermophilus (Fogarty & Kelly 1979). The production of proteolytic enzymes is repressed by amino acids in many microorganisms but the proteases may also be repressed by glucose (Fogarty & Kelly 1979). However, some extracellular enzymes are also inducible. End-product inhibition of proteases by amino acids is known and the specific repressive amino acids identified in a number of cases (Fogarty & Kelly 1979).

The second stage in Gujer and Zehnder's (1983) general outline of the digestion process is the fermentation of acids and sugars, giving rise to the intermediary products, acetate and hydrogen (Figure 4). Acetate is the most important compound quantitatively produced in organics fermentations by bacteria, with propionate production of secondary consequence (Toerien & Hattingh 1969; Sorensen et al. 1981). Only a limited number of microorganisms appear to mediate the further breakdown of propionate under anaerobic conditions (Widdell & Pfennig 1982). The anaerobic degradation of amino acids has been examined in terms of the interactions between methanogenic and amino acid-degrading bacteria (Verstraete et al. 1981; Nagase & Matsuo 1982): certain amino acids were reported to degrade oxidatively (cf. Figure 4) by dehydrogenation, with the associated methanogens acting as hydrogen acceptors. Acidic end-products and ammonia are formed from the amino

or amide groups of amino acids. The catabolism of these organic compounds is mediated by a large number of both obligatory and facultatively anaerobic organisms including clostridia, mycoplasmas and streptococci and the process utilises single amino acids, pairs of amino acids or a single amino acid in conjunction with a non-nitrogenous compound (Cooney 1981).

Acetate is the main fermentation product under stable digester conditions but it can also be produced by fatty acid oxidation. In reactors treating sewage wastes, around 30% of the overall solids content of the digestible influent may be fats; in animal wastes fats comprise about 5 - 15% of the total solids (Hobson 1982). The majority of the fats are triglycerides, i.e. glycerol substituted by fatty acids, and β-oxidation has been reported to be the mechanism of degradation of the fatty acids (Gujer & Zehnder 1983) to release an acetate molecule at each reaction. The cyclic pathway of β-oxidation is repeated until the saturated fatty acid is completely converted to C_2-units. Where a saturated fatty acid containing an odd number of carbon atoms is to be degraded, the last compound will be a C_3-unit or malonyl-CoA. This is an energetically unfavourable reaction and the presence of two bacteria is required (McInerney et al. 1979; Boon & Bryant 1980).

Those microorganisms which produce acetate from propionate, butyrate and other higher fatty acids form the group known as the obligate hydrogen producing acetogenic (OHPA) bacteria (Verstraete et al. 1981). The hydrogen and acetate synthesised by the metabolism of the OHPA digester population have been estimated to provide the

substrate for 54% of the total methane produced in anaerobic reactors (Kaspar & Wuhrmann 1978). These bacteria are intolerant of pH fluctuations and must be maintained at neutral pH for maximum efficiency; their doubling times are of the order of 2 - 6 days (McInerney et al. 1979; Boon & Bryant 1980), i.e. longer in general than even those of the methanogens. The syntrophic association of OHPA and methanogenic bacteria allows the latter to assimilate the hydrogen produced by the former.

Butyrate and propionate, as well as acetate, are thus converted to methane, the most reduced organic molecule. Methanogenesis is a conversion of hydrogen and carbon dioxide by the methane-producing bacteria, generally considered to be the most oxygen-sensitive of the bacteria known and hence the most strictly anaerobic.

The isolation and cultivation of methanogens depend upon procedures which produce strictly anaerobic conditions. Those techniques developed from the pioneering work of Hungate (1950;1969; Latham & Wolin 1978) have proved the most successful. Methanogens have also been presumptively identified by the use of fluorescence microscopy, which utilises the properties of the chromophores F_{420} and F_{342} (Mink & Duggan 1977; Doddema & Vogels 1978) found in all methanogenic species.

The methanogens cannot utilise complex organic compounds and their energy metabolism is directed towards a system that produces methane as the only significant end-product. All methanogenic bacteria examined to date oxidise hydrogen and reduce carbon dioxide to methane. Some species metabolise formate, while the members of

Methanosarcina will also utilise methanol, methylamine (dimethyl-, trimethly- and ethyldimethylamine) and acetate as sole electron donor for growth and methane production (Balch et al. 1979).

The importance of sulphate-reducing microorganisms in organic polymer degradation to methane in anaerobic reactors cannot be underestimated. Species of the genera Desulfovibrio and Desulfotomaculum are routinely isolated from digesters (Zeikus 1979) and populations of 10^4 sulphate reducers per ml of reactor fluid have been obtained (Toerien et al. 1968). Complex carbon compounds can be catabolised by many of these species in the presence of excess sulphate, or in situations where sulphate is limited but where hydrogen oxidising bacteria such as the methanogens are present (Bryant et al. 1972). The sulphate-reducing bacteria are known to grow via the coupled oxidation of immediate methanogen energy sources (i.e. acetate and hydrogen) to the reduction of sulphate (Widdell & Pfennig 1982).

One of the main reasons for the paucity of information on the various stages of anaerobic digestion is the difficulty in the cultivation of strictly anaerobic microorganisms; this difficulty can be traced to the extreme toxicity of oxygen to many anaerobic genera. Aerobic manipulation can be tolerated for short periods by some strict anaerobes (including methanogens) provided the culture media have been pre-reduced under anaerobic conditions (Patel et al. 1984).

1.3 THE BIOMASS OF ANAEROBIC REACTORS

Several anaerobic reactor types are utilised for waste treatment by biological means; these can be broadly divided into two groups, namely the fixed film reactors and the non-attached growth systems. The biomass of the former comprises bacteria attached as films to inert supportive media; the latter depend for their operation on the metabolic activity of microorganisms suspended as flocs or granules in the reactor vessel.

Almost any surface immersed in a fluid medium can be colonised by bacterial cells. The attached organisms grow and proliferate and many manufacture extracellular polymeric substances which form a structural matrix around the cell and beyond, eventually producing a structured mat of interlinked fibres, or biofilm. The generation of the biofilm on a surface under conditions of fluid flow is the result of several biological processes (Trulear & Characklis 1982; Characklis & Cooksey 1983), primarily:

1. transport of organic molecules, and their adsorption to the surface;
2. transport of microorganisms to the surface;
3. attachment of the bacteria by the recognised two-stage adhesion process, i.e. (i) rapid initial approach of cell to surface, influenced by electrostatic repulsion at long range and van der Waals attraction at short range, and (ii) firm attachment of cells by polymer bridging and steric interactions, a slower process;
4. production of the biofilm by the metabolic activity of the attached microorganisms; and,
5. detachment of areas of mature biofilm by hydraulic

forces (Figure 5).

Biofilms are produced on a wide variety of plastic, metal, ceramic and other components upon which the bacterial cells are typically enmeshed in the condensed remnants of their exopolysaccharide glycocalyces. Thick, adherent biofilms have been shown to protect their indigenous microflora from potentially harmful materials such as antibiotics at concentrations many times higher than planktonic in vitro Minimum Inhibitory Concentration (MIC) levels (Marrie et al. 1982). Detailed studies in pathogenic and industrial systems of glycocalyx-enclosed bacteria have indicated that the cells are protected to a marked extent because antibiotic and chemical agents cannot penetrate the anionic matrix of the enveloping glycocalyces (Costerton et al. 1981; Ruseska et al. 1982).

Flocculation is of practical significance in the treatment of wastes, as flocculated microstructures are easily collectable by sedimentation from separated streams in a wastewater treatment plant. The phenomenon is of particular importance in the contact and carrier-assisted contact processes, and also in the upflow sludge blanket reactor system. The formation of flocculated structures can occur by several means: these are listed in Table 4. Although controversy exists as to the actual mechanism operating, the general consensus of opinion points to elucidation of the phenomenon by the concepts of colloid science.

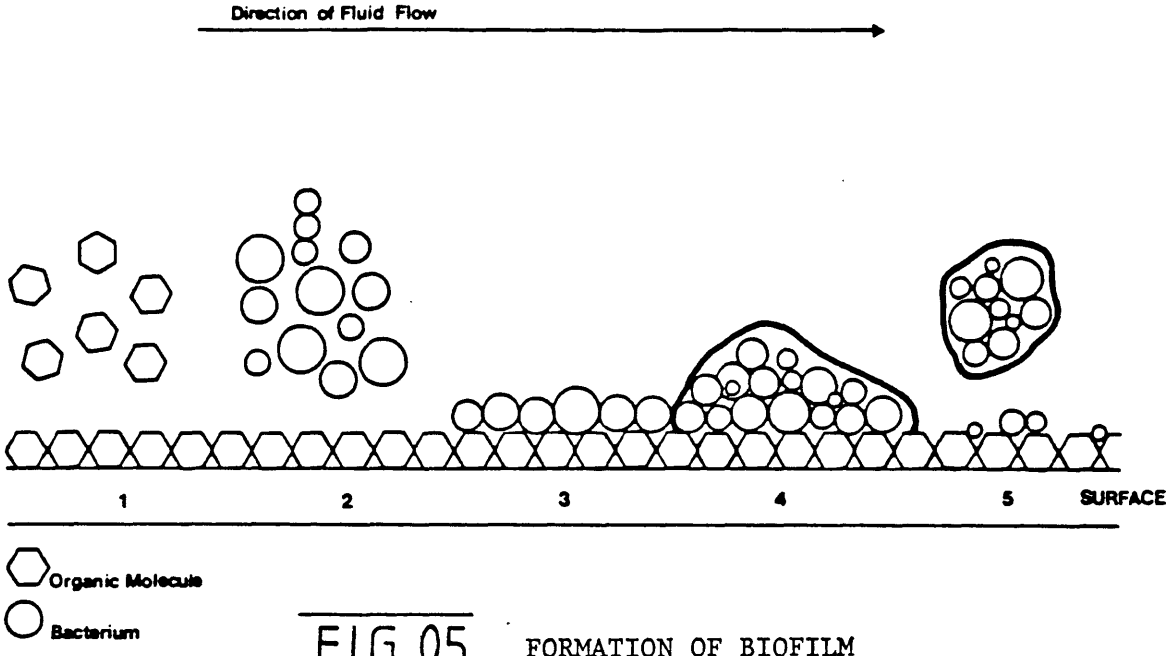
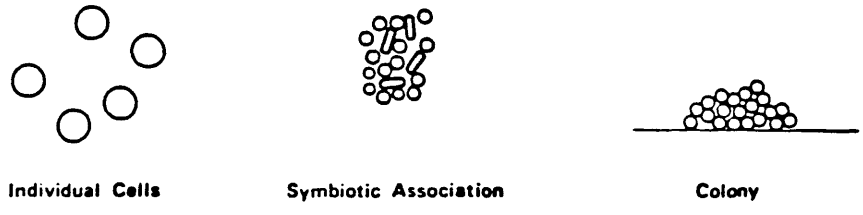


FIG. 05 FORMATION OF BIOFILM
(after Trulear & Characklis, 1982)



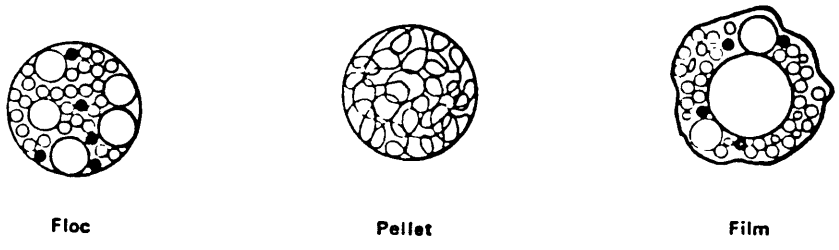


FIG. 06 FORMS OF BIOMASS

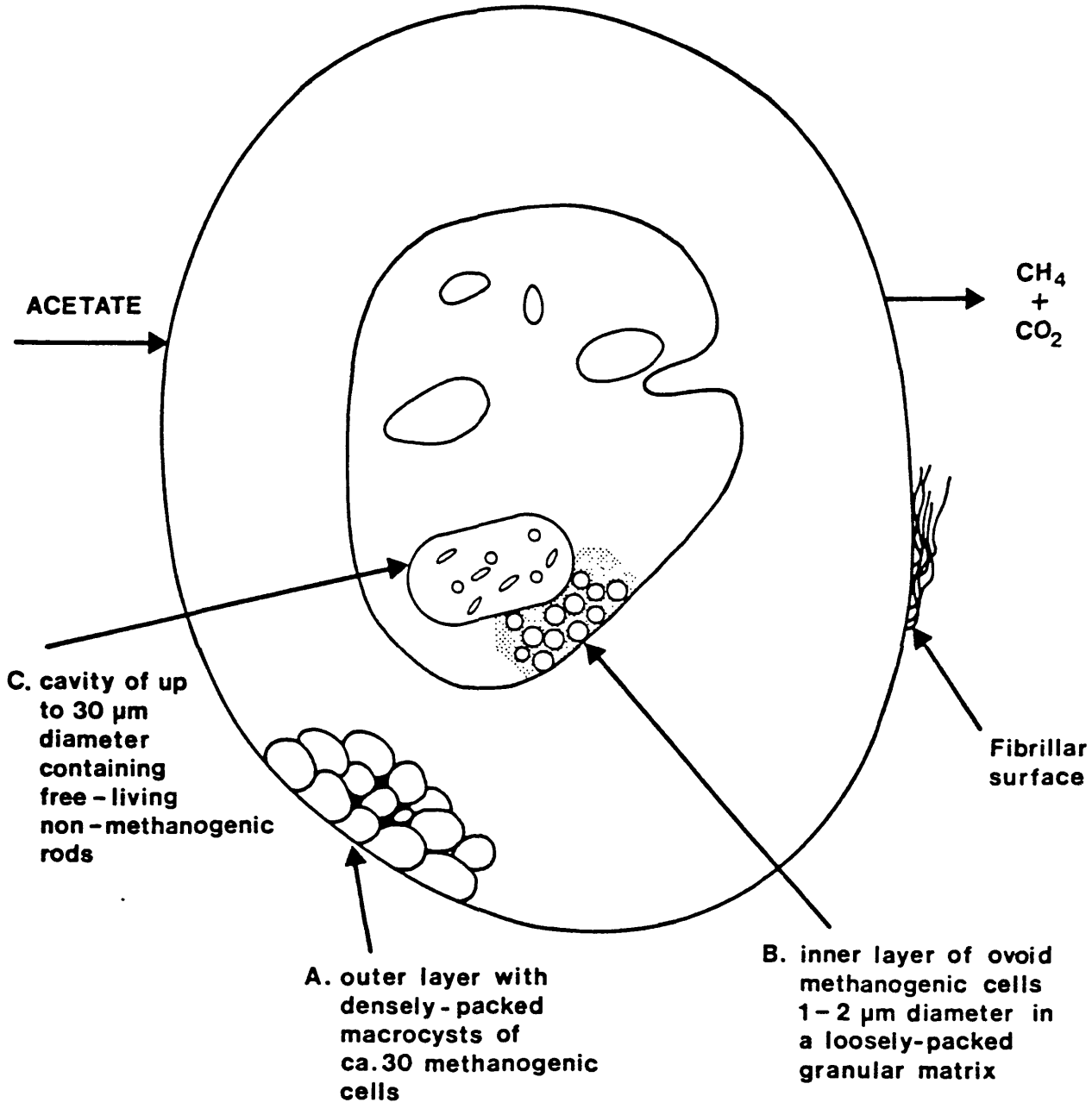
TABLE 4 MAJOR FACTORS FAVOURING BACTERIAL AGGREGATION (after Ash (1979))

Gel entrapment	Viscous effect
Incipient flocculation	Interaction between adsorbed or attached microorganisms - short range forces
Charge-mosaic interaction	Flocculation by cationic polyelectrolytes
Polymer bridging	Flocculation by polymer addition - short range forces

The phenomenon of microbial pellet or granule formation has been recorded in many instances (Cohen et al. 1979; Lettinga et al. 1979a; Hulsoff-Pol et al. 1983; Ross 1984), but in the treatment of wastewater, granulation appears to be restricted to upflow anaerobic sludge blanket (UASB) reactor types, and generally to those treating carbohydrate-rich or VFA-type wastes (Ross 1984). Films and flocs are continuous but pellets may be networks of filaments (Figure 6). An examination of the bacterial morphology of pellets from a full-scale upflow anaerobic clarigester by scanning electron microscopy revealed rod-like and filamentous microbial forms and also the presence of extracellular polymer (Ross 1984). The pellets were observed to be held intact by fibrous strands interwoven with the component granule bacteria; the network of strands was generally composed of polysaccharide.

The morphology and ultrastructure of thermophilic granules have been investigated by light and electron microscopy (Bochem et al. 1982). Three distinct strata could be visualised in pellets of 1 - 3 mm in diameter (Figure 7).

The importance of extracellular polymeric material to



REPRESENTATION OF A GRANULAR CONSORTIUM

(after Bochem et al. 1982).

FIG.07

adhesion and film and floc formation had already been noted; the aggregation of bacteria into pellets under the low-shear conditions of the UASB reactor system may also be strongly influenced by polymers (Ross 1984) as microbial agglutination and accumulation of extracellular material have been shown to be related (Brown & Lester 1979).

Pellets, therefore, appear to be granular consortia of various types of bacteria, whose interspecific relationships have not been fully clarified and whose formation depends critically upon wastestream composition and digester conditions.

The various forms of biomass presently utilised in anaerobic waste treatment are outlined in Table 5.

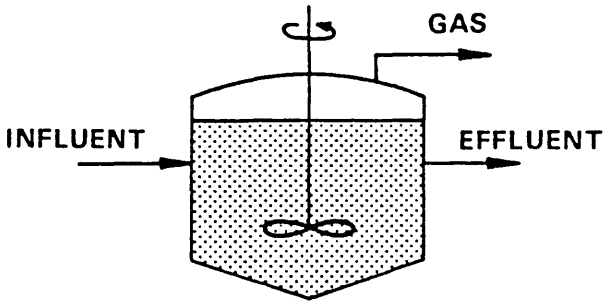
TABLE 5 BIOMASS FORMS IN ANAEROBIC REACTORS

Systems of Biomass Retention	Biomass Form	Anaerobic Reactor Type
Sedimentation	Floc	Contact process
Sedimentation	Pellet/Granule	UASB
Adsorption by non-porous inert support medium	Biofilm	Anaerobic filter RBC Expanded bed Fluidised bed
Entrapment by porous inert support medium	Biofilm	Anaerobic filter Fluidised bed

1.4 ANAEROBIC REACTOR SYSTEMS

In common with many of the anaerobic bioreactor systems, the simple mix digester or continuously stirred tank reactor (CSTR) (Figure 8) was developed from its

FIG.08



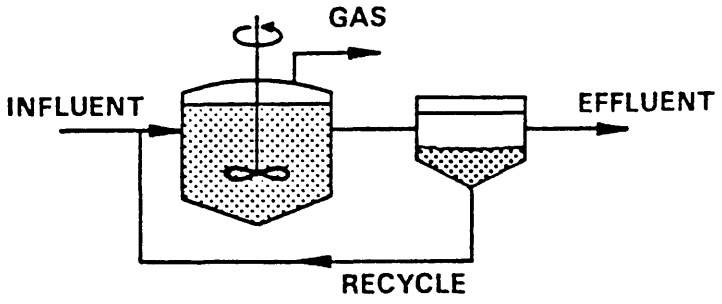
stirred tank reactor

aerobic counterpart.

The CSTR is the most simple of the anaerobic digesters in common use. The SRT and the HRT in this type of reactor are equal and tend to be protracted; high rate anaerobic treatments using the CSTR are therefore not possible. These systems have however been utilised successfully for the stabilisation of sewage sludge and the conversion of industrial wastewaters which contain high solids concentrations, such as crop residues (Morris 1979).

The Contact or recycled flocs process comprises a continuously-fed, completely mixed reactor stage followed by solids/liquid separation (Figure 9). A degasification step is frequently included in system design. The effluent is discharged from the settling device and the settled biomass returned to the digester vessel, where it is mixed

FIG.09



contact process

with the incoming feed (Sutton et al. 1982). Reinoculation of a well-acclimated sludge can maintain optimum stabilisation of industrial wastewaters, which, unlike sewage sludges for example, do not generally contain a high proportion of microflora.

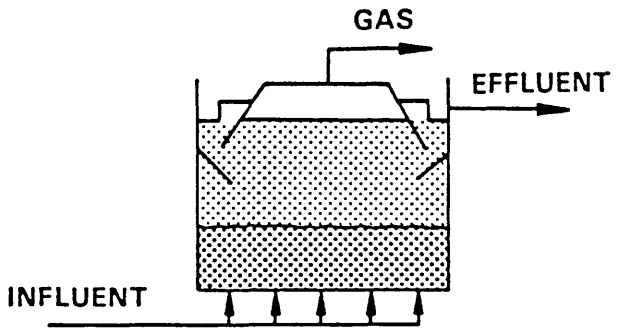
The anaerobic contact process was initially developed to treat meat packing waste and has subsequently been used for food processing and other wastes. The process was one of the first of many anaerobic digestion systems to incorporate the retention of microorganisms in the digester independent of HRT.

The upflow anaerobic sludge blanket (UASB) reactor (Figure 10) was initially developed for widespread use in the Netherlands (Lettinga et al. 1980). Inert media are generally absent from the system and the biomass is maintained in suspension by gas bubbles. The bacteria develop as a flocculant mass in an upward-flowing wastestream. The microbial blanket is retained by its own mass and by baffles or screens forming the settler unit in the upper portion of the reaction vessel, whilst gas and liquid escape from the top of the tank (Henze & Harrremoes 1983).

One of the two fundamental design principles for maintenance of high sludge retention in the UASB is founded on sludge with improved sedimentation properties. Larger granules form the sludge bed or lower portion of the reactor, developing after a few months of system operation (Christensen et al. 1984). The UASB system is mixed by hydraulic upflow and rising gas bubbles and improved agitation can be achieved by the use of several influent ports to deliver feed to the vessel. COD removal occurs throughout the bed and blanket (Christensen et al. 1984). The sludge concentration in the blanket is in the region of 10-30g VSS l^{-1} and the average reactor sludge concentration is about 20-40g VSS l^{-1} (Heijnen 1983). An investigation into the effect of sludge loading rate on granulation in UASB systems indicated that pellets formed only at loading rates in excess of 0.6 kg COD kg^{-1} VSS d^{-1}, while at 0.3 kg COD kg^{-1} VSS d^{-1} bulking and washout occurred (Hulsoff-Pol et al. 1983).

The second main design principle of the UASB is in the

FIG.10



sludge blanket reactor

installation of a liquids/solids separating or settling device in the upper part of the reaction vessel. The smaller particle size and flocculation characteristics of the blanket zone give rise to a settling rate inferior to that found in the bed, thus to permit retention of the blanket sludge, the applied liquid velocity in the settler unit should be relatively low (0.5-1.0 mh^{-1}); higher liquid velocities tend to produce unacceptable sludge losses (Christensen et al. 1984). However, these lower velocities allow the accumulation of any sediment from the influent wastewater in the reactor and in the sludge, resulting in decreased sludge activity. The UASB is not, therefore, an effective treatment process for wastewater containing high suspended solids concentrations and organics in the waste must possess a proportionately high degree of solubility.

The total suspended solids (TSS) should be limited ideally to approximately 500 mg l^{-1} (Christensen et al. 1984).

One of the most serious limitations of the sludge blanket process can be the considerable time involved in the initiation of digestion. The washout of sludge during the initial phases of operation is often significant and the consequent loss of net bacterial growth with the effluent can engender a depression in the retained sludge volume and stagnation in the gas production phase of the reaction (Hulsoff-Pol et al. 1983). Wastewater composition plays a major part in the operation of UASB reactors; granulation of sludge proceeded satisfactorily in one investigation when yeast, sugar beet and potato wastes formed the substrates, whereas problems arose with distillery, corn starch and rendering wastes (Hulsoff-Pol et al. 1983).

Initial seeding with an active digester sludge is a prerequisite for effective start-up of a UASB, as are prevention of pH and toxic shocks, gradual loading increases and extended HRTs. Start-up therefore is dependent upon an equilibrium between loading and washout as well as the selection of a suitable seed sludge, wastewater composition and careful system management; the addition of fine sand or other solids is not uncommon as a start-up aid (Henze & Harremoes 1983).

The UASB was reported to perform well in both the thermophilic and the mesophilic ranges (Schwartz et al. 1981), although pH in the thermophilic reactors employed was slightly higher than the optimum range. Substantial buffering developed in the reactors within a few weeks

subsequent to start-up and no further pH adjustment to feedstock was required. Some investigators have reported the advantages in maintaining pH at or near neutrality in UASB reactors (Lettinga et al. 1979a; Christensen et al. 1984); reduction in COD removal is the frequent result of pH fluctuations. Thermophilic UASB systems typically produce a gas with a methane content of approximately 3-4% less than that of their mesophilic counterparts. A comparison of 35^{o}C and 50^{o}C operations in UASB reactors suggested that the systems maintained in the mesophilic range were more efficient in terms of COD and BOD removal and TSS reduction at organic loadings greater than 18g COD l^{-1} d^{-1} (Schwartz et al. 1981). A sucrose/glucose influent applied to UASB digesters under thermophilic conditions and at a loading rate of 45kg COD m^{-3} d^{-1} was treated with an efficiency of 80% within three months of system initiation (Wiegant et al. 1983).

Increased loading is tolerated with little loss of stability by UASB reactors but variations in COD and BOD removal with increasing organic loading is not always consistent (Schwartz et al. 1981; Ronda 1986) and erratic TSS removal rates have been observed (Christensen et al. 1984). High suspended solids concentrations often recorded in sludge blanket system effluents may be in part a result of the design of the gas/solids separator, or intermittent blockage of some parts of the settler apparatus. Lane (1983) reported that UASB efficiency improved considerably as the organic load increased and postulated that this was an effect of the conditioning or adaptation of the sludge with time. At a loading rate of 11.15g COD l^{-1} d^{-1} and an

HRT of 7 days, 95% COD removal was achieved. The greatest washout rate (and therefore lowest sludge concentration) in a typical UASB system was observed to occur at low organic loading rates (Hulsoff-Pol et al. 1983).

One of the major problems of the UASB reactor design has been its sensitivity to feed interruptions: generally, a complete recycle of the entire system is necessary if process or instrumentation failure occurs (Lettinga et al. 1981; Lane 1983; Christensen et al. 1984). Although feed interruption has a severe adverse effect on the system, shock-loading of substrate appears to be better tolerated; in a methanol-converting UASB process, shock loads were observed to have a stimulatory effect on reactor activity (Lettinga et al. 1979a).

A number of wastestreams from various sources are amenable to treatment by the UASB process (Sanchez-Riera et al. 1985; Sax 1985; Rinzema et al. 1986). Due to the considerable progress made in the technical aspects of the UASB, the practical feasibility of the process has been proved for the treatment of wastewaters from the sugar beet industry (Lettinga et al. 1979a) and alcoholic waste from chemical sources (Lettinga et al. 1981). COD loadings of between 4 and 14 kg m^{-3} d^{-1} of sugar beet waste with a COD of 5000-9000 mg l^{-1} have been applied to a UASB, with 65-95% COD reduction obtained (Lettinga et al. 1981). In a system treating acetate-rich wastes, lower removals of up to 70% were noted and 1.6 kg COD m^{-3} d^{-1} was found to be optimum for biogas production; the system was self-scrubbing and the methane content of the gas reached 99% (Godwin et al. 1982). Filtered slaughterhouse wastes were

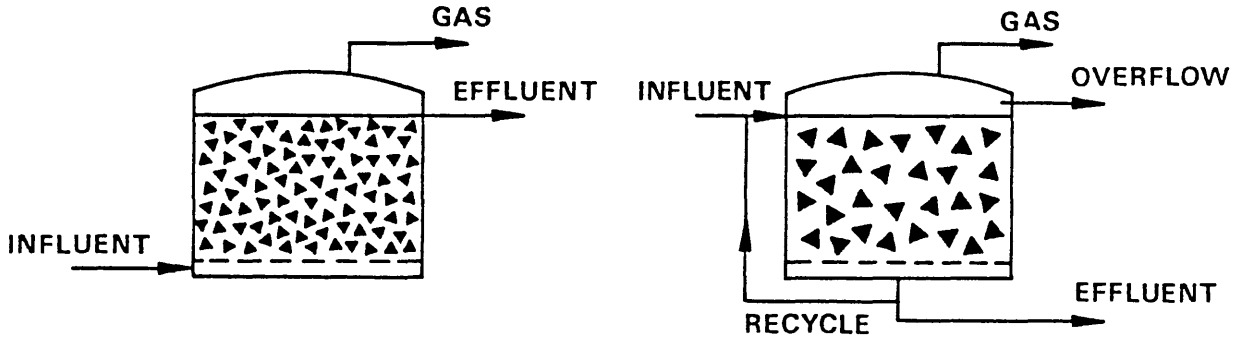
treated with 80% efficiency in a pilot-scale UASB at temperatures below 20°C (Sayed et al. 1981) although in general mesophilic and thermophilic operations are typical of these type of reactors.

The UASB configuration has been estimated to be particularly suited to the treatment of wastes with low suspended solids and high dissolved organic substances such as sugar wastes (Christensen et al. 1984), although a major problem in such systems is the high ammonia content which can be deleterious to microbial pellet production and thus to full efficiency of operation (Stafford & Etheridge 1983).

The settling and recycle of biomass present difficulties in the anaerobic treatment systems which depend upon freely suspended bacterial growth. Several techniques have elaborated on these processes by immobilisation of the biomass on or around carrier media. The anaerobic filter and rotating biological contactor systems require a relatively large quantity of inert media whereas the carrier-assisted contact process utilises very little (Henze & Harremoes 1983).

The wastewater in the anaerobic filter (fixed bed, packed bed) system passes the reactor usually with vertical flow, either upflow or downflow (Figure 11) although a horizontal flow unit has been described (Landine et al. 1982). The early investigations of upflow media processes in relation to the anaerobic conversion of wastes demonstrated that solids removal and gasification of the waste was feasible. The anaerobic filter assembly was first assessed by Coulter et al. (1957) and further

FIG.11



anaerobic filter (upflow configuration)

anaerobic filter (downflow configuration)

developed by the work of Young and McCarty (1969); these workers illustrated in their extensive laboratory studies the potential of upflow, fixed film anaerobic bioreactors for the treatment of dilute organic wastestreams and the production of biogas of methane content of up to 75%.

The microorganisms in the anaerobic filter attach to an inert medium or become entrapped. In the upflow design (Figure 11) the water flows upward through the support matrix and biogas is collected at the top. The system can function at ambient temperatures or be heated, or a hot wastestream may be utilised to maintain reactor temperature at a specified level. The bacterial mass in and on the inert medium rapidly degrades the substrates in the wastestream and although the biomass tends to disentrain at intervals its overall detention time in the digester may be

of the order of 20 days, thus permitting the growth of methanogens. Once a suitable microflora has developed to a sufficient degree it can be maintained permanently in the reactor to ensure continuous growth (Hobson 1982).

Various media types have been employed in both upflow and downflow anaerobic filters, of average diameter 0.2mm to 6.0cm and of both porous and non-porous structure (Huysman et al. 1983). Downflow filters may suffer blockages if an excessively small medium is employed (Cooper & Wheeldon 1981) and to minimise this, filter media tend to have relatively large diameters (>2.0cm). The surface area of medium per m^3 of reactor is therefore limited to values of approximately 200 m^2 m^{-3} (Jewell 1981). Due to this restricted area the sludge grows in thick layers of the order of 1-4mm around the packing particles and diffusion limitation probably occurs to some extent. The sludge concentration has been found to be between 10-20g VSS l^{-1} and the filter may still be susceptible to blockages from solids (Heijnen 1983).

The surface roughness of the filter packing media and the degree of porosity, in addition to pore size, affect the rate of colonisation by bacteria. A variety of materials have been examined as supporting matrices for use in downflow reactors by van den Berg and Kennedy (1981). These included polyvinyl chloride sheets, potters' clay, red drain tile clay, needle punched polyester and glass. Reticulated polyurethane foam was considered an excellent colonisation matrix (Huysman et al. 1983) although Fynn and Whitmore (1984) reported that the weak binding forces between the methanogens and such particles

could impose a limit on process intensification in anaerobic filters. Media used in full-scale and pilot tests have ranged from rock to a number of commercially available plastic and ceramic packings (Chian & de Walle 1977), in addition to a variety of materials such as cloth and brick (Young & Dahab 1982), gravel (Sachs et al. 1982), crushed stone (Anderson & Ibrahim 1978), Raschig rings (Plummer et al. 1968), anthracite and granular activated carbon (Khan et al. 1982). It has been indicated that small-diameter, loose-fill media should not be used with high strength carbohydrate wastes because of the rapid increase in biomass synthesis with this type of effluent and hence the potential for plugging (Young & Dahab 1982).

Descriptions of the operation and performance of up- and downflow filters have been provided by several investigators. The 'Anflow' system was developed as an energy-conserving wastewater treatment and a demonstration pilot plant of the upflow type to treat low strength, low temperature wastes was reported (Genung et al. 1979). The anaerobic filters of Schwartz et al. (1981), using a feed of thermally conditioned sludge decant liquors were claimed to perform well at both mesophilic and thermophilic temperatures. Changes in organic load did not produce instability and recovery after months of downtime was stated to be excellent. Methane was produced at steady state at around 0.33 m^3 kg^{-1} COD removed and the total gas evolved contained 60-68% methane. The mesophilic reactor did not suffer blockage although the quartzite media were coated in a substantial layer of solids.

The temperature range of the downflow anaerobic filter

is generally considered to be from 10-60^{o}C although most investigations have been performed at 35^{o}C. Messing (1983) found no advantage in operating a thermophilic (55^{o}C) over a mesophilic system and Duff and Kennedy (1982) recorded instability of thermophilic downflow reactors at 30-40 kg COD m^{-3} d^{-1}, whether hydraulically or organically overloaded. Stafford and Etheridge (1983) have indicated that reactors operating with extended SRT can be maintained at lower temperatures but strict temperature controls are paramount to system efficiency.

Waste types treated in upflow filter systems include domestic sewage, molasses distillery, pharmaceutical, vegetable processing, abattoir and synthetic stillage (Perkins et al. 1975; Braun & Huss 1982; Genung et al. 1982; Sachs et al. 1982; Sanchez-Riera et al. 1982; Young & Dahab 1983). The treatment of both strong and weak organic effluents has been demonstrated (Newell 1981) with up to 85% COD removal, while 90-99% COD removal from dairy wastes was achieved (Stafford & Etheridge 1983). Using a synthetic filter medium of high surface area Kobayashi et al. (1983) investigated the treatment of low strength domestic wastewater of 288mg COD l^{-1}. The average BOD removal rate was 79% and the COD removal 73%. Removal efficiencies showed little sensitivity to daily fluctuations in influent wastewater quality.

Downflow anaerobic filters have been employed to treat concentrated wastes of up to 130g COD l^{-1} (Messing 1983). Treatment of petrochemical wastes, with COD reductions of 93-95% have also been documented (Britz et al. 1983). Optimum retention time was 2.3 days at a loading rate of

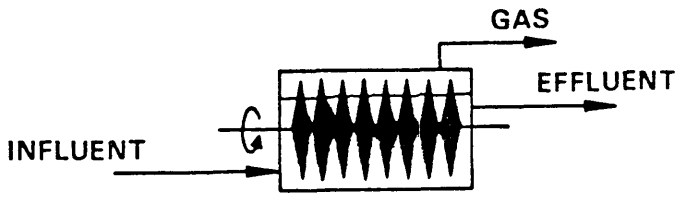
4.7kg COD m^{-3} d^{-1} with 0.88m^3 m^{-3} d^{-1} biogas produced, over 90% of which was methane. Van den Berg and Lentz (1979), in a comparison between upflow and downflow filters, observed that the differences in performance of the two types were negligible, both achieving COD removals of the order of 90% at 0.5-2.6 days HRT and at loadings of 3-15kg VS m^{-3} d^{-1} of bean blanching wastes. However, basic operational parameters were reported to differ, the downflow systems acting as fixed bed processes, while the upflow reactors appeared to operate as partially expanded or fluidised bed systems.

Anaerobic filters can resist high shock loads (Kennedy & van den Berg 1982) although their loading capacities are less than biofilm systems such as the fluidised bed, as a consequence of the larger sized media required for minimisation of clogging and short-circuiting. The buffering capacity of the wastestream should, moreover, be sufficiently high to counteract drastic reductions in pH which may cause system failure. The relatively rapid responses observed to increased loading rates suggest amenability to intermittent operation; this has been verified by Young and Dahab (1982) and others: periods without feed ranging from days to months have been followed by return to efficient treatment capacity in a few weeks (Taylor 1972).

Large shock loadings of toxic materials are frequently accommodated by filters, primarily because of the biofilm nature of the microflora; acclimation to or recovery from exposure to compounds such as chloroform and formaldehyde have been documented (Parkin et al. 1981).

The rotating biological contactor (RBC) is also a fixed bed reactor and was first described in 1928 by the filing of the original patent (Borchardt 1971). However it was only with the emergence of plastics as effective and

FIG.12



rotating biological contactor

inexpensive lightweight supporting media that the aerobic process became widespread in wastewater treatment; the anaerobic system is less widely documented, but is potentially superior in a number of respects including reactor size and loading rates (Friedman & Tait 1980; Laquidara et al. 1985). In the RBC, microorganisms attach to the inert plastic media to form a biofilm; the support medium in a disc-array configuration is partly or fully submerged and rotates slowly on a horizontal axis in a vessel through which the wastestream flows (Figure 12). As the medium rotates, the microbial film is thus exposed to the nutrients in the feedstock. The velocity of revolution provides some control over biofilm thickness (Winkler

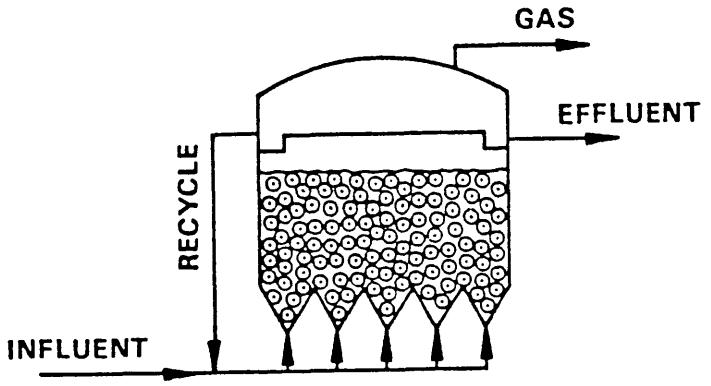
1981).

The effective and reliable treatment of industrial and other wastes by biological means necessarily requires a system which demonstrates wide-ranging tolerance of fluctuations in operational conditions. Temperature, influent COD and pH increases and decreases will all, to varying degrees, affect the activity and viability of anaerobic bacterial populations.

The anaerobic conversion of wastes is limited in suspended growth systems by sludge activity and sludge concentration, although these limitations can be attenuated to some extent by settling, manipulation of waste composition and recycle. Fixed film stationary processes such as the anaerobic filter depend on immobilised biomass; to minimise pressure losses and clogging by solids, the inert media have large diameters which in turn limit the surface area. The biomass grows in layers of 1-4mm thick around the packing material and diffusion limitations are likely to exist (Heijnen 1983). These problems are effectively overcome in the anaerobic expanded and fluidised bed systems (Figure 13) where the smaller diameter media employed enable very high biomass concentrations to accumulate as thin films around the carrier particles. Expansion or fluidisation of the media reduces or eliminates the problems of blockage and retention times necessary for treatment can be markedly reduced.

The distinction between expansion and fluidisation is not clearly defined; such considerations as media size and liquid flow must necessarily affect the final state of the

FIG.13



expanded / fluidised bed reactor

reactor and although in general expansion occurs to about 5% of bed volume, some workers have operated reactors described as expanded beds at expansions of up to 25%.

The anaerobic expanded bed process was developed by Jewell and others as an extension of existing anaerobic systems in 1974. The reactor was considered to be the first anaerobic conversion system with the capacity to treat dilute wastestreams at ambient temperatures; Jewell (1981) attributed this ability primarily to two main factors:

1. the formation of an active biomass concentration of around 30kg m^{-3} of reactor, which was capable in theory of reaching concentrations of the order of 100kg m^{-3};
2. the trapping and filtering of fine inert particles which could result in a final effluent suspended solids

concentration of less than 5mg l^{-1}.

These determinants combined to provide an efficient and high rate biological conversion process which did not require the high fluid flow rates necessary in comparable aerobic systems and which produced low sludge volumes for disposal.

The expanded bed has been employed for the treatment of a number of wastes including molasses fermentation (Frostell 1980) and domestic (Jewell 1981; Jewell et al. 1981; Rockey & Forster 1982) and synthetic effluents (Switzenbaum & Jewell 1980). Switzenbaum and Jewell (1980) considered the expanded bed configuration to be effective for the conversion of low strength organic substrates (COD<600mg l^{-1}), operating at low temperatures of 10-20^{o}C, at high organic loading rates (>8kg COD m^{-3} d^{-1}) and at short HRTs in the region of several hours. These results were substantiated by other investigators (Jewell & Morris 1981) who further suggested that acclimation of the reactors to the influent wastestream was an additional factor in the enhancement of system stability under shock-loading and other adverse conditions. However, dilute domestic sewage, the TOC of which varied between 40 and 260 mg l^{-1} was applied to an expanded bed and the removal achieved found to be low, of the order of 17-52% (Rockey & Forster 1982) except at loadings below 0.5 kg TOC m^{-3} d^{-1}; these workers therefore concluded that under typical real-scale conditions, the expanded bed reactor would not be suitable for wastestreams of such a nature.

The operational characteristics of anaerobic fluidised bed reactors have been outlined by Cooper and Wheeldon

(1981) and Heijnen (1983). The biological fluidised bed concept was initially applied to denitrification processes and subsequently to carbonaceous and nitrogenous oxidation systems (Jeris et al. 1974; Jeris et al. 1977). The utilisation of anaerobic fluidised beds for denitrification of both low and high nitrate concentrations in sewage and nuclear fuel processing wastes has been reported by Francis and others (1975;1977).

The influent feed to the typical fluidised bed enters at the base of the reactor (Figure 13). Even flow distribution through the reactor cross-section, especially in the zone close to the feed entry point, can, however, present a design problem in fluidised bed systems (as it also does in packed bed units) and some form of feed distributor is requisite. In one investigation, a layer of gravel at the reactor base was employed (Bull et al. 1982) but this proved unsatisfactory due to frequent blockage. It was therefore replaced by a conical distributor; the influent was orientated downwards into the base of an angled cone, resulting in a dynamic upwash in the lower part of the vessel.

The bacteria in fluidised beds attach to small diameter media, typically sand (Bull et al. 1982; Switzenbaum et al. 1984). The high vertical velocity of the wastestream to be treated expands the bed to a point beyond which the net downward gravitational force is equalled by the frictional drag. An extremely high degree of recycle is required and single carrier grains do not have a fixed position within the bed but each particle tends to remain nonetheless within a restricted location.

Other anaerobic digesters such as the filter assembly must contain large supportive grains or microbial growth quickly clogs them: the surface area of bacterial film per unit vessel volume of these reactors and consequently the volumetric productivity are thus low. In the fluidised bed such mechanical constraints do not apply as the carrier particles can be much smaller: the reaction zone expands to accommodate increasing bacterial growth.

Inert support particles of sizes 0.5mm and 0.7mm diameter commonly in use in fluidised bed systems were compared as part of an investigation (Sutton & Li 1983). Equivalent performances of the reactors employed were attained in a shorter time from start-up using the 0.5mm sand; in addition, the unit operating with the 0.7mm particles required twice the recycle rate of the reactor utilising the smaller media for a bed expansion of 30-50%. Switzenbaum et al. (1984) substantiated these observations by demonstrating that reactor efficiency was improved by the replacement of 0.5mm sand with sand of 0.2mm diameter. A very large surface area for bacterial colonisation can be obtained by the use of small diameter media; biomass concentrations of the order of 8-40g VSS l^{-1} can be achieved, and reactor size and treatment times are hence significantly reduced (Hickey & Owens 1981).

Bacterial activity in fluidised beds, unlike that in other fixed film reactors such as anaerobic filters, has been shown to be greatest for both acetogens and methanogens in the central region of the bed (Bull et al. 1984). The mobility of the media and the high degree of recycle were believed to influence this phenomenon. In the

filter, microbial activity and substrate profiles have indicated that the majority of waste conversion occurs in the lower region of the reactor (Young & McCarty 1969; van den Berg & Lentz 1979).

Several factors have been claimed to contribute to the superiority of the fluidised bed system, all of which may be attributed to a greater or lesser extent to high biomass concentration:

1. maximum contact between biocoated media and the liquid phase comprising the wastestream;
2. liquid-film diffusional resistances due to particle motion and liquid velocities are minimal;
3. channelling, plugging and gas holdup problems are generally circumvented;
4. bacterial film thickness can be controlled and optimised; and,
5. high biomass concentrations reduce reactor size and hence land area necessary for the treatment plant.

The latter consideration renders the use of the fluidised bed as an on-site treatment highly effective; the land area requirement can be as much as 80% less than that typical of conventional anaerobic units (Cooper & Wheeldon 1981).

The problem of cell washout that complicates the operational stability of conventional digesters is less likely in a fluidised bed. According to Andrews (1982), washout is impossible as long as the superficial liquid velocity is kept below the settling velocity, although this has proved to be extremely difficult in practice. The fixed film nature of the fluidised bed permits the

maintenance of extended mean cell residence times at low HRTs without the requirement for biomass settling; organic loading and bacterial growth rate are the only two parameters controlling the mean cell residence time. A biomass concentration of 5.65kg VS m^{-3} has been reported for a fluidised bed with an SRT of 12 days and an SRT/HRT ratio of 23 (Stephenson & Lester 1986).

Wastewaters which have been treated by anaerobic fluidised beds include meat and dairy wastes, whey permeate, food processing, bakery, chemical, soft drink bottling and acid whey wastes, beet molasses and heat treatment liquor (Bull et al. 1982; Hickey & Owens 1981; Stephenson & Lester 1986).

A pilot scale fluidised reactor employed for the conversion of whole whey effluent was operated at 35^{o}C and loading rates of between 13.4 and 37.6 kg COD m^{-3} d^{-1} were applied (Hickey & Owens 1981). Reductions in COD of 84% and 72% respectively were achieved, with HRTs of 1.4 and 4.9 days. At 24^{o}C COD removals were found to be about 10% less than those obtained at the higher temperature. High strength meat and dairy wastes were treated in a fluidised bed at COD loading rates of up to 6 kg COD m^{-3} d^{-1} at 37^{o}C and 3 kg COD m^{-3} d^{-1} at 20^{o}C, with COD reductions in excess of 70% obtained (Bull et al. 1982). Moderately high strength chemical waste comprising mainly ethanol was applied to a fluidised bed at organic loads in the range 4.1-27.3 kg COD m^{-3} d^{-1} (Hickey & Owens 1981). Removal efficiencies of between 93% and 79% resulted, with 81-84% methane content in the biogas. Food processing effluent and heat treatment liquor were also effectively dealt with

in a system comprising pilot scale fluidised beds, although soft drink bottling waste, high in refined sugars was degraded more slowly (Hickey & Owens 1981).

Variations in both loading and temperature are readily accommodated by fluidised bed systems, as the results described above show. Toxic shocks appear to be equally well tolerated. A strongly organic whey waste applied to a reactor at loadings of up to 37.6kg COD m^{-3} d^{-1} resulted in increased VFA concentrations (and hence depressed pH) but no inhibition of reactor performance was manifested and the average biogas content of methane remained at 60% (Hickey & Owens 1981). The volatile acids level in the bed was found to be highly dependent upon the organic loading rate, a situation probably arising from the variable rates of substrate assimilation of the various bacterial populations within the reactor. An increase in organic loading resulted in an increase in soluble COD removal rates in the system of Boening and Larsen (1982) but overall percentage removal efficiency decreased. Biomass concentration was observed to increase with decreasing temperatures (35-12.5^{o}C), compensating for the reduced reaction rates of bacteria at lower temperatures and thus reducing the sensitivity of the system to temperature change.

The stability of an unheated (20^{o}C) fluidised bed was investigated by Bull et al. (1983b). The unit was operated on synthetic meat waste and the effects of COD, pH, hydraulic and temperature shocks were examined. Influent flowrate increases of 100% and 150% for 4 and 8 hour periods resulted in a negligible pH decrease (<0.3) with a

concomitant increase in VFA levels and increased effluent COD values of 40-50%. Raising the influent COD by 300% for short periods produced similar effects. Increased VFA levels upon increase of influent COD is a common phenomenon in anaerobic systems; the accumulation of higher fatty acids such as propionate and butyrate are frequently noted, as these are often present under overload conditions (Henze & Harremoes 1983).

The advantages of anaerobic treatment of moderate and high strength wastes, i.e. production of utilisable methane, low cell synthesis, no oxygen requirement and low nutrient requirement, are well known. However, the technical limitations of conventional digesters - large reactor volumes and long HRTs, intolerance of rapid temperature changes and high organic loading rates - are substantially reduced or eliminated by the use of high rate anaerobic configurations such as the UASB, the anaerobic filter and the fluidised bed systems. The high biomass concentrations that can be achieved enable these types of reactor to accommodate severe overload conditions, alterations in influent composition and temperature changes with great efficiency. The potential of such systems for the conversion of high strength soluble industrial wastewaters has been demonstrated, with low HRTs and thus high processing rates and high quality effluent being among the major advantages, in addition to reduced reactor volumes and consequently much smaller land area requirements than those necessary for conventional waste treatment processes.

CHAPTER 2
OBJECTIVES

Significant quantities of high strength wastewaters are generated by most industrial processes; many of these are organic and as such are potentially suitable for treatment by biological means. There have been a number of microbiological, biochemical and technological advances made in the understanding and implementation of anaerobic digestion processes with respect to wastewater treatment. The development of new and superior anaerobic reactor designs with increased tolerance to toxic and shock loadings of concentrated effluents has established a potential for the recovery of energy in the form of utilisable methane whilst reducing the pollution load of strongly organic wastes.

The anaerobic fluidised bed, the anaerobic filter and the upflow anaerobic sludge blanket (UASB) are high rate biological systems which may prove ideally suited to the conversion of many soluble, concentrated industrial effluents, as these reactors have the ability to retain large concentrations of biomass and to operate at very high organic loadings. However, despite numerous treatability studies, it is apparent that one of the most serious drawbacks of high rate anaerobic systems at their present stage of development is the time required to start up the process. The factors which affect start-up, biomass retention and distribution and reactor stability are still only partially understood. The long term stability of such reactors when subject to shock loadings and intermittent operation also requires elucidation. In addition, as many

industrial effluents are subject to variations in composition, pH and flow rates and may contain bacterially inhibiting substances, the limitations of the practical application of high rate anaerobic bioreactors to the treatment of such wastes also requires assessment.

Since the knowledge of many aspects of anaerobic treatment processes is still incomplete, the following objectives in this study were defined:

1. To compare the effects of various regimes on the rapid start-up and biomass attachment of anaerobic fluidised beds and to assess the biological and operational stabilities of these reactors under conditions of shear stress, nutrient addition and intermittent operation.
2. To examine the potential of the anaerobic fluidised bed for acclimation to recalcitrant industrial wastewaters and to assess and compare the operational capabilities of fluidised beds, anaerobic filters and UASB reactors during start-up on high strength, potentially toxic industrial effluents.
3. To examine the difficulties imposed by various high strength industrial wastes on the start-up and operational efficiencies of anaerobic fluidised beds, with regard to substrate composition and bacterial activity.

CHAPTER 3
MATERIALS AND METHODS

3.01 LABORATORY-SCALE ANAEROBIC FLUIDISED BED REACTORS

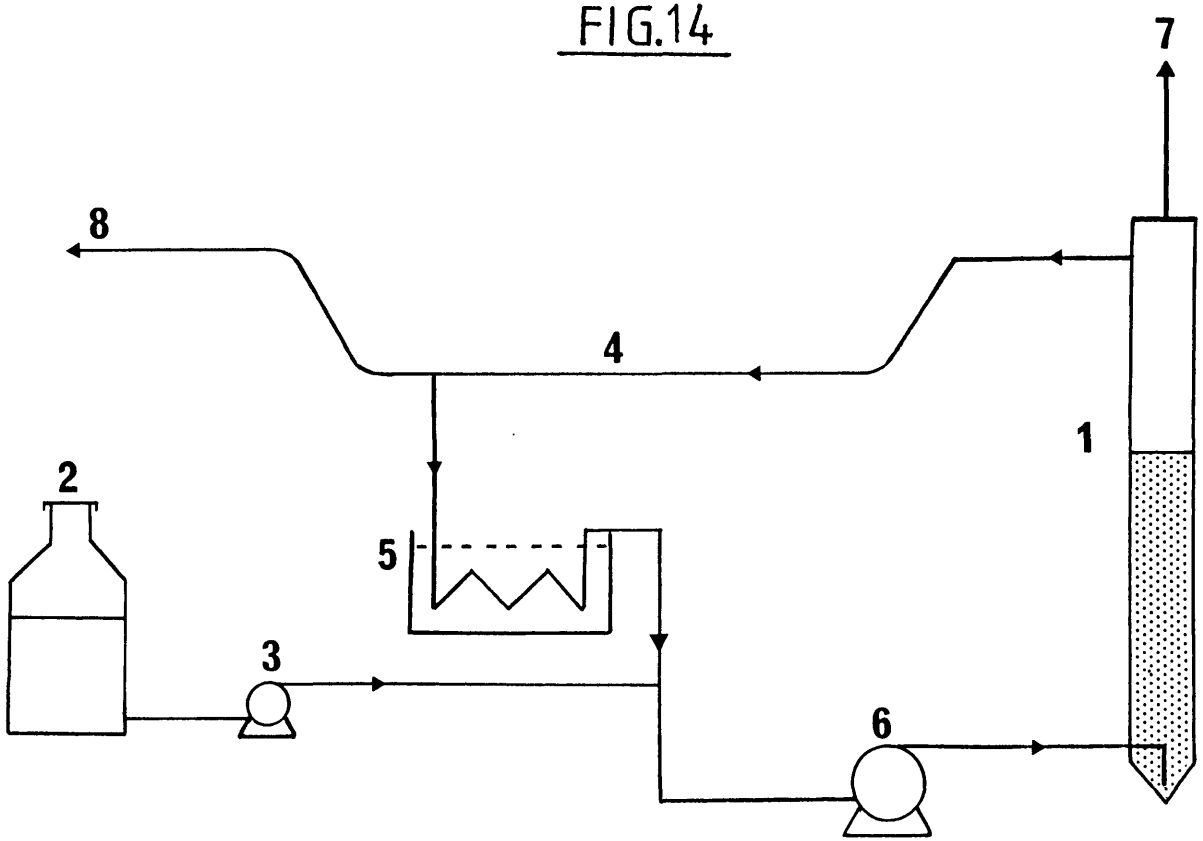
3.01.1 CONSTRUCTION OF 2L ANAEROBIC FLUIDISED BEDS

Four 2l anaerobic fluidised beds were employed. The system consisted of a fluidised bed column, a recycle pumpline, a feed system and an overflow arrangement. A schematic representation of the assembly is shown in Figure 14.

The reactor body was constructed of extruded acrylic tubing (Visijar-Tuckers Plastics, Croydon, U.K.) of height 1m, i.d. 50mm and wall thickness 5mm. The base of the column was connected by a 12 TPI screw thread joint to a machined acrylic boss complete with a plastic O-ring seal. The boss was machined internally to a conical shape to facilitate the even upward distribution of recycled and influent liquid (Figure 15). A stainless steel inlet pipe with a 5mm i.d. was bent downwards through an angle of 90^{o} into the apex of the cone. The top of the reactor was connected to the column in a manner similar to the basal connection, by a machined acrylic boss. The latter provided for gas draw-off with a 9mm i.d. acrylic outlet pipe to allow connection of the gas discharge assembly (Figure 15).

The effluent liquid left the reactor at a level 15cm below the gas draw-off outlet and passed into a U-tube of 5mm i.d. flexible polyvinylchloride (pvc) tubing (Figure 14). The effluent overflow side of this U-tube was raised in order that a positive pressure maintained the liquid level in the reactor column above that of the effluent

FIG.14



1 fluidised bed
2 waste vessel
3 feed pump
4 U-tube
5 heated water bath
6 recycle pump
7 effluent gases
8 treated effluent

SCHEMATIC REPRESENTATION OF THE 2 1 FLUIDISED BED ASSEMBLY

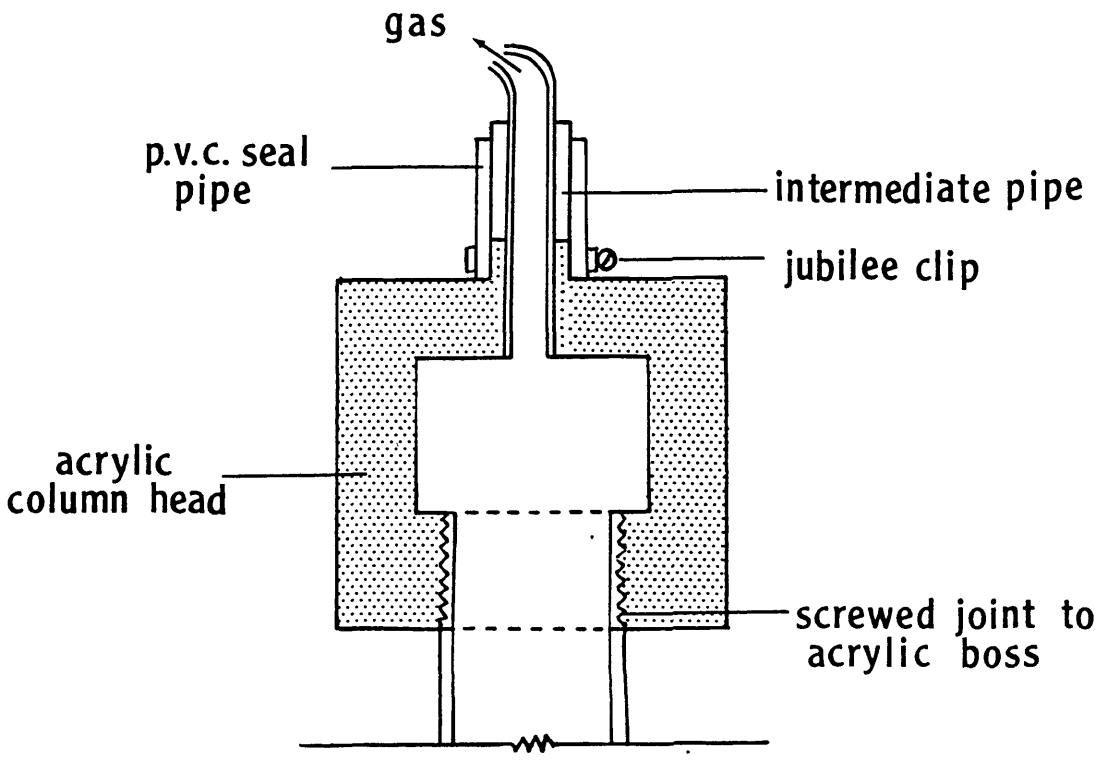
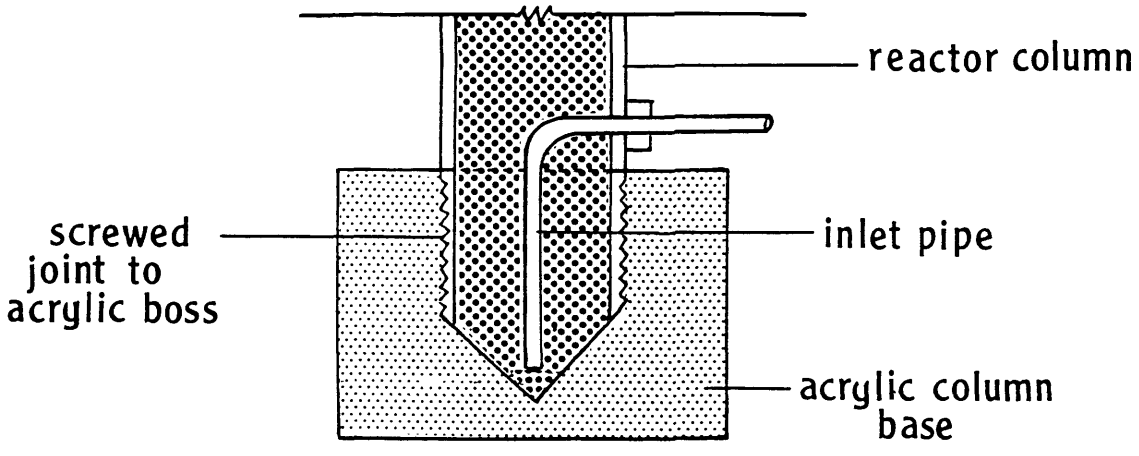


FIG.15



HEAD AND BASE ARRANGEMENT OF THE 2 1 FLUIDISED BED

outlet.

A recycle line of 5mm i.d. flexible pvc tubing was attached by a T-piece connector to the U-loop to return the recycled flow to the base of the column. Within this recycle line a 2m length of tubing was wound into a coil and placed in a heated water bath (Model SB2, Grant Instruments, Cambridge, U.K.). The water surface was insulated with polystyrene beads to minimise heat loss and evaporation. Also within the recycle line was attached a 2m length of silicone rubber tubing of 6.5mm i.d. (Esco Rubber, Bargoed, Wales) which was fed through the head of a peristaltic pump (HRSV/214 H.R. Flow Inducer, Watson-Marlow, Falmouth, U.K.). The pump was fitted with variable speed controllers which provided the degree of recycle required. The silicone tubing in the pump head was examined daily for signs of wear and both tubing and pump head rollers lubricated with glycerol to extend tubing life. A fresh section of tubing was positioned through the head each 7 days. A polypropylene non-return valve (Gallenkamp, Loughborough, U.K.) was fitted in the recycle line 150mm from the column inlet to prevent loss of the reactor bed in the event of failure or tubing rupture.

Influent wastewater was pumped from a storage vessel into the recycle pipeline (Figure 14) using a peristaltic pump (Chem-Lab CPP 30, U.K.). Flow rated pvc tubing (Hampshire Labaids Ltd., U.K.) of selected size was used in the pump head to provide the required feed rates. The tubing was connected on the suction and delivery sides of the pump to 2.5mm i.d pvc tubing.

PVC tubing of 5mm i.d. was used for effluent gas draw-

off from the head of the reactor column (Figure 15). Gas production was measured by the displacement of acidified water from an inverted 1l measuring cylinder. Samples for gas analysis were removed from the gas draw-off tubing using precision 100ml syringes (Hamilton Bonaduz AG).

All tubing connections were made by means of polypropylene push-fit connectors (Gallenkamp).

3.01.1.1 BIOMASS SUPPORT MATERIAL

Each reactor was filled to a height of 45cm with Redhill 50 silica sand of 0.35mm mean diameter (British Industrial Sands Ltd., Surrey, U.K.). The use of silica sand of relatively small diameter allowed fluidisation of the bed with a low upflow velocity from the recirculation system.

3.01.1.2 OPERATION OF 2L FLUIDISED BEDS

Each reactor was filled with filtered secondary sludge digestion supernatant liquor (Monkmoor Water Pollution Control Works, Shrewsbury, U.K.). Air was removed from the system by filling the tubing with liquor. The recycle pump (Figure 14) was then started up and the pump speed adjusted to provide a 30% (15cm) fluidisation of the sand bed, measured on the surface of the sand. A recycle velocity of $7.4mh^{-1}$ was required to provide this degree of expansion. The temperature in the water bath was subsequently adjusted to produce a liquid temperature in the reactors of $36\pm2^{o}C$.

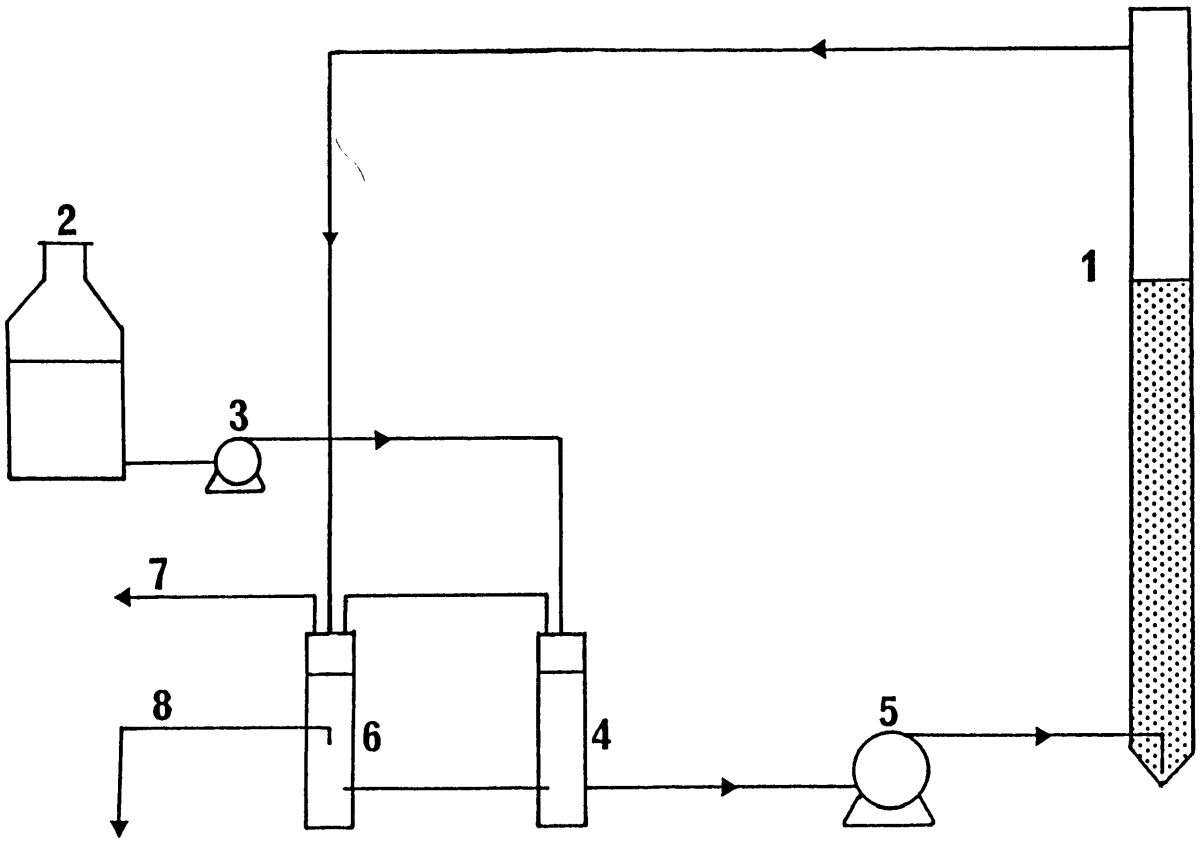
3.01.2 CONSTRUCTION OF 4L ANAEROBIC FLUIDISED BEDS

The second version of the anaerobic fluidised bed system was 4l in volume and comprised a reactor column, recycle and overflow chambers, and recyle and feed systems. A schematic arrangement is presented in Figure 16. Four such units were utilised.

The reactor body, constructed of extruded acrylic tubing of 50mm i.d. and 5mm wall thickness, was 2m in height. The base and top of the column were similar in form to those employed in the 2l reactors (Figure 15) with one important difference: the top acrylic boss was not provided with an extruding gas outlet but was pierced with a 13mm diameter circular opening, which was sealed with a rubber bung, to enclose the gas space above the liquid level of the system. The opening was included to provide a release of gas and/or liquid in the event of undue pressure build-up in the system. Effluent gas therefore left the top of the column with the effluent liquid through a 5mm i.d. steel effluent port situated 4cm below the screw thread. The effluent passed down a 2m length of 5mm i.d. flexible pvc tubing, which contained within its length a 3-way, 4mm polypropylene stopcock (Nalgene, BDH Apparatus Div., Dagenham, U.K.), and flowed into the overflow chamber (Figure 16).

The overflow and recycle chambers were constructed from extruded acrylic tubing and were of height 300mm, 60mm i.d. and wall thickness 5mm. The chambers were connected to each other 50mm from their bases by a length of 5mm i.d. pvc tubing. The overflow chamber included an L-shaped glass tubing overflow 200mm from the base and leading to

FIG.16



1 fluidised bed
2 waste vessel
3 feed pump
4 recycle chamber
5 recycle pump
6 overflow chamber
7 effluent gases
8 treated effluent

SCHEMATIC REPRESENTATION OF THE 4 1 FLUIDISED BED ASSEMBLY

waste via a length of silicone tubing.

The wastewater in use was pumped from the storage vessel to the recycle chamber using an LKB Multi-Perpex pump (LKB Ltd.,Croydon, U.K.). Silicone tubing lengths of 3mm i.d. (Jencons, Leighton Buzzard, U.K.) and 1mm i.d. (Esco Rubber) were used to connect 10l or 20l storage pyrex aspirators to the recycle chamber: the size selected for the pump head depended upon the feed flow rate required. The recycle and overflow chambers were both used to ensure that the influent passed through the reactor rather than flowing directly out with the effluent. The tops of both chambers were sealed with rubber bungs, through which holes had been pierced and short lengths of 6mm o.d. pyrex tubing inserted to receive wastewater. The pressure was equalised between each chamber by an interconnecting pvc tube, and a third bore in the overflow chamber allowed the escape of the gases produced (Figure 16).

The mixed recycled effluent and influent wastewater was pumped upwards through the fluidised bed by a Watson-Marlow HRSV 214 flow inducer fitted with a length of 6.3mm i.d. silicone rubber tubing (Esco Rubber) at a rate sufficient to maintain the required fluidisation. The tubing in the pump head and the head rollers were lubricated daily with glycerol and a new section of tubing moved into place twice weekly.

Initially, a coil of 5mm i.d. pvc tubing, inserted between the recycle chamber and the recycle pump, was placed in a water bath controlled by a chiller

thermocirculator unit (Churchill Instruments, Uxbridge, U.K.). The temperature of the water bath was adjusted to maintain the required temperature of 36±2°C within the fluidised bed. Subsequent to this, three flat electrical heating belts (Boots Co., Nottingham, U.K.) were placed around the reactor column to provide the desired temperature.

Sampling inlets were set into the reactor at regular intervals. These consisted of a stainless steel tube of 3mm i.d. inserted through the reactor wall and fixed in place by a brass ferrule arrangement. Silicone tubing was attached to the external section of steel tubing and clamped off tightly to prevent leakage.

Gas production was initially measured by the displacement of acidified water from an inverted 1l polypropylene measuring cylinder. Gas samples were collected by syringe from a section of silicone rubber tubing, 6.3mm i.d., attached to the end of the pvc gas effluent line, and from which a glass L-piece directed effluent gases under the lip of the inverted cylinder. An enclosed gas collection system was then fitted: gas was measured by the displacement of acidified water and was measured at atmospheric pressure by the use of a balancing reservoir. Gas samples were removed via a silicone rubber septum fitted into the top of the gas collector.

The tubing connections were made by polypropylene push-fit connectors and a polypropylene non-return valve (Gallenkamp) was fitted 150mm from the reactor column inlet to prevent loss of column contents in the event of failure.

3.01.2.1 BIOMASS SUPPORT MATERIAL

One litre of B15 Redhill 65 silica sand, of mean diameter 0.22mm (British Industrial Sands Ltd.) was placed in each fluidised bed column, providing an active (expanded) volume of 2.4l.

3.01.2.2 OPERATION OF 4L FLUIDISED BEDS

Each reactor was filled with water or a water:feed mixture and air removed from all the tubing by filling with liquid. The recycle and overflow chambers were filled to an approximate height of 150mm. The recycle pump was then started up and the pump speed adjusted to provide the expansion required. The temperature of the system was maintained by adjustment of the heated water bath or by the use of heating belts. Anaerobically digested sewage sludge (Hogsmill Valley Water Pollution Control Works, Thames Water Authority) was used as seed and injected into each column via the lowest sampling port.

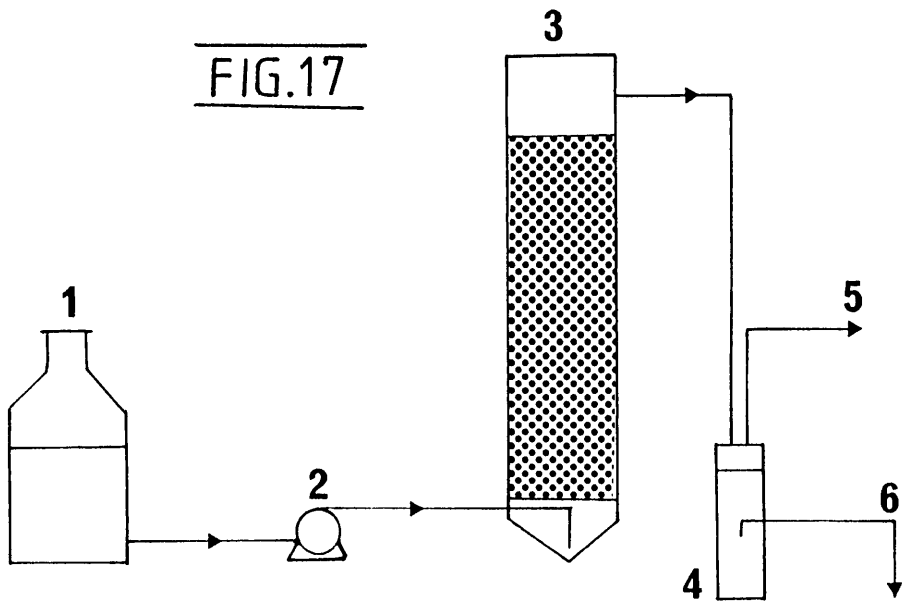
3.02 LABORATORY SCALE ANAEROBIC FILTERS

3.02.1 CONSTRUCTION OF THE ANAEROBIC FILTER SYSTEM

The anaerobic filter system comprised a columnar reactor and an overflow chamber. Recycle of effluent was not employed. A schematic representation of the filter is shown in Figure 17. Two anaerobic filter systems were constructed.

The reactor body was constructed from extruded acrylic tubing (Visijar Tuckers Plastics) of 1m in height, 88mm i.d. and 6mm wall thickness. The solid detachable acrylic base was 75mm in height and was machined internally to a

FIG.17



1 waste vessel

2 feed pump

3 anaerobic filter

4 overflow chamber

5 effluent gas

6 treated waste

SCHEMATIC REPRESENTATION OF THE ANAEROBIC FILTER ASSEMBLY

conical shape to facilitate an even upward distribution of influent wastewater. Above the cone an acrylic dispersion plate, incorporating 28 evenly distributed perforations 5mm in diameter, was fixed to support the media bed and allow passage of the influent. The base section was attached to a perspex collar around the reactor body by means of a 6 screw and wingnut arrangement (Figure 18) complete with a rubber gasket seal.

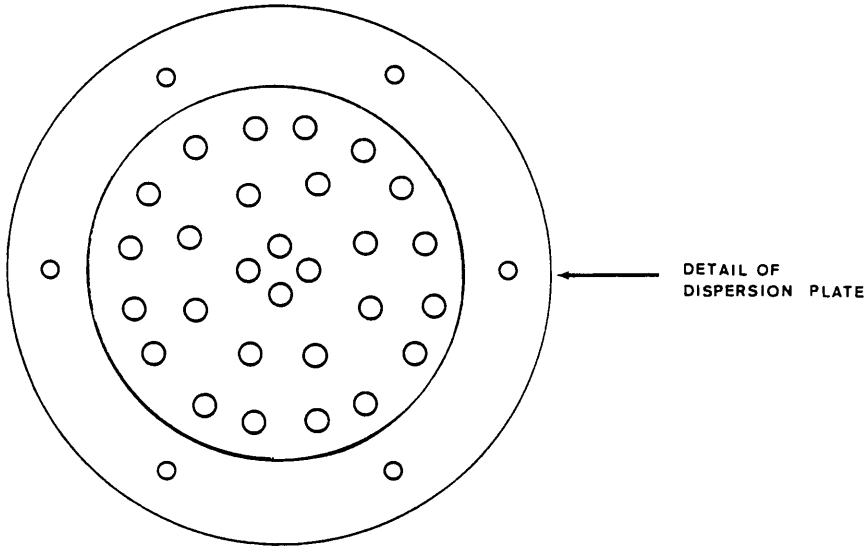
Dispersion rings of 5mm thickness and 2mm width were attached within the reactor body at heights of 0.3m and 0.6m to prevent short-circuiting at the media/column boundary. A stainless steel influent pipe of 5mm i.d. was let into the base of the reactor and bent downwards through 90^{o} into the cone to assist influent dispersion (Figure 18). The detachable top of the filter comprised a flat plate 150mm in diameter attached to a second collar around the reactor body by a rubber gasket seal and a 6 screw and wingnut arrangement (Figure 19).

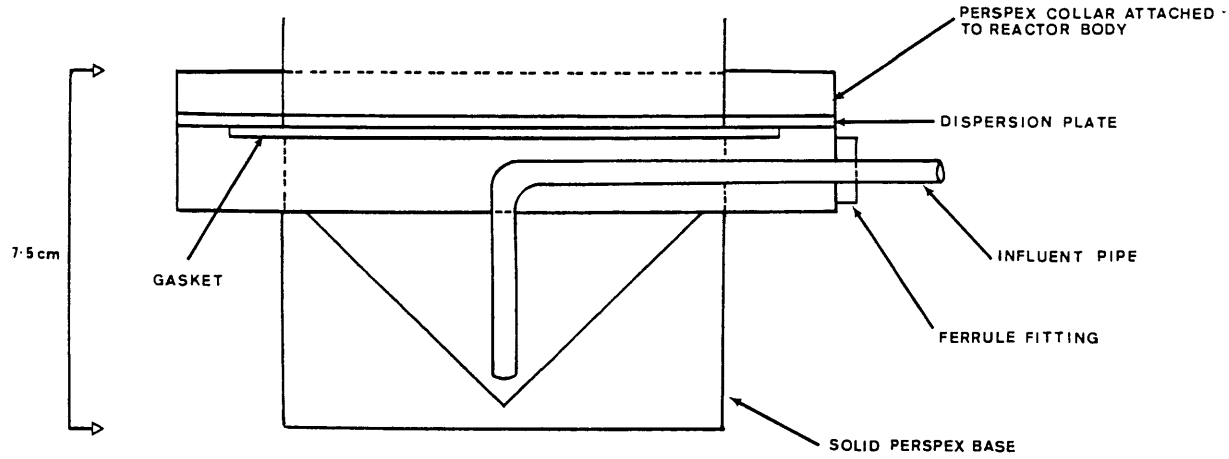
Sampling ports were set into the body of the reactor at 20cm and 50cm heights from the base (Figure 19). These were similar in structure to those employed in the 4l anaerobic fluidised beds (section 3.01.2) and reached the centre of the filter column.

Gas and effluent left the filter by a 5mm i.d. stainless steel effluent port 4cm from the top and was carried by 5mm i.d. flexible pvc tubing to an overflow chamber. A 3-way, 4mm polypropylene stopcock (Nalgene) was inserted approximately half way along this length of tubing. The overflow chamber was constructed from extruded acrylic tubing and was 340mm in height and of 50mm i.d..

FIG. 18

ANAEROBIC FILTER - BASE DETAIL





THE ANAEROBIC FILTER

KEY: S.P. = SAMPLING PORT
D.R. = DISPERSION RING

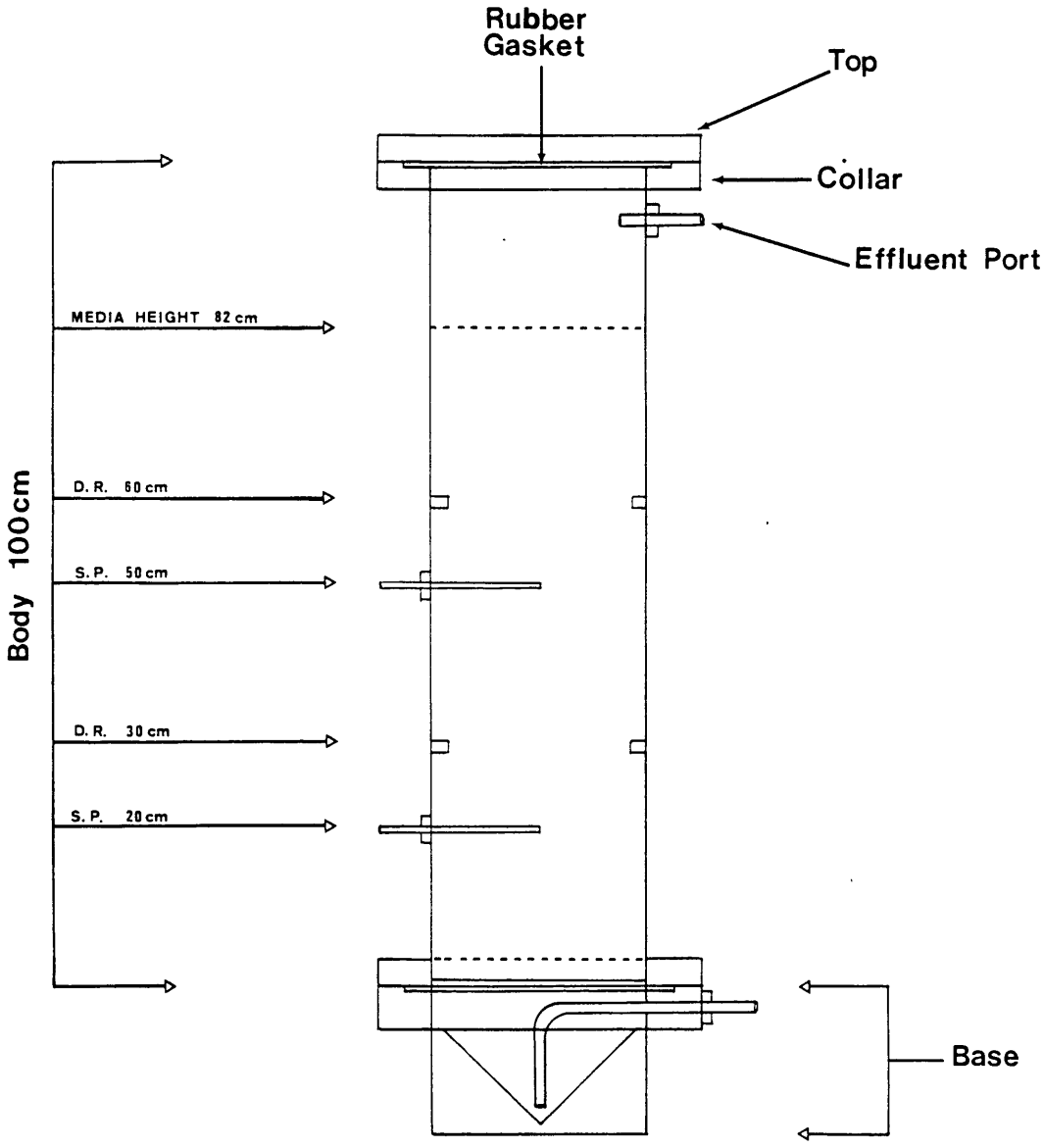


FIG.19

An L-shaped stainless steel overflow was situated 200mm from the base of the chamber, from whence the treated effluent was removed to waste. The chamber was sealed by a rubber bung, which was pierced to allow the escape of effluent gas.

The influent wastewater was pumped upwards through the reactor by an LKB Multi-Perpex pump (LKB Ltd.) using 3mm i.d. silicone tubing (Jencons). The latter was connected on the suction side of the pump to the 10l aspirator of influent substrate and on the delivery side to a polypropylene non-return valve (Gallenkamp), which was placed 150mm from the influent port at the base of the anaerobic filter. A length of silicone tubing, 6.3mm i.d., connected the valve to the influent port. Two heating belts (Boots Co.) were used to maintain reactor temperature at 36±2^{o}C.

Gas was measured at atmospheric pressure by displacement of acidified water from a cylindrical glass vessel, attached to which was a balancing reservoir. Gas samples were taken via a silicone rubber septum fitted into the top of the gas collector.

3.02.1.1 BIOMASS SUPPORT MATERIAL

The support media consisted of almost-spherical particles of 'lytag', a lightweight rocky aggregate, of diameters in the range 5 to 13mm (Lytag Ltd., Tilbury, U.K.). These media were placed in the reactor, supported by the dispersion plate, to a height of 82cm, and thus provided an active volume of 5l.

3.02.1.2 OPERATION OF ANAEROBIC FILTERS

Each reactor was filled with a water:feed mixture. The temperature was maintained by the application of heating belts. Anaerobically digested sewage sludge (Hogsmill Valley Water Pollution Control Works, Thames Water Authority) was used as seed and injected into each column via the sampling ports.

3.03 LABORATORY SCALE UPFLOW ANAEROBIC SLUDGE BLANKETS

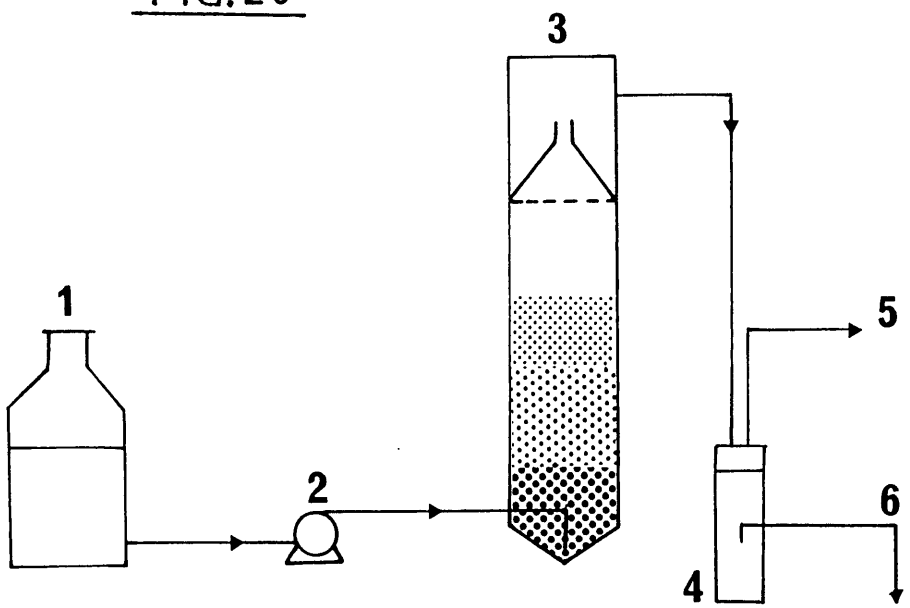
3.03.1 CONSTRUCTION OF THE UPFLOW ANAEROBIC SLUDGE BLANKET (UASB) SYSTEM

The upflow anaerobic sludge blanket (UASB) arrangement comprised a reactor column and an overflow chamber. Recycle of effluent and biomass was not employed. The UASB system is shown schematically in Figure 20. Two such units were constructed.

The reactor body was constructed from extruded acrylic tubing of 1m in height, 88mm i.d. and 6mm wall thickness. The solid detachable acrylic base was 70mm in height and machined internally to a conical shape. The base was attached to a perspex collar incorporated into the lower section of the reactor body by a 6 screw and wingnut arrangement, with rubber gasket seal (Figure 21). A stainless steel influent pipe of 5mm i.d. entered the base section and bent downwards through 90^{o} into the apex of the cone. This arrangement assisted influent dispersion in the system.

A support ring of 5mm width and 2mm thickness was incorporated within the body of the UASB at a height of 80cm from the base, to support a conical liquid/solids

FIG. 20



1 waste vessel

2 feed pump

3 upflow anaerobic sludge blanket

4 overflow chamber

5 effluent gas

6 treated waste

SCHEMATIC REPRESENTATION OF THE UASB ASSEMBLY

separator. The separator consisted of an inverted polyacrylamide cone having a 60^{o} wall angle and with 8 slots 0.5 x 2cm cut along the widest edge, 1cm from the ring (Figure 22). A funnel opening of 5mm was provided at the apex of the cone, through which the majority of the gas and liquid would pass. The angled wall of the separator was required to enhance the settling of the solid biomass granules, retaining them in the reactor vessel.

The top section of the UASB comprised a flat, detachable plate, 150mm in diameter, which attached via screws and wingnuts to an upper collar around the reactor body (Figure 23). A rubber gasket completed the seal.

Sampling ports were set into the reactor body at 10, 25 and 40cm heights from the base. These were similar to those described in section 3.01.2. The treated waste and effluent gases left the system 4cm from the top by means of a 5mm i.d. stainless steel effluent port, and were carried to the overflow chamber by an arrangement similar to that used in the anaerobic filter (section 3.02.1).

The influent wastewater was pumped upwards through the UASB by an LKB Multi-Perpex pump (LKB Ltd.). The connections and tubing were identical to those used in the anaerobic filter (section 3.02.1). Reactor temperature was maintained at 36±2^{o}C by the use of two heating belts (Boots Co.) wound around the body of the reactor.

Gas was measured by displacement of acidified water at atmospheric pressure. Gas samples were removed by syringe through a silicone rubber septum fitted into the top of the gas collector.

UASB - BASE DETAIL

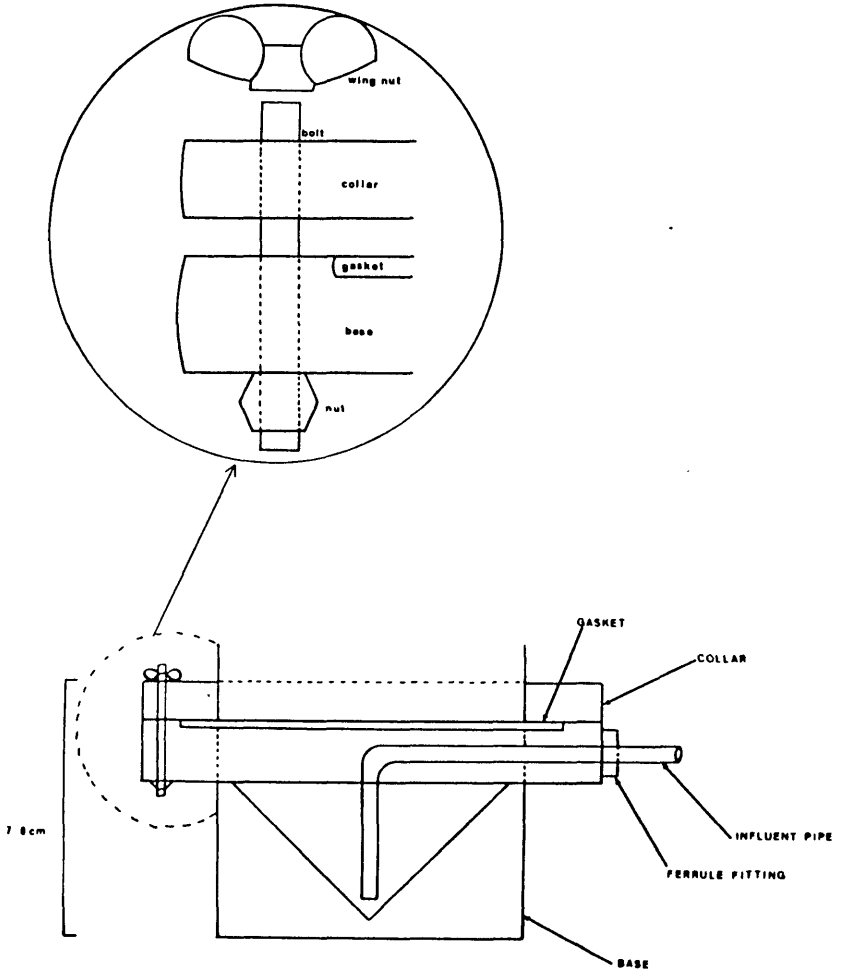


FIG. 21

UASB - Liquid/Solids Separator

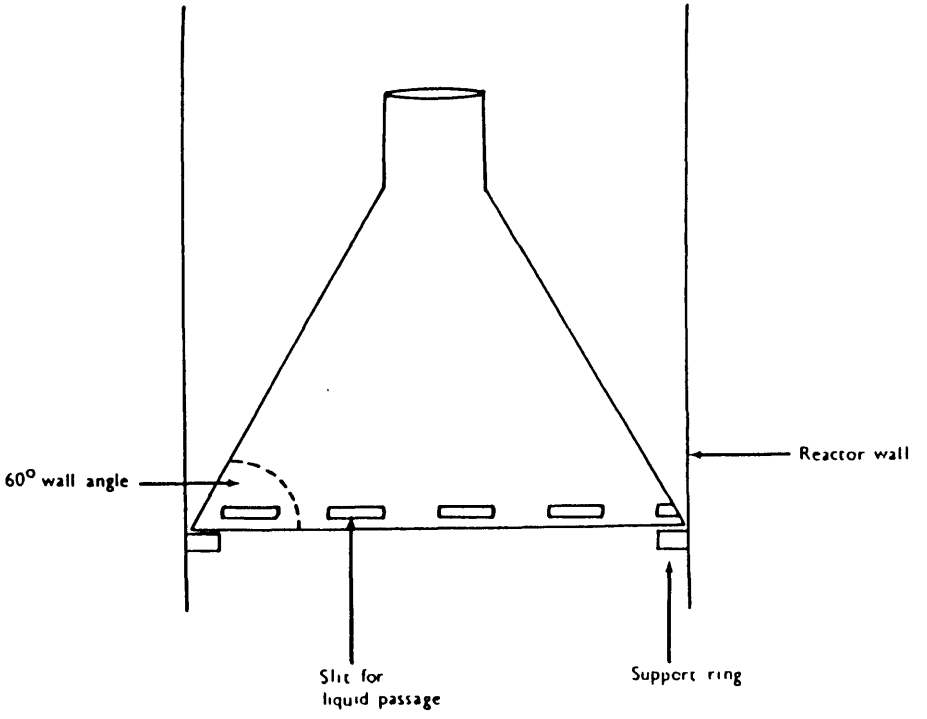


FIG. 22

THE UPFLOW ANAEROBIC SLUDGE BLANKET

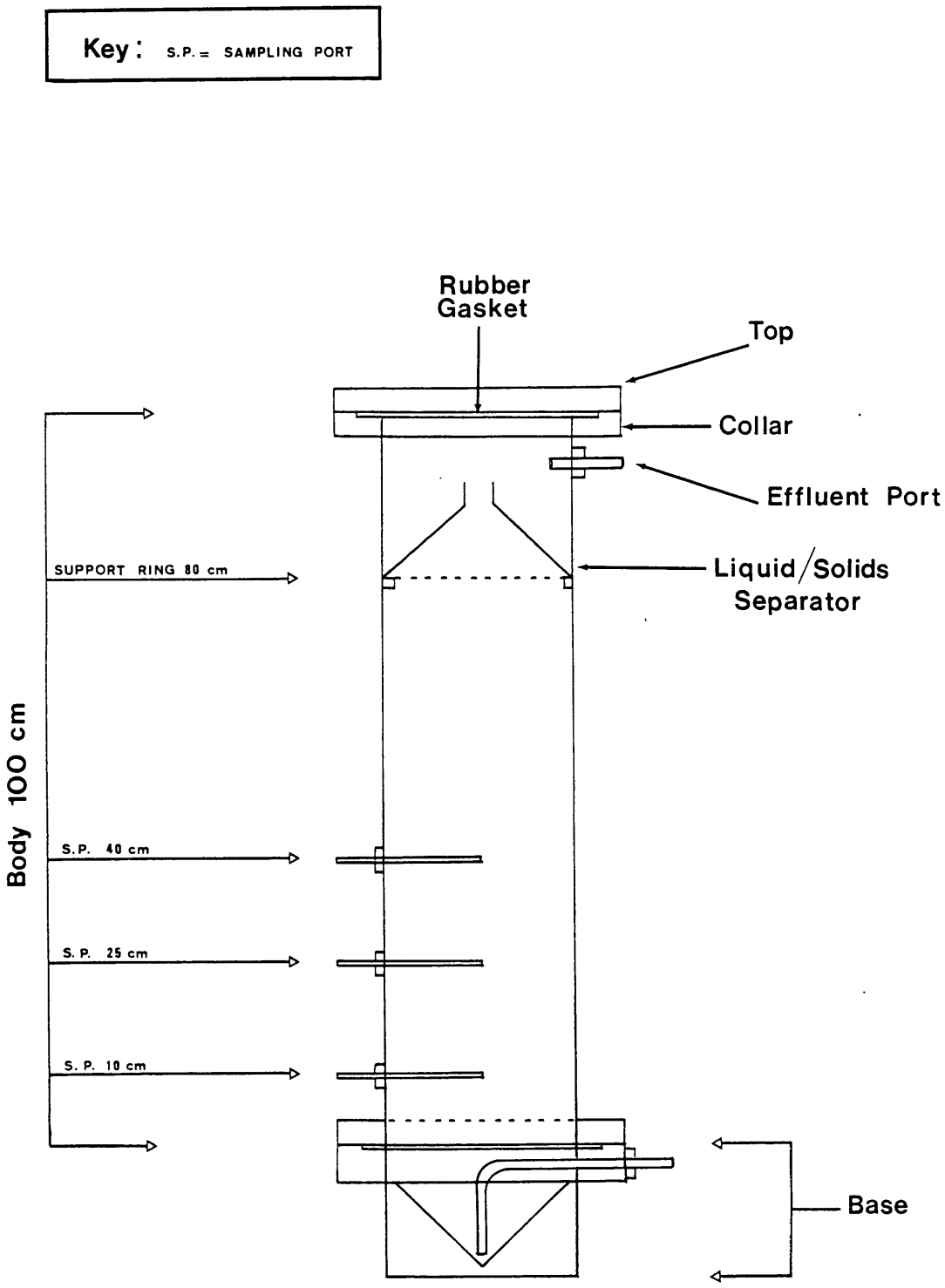


FIG. 23

3.03.1.1 BIOMASS

The seed employed in the upflow anaerobic sludge blanket was an already-granulated biomass kindly supplied by C. Davidson and Sons, Mugiemoss Mill, Aberdeen, Scotland. The granular sludge originated from pilot scale UASB reactors operating on papermill effluent. The well-developed granules were approximately 2mm in diameter, with a specific gravity in excess of 1.0.

3.03.1.2 OPERATION OF SLUDGE BLANKET REACTORS

Each reactor was filled with a water:feed mixture. The temperature was maintained by the use of heating belts. The seed inoculum was introduced into the reactor column through the top.

3.04 EFFLUENT SAMPLING PROCEDURES

Effluent samples from the 2l fluidised bed systems were removed from the effluent overflow of the U-tube (section 3.01). Samples of 60 to 100ml were collected directly into standard 250ml polythene sample bottles with screw-on polythene caps over a period of 2 to 4 hours, depending on the influent flow rate.

Effluent samples were removed from the effluent overflow line of the 4l fluidised bed systems by means of the 3-way stopcock inserted into the line (section 3.01.2). Samples of 20 to 30ml were removed at approximately the same time each day into 40ml glass sampling tubes with push-on polypropylene stoppers.

Effluent samples from the anaerobic filters and the upflow anaerobic sludge blankets were removed into 40ml

glass sampling tubes with push-on stoppers. A sample of 20 to 30ml was taken over a period of several minutes daily from the stopcock situated in the overflow line of each system (sections 3.02.2 and 3.03.2).

3.05 SYNTHETIC AND INDUSTRIAL WASTEWATERS

3.05.1 SYNTHETIC WASTEWATERS

A meat wastewater based on meat extract, glycerol and essential inorganic nutrients was used. In addition a synthetic glucose effluent based on that of Anderson and Donnelly (1978) was employed. The composition of each waste is given in Table 6 together with the composition of a trace element solution, modified from that of Wiegant et al. (1983), with which each synthetic waste was supplemented at a concentration of 0.05ml l^{-1}, or as required. Except where stated, all chemicals used were of Analar grade (BDH Chemicals Ltd., U.K.).

Each wastewater was prepared in 10l or 20l volumes with distilled water. In one phase of the study, the meat-based effluent was prepared at five times the given concentration and up to half the 'Lab Lemco' component replaced by its COD equivalent of methanol; where a substrate with methanol was used, the NH_4Cl concentration was adjusted to 50%. These measures were employed to enhance methanogenic growth (Bull et al. 1983c). The wastes were autoclaved at 121°C for 1 hour to maintain a consistent influent quality. The synthetic glucose waste had a nominal COD of 12000mg l^{-1} and the meat waste a COD of 2500mg l^{-1}.

TABLE 6 COMPOSITION OF SYNTHETIC WASTEWATERS AND TRACE ELEMENT SOLUTION

CONSTITUENT	CONCENTRATION (mg l^{-1})
Glucose-based wastewater	
Glucose	8000
Peptone (Oxoid L39)	2400
Oxoid 'Lab Lemco' powder (L34)	800
Potassium dihydrogen orthophosphate	240
Sodium hydrogen carbonate	320
Calcium chloride (hydrated)	22
Magnesium chloride (hydrated)	48
Meat-based wastewater	
Oxoid 'Lab Lemco' powder (L34)	1950
Glycerol	200
Ammonium chloride	360
Sodium chloride	50
Potassium dihydrogen orthophosphate	30
Calcium chloride (hydrated)	24
Magnesium sulphate (hydrated)	7.5
Trace element solution	
Cobalt nitrate	0.1
Ferrous chloride	0.1
Ethylenediaminetetraacetic acid (EDTA)	0.05
Manganous chloride	0.025
Sodium selenate	0.005
Ammonium molybdate	0.0045
Boric acid	0.0025
Zinc chloride	0.0025
Nickel chloride	0.0025
Cupric nitrate	0.0015

3.05.2 PHARMACEUTICAL WASTEWATERS

Two pharmaceutical wastewaters were made available for this study. Both originated as aqueous filtrates from the manufacture of therapeutic chemicals. Analyses were carried out to determine the COD concentrations (section 3.06.01) and ammonia-nitrogen (section 3.06.09), chloride (section 3.06.10) and total phosphorus (section 3.06.11) content of the effluents. Their major characteristics are given in Table 7. The effluents were stored in 20l polyethylene carboys (Nalgene) until required, whereupon they were diluted and dispensed into 10l pyrex aspirators. The wastes were diluted with distilled water to the appropriate COD concentrations and their pH values adjusted to 7.0 by the addition of NaOH. Both wastes were fully soluble and contained negligible suspended solids.

TABLE 7 COMPOSITION OF PHARMACEUTICAL EFFLUENTS

WASTE	PRINCIPAL CHARACTERISTICS	
	COMPONENT	CONCENTRATION
A	water	
	n-propanol	
	NaCl	4950 $mg\,l^{-1}$
	NH_3-N	147 $mg\,l^{-1}$
	total P	51 $mg\,l^{-1}$
	pH	5.0
	COD	174000 $mg\,l^{-1}$
B	water	
	dimethlyformamide	30%
	NaCl	4125 $mg\,l^{-1}$
	NH_3-N	15 $mg\,l^{-1}$
	total P	not detectable
	pH	6.0
	COD	325000 $mg\,l^{-1}$

3.05.3 FRUIT PROCESSING EFFLUENT

The fruit processing effluent was obtained from a

factory which produces jams, and fruit for pie fillings and yoghurt making. The wastewater arose from plant washouts at the end of production runs, accidental spillages, prewashing of containers and the release of cooling waters from a vacuum plant for jam production. The effluent was high in sugars and contained the preservative sodium metabisulphate, starch and pectin.

The effluent is drained by gravity to an underground collection sump from where it is pumped through a wedge wire screen to remove large pieces of solid matter (Figure 24). The flow then passes through a pH correction tank that is automatically controlled by a pH probe. Sodium bicarbonate is used for pH correction. Flow measurement is provided by means of a vee-notch and ultrasonic level detector at the outlet of the pH correction tank.

Samples of fruit processing wastewater were taken from the outlet of the vee-notch chamber (Figure 24). Samples of 10l volume were removed at intervals of 7 to 10 days. The variation in sample characteristics are shown in Table 8.

TABLE 8 COMPOSITION OF FRUIT PROCESSING EFFLUENT

Parameter	Range	Mean
pH	4.0 - 7.4	5.2
COD (mgl^{-1})	5800 - 13100	7900
Suspended solids (mgl^{-1})	180 - 520	350

The pH of the influent was adjusted where necessary to a value in the range 6.5-7.0 by the addition of sodium bicarbonate prior to feeding to the anaerobic reactor.

The effluent samples were stored in and dispensed from 10l polyethylene containers, diluted as required with distilled water.

3.05.4 SOFT DRINKS MANUFACTURING EFFLUENT

The soft drinks wastewater was obtained from a factory which produces carbonated and concentrated soft drinks. The effluent originated from production line start-up operations, where the product was deliberately wasted to obtain product uniformity before bottle filling, spillage during bottle filling, accidental spillages and cleaning down operations after a production run. The wastewater contained sugar or saccharine sweeteners, citric or malic acid, flavourings, colourings and the preserving agents sodium benzoate (E211) and sodium metabisulphate (E223). Biocidal cleaning agents based on quaternary ammonium compounds or chlorine based compounds, and added detergents also contributed to the waste load.

The production line effluent gravitates through a drainage system to a collection tank. It is then pumped up through a tank at ground level where a solution of sodium carbonate is added for pH correction (Figure 25). The volume of flow through a vee-notch is measured ultrasonically.

Effluent samples were removed from the outlet of the vee-notch tank (Figure 25). Samples of 10l volume were taken at 7 to 10 day intervals. Sample characteristics varied in the manner shown in Table 9.

Sodium bicarbonate was added to the influent feeds to the anaerobic system where necessary to adjust the pH to a

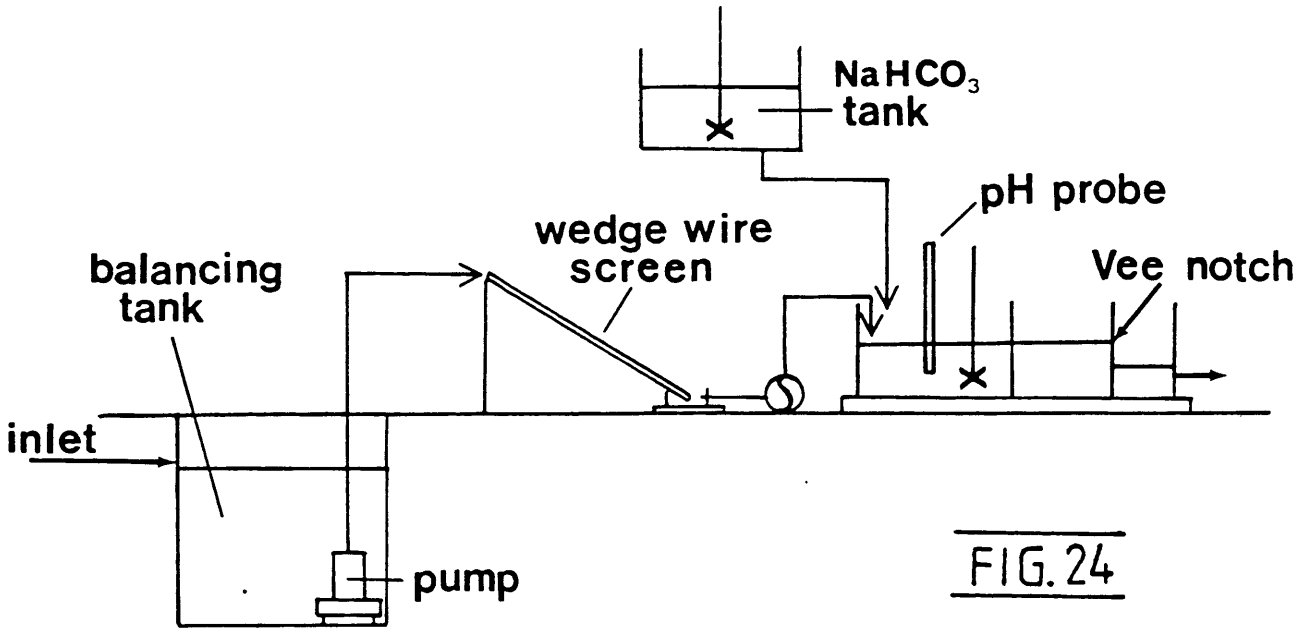


FIG. 24

SCHEMATIC ARRANGEMENT OF IN-PLANT TREATMENT OF FRUIT PROCESSING EFFLUENT

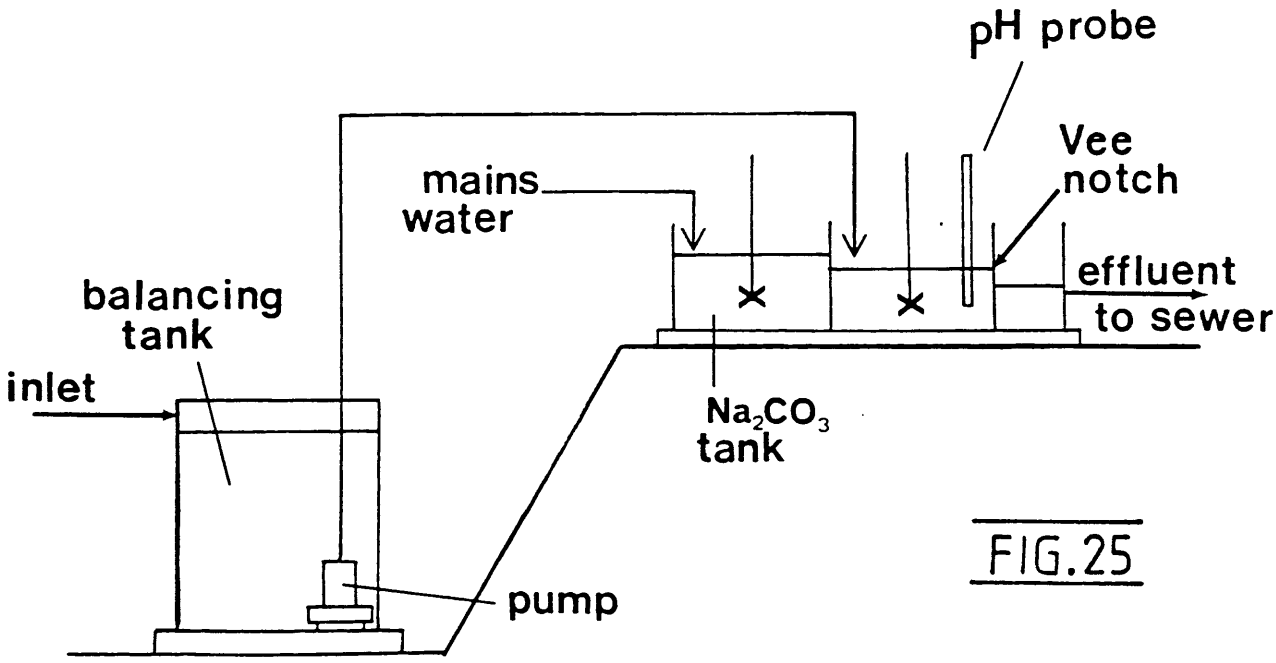


FIG. 25

SCHEMATIC ARRANGEMENT OF IN-PLANT TREATMENT OF SOFT DRINKS MANUFACTURING EFFLUENT

value between 6.5-7.0.

The effluent samples were stored in 10l polyethylene containers and diluted when required with distilled water.

TABLE 9 COMPOSITION OF SOFT DRINK PROCESSING EFFLUENT

Parameter	Range	Mean
pH	3.7 - 6.7	5.7
COD (mgl^{-1})	9000 - 15000	11500
Suspended solids (mgl^{-1})	160 - 360	280

3.06 ANALYTICAL METHODS

3.06.01 CHEMICAL OXYGEN DEMAND

The technique employed was based on the standard Methods for the Examination of Waters and Associated Materials (1977) and incorporated minor modifications. The size of sample taken was in accordance with Table 10.

TABLE 10. SIZE OF SAMPLE FOR COD ANALYSIS

COD range (mgl^{-1})	Sample size (ml)
<200	5
200-600	2.5
>600	<1

The sample was generally filtered and diluted to 5ml with distilled water if necessary.

Oxidising reagent was prepared by mixing 400ml potassium dichromate solution (7.6618gl^{-1} in distilled water) with 1500ml of silver sulphate solution (10gl^{-1} in concentrated sulphuric acid). The sample and 9.5ml of oxidising reagent were added to a 250ml round bottomed

flask (Quickfit, Corning, U.K.), together with 0.5ml of mercuric sulphate solution ($200gl^{-1}$ in 10% (v/v) sulphuric acid) to prevent chloride precipitation. A distilled water blank was also prepared. The sample was boiled under reflux for two hours.

After cooling, 40ml distilled water was added to the flask through the condenser and the sample titrated against 0.0625M ferrous ammonium sulphate in 2% (v/v) sulphuric acid. Ferrous phenanthroline was used as indicator.

The COD of the sample was estimated from the relationship:

$$COD = \frac{(B - S) \times 500}{V} \qquad (10)$$

where: B = blank titration (ml)

S = sample titration (ml)

V = sample volume (ml)

3.06.02 pH

Sample pH was determined electrometrically using an Orion Research Model 701 pH meter, with facility for temperature compensation. Meter standardisation was carried out prior to each determination using a pH 7.0 buffer solution (Gallenkamp). The latter was renewed weekly.

3.06.03 SUSPENDED SOLIDS

Suspended solids concentrations were determined using the standard Methods for the Examination of Waters and Associated Materials (1980). Effluent samples were filtered using Whatman Glass Fibre GF/C grade 7.0cm

filters. Filter papers were heated at 105°C and dried to constant weight.

3.06.04 VOLATILE SOLIDS

Volatile solids concentrations (VS) in the anaerobic fluidised beds were estimated using a method modified from that of Stephenson and Lester (1986). A known volume of sand support media was removed from the bed, transferred to a 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 minutes. After cooling to constant weight, volatile solids were determined from:

$$VS\ (mgl^{-1}) = \frac{M_{105} - M_{500}}{S} \times 1000 \qquad (11)$$

where: M_{105} = mass after heating at 105°C (mg)

M_{500} = mass after heating at 500°C (mg)

S = sample size (ml)

3.06.05 TOTAL VOLATILE ACIDS

The concentration of total volatile acids in a sample was determined by the colorimetric method recommended in Methods for the Examination of Waters and Associated Materials (1979). Absorbance was measured at 500nm with a 1nm slit width using 10mm glass cells (Frost Instruments, Wokingham, U.K.) on a Pye Unicam Model SP8-100 UV-visible spectrophotometer. Working standards were prepared from a 10000mgl^{-1} stock acetate solution and calibration was carried out using a distilled water blank.

3.06.06 INDIVIDUAL VOLATILE ACIDS

Individual volatile acids concentrations were determined using a Phil ips Pye Unicam GCD gas chromatograph. A 1.83m, 2mm i.d. glass column containing a 10% free fatty acid phase on a 100-200 mesh chromosorb W.H.P. support was used. Nitrogen was used as the carrier gas at a flow rate of 120ml min^{-1}. Hydrogen and air were used for the detector gases at flow rates of 150ml min^{-1} and 375ml min^{-1} respectively. The chromatograph conditions are included in Table 11.

The injection volume of sample was 5µl. Prior to each sample injection, the column was conditioned with one 8µl injection of 50% (v/v) formic acid. The latter polarised the column and optimised the resolution of propionic and iso-butyric acids and reduced peak tailing. A volatile acids calibration standard (BDH Chemicals Ltd., Poole, U.K.), the composition of which is shown in Table 12, was used. The detector output was recorded on a Perkin Elmer 023 chart recorder and acid concentrations were determined by comparison of peak heights.

Some samples were analysed using a Perkin Elmer Sigma 2 gas chromatograph with flame ionisation detector. This was coupled to a Perkin Elmer LC1-100 integrator to give direct read-out of individual volatile acids concentrations.

3.06.07 GAS COMPOSITION

The method used was that of Kirk et al. (1982) and employed a modified gas chromatograph (Gallenkamp). The instrument was adapted to contain two parallel packed columns containing chromosorb 102 and molecular sieve 5A

TABLE 11 GAS CHROMATOGRAPH SETTINGS FOR VOLATILE ACIDS ANALYSIS

Injection temperature	250°C
Detector temperature	300°C
Initial temperature	120°C
Final temperature	170°C
Initial time	4 min
Rate of rise	4^{o} min^{-1}

TABLE 12 COMPOSITION OF VOLATILE ACIDS CALIBRATION STANDARD

ACID	CONCENTRATION (mgL^{-1})
Acetic	200
Propionic	100
n - Butyric	50
n - Hexanoic	50
i - Butyric	20
n - Valeric	20
i - Valeric	20

(Phasesep Ltd., Queensferry, Clwd), each passing through opposing cells of a thermal conductivity detector. The chromatograph was calibrated using 100% methane (BOC Ltd., Special Gases). Methane was determined by injection of 500µl of effluent gas by a glass gastight syringe into the chromosorb 102 column. Traces were recorded on a Servogor 120 chart recorder (Servoscript, Croydon, U.K.) and comparisons of peak heights made.

3.06.08 RELATIVE METHANE FORMING ACTIVITY

Methane production was determined using a method modified from van den Berg and Lentz (1979). A solids sample of approximately 3ml was incubated at 37^{o}C in 50ml of a 1000mgl^{-1} acetic acid solution adjusted to pH 7.4±0.2 with ammonium hydroxide solution. Reagent bottles of 125ml with rubber serum caps of appropriate size (Gallenkamp) were utilised. The bottles were gassed using oxygen-free nitrogen (BOC gases, U.K.) for 10 minutes at a flow rate of 0.5ml min^{-1} both before and after seed and substrate addition. Gas pressures within the bottles were brought to atmospheric by inserting a syringe, and the bottles incubated for 2 days. Gas production was measured by inserting a lubricated syringe through the serum cap: any gas pressure within the bottle caused the syringe plunger to move upwards.

Gas composition was analysed as described in section 3.06.07.

3.06.09 AMMONIA-NITROGEN

The ammonia-nitrogen content of wastewaters was

determined using the standard phenate method recommended by the American Public Health Association (1985). The method is based on the production of intensely blue indophenol, formed by the reaction of ammonia, hypochlorite and phenol, catalysed by a manganous salt. A Pye Unicam Model SP8-100 UV-visible spectrophotometer was employed at 630nm with a 1nm slit width and using 20mm glass cells (Frost Instruments) to determine sample concentrations.

3.06.10 SODIUM CHLORIDE

Sodium chloride concentrations were estimated by the argentometric method for chloride detection in accordance with the procedures outlined by the American Public Health Association Standard Methods (1985). A calibration curve was prepared using dilutions of a standard sodium chloride solution. Samples of 100ml in 250ml Erlenmeyer flasks were adjusted to pH 7-10 and 1ml potassium chromate indicator added. Each sample was titrated with a standard silver nitrate titrant to the end point. A reagent blank was established and the concentration of sodium chloride calculated by the following:

$$mgNaCl\ l^{-1} = \frac{(A-B) \times N \times 35450}{V_S} \times 1.65 \quad (12)$$

where: A = titration volume for sample (ml)

B = titration volume for blank (ml)

N = normality of silver nitrate

V_S = sample volume (ml)

3.06.11 TOTAL PHOSPHORUS

The total phosphorus content of samples was determined by the mild digestion followed by colorimetry procedure recommended in Methods for the Examination of Waters and Associated Materials (1980). The samples were neutralised and digested with sulphuric acid. Following cooling and a further neutralisation stage, colorimetric analysis was performed using a Pye Unicam Model SP8-100 UV-visible spectrophotometer at 882nm, with 1nm slit width and 40mm glass cells (Frost Instruments).

3.06.12 GLASSWARE

All glassware used was cleaned by soaking in a 5% (v/v) solution of 'Decon 90' detergent (BDH Chemicals Ltd.) for 24 hours, unless otherwise stated. Thorough rinsing with distilled water followed this step. Polypropylene containers were similarly cleaned.

3.07 PRODUCTION OF NATURAL POLYMER

Pure cultures of a polymer-forming capsulated strain of Klebsiella aerogenes, NCTC 8172 (National Collection of Type Cultures, Central Public Health Laboratory, London) were grown as batch cultures in a shaking incubator at 70 rpm and at a temperature of 25±2^{o}C. Two successive subcultures of 125ml in 2l conical flasks were made prior to inoculation of three 200ml cultures. The latter were harvested after 5 days. Batch culture was also carried out using an LKB 1601 Ultraferm fermentation system (LKB Instruments Ltd., Selsdon, Surrey) operated at a temperature of 25±2^{o}C, a pH of 6.8 and a dissolved oxygen

concentration of approximately 6.3mg l^{-1}.

The medium employed to promote extracellular polymer production by K. aerogenes was that of Rudd et al. (1982). It contained the AR components listed in Table 13.

Culture samples for a viable count were serially diluted in 0.1M phosphate buffer, plated in triplicate onto casitone-glycerol-yeast extract agar (Pike & Carrington 1972) and incubated at 25°C for 24 hours.

Cell dry weight was determined by filtering samples through a 0.22µm micropore cellulose acetate membrane (Amicon, Woking, Surrey) and weighing the residue subsequent to drying to constant weight at 105°C.

TABLE 13. COMPOSITION OF K. aerogenes CULTURE MEDIUM

Compound	Concentration (mgl^{-1})
sucrose	10000
ammonium sulphate	300
sodium dihydrogen phosphate	2000
dipotassium hydrogen phosphate	2000
potassium sulphate	1000
sodium chloride	1000
magnesium sulphate	200
calcium chloride	20
ferrous sulphate	1

3.08 SELECTION OF SYNTHETIC POLYMER

The effectiveness of two synthetic flocculants added to digested sewage sludge (Hogsmill Valley Water Pollution Control Works) at varying dosages were compared in terms of supernatant clarification and speed of sludge settlement.

Four different volumes, 0.5, 1.0, 1.5 and 2.0 ml, of Millfloc C_{30} or Millfloc C_{50} (Crosmill Flocculants Ltd., Goostrey, Cheshire) polymer solutions (0.5% of active flocculant) were added respectively to two series of five

beakers each containing 500ml of a 20% (v/v) solution of digested sewage sludge. The fifth beaker in each series was left unamended by polymer and was used as a control. Flocculation was achieved by stirring on a sedimentation jar test apparatus (Aztec Environmental Control, Ltd.). Stirring for 10s at 400 rpm was followed by further agitation at 50 rpm for 2 min. After a settling period of 15 min, samples of supernatant were removed and their absorbances measured at 420nm on a Pye Unicam Model SP8-100 UV-visible spectrophotometer, using 10mm glass cells (Frost Instruments) at a 1nm slit width.

3.09 DETERMINATION OF EFFECTS OF POLYMER ADDITION ON MICROBIAL ATTACHMENT

The effects of the addition of natural and synthetic polymers on the attachment of microorganisms to the surfaces of sand particles were determined by the following: three series of five replicate test tubes containing 5ml of sand were amended with 10ml of the digested sludge solution used in section 3.08. These were designed to simulate the final concentrations of each which were to be used in the fluidised bed reactor (section 3.01.2). Volumes of 0.4ml of C_{30} or C_{50} synthetic polymer solutions or K. aerogenes suspension were added to one set of replicates and the tubes sealed by rubber stoppers. The tubes were then quickly inverted four times to obtain adequate dispersion of the flocculant. An additional series of tubes without polymer was included as control.

After a settling period of 15 min, supernatant samples were removed and their absorbances measured as above.

3.10 BATCH TESTING OF PHARMACEUTICAL WASTES

Two pharmaceutical wastes, A and B (section 3.05.2), were tested for anaerobic biodegradability in the following manner.

Duplicate batches of 125ml reagent bottles with rubber serum caps of appropriate size (Gallenkamp) were utilised. The bottles were gassed using oxygen-free nitrogen (BOC gases, U.K.) for 15 min at a flow rate of approximately 0.5 l min^{-1} prior to seed and substrate addition. An easily-degradable glucose-based substrate, as described in section 3.05.1, was prepared and diluted with distilled water to 2500mg COD l^{-1}. The substrate also contained a trace mineral solution (section 3.05.1) at a concentration of 0.05ml l^{-1}. The wastes A and B were diluted to 2500mg COD l^{-1} and neutralised to pH 7.0 with NaOH.

Samples of glucose substrate and pharmaceutical waste plus substrate were prepared and added to each reagent bottle as indicated in Table 14, whilst gassing. The bottles were equilibrated to 37°C in a heated water bath (Grant Instruments Ltd.). Ten ml of anaerobically digested sewage sludge were then transferred to each bottle. Each serum cap was inserted whilst simultaneously removing the gas flushing needle. The bottles were then equilibrated for one hour and the gas pressures within them brought to ambient by inserting a syringe.

The bottles were then incubated for 30 days at 37°C. After the incubation period the final percentage of methane in the effluent gas of each bottle was assayed using the technique outlined in section 3.06.07.

TABLE 14 BATCH TESTING OF PHARMACEUTICAL WASTES : PREPARATION OF REAGENT BOTTLES

BOTTLE*	WASTE A (%)	WASTE B (%)	GLUCOSE WASTE (%)	TOTAL COD (mgL^{-1})	SEED (ml)	TOTAL VOLUME (ml)
1	0	0	100	2500	10	60
2	0	20	80	2500	10	60
3	0	40	60	2500	10	60
4	0	60	40	2500	10	60
5	0	80	20	2500	10	60
6	0	100	0	2500	10	60
7	20	0	80	2500	10	60
8	40	0	60	2500	10	60
9	60	0	40	2500	10	60
10	80	0	20	2500	10	60
11	100	0	0	2500	10	60

*Each bottle prepared in duplicate

CHAPTER 4
RESULTS

4.01 EFFECTS OF SYNTHETIC POLYMER ADDITION ON SLUDGE FLOCCULATION

The flocculation capacities of the synthetic polymers C_{30} and C_{50} were compared in duplicate jar tests (section 3.08). The supernatant turbidity of sludge samples amended with varying concentrations of each flocculant is shown in Figure 26. An increase in polymer concentration in the system caused a corresponding decrease in supernatant turbidity. The effect was less marked at polymer solution concentrations in excess of 2.0mg l^{-1} however, therefore 2.0mg l^{-1} was considered to be the optimum polymer solution concentration. At this value there was a difference of 15% in turbidity by flocculants C_{30} and C_{50}, indicating that the latter encouraged superior sludge settleability.

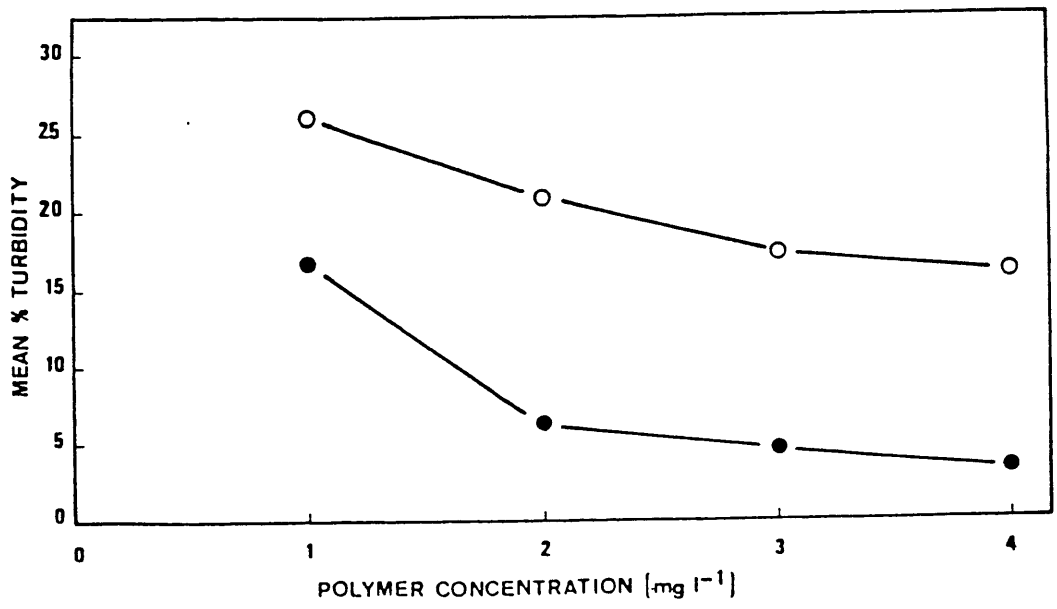


FIG.26 Supernatant turbidity of sludge samples amended with flocculant polymers C_{30} (o) and C_{50} (●).

4.02 POLYMER-AIDED ATTACHMENT OF SLUDGE TO SAND CARRIER MEDIUM

The effect of both natural and synthetic polymers on microbial attachment to media surfaces was investigated by the addition of the polymers to sand-sludge admixtures (section 3.09). Optimum concentrations of 2.0mg l^{-1} of the C_{30} and C_{50} flocculant solutions were utilised in replicate jar tests. As the culture of Klebsiella aerogenes (section 3.07) tended to form a colloidal suspension which contributed to the turbidity of the sludge solution, a blank of the culture was prepared and its absorbance subtracted from that of the test K.aerogenes culture.

The results are shown in Table 15. These indicated that the polymer C_{50} could achieve a lower supernatant turbidity than polymer C_{30} and the K.aerogenes culture.

TABLE 15. COMPARISON OF SUPERNATANT TURBIDITY OF SLUDGE-SAND MIXTURES AMENDED WITH POLYMERS

Flocculant	Mean Absorbance* (X)	Standard Deviation (s)	Turbidity %
Blank	0.395	0.024	100
C_{30}	0.237	0.041	60
C_{50}	0.150	0.041	38
K.aerogenes Blank	0.608	0.048	60
K.aerogenes	0.371	0.081	

* of 5 replicates

The results of paired t-tests applied to the mean absorbances obtained indicated that the observed differences between the synthetic flocculants were

statistically significant ($P<0.01\%$), as were the differences between the K.aerogenes culture and the blank ($P<0.01\%$).

The addition of polymer was hence considered conducive to sludge settlement and to bacterial attachment and C_{50} was superior to C_{30} in both respects. Polymer C_{50} and K.aerogenes were utilised in the subsequent start-up operations.

4.03 START-UP OF ANAEROBIC FLUIDISED BEDS

4.03.1 START-UP PROCEDURES

The anaerobic fluidised bed reactor construction described in section 3.01.2 and the meat based waste with trace element addition (section 3.05.1) were used. The flowrate to each system was adjusted to maintain a bed expansion of 20%. The wastewater exerted a nominal COD of 2500mg l^{-1}. Where variable influent strength was required, the feed was prepared at five times the above concentration and then diluted with distilled water to provide final influent concentrations ranging from 1000-11600mg l^{-1}.

The four fluidised beds were filled with the meat waste and methanol solution at 2500mg l^{-1}, seeded with 30ml of digested sewage sludge and polymer where appropriate and operated at 37°C in closed system for 48 hours prior to feeding. Reactor 1 acted as control. Synthetic polymer C_{50} was added to the second system and Klebsiella aerogenes suspension to the third (section 4.02). The fourth system was maintained under complete anaerobiosis by continuous purging of the recycle chamber and feed aspirator with oxygen-free nitrogen at a pressure

of approximately 1.38 x 10^4Pa.

Start-up involved stepwise increases in hydraulic loading but incorporated variable organic loading. The latter was achieved by varying the influent strength and was regarded as more representative of real world conditions, since pumping rates are in general more controllable than influent composition and strength. In addition, up to half of the COD of the 'Lab Lemco' component of the feed was replaced by its COD equivalent of methanol (section 3.05.1). The loading regime is given in Table 16. From day 45 to day 50 of start-up the organic loading rate was held constant to ascertain that the reactors had reached steady state.

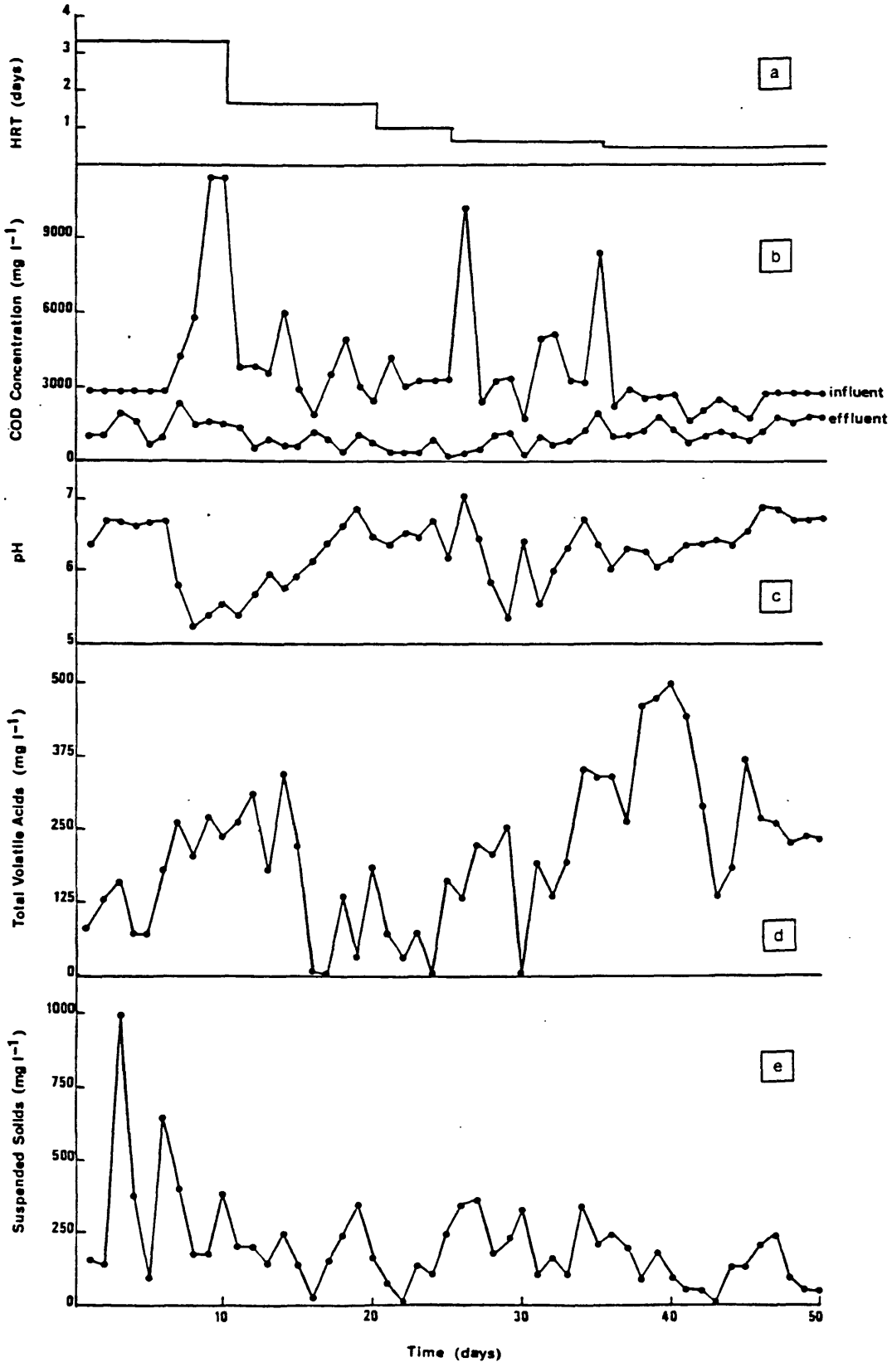
4.03.2 INFLUENCE OF VARIABLE LOADING ON THE START-UP AND PERFORMANCE OF AN ANAEROBIC FLUIDISED BED

Influent and effluent COD concentrations, pH values and total volatile acids (TVA) and suspended solids concentrations for the control system throughout the experiment are presented in Figure 27. Increased COD loading generally resulted in a concomitant increase in effluent COD (Figure 27 (b)), with the reactor responding rapidly to transient organic shocks over the initial HRTs of 3.33 to 1.67d. As HRT decreased below 1.67d, however, an increase in mean percentage COD removal was exhibited (Table 17). COD removal stabilised towards the end of the start-up period, when constant loading was introduced, although the removal rate was lower than that exhibited during influent variation, probably because of the short final HRT applied, and adjustment of the feed components.

Table 16 Influent parameters during the start-up of anaerobic fluidised bed reactors

Day	Influent Flow Rate($l d^{-1}$)	HRT (d)	Organic loading rate (kg COD m^{-3} d^{-1})		Mean Influent COD ($mg l^{-1}$)	Wastewater Composition	
			mean	range		CH_3OH (% COD)	NH_4Cl (% Final Conc.)
0	0.75	3.33	1.36	0.91-3.63	4361	50	50
10	1.5	1.67	2.77	1.16-7.26	4434	50	50
20	2.5	1.00	3.41	2.53-4.38	3270	50	50
25	4.0	0.63	6.99	2.75-16.78	4085	25	50
35	4.3	0.54	5.34	3.12-15.54	2845	0	100
50	4.3	0.53	5.30	-	2900	0	100

FIG.27



Effect of variable organic loading on effluent quality during start-up of an anaerobic fluidised bed reactor.

During the first ten days of operation, the fluidised bed was subjected to a peak influent COD concentration of 11.6g l^{-1}. The system responded with effluent COD values of 0.75-1.5g l^{-1} over the following three days. A maximum COD removal efficiency of 97% was recorded when the influent COD concentration was 3.3g l^{-1} and the HRT was 0.63d.

TABLE 17. MEAN COD REMOVAL AND EFFLUENT TOTAL VOLATILE ACIDS (TVA), SUSPENDED SOLIDS (SS) AND VOLATILE SOLIDS (VS) CONCENTRATIONS IN AN ANAEROBIC FLUIDISED BED DURING START-UP

HRT (d)	Mean COD removal (%)	Mean TVA (mg l^{-1})	Mean SS (mg l^{-1})	VS (mg l^{-1})
3.33	60	159	350	1650
1.67	78	173	209	1250
1.00	82	72	98	2250
0.63	81	188	253	3450
0.54	54	313	107	4400

Throughout start-up, the pH, TVA and suspended solids concentrations fluctuated to a marked extent (Figure 27). The TVA concentrations were lower when effluent COD values were also low. Severe COD overloading of the reactor resulted in short-term fluctuations in TVA concentrations, and hence pH, with TVA levels increasing 6-fold but returning to normal concentrations within 48 hours after overload. The accumulation of TVA during periods of marked reactor instability was common, with COD removal decreasing rapidly from 80% to less than 50% at increased acids concentration. Reactor pH decreased from 6.4 prior to shock loading to 5.9 under shock load conditions (Figure

27(c)). When the COD loading was stabilised at day 45, a pH value of 6.9 was ultimately achieved.

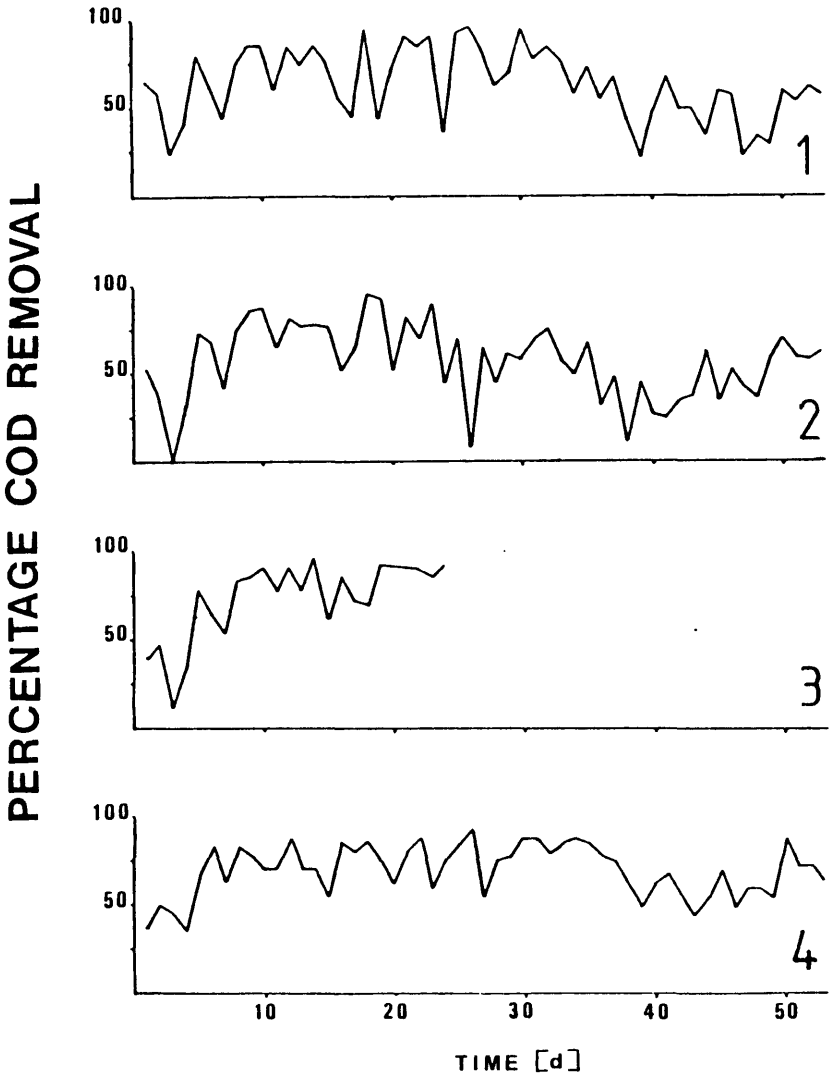
Effluent solids concentration increased initially up to 1000mg l^{-1} (Figure 27(e)) then fluctuated within the range 350-375mg l^{-1} during the first hydraulic retention period. However, after day 24, the effluent suspended solids were low in comparison to the attached volatile solids. The fluctuations observed in effluent solids concentration during start-up appeared to be more directly related to the variable nature of the influent (Figure 27(b) and (e)) than to any inherent instability of the system itself. This was evident when the feed was returned to a constant strength on day 45 at the end of the stepped loading regime, at which point the effluent characteristics stabilised, indicating that steady state had been reached.

4.03.3 COMPARISON OF FOUR START-UP REGIMES ON THE PROCESS PERFORMANCES OF ANAEROBIC FLUIDISED BEDS

An examination into the effects of the addition of synthetic (reactor 2) and natural (reactor 3) polymers and the maintenance of anaerobic conditions (reactor 4) on the start-up of anaerobic fluidised bed systems was undertaken. The results were compared to those of the control system (1) (section 4.03.2) and are shown in Figures 28 to 31. Data for system 3 have been given up to day 25 only, as this reactor suffered a major and irreparable breakdown at this point, and previous minor operational interruptions rendered the system unreliable.

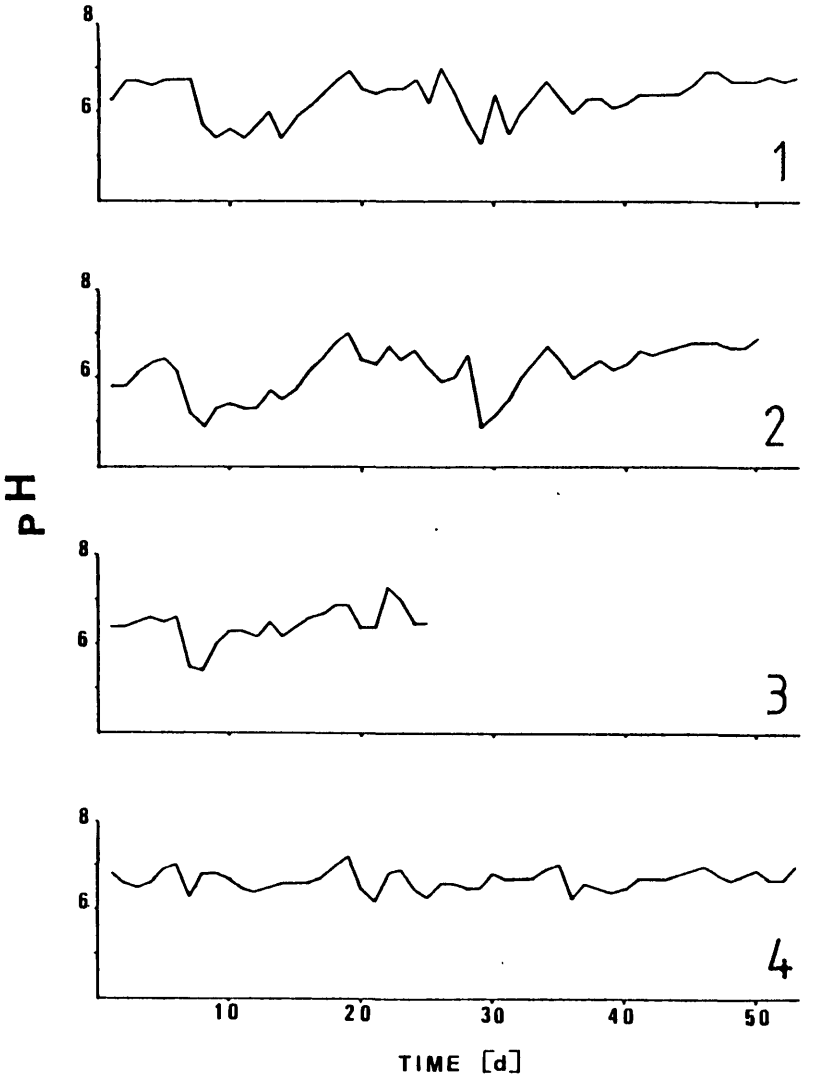
The four systems displayed marked variations in most of the process parameters considered, although a common

FIG. 28



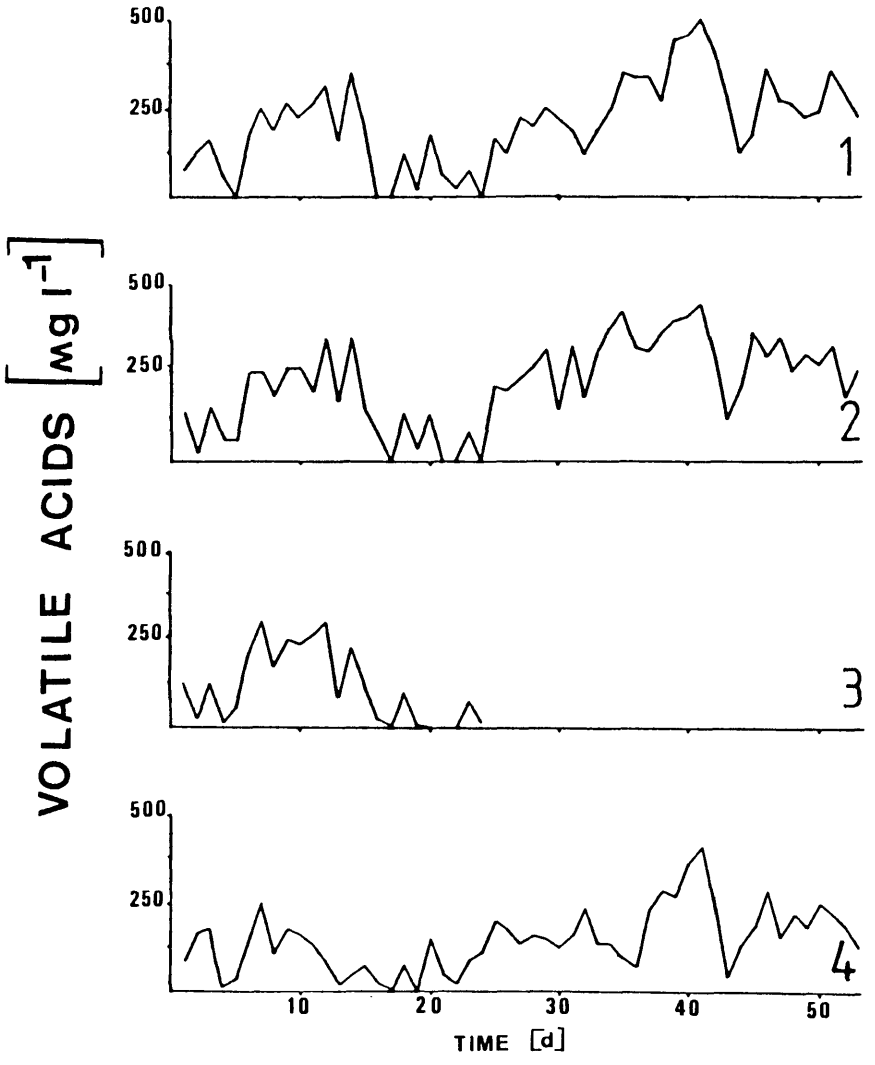
Comparison of four start-up regimes on COD removal efficiency in anaerobic fluidised beds.

FIG. 29



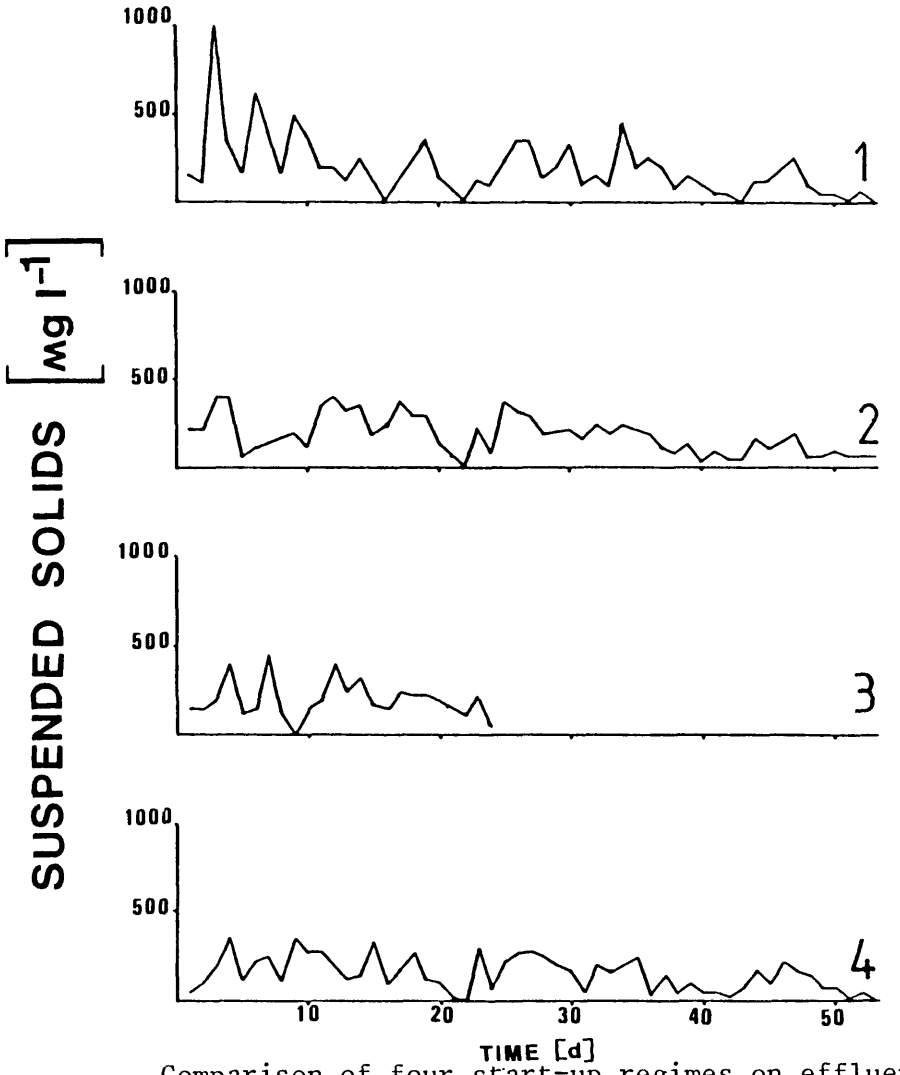
Comparison of four start-up regimes on pH of anaerobic fluidised beds.

FIG. 30



Comparison of four start-up regimes on total volatile acids concentration in anaerobic fluidised beds.

FIG. 31



Comparison of four start-up regimes on effluent suspended solids concentration in anaerobic fluidised beds.

pattern of recovery after overloading was observed. The mean COD removal efficiency of reactor 2 (Figure 28), amended with synthetic polymer, increased from 55% to 74% during the first two HRTs applied. When the HRT was reduced to 1d (Table 16) and the mean loading rate applied increased to 3.41kg COD m^{-3} d^{-1}, COD removal dropped to 57%. In reactor 3, augmented with K.aerogenes, the COD removal efficiency reached values greater than those exhibited by the control (Figure 28). The pattern of COD removal in reactor 4, under nitrogen purge, was similar to the other systems but the fluctuations in removal observed were much less marked, and efficiencies in excess of 70% were maintained during the major part of start-up.

The pH values in all four reactors decreased after 5 days of operation, apparently in response to increased influent COD concentrations (Figure 29). Reactor 4 sustained the pH drop most efficiently and the rapid recovery observed in the system, maintained throughout start-up, indicated good buffering capacity. However, the reactor amended with natural polymer recovered more rapidly than the control and the synthetic polymer-amended systems. The patterns of pH values obtained tended to mirror the production and accumulation of volatile acids (Figure 30) in the control and polymer-amended beds. Subsequent to initial COD overloading, relatively constant acids concentrations in the range 100-250mg l^{-1} were recorded. This was succeeded by gradual acids accumulation as the mean COD loading was increased. The accumulation of volatile acids after day 15 in the nitrogen-purged system (4) was not reflected in effluent pH, further demonstrating

the good buffering capacity of this reactor.

Effluent suspended solids values over start-up for the four fluidised beds are given in Figure 31. These were relatively low over the period, although considerable initial washout was exhibited by the control system. The latter indicated that both polymer addition and complete anaerobiosis were conducive to bacterial solids retention.

4.03.4 ATTACHMENT OF SEED SLUDGE TO SAND MEDIUM DURING REACTOR START-UP

The effect on bacterial attachment of start-up regimes incorporating stepped hydraulic and variable organic loadings, in addition to polymer amendment and nitrogen purge, were investigated. The results given are examined in respect of systems 1, 2 and 4, as these completed the operational run (section 4.03.3). The values obtained for system 3, amended with natural polymer, are included where appropriate for comparison only.

The attached biomass concentration in the fluidised beds, measured as volatile solids, are shown in Table 18. Volatile solids concentrations were found to be generally greater during the latter stages of start-up, from days 35-50, except for reactor 2, where initial biomass retention was high ($3800 mg l^{-1}$). In systems 1 and 4, volatile solids concentrations fluctuated more near the bases of the reactors over start-up, but a greater concentration of biomass was retained at the end of the period in the upper parts of the beds (Figure 32). In contrast, the polymer-amended reactor (2) exhibited less variation in volatile solids with time, but the final attached biomass

TABLE 18 RELATIONSHIP OF EFFLUENT SUSPENDED SOLIDS (SS) TO ATTACHED VOLATILE SOLIDS (VS) DURING START-UP OF FLUIDISED BED REACTORS

Days from start-up	Mean attached VS ($mg\ l^{-1}$)			Mean effluent SS ($mg\ l^{-1}$)				Mean effluent SS ($mg\ d^{-1}$)				Washout of SS d^{-1} as % of VS			
	1	2	4	1	2	3	4	1	2	3	4	1	2	3	4
10	1650	3800	2000	350	259	225	180	263	194	169	135	15.9	5.1	7.3	6.8
20	1250	3000	1800	209	306	299	207	314	459	449	311	25.1	15.3		17.3
25	2250	2950	1400	98	124	137	110	245	310	343	275	10.9	10.5		19.6
35	3450	3150	1850	253	263		212	1012	1052		848	29.3	33.4		45.8
50	4400	2500	4950	107	123		105	460	529		452	10.5	21.2		9.1

1. Control reactor
2. Reactor amended with synthetic polymer
3. Reactor amended with K. aerogenes polymer
4. Reactor maintained anaerobically

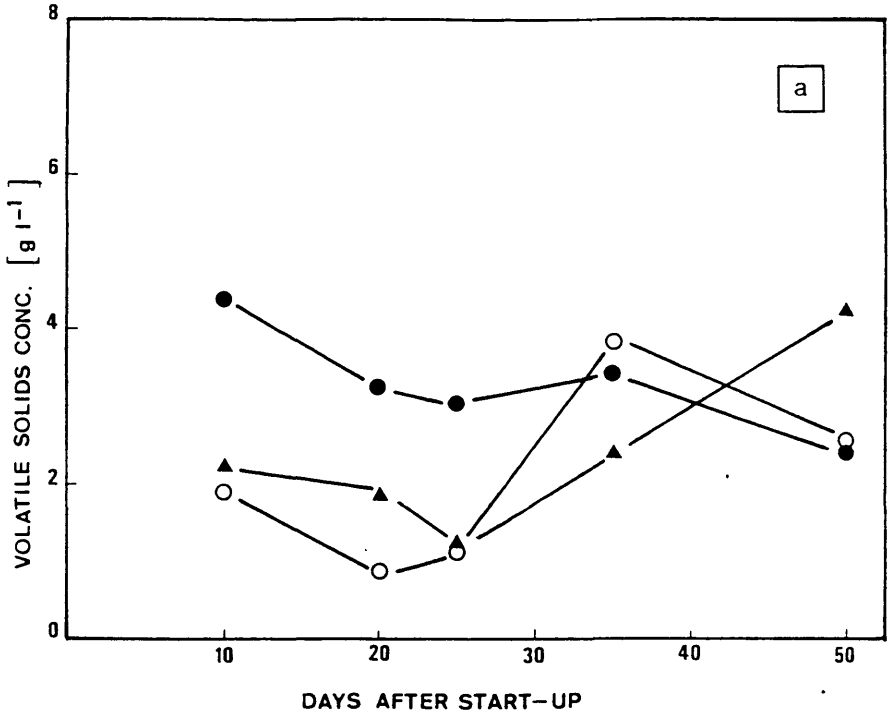
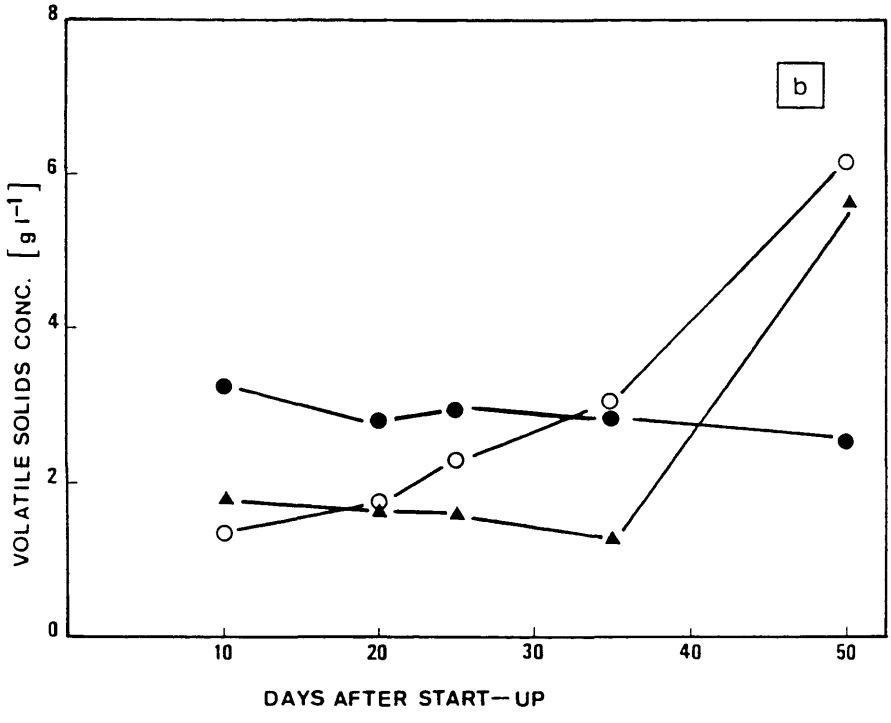


FIG. 32



Biomass accumulation at heights of (a) 15 cm and (b) 55 cm within anaerobic fluidised beds during start-up. Control reactor (○); polymer-amended reactor (●); N_2-purged reactor (▲).

concentration of 2500mg l^{-1} was lower than those of systems 1 and 4. Greater microbial attachment was thus found in the control and the nitrogen-purged reactors at short HRTs and increased organic loading rates but these conditions at the end of start-up were detrimental to the attachment of the microflora of the polymer-amended system.

The mean effluent suspended solids of the reactors are given in Table 18. The control system (1) evinced considerable solids washout during the ten days after seeding, with a peak loss of 1000mg l^{-1} on day 3. The mean biomass lost per day corresponded to 16% of the attached solids in the reactor. In contrast, systems 2 and 4 produced relatively low effluent suspended solids, of the order of 260 and 180mg l^{-1} respectively during this period, the washout per day comprising only 5.1% and 6.8% of the concentration of biomass retained on the support medium (Table 18). The increase in influent flow rate from 2.5 to 4.0 ld^{-1} and the corresponding reduction in HRT from 1d to 0.63d (Table 16) caused increased shear and hence the greatest biomass washout between days 26-35. After this, stabilisation occurred and washout was low in relation to volatile solids concentration, indicating good microbial attachment to the sand particles.

4.03.5 EFFECT OF BIOMASS ATTACHMENT ON REACTOR PERFORMANCE

The performance data for the three fully operational systems in terms of COD removal are given in Table 19. In general all the reactors displayed wide variations in effluent COD, which largely corresponded to fluctuations in the influent COD concentrations and hence the organic

TABLE 19 Variations in organic loading rate and COD removal during start-up of fluidised bed reactors under different regimes

HRT	Mean percentage COD removal				Variation in COD removal (%RSD)				Variation in organic loading rate (%RSD)
	1	2	3	4	1	2	3	4	
3.33	60	51	58	59	30	49	41	31	63
1.67	78	78	82	76	24	17	13	12	60
1.00	82	69	89	74	10	25	4	15	18
0.63	81	66		84	16	18		8	56
0.54	54	51		64	28	31		17	44
0.54[a]	69	64		67	5	3		7	--

a Steady state conditions after 5 days constant organic loading

%RSD expresses standard deviation as a % of the mean (%RSD = /x x 100)

loading rate. However, a comparison of the variability of the organic loading rate and COD removals at each HRT during start-up indicated that the reactors had effected considerable reductions in the degree of fluctuation in the organic strength of the waste (Table19). The nitrogen-purged system (4) appeared to be superior in this respect, the % RSD of COD removal being much lower than that of the organic loading rate. Reactor 2, amended with synthetic polymer, showed a lesser ability to compensate for influent COD peaks than the other two systems.

During the first 20 days of start-up, similar mean COD removals were displayed by all three systems with mean percentage removal increasing by approximately 20% as the HRT decreased and organic loading increased. Mean volatile solids concentrations also decreased at the second HRT (Table 18) suggesting that the non-active biomass had washed out of the systems and a good degree of adaptation to the substrate had been achieved by the remainder. At an HRT of 1d and a mean organic loading rate of 3.41kg COD m^{-3} d^{-1}, the mean COD removal efficiencies of the control (1) and the gas-purged system (4) remained similar to those of the previous HRT. The polymer-amended reactor, however, exhibited a reduction in COD removal, although the concentration of attached biomass in the system was greater than that in the other reactors (Table 18). This suggested that a population imbalance was occurring within the system, with retention of inactive biomass.

Fluidised beds 1, 2 and 4 exhibited lower mean COD removals at the final HRT of 0.54d despite the decrease in the mean organic load applied. However, the variability in

organic load was high and the wastewater had been amended, by increase of NH_4Cl and replacement of methanol (section 3.05.1) to its final composition. The variability in COD removal during the steady state phase at constant organic loading was much reduced, suggesting that most of the previous variability was due to influent fluctuations and not process instability. A similar mean percentage COD removal was exhibited by the control and N_2-purged reactors.

The pH of the reactors during the first HRT fluctuated around an initial value of 6.8, within the ranges of 5.19-6.24, 4.90-6.37 and 6.33-6.96 for beds 1, 2 and 4 respectively. Hence the greatest pH imbalance occurred in reactor 2, which contained the greatest concentration of attached biomass (Table 18). The reactors exhibited daily fluctuations in pH of up to 1.0 unit over start-up, but recovery times were variable: system 4 returned to pH 6.40 from 5.54 in 24 hours although the polymer-amended bed required 10 days and the control 12 days to recover from pH decreases to 4.90 and 5.74 respectively. It appeared that the microflora of reactors 1 and 2 compensated less well for pH oscillations than the nitrogen-purged reactor and response in the former to drastic pH decreases were slow.

Accumulation of TVA in reactors 1 and 4 generally corresponded to the concentrations of volatile solids present, with the greatest mean TVA levels of 313mg l^{-1} and 227mg l^{-1} produced in 1 and 4 respectively at biomass concentrations of 4400 and 4950mg VS l^{-1}. In the polymer-amended reactor the highest concentration of TVA, 318mg l^{-1}, was produced at the lowest volatile solids

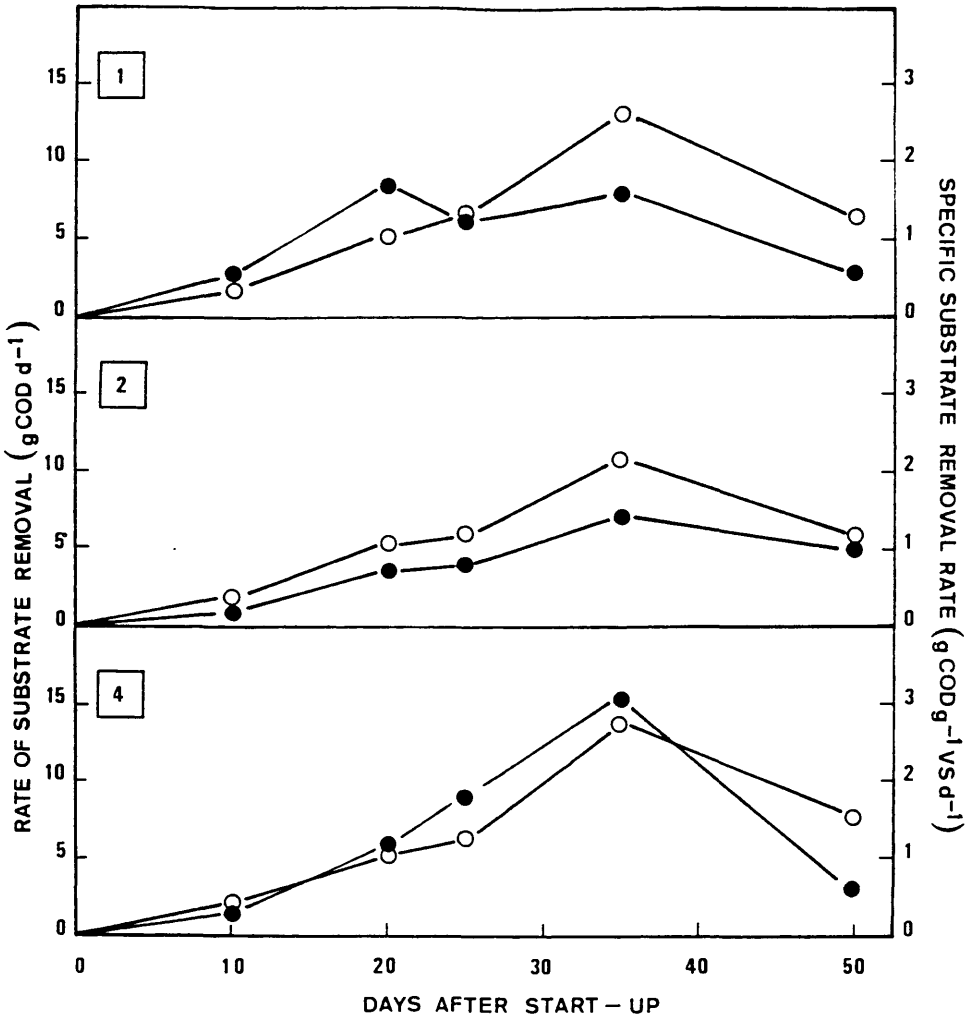
concentration of 2500mg l^{-1}, indicating that the majority of the attached microbial population were acid-producing bacteria, the acid-utilisers being present in insufficient numbers to control the excess TVA accumulation.

The rate of substrate removal by the attached biomass was similar in the three systems (Figure 33), increasing as HRT decreased and mean organic load increased, until the final HRT of 0.54d, which corresponded to a reduction in mean organic loading (Table 16). When estimated in relation to volatile solids concentration in the reactors, it was shown that the system maintained anaerobically since initiation evinced superior substrate removal rates per g VS at the greatest organic loading rate (Figure 33).

Gas production was low and erratic throughout start-up in the three systems, but was measured quantitatively at the final COD loading of 5.3kg COD m^{-3} d^{-1} and HRT of 0.53d. The results are shown in Table 20. Reactors 1 and 2 produced lower volumes of gas per day than the nitrogen-purged system and these gases contained lower proportions of methane.

TABLE 20. GAS PRODUCTION IN ANAEROBIC FLUIDISED BEDS DURING START-UP AT A COD LOADING OF 5.3kg COD m^{-3} d^{-1} AND AN HRT OF 0.53d.

Reactor	Total gas volume $m^3m^{-3}d^{-1}$	% Methane	Total methane volume $m^3m^{-3}d^{-1}$
1	0.92	30	0.28
2	0.33	16	0.05
4	1.25	51	0.64



Rate of substrate removal (o) and specific rate of removal (●) in anaerobic fluidised bed reactors during start-up. Control reactor (1); polymer-amended reactor (2); N_2-purged reactor (4).

FIG.33

4.04 EFFECT OF BED EXPANSION ON BIOMASS ATTACHMENT AND REACTOR PERFORMANCE IN THREE ANAEROBIC FLUIDISED BEDS

The hydraulic loading in the three fluidised beds which were started up as described in section 4.03 was adjusted to provide sand bed expansions of 5, 10, 15, 20, 25 and 30%. A constant HRT of 13h and a constant organic loading rate of 5.3kg COD m^{-3} d^{-1} were maintained by adjustment of the feed flow rate (Table 21). The reactors were allowed to stabilise at each new expansion for 10 days and then operated for 5 days at the given expansion. The organic removal efficiencies achieved by each of the three systems over a range of expansions are shown in Figure 34. The COD reductions over the course of the experiment followed a similar pattern, with the control reactor exhibiting the highest COD removal efficiencies overall. Marked fluctuations in COD removal were evident in all systems at the 5% expansion, with the gas-purged reactor experiencing the greatest reduction in conversion efficiency, which was not fully recovered at the higher expansions. However, the variations evident in mean % COD removals between expansion levels 10 to 30% were less pronounced in the nitrogen-purged system than in the other two reactors, indicating that this system was capable of maintaining a stable effluent quality despite increasing shear stresses within the bed.

Bed disruption was a common occurrence during the 5% expansion phase, resulting from the presence of entrapped gas bubbles within the bed. The reduced mobility of sand particles at 5% expansion prevented rapid disentrainment of small bubbles and appeared to enhance their coalescence

TABLE 21 FLUIDISED BED REACTOR PARAMETERS DURING VARIABLE BED EXPANSION

Bed Expansion (%)	Fluidised Volume (l)	Influent Flow Rate ($l\ d^{-1}$)	H R T (d)	Organic Loading Rate (kg COD $m^{-3}\ d^{-1}$)	Liquid Upflow ($l\ h^{-1}$)
5	2.06	3.79	0.544	5.3	9.96
10	2.16	3.97	0.544	5.3	11.16
15	2.25	4.14	0.544	5.3	15.00
20	2.35	4.32	0.544	5.3	17.88
25	2.45	4.50	0.544	5.3	19.92
30	2.55	4.69	0.544	5.3	24.00

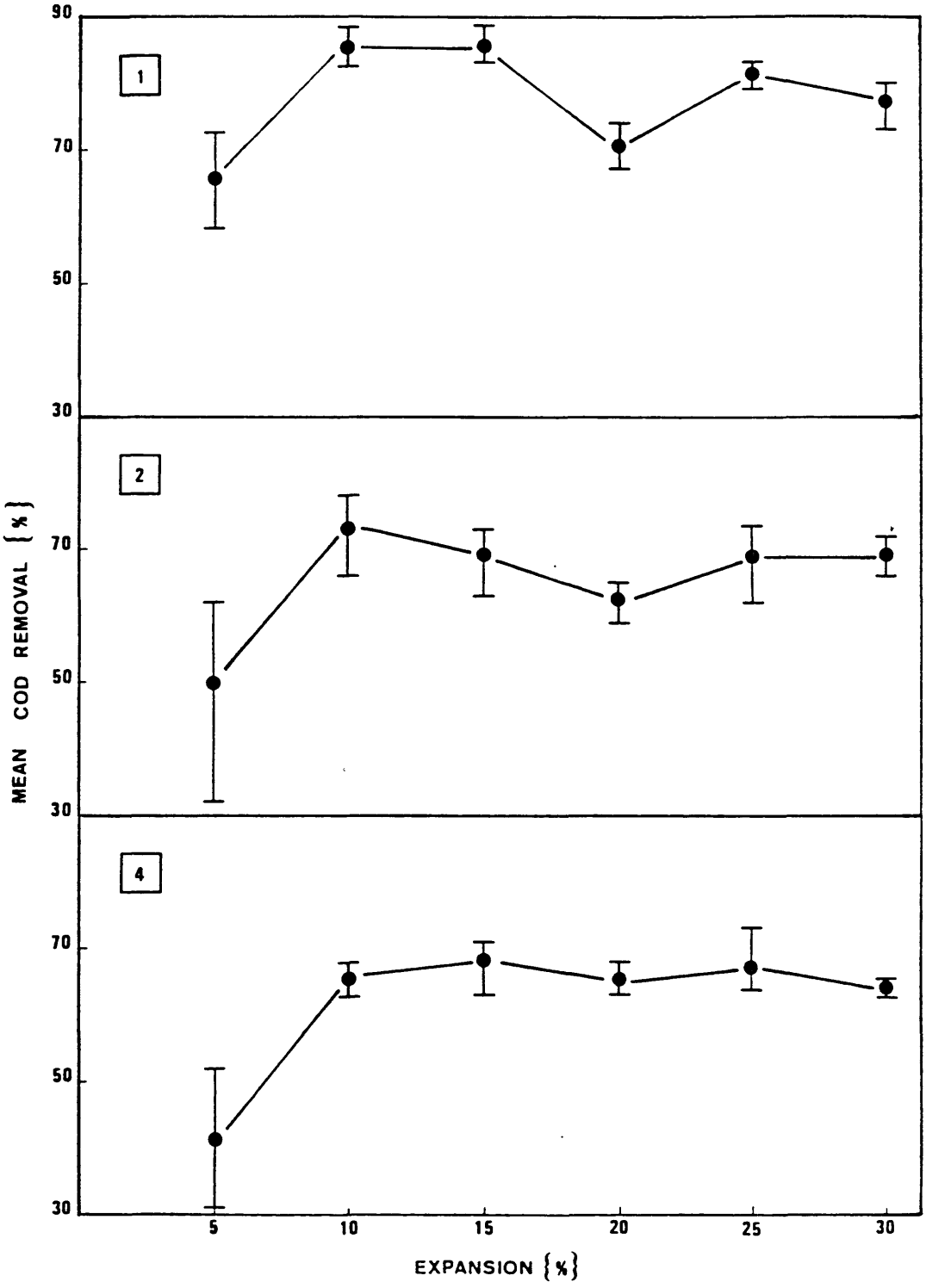


FIG.34 Mean % COD removals achieved by anaerobic fluidised beds operating at varying bed expansions. Control reactor (1); polymer-amended reactor (2); N_2-purged reactor (4).

Range bars indicate maximum and minimum % COD removals.

into larger bubbles. Upon release, these large bubbles caused carry-over of sand and attached biomass into the effluent.

All three systems exhibited large decreases in volatile solids concentrations between the 5% and 10% expansions (Figure 35) although concomitant increases in suspended solids were not apparent, suggesting that the sloughed biomass washed out of the reactors and did not remain suspended after the initial decrease. Biomass concentrations remained relatively steady at expansions in the range 10-30% although the reactors showed slightly raised volatile solids levels at the 25% fluidisation rate, indicating that this expansion was optimum for biomass retention, although optimum % COD removal occurred at 10 or 15% expansion (Figure 34).

Mean pH values during the expansion phase remained relatively constant within the range 6.53-7.17, although some fluctuations in TVA concentrations at lower expansions occurred. However, TVA in the three systems ranged from 161-285mg l^{-1} during this phase of the study, indicating microbial stability within the fluidised beds.

Gas production throughout the expansion phase was very erratic due to the bed disruption and biomass loss experienced at 5%. Generally, greater bed expansions resulted in larger volumes of gas comprising a higher percentage of methane. Offgas of 70% methane was obtained in the control at 30% expansion, whilst all three systems produced the lowest measurable gas volumes at 15% expansion. The methane content of the offgas of the polymer-amended reactor (2) was noticeably low, from 4.5-

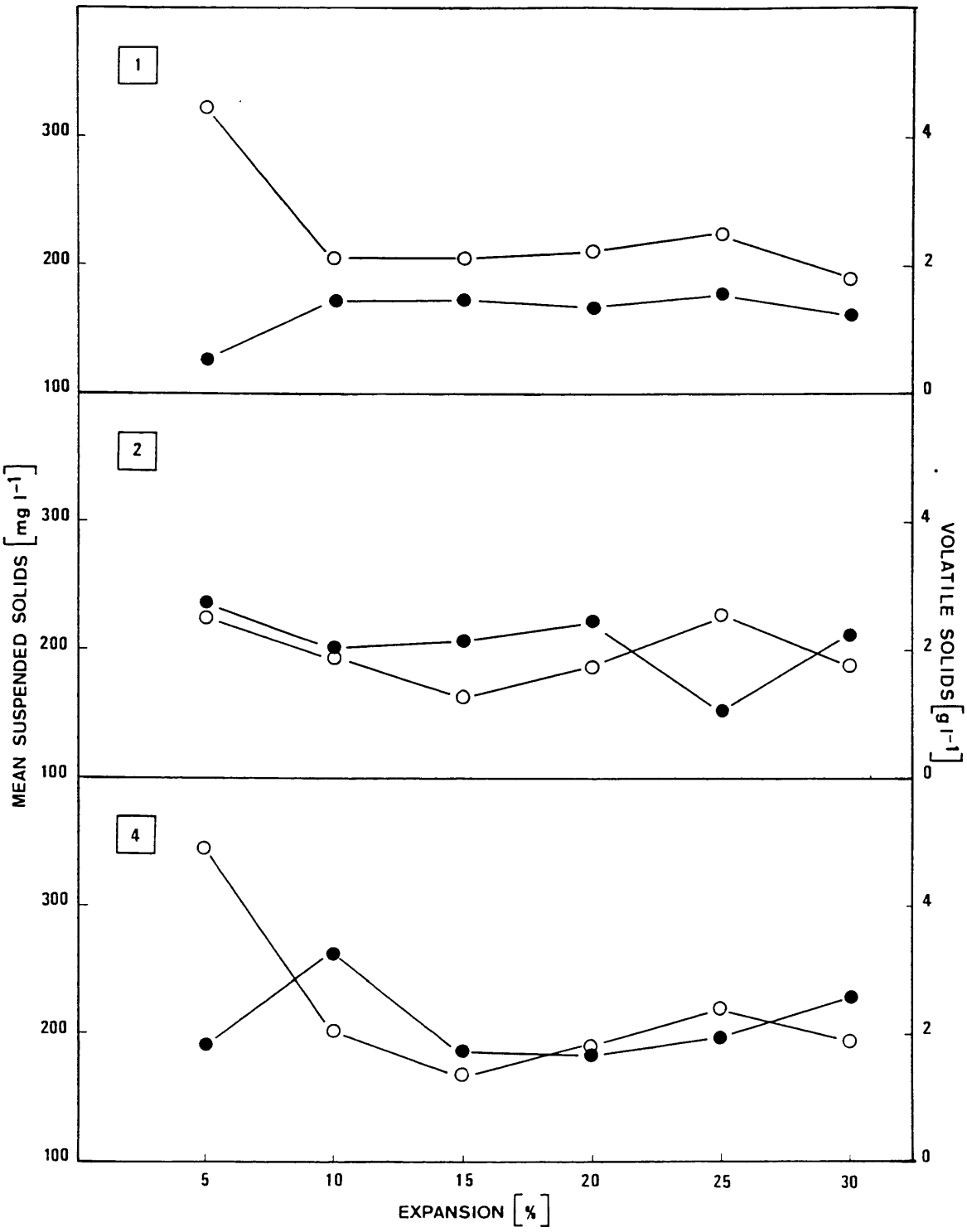


FIG.35 Suspended (●) and volatile (○) solids concentrations in anaerobic fluidised beds operated at varying bed expansions. Control reactor (1); polymer-amended reactor (2); N_2-purged reactor (4).

14.0% over the range of expansions from 15-30% compared to 5.2-70.0% and 8.8-52.5% for reactors 1 and 4 respectively.

Low methane levels may have been the result of the presence of a population of sulphate reducing bacteria in the systems, as H_2S was qualitatively detected. Accurate estimations of gas volume were difficult to make over the expansion phase, however, as gas production proved to be irregular. The COD removal exhibited at the applied loading rate of 5.3kg COD m^{-3} d^{-1} should have produced a greater yield of methane than that actually obtained. The discrepancy probably arose both from the erratic nature of gas production and the loss of biomass as effluent suspended solids, as soluble effluent COD only was determined.

4.05 INFLUENCE OF INCREASING TRACE ELEMENT ADDITION ON THE PERFORMANCE OF ANAEROBIC FLUIDISED BEDS

Two fluidised bed reactors operating at steady state on synthetic meat waste (section 3.05.1) at an organic loading rate of 4.5kg COD m^{-3} d^{-1} and an HRT of 0.54d were employed to investigate the effects of increasing trace elements on reactor operational parameters. During normal operation of the fluidised beds on the meat waste the trace element solution (Table 6) was present at 0.05ml l^{-1}. For the duration of this study the trace element solution was added to the feed at concentrations in the range 0.1 to 2.4 ml l^{-1} (Table 22). The quantities of trace element compounds present in the reactors at the initial and final stages of the study are shown in Table 23.

The effect on reactor performance of trace compound

TABLE 22 ADDITION OF INCREASING CONCENTRATIONS OF TRACE ELEMENT SOLUTION TO ANAEROBIC FLUIDISED BEDS

Day	Concentration of trace element solution (ml l^{-1})
1-4	0.1
5-8	0.2
9-12	0.4
13-16	0.6
17-20	0.8
21-24	1.0
25-27	1.2
28-30	1.4
31-33	1.6
34-36	1.8
37-39	2.0
40-42	2.2
43-46	2.4

TABLE 23 CONCENTRATION OF TRACE ELEMENT COMPOUNDS PRESENT IN SOLUTIONS ADDED TO FLUIDISED BEDS

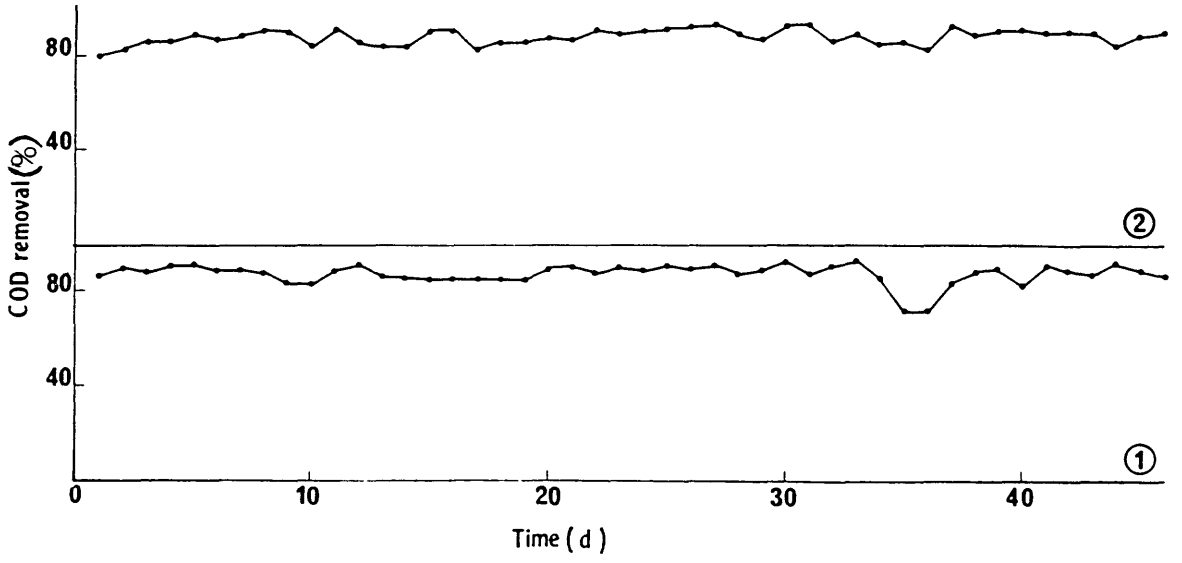
Trace element compound	Amount present	
	0.1ml ($\mu g\ l^{-1}$)	2.4ml ($\mu g\ l^{-1}$)
cobalt nitrate	0.01	0.24
ferrous chloride	0.01	0.24
ethylenediaminetetraacetic acid	0.005	0.12
manganous chloride	0.0025	0.06
sodium selenate	0.0005	0.012
ammonium molybdate	0.00045	0.0108
boric acid	0.00025	0.006
zinc chloride	0.00025	0.006
nickel chloride	0.00025	0.006
cupric nitrate	0.00015	0.0036

addition are presented in Figures 36 to 40. The efficiency of COD removal in both systems remained in the range 80% to 94% until day 35, when reactor 1 experienced a decrease in removal efficiency to 72% (Figure 36). This was corrected within two days and operational stability maintained until the end of the study. Suspended solids concentrations in the two fluidised beds fluctuated between 65 and 250mg l^{-1}, with mean values of 156 and 157mg l^{-1} for systems 1 and 2 respectively. However, fluctuations in the first reactor were less pronounced between days 10 and 30 (Figure 37).

The pH values of both fluidised beds remained near neutral throughout the experimental period (Figure 38), indicating that the good buffering capacities within both reactors were unaffected by increasing trace elements. However, the TVA concentrations of both systems decreased to values of less than 50mg l^{-1} (Figure 39), suggesting that either acid production was decreasing or that volatile acids were being utilised at a rate greater than before.

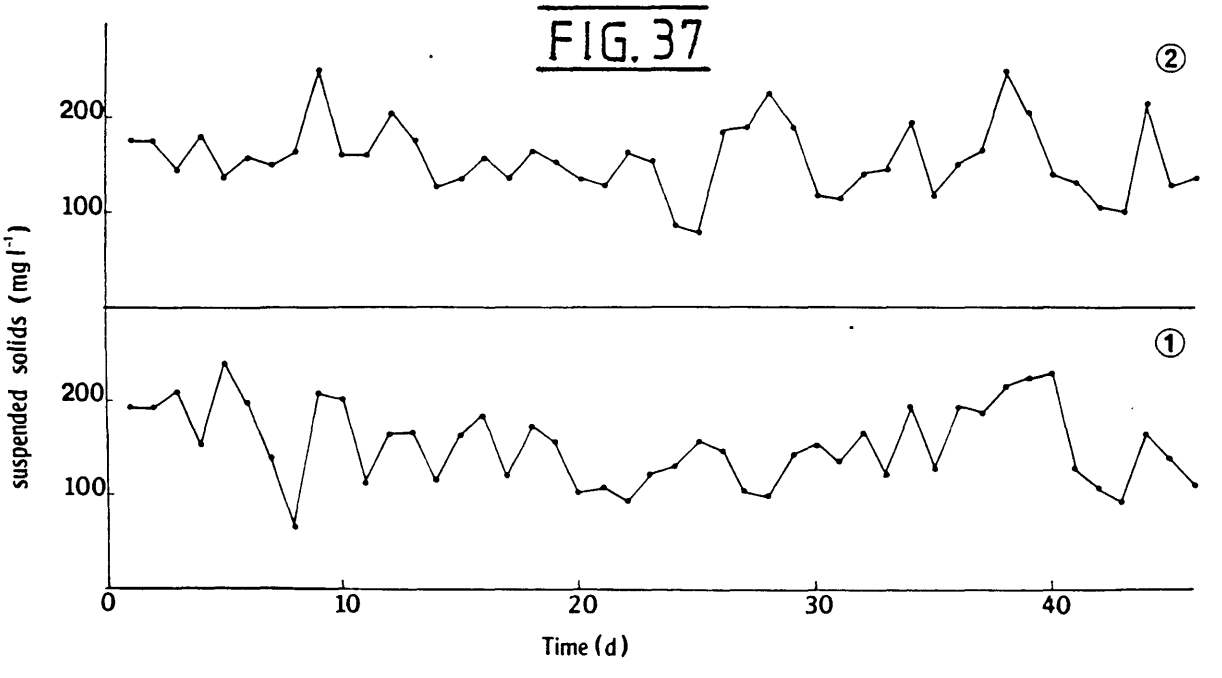
The methane levels produced in the two beds are shown in Figure 40. A marked increase in methane production in both systems was evident from day 24. In the first reactor the mean volume of methane produced from day 1 to day 23 was 0.137m^3 kg^{-1}COD m^{-3} d^{-1}, whereas the mean volume generated between days 24-46 was 0.186m^3 kg^{-1}COD m^{-3} d^{-1}. The corresponding values for the second fluidised bed were 0.155 and 0.261m^3CH_4 kg^{-1}COD m^{-3} d^{-1} respectively. The increases in biogas production became apparent after day 30 when the concentration of trace element solution had been increased to 1.6ml l^{-1}.

FIG.36



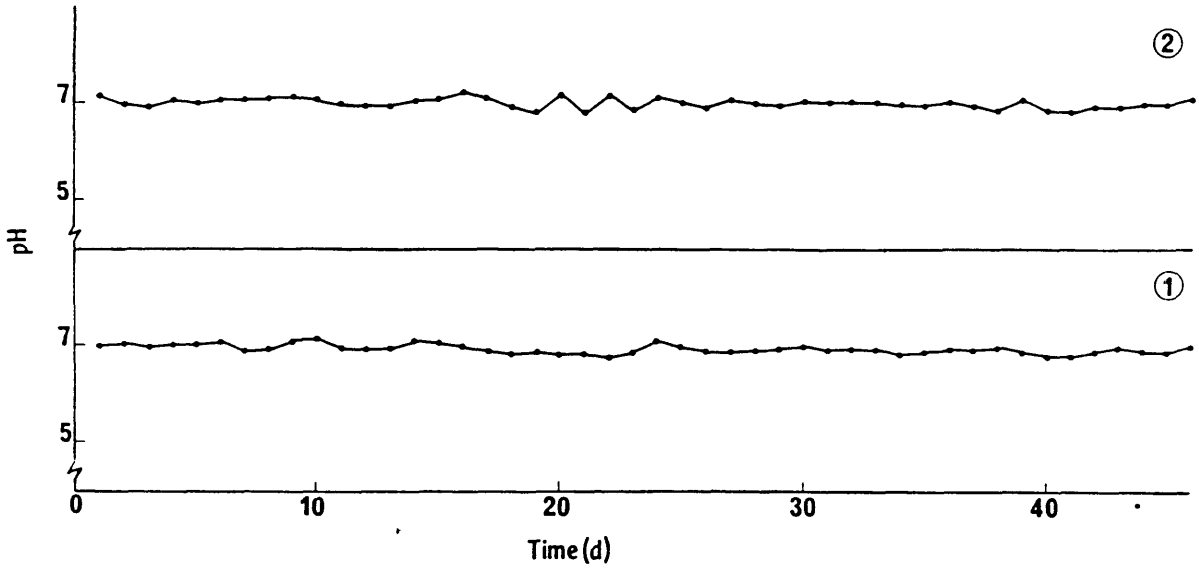
COD removal during increasing trace element addition in two anaerobic fluidised beds.

FIG. 37



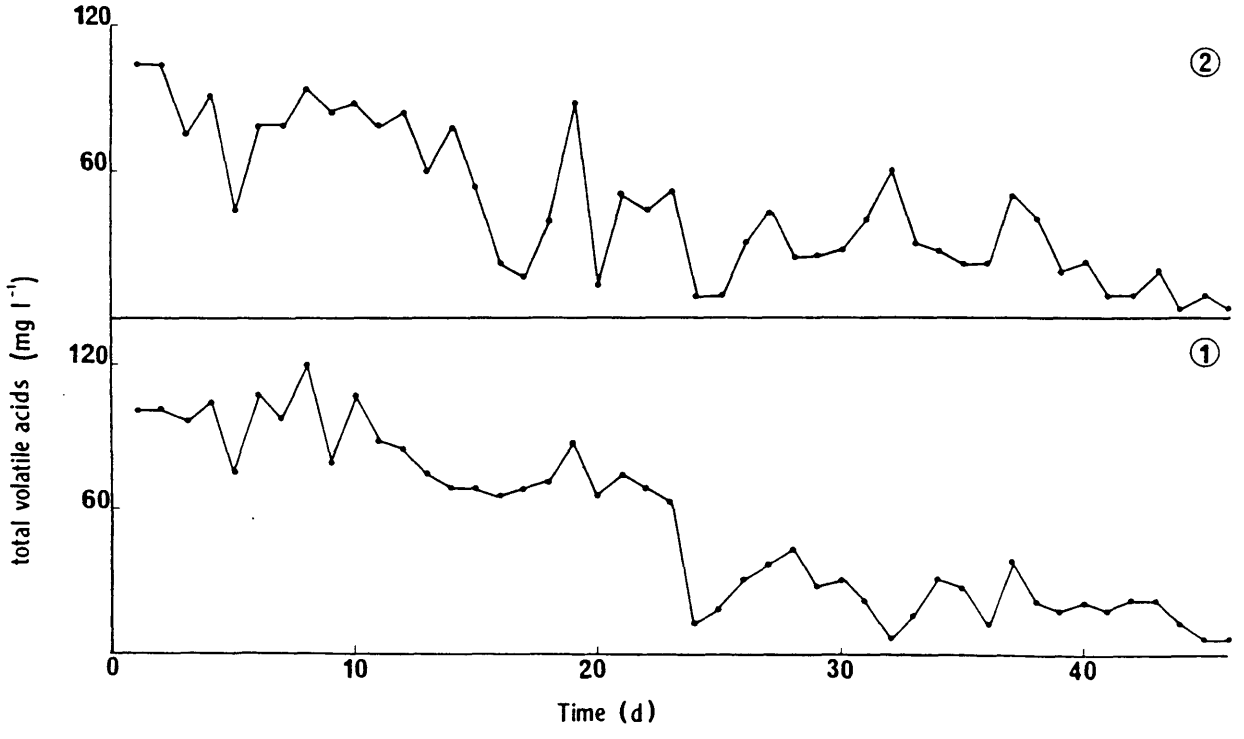
Suspended solids concentration during increasing trace element addition in two anaerobic fluidised beds.

FIG. 38



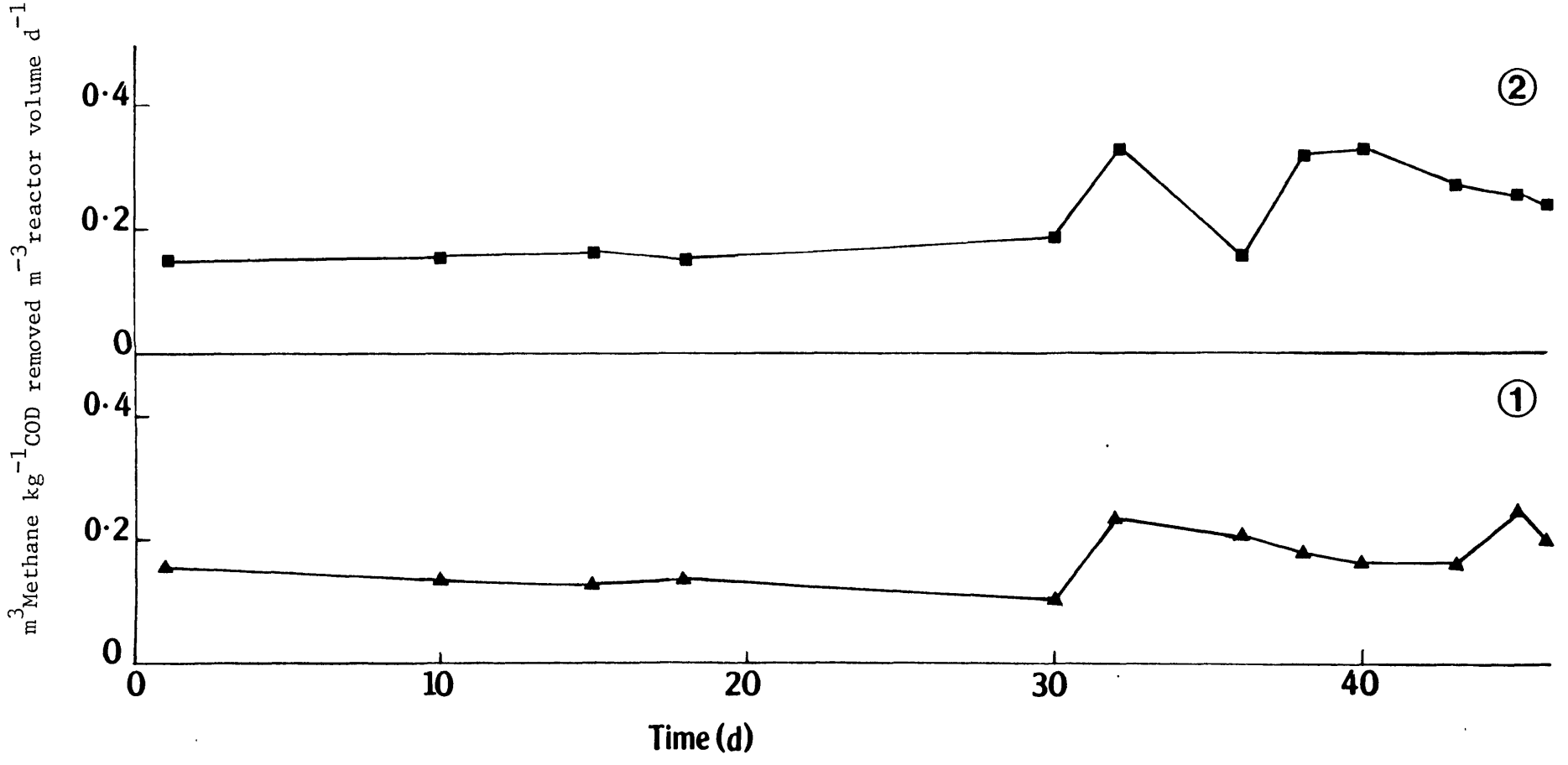
pH during increasing trace element addition in two anaerobic fluidised beds.

FIG. 39



Total volatile acids concentrations during increasing trace element addition in two anaerobic fluidised beds.

FIG.40



Methane production during increasing trace element addition in two anaerobic fluidised beds.

4.06 EFFECT OF VARYING PERIODS OF DOWNTIME ON THE START-UP OF ANAEROBIC FLUIDISED BEDS

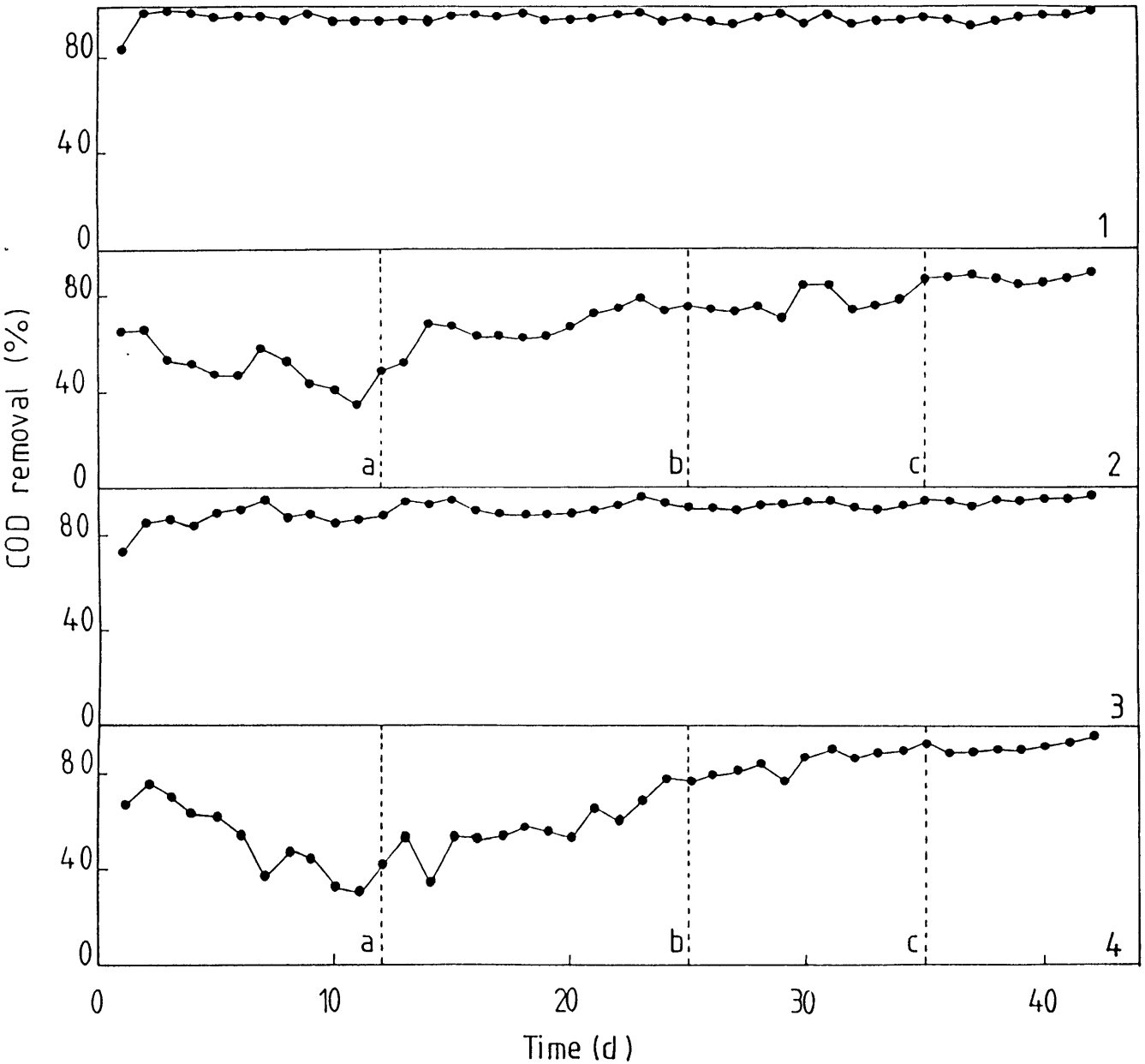
The operational capabilities of four anaerobic fluidised beds restarted after periods of downtime of 39 and 89 days were determined. The reactors had been operating at steady state on synthetic meat wastewater of 2500mg COD l^{-1} (section 3.05.1), at an organic loading rate of 4.5kg COD m^{-3} d^{-1} and with an HRT of 0.53d prior to shutdown.

At restart, the previous steady state organic loading rate was applied. However, it was necessary to alter this rate during the restart of the pair of reactors that had been shut down for 89 days. The reduction in organic loading was obtained by reducing the COD concentration of the influent synthetic meat wastewater, as shown in Table 24.

The restart of four fluidised beds at full loading rates after two different periods of downtime presented immediate operational contrasts. All four units were restarted on day 0, and at day 1 exhibited COD removals of over 50% (Figure 41). In reactors 1 and 3, which had experienced a 39 day downtime, COD removal increased and remained above 90% for the entire period of the experiment. In systems 2 and 4, however, which had undergone a downtime period of 89 days, COD removal decreased to levels approaching 30%. The organic loading rate to these reactors was hence decreased from 4.5 to 2.5kg COD m^3 d^{-1} to combat this. As COD removal efficiency increased (Figure 41) the organic loading was stepped up accordingly to 3.5kg COD m^{-3} d^{-1} on day 25 of restart, and to the full

TABLE 24 Influent parameters of 4 anaerobic fluidised beds during restart following periods of downtime

Day	Influent Flow Rate $l d^{-1}$	HRT d	Organic loading Rate (kg COD m^{-3} d^{-1})				Influent COD concentration ($mg l^{-1}$)			
			1	2	3	4	1	2	3	4
0-11	4.32	0.56	4.5	4.5	4.5	4.5	2500	2500	2500	2500
12-25	4.32	0.56	4.5	2.5	4.5	2.5	2500	1390	2500	1390
26-34	4.32	0.56	4.5	3.5	4.5	3.5	2500	1940	2500	1940
35-42	4.32	0.56	4.5	4.5	4.5	4.5	2500	2500	2500	2500



Percentage removal of COD in 4 fluidised beds started up after 39 days (beds 1 and 3) and 89 days (beds 2 and 4) downtime. Loading change to 2.5 kg COD m^{-3} d^{-1} (a), 3.5 kg COD m^{-3} d^{-1} (b) and 4.5 kg COD m^{-3} d^{-1} (c).

FIG. 41

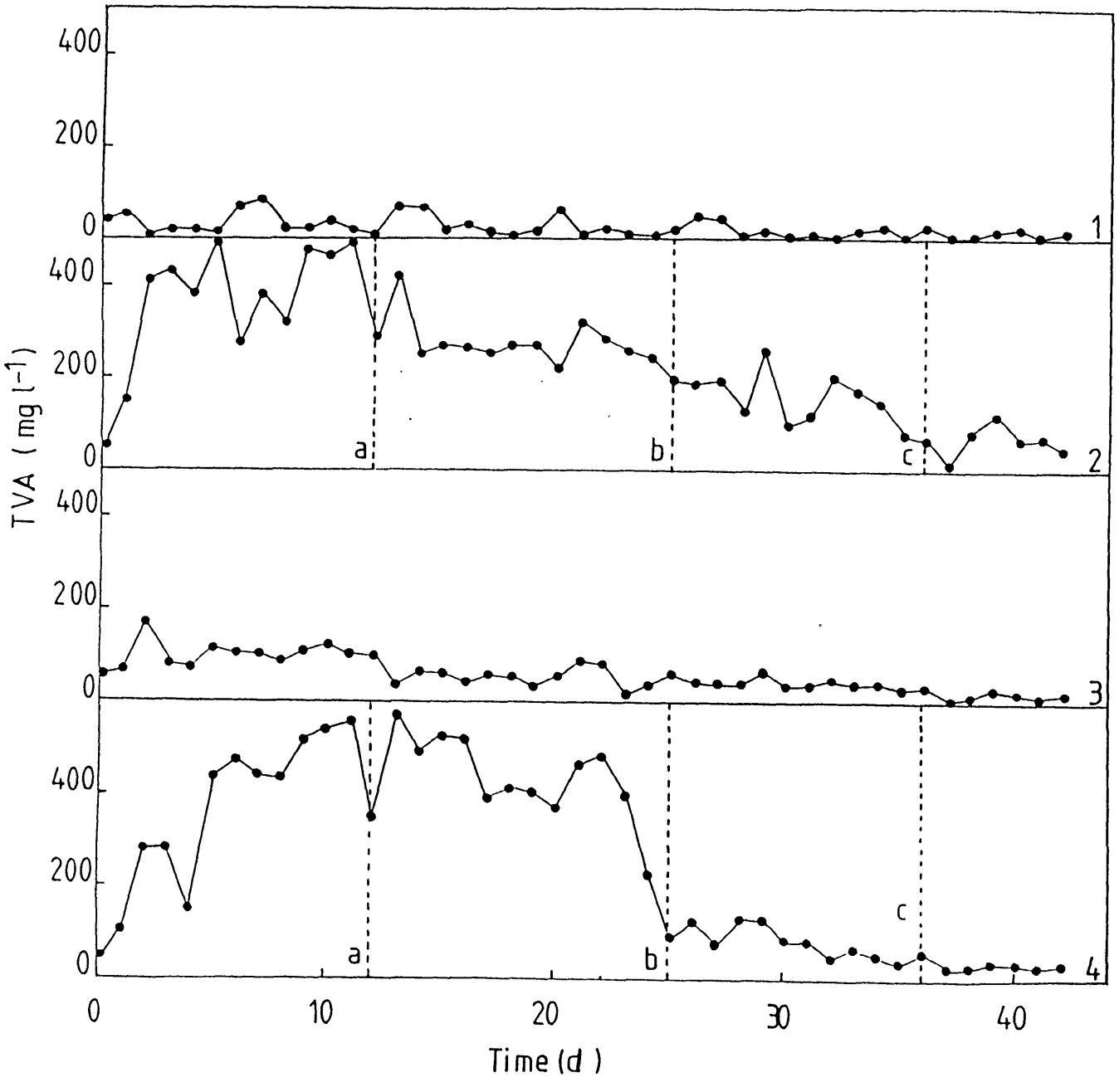
loading on day 35 (Table 24). Removal of COD had reached values in excess of 90% by the end of the restart period at day 42 (Figure 41).

The TVA concentrations within the fluidised beds (Figure 42) indicated the effects of restart upon the biomass of each reactor. In the units started-up after 89 days, acid production rose rapidly and continued at high concentrations until stabilisation occurred at the final load changes (Figure 42). In the other two systems, TVA concentrations remained below 200mg l^{-1} throughout the period. The gas data presented in Figures 43 and 44 show that the mean methane production for reactors 2 and 4 remained low after restart and the methane content of the offgases was also lowest in these systems (Figure 44). The fluidised beds which had been shut down for 39 days, however, showed no interruption in gas production. In contrast, the suspended and volatile attached solids concentrations (Figures 45 and 46) remained relatively similar for all four systems throughout the study.

The difference in the operational performances of the two pairs of reactors was quite marked, those units with the shorter period of downtime restarting at full potential within one day and the beds after 89 days of downtime requiring more time and a reduction in organic loading before full capacity could be reached.

4.07 BACTERIAL ACTIVITY PROFILE ALONG THE LENGTH OF TWO ANAEROBIC FLUIDISED BEDS

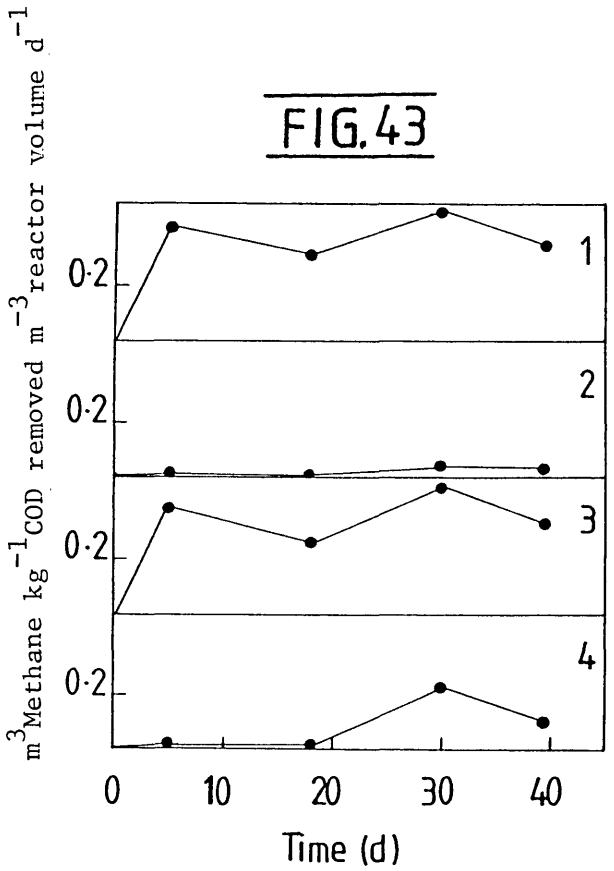
Subsequent to the investigation detailed in section 4.06, reactors 1 and 2 were allowed to stabilise for 10



Total volatile acids concentrations in 4 fluidised beds started up after 39 days (beds 1 and 3) and 89 days (beds 2 and 4) downtime. Loading change to 2.5 kg COD m^{-3} d^{-1} (a), 3.5 kg COD m^{-3} d^{-1} (b) and 4.5 kg COD m^{-3} d^{-1} (c).

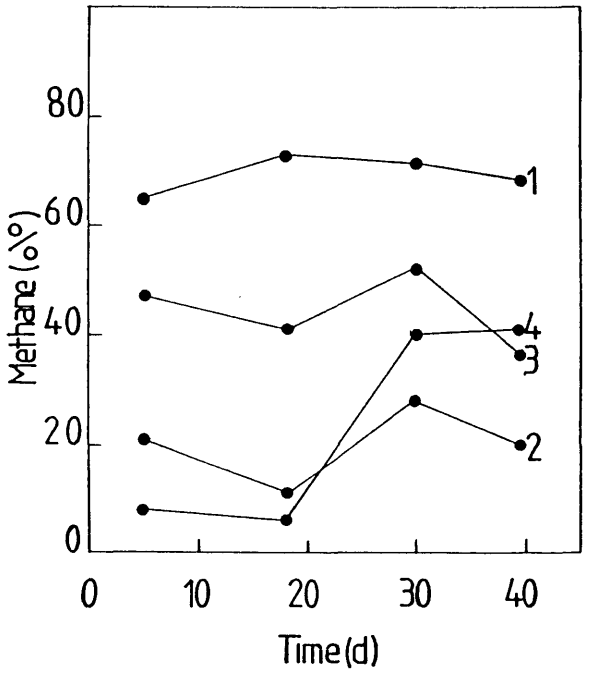
FIG. 42

FIG.43

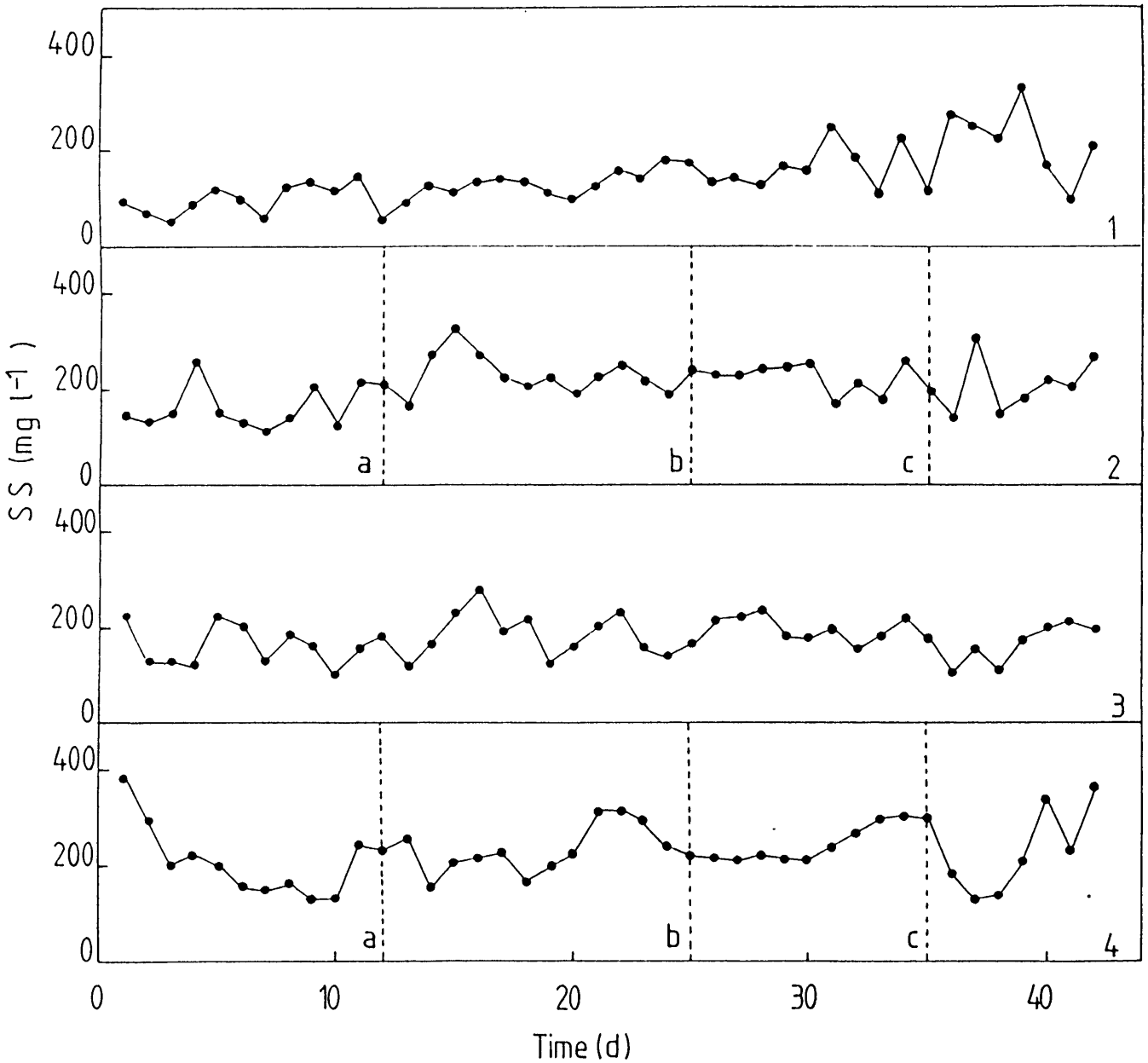


Mean methane production in 4 fluidised beds started up after 39 days (beds 1 and 3) and 89 days (beds 2 and 4) downtime.

FIG.44



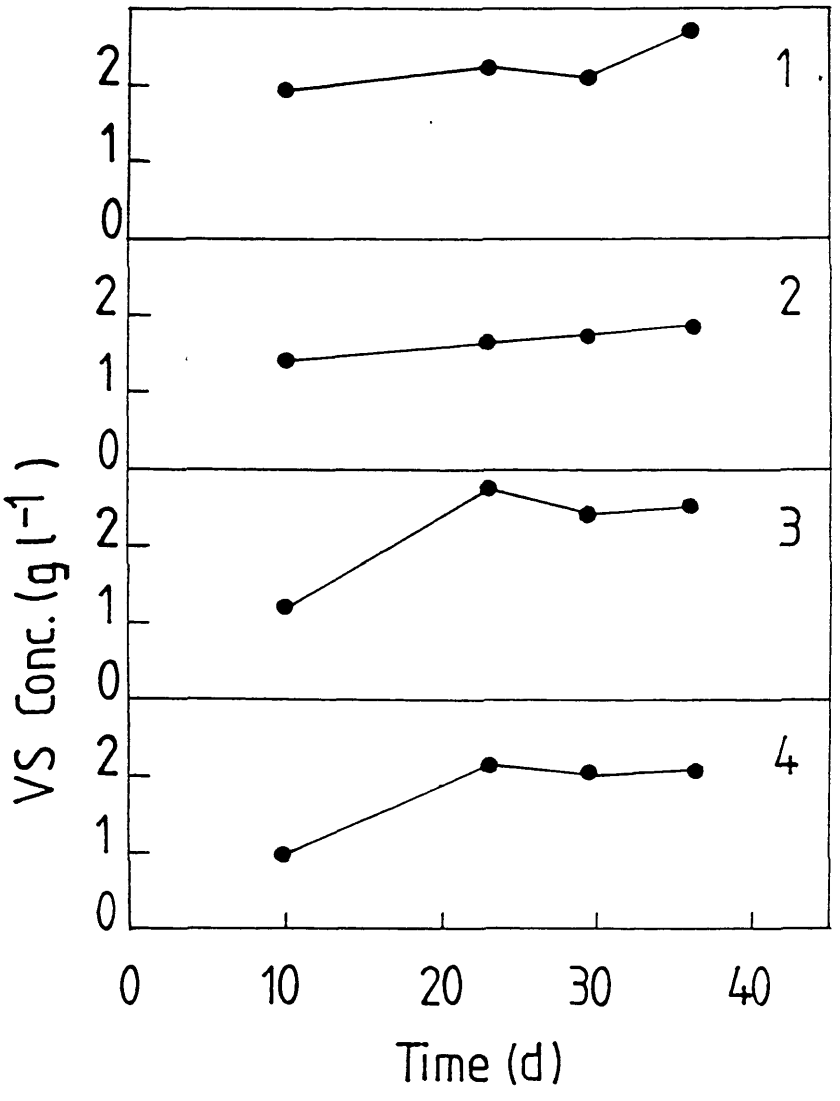
Mean percentage methane in 4 fluidised beds started up after 39 days (beds 1 and 3) and 89 days (beds 2 and 4) downtime.



Suspended solids concentration in 4 fluidised beds started up after 39 days (beds 1 and 3) and 89 days (beds 2 and 4) downtime. Loading change to 2.5 kg COD m^{-3} d^{-1} (a), 3.5 kg COD m^{-3} d^{-1} (b) and 4.5 kg COD m^{-3} d^{-1} (c).

FIG.45

FIG.46



Volatile solids concentrations in 4 fluidised beds started up after 39 days (beds 1 and 3) and 89 days (beds 2 and 4) downtime.

days and analyses were carried out to determine the bacterial activity along the length of the reactor columns. At the final organic loading rate of 4.5kg COD m^{-3} d^{-1}, samples of approximately 5ml were removed via the sampling ports of the fluidised beds (section 3.01.2) at bed heights of 15, 55, 95 and 142cm, and 15, 45, 117 and 147cm for reactors 1 and 2 respectively. The solids were allowed to settle and the liquor was removed and analysed. The solids were also analysed for attached volatile materials (section 3.06.04). Relative methane-forming activity was determined in the two reactors by removing 3ml samples via the sampling ports and incubating them (section 3.06.08).

Figures 47 to 49 show the results of the analyses carried out. The COD removal efficiency was essentially the same in all portions of the bed, a situation which may be ascribed to the high recycle ratio inherent in the system. However, TVA concentrations were greatest in the central and upper regions of both beds (Figure 47). Suspended solids concentrations were lower at the bottom and top regions (Figure 47) although volatile attached solids were markedly more concentrated at the top of both fluidised beds, remaining relatively constant in the lower and central sections (Figure 48).

The percentage of methane in the biogas produced by samples removed from the length of the reactor bed and incubated is shown in Figure 49. Those samples from the lower portions of the two fluidised beds both produced offgases comprising low proportions of methane, whereas the central and upper portions of the two units were capable of producing a greater percentage of methane.

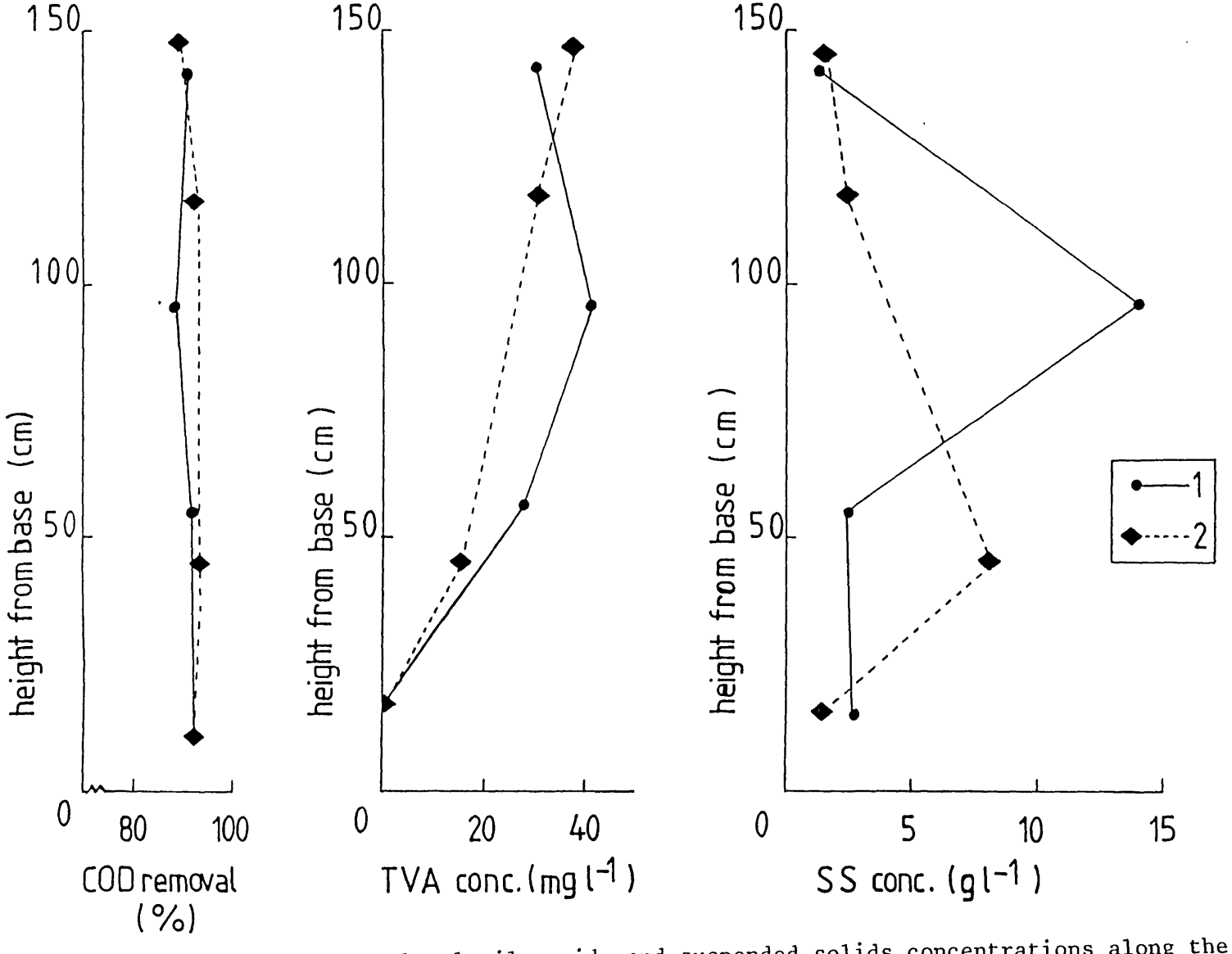


FIG.47

COD removal and total volatile acids and suspended solids concentrations along the length of fluidised beds 1 and 2 at steady state; bed 1 (●); bed 2 (◆).

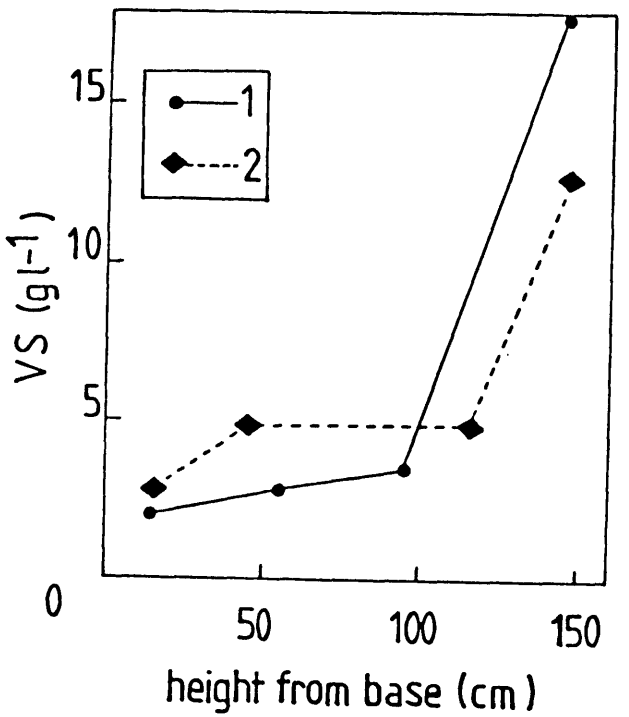


FIG.48 Volatile solids concentrations along the length of fluidised beds 1 and 2 at steady state; bed 1 (●); bed 2 (◆).

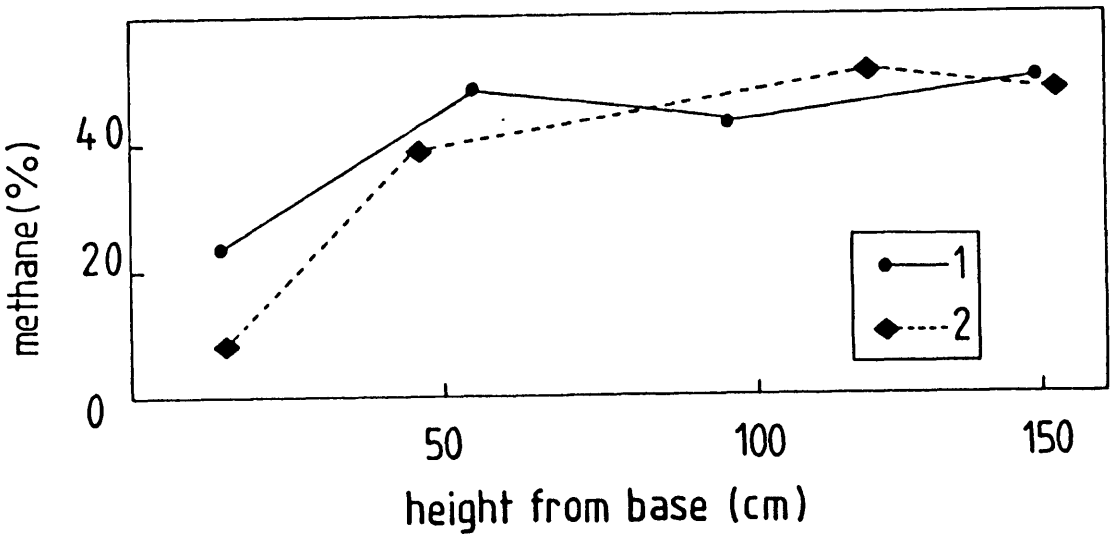


FIG.49 Percentage methane production of samples taken from different heights in fluidised beds 1 and 2 at steady state; bed 1 (●); bed 2 (◆).

4.08 ACCLIMATION OF ANAEROBIC FLUIDISED BEDS TO TWO PHARMACEUTICAL WASTES

4.08.1 BIODEGRADATION TESTS

The acclimation of anaerobic fluidised beds to two pharmaceutical wastewaters (section 3.05.2) gradually introduced with the influent feed was examined. However, to test if the wastes were degradable anaerobically, batch testing was carried out as described in section 3.10. The two pharmaceutical wastes were designated A and B; A contained n-propanol as the major component and B contained dimethylformamide (DMF) as the major component.

Without exception, those bottles containing some part of the COD as industrial waste (section 3.10) produced offgases of a higher percentage methane than the control bottles, which contained no pharmaceutical wastes (Figure 50). These results suggested that both wastes were potentially biodegradable anaerobically and that the seed inocula were active. Industrial waste B, which comprised 30% DMF, produced an offgas containing a greater proportion of methane than the gas generated by waste A; these results suggested that DMF was more amenable to anaerobic degradation in the presence of the given bacterial population than propanol. Both controls produced offgases of less than 10% methane, although carbon dioxide production was qualitatively noted in both cases.

4.08.2 ACCLIMATION TO PHARMACEUTICAL WASTES IN FLUIDISED BEDS

The four 4l laboratory scale fluidised bed reactors which had been operating at steady state at 37^{o}C on the

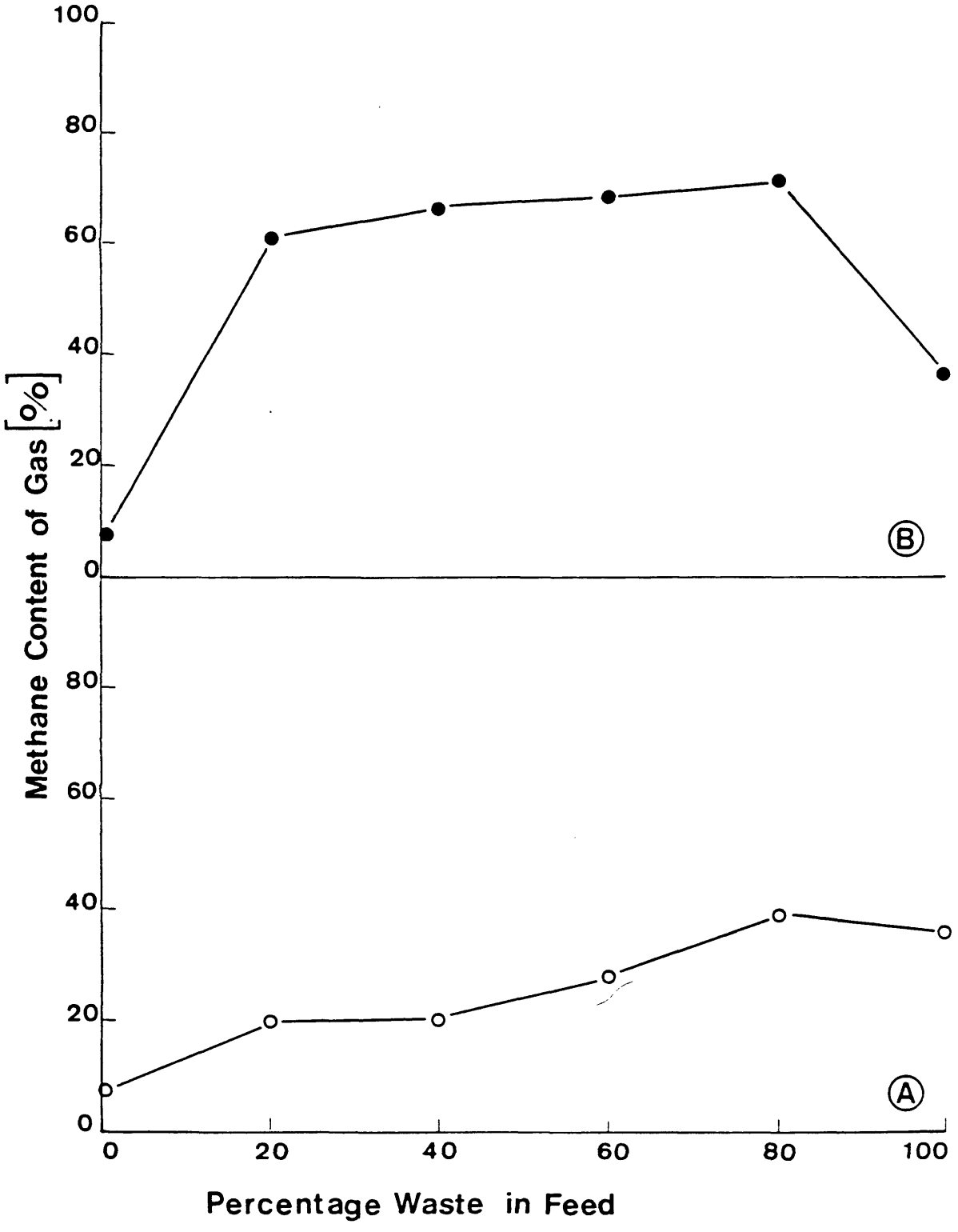


FIG. 50

Percentage of methane produced in batch samples containing increasing proportions of propanol-containing (A) and dimethylformamide-containing (B) pharmaceutical waste.

synthetic meat waste were each supplied with an influent organic loading of 4.5kg COD m^{-3} d^{-1}. The reactors were operated at an HRT of 0.53d. These conditions were kept constant throughout the experimental period. Two of the reactors were used as controls and maintained at steady state on the meat waste, which provided an influent COD of 2500mg l^{-1}. One of the remaining two units was fed with pharmaceutical waste A and the other with pharmaceutical waste B, both diluted to give concentrations of COD of 2500mg l^{-1}. Initially 10% of the COD contributed by the synthetic substrate was replaced by either A or B pharmaceutical waste COD. Subsequently, the pharmaceutical waste contributions were increased by 10% increments until 100% industrial waste COD was applied.

Figures 51 to 57 summarise the performances of the four anaerobic fluidised beds. Table 25 indicates the periods of adjustment to a new feed composition for the two experimental beds. The changes in influent composition were made in response to evidence of stability of COD removal or TVA production, or efficiency of gas production.

At day 0, all four columns exhibited COD removals in excess of 90%. These high removal rates continued in the control systems throughout the course of the experiment (Figure 51). COD removal in the reactor treating the propanol waste remained relatively constant over the acclimation period, until the system experienced an influent of 100% pharmaceutical waste: removal of COD dropped abruptly to approximately 50% and remained at that level for the duration of the study. The sudden reduction in COD removal was accompanied by a substantial increase

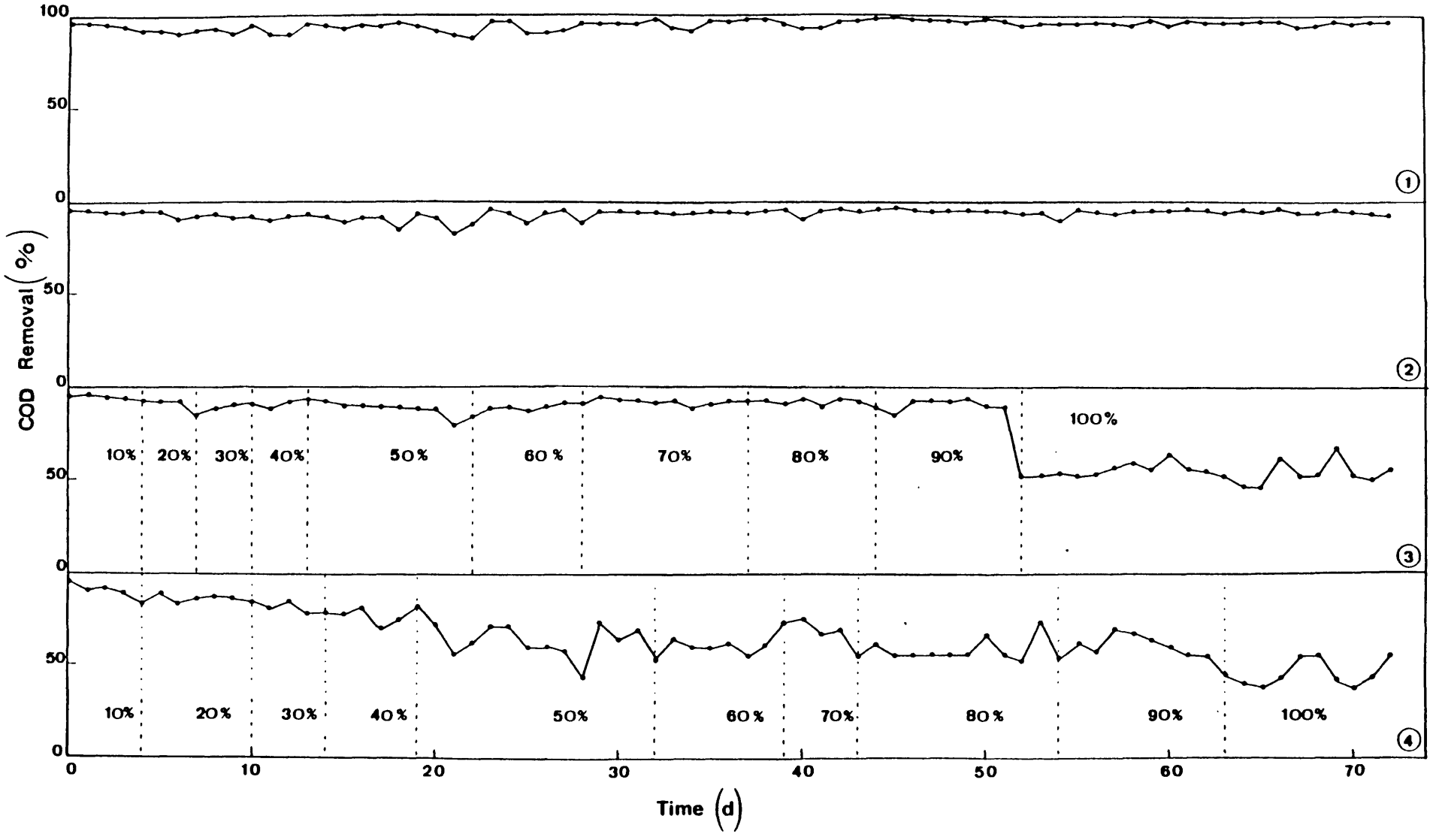


FIG.51

Percentage COD removal in fluidised beds during acclimation to pharmaceutical wastes: controls (1) and (2); propanol-containing waste (3); dimethylformamide-containing waste (4).

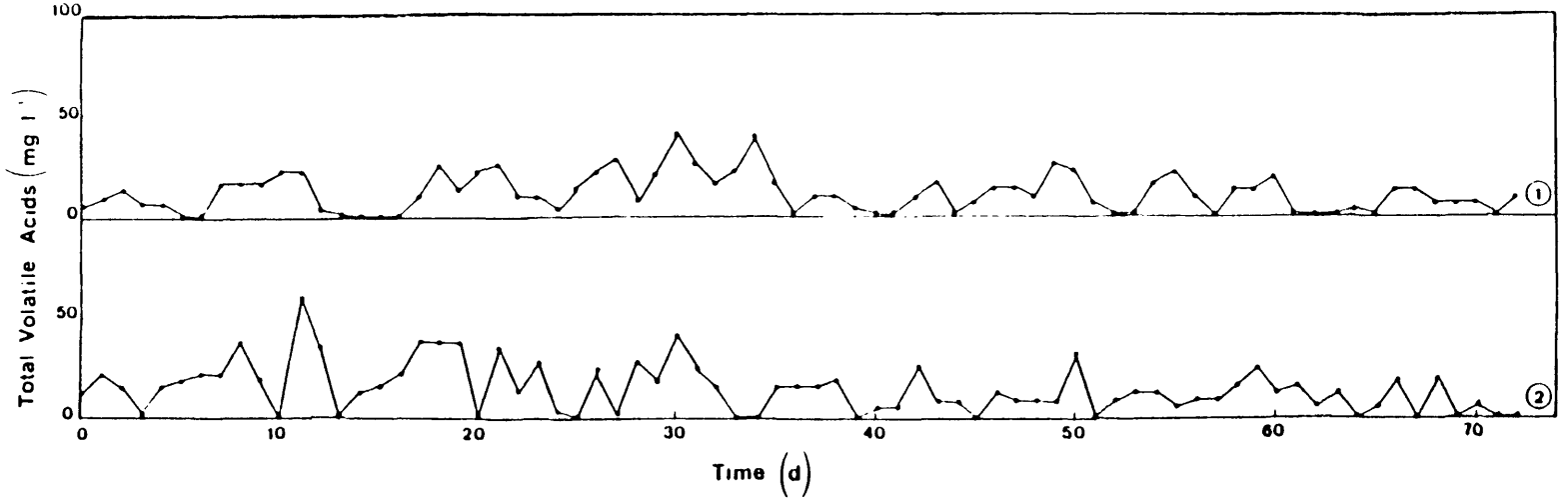


FIG. 52 Total volatile acids production in control anaerobic fluidised beds operating on meat waste.

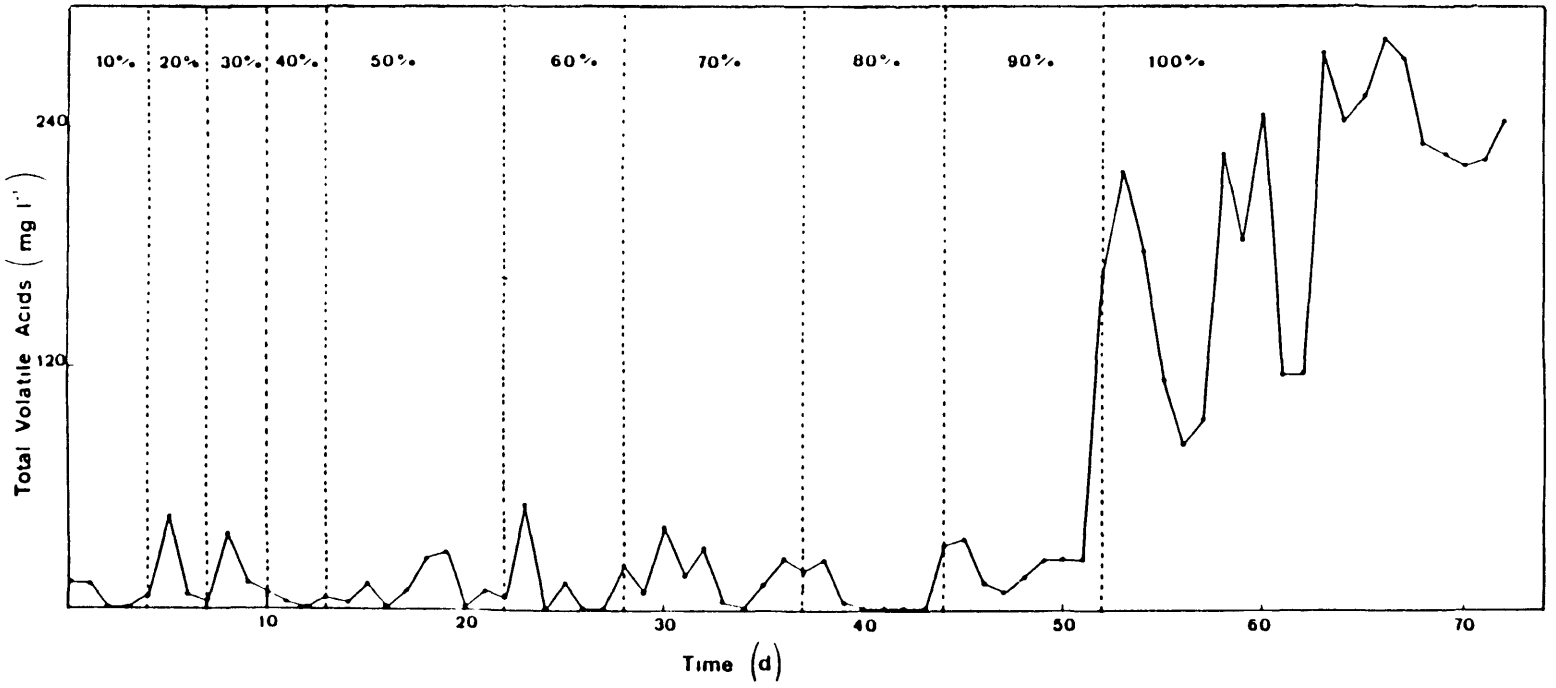


FIG. 53 Total volatile acids production during acclimation of an anaerobic fluidised bed to a propanol-containing pharmaceutical waste.

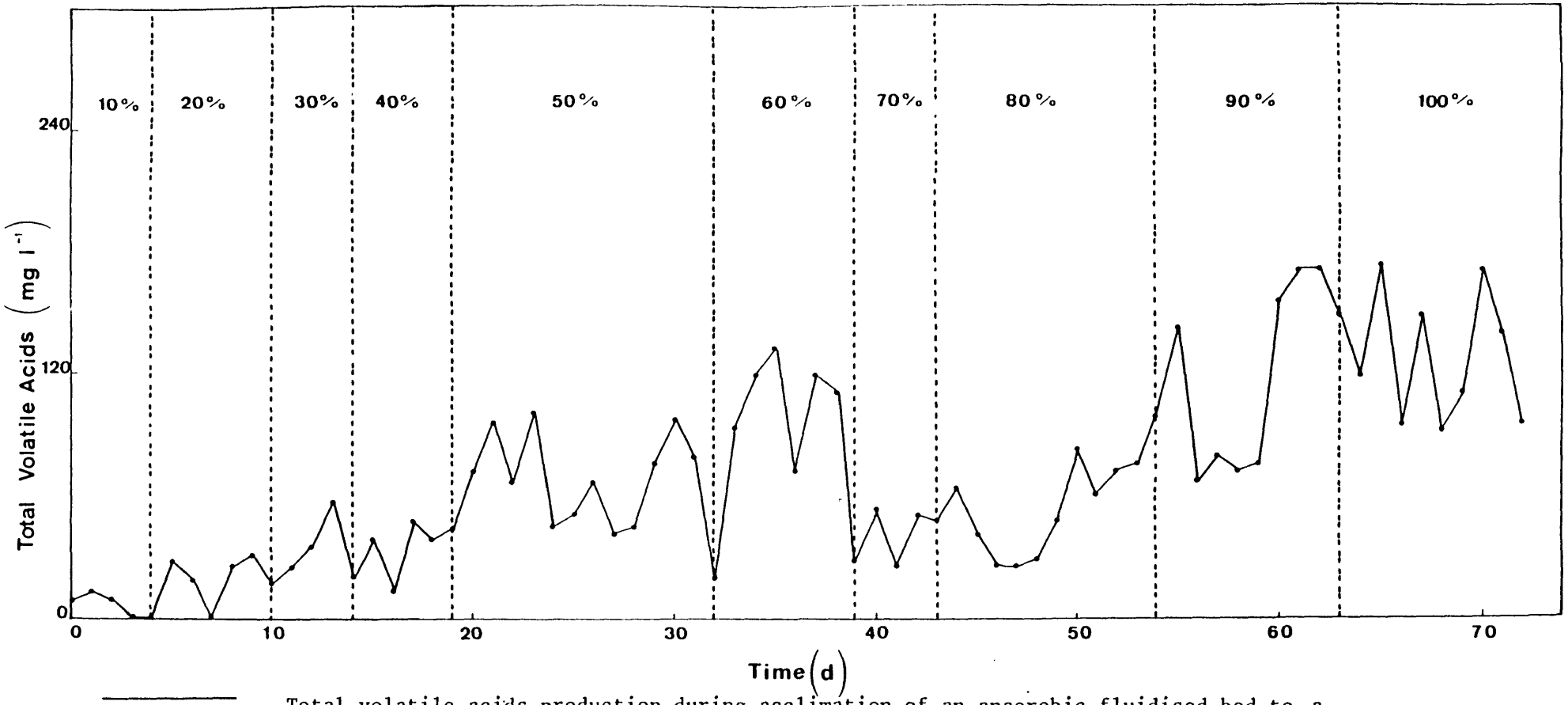


FIG.54 Total volatile acids production during acclimation of an anaerobic fluidised bed to a dimethylformamide-containing pharmaceutical waste.

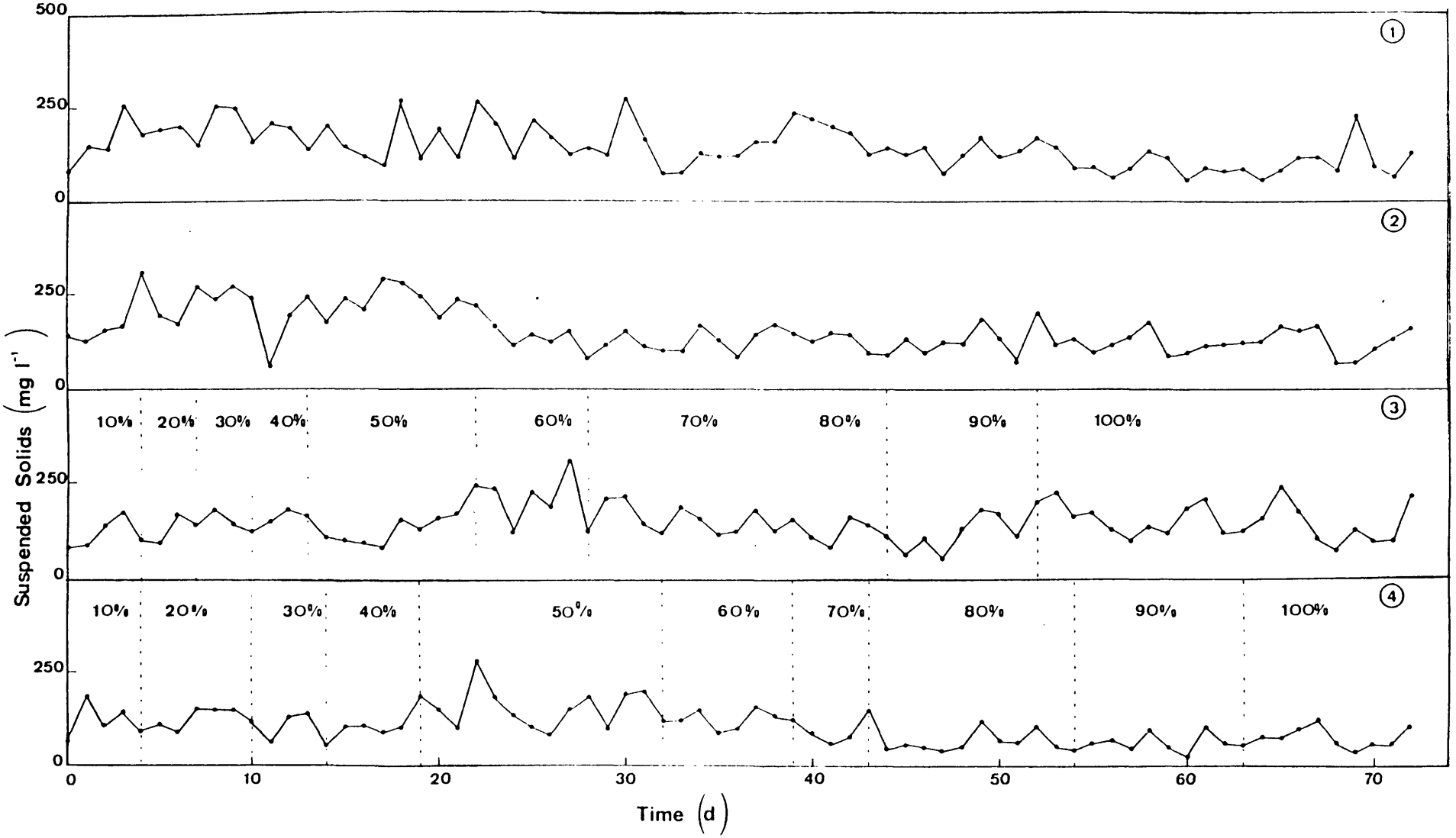
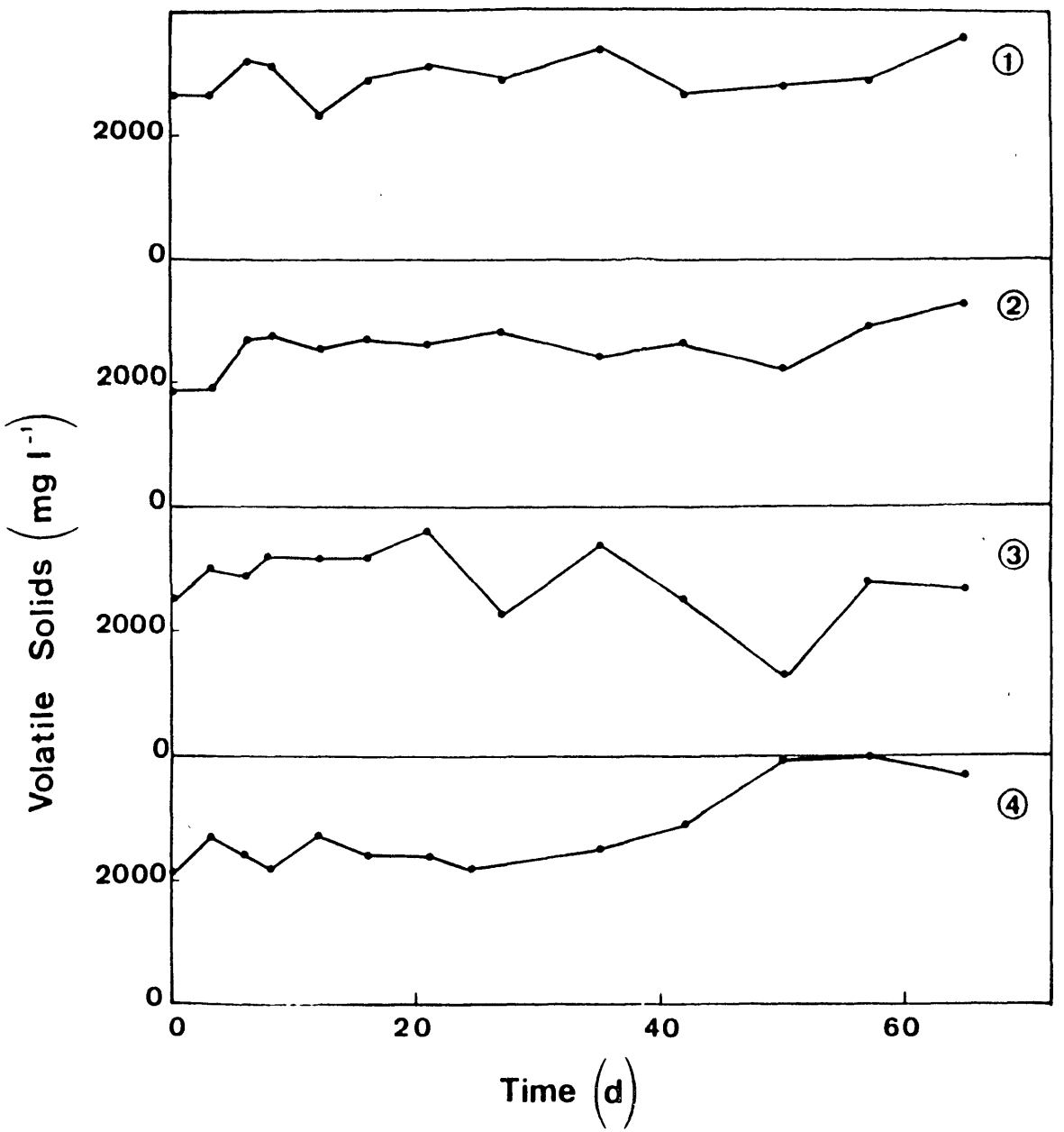


FIG. 55 Suspended solids production in fluidised beds during acclimation to pharmaceutical wastes: Controls (1) and (2); propanol-containing waste (3); dimethylformamide-containing waste (4).



Volatile solids concentrations in fluidised beds during acclimation to pharmaceutical wastes: controls (1) and (2); propanol-containing waste (3); dimethylformamide-containing waste (4).

FIG. 56

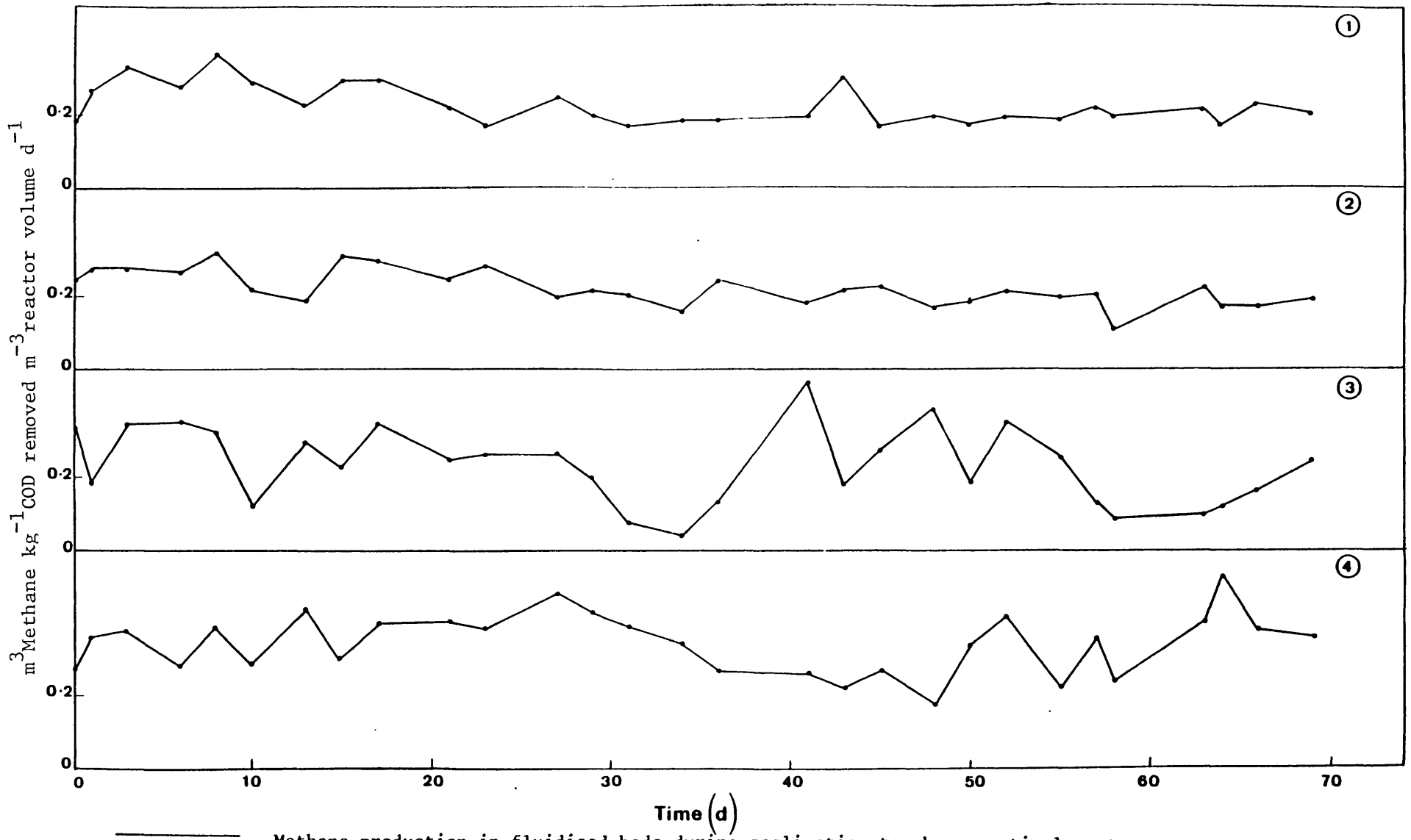


FIG.57 Methane production in fluidised beds during acclimation to pharmaceutical wastes: controls (1) and (2): propanol-containing waste (3); dimethylformamide-containing waste (4).

in TVA production compared to the control systems in the reactor treating waste A at the change-over to 100% pharmaceutical waste (Figures 52 and 53). Previously low levels of around 20mg l^{-1} were followed by effluent concentrations of over 200mg l^{-1}, which continued throughout the remainder of the study.

TABLE 25. FEED COMPOSITION CHANGES IN FLUIDISED BEDS DURING ACCLIMATION TO PHARMACEUTICAL WASTES A AND B

Waste	Days	% Pharmaceutical waste in influent
A	1 - 3	10
	4 - 6	20
	7 - 9	30
	10 - 12	40
	13 - 21	50
	22 - 27	60
	28 - 36	70
	37 - 43	80
	44 - 51	90
	52 - 72	100
B	1 - 3	10
	4 - 9	20
	10 - 13	30
	14 - 18	40
	19 - 31	50
	32 - 38	60
	39 - 42	70
	43 - 53	80
	54 - 62	90
	63 - 72	100

The pH levels within the n-propanol-fed system remained relatively stable, the lowest value of 5.48 occurring on day 59. This was corrected within one day by the addition of sodium bicarbonate to pH 6.48. Suspended solids concentrations fluctuated to a small extent daily but no significant deviations from the control values were observed (Figure 55). Volatile attached solids levels decreased between days 35 and 59 although no obvious loss of activity was noted, and increased again from day 51,

indicating that at an input of 100% industrial waste, biomass losses were not occurring. Methane production in this reactor did, however, drop slightly from day 52 onwards (Figure 57).

Gas production in the reactor fed with waste A was erratic throughout the course of the operation, peaking at $0.46m^3CH_4\ kg^{-1}COD\ d^{-1}$ (Figure 57). Mean methane production in this system was $0.277m^3\ kg^{-1}COD\ d^{-1}$, which was very similar to the mean methane yields of the controls - 0.222 and $0.214m^3\ kg^{-1}COD\ d^{-1}$ respectively. These gas production rates are not high. However, as the steady state COD removal exceeded 90% for the major part of the study, the operational conditions were not altered.

The reactor acclimating to the DMF-containing waste exhibited rather different behaviour. COD removal (Figure 51) decreased gradually throughout the acclimation period, culminating in a mean COD removal efficiency of 45% at 100% waste B. This suggested that a portion of the waste was not anaerobically biodegradable under the conditions prevalent in the fluidised bed system. Assays for DMF were not carried out during this study and hence its fate under the anaerobic conditions prevalent in this system could not be elucidated. However, methane generation in reactor 4 exceeded that of the controls (Figure 57), with a mean production rate of $0.344m^3\ kg^{-1}COD\ d^{-1}$.

The TVA concentrations produced in the DMF-fed reactor were greater than those of the controls and were generally erratic (Figure 54) although maximum TVA values did not exceed $180mg\ l^{-1}$. High levels of acid in an anaerobic digester system tend to suggest imbalance but this was

unlikely to be the case here: pH values ranged between 6.74 and 7.64 throughout the period of the experiment, with a bias towards high rather than low pH. Suspended solids concentrations generally remained below 200mg l^{-1} (Figure 55) and attached volatile solids concentrations were stable until about day 35, when they began to increase to a consistently high value of almost 4000mg l^{-1} (Figure 56).

The propanol-based waste (A) was originally colourless and the treated effluent, after filtration (to remove suspended solids) was also colourless. In contrast, waste B was of a strong yellow-brown colour undiluted and retained the colour in a weaker form when diluted to 2500mg COD l^{-1}. The colour could not be removed by a 0.22µm membrane. Upon treatment, however, the effluent was completely clear: the colour causing compound was evidently degraded or otherwise altered by the fluidised bed system.

4.09 START-UP OF THREE TYPES OF ANAEROBIC BIOREACTOR ON PHARMACEUTICAL WASTES

4.09.1 START-UP PROCEDURES

Two of the 41 fluidised beds (section 3.01.2), two anaerobic filters (section 3.02) and two upflow anaerobic sludge blanket (UASB) reactors (section 3.03) were started up on the two pharmaceutical wastes A and B (section 3.05.2). The six reactors were each filled with a 1:1 water and feed solution at the first feed dilution of 1302mg COD l^{-1}. The fluidised bed systems were then fluidised by expanding the sand bed to 20% and a 40ml inoculum of anaerobically digested sewage sludge (section 3.01.2.2) was injected into each column. Each of the two

anaerobic filters were seeded to 10% of their active volume with 500ml anaerobically digested municipal sludge (section 3.02.1.2). This volume of inoculum was chosen as larger quantities of seed sludge may have caused blockage of the voidage in the media and reduced reactor effectiveness. The UASB systems were also seeded at 10% of their active volume, using granulated biomass obtained from pilot scale UASB reactors treating papermill effluent (section 3.03.1.1). All six reactors were left in closed system for 48 hours to enhance biomass attachment in the filters and fluidised beds, and biomass settling in the UASB reactors.

All six reactors were started-up on pharmaceutical wastes A and B, each reactor receiving either the n-propanol-containing (A) or the dimethylformamide (DMF)-containing (B) effluent. The start-up was carried out following the stepped organic loading procedures adopted in section 4.03 to produce the most rapid biomass development. Organic loadings were increased by increasing influent COD concentrations and, at the first step, by decreasing the HRT when effluent characteristics of reactor performance warranted the increase (Table 26). In the case of the n-propanol waste, organic loading was not continued beyond 2.0kg COD m^{-3} d^{-1} as the reactors were responding unfavourably. The DMF-based effluent was applied until a loading of 3.0kg COD m^{-3} d^{-1} was achieved.

One of each pair of reactors was fed with the propanol-containing waste and the other with the DMF-containing waste, in order to provide a comparison between the anaerobic systems as well as between wastewaters. Tables 27 to 29 summarise the reactors' performances throughout

TABLE 26 : OPERATIONAL PARAMETERS OF 6 ANAEROBIC REACTORS STARTING UP ON PROPANOL-CONTAINING (A) AND DIMETHYLFORMAMIDE-CONTAINING (B) PHARMACEUTICAL WASTES.

REACTOR	WASTE	DAYS	ORGANIC LOADING $KgCODm^{-3}d^{-1}$	INFLUENT COD mgl^{-1}	HRT d	FEED FLOW RATE ld^{-1}
Anaerobic filter	A	0-16	0.75	1302	1.74	2.88
		17-47	1.50	1500	1.00	5.00
		48-69	2.00	2000	1.00	5.00
Anaerobic filter	B	0-16	0.75	1302	1.74	2.88
		17-47	1.50	1500	1.00	5.00
		48-64	2.00	2000	1.00	5.00
		65-75	3.00	3000	1.00	5.00
UASB	A	0-6	0.75	1302	1.74	2.88
		7-17	1.50	1500	1.00	5.00
		18-50	2.00	2000	1.00	5.00
UASB	B	0-6	0.75	1302	1.74	2.88
		7-17	1.50	1500	1.00	5.00
		18-34	2.00	2000	1.00	5.00
		35-50	3.00	3000	1.00	5.00
Fluidised Bed	A	0-16	0.75	1302	1.74	1.38
		17-47	1.50	1500	1.00	2.40
		48-69	2.00	2000	1.00	2.40
Fluidised bed	B	0-16	0.75	1302	1.74	1.38
		17-47	1.50	1500	1.00	2.40
		48-64	2.00	2000	1.00	2.40
		65-75	3.00	3000	1.00	2.40

start-up.

4.09.2 START-UP OF ANAEROBIC FILTERS ON PHARMACEUTICAL WASTES

At the lowest loading rates of 0.75kg COD m^{-3} d^{-1}, the anaerobic filters produced effluent TVA concentrations of below 200mg l^{-1} and suspended solids concentrations of less than 130mg l^{-1} (Table 27). The pH of the filter fed on n-propanol-based waste (A) varied by only one unit throughout the experimental period. The second filter, utilising DMF-containing waste (B) exhibited a high pH level initially and this was maintained during the study. Table 26 indicates that the filter fed on the DMF-based effluent achieved a greater final organic loading rate than did the other system, in addition to a higher COD removal. The n-propanol-fed system exhibited a pH value consistently below 7.0 and achieved a lower final organic loading rate and degree of COD removal efficiency than did the second filter (Tables 26-27), suggesting that low pH and low operational efficiency were interrelated. Total volatile acids concentrations in this filter were greater than those of the DMF-fed system, although not exceeding 233mg l^{-1}. Effluent suspended solids concentrations in both anaerobic filters did not exceed 148mg l^{-1} during the study, those in the DMF-fed reactor being less than those observed in the n-propanol-fed system.

The greatest disparity between the performances of the two filters was seen in COD removal efficiency (Table 27). This variability was probably due to the natures of the two pharmaceutical wastes and the pH levels maintained in the

TABLE 27 : OPERATIONAL PERFORMANCES OF 2 ANAEROBIC FILTERS DURING START-UP ON PROPANOL-CONTAINING (A) AND DIMETHYLFORMAMIDE-CONTAINING (B) PHARMACEUTICAL WASTEWATERS

FILTER	*DAYS	MEAN pH	MEAN SUSPENDED SOLIDS mgl^{-1}	MEAN TOTAL VOLATILE ACIDS mgl^{-1}	MEAN % REMOVAL COD
A	0-6	6.97	127	115	54
	7-13	6.76	107	160	44
	14-20	6.51	62	184	48
	21-27	5.98	81	150	40
	28-34	6.47	85	98	44
	35-41	5.78	100	141	34
	42-48	5.83	91	194	40
	49-55	5.99	123	233	35
	56-62	5.89	148	228	28
	63-69	6.05	136	213	32
B	0-6	7.92	69	63	57
	7-13	8.40	44	107	41
	14-20	8.51	48	116	47
	21-27	8.58	35	92	43
	28-34	8.70	40	80	45
	35-41	7.59	43	107	43
	42-48	7.13	120	141	48
	49-55	7.37	88	114	55
	56-62	7.20	88	116	56
	63-69	7.59	113	104	54
	+70-75	7.57	118	135	54

* Mean values of 7 days

\+ Mean values of 6 days

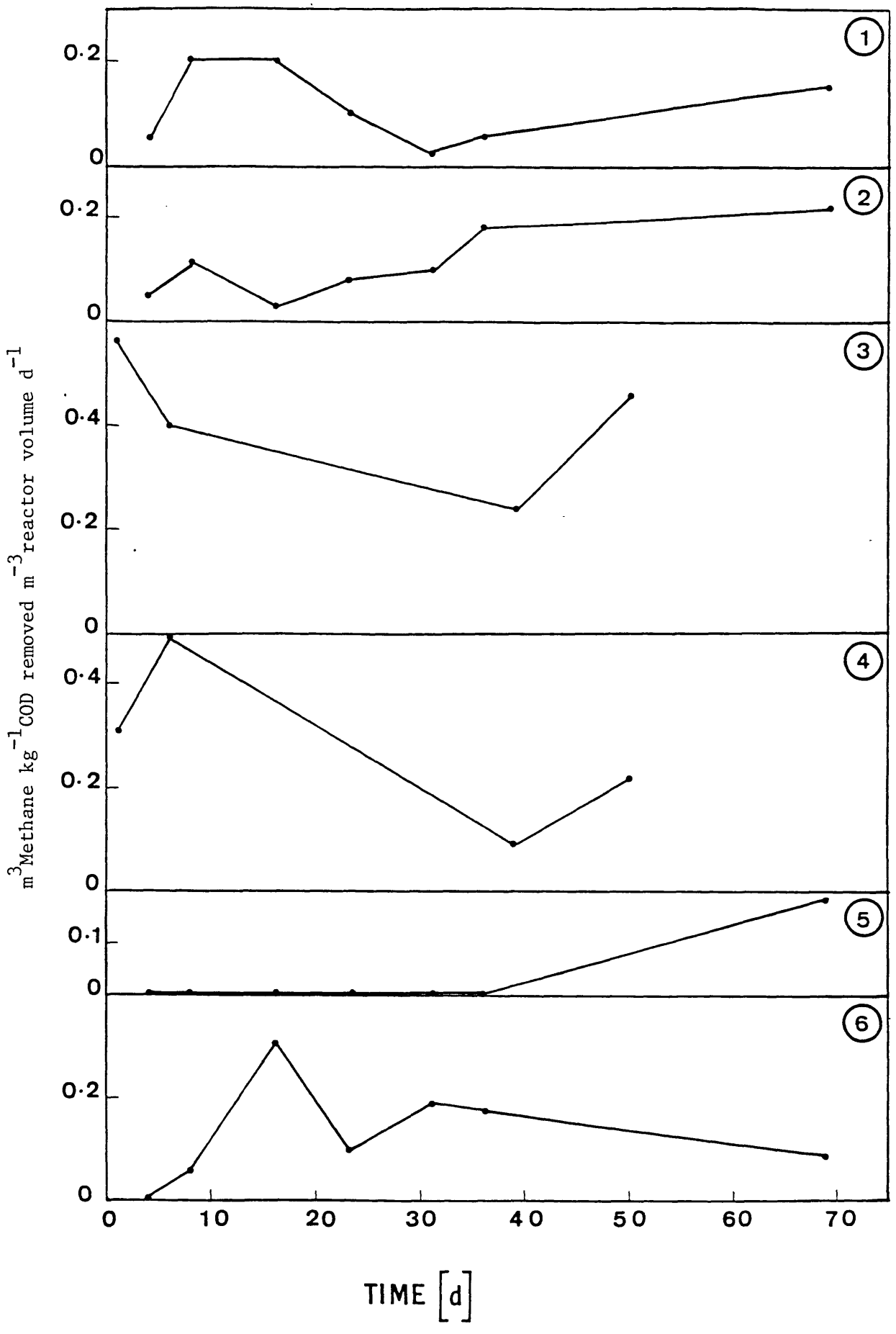


FIG. 58

Methane production in 6 reactors treating propanol-containing (A) and dimethylformamide-containing (B) pharmaceutical wastes: (1) anaerobic filter fed on A; (2) anaerobic filter fed on B; (3) UASB fed on A; (4) UASB fed on B; (5)fluidised bed fed on A; (6) fluidised bed fed on B.

systems. The latter in anaerobic filter A probably resulted from the conversion of the propanol component of the feed to propionic acid. The second filter exhibited COD removal efficiencies in excess of 50% at the final loading rate of 3.0kg COD m^{-3} d^{-1} (Table 27). As this removal was maintained from the initiation of start-up, the waste either contained an undegradable fraction, or some waste component was depressing the activity of a part of the microbial population. Gas production in both systems was erratic throughout (Figure 58), although the final levels of methane achieved in the DMF-fed filter exceeded the methane obtained in the other.

4.09.3 START-UP OF UPFLOW ANAEROBIC SLUDGE BLANKETS ON PHARMACEUTICAL WASTES

The UASB reactors were started up later than the filters and fluidised beds in this study since organic loading to both systems could be increased rapidly (Table 28) as COD removal efficiencies were initially high. Total volatile acids concentrations in the reactors were similar to those found in the anaerobic filters, being slightly lower in the UASB treating the DMF-containing waste. The pH levels in the n-propanol-fed reactor tended towards the acidic, whereas the pH values recorded for the second UASB were greater, although not exceeding 8.00, suggesting that for pH, the UASB was more stable than the anaerobic filter system, with good buffering capacity being developed.

The suspended solids concentrations of both UASB reactors were initially almost four times greater than those levels obtained in the filters (Table 28). However,

TABLE 28 : OPERATIONAL PERFORMANCES OF 2 UASB REACTORS DURING START-UP ON PROPANOL-CONTAINING (A) AND DIMETHYLFORMAMIDE-CONTAINING (B) PHARMACEUTICAL WASTEWATERS

UASB	*DAYS	MEAN pH	MEAN SUSPENDED SOLIDS mgl^{-1}	MEAN TOTAL VOLATILE ACIDS mgl^{-1}	MEAN % REMOVAL COD
A	0-6	6.60	458	22	69
	7-13	5.87	295	108	51
	14-20	6.51	295	88	64
	21-27	5.99	207	155	44
	28-34	5.87	164	225	33
	35-41	6.10	146	204	36
	42-48	6.23	117	281	30
	49-50+	6.35	94	139	26
B	0-6	6.83	401	19	68
	7-13	7.08	243	45	59
	14-20	7.35	153	37	61
	21-27	7.30	234	65	62
	28-34	7.35	202	132	56
	35-41	7.78	107	191	53
	42-48	7.81	62	189	47
	49-50+	7.91	49	123	50

* Mean values of 7 days

\+ Mean values of 2 days

as start-up progressed, the suspended solids in both UASB reactors settled to levels lower than those of the packed bed reactors, indicating the good settleability of the biomass. The initial washout of material may have been caused by the transition from the papermill waste upon which the granular sludge had been grown to the pharmaceutical waste.

The COD removal efficiencies at the final loading rates (Table 28) in both UASB systems were similar to those of the filters and were therefore probably functions of wastewater composition rather than reactor operation. Initial methane production in the UASB reactors was much greater than that observed in the other reactors (Figure 58) but decreased as start-up progressed. The effects of the waste on the biomass hence became more pronounced as organic loading increased. However, methane production increased again towards the end of the experimental period, indicating that some tolerance to the pharmaceutical waste was developing.

4.09.4 START-UP OF ANAEROBIC FLUIDISED BEDS ON PHARMACEUTICAL WASTES

The fluidised beds, at the lowest loading rate of 0.75kg COD m^{-3} d^{-1}, exhibited moderate TVA concentrations in the range 22 to 211mg l^{-1}. Suspended solids concentrations were also low in both beds (Table 29) and remained below 100mg l^{-1} throughout the study in the DMF-fed unit, although a slight increase in mean suspended solids was observed in the n-propanol-fed reactor towards the end of the experimental period (Table 29). The pH of

TABLE 29 : OPERATIONAL PERFORMANCES OF 2 ANAEROBIC FLUIDISED BEDS DURING START-UP ON PROPANOL-CONTAINING (A) AND DIMETHYLFORMAMIDE-CONTAINING (B) PHARMACEUTICAL WASTEWATERS

FLUIDISED BED	*DAYS	MEAN pH	MEAN SUSPENDED SOLIDS mgl^{-1}	MEAN TOTAL VOLATILE ACIDS mgl^{-1}	MEAN % REMOVAL COD
A	0-6	6.98	90	65	69
	7-13	6.72	75	129	39
	14-20	6.72	68	160	49
	21-27	6.77	89	134	42
	28-34	7.07	91	109	53
	35-41	6.78	138	88	48
	42-48	7.00	106	188	38
	49-55	7.02	113	255	41
	56-62	6.92	144	214	39
	63-69	7.16	188	199	40
B	0-6	7.22	100	35	75
	7-13	7.21	49	49	58
	14-20	7.13	41	58	52
	21-27	7.14	61	62	54
	28-34	7.23	48	62	55
	35-41	7.21	39	51	53
	42-48	7.16	48	81	47
	49-55	7.52	62	115	54
	56-62	7.44	83	121	58
	63-69	7.84	76	174	50
	+70-75	7.48	64	184	51

* Mean values of 7 days

\+ Mean values of 6 days

the fluidised beds remained close to neutral throughout the start-up, although, in common with both filters and UASB reactors, the DMF-fed system maintained a pH which tended towards basic.

The COD removal efficiencies of both fluidised beds decreased as the organic loading increased (Table 29), the propanol-fed system (A) deteriorating to a greater extent than the other. At the final organic loading of 3.0kg COD m^{-3} d^{-1}, a removal of 50% was achieved in reactor B whereas reactor A, at a final loading of 2.0kg COD m^{-3} d^{-1}, was removing only 40% of the influent COD.

Methane production rates in both fluidised beds were poorer overall than those obtained in the filters and the UASB systems (Figure 58). However, the second reactor, utilising DMF-containing waste, produced methane during the major part of the start-up programme.

4.09.5 COMPARISON OF OPERATIONAL PERFORMANCES OF THREE ANAEROBIC REACTOR TYPES STARTING UP ON PHARMACEUTICAL WASTES

There were several differences in the operational performances of the three reactor configurations during start-up on the recalcitrant pharmaceutical wastes. This reflected both the variable natures of the effluents and the reactor assemblies. The UASB reactors started up much more rapidly than both the anaerobic filters and the fluidised beds as a consequence of the already-granulated biomass used as seed. Initially high COD removals and gas production were observed in both UASB systems although at the higher organic loadings subsequently applied, the advantage was lessened. However,the top loading of

2.0kg COD $m^{-3}d^{-1}$ propanol-based waste was achieved at day 18 in the first UASB reactor and the final loading of 3.0kg COD $m^{-3}d^{-1}$ was applied at day 35 in the DMF-fed UASB (Table 26). Similar loading increases were made at days 45 and 65 for n-propanol and DMF wastes respectively in both the filters and the fluidised beds.

Generally, start-up was difficult and prone to instability upon changes in organic loading rate. The greatest decreases in COD removal efficiencies in all three reactor configurations were recorded upon the change from an organic loading rate of 0.75kg COD m^{-3} d^{-1} to a rate of 1.5kg COD m^{-3} d^{-1} (Tables 27 to 29). This change of loading rate was accompanied by a decrease in HRT from 1.74d to 1d (Table 26). The latter was therefore probably a significant factor in the reduction of COD removal efficiency at this stage, although the UASB digesters exhibited a greater tolerance to the operational changes than the other reactors. The final COD removal efficiencies were similar in all three systems for the DMF-containing waste (Tables 27-29) although for the n-propanol-based feed, the fluidised bed reactor removed 40% of the influent COD, whilst the filter and UASB systems removed 32% and 26% respectively. These results were indicative of the ameliorating influence of the high recycle of the fluidised system, whereby any potentially toxic waste component is diluted by mixing with the recycled liquid.

The fluidised bed configuration was superior to the other reactor systems in the maintenance of pH levels, probably as a result of the high fluid recycle employed.

The filter was less stable to pH fluctuation than the UASB although neither design incorporated a recycle facility. The TVA concentrations present during start-up were, however, similar for the three configurations. Effluent suspended solids concentrations remained below 200mg l^{-1} throughout the study in the fluidised bed and filter systems, whereas initially high solids washout was evident in the UASB reactors (Table 28). The latter stabilised after the final organic loading rate changes, as the non-settling biomass was removed to leave granular sludge of good settleability in the vessels.

Methane production was greatest in the sludge blanket configuration as the pelletised biomass used probably contained an active population of methanogens. Methane production in the filters increased towards the end of the study (Figure 58) as acclimation to the wastes occurred. However, although the fluidised beds also exhibited an increase in methane generation as start-up progressed, the mean volumes of gas obtained were lower than those of the other systems and were probably a function of the low initial biomass inoculum applied to the beds (section 4.09.1).

The propanol-containing effluent appeared to be less amenable to anaerobic digestion than the DMF-containing wastewater in all three reactor types and could not be applied at similar organic loading rates. However, the operational parameters of the fluidised beds, anaerobic filters and UASB reactors remained stable at the final loading rates and HRTs applied, with mean TVA levels of less than 250mg l^{-1} and suspended solids concentrations

below 150mg l^{-1} recorded for five of the six systems (Tables 27-29).

4.10 START-UP OF FOUR ANAEROBIC FLUIDISED BEDS ON SYNTHETIC AND INDUSTRIAL WASTEWATERS

4.10.1 START-UP PROCEDURES

Four 2l anaerobic fluidised beds, the construction of which were described in section 3.01.1, were started up on synthetic glucose wastewater (section 3.05.1), propanol-containing pharmaceutical waste (section 3.05.2), fruit processing effluent (section 3.05.3) and soft drinks manufacturing effluent (section 3.05.4). The recycle flowrate to each reactor was adjusted to provide a bed expansion of 30% and the systems were operated at 36±2°C.

The four 2l fluidised beds were situated near the factories supplying the soft drinks and fruit processing wastewaters and effluent samples were analysed at the local water authority (Severn-Trent) laboratories. The reactors were started-up using the stepped loading techniques outlined in Table 30. Each reactor was fed on one of the wastes available. The industrial wastes were diluted with distilled water as appropriate, although at the highest organic loading rates (Table 30) the influent samples of factory waste were obtained at COD concentrations which required adjustment of the influent flow rate to produce the required organic loading: this resulted in changes to the HRT. The synthetic waste was applied undiluted to the relevant fluidised bed to prevent rapid in-use deterioration of the feed. A low flow rate and hence an extended HRT were employed to produce the required organic

TABLE 30 : OPERATIONAL PARAMETERS OF 4 FLUIDISED BED REACTORS STARTING UP ON SYNTHETIC AND INDUSTRIAL WASTEWATERS

REACTOR AND WASTE	DAYS	ORGANIC LOADING $KgCODm^{-3}d^{-1}$	INFLUENT COD mgl^{-1}	HRT d	FEED FLOW RATE ld^{-1}
1. Synthetic glucose	1-13	1.5	12400	8.18	0.14
	14-25	3.0	12400	3.56	0.33
	26-33	4.5	11500	2.56	0.46
	34-63	7.5	11500	1.54	0.76
2. Fruit processing	1-13	1.5	3000.	1.95	0.60
	14-25	3.0	6000	1.95	0.60
	26-33	4.5	9000	1.95	0.60
	34-63	7.5	7700	1.02	1.15
3. Soft drink manufacturing	1-13	1.5	3000	1.95	0.60
	14-25	3.0	6000	1.95	0.60
	26-33	4.5	9000	1.95	0.60
	34-63	7.5	10000	1.36	0.86
4. Pharmaceutical	1-13	1.5	3000	1.95	0.60
	14-25	3.0	6000	1.95	0.60
	26-33	4.5	9000	1.95	0.60
	34-63	7.5	15000	1.95	0.60

loading rate.

4.10.2 COMPARISON OF OPERATIONAL PERFORMANCES OF FOUR FLUIDISED BEDS STARTING UP ON SYNTHETIC AND INDUSTRIAL WASTEWATERS

The operational performances of the four fluidised beds during start-up are given in Table 31. The digested sludge supernatant liquor used as seed for the four reactors provided more initial biomass than did the inoculum used in the 4l fluidised beds (section 4.09.4). However, suspended solids concentrations in those systems treating the industrial wastes did not exceed 270mg l^{-1} (Table 31). In contrast, the reactor fed with the synthetic glucose-based waste produced effluent suspended solids concentrations in excess of 600mg l^{-1} at the higher organic loading rates.

The COD removal efficiencies of the four systems differed markedly. The easily-degradable glucose substrate was removed at high efficiency whilst the industrial wastes proved recalcitrant (Table 31). Only at the top loading rate of 7.5kg COD m^{-3} d^{-1} did the effluent COD concentrations in the glucose-fed fluidised bed increase. This decrease in COD removal was accompanied by an increase in TVA concentration. However, the COD removal efficiency of the reactor increased slightly at the end of the experimental period, whilst the TVA concentrations of the system decreased from 1300 to 650mg l^{-1} (Table 31).

A similar pattern was seen in the fluidised bed treating pharmaceutical effluent. This waste was converted much less readily, as had already been noted from the previous experiments (section 4.09). Low pH levels were a

TABLE 31 : OPERATIONAL PERFORMANCES OF 4 ANAEROBIC FLUIDISED BEDS DURING START-UP ON HIGH STRENGTH WASTES

REACTOR AND WASTE	DAYS	MEAN pH	MEAN SUSPENDED SOLIDS mgl^{-1}	MEAN TOTAL VOLATILE ACIDS mgl^{-1}	MEAN % REMOVAL COD
1. Synthetic glucose	0-6	5.46	487	1686	67
	7-13	4.91	490	973	69
	14-20	7.77	457	1664	64
	21-27	8.53	683	1107	67
	28-34	8.44	637	1695	68
	35-41	7.30	487	977	66
	42-48	6.49	368	1300	56
	49-55	6.81	364	650	59
2. Fruit processing	0-6	4.39	130	743	26
	7-13	4.20	87	943	24
	14-20	7.51	247	2450	13
	21-27	8.36	192	1907	50
	28-34	7.50	216	2393	51
	35-41	7.04	253	2157	35
	42-48	6.76	246	1379	41
	49-55	6.50	168	850	25
3. Soft drink manufacturing	0-6	4.93	96	586	29
	7-13	4.41	119	414	36
	14-20	7.67	262	236	16
	21-27	8.41	202	550	37
	28-34	7.60	122	686	79
	35-41	5.79	167	1129	68
	42-48	6.94	104	900	67
	49-55	6.81	216	550	55
4. Pharmaceutical	0-6	6.21	68	382	39
	7-13	6.09	79	239	39
	14-20	6.96	122	296	24
	21-27	6.37	111	357	49
	28-34	5.96	120	639	50
	35-41	5.63	112	775	37
	42-48	6.77	102	629	44
	49-55	6.54	68	542	78

feature of the system and COD removals were inefficient even at the initial low organic loadings. However, a decrease in TVA concentration during the final week of operation was accompanied by a substantial increase in COD removing ability (Table 31).

The performance of the fluidised bed treating the fruit processing effluent was not as efficient as the other units in terms of substrate removal (Table 31). Slight improvements were observed at the 4.5kg COD m^{-3} d^{-1} organic loading rate but this dropped sharply when loading was increased to 7.5kg COD m^{-3} d^{-1} (day 34). Reactor efficiency did not recover although TVA concentrations improved at the end of the operational run. Similar patterns were observed for the soft drink manufacturing effluent, although final COD removal efficiencies reached 55%.

The rates of gas production in the four systems were measured in two phases towards the end of start-up. Gas production was low but consistent for the reactors (Table 32) and increased with increasing COD loading. Gas analysis was not undertaken as problems in transporting samples for gas analysis from the site to the laboratory without leakage were encountered.

TABLE 32. GAS PRODUCTION DURING START-UP OF ANAEROBIC FLUIDISED BEDS ON HIGH STRENGTH WASTES

Reactor	Waste	Gas production $m^3m^{-3}d^{-1}$	
		Days 26-33	Days 34-52
1	Glucose	0.20	0.25
2	Fruit processing	0.28	0.35
3	Soft drinks manufacturing	0.25	0.31
4	Pharmaceutical	0.22	0.28

4.10.3 VOLATILE ACIDS PRODUCTION IN FOUR FLUIDISED BEDS STARTING UP ON SYNTHETIC AND INDUSTRIAL WASTEWATERS

Analyses of individual volatile acids (section 3.06.06) were carried out for the 21 fluidised bed reactors. Each system exhibited pronounced variations. Figures 59 to 62 show the results of individual volatile acids analysis. The concentrations of acids produced by all four fluidised beds during the start-up operations indicated that the acetogenic bacteria were the predominant flora throughout the experimental period, and for those reactors treating glucose, fruit processing and soft drinks manufacturing effluents, acetate was the principal result of their activity (Figures 59 to 61). Propionate was the major volatile acid generated in the reactor fed on pharmaceutical waste (Figure 62), probably as a result of the high n-propanol content of the feed.

The fluidised beds utilising fruit waste (Figure 60) and pharmaceutical waste (Figure 62) exhibited the greatest concentrations of propionic acid during start-up. In the former, propionate levels exceeded those of acetate during the last phase of the experiment, subsequent to the final loading increase. In the case of the pharmaceutical-fed reactor, propionic acid levels increased with COD loading throughout start-up ,as a result of increasing levels of the n-propanol component of the feed. However, propionate concentrations did not fall below 400mg l^{-1} at the end of the operational run (Figure 62).

The reactor treating soft drinks effluent produced much lower concentrations of volatile acids than those systems converting glucose and fruit processing wastes

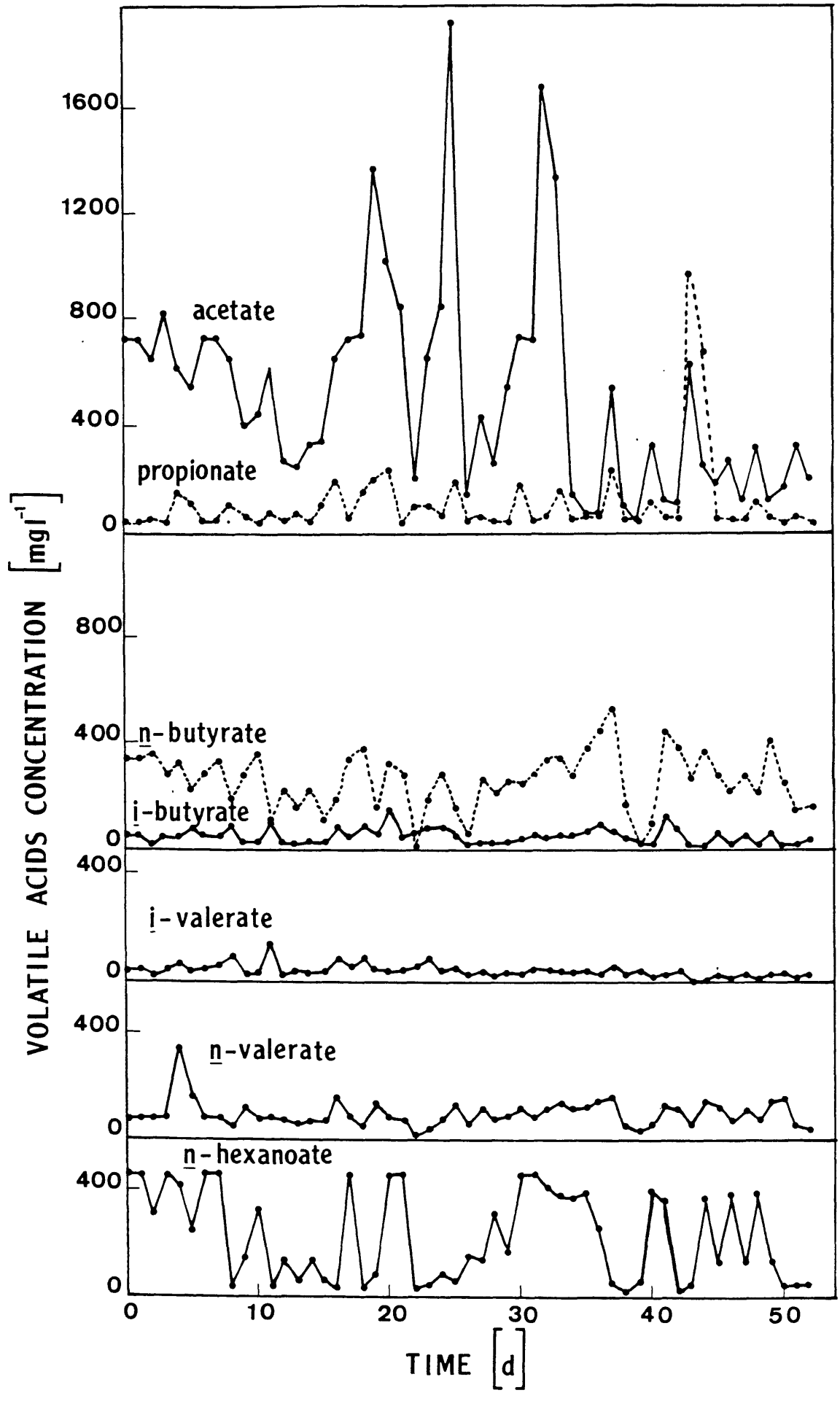


FIG.59 Volatile acids concentrations during the start-up of an anaerobic fluidised bed on glucose waste.

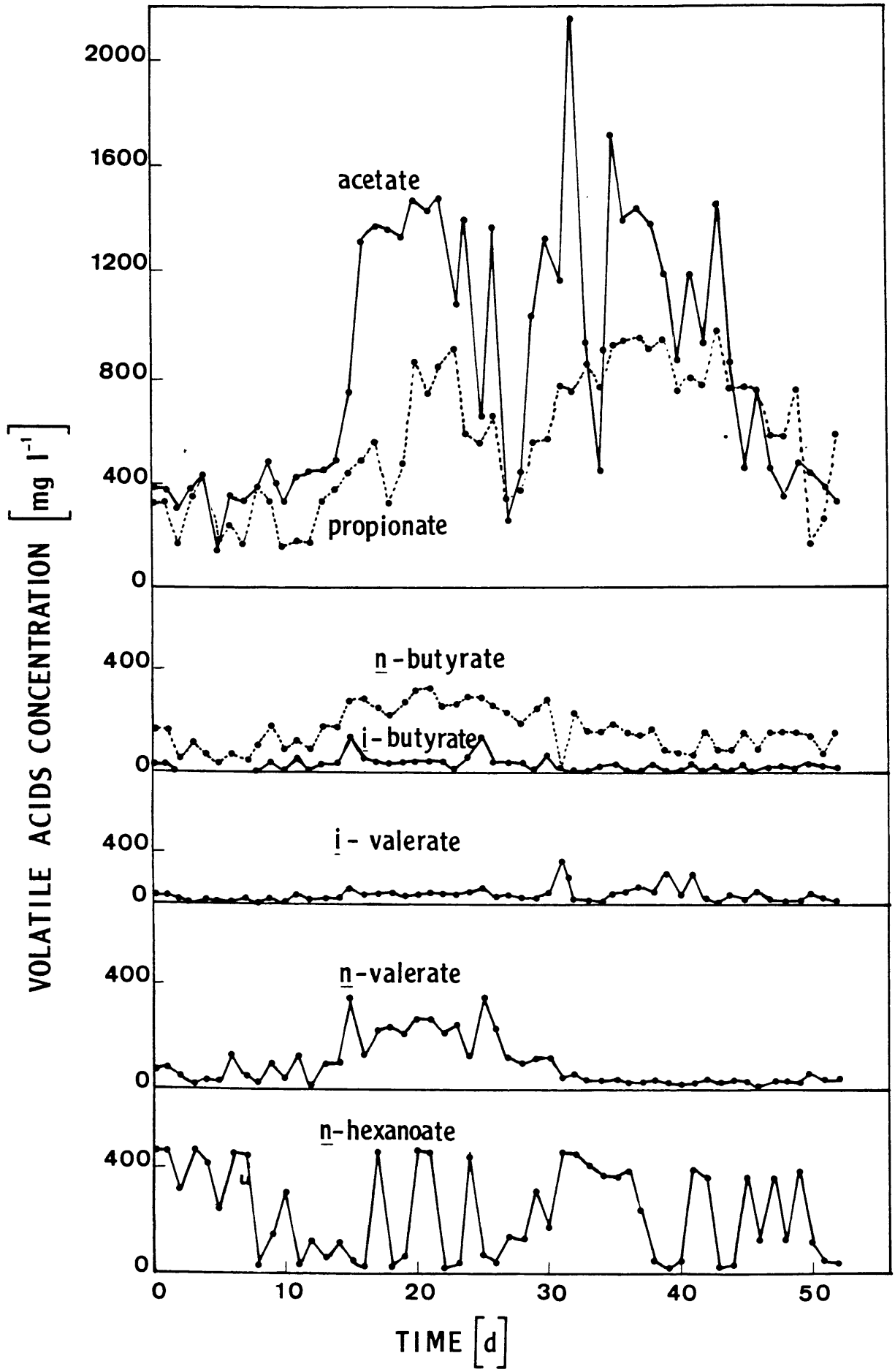


FIG. 60 Volatile acids concentrations during the start-up of an anaerobic fluidised bed on fruit processing waste.

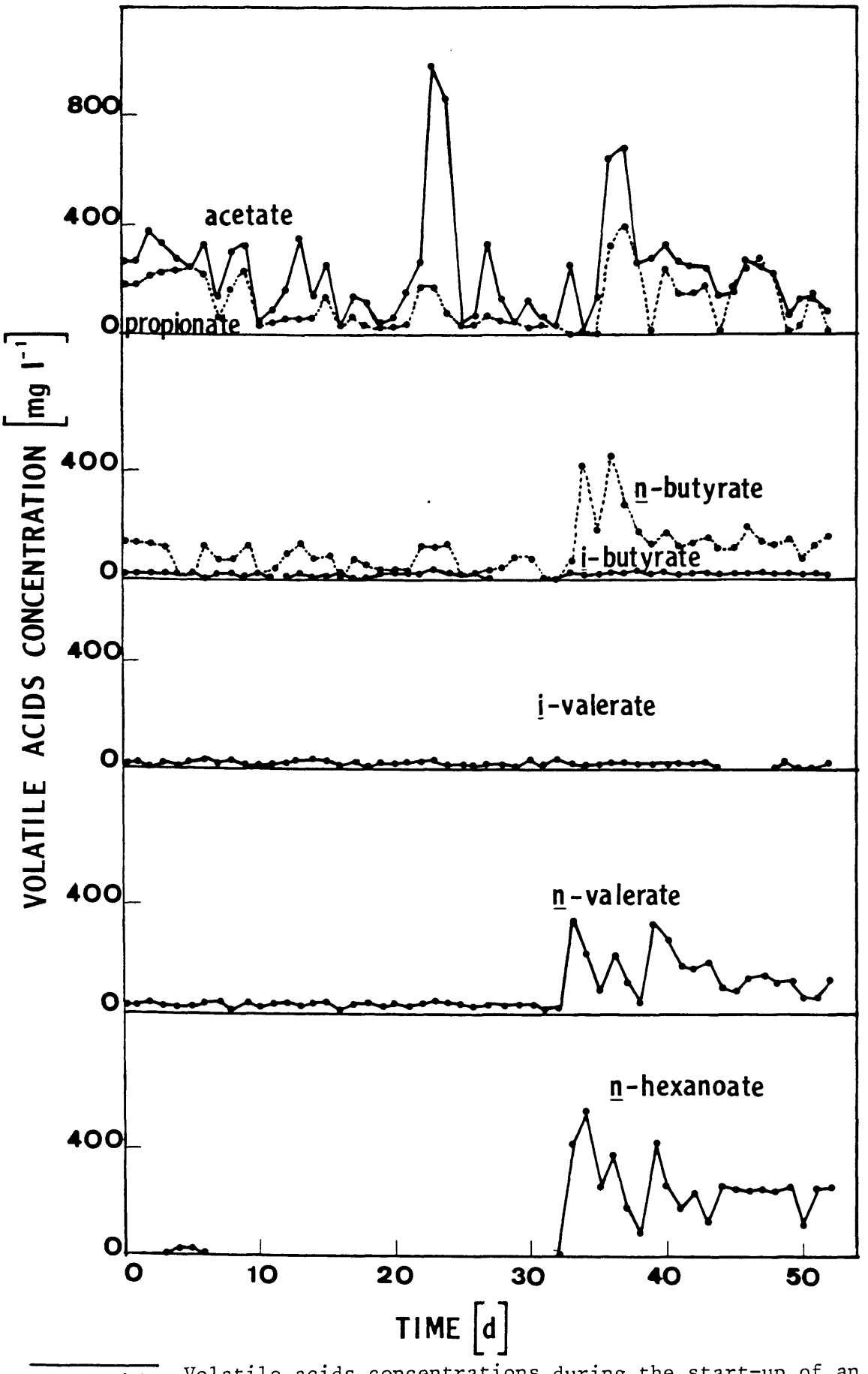


FIG.61 Volatile acids concentrations during the start-up of an anaerobic fluidised bed on soft drinks manufacturing waste.

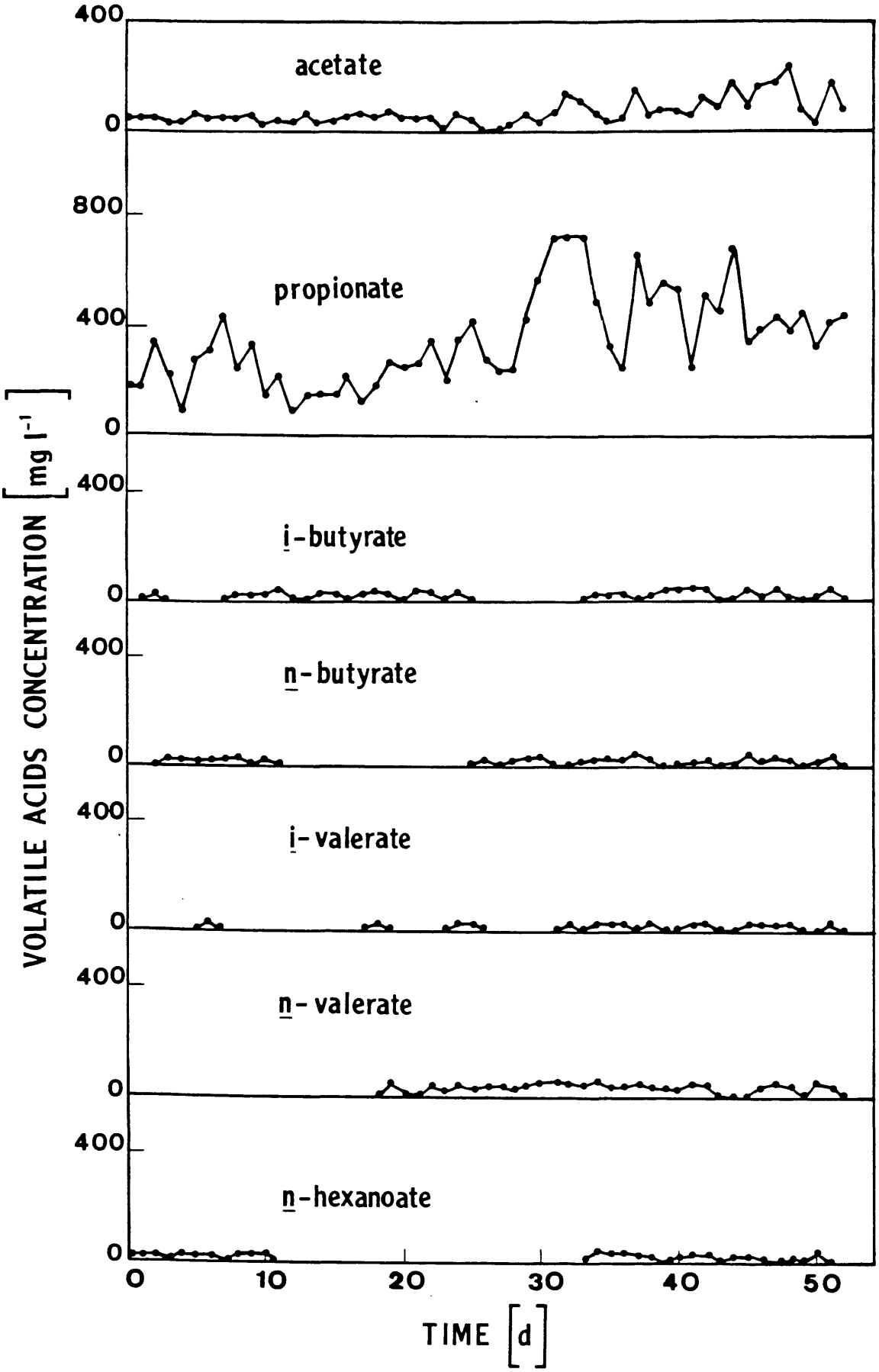


FIG.62 Volatile acids concentrations during the start-up of an anaerobic fluidised bed on pharmaceutical waste.

(Figure 60). The concentrations of n-butyrate in the systems treating glucose, fruit processing and soft drinks wastewaters fluctuated between 200 and 400mg l^{-1}. Hexanoic acid was present in all four fluidised beds, appearing suddenly in the effluent of the soft drinks waste fed reactor (Figure 61) when loading was increased to 7.5kg COD m^{-3} d^{-1}. This may be attributed to the shock load applied, as the concentration of hexanoate decreased subsequent to this, although COD removal decreased also. However, in all four systems, total volatile acids concentrations decreased towards the end of the experimental period, indicating that digester conditions had stabilised.

The type and concentration of volatile acids produced during the anaerobic digestion process appeared to be directly influenced by the substrate applied to the systems, as the sugar-rich glucose and fruit processing wastes gave rise to products such as acetate, whereas the propanol-containing pharmaceutical effluent stimulated propionate production.

CHAPTER 5
DISCUSSION

Anaerobic biological systems have been extensively employed to treat sewage sludges of high solids content. Recently developed systems such as the anaerobic filter (Mosey et al. 1978), the upflow anaerobic sludge blanket (Lettinga et al. 1980) and the expanded and fluidised bed (Francis & Callahan 1975) configurations have been established for the anaerobic conversion of primarily soluble effluents. The anaerobic fluidised bed reactor has been found to be well-suited to meso- and thermophilic treatment of both low and high strength wastewaters (Jewell et al. 1981; Bull et al. 1983a), with most of the published data relating to operation under steady state conditions.

One of the most serious drawbacks of anaerobic processes is the protracted period required for start-up, a condition attributed to the slow growth rates of anaerobic bacteria and hence the slow accumulation of active biofilm on the carrier media (van den Berg et al. 1980a). The situation is aggravated by the lack of information available on the factors affecting biomass retention and distribution within reactor systems. During start-up, the anaerobic reactor remains in the non-steady or quasi-steady state until the biofilm is fully developed. Once stabilised at high biomass concentrations, the fluidised bed can tolerate very high organic loading rates and transient shock loads (Bull et al. 1983b). The objectives of start-up are thus to ensure good biomass attachment and subsequently, efficient microbial conversion of the organic component of the wastestream.

A stepped organic loading regime during start-up has been shown to provide conditions conducive to rapid biofilm development (Bull et al. 1983c; Rudd et al. 1985) and high biomass concentrations. The initial study reported here utilised stepped hydraulic loading but variable organic loading, simulating industrial conditions of controlled influent supply but variable influent strength. An investigation into the effects of addition of a natural polymer and a synthetic cationic polymer to encourage microbial attachment to the support medium was also undertaken. Another aim of the study was to determine the benefits to start-up, if any, of the maintenance of strict anaerobiosis within the reactor system. The limiting redox potential (Eh) for methanogenic growth is -330mV (Cooney 1981); oxygen is thus a potent inhibitor of methanogenesis.

Variable values in effluent COD resulted from the variable influent substrate concentrations supplied to the four anaerobic fluidised beds utilised. Such high effluent COD values are the result of reduced metabolisation of biodegradable compounds. A microbial culture must increase its growth rate in order to tolerate organic overload, or selection of a suitable microbial population must occur. It has been suggested that different methanogenic species are selected according to the loading conditions (Hulsoff-Pol et al. 1983), with low organic loads stimulating the predominance of filamentous species of Methanothrix and high organic loads the predominance of rod-like forms of Methanosarcina. Although both are acetoclastic methanogens, species of Methanosarcina exhibit a more versatile utilisation of substrate, since in addition to

acetate and hydrogen they also use methanol and methylamines (Balch et al. 1979). However, the filamentous Methanothrix has a great affinity for inert support material and may be retained within the system more efficiently, although it has been shown that Methanosarcina can replace Methanothrix as the dominant methanogen as the HRT of the system decreases below 4 days (Wang et al. 1986).

The mean COD removal efficiency of 70-80% achieved in the control system was slightly low compared to the values of 74-85% reported by Kennedy and Droste (1983) and considerably lower than the final COD removal efficiencies of 90% reported by Bull et al. (1983c). This was probably due to the highly variable loading conditions during the early stages of start-up which did not allow the development of a stable, healthy biofilm. As with other anaerobic studies on fluidised bed treatment (Barnes et al. 1983) and other systems such as UASB reactors (Lettinga et al. 1980) the stability and response of the reactors can be judged from overall performance of the system and the concentration of intermediates and end products. The recovery of the fluidised bed system from instability suggests that it is suitable for the treatment of wastes of the variable influent COD compositions often associated with industrial effluents. However, for maximum substrate removal efficiency and to reduce the start-up period, some type of load equalisation is obviously desirable.

A comparison of the performances of the anaerobic fluidised beds started using different regimes indicated that microbial attachment was aided by synthetic polymer

addition. Initial biomass retention was enhanced but the comparatively poor process performance exhibited suggested that the added polymer interfered with the selection and establishment of an appropriate bacterial population by preventing normal microbial succession. Increased biomass accumulation as a result of increased organic loading is a general phenomenon in fixed film systems (Switzenbaum & Jewell 1980; Jewell et al. 1981; Schraa & Jewell 1984) and the performance of the reactor amended with synthetic polymer indicated that the initial high attachment rate of a heterogeneous population was ultimately detrimental, perhaps establishing the predominance of unsuitable species. Unless carefully managed, the addition of flocculant polymers could create selection problems, although some successful studies have been reported (Salkinoja-Salonen et al. 1983). However, the use of additives to promote attachment can be avoided if reactor conditions are optimal for the growth of microorganisms of good aggregating capacity.

The reactor amended with the suspension of natural polymer together with the polymer-producing organisms (Klebsiella aerogenes) proved to be more stable than the reactor treated with synthetic polymer, when the COD loading to the system was increased. The COD removal efficiency improved as start-up progressed and the near-neutral pH values and low volatile acids concentrations maintained within the system indicated that the additional K. aerogenes suspension was beneficial to the attachment and development of the bacterial population. However, under certain conditions the production and excretion of

biopolymer into the medium by K. aerogenes may not occur. In such circumstances attachment will be dependent upon the amount of biopolymer present during seeding. Another important aspect to be considered relates to the ecology of the biofilm, which may be significantly influenced by the interaction of the microbial population dispersed in the fluid and the population attached at the surface, especially in a fluid-flow system (Characklis & Cooksey 1983).

Start-up procedures are enhanced by the provision of an oxygen-free atmosphere: the buffering capacity readily achieved and sustained by the nitrogen-purged system used in the present study maintained the pH in a range conducive to methanogenic growth (6.3-7.4) (Balch et al. 1979). Fluctuations in suspended solids and total volatile acids (TVA) concentrations were much less pronounced and COD removal efficiency was finally greater in the reactor purged with nitrogen than in the other systems, observations in agreement with previous studies which have indicated that a strict control of redox potential is necessary for the maintenance of reactor performance (Kennedy & Droste 1983; Lettinga et al. 1983). The superior performance of the completely anaerobic system at low volatile solids concentrations indicates that the quality of the attached biomass is a more important factor than the quantity present during start-up.

Variability in influent organic loading appeared to exacerbate the formation of an ill-balanced microbial population within the biofilm matrix of the polymer-amended reactor and led to process instability. Other studies

(Bull et al. 1983c; Rudd et al. 1985) have demonstrated that constant influent strength is conducive to the formation of high concentrations of active biomass. The concentration of substrate applied to a system influences microbial growth (Brunetti et al. 1983) and the frequent build-up of inhibiting concentrations of TVA evident in this study were probably the result of the faster growth of acidifying bacteria compared with that of the obligate hydrogen-producing acetogenic (OHPA) bacteria and the acetoclastic methanogens. Subsequent variable influent loadings prevented the complete restoration of balanced digestion, resulting in low pH levels as buffering capacity was overcome. Low pH values inhibit or prevent methane production and long periods are often necessary to restore system balance due to the low growth rates of the OHPA bacteria and the methanogens (Hulsoff-Pol et al. 1983).

Efficiency of operation in fixed film reactors depends greatly on good hydraulic distribution and maximal substrate availability to the reactor microorganisms. The minimum linear velocity of the system is that velocity which permits these conditions. As the fluid flow rate increases above the minimum linear velocity, biofilm accumulates on the carrier media (Trulear & Characklis 1982). However, as fluid velocity increases at higher flow rates, biofilm accumulation decreases and detachment can occur, a condition which eventually culminates in process failure.

The influence of the shear stresses on attached microflora caused by the degree of expansion of the sand medium comprising the reactor bed was examined in anaerobic

fluidised beds under steady state conditions. Microbial attachment and efficiency were impaired at the lowest bed expansion of 5%. The nitrogen-purged reactor, which had performed particularly well at start-up, experienced the greatest loss of attached biomass of the three systems examined at this expansion, with a consequent reduction in performance. The well-adapted population of this reactor, having grown up in a completely anaerobic environment throughout start-up, may have been less diverse than that of the control system and hence more sensitive to major disruptive influences. Although reactor performance was regained, the increase in attached biomass was not great enough to return the nitrogen-purged system to optimum efficiency. However, increased biomass concentrations at expansions in the range 10-30% indicated that either increase of shear stress in all three systems did not cause extensive sloughing of the biofilm, or that microbial removal and deposition rates remained similar. Biofilm detachment increases with fluid velocity and mass of attached biofilm and is dependent upon the fluid shear stress at the fluid-biofilm interface (Trulear & Characklis 1982). The expansions used, therefore, were not sufficient to cause massive removal of attached biomass, although slight decreases in volatile solids concentrations between 25% and 30% suggested that at expansions in excess of 25% biofilm detachment would exceed deposition by the fluid flow regime.

In addition, greater expansions facilitated removal of gas as small bubbles which could rise easily through the spaces in the bed. Increased TVA concentrations at lower

expansions indicated accumulation of acids and hence suppressed methanogenic activity in the reactors, but good buffering capacities were sustained as fluidisation increased. The optimum expansions for methane evolution were 20% and 30%, with a fall in the percentage of methane obtained at the 25% expansion. A small shift in the anaerobic population at this step may have occurred, causing an increase in the numbers of non-methanogenic species, as volatile solids concentrations were also observed to increase at the 25% expansion. However, fluidised beds appear to possess microbial populations that are relatively stable to increasing shear stresses.

It has been recognised that the maximum volume of biofilm attached to the media surface in a turbulent flow regime could be limited by fluid shear stress: the increase of shear removal rate was found to be proportional to interfacial fluid shear stress and, at high film volumes, detachment approached infinity (Trulear & Characklis 1982; Characklis & Cooksey 1983). The critical shear stress in fixed biofilm systems is that value at which all the cells are removed; the proportion remaining after a specified period of shear is indicated by the adhesion number (Powell & Slater 1982). The provision of settling time in a reactor system prior to operational processes tends to increase the level of shear stress necessary for film removal from media surfaces as the microflora have formed stable linkages; Gram-positive bacteria are more easily removed than those with Gram-negative cell walls in complex media, as the outer surfaces of the latter probably enhance adhesion (Rogers 1979). Frictional resistance to flow is

characteristic of biofilm-dependent reactor processes. This resistance has been observed to increase where filamentous forms of bacteria predominate, possibly as a result of the dissipation of energy by the fibrillar structure (Trulear & Characklis 1982).

During the accumulation process at the initiation of digester operation, and as the reactor proceeds towards steady state, a succession of microbial communities occurs (Hobson & Shaw 1974). The fluid shear stress in the system may enhance this process by shearing and sloughing off microorganisms displaced by other competitive groups. The decay of bacteria within the film also affects biomass loss rate; the steady state biofilm model of Rittmann and McCarty (1980a;1980b) has been shown to apply also to situations of significant shear-stress losses (Rittmann 1982). The model predicts the conditions under which shear-stress loss is likely to be important.

The identification of specific nutrient requirements for stable bacterial growth can be a time-consuming process and little has been reported relative to anaerobic reactors. The influence on fluidised bed reactor performance of increasing the concentrations of trace elements in the influent substrate was examined. The good buffering capacity of the reactors was unaffected, as was COD removal ability, by trace elements increase. However, a decrease in TVA levels concomitant with an increase in methane production indicated either that improved rates of acids conversion to methane was occurring or that the reactor populations had shifted slightly in favour of the

methanogenic rather than the acidogenic microflora.

The stimulation of acetate conversion to methane and carbon dioxide in a mixed population of anaerobic fixed film digester bacteria was investigated by Murray and van den Berg (1981). The addition of nickel, cobalt and molybdenum increased total gas (including methane) production by 42% and allowed greater volumes of food processing waste to be effectively treated by decreasing the reactor residence time. These effects were considered the result of the accumulation of a thicker methanogenic film. The addition of nickel to a culture of methanogens was reported to increase the level of acetate utilisation. Where this stimulatory effect was observed, Methanosarcina spp. were dominant (Speece et al. 1983). Nitrate reduction and COD utilisation increased significantly when molybdenum and selenium were added to an anaerobic system (Chakrabarti & Jones 1983) and sulphate reducers were observed to require biotin and yeast extract for function (Ueki & Suto 1981). The present study incorporated nickel, molybdenum and selenium but biotin and yeast extract were not added. However, the meat-based substrate employed contained these supplements.

The presence of trace metals such as molybdenum, selenium, tungsten and nickel is probably necessary for the activity of several enzyme systems. The facultative anaerobe Escherichia coli is capable of obtaining energy for growth from electron-transport-dependent ATP synthesis under anaerobic conditions using either fumarate or nitrate as a terminal electron acceptor (Haddock 1977). The two anaerobic electron transport chains utilising nitrate and

fumarate appear to share common components. Under anaerobic conditions of molybdenum-limitation, the synthesis of the enzymes formate dehydrogenase and nitrate reductase by E. coli is decreased, as both of these enzymes need molybdenum as a cofactor for activity. The dehydrogenase also requires selenium and limitation of this element under anaerobiosis results in a decrease in the enzyme activity (Lester & de Moss 1971).

Tungsten acts as a competitive inhibitor of molybdenum in many biological systems (Scott & de Moss 1976). The anaerobic growth of E. coli in a medium containing tungstate resulted in the synthesis of inactive formate dehydrogenase and nitrate reductase enzymes. Subsequent incubation of the E. coli cells with molybdenum under non-growing conditions led to the rapid activation of both the enzyme systems. Investigations into the requirements of anaerobic bacteria for trace elements have resulted in the discovery of several selenium-, tungsten- and nickel-enzymes (Table 33). Both selenium and nickel are often found as contaminants in culture media, the former being present in significant quantities in commercially-produced sodium- and hydrogen-sulphide and the latter in iron salts (Thauer & Morris 1984). However, Speece et al. (1986) consider that even the presence of detectable concentrations of soluble trace metals cannot be equated with adequate bioavailability: the case histories examined by these workers suggest that Fe++ in particular can be limiting when the soluble concentration appears adequate. Nonetheless, the work carried out in the present study does indicate that trace elements in sufficient quantities do

Table 33 Some Trace Element-Dependent Enzymes of Anaerobic Bacteria

Element	Enzyme	Anaerobic Microorganism	Reference
Selenium	Formate dehydrogenase	Acetogenic bacteria	Wagner & Andreesen (1977) Ljungdahl & Andreesen (1978)
		Methanococcus vanielii	Jones & Stadtman (1981)
	Glycine reductase	Several clostridia	Turner & Stadtman (1973) Stadtman (1978) Dürre & Andreesen (1982)
	Hydrogenase	Methanococcus vanielii	Yamazaki (1982)
	Nicotinic acid hydroxylase	Cl. barkeri	Imhoff & Andreesen (1979)
	Xanthine dehydrogenase	Some clostridia	Wagner & Andreesen (1979) Dürre et al (1981) Dürre & Andreesen (1982)
Tungsten	Formate dehydrogenase	Acetogenic bacteria	Andreesen & Ljungdahl (1973) Andreesen et al (1974) Leonhardt & Andreesen (1977)
		Methanococcus spp.	Jones & Stadtman (1976)
		Cl.thermoaceticum	Ljungdahl & Andreesen (1978) Yamamoto et al (1983)
Nickel	Carbon monoxide dehydrogenase	Cl. pasteurianum	Diekert et al (1979) Drake (1982)
		Cl. thermoaceticum	Drake et al (1980) Diekert & Ritter (1983)
		Acetobacterium woodii	Diekert & Ritter (1982)
	Hydrogenase	Methanobacterium thermoautotrophicum	Graf & Thauer (1981) Albracht (1982) Jacobson et al (1982)
		Vibrio succinogenes	Unden et al (1982)
		Desulfovibrio gigas	Cammack et al (1982)
		D. desulfuricans	Krüger et al (1982)
	Methyl-CoM methyl reductase	Methanogenic bacteria	Diekert et al (1980) Whitman & Wolfe (1980) Ellefson et al (1982)

improve the conversion of substrate to methane, although the precise mechanisms of the changes occurring were not clear.

If the waste to be treated by anaerobic degradation is not rich in micronutrients, supplementation, either chemically or by the addition of another wastestream may be necessary. Speece et al. (1986) recommend supplementation of Fe++, Co++ and Ni++, vitamin B_{12}, acetate and mercaptoethanesulphonic acid. However, the design and optimisation of the growth environment should include consideration of the technical and economic constraints imposed by the nature and quantity of the waste to be converted.

One of the most basic considerations in the design of industrial anaerobic digesters is that of their suitability for the wastes to be treated. If the wastes under consideration are generated by industries which operate seasonal campaigns, e.g. sugar beet, or run otherwise non-continuously, the anaerobic reactors chosen must be capable of tolerating periods of downtime ranging from days to months.

Under steady state conditions, the fluidised bed has been shown to tolerate variable loadings and temperature, pH and COD shocks well (Bull et al. 1982; 1983b; Stephenson & Lester 1986). The effects of long periods of non-operation on reactor performance are not so generally well-documented, although data for other high rate anaerobic digesters have been recorded. The UASB reactor, for example, is considered stable after periods of downtime, and start-up after a feed-interruption of a few weeks has

been reported to present no problems, with gas values reaching acceptable levels some hours after the start of feeding (Lettinga et al. 1980). Intermittent operation of the UASB may result in process failure, however (Schwartz et al. 1981). Conventional digesters and activated sludge units cannot generally tolerate interrupted operations (Young 1980).

The effects on the performance of anaerobic fluidised beds of periods of downtime of 39 and 89 days were examined in the current study. Full operational performance was retained after the shorter downtime period but restart required manipulation after the 89 day shutdown due to poor COD removal and high volatile acids production. Anaerobic filter reactors have been shown to require 3 to 4 days to recover their COD removal capacity after a 14 day shutdown (Young 1980), although 6 months downtime required only a 16 day restart period (Schwartz et al. 1981). This suggests that different configurations of high rate digester respond differently to periods of downtime, although the response is generally rapid and efficient for attached-biomass systems. Size of reactor, amount of biomass present, waste-type and length of in-use time before shutdown may also be important factors. In the current study, two reactors had operated continuously for almost 8 months, and another two units for 6 months, on meat waste. A thermophilic anaerobic filter reactor that had been operated continuously for almost 18 months on thermally conditioned sludge decant liquor, and was then shut down for 4 months became active upon restart and digestion efficiency was regained in 2 days (Schwartz et al. 1981).

In those fluidised beds restarted after 39 days downtime, TVA concentrations remained low throughout the experimental period, whereas acid production rates rose rapidly and continued at high levels in the second pair of beds until stabilisation at the final load change, at day 35, had occurred (section 4.06). Mean methane production in those reactors restarted after 89 days was also low. It is probable that the acidogenic population within fluidised beds survive for longer periods than the methanogenic population, or, alternatively, that the acidogens adapt more quickly to changing conditions such as abrupt start-up at high organic loading rates after a period of starvation or the sudden turbulence and shear stresses imposed by fluidisation. Such adaptation could be a function of the faster growth rates of the majority of acidogenic bacteria over methanogens (Verstraete et al. 1981) or the ability of some acid-producing bacteria to form spores and thus survive adverse conditions. The increased TVA concentrations noted above for the reactors after 89 days downtime were more likely to have been caused by a shift in microbial population than by any loss of biomass, as little difference was observed between the four reactors in solids concentrations.

In the few previous reports of restart after downtime in similar reactors (such as the anaerobic filter), longer periods of shutdown also required more extended restarting periods (Young 1980; Schwartz et al. 1981). The differences in these restart times between reactor types may be in part accounted for by the different flow regimes employed, the mobilisation of the support matrix and the

high recycle ratios of fluidised beds. A critical period may exist during downtime in anaerobic systems whereby, to retain the complete operational ability and maintain the microbial balance, temporary restart is advisable. This critical period for the systems under investigation was found to be between 39 and 89 days.

At the end of the restart investigation, after a period of stabilisation, samples of bed were removed from two of the reactors along the length of each column and analysed to provide profiles of the bacterial activity through the length of the beds at steady state. The sand support medium utilised is theoretically completely mixed but the 10 to 30% expansions typical of such reactors and the ranges of particle sizes found may produce a situation whereby biomass distribution and activity are non-uniform. Determinations were thus made of the concentration and activity of both the suspended and the attached biomass within the steady state system.

The amount of COD removed was essentially the same in all portions of the bed, a situation which may be ascribed to the high recycle ratio inherent in the fluidised bed system. Total volatile acids concentration, however, was greatest in the central and upper regions of both beds, although Bull et al. (1984) found, at a similar loading, that acids concentrations in fluidised beds changed little up the reactor column. The localised acid production may have been the result of the concentrations of acetogens immobilised in the upper bed sections, and indicates that the sand media are not subject to random distribution or movement throughout the extent of the bed. Suspended

solids concentrations were lower at the bottom and top regions, possibly as a result of the flow patterns of the inlet (base) and solid/liquid interface (top) regions of the bed. Volatile attached solids were markedly more concentrated at the top of both fluidised beds, although remaining relatively constant in the lower and central sections. Rockey and Forster (1985) indicated that the greatest biofilm thickness developed at the top of an expanded bed reactor. Bull et al. (1984) however, found that volatile solids concentration increased up the height of a fluidised bed, but differences in carbon source and loading may have been responsible for these observations.

The proportions of methane in the biogas produced by samples removed from the length of the reactor beds indicated that the central and upper portions of the two units were capable of producing offgases comprising a higher percentage of methane. This indicated either that the methanogenic bacteria of the population were present in greater numbers at the top of the reactor bed, or that they were more active in this part of the system. Total volatile acids concentration increased up the length of the bed, reaching maximum in the top third section. The acidogens were therefore also more active in the upper portions of the bed, or were present in greater numbers, and the acids they produced, serving as substrates for the methanogens, induced a similar increase in methanogenesis in the upper reactor regions. In addition, as suspended solids concentrations were greatest in the central region of the fluidised bed and volatile attached solids concentrations highest at the top of the bed, it

appears that the attached biomass is more active than the suspended, or unattached, portion, and has a more important role in COD reduction and acids and methane production.

Organic wastes with high oxygen demand are generated by a large number of industries. Many wastestreams, however, especially those from chemical or related manufacturing operations, can contain varying levels of potentially toxic materials which may inhibit the anaerobic digestion process. In some instances the effects of introducing such a stream into a biological system of unacclimated microorganisms may be highly deleterious, even if the waste is known to be biodegradable. With acclimation, the toxic effects may be greatly reduced or eliminated (Parkin & Speece 1983).

The occurrence of toxic waste compounds is a common feature of the pharmaceutical industry and many hazardous aqueous streams produced must be removed in drums for off-site disposal. The processing of organic pharmaceutical wastes using biological systems may prove to be an effective and economic alternative, if microbial populations can be successfully acclimated to particular effluents. Pharmaceutical wastes have been successfully treated using the anaerobic filter reactor (Sachs et al. 1978; 1982) and acclimation to particular compounds has been demonstrated (Lin Chou et al. 1979).

Two pharmaceutical wastes were applied to fluidised beds. The principal constituent of the first waste was n-propanol and the second contained 30% dimethylformamide (DMF), a potentially carcinogenic, embryotoxic and

hepatotoxic solvent (Fishbein 1981). The levels of COD removal in the reactor treating the n-propanol-containing waste remained relatively constant over the acclimation period, until the system experienced an influent of 100% pharmaceutical waste. In an acclimation investigation by Lin Chou et al. (1979), completely mixed systems were used to study the utilisation efficiency of propanol and other petrochemical components. The propanol was utilised with an efficiency of 41% in the completely mixed system, which had a 20d HRT and which received the daily addition of an inorganic salt solution; the fluidised bed in the present study exhibited 50% COD removal, although the pharmaceutical waste was not wholly composed of n-propanol. The cause of the reduction in COD removal efficiency observed in the current investigation may have been partly related to the removal of essential trace elements by the withdrawal of the meat waste portion of the feed: trace elements are necessary for anaerobic digestion. However, the propanol-containing wastewater also contained sodium chloride, although in the diluted waste used the sodium ion was present at a concentration of less than the 4000mg l^{-1} considered to be toxic by Kugelman and McCarty (1965). A change in microbial population rather than a gradual die-off of biomass is the most likely explanation as the sudden decrease in COD removal was accompanied by a large TVA concentration increase although effluent suspended solids remained unchanged. The slight drop in methane production in this reactor at the end of the acclimation period also suggested population imbalance in the system as the increase in TVA accumulation at 100% of the propanol-

containing waste indicated that the acetogens were active but that the acids they produced were not being removed and converted to methane at the levels previously observed.

The gas production rates were low; the anaerobic filters of Seeler and Jennett (1978), acclimating to pharmaceutical waste but using sodium acetate as the main substrate, also exhibited low gas production rates. These were attributed to the low growth rates of the microbial population on acetate and the substrate was accordingly switched to methanol. However, as the steady state COD removal in this study was excellent the synthetic meat waste in use was retained. Low gas production is commonly found in anaerobic systems under stressed conditions.

The fluidised bed acclimating to the DMF-containing pharmaceutical waste exhibited behaviour different to the above, with COD removal decreasing gradually throughout the acclimation period. A part of the wastestream was therefore not anaerobically biodegradable under existing operating conditions and may have been exerting increasing toxic effects. Dimethylformamide has been reported to be biodegradable in completely mixed activated sludge systems (Carter & Young 1984) but acclimation was necessary; some ammonia was produced during microbial metabolism and some nitrogen was incorporated into cellular material. The fate of DMF under the anaerobic conditions prevalent in the fluidised bed was not investigated in this study. However, some Methanosarcinaceae are known to use the related compound dimethylamine (DMA) as a sole substrate (Balch et al. 1979) and as methane generation in the system exceeded that of the controls, the methanogens in the system

were active despite rather low COD removals.

The behaviour of the fluidised beds indicated that both high strength pharmaceutical wastes were partially amenable to anaerobic digestion and could be treated after acclimation at concentrations of at least 2500mg l^{-1} without the addition of bacterial supplements such as nitrogen, phosphorus and trace elements. The short HRTs of the reactors, coupled with the dilution effect of high recycle, ensures high rate treatment with short exposure to most potentially damaging portions of the influent. Sachs et al. (1978), also treating a pharmaceutical waste by anaerobic reactor (filter), obtained 70 to 80% COD removals at a waste concentration of 2000mg l^{-1} and an HRT of 1.5d, but these workers added nitrogen and phosphorus to support anaerobic growth. Such additions may have aided the metabolism of the propanol-based waste as COD removal efficiency dropped only at the 100% waste input stage, indicating a limitation of nutrients that had been previously supplied by the meat waste and trace element solution. The DMF-based waste was unlikely to have benefited from a similar supplementation, as COD removal efficiency decreased from the outset of the experiment and continued to decrease as the proportion of this waste in the feed increased. The limitation to degradation in this case was probably a recalcitrant material present as part of the waste, but a toxic effect may have been exerted on a particular section of the microbial population within the reactor.

It was noted that the DMF-containing waste, which in its original form was of a strong yellow-brown colour,

was rendered colourless by anaerobic fluidised bed treatment (section 4.08). It has been shown that almost no degradation of the organic colourants anthraquinone and certain azo dyes occurs in activated sludge, whereas under anaerobic conditions the azo dyes are reduced to colourless constituents believed to be the corresponding amines (Meyer 1981). These degradations were reported to have been mediated by various microorganisms possessing relatively non-specific enzymes. A similar effect may also have occurred in the anaerobic systems utilised in the current study. The results obtained indicate the potential of anaerobic systems in the treatment of highly coloured effluents, such as those from textile finishing.

The difficulties apparent in the degradation and removal of many recalcitrant materials in bacterial systems may have several bases. The excessive size of some molecules, many of which contain condensed aromatic ring structures, require the combined activities of a variety of microbial species for breakdown into substituents suitable for microbial assimilation. Furthermore, the form of substituent groupings and the existence of unusual linkages and bondings are properties which tend to make some organic molecules less than amenable as microbial substrates (Lin Chou et al. 1979), as novel enzyme systems may be necessary to attack these successfully. Organic molecules such as solvents and phenolic compounds are, in addition, inhibitory in many cases to bacterial systems and enzymes (Lin Chou et al. 1979; Harper et al. 1984).

Adaptation to a toxicant or recalcitrant is an important consideration in microbial systems; the reactor

bacteria possessing enzymes that are capable of the catabolism, partial or complete, of an alien molecule, may be slow growing or extremely sensitive and appear later in the digestion process. Anaerobic cultures have been reported to recover from shock doses of toxicants after several months have elapsed (Parkin & Speece 1982). Under conditions of the strictest anaerobiosis, the biodegradability of eleven aromatic derivatives of the complex polymer lignin was demonstrated (Healy & Young 1978). Acclimation procedures carried out using a heterogeneous microbial population and a particular aromatic substrate resulted in bacteria simultaneously acclimating to other aromatic substrates possessing similar substituent group arrangements on the aromatic ring.

The above observation is an indication of the remarkable versatility of microbial enzyme systems, which enables the organisms possessing them to deal with naturally occurring complex molecules. The acclimation characteristics and degradation rates of various petrochemical compounds metabolised by microbial cultures were found to be affected by the structure and position of functional groups (Lin Chou et al. 1979). The list of petrochemicals catabolised by bacteria after acclimation procedures had been completed included acetaldehyde, catechol, vinyl acetate, nitrobenzene and phthalic acid. Those compounds with ester, hydroxyl and carboxyl groups acclimated more rapidly than those possessing carbonyl, amino or chloro groups and in addition exhibited more rapid degradation rates; 2-propanol was found to have a greater utilisation efficiency than propanol.

The microbial degradation pathways of recalcitrant organics such as the ligno-aromatic compounds have been analysed and decomposition models outlined (Evans 1977; Healy et al. 1980; Verstraete et al. 1981). Investigations have been by no means exhaustive and although lignin itself must be depolymerised or chemically modified prior to anoxic degradation (Zeikus et al. 1982), a diversity of these compounds are probably amenable to anaerobic breakdown to methane and carbon dioxide.

One of the few significant drawbacks that is frequently found in the application of anaerobic treatment to wastewaters is the difficulty of start-up procedures, which can be time-consuming and unproductive. The major problem often concerns the establishment of the most suitable microbial population for the waste to be treated, especially where the waste is a complex and recalcitrant industrial stream. Once the biomass is established, reactor operation is generally quite stable (Salkinoja-Salonen et al. 1983).

Anaerobic bacteria exhibit growth rates that are much slower than those of aerobes (Verstraete et al. 1981); adequate seeding of the anaerobic reactor at start-up is thus more critical than is the case for aerobic systems. In fixed film reactors, a quantity of seed that is at least 10% of the reactor volume has been recommended (Salkinoja-Salonen et al. 1983), otherwise start-up may fail or build-up to steady state may be prolonged. A seed inoculum of 30-50% has been claimed to reduce the start-up time

required in anaerobic systems to a considerable extent. However, in the present study a small inoculum volume was employed to start up fluidised beds as excessive amounts of seed have caused serious problems in some high rate processes (Brunetti et al. 1983). In addition, small inocula have been successfully employed for the start-up of other fluidised bed systems (Bull et al. 1982; Stephenson & Lester 1986). The quantity of seed utilised in the anaerobic filters employed comprised 10% of the reactor volume, although 30% was recommended by Wilkie and Colleran (1984) for filter systems. A 10% pre-granulated seed was used in the UASB reactors, although higher inocula are generally more effective (Salkinoja-Salonen et al. 1983).

The pharmaceutical wastes treated in the three reactor configurations contained utilisable COD, but ammonia and phosphorus concentrations were low and trace nutrients were not supplied. As was observed in the previous acclimation studies, the dimethylformamide (DMF)- containing waste was more readily-tolerated than the n-propanol-containing effluent (section 4.09). The levels of pH in the reactor units also followed patterns similar to those recorded in the acclimation investigation, those systems treating the propanol-based waste exhibiting low pH values. Young (1980) has observed that anaerobic filters are most sensitive to pH changes during the start-up period and that a decrease in pH to 6.5 or less can increase the time required for start-up. Those reactors starting-up on the propanol effluent achieved both lower final organic loading rates and lower COD removal efficiencies than their counterparts treating the DMF-based waste, confirming an

interrelationship between low pH and low operational efficiency.

In fixed film systems such as the anaerobic filter, initial biofilm attachment to carrier media is the primary and perhaps the most difficult aspect of start-up; pH shocks in particular can alter the charge balance of the system, thus interfering with adhesive and cohesive binding forces (Schwartz et al. 1981). The TVA levels observed in the anaerobic filters in this study were comparable with those reported by Seeler and Jennett (1978) during start-up, although the latter introduced pharmaceutical waste into their anaerobic filter gradually, with sodium acetate and then methanol as the initial substrates. Effluent suspended solids concentrations in the reactors used in the current study were also in agreement with those values reported in other investigations using pharmaceutical wastes (Sachs et al. 1978; 1982; Seeler & Jennett 1978).

In the UASB systems, COD removal efficiencies were originally high, a factor which can be attributed to the active granulated biomass used as seed (Lettinga et al. 1979a; 1979b; 1981). Total volatile acids levels were similar to those in both the filters and the fluidised beds, although Schwartz et al. (1981) reported that sludge blanket reactors generally exhibit lower TVA concentrations than anaerobic filters. However, effluent suspended solids were initially almost four times as great in the UASB reactors as in the other systems. The start-up of non-attached biomass processes and those systems which rely on suspended as well as attached microflora is prone to washout of biomass during the initial phase of the

procedure (Heijnen 1983). Experiments with the UASB reactor in particular reveal significant losses of active microbial mass during this stage; the amount of seed sludge retained in the reactor vessel is small but of superior settleability. Recycle of effluent solids is not considered advantageous as washout of seed sludge returned to the UASB by recycle has been noted to recur within a few days (Hulsoff-Pol et al. 1983). The specific activity of the sludge removed from the reactor under such circumstances has been found to be similar to that of the sludge retained, i.e. a portion of the net microbial growth occurring during the initial phases of start-up is also removed with the effluent.

The COD removal efficiencies of the fluidised bed reactors proved to be superior to the UASB and filter systems at the final organic loading rates of pharmaceutical wastes. However, methane production rates in the fluidised beds were lower, indicating that the methanogenic populations in these systems were less active despite the ameliorating influences of high recycle. The low seed inoculum used at the initiation of start-up may have contributed to the low gas production obtained: methanogenic bacteria have relatively long generation times of 0.2-0.5 days (Verstraete et al. 1981) and consequently give a poor sludge yield. Four to eight months may be required for the attainment of a microbial steady state in suspended biomass systems, which are more subject to biomass washout than their fixed film counterparts. In a thermophilic operation, with an even smaller sludge yield, considerably longer start-up periods - in some cases

exceeding a year - may be necessary (Henze & Harremoes 1983). Using a thermophilic expanded bed, however, steady state conditions after 5 months of operation were reported by Schraa and Jewell (1984) and less than 3 months were necessary to reach steady state in the anaerobic mesophilic fluidised bed system of Rudd et al. (1985). In contrast, the conventional digester utilised by Rimkus and others (1982) evinced erratic VFA levels for the first 18 months of operation.

An appraisal of the start-up of anaerobic fluidised beds was made by Bull et al. (1983c) using four regimes: two of the reactors were loaded continuously and two were step-loaded over a period of 40 days; methanol replaced 50% of the COD load in one continuously-loaded and one step-loaded system. In all the units, COD reductions in excess of 90% were attained at loadings of 4.8kg COD m^{-3} d^{-1} within 40 days. The most efficient start-up regime was considered to be the stepped-loading process with initial addition of methanol as a substrate to enhance the growth of methanogenic bacteria. The absence of operational difficulties using this system indicated that much greater COD loadings can be applied to fluidised beds with resultant rapid start-up and continued reactor efficiency if the substrate and operating conditions are suitable. The slow and inefficient start-up of the three reactor configurations and the variability of the final COD removal efficiencies obtained for the propanol-based and the DMF-containing effluents used here were probably due to the natures of the two pharmaceutical wastes. The utilisation of propanol under anaerobic conditions has been

documented (Lin Chou et al. 1979) but full degradation did not occur. The conversion of the propanol component of the feed to propionic acid seems likely and propionate has often been implicated as a major cause of digester failure (McCarty & Brosseau 1963; Hobson & Shaw 1976). In addition, low pH values are detrimental to the degradation of the common volatile fatty acids (Andrews 1969).

Start-up is an extremely sensitive period in anaerobic reactor operation and although this was achieved using recalcitrant pharmaceutical wastes without benefit of nutrient or trace element addition, improved performances would probably have been achieved by start-up on an easily-assimilable waste, followed by the gradual introduction of the recalcitrant effluent. Nel et al. (1984) achieved COD reductions of 83% during the start-up of a UASB system on a petrochemical wastewater, although short HRTs (1.48d) were finally detrimental to the reactor. The anaerobic sludge used as seed was mixed with an anaerobic enrichment agar medium, which appeared to enhance the formation of characteristic UASB pellets. An anaerobic filter was started up on a glucose solution, which was progressively replaced by textile desizing and scouring wastewater (Athanasopoulos 1986). Four months were required for full operation, although COD removals fluctuated in the range 60-90% as the influent composition tended to vary. The top loading achieved was 2.75kg COD m^{-3} d^{-1}, results similar to those achieved in the present study, although the reported biogas yield comprised 70-80% methane.

The propanol-containing pharmaceutical waste was utilised, with a synthetic glucose waste, a soft drinks

manufacturing waste and a fruit processing effluent to start up four 2l anaerobic fluidised bed rectors. Modifications of the stepped loading regime adopted in the study have been used successfully in other anaerobic start-up operations (Bull et al. 1983c; Rudd et al. 1985; Stephenson & Lester 1986).

The regime employed encouraged rapid start-up in the fluidised bed fed on the glucose substrate and the pharmaceutical waste, both systems exhibiting evidence of methanogenesis towards the end of the start-up period. The consistent COD removal combined with declining TVA levels in the reactor treating fruit processing effluent also indicated that methanogenesis was probably occurring, although the performance of this bed was not as efficient as the other units in terms of substrate removal.

The performances of the four fluidised beds at the end of the experimental period is compared in Table 34 with published data for anaerobic systems treating similar wastes. The results obtained here compare favourably with those of other investigations, although the mean COD removal efficiencies for both the fruit processing and the soft drinks manufacturing effluents were slightly lower than were expected. Both factories investigated in the current study produce wastes that are high in sugar content and hence should be easily degradable. However, the soft drinks manufacturing effluent also contained added citric or malic acids to maintain the sugar/acid balance in the product, other flavourings and sodium metabisulphate as preservative. Additionally, biocidal cleaning agents based on quaternary ammonium compounds or chlorine-based

TABLE 34 COMPARISON OF ANAEROBIC REACTOR SYSTEMS TREATING INDUSTRIAL WASTEWATERS

REACTOR TYPE	WASTE TYPE	ORGANIC LOADING RATE ($kgCODm^{-3}d^{-1}$)	% COD REMOVAL	REFERENCE
Anaerobic fluidised bed	Food processing	3 - 24	75 - 86	Jeris (1983)
	Chemical	4 - 27	79 - 93	Jeris (1983)
	Soft drinks	4 - 19	68 - 90	Jeris (1983)
	Fruit processing	7.5	45	current study
	Soft drinks	7.5	63	current study
	Pharmaceutical	7.5	84	current study
	Glucose	7.5	65	current study
Anaerobic filter	Pharmaceutical	0.4 - 3.5	94 - 98	Jennett & Dennis (1975)
	Fruit processing	6.4	58	van den Berg & Kennedy (1982
UASB	Fruit peel	11.2	95	Lane (1984)
Contact reactor	Fruit peel	3.4	87	Lane (1983)

compounds were used in the plant after each production run and formed part of the effluent. Chlorine is often used as a disinfectant in the production line at bottling plants. The inhibitory effects of chlorine in conjunction with several soft drinks additives has been reported by Witkowski and Jeris (1984). In addition, Hickey and Owens (1981) found that the degradation rates of soft drink bottling effluent were slow, despite the high content of refined sugars in the waste. The presence of the bacterial inhibitors in the soft drink product itself were held to be responsible for this effect (Blanc et al. 1978).

The fruit processing plant also used sodium metabisulphate as preservative and cleaning compounds appeared in plant wastewater. In addition, sulphate and sulphide were present in the molasses and pectin used in the production processes. The sulphates and other oxidised compounds of sulphur are easily reduced to sulphide under the conditions prevalent in anaerobic digesters (Henze & Harremoes 1983). Methionine and cysteine, the sulphur-containing amino acids of protein, can also undergo degradation to sulphide. These compounds are significant when anaerobic treatment is considered for industrial processes which tend to produce large quantities of sulphides in their wastestreams: one of the major problems in treating high sulphate waters is the competition between sulphate-reducing and methanogenic bacteria (Rinzema et al. 1986). Desulfovibrio and other sulphate-reducing genera form sulphides from sulphates and some of the fermentative microorganisms use sulphur-containing amino acids to produce sulphides. In completely mixed reactors and

anaerobic filters, sulphide concentrations of 1500mg l^{-1} proved to be toxic to the processes but the effects were reported to be partially reversible by return to normal operating conditions and complete eventual recovery was observed (Parkin & Speece 1983).

The toxicity of sulphide is closely related to the free H_2S concentration and at a pH value of less than 6.5, toxicity is increased (Henze & Harremoes 1983). However, Saerner (1986) has proposed several measures for use at pilot scale levels for control of H_2S in anaerobic systems. These include sulphide precipitation by heavy metals, gas scrubbing in buffered water to produce elemental sulphur, and pH control. The latter is achieved by alkali addition to bring digester pH within the range 7.5-8.0: the levels of pH and sulphide ion (HS^-) increase and the H_2S content of the system falls.

High effluent suspended solids in the fluidised bed fed with synthetic glucose-based waste probably resulted from excessive biomass growth on the easily-degradable substrate, followed by sloughing and washout of biofilm due to shear forces within the bed. Alternatively, high solids concentrations may have been caused by the HRT reduction at successive stages during the start-up of this system. The COD removal efficiency of the reactor was much greater initially than those of the other units, a factor tending to support the efficacy of extended HRTs during the initial phases to allow time for the development of suitable bacterial populations. The high initial COD removal efficiencies in the glucose-fed reactor when compared to the other three systems indicated that the reactor bacteria

were readily acclimating to the the feed.

High strength, soluble, biodegradable substrates such as glucose, sucrose and carbohydrate wastes are readily removed in anaerobic bioreactors. A full scale anaerobic fluidised bed fed on a synthetic glucose waste achieved COD removal efficiencies in excess of 90% (Chen et al. 1985) at a loading of 35kg COD m^{-3} d^{-1} after 120 days. A UASB system treating sucrose waste reached a similar removal efficiency within 69 days at an organic loading of 12.8kg COD m^{-3} d^{-1} (Mendez Pampin et al. 1986) although the granular sludge produced in the reactor deteriorated and filamentous biomass resulted. The soluble carbohydrate (fructose) COD applied to an anaerobic filter at a loading of 7.5kg COD m^{-3} d^{-1} was removed at an efficiency of 85-90% although effluent solids concentrations remained within the range 50 to 200mg l^{-1}, unlike the solids levels obtained in the fluidised bed reported here. However, recycle was not practised in the filter and shear forces must consequently have been of less importance in that system.

The most notable differences between the performances of the four beds in this part of the study and the fluidised beds, anaerobic filters and upflow anaerobic sludge blankets discussed above were the levels of TVA produced. The high concentrations of volatile acids found in the smaller fluidised beds were to some extent reflected by the reduced buffering capacities achieved in these systems and were also likely to have been influenced by the greater organic loadings applied. Analyses of individual volatile acids were carried out for the 21 reactors and each system exhibited degrees of variation.

The accumulation of volatile acids usually occurs during digestion at times of shock loading or at periods of reactor instability (Kennedy & van den Berg 1982) and the start-up of anaerobic systems is generally considered to be a highly unstable phase. The fluidised beds utilising fruit waste and pharmaceutical waste exhibited the greatest concentrations of propionic acid during start-up. In the former, propionate levels exceeded those of acetate during the last phase of the experiment, after the final loading increase. It has been reported that during elevated acids production due to organic loading increases, propionate levels tend to remain high for a long period, whereas acetate levels fall off more quickly (Barnes et al. 1983). Propionate levels increased with COD loading throughout start-up in the pharmaceutical waste-fed system, probably as a result of increasing levels of the n-propanol component of the feed. The anaerobe Selenomonas ruminantium **var.** lactylitica has been shown to produce propionate as the major fermentation product (Mulder et al. 1986). However, propionate concentrations did not decrease substantially at the end of start-up, but only a limited number of microorganisms are reported to mediate the further breakdown of this acid under anaerobic conditions (Widdell & Pfennig 1982).

The reactor treating soft drinks effluent produced much lower concentrations of TVA compared to the other systems. This may be accounted for, as has already been noted, by the presence of bacterial inhibitors in the wastewater, which can affect the rate of acid production by the reactor microflora (Blanc et al. 1978). In some cases,

the acetogenic bacteria have been found to be more sensitive than the methanogens to inhibition and thus may be the rate-limiting organisms in anaerobic digestion (McCarty & Brosseau 1963). The lower overall levels of acids produced were likely to be the result of the low nutrient quality of this effluent, which would tend to produce bacterial flora of limited degradative ability.

Anaerobic reactor instability is generally manifested by a marked and rapid increase in VFA concentrations; this often indicates the failure of the methanogenic population due to other environmental disruptions such as shock loadings, nutrient depletion or infiltration by inhibitory substances. Acetate has been described as the least toxic of the volatile acids (Ianotti & Fischer 1984), whilst propionate has often been implicated as the main effector of digester failure (McCarty & Brosseau 1963; Hobson & Shaw 1976). Propionate was found to be more inhibitory than butyrate for Bacteroides but the reverse applied in the case of Peptostreptococcus (Ianotti & Fischer 1984). Methanogens were demonstrated to be inhibited at propionate concentrations in excess of 3000mg l^{-1}, although this effect could be overcome by acclimation (McCarty & Brosseau 1963). The methanogen Methanobacterium formicicum was reported to tolerate both acetate and butyrate at concentrations of up to 10000mg l^{-1} (Hobson & Shaw 1976) although variable inhibitory levels for propionate of 1000mg l^{-1} (Ianotti & Fischer 1984) and 5000mg l^{-1} (Hobson & Shaw 1976) have been recorded.

In any system that is overloaded and hence contains high concentrations of VFAs, the digestion process may be

inhibited by the VFAs themselves and also by any alcohols remaining undegraded. A low pH is more detrimental to VFA degradation than it is to methane formation from methanol as the latter does not dissociate within the pH range 3.0-8.0; according to Andrews (1969), conversion rate inhibition by the volatile acids at acidic pH values can be attributed to the existence of unionised VFAs in significant quantities in the system. These unionised acids are present in amounts dependent upon the total concentration of VFAs in solution. The undissociated nature of these acids allow them to penetrate the bacterial cell membrane more efficiently than their ionised counterparts, and once assimilated, induce an intracellular decrease in pH and hence a decrease in microbial metabolic rate.

A pH-dependent equilibrium exists between the ionised and unionised components of the volatile acids:

$$CH_3COOH \longleftrightarrow CH_3COO^- + H^+ \qquad (10)$$

As the pH value drops, equilibrium shifts to the left, resulting in an increase in the concentration of unionised volatile acids (UVA). A value of 1.73×10^{-5} has been given for the ionisation constant at $35^{o}C$. Digester failure becomes increasingly more likely as the concentration of UVA rises above 10mg l^{-1} (Kroeker et al. 1979). These results are indicative of a more direct functional relationship between the concentrations of UVAs and the level of toxicity (defined as a total cessation of metabolic activity) within the digester system.

The type and concentration of TVAs produced during the current study appeared to be directly influenced by the

substrate applied to the systems. Sugar-rich wastes such as glucose and fruit processing are easily assimilated by microorganisms and give rise to products such as acetate. More recalcitrant wastes such as propanol-containing pharmaceutical effluent appear to select for microbial populations capable of specific conversions. Other components of reactor substrates may produce specific breakdown products and alterations in influent composition may trigger changes in bacterial populations or reactions.

The reactions of the anaerobic reactors utilised in the preceding two investigations were variable, reflecting both the variable natures of the influents and the reactor configurations. The anaerobic filter reactors exhibited slightly superior COD removing abilities to the UASB systems, although the latter could be started up more rapidly; the fluidised beds were superior to both systems in COD removal, a situation probably resulting from the recycle mode of operation in these reactors. In addition, pH was more controllable in the 4l fluidised beds than in the other systems. Methane production was greatest in the UASB reactors throughout the start-up period, as the seed used was already granulated and had therefore probably formed the symbiotic consortia necessary for the complete conversion of substrate to product.

Four pilot scale high rate anaerobic reactors, including the fluidised bed, upflow anaerobic filter and UASB configurations, were evaluated by Jovanovic et al. (1986). The reactors were operated under pseudo-steady state conditions using brewery effluent. Varying influent

concentrations and HRTs were employed. These workers found that no significant differences in solids and methane yield coefficients existed, although COD removal efficiency was superior in the fluidised bed. In addition, the upflow filter achieved a greater degree of COD removal than the UASB, results in agreement with those of the present study. It was demonstrated that COD removal was predominantly affected by organic loading in the pilot systems, although the anaerobic filter and the UASB were subject to a smaller effect of retention time. Retention time is an important consideration in anaerobic systems, especially where no recycle facility exists. The fluidised bed system depends upon a high degree of recycle for operation, but this constraint does not necessarily apply to the anaerobic filter and UASB configurations. Recycle was not employed in the latter reactor types in the present investigation although the HRTs of these systems were the same as those of the fluidised beds. However, the high rate bioreactors all employ the principle of high biomass concentrations and hence extended SRTs, to alleviate the necessity for the long HRTs that constituted the major drawback of older anaerobic systems.

The operating performances of various anaerobic systems treating synthetic and industrial wastewaters are described in Table 35. High rate bioreactors have been shown to be superior in most respects to those systems requiring both extended solids and hydraulic retention times: high rate systems operate at short HRTs and can tolerate high organic loadings. In general, synthetic wastes are more amenable to degradation as their

TABLE 35 OPERATING PARAMETERS AND PERFORMANCES OF ANAEROBIC REACTORS TREATING VARIOUS WASTES

REACTOR TYPE	LOADING RATE ($kgCODm^{-3}d^{-1}$)	HRT (d)	WASTE TYPE	% COD REDUCTION	TEMP. ^{o}C	REFERENCE
Anaerobic	6.7	2.5	Sewage sludge	65	35	Schwartz et al (1981)
Filter	38	0.5	Sewage sludge	50	35	Schwartz et al (1981)
	17.2	1.0	Sewage sludge	60	50	Schwartz et. al (1981)
	0.5	3.0	Alcohol stillage	84	30	Dahab & Young (1981)
	2	1.5	Alcohol stillage	74	30	Dahab & Young (1981)
	9.3	11.8	Pear peeling	64	35	van den Berg et al (1981)
Expanded						
Bed	30	0.17	Sucrose	80	55	Schraa & Jewell (1984)
	16	0.19	Sucrose	48	55	Schraa & Jewell (1984)
	2.4	0.21	Glucose/Yeast extract	90	22	Jewell et al (1981)
	24	0.02	Glucose/Yeast extract	45	10	Jewell et al (1981)
UASB	5	4.0	Sewage sludge	68	35	Schwartz et al (1981)
	18	1.0	Sewage sludge	60	35	Schwartz et al (1981)
	5	4.0	Sewage sludge	55	50	Schwartz et al (1981)
	11.2	7.0	Citrus peel press liquor	95	37	Lane (1983)

CONT'D.

REACTOR TYPE	LOADING RATE ($kgCODm^{-3}d^{-1}$)	HRT	WASTE TYPE	% COD REDUCTION	TEMP. ^{o}C	REFERENCE
Fluidised	3.5	2.06	Food processing	86	36	Jeris (1983)
Bed	24.1	0.31	Food processing	75	36	Jeris (1983)
	13.4	4.9	Whole whey	84	35	Hickey & Owens (1981)
	37.6	4.9	Whole whey	72	35	Hickey & Owens (1981)
	15	3.5	Whole whey	71	24	Hickey & Owens (1981)
	36.8	1.5	Whole whey	65	24	Hickey & Owens (1981)
	4.8	0.53	Synthetic meat	71	37	Stephenson & Lester (1986)
	9.5	0.53	Synthetic meat	76	37	Stephenson & Lester (1986)
	6	1.67	Glucose	90	25	Bull et al (1984)
	12	1.67	Glucose	65	25	Bull et al (1984)
	18	1.67	Glucose	7.5	25	Bull et al (1984)
Contact	3.4	-	Fruit	87	36	Lane (1984)
Reactor	10	5.5	Rum stillage	80	35	van den Berg et al (1981)
CSTR	2.8	26.7	Citrus peel press liquor	80	37	Lane (1983)
	1.2	8.5	Bean blanching	70	35	van den Berg et al (1981)
	18.5	0.14	Synthetic meat	18	37	Stephenson & Lester (1986)
	9.2	0.13	Synthetic meat	12	37	Stephenson & Lester (1986)
RBC	0.0195*	0.73	Synthetic	96	35	Tait & Friedman (1980)
CASBER	3.9	2.4	Synthetic molasses	90	35	Martensson & Frostell (1983)

*as $kg\ m^{-2}d^{-1}$

characteristics can be controlled. Industrial effluents can contain numerous recalcitrant and/or toxic moieties, the control of which is either limited or non-existent and to which anaerobic systems must acclimate, with or without the aid of nutrients or other additions.

The view that anaerobic systems possess an inherent disadvantage in their intolerance of the long-term and transient toxicities of many industrial wastewaters has frequently limited the introduction of anaerobic treatment processes. The low cell yield coefficient of anaerobic microorganisms may be disadvantageous to recovery from or acclimation to wastestream toxicants in conventional digester operations, but the protracted SRTs attainable in the high rate reactors gives these systems an intrinsic advantage over traditional suspended biomass reactors.

Detailed appraisals of those systems already in existence have provided some of the data necessary for a fuller understanding of the potentials of anaerobic wastewater treatment in high rate bioreactors. Much work remains to be done, especially with regard to fundamental aspects of anaerobic microbiology and optimum design and operation of reactor systems. As the technology evolves, more design configurations of varying complexity will be produced to increase the practical biological conversion operations already in existence. New applications of anaerobic biotreatment will present novel problems which must be dealt with within the spheres of economics, microbiology and engineering.

CHAPTER 6
CONCLUSIONS

1. The addition of natural and synthetic polymers to improve the association of biomass with sand particles was found to be effective in jar tests. The synthetic polymer C_{50} was superior to the polymer C_{30}. The biopolymer produced by Klebsiella aerogenes also improved bacterial attachment.
2. Start-up is an extremely sensitive phase in the operation of anaerobic biological systems. However, rapid recovery from high degrees of influent variation indicated that a stepped increase loading regime aided rapid start-up, although influent equalisation prior to feeding was considered to be desirable.
3. The provision of completely anaerobic conditions from day one encouraged efficient start-up, assisted the development of a suitable microbial population and ameliorated the effects of influent variation in anaerobic fluidised beds.
4. The addition of natural polymer and polymer-producing microorganisms was potentially advantageous to the biomass retention and process performance of anaerobic fluidised beds.
5. Bacterial attachment was promoted by the addition of synthetic flocculant polymer but the evolution of a stable and well-adapted microbial population may have been inhibited.
6. Reactor performances under increasing rates of fluidisation indicated stability within a range of moderate expansions. An expansion of 5% caused bed

disruption. The optimum expansion for COD removal efficiency was 15%, whereas gas production was optimal at 30% fluidisation.

7. The increase of trace element concentrations in anaerobic fluidised beds did not affect pH or COD removal ability. Total volatile acids levels decreased and methane production increased, indicating that either the conversion of acids to methane had improved or that a population shift favouring the domination of methanogenic flora had occurred.

8. The performance of anaerobic fluidised beds upon restart depended upon the period of downtime undergone. Full activity could be resumed within one day if downtime was less than a critical length of time for the system: in the case of the systems examined, the critical period was between 39 and 89 days.

9. Extended downtime resulted in the loss of the methanogenic activity of the biomass, although acidogenic activity was retained; a population shift may have occurred.

10. Bacterial activity profiles at steady state indicated that methanogenic and acidogenic activities were greatest in the upper third sections of the reactor beds. Attached biomass appeared to be more active than suspended biomass.

11. Biodegradation tests carried out on two pharmaceutical wastes, one containing n-propanol and the other dimethylformamide as the major constituents, indicated that both wastes were degradable anaerobically.

12. Anaerobic fluidised beds could be successfully acclimated to two pharmaceutical wastes, one containing propanol (A) and the other dimethylformamide (B) as principal constituents, at organic loadings of 4.5kg COD m^{-3} d^{-1} and HRTs of 0.53d. Final COD removal efficiencies of 54% and 45% for wastes A and B respectively were achieved without nutrient or trace element supplementation.
13. At inputs of 100% of n-propanol-based waste of 2500mg COD l^{-1}, biomass was not lost but the acids produced in the system increased and methane production decreased, probably as a result of depressed methanogenic activity.
14. The dimethylformamide-containing waste employed as substrate in anaerobic fluidised beds contained a fraction which was either non-biodegradable or toxic under the test conditions used, but the methane generated as a result of the degradation exceeded that of the controls, indicating that the substrate was amenable to methanogenic metabolism.
15. Anaerobic fluidised beds were found to be superior to both anaerobic filters and upflow anaerobic sludge blankets in COD removal efficiency and pH stability, although methane production was greater in the UASB system.
16. Recalcitrant wastestreams such as fruit processing, soft drinks manufacturing and pharmaceutical effluents could be used to start up high rate anaerobic bioreactors. Organic loadings of up to 7.5kg COD $m^{-3}d^{-1}$ could be successfully applied to

fluidised beds, although the bacterial inhibitors present as part of the fruit processing and soft drinks manufacturing streams reduced the full effectiveness of these systems. The pharmaceutical wastes utilised also contained substances inhibitory to breakdown, although the dimethylformamide-based waste was more amenable to conversion than the n-propanol-containing effluent. Acclimation of the latter was observed in a 2l fluidised bed and 78% COD removal could be achieved at a loading of 7.5kg COD m^{-3} d^{-1}.

17. The levels and types of volatile acids produced during anaerobic digestion were found to relate closely to the composition of the substrate applied to the system, with sugar-rich wastes giving rise to high effluent concentrations of acetate and propanol-containing wastes causing high effluent levels of propionic acid.

18. Start-up is a sensitive period in anaerobic reactor operation and although this was achieved using recalcitrant industrial wastes without benefit of nutrient or trace element addition, improved performances would likely have been attained by start-up on an easily-assimilable waste, followed by the gradual introduction of the recalcitrant effluent.

19. The anaerobic fluidised bed reactor, the anaerobic filter and the upflow anaerobic sludge blanket system have been shown to have great potential for the treatment of high strength soluble industrial wastewaters, including many recalcitrant streams.

However, due to the moderate performances achieved using certain industrial wastes, the use of these high rate treatment systems may have to be confined to pretreatment processes in some instances. With the variety of high rate reactor designs available and the amenability of such systems to modification, units may be replaced or upgraded as required to achieve increased stability, higher loading capacities and greater process efficiencies.

REFERENCES

ALBRACHT, S.P.J., GRAF, E.J. & THAUER, R.K. (1982) FEBS Lett. 140:311-313

AMERICAN PUBLIC HEALTH ASSOC. (1985) Standard Methods for the Examination of Water and Wastewater (15th ed.), Washington, D.C., U.S.A.

ANDERSON, G.K. & DONNELLY, T. (1978) Anaerobic contact digestion for treating high strength soluble wastes. In G. Mattock (ed.) New processes of wastewater treatment and recovery. Ellis Horwood, Chichester, p75-96

ANDERSON, G.K. & IBRAHIM, A.B. (1978) Prog. Water Technol. 10:237-253

ANDREESEN, J.R. & LJUNGDAL, L.G. (1973) J. Bacteriol. 116:867-873

ANDREESEN, J.R., EL GHAZZAWI, E. & GOTTSCHALK, G. (1974) Arch. Microbiol. 96:103-118

ANDREWS, G.F. (1982) Biotechnol. Bioeng. 24:2013-2030

ANDREWS, J.C. (1969) J. Sanit. Eng. Div. ASCE 95:95-116

ASH, S.G. (1979) Adhesion of microorganisms in fermentation processes. In: D.C. Ellwood, J. Melling & P. Rutter (eds.) Adhesion of microorganisms to surfaces. Academic Press, London, p57-86

ATHANASOPOULOS, N. (1986) Biotechnol. Lett. 8:377-378

BALCH, W.E, FOX, G.E., MAGRUM, L.J., WOESE, C.R. & WOLFE, R.S. (1979) Microbiol. Rev. 43:260-296

BARNES, D., BLISS, P.J., GRAUER, B., KUO, C.H. & ROBINS, K. (1983) Environ. Technol. Lett. 4:195-202

BLANC, F.C., O'SHAUGHNESSY, J.C & MILLER, C.H. (1978) Treatment of bottling plant wastewater with rotating biological contactors. In: Proc. 33rd Ind. Waste Conf., Purdue Univ., Lafayette, Indiana. Ann Arbor Science, Ann Arbor, Michigan, p614-623

BOCHEM, H.P., SCHOBERTH, S.M., SPREY, B. & WENGHER, P. (1982) Can. J. Microbiol. 28:500-510

BOENING, P.H. & LARSEN, V.F. (1982) Biotechnol. Bioeng. 24:2539-2556

BOONE D.R. & BRYANT, M.P. (1980) Appl. Environ. Microbiol. 40:626-632

BORCHARDT, J.T. (1971) Biological waste treatment using rotating discs. In: R.P. Canale (ed.) Biological waste treatment. Wiley-Interscience, New York.

BRAUN, R. & HUSS,S. (1982) Process Biochem. 17:25-27

BRITZ, T.J., BOTES, P.J. & MEYER, L.C. (1983) Biotechnol. Lett. 5:113-118

BROWN, M.J. & LESTER, J.N. (1979) Water Res. 13:817-837

BRUNETTI, A., BOARI, G., PASSINO, R. & ROZZI, A. (1983) Physico-chemical factors affecting starting-up UASB reactors. In: Proc. Eur. Symp. on Anaerobic Wastewater Treatment, Noordwijkerhaut, Netherlands, p317-334

BRYANT, M.P., CAMPBELL, L.C., REDDY, C. & CRABILL, M. (1972) Appl. Environ. Microbiol. 33:1162-1169

BULL, M.A., STERRITT, R.M. & LESTER, J.N. (1982) Trans. I. Chem. E. 60:373-376

BULL, M.A., STERRITT, R.M. & LESTER, J.N. (1983a) Water Res. 17:1563-1568

BULL, M.A., STERRITT, R.M. & LESTER, J.N. (1983b) J. Chem. Technol. Biotechnol. 33B:221-230

BULL, M.A., STERRITT, R.M. & LESTER, J.N. (1983c) Biotechnol. Lett. 5:333-338

BULL. M.A., STERRITT, R.M. & LESTER, J.N. (1984) Water Res. 18:1017-1020

CAMMACK, R., PATIL, D., AGUIRRE, R. & HATCHIKIAN, E.C. (1982) FEBS Lett. 142:289-292

CARTER, J.L. & YOUNG, D.A. (1984) Biodegradation of chemical plant wastewater containing dimethyl-formamide. In: Proc. 38th Ind. Waste Conf. Purdue Univ., Lafayette, Indiana. Ann Arbor Science, Ann Arbor, Michigan, p481-486

CHAKRABARTI, T. & JONES, P.H. (1983) Water Res. 17:931-936

CHARACKLIS, W.G. & COOKSEY, K.E. (1983) Adv. Appl. Microbiol. 29:93-138

CHEN, S.J., LI, C.T. & SHIEH, W.K. (1985) Performance evaluation of the anaerobic fluidised bed biofilm reactor: methane production from glucose. In: Proc. 40th Ind. Waste Conf. Purdue Univ., Lafayette, Indiana. Ann Arbor Science, Ann Arbor, Michigan, p925-936

CHIAN, E.S.K. & DE WALLE, F.B. (1977) Water Res. 11:295-304

CHIN, M. (1983) Appl. Environ. Microbiol. 45:1271-1276

CHRISTENSEN, D.R., GERICK, J.A. & EBLEN, J.E. (1984) J. Water Pollut. Control Fed. 56:1059-1062

COHEN, A., ZOETEMEYER, R.J., VAN DEURSEN, A. & VAN ANDEL, J.G. (1979) Water Res. 13:571-580

COHEN, A., BREURE, A.M., VAN ANDEL, J.G. & VAN DEURSEN, A. (1982) Water Res. 16:449-455

COONEY, C.L. (1981) Growth of microorganisms. In: H-J Rehm & G. Reed (eds.) Biotechnology, a comprehensive treatise in 8 volumes. Volume 1: Microbial fundamentals. Verlag-Chemie, Weinheim-Deerfield Beach, Florida-Basel, p73-112

COOPER, P.F. & WHEELDON, D.H.V. (1981) Complete treatment of sewage in a 2-fluidised bed system. In: P.F. Cooper & B. Atkinson (eds.) Biological fluidised bed treatment of water and wastewater. Ellis Horwood, Chichester, p121-144

COSTERTON, J.W., IRVIN, R.T. & CHENG, K-J (1981) Ann. Rev. Microbiol. 35:399-424

COULTER, J.B., SONEDA,S. & ETTINGER, M.B. (1957) Sewage & Ind. Wastes 29:468-477

CROSS, W.H., CHIAN, E.S.K., POHLAND, F.G., HARPER, S., KHARKAR, S., CHENG, S.S. & LU, F. (1982) Biotechnol. Bioeng. Symp. 12:349-363

DAHAB, M.F. & YOUNG, J.C. (1981) Biotechnol. Bioeng. Symp. 11:381-397

DIEKERT, G.B., GRAF, E.G. & THAUER, R.K. (1979) Arch. Microbiol. 122:117-120

DIEKERT, G., KLEE, B. & THAUER, R.K. (1980) Arch. Microbiol. 124:103-106

DIEKERT, G.B. & RITTER, M. (1982) J. Bacteriol. 151:1043-1045

DIEKERT, G. & RITTER, M. (1983) FEBS Lett. 151:41-44

DODDEMA, H.J. & VOGELS, G.D. (1978) Appl. Environ. Microbiol. 36:752-754

DRAKE, H.L., HU, S-I. & WOOD, H.G (1980) J. Biol. Chem. 255:7174-7180

DRAKE, H.L. (1982) J. Bacteriol. 149:561-566

DUFF, S.J.B. & KENNEDY, K.J. (1982) Biotechnol. Lett. 4:815-820

DURRE, P., ANDERSCH, W. & ANDREESEN, J.R. (1981) Int. J. Systematic Bacteriol. 31:184-194

DURRE, P. & ANDREESEN, J.R. (1982) J. Gen. Microbiol. 128:1457-1466

EASTMAN, J.A. & FERGUSON, J.F. (1981) J. Water Pollut. Control Fed. 53:352-366

ELLEFSON, W.L., WHITMAN, W.B. & WOLFE, R.S. (1982) Proc. of the Nat. Acad. Sci., U.S.A. 79:3707-3710

EVANS, W.C. (1977) Nature, London 270:17-22

FISHBEIN, L. (1981) Sci. of the Total Environ. 17:97-110

FOGARTY, W.M. & KELLY, C.T. (1979) Developments in microbial extracellular enzymes. In: A. Wiseman (ed.) Topics in enzyme and fermentation technology volume 3, Ellis Horwood, Chichester, p45-102

FRANCIS, C.W. & CALLAHAN, M.W. (1975) J. Environ. Quality 4:153-163

FRANCIS, C.W. & MALONE, C.D. (1977) Prog. Water Technol. 8:687

FRIEDMAN, A.A. & TAIT, S.J. (1980) J. Water Pollut. Control Fed. 8:2257-2269

FYNN, G.H. & WHITMORE, T.N. (1984) Biotechnol. Lett. 6:81-86

GENUNG, R.K., MILLION, D.L., HANCHER, C.W. & PITT JNR., W.W. (1979) Biotechnol. Bioeng. Symp. 8:329-344

GENUNG, R.K., HANCHER, C.W., RIVERA, A.L. & HARRIS, M.T. (1982) Biotechnol. Bioeng. Symp. 12:365-380

GODWIN, S.J., WASE, D.A.J. & FORSTER, C.F. (1982) Process Biochem. 17:33-34,45

GRAF, E.G. & THAUER, R.K. (1981) FEBS Lett. 136:165-169

GUJER, W. & ZEHNDER, A.J.B. (1983) Water Sci. Technol. 15:127-167

HADDOCK, B.A. (1977) The isolation of phenotypic and genotypic variants for the functional characterisation of bacterial oxidative phosphorylation. In: B.A. Haddock & W. A. Hamilton (eds.) Microbial Energetics: 27th Symp. of Soc. for Gen. Microbiol., Imperial Coll., London. Cambridge Univ. Press, p95-120

HARPER, S.R., CROSS, W.H., POHLAND, F.G. & CHIAN, E.S.K. (1984) Biotechnol. Bioeng. Symp. 13:401-420

HEALY, J.B. & YOUNG, L.Y. (1978) Appl. Environ. Microbiol. 35:216-218

HEALY, J.B., YOUNG, L.Y. & REINHARD, M. (1980) Appl. Environ. Microbiol. 39:436-444

HEERTJES, P.M. & VAN DER MEER, R.R. (1979) Comparison of different methods for anaerobic treatment of dilute wastewaters.Proc. 34th Ind. Waste Conf. Purdue Univ., Lafayette, Indiana. Ann Arbor Science, Ann Arbor, Michigan, p790-804

HEIJNEN, J.J. (1983) Development of a high rate fluidised bed biogas reactor. Proc. Eur. Symp. on Anaerobic Wastewater Treatment, Noordwijkerhaut, Netherlands, p259-274

HENZE, M. & HARREMOES, P. (1983) Water Sci. Technol. 15:1-102

HICKEY, R.F. & OWENS, R.W. (1981) Biotechnol. Bioeng. Symp. 11:399-413

HOBSON, P.N. (1982) Production of biogas from agricultural wastes. In: N.S. Subba Rao (ed.) Advances in agricultural microbiology. Butterworth Scientific, London, p523-550

HOBSON, P.N. (1983) J. Chem. Technol. Biotechnol. 33B:1-20

HOBSON, P.N. & MCDONALD, I. (1980) J. Chem. Technol. Biotechnol. 30:405-408

HOBSON, P.N. & SHAW, B.G. (1974) Water Res. 8:507-516

HOBSON, P.N. & SHAW, B.J. (1976) Water Res. 10:849-852

HUBER, H., THOMM, M., KONIG, H., THIES, G. & STETTER, K.O. (1982) Arch. Microbiol. 132:47-50

HULSOFF-POL, L.W., DE ZEEUW, W.J., VELZEBOER, C.T.M. & LETTINGA, G. (1983) Water Sci. Technol. 15:291-304

HUNGATE, R.E. (1950) Bacteriol. Rev. 14:1-49

HUNGATE. R.E. (1967) Arch. Microbiol. 59:158-164

HUNGATE, R.E. (1969) A roll tube method for the cultivation of strict anaerobes. In: J.R. Norris & D.W. Ribbons (eds.) Methods in Microbiology Vol. 3B. Academic Press Inc., New York, p117-132

HUYSMAN, P., VAN MEENEN, P., VAN ASSCHE, P. & VERSTRAETE, W. (1983) Biotechnol. Lett. 5:643-648

IANNOTTI, E.L. & FISCHER, J.R. (1984) Effects of ammonia, volatile acids. pH and sodium on growth of bacteria isolated from a swine manure digester. In: Proc. 40th Gen. Meeting of the Soc. for Ind. Microbiol., Sarasota, Florida. Victor Graphics, Maryland, p741-747

IMHOFF, D. & ANDREESEN, J.R. (1979) FEMS Microbiol. Lett. 5:155-158

JACOBSON, F.S., DANIELS, L.,FOX, J.A., WALSH, C.T. & ORME-JOHNSON, W.H. (1982) J. Biol. Chem. 257:3385-3388

JENNET, J.C. & DENNIS, N.D. (1975) J. Water Pollut. Control Fed. 47:104-121

JERIS, J.S. (1983) Water Sci. Technol. 15:167-176

JERIS, J.S., BEER, C. & MUELLER, J.A. (1974) J. Water Pollut. Control Fed. 46:2118-2128

JERIS, J.S., OWENS, R.W. & HICKEY, R. (1977) J. Water Pollut. Control Fed. 49:816-831

JEWELL, W.J. (1981) Development of the attached microbial film expanded bed process for aerobic and anaerobic waste treatment. In: P.F. Cooper & B. Atkinson (eds.) Biological fluidised bed treatment of water and wastewater. Ellis Horwood, Chichester, p251

JEWELL, W.J. & CUMMINGS, R.J. (1984) J. Food Sci. 49:407-414

JEWELL, W.J. & MORRIS, J.W. (1981) Influence of varying temperature, flowrate and substrate concentration on the anaerobic attached film expanded bed process. In: Proc. 36th Ind. Waste Conf. Purdue Univ., Lafayette, Indiana. Ann Arbor Science, Ann Arbor, Michigan, p655-664

JEWELL, W.J., SWITZENBAUM, M.S. & MORRIS, J.W. (1981) J. Water Pollut. Control Fed. 53:482-490

JONES, J.B. & STADTMAN, T.C. (1976) Methanococcus vanielii: growth and metabolism on formate. In: H.G. Schlegel, G. Gottschalk & N. Pfennig (eds.) Symp. on microbial production and utilisation of gases (H_2, CH_4, CO). p199-215

JONES, J.B. & STADTMAN, T.C. (1981) J. Biol. Chem. 256:656-663

JOVANOVIC, M., MURPHY, K.L. & HALL, E.R. (1986) Parallel evolution of high rate anaerobic treatment processes: retention time and concentration effects. In: Proc. EWPCA Water Treatment Conf. Aquatech '86: Anaerobic treatment, a grown-up technology, Amsterdam, Netherlands, p145-158

KASPAR, H.F. & WUHRMANN, K. (1978) Appl. Eviron. Microbiol. 36:1-7

KENNEDY, K.J. & DROSTE, R.L. (1983) Effect of influent concentration on the start-up of anaerobic downflow stationary fixed-film (DSFF) reactors. In: Proc. 38th Ind. Waste Conf. Purdue Univ., Lafayette, Indiana. Ann Arbor Science, Ann Arbor, Michigan, p615-628

KENNEDY, K.J. & VAN DEN BERG, L. (1982) Biotechnol. Lett. 4:171-176

KHAN, K.A., SUIDAN, M.T. & CROSS, W.H. (1982) J. Env. Eng. Div. ASCE 108:269-285

KIRK, P.W.W., LESTER, J.N. & PERRY, R. (1982) Water Res. 16:973-980

KOBAYASHI, H.A., STENSTROM, M.K. & MAH, R.A. (1983) Water Res. 17:903-909

KRUGER, H-J., HUYNH, B.H., LJUNGDAHL, P.O., XAVIER, A.V., DER VARTANIAN, D.V., MOURA, I., PECK JNR., H.D., TEIXEIRA, M., MOURA, J.J.G. & LE GALL, J. (1982) J. Biol. Chem. 257:1462-1463

KUGELMAN, I.J. & MCCARTY, P.L. (1965) J. Water Pollut. Control Fed. 37:97-116

LANDINE, R.C., VIRARAGHAVEN, T., COCCI, A.A., BROWN, G.J. & LIN, K.C. (1982) J. Water Pollut. Control Fed. 54:102-110

LANE, A.G. (1983) Environ. Technol. Lett. 4:349-352

LANE, A.G. (1984) Environ. Technol. Lett. 5:141-150

LAQUIDARA, M.J., BLANC, F.C. & O'SHAUGHNESSY, J.C. (1985) Development of biofilm in the anaerobic rotating biological contactor. In: Proc. 40th Ind. Waste Conf. Purdue Univ., Lafayette, Indiana. Ann Arbor Science, Ann Arbor, Michigan, p949-958

LATHAM, M.J. & WOLIN, M.J. (1978) Use of a serum bottle technique to study interactions between strict anaerobes in mixed cultures. In: D.W. Lovelock & R. Davis (eds.) Techniques for the study of mixed populations, Academic Press, London, p113-124

LAWRENCE, A.W. & MCCARTY. P.C. (1969) J. Water Pollut. Control Fed. 42:R1-R17

LEHMAN, V. & WELLINGER, A. (1981) Biogas production from full-scale on-farm digesters. In: F. Vogt (ed.) Energy conservation and use of renewable energies in the bio-industries. Pergamon Press, Oxford, p353-363

LEONHARDT, U. & ANDREESEN, J.R. (1977) Arch. Microbiol. 115:277-284

LESTER, R.L. & DE MOSS, J.A. (1971) J. Bacteriol. 105:1006-1014

LETTINGA, G., VAN DER GEEST, A.Th., HOBMA, S. & VAN DER LAAN, J. (1979a) Water Res. 13:725-737

LETTINGA, G., VAN VELSEN, A.F.M., DE ZEEUW, W. & HOBMA, S. (1979b) Feasibility of the upflow anaerobic sludge blanket (UASB) process. In: Proc. 1979 Nat. Conf. on Environ. Eng. ASCE, San Francisco, U.S.A. p35-45

LETTINGA, G., VAN VELSEN, A.F.M., HOBMA, S.W., DE ZEEUW, W. & KLAPWIJK, A. (1980) Biotechnol. Bioeng. 22:699-734

LETTINGA, G., DE ZEEUW, W. & OUBORG, E. (1981) Water Res. 15:171-182

LETTINGA, G., HOBMA, S., HULSOFF-POL, L.W., DE ZEEUW, W., DE JONG, P., GRIN, P. & ROERSMA, R. (1983) Water Sci. Technol. 15:177-195

LIN CHOU, W., SPEECE, R.E. & SIDDIQI, R.H. (1979) Biotechnol. Bioeng. Symp. 8:391-414

LJUNGDAHL, L.G. & ANDREESEN, J.R. (1978) Formate dehydrogenase, a selenium-tungsten enzyme from Clostridium thermoaceticum. In: S. Fleischer & L. Parker (eds.) Methods in enzymology Vol. 53. Academic Press, New York-London, p360-372

MACKIE, R.I. & BRYANT, M.P. (1981) Appl. Environ. Microbiol. 41:1363-1373

MAH, R.A. & SUSSMAN, C. (1968) Appl. Microbiol. 16:358-361

MARRIE, T.J., NELLIGAN, J. & COSTERTON, J.W. (1982) Circulation 66:1339-1341

MARTENSSON, L. & FROSTELL, B. (1983) Water Sci. Technol. 15:233-246

MASSEY, M.C. & POHLAND, F.G. (1978) J. Water Pollut. Control Fed. 50:2204-2222

MCCARTY, P.L. & BROSSEAU, M.H. (1963) Effects of high concentration of individual volatile fatty acids on anaerobic treatment. In: Proc. 18th Ind. Waste Conf. Purdue Univ., Lafayette, Indiana. Ann Arbor Science, Ann Arbor, Michigan, p283-296

MCCONVILLE, T. & MAIER, W.J. (1979) Biotechnol. Bioeng. Symp. 8:345-359

MCINERNEY, M.J., BRYANT, M.P. & PFENNIG, N. (1979) Arch. Microbiol. 122:129-135

MENDEZ-PAMPIN, R.J., SIERRA ALVEREZ, R., HULSOFF-POL, L.W. & LETTINGA, G. (1986) Start-up of a one phase UASB reactor on a sucrose waste. In: Proc. EWPCA Water Treatment Conf. Aquatech '86: Anaerobic treatment, a grown-up technology, Amsterdam, Netherlands, p 698-702

MESSING, R.A. (1983) Ann. Reports Fermentation Processes 6:23-33

METHODS FOR THE EXAMINATION OF WATERS AND ASSOCIATED MATERIALS. Chemical Oxygen Demand (Dichromate Value) of Polluted and Waste Waters 1977. HMSO, London.

METHODS FOR THE EXAMINATIONS OF WATER AND ASSOCIATED MATERIALS. Determination of Volatile Fatty Acids in Sewage Sludge 1979. HMSO, London.

METHODS FOR THE EXAMINATION OF WATERS AND ASSOCIATED MATERIALS. Phosphorus in Waters, Effluents and Sewages 1980. HMSO, London.

METHODS FOR THE EXAMINATION OF WATERS AND ASSOCIATED MATERIALS. Suspended, Settleable and Total Dissolved Solids in Waters and Effluents 1980. HMSO, London.

MEYER, U. (1981) FEMS Symp. 12:371-385

MINK, R.W. & DUGAN, P.R. (1977) Appl. Environ. Microbiol. 27:985-987

MONOD, J. (1949) Ann. Rev. Microbiol. 3:371-394

MORRIS, J.E. (1979) The digestion of crop residues - an example from the Far East. In: Proc. 1st. Int. Symp. on Anaerobic Digestion, Cardiff, U.K., Applied Science Publishers, London p289-302

MORRIS. J.W. & JEWELL. W.J. (1981) Organic particulate removal with the anaerobic attached-film expanded-bed process. In: Proc. 36th Ind. Waste Conf. Purdue Univ., Lafayette, Indiana. Ann Arbor Science, Ann Arbor, Michigan, p621-630

MOSEY, F.E. (1978) Water Pollut. Control 77:370-378

MULDER, R., VERKUIJLEN, J. & VAN ANDEL, J.G. (1986) Identification of organisms and biopolymers isolated from bacterial aggregates formed in a glucose acidifying reactor. In: Proc. EWPCA Water Treatment Conf. Aquatech '86: Anaerobic treatment, a grown-up technology. Amsterdam, Netherlands, p.629-632

MURRAY, W.D. & VAN DEN BERG, L. (1981) Appl. Environ. Microbiol. 42:502-505

NAGASE, M. & MATSUO, T. (1982) Biotechnol. Bioeng. 24:2227-2239

NEL, L.H., DE HAAST, J. & BRITZ, T.J. (1984) Biotechnol. Lett. 6:741-746

NEWELL, P.J. (1981) The use of a high rate contact reactor for energy production and waste treatment from intensive livestock units. In: F. Vogt (ed.) Energy conservation and use of renewable energies in the bio-industries. Pergamon, Oxford, p395-407

NORRMAN, J. (1983) Water Sci. Technol. 15:247-259

NOVAK, J.T. & CARLSON, D.A. (1970) J. Water Pollut. Control Fed. 42:1932-1943

NUTT, S.G., MELCER, H. & PRIES, J.H. (1984) J. Water Pollut. Control Fed. 56:851-857

PARKIN, G.F. & SPEECE, R.E. (1982) J. Environ. Eng. Div. ASCE 108(EE3):525-531

PARKIN, G.F. & SPEECE, R.E. (1983) Water Sci. Technol. 15:261-289

PARKIN, G.F., SPEECE, R.E. & YANG, C.H.J. (1981) A comparison of the response of methanogens to toxicants: anaerobic filter vs. suspended growth systems. In: Proc. Seminar/Workshop on Anaerobic Filters: An energy plus for wastewater treatment. Argonne Nat. Laboratory, Argonne, Illinois, p37-57

PATEL, G.B., ROTH, L.A. & AGNEW B.J. (1984) Can. J. Microbiol. 30:228-235

PERKINS, C.W., TSUGITA, L.D. & TEKIPPE, R.J. (1975) Source control and biological treatment of artichoke processing plant. In: Proc. 30th Ind. Waste Conf. Purdue Univ., Lafayette, Indiana. Ann Arbor Science, Ann Arbor, Michigan, p192-197

PIKE, E.B. & CARRINGTON, E.G. (1972) Water Pollut. Control 71:583-605

PLUMMER, A.H., MALINA, J.F. & ECKENFELDER, W.W. (1968) Stabilization of low carbohydrate waste by an anaerobic submerged filter. Proc. 23rd Ind. Waste Conf. Purdue Univ., Lafayette, Indiana. Ann Arbor Science, Ann Arbor, Michigan, p462-473

POWELL, G.E., HILTON, M.G., ARCHER, D.B. & KIRSOP, B.H. (1983) J. Chem. Technol. Biotechnol. 33B:209-215

POWELL, M.S. & SLATER, N.K.H. (1982) Biotechnol. Bioeng. 24:2527-2537

RIMKUS, R.R., RYAN, J.M. & COOK, E.J. (1982) J. Water Pollut. Control Fed. 54:1447-1457

RINZEMA, A., PAARDEKOOPER, A.H., DE VEGT, A.L. & LETTINGA, G. (1986) Anaerobic treatment of edible oil refinery wastewater in granular sludge UASB reactors. In: Proc. EWPCA Water Treatment Conf. Aquatech '86: Anaerobic treatment, a grown-up technology. Amsterdam, Netherlands, p205-218

RITTMANN, B.E. (1982) Biotechnol. Bioeng. 24:501-506

RITTMANN, B.E. & MCCARTY, P.L. (1980a) Biotechnol. Bioeng. 22:2343-2357

RITTMANN, B.E. & MCCARTY, P.L. (1980b) Biotechnol. Bioeng. 22:2359-2373

ROCKEY, J.S. & FORSTER, C.F. (1982) Environ. Technol. Lett. 3:487-496

ROCKEY, J.S. & FORSTER, C.F. (1985) Environ. Technol. Lett. 6:115-122

ROGERS, H.J. (1979) Adhesion of microorganisms to surfaces: some general considerations on the role of the envelope. In: D.C. Ellwood, J. Melling & P. Rutter (eds.) Adhesion of microorganisms to surfaces. Academic Press, London, p29-55

RONDA, F., SIERRA, R. & FDZ-PALANCO,F. (1986) Stability behaviour of an UASB reactor. Organic loading rate and temperature disturbance. In: Proc. EWPCA Water Treatment Conf. Aquatech '86: Anaerobic treatment, a grown-up technology. Amsterdam, Netherlands, p764-767

ROSS, W.R. (1984) Water SA 10:197-204

RUDD, T., STERRITT, R.M. & LESTER, J.N. (1982) Eur. J. Appl. Microbiol. Biotechnol. 16:23-27

RUDD,T., HICKS, S.J. & LESTER, J.N. (1985) Environ. Technol. Lett. 6:209-224

RUSESKA, I., ROBBINS, J., COSTERTON, J.W. & LASHEN, E.S. (1982) Oil Gas J. 80:253-264

SACHS, E.F., JENNETT, J.C. & RAND, M.C. (1978) Anaerobic treatment of synthesized organic chemical pharmaceutical wastes. In: Proc. 33rd Ind. Waste Conf. Purdue Univ., Lafayette, Indiana. Ann Arbor Science, Ann Arbor, Michigan, p507-514

SACHS, E.F., JENNETT, J.C. & RAND, M.C. (1982) J. Environ. Eng. Div. ASCE 108:297-314

SAERNER, E. (1986) Influence and control of H_2S on full scale plants and pilot plant experiments. In: Proc. EWPCA Water Treatment Conf. Aquatech '86: Anaerobic treatment, a grown-up technology. Amsterdam, Netherlands, p189-294

SALKINOJA-SALONEN, M.S., NUYS, E-J., SUTTON, P.M., VAN DEN BERG, L. & WHEATLEY, A.D. (1983) Water Sci. Technol. 15:305-308

SANCHEZ-RIERA, F., VALZ-GIANINET, S., GALLIERI, D. & SINERIZ, F. (1982) Biotechnol. Lett. 4:127-132

SANCHEZ-RIERA, F., CORDOBA, P. & SINERIZ, F. (1985) Biotechnol. Bioeng. 27:1710-1716

SAX, R.I. (1985) Anaerobic pretreatment of brewery wastewater on the industrial scale. In: Proc. 40th Ind. Waste Conf. Purdue Univ., Lafayette, Indiana. Ann Arbor Science, Ann Arbor, Michigan, p59-68

SAYED, S., DE ZEEUW, W., LETTINGA, G., RAS, M. & ZEEVALINK, J. (1981) Anaerobic treatment of slaughterhouse waste. Proc. 2nd Int. Symp. on Anaerobic Digestion, Travemunde, Germany.

SCHIEFINGER, C.C., LINEHAN, B. & WOLIN, M.J. (1975) Appl. Microbiol. 29:480-483

SCHRAA,G. & JEWELL, W.J. (1984) J. Water Pollut. Control Fed. 56:226-232

SCHWARTZ, L.J., DE BAERE, L.A. & LANZ, R.W. (1981) Biotechnol. Bioeng. Symp. 11:463-479

SCOTT, R. (1979) Rennets and cheese. In: A. Wiseman (ed.) Topics in enzyme and fermentation technology Vol. 3. Ellis Horwood, Chichester,p103-169

SCOTT, R.H. & DE MOSS, J.A. (1976) J. Bacteriol. 126:478-486

SEELER, T.A. & JENNETT, J.C. (1978) Treatment of wastewater from a chemically synthesized pharmaceutical manufacturing process with the anaerobic filter. In: Proc. 33rd Ind. Waste Conf. Purdue Univ., Lafayette, Indiana. Ann Arbor Science, Ann Arbor, Michigan, p686-695

SMITH, M.R. & MAH, R.A. (1978) Appl. Environ. Microbiol. 36:870-879

SORENSEN, J., CHRISTIENSEN, D. & JORGENSEN, B.B. (1981) Appl. Environ. Microbiol. 42:5-11

SPEECE, R.E. (1983) Environ. Sci. Technol. 17:416-427

SPEECE, R.E., PARKIN, G.F. & GALLAGHER, D. (1983) Water Res. 17:677-683

SPEECE, R.E., PARKIN, G.F., TAKASHIMA, M. & BHATTACHARYA,S. (1986) Trace nutrient requirements of anaerobic digestion. In: Proc. EWPCA Water Treatment Conf. Aquatech '86: Anaerobic treatment, a grown-up technology. Amsterdam, Netherlands, p175-188

STADTMAN, T.C. (1978) Selenium-dependent clostridial glycine reductase. In: S. Fleischer & L. Packer (eds.) Methods in enzymology vol. 53. Academic Press, New York-London, p373-382

STAFFORD, D.A. & ETHERIDGE, S.P. (1983) I. Chem. E. Symp. 77:141-147

STEPHENSON, T. & LESTER, J.N. (1986) Biotechnol. Bioeng. 28:372-380

SUTTON, P.M. & LI, A. (1981) Anitron and oxitron systems: high rate anaerobic and aerobic biological treatment systems for industry. In: Proc. 36th Ind. Waste Conf. Purdue Univ., Lafayette, Indiana. Ann Arbor Science, Ann Arbor, Michigan, p665-677

SUTTON, P.M. & LI, A. (1983) Water Sci. Technol. 15:333-344

SUTTON, P.M., LI, A., EVANS, R.R. & KORCHIN, S. (1982) Dorr-Oliver's fixed film and suspended growth anaerobic systems for industrial wastewater treatment and energy recovery. In: Proc. 37th Ind. Waste Conf. Purdue Univ., Lafayette, Indiana. Ann Arbor Science, Ann Arbor, Michigan, p667-675

SWITZENBAUM, M.S. & JEWELL, W.J. (1980) J. Water Pollut. Control Fed. 52:1953-1965

SWITZENBAUM, M.S. & DANSKIN, S.C. (1981) Anaerobic expanded bed treatment of whey. In: Proc. 36th Ind. Waste Conf. Purdue Univ., Lafayette, Indiana. Ann Arbor Science, Ann Arbor, Michigan, p414-424

SWITZENBAUM, M.S., SHEEHAN, K.C. & HICKEY, R.F. (1984) Environ. Technol. Lett. 5:189-200

TAIT, S.J. & FRIEDMAN, A.A. (1980) J. Water Pollut. Control Fed. 52:2257-2269

TAYLOR, D.W. (1972) Full scale anaerobic trickling filter evaluation. In: Proc. 3rd Nat. Symp. on Food Processing Wastes. Report no. EPA-R2-72-018, U.S. Environmental Protection Agency, Washington, D.C.

THAUER, R.K. & MORRIS, J.G. (1984) Metabolism of chemotrophic anaerobes: old views and new perspectives. In: D.P. Kelly & N.G. Carr (eds.) Proc. 36th Symp. Part II of the Soc. for Gen. Microbiol., Univ. of Warwick, U.K.. Cambridge Univ. Press, Cambridge, p123-168

TOERIEN,D.F. & HATTINGH, W.H.J. (1969) Water Res. 3:385-416

TOERIEN, D.F., THIEL, P.G. & HATTINGH, W.H.J. (1968) Water Res. 2:505-509

TRULEAR, M.G. & CHARACKLIS, W.G. (1982) J. Water Pollut. Control Fed. 54:1288-1301

TURNER, D.C. & STADTMAN, T.C. (1973) Arch. Biochem. Biophys. 154:366-381

UEKI, A. & SUTO, T. (1981) J. Gen. Appl. Microbiol. 29:229-237

UNDEN, G., BOCHER, R., KNECHT, J. & KROGER, A. (1982) FEBS Lett. 145:230-234

VAN DEN BERG, L. & LENTZ, C.P. (1978) Food processing waste treatment by anaerobic digestion. In: Proc. 32nd Ind. Waste Conf. Purdue Univ., Lafayette, Indiana. Ann Arbor Science, Ann Arbor, Michigan, p252-258

VAN DEN BERG, L. & LENTZ, C.P. (1979) Comparison between up and downflow anaerobic fixed film reactors of varying surface-to-volume ratios for the treatment of bean blanching wastes. In: Proc. 34th Ind. Waste Conf. Purdue Univ., Lafayette, Indiana. Ann Arbor Science, Ann Arbor, Michigan, p319-325

VAN DEN BERG, L., LAMB, K.A., MURRAY, W.D. & ARMSTRONG, D.W. (1980a) J. Appl. Bacteriol. 48:437-447

VAN DEN BERG, L., LENTZ, C.P. & ARMSTRONG, D.W. (1980b) Anaerobic waste treatment efficiency comparisons between fixed film reactors, contact digesters and fully mixed continuously fed digesters. In: Proc. 35th Ind. Waste Conf. Purdue Univ., Lafayette, Indiana. Ann Arbor Science, Ann Arbor, Michigan, p788-793

VAN DEN BERG, L., KENNEDY, K.J. & HAMODA, M.F. (1981) Effect of type of waste on performance of anaerobic fixed film and upflow sludge bed reactors. In: Proc. 36th Ind. Waste Conf. Purdue Univ., Lafayette, Indiana. Ann Arbor Science, Ann Arbor, Michigan, p686-692

VAN DEN BERG, L. & KENNEDY, K.J. (1981) Biotechnol. Lett. 3:165-170

VAN DEN BERG, L. & KENNEDY, K.J. (1982) J. Chem. Technol. Biotechnol. 32:427-432

VERSTRAETE, W. (1983) Biomethanation of wastes: perspectives and potentials. In: Biotech '83: Proc. Int. Conf. on the Commercial Applications and Implications of Biotechnology. Online, p725-742

VERSTRAETE, W., DE BAERE, L. & ROZZI, A. (1981) Trib. Cebedeau 34:367-375

WAGNER, R. & ANDREESEN, J.R. (1977) Arch. Microbiol. 114:219-224

WAGNER, R. & ANDREESEN, J.R. (1979) Arch. Microbiol. 121:255-260

WANDREY, C. & AIVASIDIS, A. (1983) Ann. N.Y. Academy Sci. 413:489-500

WANG, S.C., TSENG, I.C. & WANG, L.C. (1986) The characteristics of Methanosarcina and Methanothrix in an anaerobic digester. In: Proc. EWPCA Water Treatment Conf. Aquatech '86: Anaerobic treatment, a grown-up technology. Amsterdam, Netherlands, p29-38

WHEATLEY, A (1983) Biomethanation and by-product recovery from effluents. In: Biotech 83: Proc. Int. Conf. on the Commercial Applications and Implications of Biotechnology. Online, p761-780

WHITMAN, W.B. & WOLFE, R.S. (1980) Biochem. Biophys. Res. Communications 92:1196-1201

WIDDELL, F. & PFENNIG, N. (1982) Arch. Microbiol. 131:360-365

WIEGANT, W.M., CLAASSEN, J.A., BORGHANS, A.J.M.L. & LETTINGA, G. (1983) High rate thermophilic anaerobic digestion for the generation of methane from organic wastes. In: Proc. Eur. Symp. on Anaerobic Wastewater Treatment, Noordwijkerhaut, Netherlands, p392-410

WILDGRUBER, G., THOMM, M., KONIG, H., OBER, K., RICCHIUTO, T. & STETTER, K.O. (1982) Arch. Microbiol. 132:31-36

WILKIE, A. & COLLERAN, E. (1984) Biotechnol. Lett. 6:735-740

WINFREY, M.R., NELSON, D.R., KLEVICKIS, S.C. & ZEIKUS, J.G. (1977) Appl. Environ. Microbiol. 33:312-318

WINKLER, M. (1981) Biological treatment of wastewater. Ellis Horwood, Chichester, p211-212

WITKOWSKI, P.J. & JERIS, J.S. (1984) Anaerobic toxicity of several soft drink additives. In: Proc. 38th Ind. Waste Conf. Purdue Univ., Lafayette, Indiana. Ann Arbor Science, Ann Arbor, Michigan, p839-845

YAMAMOTO, I., SAIKA, T., LIU, S-M. & LJUNGDAHL, L.G. (1983) J. Biol. Chem. 258:1826-1832

YAMAZAKI, S. (1982) J. Biol, Chem. 257:7926-7929

YOUNG, J.C. (1980) Performance of anaerobic filters under transient loading and operating conditions. In: Proc. Seminar/Workshop: Anaerobic filters:an energy plus for wastewater treatment, Florida, U.S.A. p159-170

YOUNG, J.C. & MCCARTY, P.L. (1969) J. Water Pollut. Control Fed. 41:R160-R173

YOUNG, J.C. & DAHAB, M.F. (1982) Biotechnol. Bioeng. Symp. 12:303-316

YOUNG, J.C. & DAHAB, M.F. (1983) Water Sci. Technol. 15:369-383

ZEIKUS, J.G. (1979) Microbial populations in digesters. In: Proc. 1st Int. Symp. on Anaerobic Digestion, Cardiff, U.K.. Applied Science Publishers Ltd., London, p61-90

ZEIKUS, J.G., WELLSTEIN, A.L. & KIRK, T.K. (1982) FEMS Microbiol. Lett. 15:193-197