RETICULATED FOAM AS A BIOMASS SUPPORT MEDIUM IN THE ANAEROBIC DIGESTION OF AN INDUSTRIAL WASTEWATER

by

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ABSTRACT

This work reports the pilot-scale investigation of various anaerobic reactor systems treating a fruit washing wastewater. An open cell reticulated foam was used as a biomass support media (BSM). The foam pads (25 mm cubes) were randomly packed in the 2.5 m^{-3} reactor with an unpacked section beneath the bed.

Four general operational regimes were evaluated. These were: single and two stage operation, with and without effluent recycle. Performance was monitored throughout each run in terms of maximum COD loading rate and minimum attainable hydraulic retention time. Biomass concentrations, both within the media and freely suspended between the biomass support particles were measured on samples from each operating regime, their acetoclastic activity being determined in a laboratory test. A method was developed to ascertain whether a difference in biomass activity existed between the outside of an individual biomass support particle and at the centre of the particle, using a radioactively labelled substrate.

It was concluded that a two stage system without recycle provided the best performance with respect to the the maximum attainable loading rate (11.6 $kgCOD.m^{-3}.day$). This was approximately twice that for any of the other systems tested. The minimum hydraulic retention time corresponding to this loading was approximately 1.0 d. The superior performance of the two stage system without recycle was attributed to the increased acetoclastic populations brought about by the pre-acidified feed and the plug flow removal kinetics exhibited in reactors without recycle. Two stage systems produced higher levels of biomass in the reactor than their single stage counterparts and a large proportion of the total biomass inventory was present as suspended growth in systems without recycle. Tracer studies showed that the actual HRT was much less than that calculated from flow rate and reactor volume, indicating that large areas of the reactor were not accessible to the substrate.

Experiments investigating activity gradients in the BSM indicated that a significant difference existed between the acetoclastic activity of biomass at the centre of a colonized particle and that on the surface. It may be concluded that substrate diffusional limitations played an important role in determining the performance of this type of biomass support. Electron microscope examination of BSP fragments gave little information other than the existence of both attached and suspended growth. Most of the biomass was present as a dense fibrillar network.

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Abbreviations

The following abbreviations have been used in this thesis and are given below:

1S+RC	Single stage with recycle
2S+RC	Two stage with recycle
1SNORC	Single stage without recycle
2SNORC	Two stage without recycle
AAFEB	Anaerobic attached film expanded bed
APHA	American Public Health Association
BSM	Biomass Support Media
COD	Chemical Oxygen Demand
DG^o	Standard free energy of reaction
DSFFR	Downflow stationary fixed film reactor
ELISA	Enzyme-Linked Immunosorbant Assay
F_{420}	A co-enzyme in methanogenesis
GLC	Gas-Liquid Chromatography
HRT	Hydraulic Retention Time
PD	Proportional derivative (control action)
pH_2	Hydrogen partial pressure
PPI	Pores per inch
RTD	Residence time distribution
SEM	Scanning electron microscope/micrograph
SRB	Sulphate reducing bacteria
SRT	Solids retention time
TEM	Transmission electron microscope/micrograph
UASB	Upflow-anaerobic sludge blanket
VFA	Volatile fatty acid

CHAPTER ONE: INTRODUCTION

Of the avaliable options for municipal and industrial wastewater treatment, anaerobic digestion is perhaps unique in its potential for producing useable energy. This feature of the process, together with the high cost of disposal of some industrial wastewaters, has led to a considerable amount of research in the area, particularly during the past few decades.

The production of methane gas from organic material is not a new discovery. Volta detected methane in marsh gas in the eighteenth century and, as a result of this, various ideas were considered for its use. Cameron described the use of a septic tank for providing methane to light the city of Exeter in 1890. Later Karl Imhoff described a two compartment tank for the treatment of sewage in which the first was used for settlement of solids and the second in which the solids were anaerobically digested.

One of the earliest separate digesters ever built was in Birmingham in 1911. Nowadays many of the larger sewage works have digesters and some are entirely self sufficient in their energy needs for pumping and aeration.

Further developments during the 1960s, involving the efficiency of biomass retention, have led to the application of the process to industrial wastewaters of various strengths. Cillie *et al.* (1969) reported that wastes with a chemical oxygen demand (COD) greater than 4000 mg/l may be

treated anaerobically at a lower cost than by aerobic methods. For waste strengths above 20,000 mg COD/l anaerobic methods cost 1/4 of the equivalent aerobic cost. Cillie's analysis was for a conventional stirred tank digester, and the use of a process with biomass retention would tend to make treatment of even weaker wastes profitable. Process intensification leads to higher throughputs and therefore lower plant costs and may be achieved by several methods ranging from the use of conventional trickling filter packings to specifically designed packings such as Flocor etc.

The upflow anaerobic sludge blanket reactor, in which process operation encourages the formation of natural biomass aggregates, has been greatly researched but it seems as though the formation of biomass granules is difficult to predict. The use of a specifically optimized packing may make it possible to predict biomass inventory and activity more accurately, thus enabling refined design procedures to be developed and used. The use of reticulated foam as a biomass support has the advantage that it can be manufactured to a range of specifications and its size optimized for a particular application. It has been reported in fermenters and aerobic treatment systems (Atkinson *et al.*, 1974; Walker and Austin, 1981).

Investigations of the complex microbiological and biochemical aspects of the process have revealed many of the characteristics of the individual and associated groups of anaerobic bacteria, whilst laboratory and pilot-scale

studies have demonstrated the operational difficulties associated with the process and how they may be overcome. Integration of these areas has been slow and consequently rational design strategies for the new types of anaerobic process are limited. The main requirements are the provision of an optimum environment for the sensitive bacterial populations that proliferate and a process that affords some degree of protection from process disturbances. The primary consideration is always financial and each particular problem has to be evaluated separately.

The anaerobic digestion process, as a waste treatment method, offers several advantages over conventional aerobic treatment methods; these include:

(i) a higher organic throughput, and therefore a smaller reactor volume;

(ii) only small amounts of organic material are converted to biomass, so limiting the problems associated with further sludge disposal;

(iii) there is no need for energy and equipment to transfer oxygen to the waste;

(iv) the methane produced is a valuable source of energy (table 1.1) both for operating the process itself and other operations (e.g. electricity generation);

(v) the nutrient requirements are lower than for aerobic processes; and

(vi) odour problems are effectively eliminated by the need for closed reactors.

These may be offset by the potential disadvantages:

(i) the slow growth of the methane producing bacteria;

(ii) the effect of changes in process parameters and the presence of inhibitory substances on the sensitive bacterial populations;

(iii) the requirement of skilled process operation; and

(iv) the need for energy to heat the process (generally coming from the gas produced).

	FUEL	VOLUME OF EQUIVALENT FUEL
1m3 BIOGAS @ 622 M J	NATURAL GAS	0.571m3
	LIQUID BUTANE	0.868 litres
	PETROL	0.704 litres
	DIESEL OIL	0.621 litres

Table 1.1 Volumes of other fuels with calorific value equivalent to 1m3 of Biogas (Meynell, 1982)

This study attempts to evaluate and explain the performance of an anaerobic reactor containing a reticulated foam biomass support medium (BSM) in the treatment of a high strength industrial wastewater. Due to the complex nature of the process it is difficult to provide an exhaustive design procedure but it is hoped that this study is able to provide

sufficient information to provide guidelines for an adequate process design model.

CHAPTER TWO: LITERATURE REVIEW

2.1 The Microbiology and Biochemistry of Anaerobic Digestion

2.1.1 Introduction

The fermentation of complex organic compounds to methane and carbon dioxide requires a largely undefined, mixed population of bacterial species. Most of the present understanding of the populations involved is based on the studies of bacteria isolated from sewage sludge digesters or from the rumen of some animals (Hobson *et al.*, 1974; Hungate, 1966). Until recently the fermentation was considered to proceed in two steps, the acid forming and methane forming stages. The first stage comprises the hydrolysis of organic polymers and their conversion to organic acids, alcohols, carbon dioxide and hydrogen, and in the second stage the methane bacteria convert these compounds to the final products of methane and carbon dioxide. The discovery that methanogenic bacteria were unable to catabolize alcohols other than methanol, or organic acids other than acetate or formate indicated that there must be at least three groups of bacteria responsible for the decomposition of organic material into the final products (Bryant *et al.*, 1967).

Several nomenclatures have been proposed for the various stages in the decomposition of a complex substrate. It is usually described in three stages (fig.2.1): (1)

hydrolysis and acidification, (2) acetogenesis, and (3) methanogenesis. More recent work (Bryant, 1976; Zeikus, 1980) has indicated that at least four trophic groups of bacteria can be distinguished; the acidogenic bacteria, the proton-reducing acetogenic bacteria, the homo-acetogenic bacteria and the methane bacteria.

The overall rate limiting step in the degradation of a complex substrate to methane is dependent on its composition. In the digestion of largely soluble compounds this has been identified as the methanogenesis of volatile fatty acids (Lawrence and McCarty, 1969; Ghosh and Pohland, 1974; Kaspar and Whurmann, 1978a), whereas for partially insoluble wastes, such as pig slurry and sewage sludge, particulate solubilization can be rate limiting (Boone, 1982; Eastman and Ferguson, 1981). A greater understanding of the complex symbiotic associations of the various groups of bacteria has only recently been elucidated and this together with an appreciation of the other environmental requirements of the micro-organisms, has been essential in the successful design and operation of reliable anaerobic digestion systems.

2.1.2. The Degradation of Polymers and The Acidogenic Bacteria.

The first stage of the anaerobic breakdown involves various species of fermentative bacteria and different enzymes, which as a complex metabolic group, hydrolyse polysaccharides such as cellulose and other large organic

molecules using extracellular enzymes such as cellobiase, amylase, protease and lipase. The bacteria responsible for acidogenesis are facultative and genera such as *Bacteroides, Clostridia, Bifidobacteria* and other Gram-positive and Gram-negative rods have been isolated from sewage sludges. Populations of 10^{10}-10^{11} per gram of volatile solids are common (Toerien, 1967). The products from this stage, mainly sugars and amino acids, are then fermented to a wide spectrum of products, mainly acetate, propionate, butyrate, hydrogen, carbon dioxide, lactate, valerate, ethanol, ammonia and sulphide, for example:

$$C_6H_{12}O_6 + 2H_2O = 2CH_3COOH + 2CO_2 + 4H_2 \quad (1)$$

$$C_6H_{12}O_6 = CH_3CH_2COOH + 2CO_2 + 2H_2 \quad (2)$$

$$C_6H_{12}O_6 + 2H_2 = 2CH_3CH_2COOH + 2H_2O + CO_2 \quad (3)$$

Succinate produced by some bacteria is decarboxylated to yield propionate (Scheifinger and Wolin, 1973). The various proportions of these products, and hence the digestion process, are carefully controlled by the presence of hydrogen in the reactor (Bryant, 1979; Thauer, 1977). This has been shown in theory (Mosey and Foulkes, 1983) and by experimental methods. Kaspar and Whurmann (1978b) reported an increase in volatile acids in the reactor liquor upon increasing the hydrogen partial pressure above digesting sludge from 0.001 to 0.01 atm. Increases from 0.001 to 0.015 atm. resulted in a linear increase in propionic acid concentration despite the stimulation of both

methane production and acetate turnover. This may be explained as follows: generally the Embden-Meyer pathway (fig.2.2) provides the main route for the conversion of glucose to organic acids. The acid forming bacteria use this pathway to obtain energy from the oxidation of glucose to acetate. The hydrogen concentration regulates the overall conversion by throttling the various reactions at different positions in the pathway. During the course of this oxidation hydrogen atoms are transferred first to the carrier molecule (the oxidised form of nicotinamide adenine dinucloetide (NAD)), converting it to the reduced form (NADH) and H^+, released into solution as dissolved hydrogen gas. In order for this catabolism to proceed continuously, given an unrestricted avaliability of NAD, the NADH- produced at (A) in fig.2.2 (the substrate level phosphorylation of glyceraldehyde-3-phosphate) and at (B) (the oxidative decarboxylation of pyruvic acid to acetyl-CoA) must be regenerated. This function is accomplished by the reduction of protons to form hydrogen gas which is subsequently removed by the hydrogenotrophs (methanogens, sulphate reducing bacteria and nitrate reducing bacteria). Accumulations of hydrogen beyond the assimilative capacity of these hydrogenotrophs necessitates an alternative method of electron disposal for NADH- generation. This need is fulfilled by the fermentation of pyruvate to propionate, lactate and ethanol and/or by the fermentation of acetyl-CoA to butyric acid. Since methanogens cannot use these substrates directly, their accumulation leads to the depression of pH in the reactor and its associated problems. The availiability of NADH- is controlled by the partial

pressure of hydrogen in the reactor:

$$NADH_2 = NAD + H_2 \quad (4)$$

The free energy change for this reaction is negative when the partial pressure of hydrogen within the reactor is below 0.01 atm. (fig.2.3). The hydrogen concentration increases, for example, when stress is put on the system by overloading with organic matter or by a sudden decrease in hydraulic retention time (HRT). There is an increasing tendency for the electrons of (NADH) generated in the fermentation to be used in the conversion of pyruvate to intermediates other than acetate, carbon dioxide and hydrogen.

In an efficiently operating anaerobic digester most of the carbohydrate is fermented via acetate to carbon dioxide and hydrogen without major production of other fatty acids (Thauer and Jungermann, 1977; Bryant, 1979). Recent studies of the interactions of fermentative bacteria with H_2 utilizing methane bacteria have reinforced this theory. Pipyn and Verstraete, (1981) found that directing this primary fermentation towards ethanol and lactate production rather than acetate, may give a more constant substrate to the methane bacteria and hence permit a more efficient conversion of substrate to methane. This however is only true for processes with separated stages for acidogenic and methanogenic stages.

The acidogenic bacteria are relatively fast growing and

have typical doubling times of around 30 minutes at 35°C (Mosey and Foulkes, 1983).

2.1.3. The Hydrogen Producing Acetogenic Bacteria

Until recently it was thought that methanogenic bacteria were able to use the products from the acidogenic bacteria directly. Bryant *et al.* (1967) showed that *Methanobacta omelianskii*, previously thought of as a pure culture, was in fact a symbiotic association of two distinct species, an H_2 utilizing methanogen and a H_2 producing acetogenic bacterium. Their joint production of methane from ethanol follows eqn. 5 (DG^0 = -132.6 kJ):

$$2CH_3CH_2OH + CO_2 = 2CH_3COOH + CH_4 \qquad (5)$$

One of the species, the 'S' organism oxidises ethanol to acetate and hydrogen according to eqn. 6 (DG^0 = -6.2 kJ):

$$CH_3CH_2OH + H_2O = CH_3COOH + 2H_2 \qquad (6)$$

Since DG^0 at pH 7 is positive the equilibrium position of the reaction is to the left and the organism is unable to grow alone in ethanol. However, as the partial pressure of hydrogen is lowered, the free energy becomes progressively more negative allowing the growth of the organism in ethanol (fig.2.4). The second bacterium in the mixed culture was found to be a methanogen which could not use ethanol but used hydrogen according to eqn. 7 (DG^0 = -138.9 kJ):

$$4H_2 + CO_2 = CH_4 + 2H_2O \quad (7)$$

It can therefore be seen that the symbiosis of these two organisms is energetically favourable and they can proliferate. Propionate, longer chained fatty acids, alcohols and some aromatic compounds such as benzoate and other organic acids, produced by the acidogenic bacteria are also degraded by the acetogenic bacteria in association with methanogenic bacteria (Sahm, 1984):

$$\underset{\text{propionate}}{CH_3CH_2COOH} + 2H_2O = CH_3COOH + CO_2 + 3H_2 \quad (8)$$
$$(DG^0 = 48.1kJ)$$

In order for the energy to be available for the organism oxidising propionic acid to acetic and hydrogen, the pH_2 cannot exceed 10^{-6} atm. (Thauer and Jungermann, 1977). The maintenance of an extremely low partial pressure of hydrogen is essential for the growth of both species, and these conditions can best be achieved by intensive cell contact which allows inter-species hydrogen transfer to take place. Under these conditions the energy avaliable to the hydrogen-oxidising bacteria is reduced considerably from its value at a partial pressure of one atmosphere. This results in a low bacterial yield per mole of hydrogen oxidised. Lawrence and McCarty (1969) confirmed this when they found low growth yields for the complete methanogenesis of propionate and other fatty acids.

Experimental evidence that supports these observations has involved the specific inhibition of methane bacteria and the addition of hydrogen to actively digesting sewage

sludge (Thiel, 1969; Smith, 1970). Results in both cases showed propionate accumulation within the systems. Enrichment studies on acetogenic bacteria indicate that even under optimum conditions they grow relatively slowly with minimum doubling times of around 1.5-4.0 days, and the reactions they perform are energetically unfavourable (McInerny *et al.*, 1971; Hayes and Hall, 1981). Symbiosis enables them to exist by making their combined reactions thermodynamically more favourable.

Populations of 4.2×10^6 hydrogen producing acetogenic bacteria per ml of digester sludge have been reported (Sahm 1984). These bacteria have not been generically identified or physiologically well characterised. A new genus and species, *Syntrophobacter wolinii* that degrades propionate to acetate, CO_2 and H_2, only in co-culture with an H_2-utilizing organism has been identified by Boone and Bryant (1980), and McInerny *et al.* (1971) reports the isolation of an anaerobic bacterium *Synthrophomonas wolfi* that oxidises saturated fatty acids in association with H_2 using bacteria.

2.1.4 The Homoacetogenic Bacteria

The homoacetogenic bacteria produce acetate from hydrogen and carbon dioxide. Little is known about the functional importance of their metabolism and the interactions of homoacetogens and methanogens. Zeikus, (1980) has described homoacetogenic metabolism. He concluded that these bacteria have high thermodynamic efficiencies as

a consequence of no formation of H_2 and CO_2 during growth on multi carbon compounds. Two genera were described *Clostridium Thermoaceticum*, generally considered unable to grow on one carbon compounds and *Butyribacterium Methylotrophicum* that grows on a variety of multi carbon compounds.

2.1.5 The methane bacteria

These tertiary stage bacteria are strict anaerobes, and in mixed they culture rely on the other species present to maintain anaerobiosis. They require a lower redox potential than most other anaerobic bacteria, around -330 mV, (which corresponds to a concentration of about 1 molecule of O_2 in 10^{56} litres of water). Special techniques have been developed to grow them in defined conditions (Hungate, 1969), and several methanogens have subsequently been isolated, but only a few in pure culture. They have a limited substrate spectrum and ferment single carbon compounds, hydrogen and carbon dioxide to methane. Only a few species are able to degrade acetate to CH_4 and CO_2, and so far only three have been isolated in pure culture (*Methanosarcina barkeri, Methanococcus mazei* and *Methanothrix soengenii*). Since the free energy of conversion of acetate to CO_2 and CH_4 is small, these methanogens grow very slowly on this substrate.

$$CH_3COOH = CH_4 + CO_2 \quad (DG^0 = -32\ kJ) \qquad (9)$$

M. soengenii has a generation time of 10 days or more,

whereas *M. bakerii* grows much faster with a generation time of 2-3 days. Usually around 70% of the methane comes from the methyl group of acetate with the remainder from hydrogen and carbon dioxide (Jeris and McCarty, 1965). It has been shown that hydrogen also exerts a regulatory effect on the methanogenesis of acetate and prevents all strains of *Methanosarcina* from metabolising it (Mah, 1978). These methanogens effectively exert control of the pH by removal of volatile fatty acids and the formation of carbon dioxide. The hydrogen utilizing methane bacteria grow relatively quickly with minimum doubling times of around six hours (Mosey and Foulkes, 1983).

Morphologically these bacteria have many different cell shapes including large sarcinae, coccus groups, long cylindrical rods and short rods (Balch *et al.*, 1979). There are numerous reports of direct identification by microscopy, but results are unclear and viability can not be assesed by this method. The methane bacteria differ from classical and other eukaryotic organisms in several biochemical characteristics. Muramic acid, present in the cell wall of many bacteria, is not present in the cell wall of methanogens (Sahm, 1984), and several new co-enzymes (the nonprotein portion of an enzyme, a prosthetic group which functions as an acceptor of electrons or functional groups) have been discovered which are unique to methanogens (Vogels *et al.*, 1982). These are involved in the terminal stages of methanogenesis, and there is some evidence that methanogenic bacteria can be quantitively assessed in mixed culture by the spectrofluorimetric determination of these

compounds (Delafontaine *et al.*, 1979). In most cases the methanogenesis step is the point at which the organic pollution load is significantly reduced by the conversion of soluble chemical oxygen demand to methane, and therefore efficient COD removal equates directly with efficient methanogenesis. Most anaerobic plants treating soluble organic wastewaters are operated primarily to satisfy the requirements of this group of bacteria. Details of some methanogens, reviewed by Sahm (1984) are given in Table 2.1 and growth constants of the predominant bacterial groups are given in Table 2.1a.

2.1.6 Other Bacterial Species

Other bacterial species competing with methanogens include sulphate-reducing bacteria and denitrifying bacteria which in the presence of sulphate and nitrate can convert sulphate and nitrate to hydrogen sulphide and nitrogen gas respectively. Although the addition of high levels of nitrates and/or sulphates may have a detrimental influence on methanogenesis, in lower concentrations, these ionic species may be possibly applied to moderate the effect of excess hydrogen on a stressed anaerobic digester (Harper, 1985). Most nitrate reducing bacteria are facultative anaerobes since they will transfer electrons to oxygen, if present, or to nitrate when oxygen is absent. Denitrification starts at an oxygen partial pressure of 0.005 atm. However, sulphate-reducing bacteria are strict anaerobes, and under strict anaerobic conditions sulphate is easily reduced to hydrogen sulphide just as carbon dioxide

is converted to methane. The sequence of these three different electron acceptors, NO_3^-, SO_4^{2-}, and CO_2, follow the decreasing level of redox intensity. Therefore nitrate followed by sulphate will seriously compete with CO_2 for the electrons from organic substrates. Consequently, in mixed cultures, methane should only be formed in the absence of nitrate and sulphate (Zehnder, 1978). The presence of H_2S in digester gas can cause practical problems in its subsequent use. Sulphate reducing bacteria have demonstrated a greater affinity for hydrogen than methanogens in both pure and mixed culture (Kristjansson *et al.*, 1982).

2.1.7 Environmental Requirements of the Bacteria Associated With Anaerobic Digestion

The bacterial populations involved in anaerobic digestion require certain environmental conditions related to:

(1) temperature ;
(2) nutrients ; and
(3) absence of toxic substances

together with a suitable substrate in order that they may function efficiently in an anaerobic process.

Temperature

Temperature has a marked effect on the rate of reaction in chemical and biological systems. There are three most

commonly cited operating ranges in anaerobic digestion: cryophilic (20°C), mesophilic (20-40°C) and thermophilic (>45°C), but the majority of work seems to have been attempted in the mesophilic range. This is due largely to the economics of heating but also to the temperature sensitivity of the hydrogen-utilizing bacteria. It has been shown shown that growth and specific rate of methane production drop off below 33°C and above 40°C (Zehnder and Wuhrmann, 1977). Optimum conditions are around 35-37°C, for instance, for the hydrogen utilizing methanogen *Methanobrevibacter arbophilus* as illustrated in Figure 2.5.

Most processes can operate from 10-45° C without a major variation in the diversity of bacterial population, but above 40°C the high bacterial decay rates result in the observed yield coefficient approaching zero. van den Berg (1977) reported that continuous operation of a reactor at this temperature would be difficult with respect to start-up and slow to adapt to variations in process conditions but recent work has shown that very high loading rates have been achieved with a thermophillic sludge blanket reactor (Lettinga, 1986). The only known thermophillic methane bacterium is *Methanobacteria thermoautotrophicum* with an optimum temperature of 65-70°C. All other strains of methanogenic bacteria grow best at mesophillic temperatures. Though methane is still produced in sediments where the temperature falls below 4°C, no methane former has been isolated with optimum performance in the cryophilic range. The response of an anaerobic reactor to a shock change in temperature seems to be dependent on the degree of loading.

Jewell and Morris, (1982) reported little effect on very low loaded processes. However, it does seem that if the loading rate is high then process failure may occur, this is probably due to the fact that a shock temperature change may prove more disruptive for the methane bacteria than the relatively fast growing acidogenic bacteria. Gradual changes in process temperature of the order of one degree centigrade per day seem to be acceptable (Henze and Harremoes, 1983).

The variation of reaction rate constant has been expressed by the Arrhenius equation:

$$d(\ln K)/dT_A = E_a/RT_{A^2} \qquad (10)$$

where K = first order rate constant (s^{-1})

R = gas constant ($J\ K^{-1}\ mol^{-1}$)

E_a = activation energy ($kJ\ mol^{-1}$)

T_A = absolute temperature ($^\circ K$)

or by the following equation (Hanaki *et al.*, 1985):

$$\ln[r(T_{A,1})/(r(T_{A,2})] = -u[(1/T_{A,1})-(1/T_{A,2})] \qquad (11)$$

where $r(T_A)$ = reaction rate at $T_A\,^\circ K$

u = temperature dependency coefficient

Lawrence and McCarty (1969) report that the Monod saturation constant (K_s), but not the rate, varied with temperature (23-35°C) in the methanogenesis of acetate and found a similar relation to eqn. (11).

2.1.8 Nutrients

Anaerobic processes are often used for industrial wastewater treatment with little nutrient addition. It has been shown that the nitrogen and phosphorous contents of anaerobic sludges are approximately 10.5% N and 1.5% P by weight (Speece and McCarty, 1964). With a knowledge of the N and P constituents of the wastewater and the bacterial yield coefficients, the supplementary requirements can be calculated for any particular wastewater. Often the COD/N ratio is quoted as being a more useful process parameter than COD/N/P as the N/P ratio may be taken as 7 (Speece and McCarty, 1964). For this reason the mininum theoretical COD/N/P ratio may be assumed to be 350/7/1. van den Berg and Lentz (1977) have quoted 420/7/1 as an acceptable maximum for processes loaded in the range 0.8-1.2 kgCOD/kgVSS/day. For low loaded processes (less than 0.5 kgCOD/kgVSS/day) values of around 1000/7/1 have been reported (Benjamin *et al.*, 1981).

Other nutrients essential for the growth of anaerobic bacteria have been extensively reviewed by Henze and Harremoes, (1983) and are given in Table 2.2. The nickel requirement of methane bacteria was not realised until recently. It is essential in the synthesis of an important co-enzyme, F_{420}, in electron transfer in the terminal stages of methanogenesis (Thauer, 1981). Many industrial wastewaters contain sufficient trace elements, but their

deficiency is often reported as a reason for failure when no other explaination for slow process startup can be found.

Uncoupling of methanogenesis from growth by nutrient limitation

Two problems associated with anaerobic digestion are the length of time taken for process start-up and the production of excess biomass, which itself constitutes a waste, in fully commissioned digesters. Archer (1985) reported that methanogenesis could be coupled to cell growth during startup and uncoupled in fully operational digesters. He achieved this by the addition, or omission, of phosphate from the feed This can only be applied to wastes that are naturally deficient in phosphorus.

2.1.9 Inhibition and Toxicity in Anaerobic Systems

As with all biological processes anaerobic metabolism can be upset by toxic substances. Evaluation of the degree of toxicity can be performed by various toxicity assay procedures (Owen *et al.*, 1979; Benjamin *et al.*, 1982), but these tend to give varying results. It seems that an important property of the bacteria is their ability to acclimatise to toxic compounds. In some instances selected species have been adapted which metabolize compounds which cannot be degraded by aerobic organisms, for example chlorophenolic compounds (Salkinoja-Salonen *et al.*, 1983a). Different reactor types, for example attached growth

and plug flow systems, are able to recover more quickly from shock loads of toxic substances, than completely mixed, suspended growth systems (Parkin and Speece, 1983). Process failure most usually results from the inhibition of the methane-forming bacteria rather than the other species present. Apart from various organic substances which can impart toxicity to anaerobic systems, there are more common agents which frequently cause problems in the treatment of industrial wastes, in particular:

(1) Sulphide;
(2) Volatile acids;
(3) pH;
(4) Ammonia; and
(5) Heavy metals:

Sulphide Toxicity

Although sulphide is not present in many industrial wastewaters the non-toxic sulphite and sulphate can occur in several situations, including pulp and paper processing and distillery wastes (Webb, 1986; Carrondo *et al.*, 1983). Under normal conditions or low concentrations these compounds are non-toxic, but under anaerobic conditions they are readily converted to sulphide by the sulphate-reducing bacteria (SRB). The toxicity of sulphide is closely related to the free hydrogen sulphide concentration which itself is pH dependent, at pH < 6.5 toxicity is increased. The toxicity occurs by two methods, firstly the hydrogen sulphide produced is extremely toxic to the methane bacteria, and

secondly the sulphate reducing bacteria compete with the methane bacteria for substrate (as described above).

$$SO_4^{2-} + 4H_2 = H_2S + 2H_2O + 2OH^- \qquad DG^0 = -154 \text{ kJ}$$

$$SO_4^{2-} + CH_3COOH = H_2S + 2HCO_3 \qquad DG^0 = -47.3 \text{ kJ}$$

These reactions are energetically more favourable than the conversion of these substrates to methane. In severe cases the majority of substrate is converted to hydrogen sulphide. Inhibition has been reported at differing concentrations, again dependent on process operation and reactor type. It is widely accepted that for general operation sulphate concentrations in the influent below 0.3-0.6 kg S/m^3 may be regarded as non-inhibitory (Henze and Harremoes, 1983), depending on the pH. Free hydrogen sulphide seems to inhibit at concentrations around 0.1 kg S/m^3. The presence of this in the gas can cause problems when it is used in gas boilers and other gas handling equipment on account of the extremely corrosive nature of the sulphurous and sulphuric acids produced. Various methods have been used successfully to reduce toxicity from these compounds including the addition of iron salts to the feed, which precipitates the sulphide as insoluble metal sulphides (Jopson *et al.*, 1986). Methods for gas stripping of H_2S from the digester gas have also been tried (Anderson *et al.*, 1980), and it would seem that pure oxygen stripping may be the most cost effective option at present (Kite and Stringer, 1986)

Volatile fatty acids and pH inhibition

The volatile fatty acids (VFA) are perhaps the most common inhibitors of anaerobic systems since they are produced as intermediates in the breakdown of wastewaters to methane. The mechanism is somewhat uncertain but it is believed that the fraction of undissociated acid is the most significant factor (Andrews, 1969). The pH of the reactor and the VFA concentration are important in the identification of VFA inhibition. The toxic level for free undissociated acid has been quoted as around 1000-2000 mg/l (as acetic acid), with propionic acid being more toxic than the other acids. The toxic level for higher fatty acids seems to be of the same order (Hanaki *et al.*, 1981). Anderson *et al.* (1982) compared their results and those of other workers on VFA inhibition and conclude that undissociated VFA concentrations of around 30 mg/l (as acetate) are inhibitory. The inhibition is therefore dependent on pH (Fig. 2.6). Chang *et al.* (1983) have shown that a digester can be operated in a steady state condition independently of the residual volatile fatty acid concentration and digester pH if the un-ionized proportion of the VFA is maintained at less than 12 mg/l acetate. This is roughly of the same order of magnitude. Low pH is inhibitory, but not necessarily bactericidal (Keefer and Urtes, 1963).

The provision of pH control is sometimes necessary in the treatment of industrial wastewaters which contain strong mineral acids or alkalis. It allows maintenance of the optimum pH for the bacteria in the system, and it also

controls the balance of dissociated and undissociated acids and bases, such as the short chain fatty acids, hydrogen, sulphide and ammonia salts, which is essential for minimizing inhibition. Effluents with low nitrogen are unable to support the formation of the ammonium bicarbonate buffer in anaerobic environments. In most combined anaerobic processes (where all stages in the reaction are carried out in one reactor), the pH limits for stable operation are normally 6.5-8.5. The methane bacteria have an optimum pH of 6-8 and the acidogenic bacteria 5-6 in pure culture (Zehnder *et al.*, 1981). *Methanothrix soehngenii* has an optimum pH of 7.8 and shows no activity below pH 6.8, whereas *Methanosarcinae sp.* forms methane over the much wider range of (5-8). The adoption of a specific reactor pH will therefore determine the diversity of species present.

Many wastewaters, although biodegradable, rapidly become acidic on storage due to the action of acidogenic bacteria already present in the liquor. Adjustment of this pH is sometimes unnecessary as the acids are readily degraded by the acetogenic and methanogenic bacteria resulting in an increase in reactor pH. There are several reactions providing natural alkalinity to the system which enable the process to regulate itself to a certain extent, such as:

$CO_2 + H_2O = H^+ + HCO_3^-$ pKa = 6.4

$NH_3 + H_2O = NH_4 + OH^-$ pKa = 8.0

$H_2S = H^+ + HS^-$ pKa = 7.0

(pKa = ionization constant at 25 °C)

These conjugate acid-base pairs assist in the maintenance of the correct pH The alkalinity, often expressed as mg/l $CaCO_3$, provides an indication of the buffering capacity of the system. Suitable limits in anaerobic digestion lie between 1000-5000 mg/l (Sawyer and McCarty, 1967; Fig.2.7). Alkalinity may have to be added in some cases where the natural alkalinity of the waste is insufficient. Ferguson *et al.* (1984) studied the effect of various agents and found sodium carbonate to be the cheapest form of alkalinity when chemical cost is taken into account. The addition of lime ($Ca(OH)_2$) has posed problems on some occasions due to its tendency to form insoluble calcium precipitates and possibly causing a biomass of high inorganic content to form. For small amounts of buffering in the range 6.5-7.0 it is common to use 10% NaOH. Inhibition effects from cations can be a problem when sodium salts are used for this purpose. In most cases the requirements are below the toxicity threshold.

Ammonia Toxicity

Ammonia can be a potential inhibitor in anaerobic systems. It is believed that it is the concentration of free undissociated ammonia which is most toxic with inhibition reported at 0.1-0.2 kg N/m^3. Hill and Barth (1977) developed a dynamic model which accounted for the inhibition of anaerobic digestion by ammonia and fatty acids as it is thought that the unionized ammonia is affected in the same way as unionised VFA. Total ammonia and ammonium

concentrations as high as 5-8 kg N/m^3 can be tolerated if the pH of the reactor is within the limits of normal process operation. Inorganic and organic nitrogen compounds are rarely discharged by industry as they are valuble raw materials (Anderson *et al.*, 1982).

Heavy Metal Toxicity

Anaerobic systems, particularly sludge digesters, are vulnerable to high loadings of heavy metals. The mechanism has been reported to be due to the inhibition of enzymic activity in the metabolism of carbohydrates and fats (Mosey and Hughes, 1975). It has been demonstrated that the toxicity of heavy metal ions is directly related to the solubility product of their sulphide salts (Shaw, 1954). The toxicity is therefore dependent on the various chemical forms which the metal assumes under anaerobic conditions. It has been reported (Lawrence and McCarty, 1965) that the presence of sulphide will enable precipitation of the soluble heavy metal ions to insoluble, non-toxic metal sulphides.

Tables 2.3 and 2.4 show the inhibitory concentrations of certain organic and inorganic substances.

2.2 The Process Design Considerations Of Anaerobic Treatment

2.2.1 Introduction

The optimisation of anaerobic processes relies mainly on the provision of the following fundamental design requirements, namely;

(1) the maintenance of an environment suitable for the bacteria;
(2) good bacterial retention by effective separation of the solids retention time (SRT) from the hydraulic retention time (HRT);
(3) good contact between the bacteria and the wastewater; and
(4) the disengagement of gas bubbles from biomass to reduce the buoyant effect of gas bubbles and subsequent washout of biomass.

The bacterial species responsible for anaerobic waste stabilization must multiply at a faster rate than that at which they are washed out of the reactor. Process intensification necessitates uncoupling the HRT from the SRT. This is achieved by the entrapment of the biomass in the reactor by the use of a growth support medium or by adopting process conditions that encourage the formation of cell aggregates. The methane bacteria, being very slow growing, require a SRT in excess of around 30 days for stable process operation (Donelly, 1986).

If the bacteria are to proliferate they must be assured of good contact with the incoming wastewater. Several factors influence this, but the turbulent conditions created by gas production usually ensure good mixing.

Additional mixing may be necessary if this is insufficient. Stagnant areas of the reactor do occur, and this results in a reduction in process performance. Despite the low bacterial cell yields (compared to aerobic systems), removal of some portion of the excess biomass may become necessary after a period of operation, since the effluent solids may have increased beyond an acceptable level or short circuiting may be evident. Various methods have been suggested to alleviate this problem, but it would seem advisable to make provision at the design stage for some form of biomass removal. Camilleri (1984) reported biomass removal by gas stripping in which digester biogas is recycled to the bottom of the reactor and sparged through the bed: the turbulent conditions so induced remove biomass from the system. A similar effect could be achieved by an decrease in HRT for a short period.

For wastes with high concentrations of inert suspended material, which leads to a high inert loading, there is an increase in the inert fraction of the biomass in the reactor. The amount of active biomass is limited and subsequently the maximum loading is reduced. The reactor system may selectively retain inert material, thus greatly reducing process efficiency.

2.2.2. Conventional Anaerobic Processes

Over one hundred years ago in 1881 "an air-tight chamber" was described in France as "useful for reducing the mass and putrescible nature of suspended organic material

from municipal wastewaters" (McCabe and Eckenfelder, 1958). The first reported incidence in the U.K. was in 1897 when the local government board of the city of Exeter approved the treatment of all the city's wastewater in an anaerobic tank. This work eventually led to the development of the Imhoff tank, used to treat sludge and wastewater. In 1927 Ruhrverband installed the first heating apparatus in a separate digestion tank using the methane produced to heat the process. By the end of the 1930's anaerobic treament of municipal sludges was well established. Further developments (Stander, 1950) found that mixing greatly enhanced the rate of digestion, and this led to the development of the modern package systems. These consist essentially of a tank in which the stirring is achieved by the use of gas recirculation or sludge pumps and heating may be by internal heating coils or more commonly by external heat exchangers. The sludge gas is often used in dual-fuel boilers to maintain process temperature. Guidelines for process design were outlined by Noone (1982).

2.2.3. The Anaerobic Contact Process

The first significant advance on a simple continuously stirred tank reactor (CSTR; Fig.2.8A) was the Anaerobic Contact Process (Fig 2.8B), where a clarifier downstream of the digester was used to concentrate the biomass washed out in the effluent for its subsequent recycle to the reactor. The effective increase in the SRT/HRT ratio enabled higher treatment efficiencies, lower effluent CODs and hence some degree of process intensification. Practical problems in the

operation of these plants were experienced involving the separation of biomass and effluent, particularly in the treatment of industrial wastes. The tendency for continued gas production in the clarifier, which impaired the efficiency of operation, which led to modifications of the settler, such as, the incoporation of inclined plates (lamella type), vacuum degasification and settling combined with flocculation, and thermal shock treatment prior to sedimentation. The process is used chiefly for sewage sludge digestion (Schlegels and Kalbskopf, 1982), but some reports of successful treatment of high strength industrial wastes are evident from the sugar and food industries (Huss, 1982).

2.2.4. The Anaerobic Packed Bed Reactor/Anaerobic Filter

Anaerobic filter type reactors (Fig.2.8C) were first reported by Plummer *et al.* (1968) and Young and McCarty (1969). This work provided the basis for much of the later work in the development of many types of packed bed reactor systems. The performance of many types of packing has been investigated including rock lumps, Rashig rings, Flocor media, etc. This type of reactor usually consists of a flooded bed of the packing material through which the wastewater is passed in an upflow or downflow mode. The packing provides an inert surface on which the bacteria can be entrapped or attached. It also reduces areas of high turbulence, which cause washout, and encourages efficient sedimentation.

The biomass in such systems has been shown to exist in

three basic forms: the attached bacteria, the entrapped bacteria within voids in the reactor and the free swimming bacteria (van den Berg and Lentz, 1980; Young and Dahab, 1983). The proportions are dependent on specific reactor types and process operation methods. When operating in downflow mode film growth is reported to be mainly responsible for the retention of biomass (Van den Berg and Lentz, 1980). High concentrations of bacterial solids develop and very long SRTs of the order of 100-600 days have been reported (Plummer *et al.*, 1968). These types of reactor can be operated either with or without recycle. Flow conditions in the bed are usually dispersed plug flow. Some means for backwasing should be included in design (Young and Dahab, 1983).

The actual mechanism of methanogen retention by attachment of the bacteria in the reactor is uncertain, but is thought to be related to the surface roughness of the particle together with the overall porosity. It is most probable that other species produce a matrix which incoprates these bacteria (Murray and Van den Berg, 1981; Salkinoja-Salonen *et al.*, 1983b). During process operation a certain amount of biomass is sloughed off the support and washed out in the effluent. The majority of excess sludge is conveniently lost in this way. With some packing materials the volume of packing occupies a significant volume of the reactor, and loading rates quoted in the literature for these types are somewhat lower than for other fixed film reactors. By virtue of their hydraulic regime and the high proportion of attached growth, these types of reactors have

been shown to be more resistant to variations in process conditions, such as sudden increases in feed strength or reductions in HRT. Attached growth reactors also show a lower susceptbility to toxic substances in the feed. If this situation does occur a decrease in HRT will enable the toxic substance to be washed out of the reactor without seriously affecting the methanogens.

Fixed bed systems are unsuitable for some applications, including the treatment of wastewaters containing high concentrations of suspended solids, particularly if they are inorganic. There have been reports of short-circuiting due to clogging of the void spaces in the reactor which invariably results in a reduction in process performance (Young and Dahab, 1983).

2.2.5. Anaerobic Expanded Bed Reactors

If small inert particles, such as sand, gravel, anthracite or plastic particles, are held in suspension within a reactor system by the upflow of an influent wastewater, a large area becomes avaliable for biological growth (Fig.2.8D). The biofilm-coated particles have a high settling velocity and large surface area. A low liquid upflow velocity is employed to provide a bed expansion of around 10-20%. At this level all the particles remain in the bed. The gas production may create foaming and flotation at the top of the bed, and this may need to be controlled by some means. The thickness of biofilm can be controlled by the vsrying the degree of bed expansion. This type of

reactor has the advantage that the biomass inventory can be estimated to a reasonable level of accuracy by measurement of the bed expansion. It is claimed that these reactors allow the passage of refractory materials that would otherwise choke a packed bed reactor (Anderson and Saw, 1986). Some systems allow washout of biofilm-coated particles and treated effluent, the support is then returned to the reactor after removal of attached growth. Biomass may be effectively wasted from the reactor in this way. It is important that the distribution of influent/recycle flow is able to ensure a completely even expansion of the bed (Jewell and Morris, 1982).

2.2.6. Upflow Anaerobic Sludge Blanket Reactors

The UASB was first reported by Lettinga *et al.*, (1979). It is effectively a fixed film reactor where the natural ability of biomass to pelletize is encouraged by careful process control. After some period of operation a granular or flocculant sludge develops with pellets, or flocs, of 1-5mm diameter (Fig.2.8E). These have superior settling characteristics with a sludge volume index (SVI) of around half that for flocculant sludge (Lettinga *et al.*, 1980). The mechanism of sludge pelletization is uncertain, but is thought to be dependent on the presence of various organic salts, the selection of the correct seed sludge and waste composition. There seems to be a possibility that small particles may provide a nucleus for pellets to form (Pol *et al.*, 1983). There are several wastes which are known to readily produce a granular sludge, but workers have found

that a flocculent sludge is often produced. Startup of the process is critical, and high upflow velocities of the order of 0.05 to 0.3 m h^{-1} are essential to washout the poorly flocculating sludge and maintain dense biomass by natural selection (Lettinga *et al.*, 1979). In order to avoid the need for mechanical mixing the wastewater must be evenly distributed over the bottom of the reactor. The wastewater flows through an expanded bed of active sludge to a quiescent zone at the top of the reactor where a three phase separation device is provided for the separation of gas bubbles from sludge granules and treated wastewater. This device may be internal or external to the reactor but is most commonly an inverted funnel type arrangement. The use of lamella plates has also been reported (Shore *et al.*, 1984). The high cost of digester packing material and the concern of the long term problems of clogging often associated with packed bed reactors, make this type of system highly attractive. The successful operation of a UASB seems to be dependent on good process control and a wastewater that imparts good settling characteristics to the sludge. The majority of anaerobic processes selected for industrial wastes are of this type.

There is much further research needed on the phenomenon of pelletization, the effect of sudden increases in toxicants in the feed and the stability of biomass aggregates.

2.2.7. Fluidized Bed Reactors

The fluidized bed has many applications other than wastewater treatment. It differs from an expanded bed only in the liquid upflow velocity and degree of fluidization. Typical bed expansions are around 30% and above (with superficial upflow velocities from 6-20 m hr^{-1}. Investigations have included the use of various media such as activated carbon, sand and garnet (Shieh and Mulcahy, 1983; Li *et al.*, 1984). The use of the high recycle rates required to maintain the bed in a fluidized state may incur high pumping costs in a full scale plant, and this offsets the advantages of good mixing and temperature control characteristic of fluidized systems. At these upflow velocities gas bubbles tend not to adhere to the particles (Fig.2.8F).

2.2.8. Other Anaerobic Processes

Antonie, (1974) reported the use of a rotating disc contactor, similar to the aerobic equivalent, in which the medium can be partially or fully submerged. The velocity between the medium and wastewater gives some form of biofilm thickness control. Tait and Freidman (1980) reported the use of inert plastic media in a moving bed. The membrane anaerobic reactor , reported by Anderson *et al.* (1986), used a suspended growth reactor with an external ultramicro filtration membrane unit for solid liquid separation, and attained reactor biomass concentrations of around 30000 mg/l with effluent solids below 50 mg/l. Advantages of this type of system are that it enables high concentrations of active biomass to be maintained and it should permit faster

startup. There is a possibility that this type of system may be used in uprating an existing completely mixed reactor, and good operation is reported by Butler (1984). Bachman *et al.* (1985) reported the performance of an anaerobic baffled reactor which was a compartmentalised vessel with a plug flow hydraulic regime.

2.2.9 The Effect of Recycle

The addition of recycle to any reactor system will enable it to approach a completely mixed flow regime (Levenspiel, 1972). Recycle will also enable sudden increases in feed strength to be adequately buffered or allow the rapid dilution of toxic compounds present in the feed (Ferguson *et al.*, 1982).Other workers have reported the use of recycle to control the biofilm thickness in fixed film reactors and help reduce chanelling in fixed beds. Some degree of pH control can be achieved by recycling the effluent to increase the alkalinity of the feed, this has been reported as more successful than CO_2 stripping from the digester liqour (Ferguson *et al.*, 1984). There is evidence that high upflow velocities in a fixed bed will prevent gas holdup on the surface of the particles, which has been known to cause clogging, channeling and short circuiting. With regard to the disadvantages of the high recycle rates, the economics of the process are seriously reduced if the recycle or pumping costs are high.

2.2.9 Mixing

Some types of anaerobic reactor require additional mixing over and above that provided by the turbulent conditions created by gas production. Mixing in order to achieve better biomass/substrate contact can be accomplished by mechanical stirring and gas or liquid recirculation (Chain and DeWalle, 1977). In most cases it generally improves process performance (Lettinga, 1982). It is possible that intense mechanical mixing may have an adverse effect on the process by dispersing organisms in symbiotic relationships, for example the propionic/butyric acetogenic bacteria and the hydrogen-utilizing methane bacteria (Henze and Harremoes, 1983). It would appear that the advantages of spatial arrangement of the different bacterial groups and plug flow performance may be lost if reactors are operated in a completely mixed flow regime.

2.3 Comparison of the Various Reactors and Process Selection

Various workers have compared the numerous reactor types with respect to their organic loading and removal rates . All of the design configurations have their relative advantages and the selection of the best process is usually made on economic grounds. Lettinga (1984) observed that considerable differences exist in the maximum possible organic loading rates achievable in various processes. In some cases it is difficult to define a particular reactor configuration as COD removal is usually achieved by more than one type of entrapped biomass, for example suspended growth in a packed bed reactor or wall growth in a suspended growth reactor.

2.3.1 The Effectiveness of Biomass Retention

Lettinga (1984) considered the positive and negative aspects of biomass retention under high loading conditions. He concluded that the redispersion of sludge may reduce the sludge retention at high loadings in a UASB, and perhaps an anaerobic filter type system, due to the turbulent conditions within the bed. UASBs may suffer from pellet disintegration when shock loadings of feed occur. It would seem that the disintegration of granules at high loadings due to shear is probably insignificant. The mechanism for this has not been explained, but may be due to the rapid production of gas bubbles deep in the biomass pellet or the breakdown of pellet structure under the conditions associated with shock loading, i.e. high VFAs and low pH. In anaerobic packed bed reactors the main limiting factor is that the space occupied by carrier material renders a proportion of the total reactor volume unavaliable for use, although the selection of a highly porous medium may reduce this effect. The problem does not arise with UASB pellets as they are composed of approximately 70-80% VSS, a large proportion of which is active. The property of a high surface area of packing has been found to be important in downflow anaerobic filters and fluid bed reactors where the main mechanism of attachment is film growth. The detachment of biofilm in fixed film reactors may reduce the sludge retention capabilities further if the feed is incompletely acidified (Lettinga, 1984). Biofilm formation will be affected by shear forces in attached biomass reactors (Fynn

and Whitmore, 1984).

2.3.2 Bacteria/Substrate Contact

Undoubtedly the fluidized bed and downflow reactors offer the best conditions for bacteria/substrate contact due to good mixing and low liquid film resistance respectively. This may also be the case for UASBs, providing the incoming wastewater is adequately dispersed throughout the bottom of the sludge bed (Lettinga, 1984). Upflow filters can suffer from channeling in this respect.

2.3.3 Kinetic Factors

The influence of film or particle diffusion limitations has so far been relatively unresearched. Some experiments with granular sludge (Lettinga, 1984) have shown that particle size has little, if any, effect at high substrate concentrations. It is possible that a significant drop in substrate utilization rate may occur when the substrate concentration in the reactor bulk liquid is maintained at a relatively low level as is frequently the case in systems with high recycle ratios, for example fluidized bed systems. In these situations the concentration gradient that exists between the surrounding liquor and the conditions deep in the biomass aggregate may provide less of a driving force for the diffusion of the substrate into the particle.

The overall conclusions are that reactors achieving the highest organic loading rate (kg COD m^{-3} day^{-1}) will be

those containing the highest proportion of accessible active sludge and plug flow hydraulic regimes within the reactor. Maximum substrate utilization rates have been shown in reactor systems which separate the overall process into acid and methane forming stages, due to the greater substrate avaliability for the methane bacteria. Highest hydraulic loadings have been reported in fixed film reactors of the filter type and fluidized bed type. They will also show the greatest resistance to sudden variations in hydraulic or organic loading rate. Apart from the maximum loading rate achievable, the following factors must also be considered for the most suitable process selection:

(1) the time required for startup;
(2) stability of the process with respect to variation in process conditions and toxic substances;
(3) capability of the process to treat partially soluble wastes;
(4) capital and running costs for the plant;
(5) land requirement;
(6) skilled operator requirement; and
(7) the need for phase separation
(8) type of substrate

2.3.4 Process start-up

All anaerobic reactors seem to have startup problems. Periods of the order of 4 weeks on average seem to be needed to achieve design loading rates (Henze and Harremoes, 1983). The startup of expanded, fluidised and UASB reactors seem to present more of a problem than with other types. This is

probably due to the need to develop a natural bacterial matrix and that attached growth organisms may take more time to establish than suspended growth systems. The anaerobic contact process and anaerobic filter have been reported as the easiest to start (Anderson and Saw, 1986) The advantage of seeding the reactor with an innoculum from an operational reactor treating the same waste has been cited on several occasions; for example, the addition of a granular seed sludge to a UASB has been shown to reduce the startup from 100-300 days to 10-70 days (Rinzema, 1986). The general procedures reviewed by Henze and Harremoes (1983) (Table 2.5) seem to be a good guideline for process startup in general.

2.3.5 The Ability of High Rate Processes to Treat Wastes with High Suspended Solids.

Although the efficiency of the fluidized bed, downflow fixed film and UASB processes is low in the removal of suspended solids, the process is not seriously affected by their presence. There is some competition for bacterial attachment between a biomass support medium and dispersed matter, and consequently there may be a possible drop in the specific activity of the biomass. The upflow anaerobic filter will be highly efficient at removal of suspended solids, but process performance suffers if the bed clogs (Lettinga, 1984)

2.3.6 Process Stability

All high rate reactors are able to cope with small variations in loading rate on account of the high concentration of biomass retained in the reactor. It seems where reactors rely on the natural ability of the sludge to form aggregates the presence of toxic substances in the feed or organic overloads can alter the characteristics of the film or floc produced. Attached growth systems offer increased resistance to toxicants due to the fact that the sensitive bacterial species deep in the film are not suddenly exposed to the inhibitory conditions in the bulk liquor.

2.3.7 Capital and Running Costs

There is very little published information on the capital and operating costs of modern anaerobic plants. This will probably become avaliable as more full-scale plants are commissioned. Various factors need consideration and each particular application will be different. It is important for the value of the biogas to be accurately assessed as in some cases it may be uneconomic to recover the surplus gas or produce electricity from it. The incorporation of a support medium in the reactor will often add a significant amount to the capital cost of the plant. Factors influencing the payback period may be considered as follows:

(1) reduction in effluent charges;

(2) running costs of the plant;

(3) construction costs;

(4) benefit of the by-products;

(5) feed strength; and

(6) operating temperaure:

(7) sludge disposal costs; and

(8) pretreatment costs:

There are some cases where an industrial treatment or pretretment plant is necessary due to the threat of factory closure from the Water Authority if discharge consents are not maintained. This could be the determining factor in some cases.

2.3.8 Land Requirement

The reactors with the highest concentration of avaliable bacteria will usually require the smallest amount of land. Considerations should also be given to the area required for buffering tanks and other instrumentation and control equipment. Aspect ratio also dictates the land usage. A 2:1 aspect ratio is often selected as this is most common for tanks and cheapest to design (Wheatley and Cassell, 1985).

2.3.9 Skilled Management

All anaerobic reactors will require more attention from skilled operators compared with aerobic processes, as at present there are no satisfactory methods for the provision of automatic process control. However the process has shown potential for remote telemetric monitoring (Stafford, 1986).

2.3.10 The Need for Phase Separation

In some circumstances the separation of the two basic steps of anaerobic digestion may be advantageous. These situations include the elimination of toxic or inhibitory substances from the feed, for example sulphate present in the waste, or the buffering of variations in feed strength. Some wastes with high insoluble fractions will benefit greatly from two stage processes by reducing the inert matter fed to the methane reactor.

Since in a two-stage digestion system the acidogenic and methanogenic stages take place in separate reactors, optimum conditions can be provided for each group of organisms with the subsequent production of a good substrate for the methane bacteria, and consequently there are higher substrate utilization rates than a single stage process Cohen *et al.* (1979) made a comparison between single and two-stage processes with a soluble substrate and reported a higher biomass activity in both stages of the two stage process. Lettinga (1984) and Ghosh *et al.*, (1985) observed that improved digester stability followed the "presouring" of carbohydrate wastes. The "best" option is again most often selected on an economic basis as a two-stage system requires additional plant, control and instrumentation. Maximum organic loading rates are often quoted for the methane reactor volume, rather than the total reactor volume, which gives a better indication of process intensification.

2.3.11 Staged Anaerobic Digestion Systems

Reactor systems have been reported with staged digestion in a series of combined phase anaerobic filters (Civit *et al.*, 1984). All of the various reactor types could probably be sucessfully operated in this way. Cheung *et al.* (1986) described a series of three digesters operating in cascade, the authors concluded that at high loadings methanogenesis was limited to the final stage, and suggested that a two-stage process would be equally effective and less costly to construct. However, they concluded that further work was neccessary with other waste types before a final conclusion could be reached.

2.3.12 The Consequences of Cell Immobillisation

Atkinson (1984) reports that cell immobilisation:

(i) gives rise to a high cell concentration;

(ii) may lead to internal substrate and product gradients in microbial aggregates;

(iii) leads to heterogeneous populations within microbial aggregates;

(iv) affects overall growth and stoichiometry of reactions;

(v) permits operation beyond the critical dillution rate;

(vi) provides possibilities for the use of optimum aggregate size leading to maximum microbial activities; and

(vii) provides possibilities for the spatial location within reactors of the different microbial

populations.

All of these may be of importance in anaerobic fixed bed reactors.

2.4 The Selection of Biomass Support Media

2.4.1 Introduction

Numerous studies have shown that anaerobic packed bed reactors containing media ranging from lumps of rock to plastic packings, are most suitable for treating a wide variety of organic wastes (for example, Young and Dahab, 1983). The type of medium selected will dictate the solids retention characteristics and consequently the performance of the process. Factors influencing the choice of medium may be defined as follows:

(i) size;
(ii) porosity;
(iii) surface area;
(iv) weight and rigidity;
(v) cost;
(vi) resistance to chemical attack;
(vii) resistance to mechanical abrasion;
(viii) surface characteristics conducive to biomass retention; and
(ix) good separation of gas/liquid/biomass

It has been shown that interstitial biomass, as well as film growth, is responsible for the treatment and therefore both high porosity and high surface area per unit volume are important attributes of a biomass support medium (BSM). The total mass of the medium when empty and colonized, together with its rigidity, are also important in reactor design where deformation of the BSM may occur due to the compressive effects in a packed bed, or by the bouyancy effects associated with gas bubble holdup. The medium must be reasonably cheap, at least to make its incoporation in the reactor cost effective. It must obviously have a high resistance to chemical attack and abrasion, as the environment will contain volatile fatty acids and possibly some abrasive inorganic particles.

It has been observed that biofilms adhere more easily to rough surfaces with purturbations of the same order of magnitude as the bacteria; some workers have quoted 5 times the size of the bacterial species involved (Messing, 1982). Similarly Shimp and Pfaender (1982) reported that colonization of surfaces is favoured when microbial sized crevices are present in aerobic systems. In the case of methanogenic organisms they most likely rely on other species present to provide a matrix for attachment or entrapment (deVocht *et al.*, 1983).

The manner in which solids are transported through the medium matrix is likely to affect the withdrawal of excess biological solids from the reactor (Lettinga, 1984). This solids wasting is essential to reduce excess solids

accumulation and to help prevent dead zones within the reactor. In some cases it may become necessary to dislodge the entrapped solids to maintain good contact between the active organisms and the wastewater. If this assumption is correct, a medium of a given porosity but with a large pore diameter would be expected to show superior performance to one with the same porosity but a smaller average pore diameter.

The surface area of the medium has been reported to be of prime importance in downflow fixed bed systems using a variety of materials including glass, foamed and solid PVC, fire clay and needle punched polyester (van den Berg *et al.*, 1985), where film growth is responsible for the majority of COD removal. The researchers were unable to achieve very high loading rates, but they stated that this type of system offered extreme stability and resistance to adverse operating conditions conditions.

2.4.2 The Use of Plastic Foam as a Biomass Support Medium

The use of plastic foams has been reported in both aerobic and anaerobic treatment systems. Freeman (1961) described a novel fermenter in which aerobic micro-organisms were grown with nutrient agar supported on cellulose sponges. Process intensification was demonstrated by Atkinson *et al.*, (1979) in aerobic systems and fermenters and he concluded that the use of this type of medium in fermenters, led to high biomass concentrations, which were independent of throughput and enabled consistent biomass

concentrations. Practical commercial realisation of this was achieved by the development of the aerobic "CAPTOR" process (Walker and Austin, 1981), in which the microbial biomass was effectively immobilized on a reticulated plastic support medium. This medium comprised particles which were maintained in free suspension by aeration of the suspending wastewater. Removal of the excess biomass was achieved by squeezing out the sludge using pressure rollers. The Captor process showed better aeration efficiencies in terms of oxygen transfer rates per kWh of energy consumed over conventional activated sludge systems due, it was thought, to the longer contact time of air bubbles colliding with the support medium. High BOD removals were achieved in short retention times, but frequent cleaning of the BSM was needed to prevent anoxic zones occuring in the reactor. It was shown that by lengthening the sludge age consistent nitrification was achieved (Walker *et al.*, 1984).

The factors affecting the colonization of non-porous and porous packing materials in upflow methane reactors were investigated by Huysmen *et al.* (1983). Of the non-porous materials investigated they found that gas production was predominantly due to suspended biomass held between the voids of the packing, with surface roughness playing an important role. Of the porous materials investigated, a polyurethane non-reticulated foam was poorly colonized, while natural sponge colonized resonably well. They reported that reticulated, open cell foam in 25 x 25 x 25mm cubes was colonized both densely and rapidly. Their results showed that bacterial growth in reactors containing particles with

pore diameters of 0.43 mm (40 pores per inch, ppi) and 0.27mm (80 pores per inch) was faster than in those of 2.21 mm (10 pores per inch). They achieved maximum biomass densities of around 15 gm VSS/l using a substrate of ethanol and acetate. They also found the colonization of 10 ppi foam difficult and assumed the pore size was too large for rapid colonization. It seemed that the colonization rate was influenced by the size of the cubes since, when a reactor was filled with one piece of foam it acidified within a few days; they concluded that this was due to the fact that the diffusion of gas and nutrients in the matrix did not occur, so resulting in the uncoupling of the methanogenic associations. The use of smaller cubes of side 125 mm showed only a minor improvement in colonization, and presumably a cube of side 25mm was not rate limiting from a diffusion point of view. Polyurethane foam coated with bentonite was tested to enhance attachment by giving the neutral plastic a surface charge. The result proved to be the same as for the control. Their final conclusions were that the development of a methanogenic biofilm on a non-porous particle was determined primarily by the availability of bacterial sized crevices in the material, with the surface roughness appearing more important than surface charge. For porous materials the primary considerations were, pore size and particle porosity. With respect to diffusion limitations, for non-porous particles, unless the bacteria are attached to the outside of the matrix, the bacteria inside the surface film may face diffusion problems. The overall porosity of the particle, together with the length of the diffusion path between the cells and free interspace liquor,

must also be considered.

De Vocht *et al.*, (1983) reported on the influence of sedimentation and adhesion on the selection of methanogenic associations. They found that different process configurations using the same feed (ethanol and acetate) and inoculum (mixed digester sludge) resulted in the selection of differing predominant methanogenic species. The system, operated as a sludge blanket reactor, produced a sludge thought to contain large flocs of *Methanothrix* with few *Methanosarcina*, whereas the system when operated as a filter, using reticulated foam cubes, resulted almost exclusively in a culture of*Methanosarcina*. They found different patterns of gas production and concluded that the poorly flocculating methanogens were washed out; this resulted in a slow build-up of gas production and low sludge concentrations. The process based on matrix colonization was characterised by a rapid increase in gas production, and it was therefore assumed that biomass washout was negligible compared to the growth of *Methanosarcina* flocs in the cavities of the matrix. The resulting sludge activities in the reactor with support were higher than in the sludge blanket.

Further investigations with reticulated foam were attempted by Fynn and Whitmore (1982) who demonstrated that bacteria retained on BSMs in an enriched culture were resistant to washout. They stated that the preliminary evidence from scanning electron micrographs did not indicate any specific mechanisms involved in the attachment of the

bacteria to the support medium, but lipopolysacchride material (characteristically associated with the outer membrane or surface appendages) appeared to mediate in the process. Overall they concluded that high biomass concentrations could be achieved as compared with liquid cultures and that BSMs could be very suitable for process intensification, thus eliminating the need for solids recycle, to which the methanogens show poor resistance due to the variable settling properties of the methane producing bacterial flocs (Melchior *et al.*, 1982). In further work (Fynn and Whitmore, 1984) it was concluded that the most effective pore size, both in yielding the highest densities of methanogen colonization and in resisting washout at a dilution rate in excess of the critical dilution rate of the conventional CSTR culture, was 60 ppi, using cubes of 1 cm^3, with BSMs of larger pore sizes being significantly less effective. This is in agreement with Huysmen et al., (1983). It was found that flow velocities down to 1.5 cm/s were sufficient to remove almost completely the colonized biomass medium from the support indicating that the bacteria are easily removed from the particle. Again scanning electronmicrographs showed no evidence of attachment by polysaccharide fibres, which are found in many bacteria that adhere to surfaces (Corpe, 1980). They concluded that the methanogens were shielded from the full hydrodynamic force of the media flow, rather than entrapped within the medium. The colonized biomass was also resistant to washout when the BSM was freely suspended, provided there was little relative motion between the aqueous phase and the BSM. This could impose a limit on the potential for process intensification

in fixed or fluidised beds used for anaerobic digestion.

Both these extensive investigations produced results from well controlled laboratory digesters with feeds containing high proportions of substrates directly metabolized by methanogens. It is unlikely that most industrial wastes will be of this composition and therefore the biomass composition could be significantly different. The small quantities of inert solids also present in most wastes will probably affect colonization to a great extent (Lettinga, 1984). Fynn and Whitmore (1984) mentioned that in conventional single stage systems the other species present could lead to entrapment, and this is highly likely as the bacterial cell yield of non-methanogenic species is much higher than that of methanogens. The presence of small amounts of inert solids present in digested sludge, if used as an innoculum, may be benificial by providing a nucleus for granulation (Lettinga, 1984).

Calzada *et al.* (1984) reported the use of a non-reticulated foam as a support in the digestion of a coffee juice pulp. The pre-acidified effluent (only 50% acidified) produced a product gas containing around 90% methane at a rate of 3.42 m^3 gas/m^3 reactor/day. The methane rich gas was no doubt due to the fact that NaOH was used for pH neutralization of the feed, so resulting in the retention of carbon dioxide in the reactor liquor. They also observed that the results were higher than those produced in earlier experiments using the same substrate in upflow anaerobic tanks, and that they were good in comparison to those

obtained by Huysmen *et al.* (1983). However they do not reveal any details of the foam configuration, or the type of foam used.

2.4.3 Residence Time Distribution Theory

In an attempt to identify the degree departure from ideality of flow in a chemical reactor it is convienient to examine the residence time distribution (RTD) of the material which is flowing through the vessel. The simplest and most direct way of finding the RTD uses a physical or non-reactive tracer. If we consider the injection of a pulse of tracer at a time t=0 and measure the concentration in the effluent over a finite period until all the tracer is eluted, a response will be observed bearing some relation to the theoretical curves illustrated in Fig.2.9. The shape of the curve should enable selection of a suitable model to explain the departure from ideal (plug or completely mixed) flow.

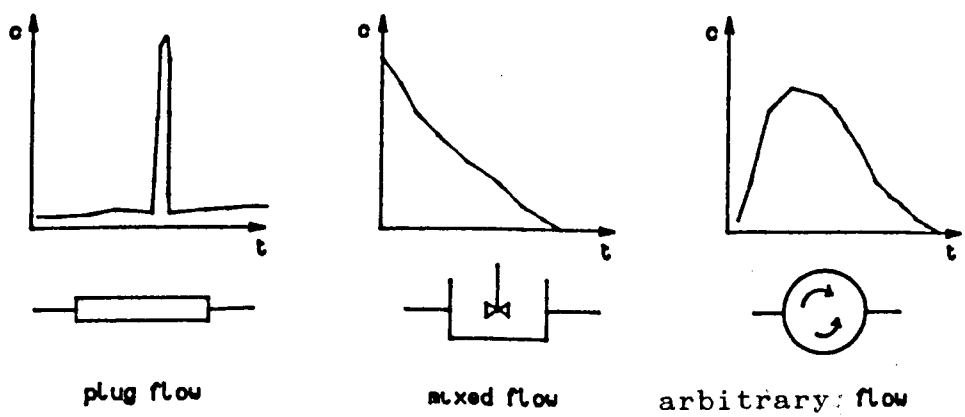


Fig 2.9 Tracer response curves for various reactor types

We may define the mean retention time of the molecules in the vessel as follows;

$$\bar{t}_{OBS} = \frac{\int tC\ dt}{\int C\ dt}$$

where $\bar{t}_{obs}$ = actual mean retention time of the system

The theoretical retention time in the system is given by:

$$\bar{t}_{th} = V/v$$

where V=theoretical reactor volume

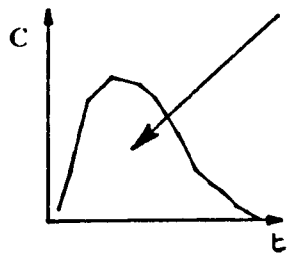
v =flow through the vessel

If there is no dead space in the reactor, then:

$$\bar{t}_{obs} \cong \bar{t}_{th} \quad \text{so} \quad \bar{t}_{obs} = V_{active}/v$$

A knowledge of the actual reactor volume thus enables the dead space to be estimated. It is convienient to normalise the scales on the concentration-time graph to give dimensionless parameters, as this enables the observed tracer curves to be matched to the results from theoretical curves for reactors for the purpose of model fitting. Considering the concentration time graph:

$$\text{area} = m/v = \int C\ dt$$

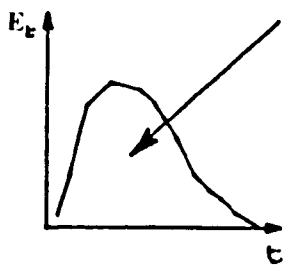


m = mass of tracer

v = flow through vessel

C = tracer concentration

we can normalise the data sets on the above curve to make the area under it equal unity:

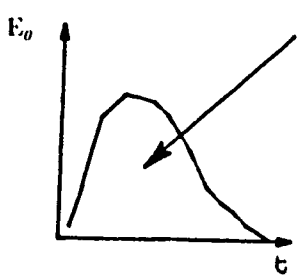


area = 1, $E_t = [v/m] \times c$

or by assuming

$$m/v = \int C \, dt$$

or, in terms of dimensionless parameters:



area = 1,

$E_\theta = t_{obs} E_t$

$= V_{obs}/v \times E_t$

$\theta = t/\bar{t} = [v/V_{obs}] \times t$

There are usually some departures from ideal flow and several models have been derived to attempt to explain this. The following are briefly described here:

(i) the dispersion model,

(ii) the tanks in series model, and

(iii) multiparameter compartment models.

2.4.3 The Dispersion Model

The dispersion model may be considered as plug flow of a fluid in a reactor upon which is superimposed some degree of backmixing (Levenspiel, 1972). The mixing processes involve a redistribution of material and may be considered statistical in nature. For molecular diffusion, in the x direction, the governing differential equation is given by Fick's Law:

$$\frac{\partial C}{\partial t} = \mathscr{D}_m \frac{\partial^2 C}{\partial x^2}$$

where Dm = the coefficient of molecular diffusion. An analogy may be drawn between this and the contribution of backmixing and may be represented by a similar equation:

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2}$$

where D_L = the coefficient of axial dispersion (this model only considers dispersion in the axial direction, not the radial direction). The basic differential equation representing this model becomes

$$\frac{\partial C}{\partial \theta} = \left(\frac{D}{uL}\right) \frac{\partial^2 C}{\partial z^2} - \frac{\partial C}{\partial z}$$

where the dimensionless group D/uL is called the vessel dispersion number and is the parameter which expresses the extent of axial dispersion:

as D/uL → 0 plug flow is approached, and

as D/ul → ∞ mixed flow is approached

This model usually represents the flow in real packed beds and tubes provided flow does not deviate not to greatly from plug flow.

2.4.3.1 Fitting the dispersion model for large elements of dispersion

If the boundary conditions at the exit and entry to the vessel are altered the observed tracer curve becomes skewed. In most cases these conditions may be described as either "open" or "closed". The term "closed" means that the inlet and outlet conditions can be considered as plug flow, thus not introducing any variance to the tracer step input. The mean and varience of an observed tracer curve may be fitted to the family of curves of the general equation:

$$\sigma_\theta^2 = \frac{\sigma^2}{\bar{t}^2} = 2\frac{D}{uL} - 2\left(\frac{D}{uL}\right)^2\left(1 - e^{-uL/D}\right)$$

with D/uL as the fitting parameter and iteratively solving for D/uL

2.4.4 The Tanks in Series Model

Another single parameter model frequently used to describe non-ideal flow is the tanks in series model. The

fluid flow through the vessel is considered as a series of equally sized stirred tanks, with the single parameter being the number of tanks in the chain N. By Laplace transforms:

$$E_\theta = (N\bar{I}_1)E = \frac{N(N\theta)^{N-1}}{(N-1)!}\, e^{-N\theta}$$

Thus the value of N may be obtained by matching the properties of the observed curves using:

$$\sigma_\theta^2 = \frac{1}{N}$$

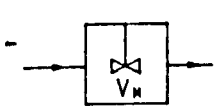
2.4.5 Multiparameter Models

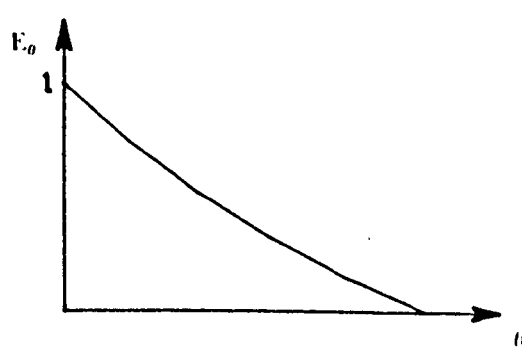
When one parameter models are unable to account satisfactorily for deviations from ideal plug or completely mixed) flow, more complex models must be adopted. These consist of different compartments interconnected by various means. Two will be considered here:

(i) mixed reactor with dead space

(ii) mixed and plug flow reactors in series

(i) Mixed reactor with dead space





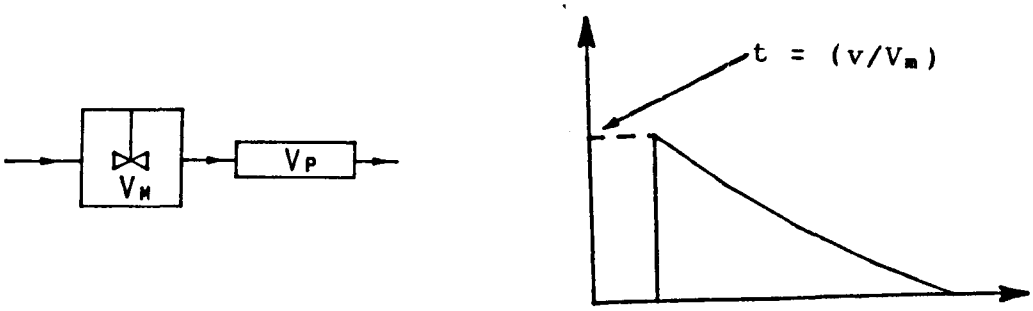
The decay portion of the observed tracer curve can be fitted to the equation:

$$E_\theta = [v/V_m]*EXP[(-v/V_m)/\theta]$$

using v/V_m as the fitting parameter this enables the V fraction to be estimated:

$$E_\theta = E_t \bar{t}$$

(ii) Mixed and plug flow reactor in series



The decay portion of the observed tracer curve (Eθ vs. θ) is:

$$E_\theta = (v/V_m)*EXP[\ (-v/V_m)t) + (V_p/V_m)$$

with v/V and vp/Vm as fitting parameters.

2.5 Parameter Definition and Analytical Measurements Applicable to Anaerobic Digestion

The term anaerobic digestion is applied to a microbiological process carried out by a largely undefined mixed population within a wide variety of types of digester, with feedstocks of different origin. These conditions may be well defined when treating a synthetic laboratory waste, but the full scale treatment of a real waste at pilot or full scale is a complex process and can only be partially defined by analysis. Describing a particular case such that it can be compared with another, or being able to make meaningful predictions for full scale operation from laboratory scale rigs, is difficult. Colin *et al.* (1983) make recommendations with regard to enabling adequate reporting of the apparatus and its performance. Colin further considered the analytical determinations required to describe a digestion process. The minimum data he considered necessary were:

(1) total solids, with or without TSS;
(2) volatile solids, with or without VSS;
(3) COD;
(4) ammoniacal nitrogen;
(5) volatile fatty acids (C2-C5);
(6) pH;
(7) total alkalinity;
(8) gas composition;
(9) specific gas production;
(10) energy flow, if full scale; and

(11) solid waste feed stock particle size;

Most workers seem to give information on all of the above as perhaps analytical methods for these determinations are well developed. Colin also gave suggestions for additional data, the majority of which is more difficult to obtain and beyond the range of a small laboratory. He finally gave recommendations for nomenclature for concentrations (biomass, substrate etc.), rates (volumetric loading rate, gas production rate) and yields (methane yield, conversion). It is most important that a set of definable parameters is agreed on, so that fair comparison of the various types of digester and process is possible.

2.6 Control of the Anaerobic Digestion Process

Various workers have made attempts to describe one or more parameters for use in the process control of anaerobic systems. This is probably to reduce the dependency on skilled process operators and so lower plant running costs. Because the overall microbiology of the process controls itself to a certain extent, process upsets are usually due to changes in the feed strength. Current monitoring methods include analysis of the feed, effluent and gas streams, but this is time consuming and the lag involved could result in process failure if, say, a toxic substance, present in the feed, was not detected early. Control problems arise from differences in the growth rates and the optimum pH of the synergistic bacteria involved in the fermentation. Symptoms of this include the accumulation of hydrogen and fatty acids

other than acetic. This imbalance must be corrected by reducing the feed rate or flushing the toxic intermediates from the reactor. The concentration of hydrogen and the types of fatty acid can therefore be used to control the feed rate. Wheatley and Cassell (1985) proposed a microprocessor-based control system capable of integrating information on pH, temperature, flow and alkalinity of the waste together with gas quality and quantity. This information would be used to control the digester feed rate, recycle, a heat exchanger and buffer addition. This system is currently under test.

Ripley *et al.* (1986) reported on the use of a modified alkalinity titration method to monitor process performance. Titration of a centrifuged sample to an endpoint of pH 5.75 (partial alkalinity, PA) and then to pH 4.3 (intermediate alkalinity, IA), makes it possible to distinguish the relative buffering contributions of both bicarbonate and volatile acid. This parameter is analogous to the volatile acid/alkalinity ratio but does not require the determination of volatile acids. Ripley reported that the successful digestion of poultry waste occurred at an IA/PA ratio below 0.3. It is a simple, inexpensive, yet sensitive technique.

The amount of hydrogen in the gas may be a sensitive guide to the metabolic state of biomass degrading wastes to methane (Gujer and Zehnder, 1983; Fernandes *et al.*, 1985; Mosey, 1985). Whitmore *et al.* (1985) found that the addition of glucose, propionate or butyrate resulted in increased hydrogen levels in the biogas and that this caused

inhibition of the degradation of volatile fatty acids and methanogenesis. They therefore concluded this may provide some form of control parameter.

Mosey and Foulkes (1983) first reported the use of hydrogen analysis in a laboratory digester using a polarographic probe. He compared the results from several U.K. sludge digesters and found that baseline values of hydrogen concentration were around 50 - 100 ppm. More recently Mosey (1985), stated that current redox probes would be unsuitable to measure the small changes in potential associated with a reduction in process performance, and he concluded that trace concentrations of H_2 are more easily detected in the biogas.

Archer (1986) reported the results of shock loading on the concentration of hydrogen in the biogas of a 6 m^3 digester treating effluent from a brewery. He stated that in normal process operation values of hydrogen from 0-15 ppm were measured. The majority of shock loadings produced a rapid increase in hydrogen concentration, peaking after 3-6 hours, but this soon declined to steady state values.

The disadvantages of using hydrogen in this way include the fact that cheap, reliable, and robust sensors are not avaliable, and that hydrogen measured in the gas phase may not be representative due to mass transfer to the liquid phase. Other gases present in the biogas may interfere with the results (e.g. H_2S, CO_2), and removal of these components could be difficult. Archer (1986) concluded that this

parameter should at present be considered only as a supplement to other more conventional indicators of process stability, and that further investigations were required together with the development of suitable probes.

Whitmore and Lloyd (1986) recount the use of mass spectrometry to monitor dissolved CH_4 and H_2 levels within thermophilic digester liquors and their use in process control by feed addition. Their results show that control was more easily achieved in continuously fed systems where the gas phase was in good contact with the liquid phase. In this instance the accumulation of inhibitory products would be diminished. The use of mass spectrometry is, at present uneconomic, and detection of hydrogen can be achieved more cheaply using polargraphic probes.

2.7 Detection and Quantification of Methanogenic Bacteria

2.7.1 Introduction

Many methods have been proposed for the estimation of the methanogenic activity of the biomass in anaerobic liquors and sludges. This activity can be directly correlated with its potential for waste stabilization. Many of the methods give nothing more than an indication, but a reliable technique would enable an anaerobic process to be more accurately controlled by monitoring the washout of methanogens from the reactor, especially during startup. A better understanding of where the bacteria proliferate in certain reactor systems and their relative activity within a

biomass aggregate would provide valuable information for both process and medium design and optimization. The methods available may be categorized as follows:

(i) identification by direct counting;
(ii) microscopic methods (fluorescence microscopy);
(iii) quantification of the various co-enzymes, unique to methanogenic bacteria;
(iv) immunosorbent assay;
(v) monitoring the uptake of radioactive substrates; and
(vi) more generalized methods based on ATP production.

2.7.2 Direct Counting Methods

On account of their extreme sensitivity to environmental oxygen, methanogens are difficult to grow compared with facultative and aerobic organisms. Recent advances in the culture of methanogens is based on the pioneering efforts of Hungate (1969). A modern technique used for the routine growth of methanogens in a pressurized atmosphere of 80% hydrogen and 20% carbon dioxide has been described by Balch *et al.* (1979). The procedure, using a modified Hungate technique, offers several advantages over conventional methods. The medium can be dispensed into culture tubes in an anaerobic chamber exposed to only low oxygen concentrations. The use of syringe transfer offers a high degree of protection from bacterial contamination and atmospheric oxygen. Methanogens are then cultivated on agar plates by the use of this type of chamber. After

innoculation the plates may be transferred to a cylinder which is pressurized to 2 atm. with a hydrogen/carbon dioxide gas mixture. Growth can then be followed by the methane production rate or a decrease in cylinder pressure.

2.7.3 Microscopic Methods

The development of microscopic methods for the detection of methanogens relies mainly on the fact that they have unique co-enzymes and co-factors which fluoresce when excited by ultra-violet light. Doddema and Vogels (1978) concluded that, although this method is useful for the detection of methane bacteria in mixed cultures, fluorescence at specific wavelengths may not be unique to methanogens. This technique has to be used with care for tentative identification only. Robinson *et al.* (1984) examined biofilms from anaerobic fixed film reactors filled with various packing materials. Their methods included light, UV, scanning and transmission electron microscopy. The two most commonly observed morphological types were long filaments, present at the film surface and sarcinae containing cysts. These were tentatively identified as belonging to the genera *Methanothrix* and *Methanosarcina* respectively. They concluded that mineral deposits were minimal for the wastewater studied (swine waste) after 12 months of process operation and not identified from the samples. The thick matrix holding the film together was believed to be a lipopolysaccharide that is commonly formed for protection and entrapment in environments with sufficient carbon (Fletcher and Floodgate, 1973).

Volcano-like structures were present in thick portions of the film, and these were probably necessary for the transport of nutrients into the biofilm and the transport of gas out. Their overall conclusions were that it was difficult to determine distinct species by these methods.

Richards and Turner (1984) compared a variety of methods for the examination of biofilms by scanning electron microscopy (SEM). They observed marked differences in micrographs from the same digester obtained with different sample preparation techniques. Their results show that fixation with glutraldehyde and drying methods may produce artefacts. They found cryo-sputtering to be the least disruptive method of sample preparation. Forster and Oakley (1985; 1986) seem to favour this type of method for assessing the relative proportions of *Methanothrix* and *Methanosarcina* in deep biofilms. In waste digesters treating complex substrates which have been seeded with sewage sludge, many other micro-organisms may be present as well as parts of insects, plant cells etc. Although microscopic techniques are useful in the tentative identification of distinct morphological types, they are unsuitable at least at present for use in quantitative estimations.

2.7.4 Assay of Co-enzymes

Methanogenic bacteria contain a number of co-enzymes and related compounds. These compounds are involved in the electron transfer reactions of methanogens (Vogels *et al.*, 1982), and are present wherever these bacteria predominate.

Various methods have been attempted to quantify these compounds to monitor methanogenesis in biomass using extraction and purification by HPLC and solvent extraction (Van Beelan *et al.*, 1983; Delafontaine *et al.*, 1979). Results of these studies showed that the correlations are good for co-enzyme F_{420}. However further work by Dolfing and Mulder (1986) suggested that their data indicated a poor correlation between F_{420} and biomass cultured on acetate. As around 70% of the methane in digestion is derived from acetate, this could mean the F_{420} content of methanogenic sludge might be unsuitable for the assessment of aceticlastic activity. Good correlations were only found with formate as the test substrate. It would seem this method is only reliable when certain substrates are utilized.

2.7.5 Enzyme Linked Immunosorbent Assay

Archer (1984) described the use of an enzyme linked immunosorbent assay (ELISA) for the detection and quantification of methanogens. Compared to co-enzyme assays, ELISA appears more specific and may be able to distinguish between different genera of bacteria. The method seems reliable for quantifying methanogens in defined cultures, but has only limited applicability to more complex systems.

2.7.6 Radioactive Substrate Incorporation

The estimation of aceticlastic activity has been attempted using radioactively labelled substrates by several

methods (White and Bobbie, 1977). Acetate incorporation into microbial lipids should mainly represent heterotrophic activity. Wright and Hobbie (1966) have detailed methods and results on the incorporation of acetate and glucose by bacteria and algae in aquatic ecosystems. Boone (1982) used an isotope dilution technique to measure acetate degradation in animal waste digesters. The population of aceticlastic methanogens can be approximated given the rate of acetate removal. The main advantage of this method is the speed with which it can be used. There are possibilities that this method may be adapted for the quantification of hydrogen utilizing methane bacteria. Uptake of the labelled substrate by non specific bacteria and other organisms can interfere and must be accounted for in the experimental design.

2.7.7 Methods Based on Gas Production

This type of method takes account of the fact that the rate of gas production is proportional to the number of methane bacteria present. The test substrate maybe selected to estimate the activity of different methanogenic species. It relies on the availability of a representative biomass sample, collected if possible under anaerobic conditions. van den Berg *et al.* (1974) described a method which records the rate of manometric liquid displacement in a Warburg type vessel using optical sensors. An analysis of the methane content of the gas is required as well. Valke and Verstraete (1983) developed a method which consists of adding increasing amounts of acetate to a series of sludge samples and determining the maximum rate of gas production

under non-limiting substrate conditions. Over this period biomass growth is minimal and acetate removal rates obey zero order kinetics. The gas is collected after passage through an NaOH solution to enable the methane volume to be read directly.

2.7.8 Other Methods

Methods have been described based on ATP assays (Holm-Hansen, 1973). These are not suitable for methanogenic bacteria (Stafford and Spensley, 1986), but may be used to quantify the viability of other species present.

2.8 Aims and Objectives

The aims and objectives of this study were:

(1) to assess the ability of open cell reticulated foam to entrap biomass in an anaerobic digester treating industrial wastewater;

(2) to evaluate media performance in various reactor operating regimes at pseudo-steady state (which may be defined as the condition in which, although feed and recycle rates are constant, the bacterial populations are in a state of constant change);

(3) to investigate the hydraulic flow regime within the different reactors used;

(4) to evaluate the performance of two stage systems;

(5) to determine the activity of the biomass and its concentration in the reactor during steady state;

(6) to examine the effect of changes in feed conditions on reactor performance;

(7) to assess the viability of the biomass within a support medium and to determine whether a substrate concentration gradient exists within the medium during steady state operation;

(8) to attempt to formulate preliminary ideas about modelling the operation of such a reactor containing media and to reinforce the model with experimentally obtained data; and

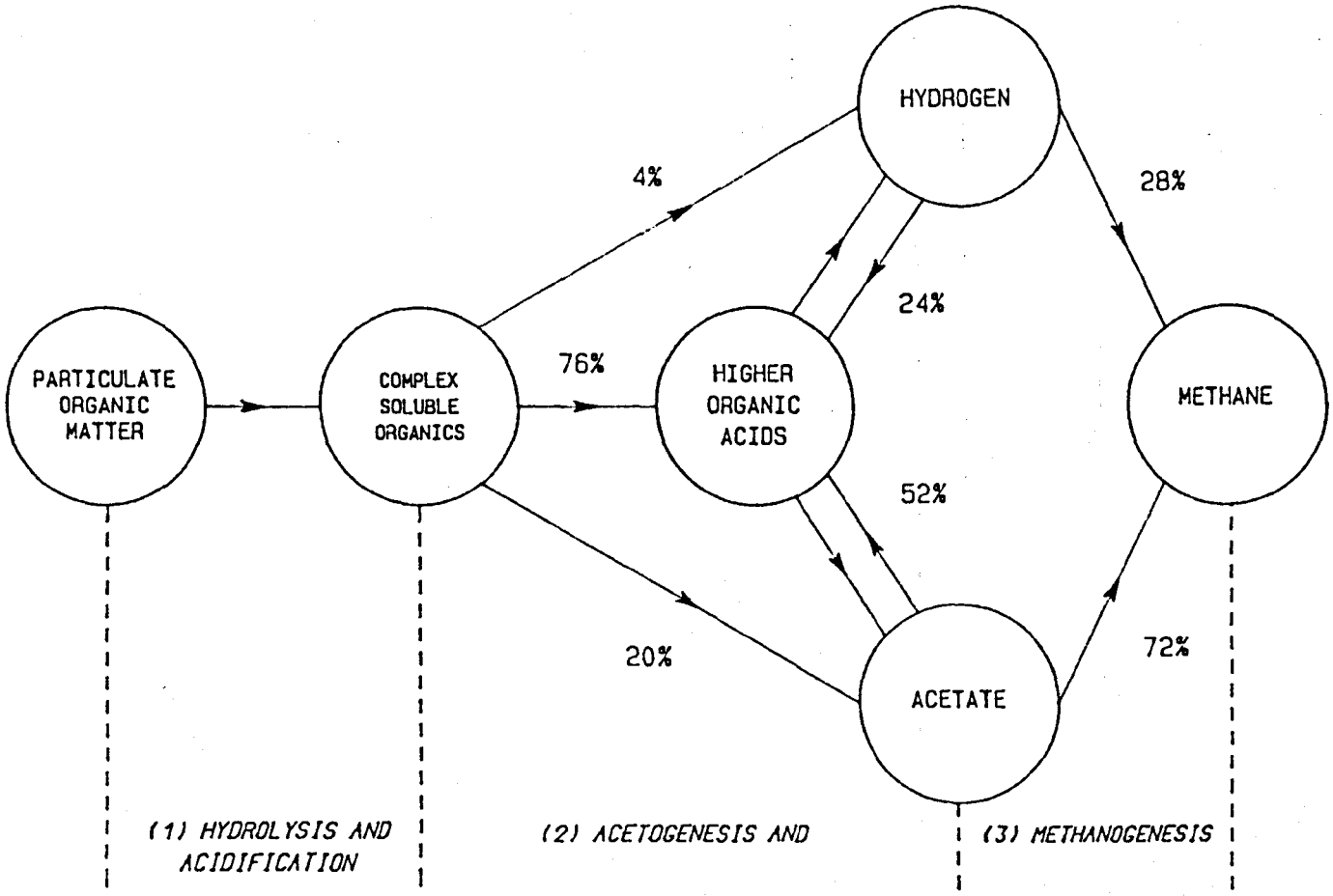


Fig. 2.1 The Stages Of Anaerobic Decomposition Of A Complex Substrate To Methane

(% represent the flow of electrons from organic compounds to methane)

(adopted from McCarty 1982)

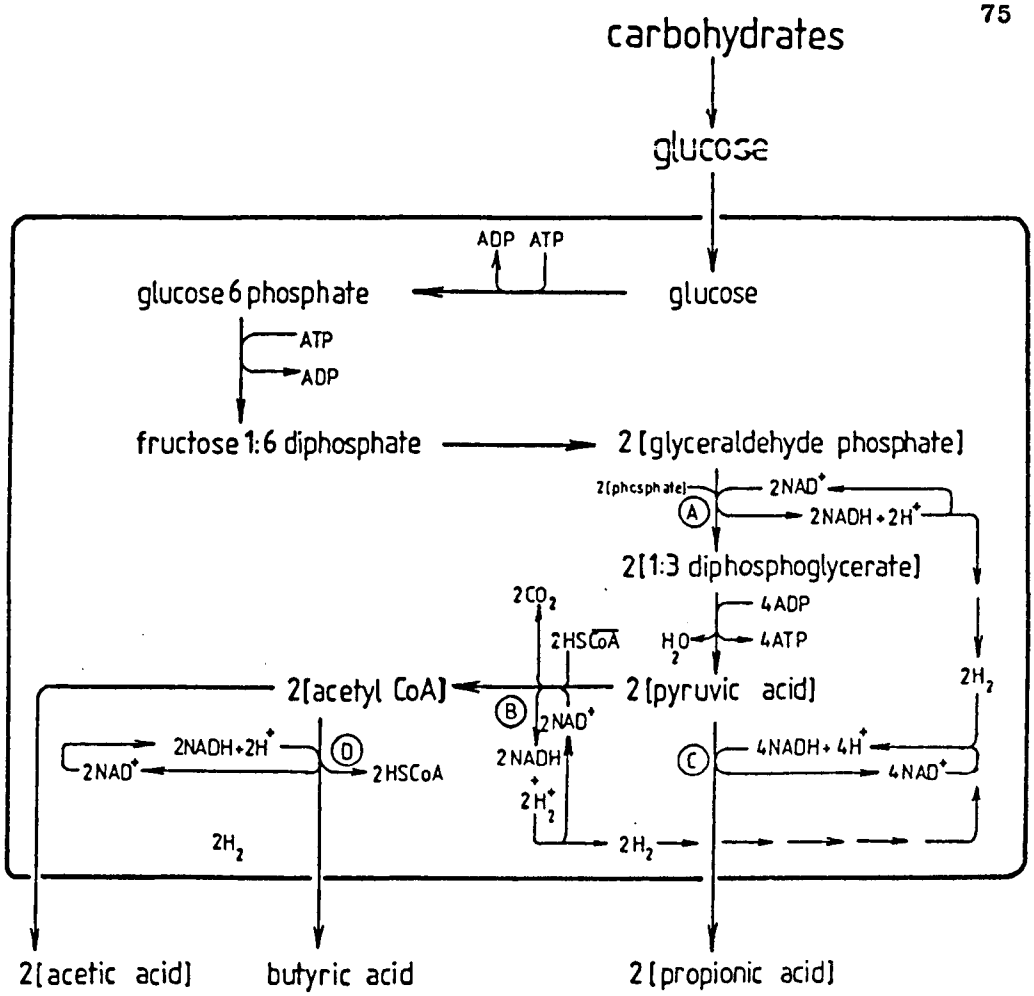


fig. 2.2 The Embden-Meyer Pathway for the degradation of glucose

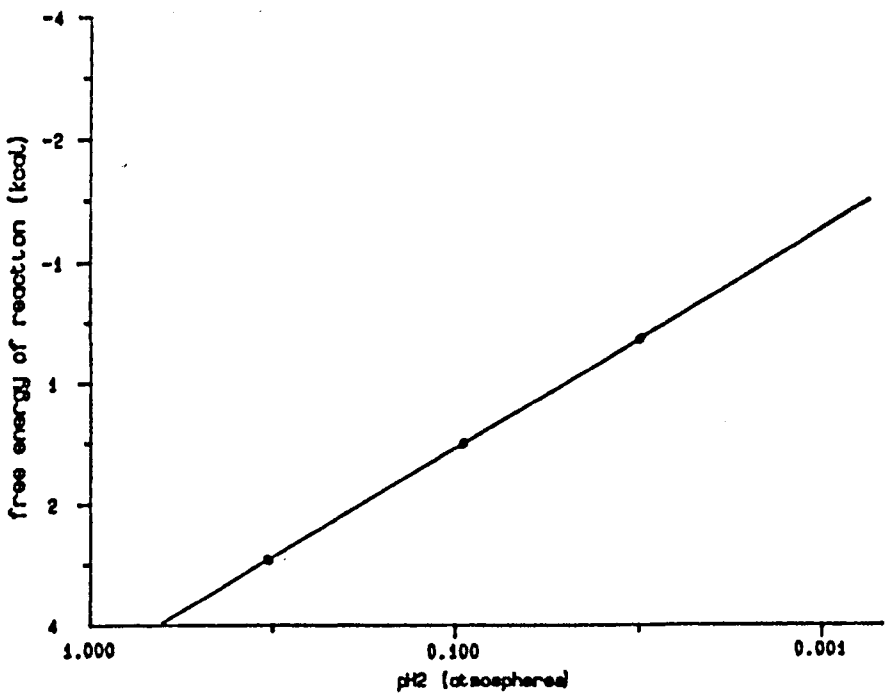


fig. 2.3 The free energy change for the oxidation of NADH

1 kcal = 4.184 kJ

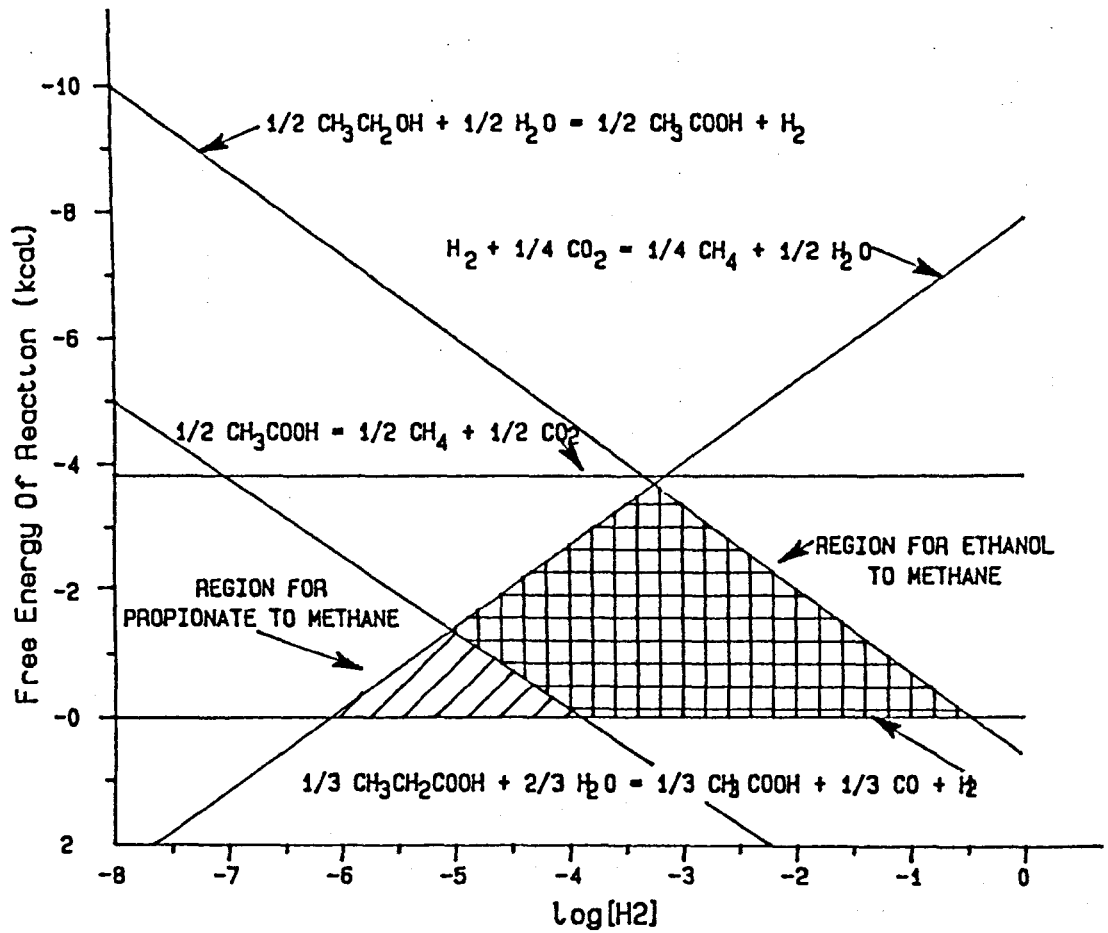


fig. 2.4 The effect of hydrogen partial pressure on the free energy of conversion of several substrates to methane

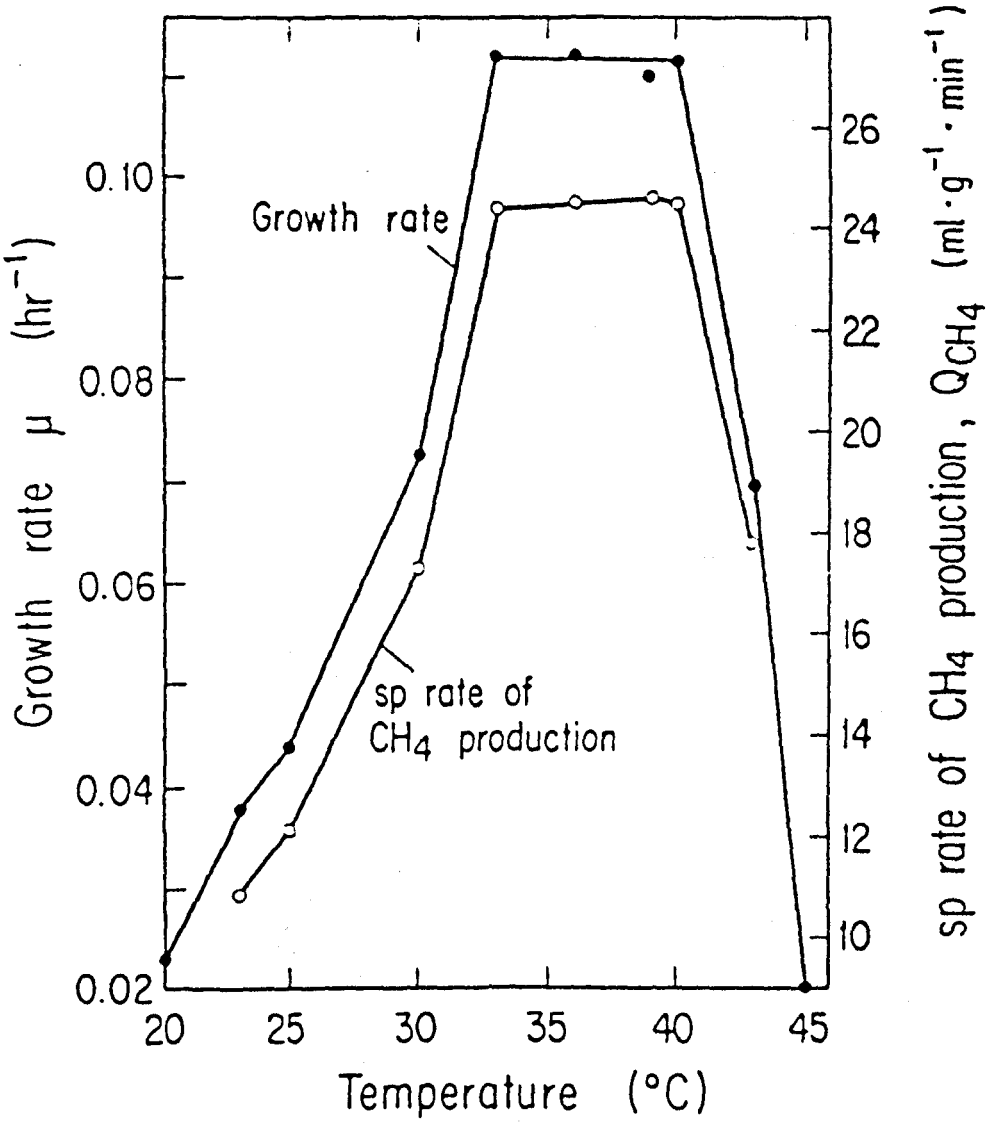


Fig. 2.5 Temperature dependency of *Methanobrevibacter arboriphilus* strain AZ (Zehnder and Wuhrmann, 1977)

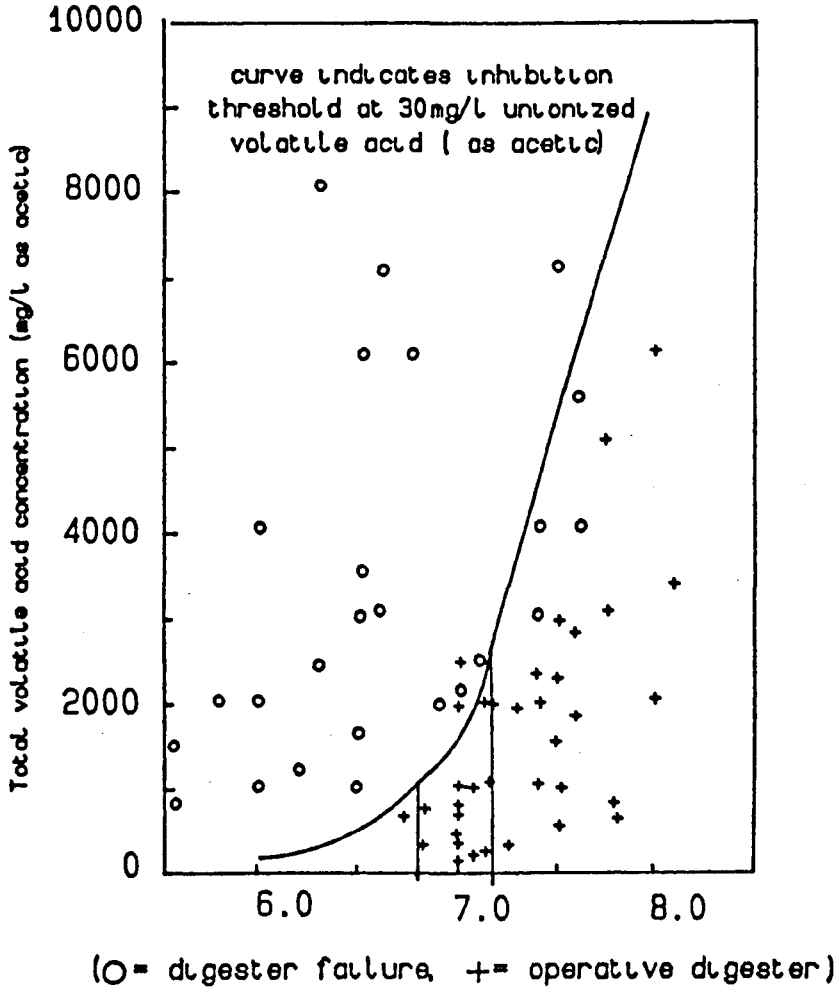


fig. 2.6 Effects of pH and total volatile acid concentration on digester stability (from Anderson et al, 1982)

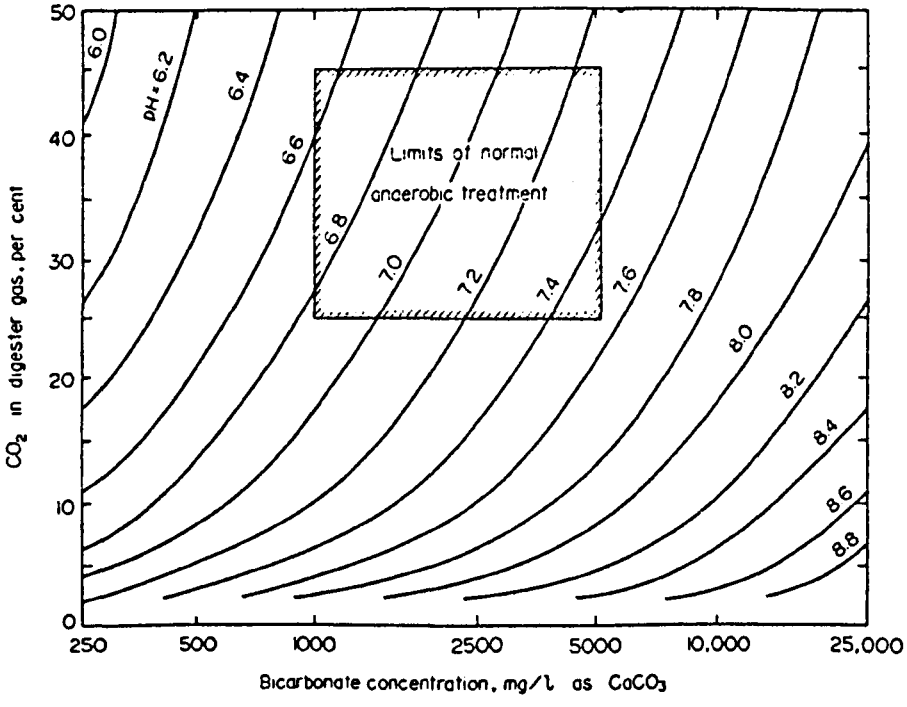


fig. 2.7 Relationship between pH, bicarbonate concentration and carbon dioxide concentration at 35 C (from McCarty 1964)

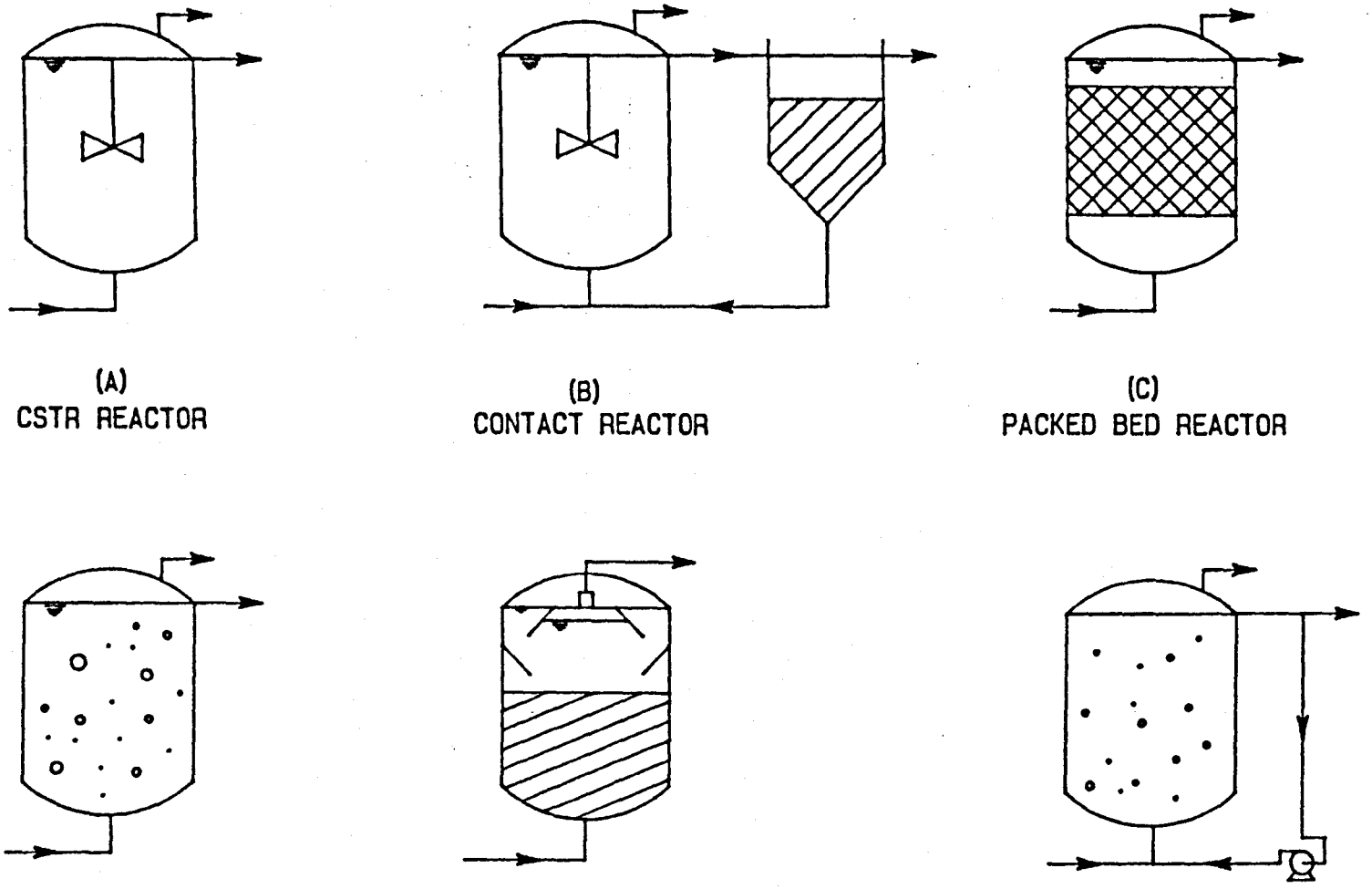


FIG. 2.8 Reactor types for anaerobic waste water treatment

SPECIES	MORPHOLOGY	SUBSTRATE
Methanobacterium	Long rods to	H_2 formate
Formicicum	filaments	H_2
bryantii		H_2
thermoautotrophicum		
.		
Methanobrevibacter	Lancet-shaped	H_2 formate
ruminatium	cocci	H_2 formate
smithii	short rods	H_2
arbophilus		
.		
Methanococcus	Motile irregular	H_2 formate
vanellii	small	H_2 formate
voltae	cocci	H_2formate
thermolithotrophicus		H_2 methanol
mazei		methylamines acetate
.		
Methanomicrobium	Motile short	H_2 formate
mobile	rods	
.		
Methanobacterium	Motile irregular	H_2formate
cariaci	small cocci	H_2 formate
marisnigri		
.		
Methanospirillum	Motile regular	H_2 formate
hungatei	curved rods	
.		
Methanosarcina	Irregular cocci	H_2acetate methanol
barkeri	as single cells	methylamines
.	packets	
Methanothrix	Rods to long	acetate
soehngenii	filaments	
.		
Methanothermus	Non-motile	H_2
fervidus	rods	

Table 2.1 Characteristics of methanogenic species in pure culture

PARAMETER / CULTURE	μ_{max} Maximum specific growth rate @ 35 C d-1	Y_{max} Maximum yield co-eff. kgVss/ kgCOD	$R_x = \mu_{max} / Y_{max}$ Maximum substrate removal rate @ 35 C kg COD/(kgVSS.day) VSS 100% active	50% active	K_s Half velocity constant kgCOD/m3
ACETIC ACID PRODUCING BACTERIA	2.0	0.15	13	7.5	0.2
METHANE PRODUCING BACTERIA	0.4	0.03	13	7.5	0.05
COMBINED CULTURE	0.4	0.18	2	1	-

Table 2.1a growth constants of the predominant bacterial groups in anaerobic digestion (henze and harremoes, 1983)

COMPOUND	BENEFICIAL CONCENTRATION IN STUDY g/L	EFFECT	LITERATURE REFERENCE
Fe ++	0.2	Precipitation of Sulphide	Speece and McCarty (1964)
		Flocculation/ biofilm structure	
	12-120 (soluble)	-	Hoban and van den Berg (1979)
Ni ++	0.01	Synthesis of F420 co-factor in methanogens	Thauer (1981)
	0.006	Increase in activity	Murray and van den Berg (1981)
Mg++	0.01-0.02	Flocculation	Lettinga et al (1980)
Ca++	0.01-0.04	Flocculation	Lettinga et al (1980)
Ba++	0.01-0.1	Flocculation	Lettinga et al (1980)
Co++	0.01	Vitamin B12	Speece and McCarty (1964)
	0.003	Increase in activity	Murray and van den Berg (1981)
SO--	0.02	Increase in activity	van den Berg (1980)

Table 2.2 Beneficial concentration of trace nutrients in anaerobic digestion (Henze and Harremoes, 1983)

SUBSTANCE	MODERATELY INHIBITORY mg/l	STRONGLY INHIBITORY mg/l
NA +	3500-5500	8000
K +	2500-4500	12000
Ca +	2500-4500	8000
Mg ++	1000-1500	3000
Ammonia-nitrogen	1500-3000	3000
Sulphide	200	200
Copper (Cu)	-	0.5 (soluble)
.	.	50-70 (total)
Chromium VI	-	3.0 (soluble)
.	.	200-260 (total)
Chromium III	-	180-420 (total)
Nickel	-	2.0 (soluble)
.	.	30 (total)
Zinc	-	1.0 (soluble)

Table 2.3 inhibitory concentrations of inorganics reported to be inhibitory to anaerobic digestion (from Parkin and Owen, 1986)

SUBSTANCE	INHIBITORY CONCENTRATION mg/l
Formaldehyde	50-200
Chloroform	0.5
Ethyl Benzene	200-1000
Ethylene Dichloride	5
Kerosene	500
Linear ABS (detergent)	1% of dry solids

Table 2.4 concentrations of various organics reported to be inhibitory to anaerobic digestion (from Parkin and Owen, 1986)

Volatile Fatty Acid concentration < 1.5-2.5 kg HAc/m3
Hydraulic Retention Time > 1 day
High alkalinity
No fluctuations in environmental conditions
Ca(OH)2 for neutralization
Calcium may promote attachment

Table 2.5 Observations and Recommendations
dduring startup of a fixed film
anaerobic reactor
(Henze and Harremoes, 1983)

CHAPTER THREE: MATERIALS AND METHODS

3.1 Background to the Site and Wastewater

The main criteria for selection of a suitable industrial waste for this project were as follows;

(1) reasonably high strength (>5000 mg/l COD);
(2) absence of suspended solids in feed;
(3) absence of inert solids in the feed;
(4) absence of toxic substances in feed;
(5) reasonable flow rate permitting low HRTs(< 0.5 days); and
(6) a wastewater of reasonably constant composition

The site selected for the study was John Morley (Importers) Ltd., Congleton, Cheshire, a company primarily concerned with the sorting, washing and packing of dried fruit imported from abroad including: sultanas, raisins and currants. Other activities included the seasonal production of confectionery mincemeat and small amounts of other foodstuffs. The waste consisted of the washwater from the production line together with the floor washings at the end of each day's production. No disinfectants, only cold water, were used for this purpose, although occasionally a mild bactericidal detergent ("Ceutegleme B" Century Oils Ltd.) was used. Domestic bleach (hypochlorite) was also used occasionally on the floors and machinery. The waste water contained large amounts of sugar together with other matter washed from the surface of the fruit. Some fruit had been

fumigated with sulphur dioxide, and many sultanas had been dipped in olive oil and potash to assist in the sun drying process. All of the fruit had been fumigated with methyl-bromide before shipment to the U.K. No evidence was found of this in the effluent and it was assumed it had been diluted to negligible levels. From the factory drains the effluent collected in a sump at the rear of the works where the solids were allowed to settle. The supernatant was pumped to the municipal sewer system in winter or used to irrigate grass fields in the summer. Periodically the sump solids were emptied by vacuum tanker. An electroflotation unit had been installed for removal of the large solids prior to field irrigation but was considered too expensive to run and seldom used. The typical daily flow was around 50 m^3/day and disposal of this cost the company approximately £30,000 per annum.

3.2 Pilot Plant Description, Design Considerations and Upstream Treatment

3.2.1 Single stage process (Figure 3.1, Plate I)

The sump, where the wastewater collected, was a below-ground concrete tank, approximately 15 m^3 in volume (3 x 1.5 x 2 m). The supernatant for the digesters was withdrawn through a stainless steel mesh filter of hole size approximately 5 mm square, the purpose of which was to remove large particles of fruit and other solid matter. A Mono MS single phase pump (Mono Pumps Ltd., Manchester), controlled by a time switch, was used to transfer this to

the feed buffering tank at a rate dependent on the demand of the digester. The feed buffering tank was approximately 3 m^3 in volume (1.2 x 2.3 x 1.1m) and fabricated from galvanized steel. Its purpose was to balance the flow from the sump and allow further separation of particulate material in the absence of larger particles. An additional filter, consisting of a block of reticulated foam was used as a final filter upstream of the methane reactor feed pump. A Watson-Marlowe MRSH peristaltic pump (Watson-Marlow Ltd., Falmouth) was found most suitable for this purpose on account of its wide range of pumping rates. An increased range of feed rate was achieved by use of a timer on the pump. Downstream of the feed pump the fresh feed was combined with the recycled liquor before the pH was corrected by the addition of aqueous NaOH. From here this stream was pumped to an industrial flow heater rated at 6 kW (Baker Electrical Ltd., Rotherham), mounted inside a housing fabricated from 4-inch diameter steel pipe with flanges welded at each end. The heater was placed externally to facilitate easy removal for inspection and cleaning without disturbing the contents of the methane reactor. The heater was controlled by a thermocouple sensor positioned in a pocket in the top of the heater assembly. Downstream of the heater the feed was pumped into the methane reactor through a flow distributor constructed from uPVC pipe (1.5 inch dia.). The branches of this were drilled with 5mm holes at 200mm centres.

The methane reactor, and associated equipment, was fabricated in 6mm steel sheet rolled and butt-welded. The

various inlet and outlets were provided with 2 inch BSP fittings and flanges. The lid was bolted to the tank body through a flange rolled from 90° angle-iron welded to the tank rim. A gas tight seal was affected by a soft-rubber gasket and other joints were sealed with silicone sealant.The internal packing was supported above the distributor on a mesh platform (10 mm sq. holes), which was itself supported on a bolted angle iron framework. The tank had an internal diameter of 1.5 m and a total depth of approximately 1.8 m. The packing was random dumped on the lower mesh and retained by a similar mesh above the bed to arrest the partial bouyancy of the biomass support medium. The water level was maintained by an open ended pipe (with mesh filter) supported in the centre of the reactor. This pipe was connected to the flanged outlet and the external pot provided a water seal to the reactor. All exposed metal surfaces were treated with a non-toxic, 2-pot epoxy paint. All exterior surfaces were lagged with 2 inch glass wool encased in aluminium, and external pipes foam-insulated.

Two identical tanks were constructed both taking feed from the same holding tank.The reactors were fabricated, moved to site, and erected by Simon-Hartley Ltd. (Etruria, Stoke-on-Trent, Staffs.)

3.2.2 Temperature Control and Monitoring

The reactor temperature was controlled with a single thermocouple probe situated at the outlet of the heater. This copper/constantan thermocouple was connected to a

temperature controller (PD control action, Anglicon Ltd., Newhaven) which provided a logic pulse output to control the solid state relays capable of switching current to the flow heater. Temperature throughout the tank contents was monitored using 8 thermocouples positioned at various points in the reactor. The output was displayed on a Farnell DT22 Digital temperature indicator (Farnell Instruments, Leeds). It was found that even under the most severe conditions, for example cold ambient temperatures and low recycle rates, no more than a 2°C temperature difference existed throughout the whole reactor.

3.2.3 pH Control

The reactor pH was achieved with a Petracourt Phm6 2-point controller (Gallenkamp Ltd., Stockton -on-Tees). The combination pH probe was mounted in the feed/recycle line and was connected directly to the controller which drove the diaphragm pump for dosing caustic soda (Prominent A0503N, Prominent Pumps Ltd., Heidelberg). Various alkali sources were tried, but it was found a "pearl" caustic soda (ICI Ltd.) was the most suitable and easily handled. The pH as measured by the probe provided an indication of the conditions within the tank, and a separate laboratory pH meter was used to determine the pH of the liquor exposed to the atmosphere. The in situ probe was calibrated monthly and showed little susceptibility to errors.

3.2.4 Pump Motor Control

The feed pump to the buffering tank and the methane reactor were both controlled by an interval timer, which enabled the pump to be switched on or off at any number of 15 minute intervals over the 24 hour period. All motors were single phase, direct on line start.

3.2.5 Gas Collection System

From the top of the reactor the gas was piped to a dry-bellows type gas meter (model no. AC175TC, International Gas Apparatus, Camberley, Surrey, temperature compensated to 60°F), using 3/4 inch uPVC pipe, via a condensate trap.

3.2.6 Recycle Off-take Point

For the purposes of this study provision was made for two take-off points from the methane reactor: one from the outflow pot (but later from the liquid space at the top of the reactor), and the other at the base of the reactor, beneath the packed bed and on the opposite side to the distributor/inlet.

All instrumentation and control apparatus was panel mounted in a site hut adjacent to the plant.

3.2.7 Reactor Packing Materials

There are several methods available for the reticulation of plastic foams. The product characteristics depend on the techniques used and the starting material. The

two most popular foams are polyester and polyether polyurethanes. The foam used in this study was of the polyester type (produced by Declon Foam Plastics Ltd., Dunstable). It was reticulated by a thermal method giving a smooth filament, as opposed to those produced by a chemical etching method which give jagged "windows" on the filaments. The polyester polurethanes are very resistant to biological attack and hydrolysis.

Of the two reactor configurations tested the space provided for the packing material, one was filled with randomly dumped 25 mm cubes of 10 pores per inch (ppi; 97% porosity); 100,000 foam pieces were used, giving a packed volume of approximately 2.00 m^3 and a total foam volume of approximately 1.56 m^3 (bed porosity approximately 78%). The other was filled with large blocks of foam , cut to shape and completely filled the packed section with no voids other than the pore space within the foam. This in effect represented a solid block of foam (10ppi).

3.2.8 Pilot Plant Startup

Various regimes have been recommended for the startup of anaerobic processes (Henze and Harremoes, 1983). Municipal digester sludge was used as an inoculum for this study as it was easily obtainable from the Kidsgrove sewage treatment works. The reactors were filled with water to operational level and heated to 35°C, the operation of recycle and feed pumps were then checked. The sludge was

transported to site in oil drums, care being taken to ensure minimum aeration of the sludge. The water in the reactors was displaced by pumping in sludge, which had been previously screened by passing it through an in-line filter fabricated from stainless steel mesh (5 mm holes sq.) . Approximately 15-20% of the water in the reactor was then displaced by the inoculum. The recycle pump was started, pumping through the bed in an attempt to disperse the sludge throughout the packed section of the reactor. A small amount (approximately 25 litres) of wastewater was pumped into the reactor, and the reactor then left to stabilize for four days. Feeding with effluent commenced on the fifth day, when the initial gas production had subsided, at a loading rate of 0.1 kg COD/m^3/day.

3.2.9 Two-stage System (Figure 3.2, Plate II)

The second stage used the reactors described in 3.2.8 with the addition of an acidification reactor in place of the feed buffering tank. In this mode of operation feed was withdrawn directly from the sump through a filter, and then combined with the recycle stream. The acidification reactor was heated with an independent immersion heater and thermostat within the tank and completely mixed by a high rate recycle pump (Mono MS single phase pump, Mono Pumps Ltd.) The tank was fabricated in polypropylene and its volume was approximately 1m^3. The method of liquid seal was similar to that in the main methanogenic reactor. No facility was provided for gas collection from the acidification reactor.

3.3 Sampling Methods and Apparratus

3.3.1 Liquid Samples

During process operation the reactor feed and effluent were monitored daily. Representative samples were taken from the feed pipe into the reactor. This was disconnected from the tank for this purpose and allowed to pump for several minutes until the disturbed solids had been flushed out. Samples of reactor effluents were taken from the outflow ports by the use of small bore flexible pipe in a siphon arrangement. Care was also taken to ensure samples were taken from the point where the outflow pot was connected to the tank to ensure all the effluent solids were recovered before settlement. It was found that one daily grab sample of feed was all that was necessary, as the feed strength varied little throughout the day; this was probably due to the effect of the feed buffering tank and the sump. Samples were analysed on the day of collection or stored at 4°C for later analysis, observing standard methods of sample preservation (Standard Methods, 1980).

3.3.2 Gaseous Samples

A rubber septum (Subaseal, Leeds) was incorporated in the gas pipe from the reactor upstream of the meter. Gas samples were withdrawn from this line with a syringe and analysed within 1-2 hours. During this time contamination of the samples from the atmosphere was found to be negligible.

3.3.3 Solids and Biomass Support Particle Samples

Various methods were attempted to withdraw samples of solids from the reactor. The most successful proved to be a long stainless steel rod approximately 5 mm dia. with a 5 mm bore plastic pipe secured to it with cable ties. The rod was calibrated in 0.5 m intervals. The probe was inserted into the reactor bed through the chimney in the lid and samples withdrawn by a siphon arrangement, initiated by a syringe. Anaerobic samples could be abstracted by this method if the receiving vessel was sealed and flushed with nitrogen prior to sampling This method gave an indication of the interstitial solids within the reactor bed if the probe was not agitated violently. If the probe was agitated a thicker sludge was obtained due to solids dislodged from the biomass support medium. Care was taken to ensure this did not occur. Examinations of colonized particles taken from the reactor showed that significant biomass could only be removed by fairly vigorous action such as squeezing the pads.

Intact pad samples were successfully removed from the top section of the reactor packed bed manually, but not from the bottom section.

3.3.4 Sampling Frequency

During normal process operation liquid samples were

taken daily, except at weekends, as grab samples of 1 litre volume. Gaseous samples were taken weekly. Solids samples of the biomass in the reactor were only taken at the end of each period of the study due to the disturbance this caused to the system. Composite samples were unnecessary on account of the buffering effects of the sump and feed buffering tank.

3.4 Analytical Methods

All total liquid samples refer to those of feed and effluent; they included settlable matter but excluded large floating particulates or submerged agglomerates. All filterable samples refer to the filtrate obtained by passing a total sample through Whatman GF/C filter papers.

3.4.1 Chemical Oxygen Demand (COD)

COD was determined by the dichromate reflux method, using suitable sample dillutions (Standard Methods, 1980). Samples not immediately analysed were preserved by acidification to pH 2 with conc. H_2SO_4 and stored at 4°C.

3.4.2 Total Solids

Total solids were determined on liquid and semi-solid samples by drying at 105°C for one hour. Samples were analysed as soon as possible after sampling. Large floating particulates or submerged agglomerates (i.e. dried fruit parts) were excluded (Standard Methods, 1980)

3.4.3 Volatile Solids

Total volatile solids were determined on liquid and semi-solid samples by ignition at 550°C for twenty minutes (Standard Methods, 1980)

3.4.4 Suspended Solids

Suspended solids were determined on liquid and sludges from the residue obtained after filtering through Whatman GF/C filter papers (70 mm dia.) and drying at 105°C for one hour (Standard Methods, 1980).

3.4.5 Volatile Suspended Solids

Volatile Suspended Solids were determined on liquid and sludge samples by ignition at 550°C of the residue obtained after passage through Whatman GF/C filter papers (70 mm dia.) for twenty minutes. All solids measurements were performed in duplicate.

3.4.6 Biomass Support Medium Solids

The determination of pad solids was achieved by removal of the biomass from a support particle in a known volume of water by violent agitation until the support matrix visually appeared biomass free. Suspended solids were then determined as described above.

3.4.7 Volatile Fatty Acids (VFA)

Total volatile fatty acids can be determined colorimetrically (Montgomery *et al.*, 1962) or by titration (James and Martin, 1952), but the use of gas liquid chromatography (GLC) (Pye Unicam model no. 304, Pye Unicam, Cambridge) enabled each individual acid to be determined to a high level of accuracy.

In this study VFA determinations were made on samples of feed, digester liquor and effluent by the method of Banfield *et al.* (1978). The addition of a small amount (10%) of pure formic acid to the samples and standards helped to prevent peak tailing and ghosting. Samples were filtered through Whatman GF/C filter paper to remove solids. Those not analysed immediately were acidified with formic acid and frozen until analysed. The sample (0.5 microlitre) was directly injected into the column using a 1 microlitre syringe (Hamilton no.7001NWG). The column, 2 m in length x 2 mm i.d., was packed with 5% Free Fatty Acid Phase (FFAP) on Chromosorb G (AW.DCMS) 80-100 mesh (Phase Separations Ltd., Queensferry, Clwyd). This was installed in a Pye Unicam 304 GLC equiped with a flame ionization detector. The column temperature was maintained at 150°C, the injector at 175°C and the detector at 200°C. The GLC was interfaced with a Pye Unicam SP3400 computing integrator to calculate peak areas for quantitative determination of each acid. The integrator was calibrated directly to give readout in acid concentrations by an external standard method. The overall accuracy was better than 5%.

3.4.8 Total and Soluble Carbohydrate

The determination of sugars and related substances was achieved by the method of Dubois *et al.* (1956). This was selected as the majority of carbohydrate in the feed was present as fructose and other low molecular weight sugars. Both total and soluble carbohydrate were measured. Glucose was used as the standard for calibration in the assay. The test may only be considered relative as the proportion of carbohydrate detected is dependent on its ability to be hydrolysed under the conditions of the test.

3.4.9 Nitrogen (as Ammonia)

Ammonia was determined by direct nesselerization following preliminary distillation (Standard Methods, 1980).

3.4.10 Nitrogen (Kjeldahl)

Kjeldahl Nitrogen was estimated from a macro-digestion followed by direct nesslerization (Standard Methods, 1980).

3.4.11 Phosphate

Phosphate was determined on filtered samples by a colorimetric method in which molybdophosphoric acid is formed and reduced by stannous chloride to molybdenum blue in aqueous solution (Standard Methods, 1980)

3.4.12 pH

A Petracourt PHM6 laboratory pH meter together with Russell (Russell pH Ltd., Auchtermuchty) combination gel-filled electrode was used for the immediate on-site determination of the pH of samples of feed and effluent. The pH in the reactor was measured in situ by the pH indicating controller. This laboratory probe was also used to calibrate the in situ probe on a regular basis.

3.4.13 Digester Gas Volume

The daily gas production was measured with a dry bellows type gas meter (described in section 3.2.5). This was temperature compensated. Variations in barometric pressure were ignored.

3.4.14 Digester Gas Composition

The digester gas was analysed for N_2, O_2, CO_2 and CH_4 by gas liquid chromatography, Pye Unicam 304 equiped with a thermal conductivity detector. Gaseous samples were obtained from the septum in plastic 50 ml syringes and directly injected in to a gas sampling valve which effectively injected a 0.5 ml sample into the column 2 m in length x 2 mm i.d. containing Carbosieve S 100/120 mesh (Phase Separations Ltd., Queensferry). The column was maintained at 150 °C with the injector at 175°C and the detector at 200°C. The carrier gas was helium at a flow rate of 40 ml/min. The

GLC was linked to a computing integrator (Pye Unicam SP3400) to quantify the results. Calibration was achieved with aerosol canisters of pure gases (Phase Separations Ltd.). Hydrogen sulphide was occasionally estimated by a titrimetric method (Sawyer and McCarty, 1967).

3.4.15 Feed and Recycle Rates

Feed and recycle rates were measured manually using a calibrated container (25 litres) and a stopwatch. Feed rate measurements were initially made at the outlet of the feed pump, at low rates of flow, and the flexible outlet pipe was positioned to give a delivery head equivalent to that in operation. At higher flowrates the volume of effluent was measured at the effluent outlet, after a period of steady flow.

3.4.16 Alkalinity

The alkalinity of feed samples was determined periodically by potentiometric titration to give estimates of total and bicarbonate alkalinity (Standard Methods, 1980)

3.4.17 Tracer Studies

The movement and hydraulic flow regime of a wastewater through a process may be quantified by the use of appropriate radio-active tracers (Iwugo and Winnicki, 1976). However, authorization is required before these man-made

isotopes can be used in the U.K. The use of dyes in tracer studies has the advantages of less delay and the fact that they may be more conveniently handled by unqualified personnel. Several fluorescent dyes have been employed for this purpose in sewage works but they only have a limited application owing to their absorption onto organic and inorganic particles (Smart and Laidlaw, 1977). Brown *et al.*, (1984) described the use of bromophenol blue as a sewage works tracer and concluded that it had the following characteristics which made it most suitable as a tracer for the purposes of this study;

(1) minimal absorbtion onto organic and inorganic particles;
(2) minimal degradation in both aerobic and anaerobic conditions;
(3) amenable to quick sensitive estimation by visible absorbance ; and
(4) high sensitivity (+10% at conc. of 20 ug/l) and suitability for a wide variety of wastes provided that the pH is >5

A modification of this method was used in this study. Five gms of water soluble bromophenol blue (Sigma Ltd., Poole) were dissolved in 1 litre of warm water. This was poured into the bottom of the reactor into the suction side of the recycle pump (gulp injection). This assisted in the rapid induction of the dye into the reactor at the beginning of the experiment. An automatic sampler (Bestel Dean model no. BD2306) was utilized to sample directly from the effluent

outlet at intervals of 1 hour. Sampling was continued for three retention times or until the absorbance fell below the limit of detection. The samples obtained were collected and checked to ensure the pH was greater than 5; this was corrected, if necessary, with 1 or 2 drops of conc. sulphuric acid to ensure that the dye was totally ionized. Fifty ml samples were filtered through Whatman GF/F filters prior to spectroscopic analysis. The absorbance of each sample was measured at 590 nm with a 1 cm light path. A sample of the reactor effluent, treated in the same way but before the addition of the dye into the reactor, was used as the blank.

3.4.18 Sludge Activity Tests

The method adopted to estimate the acetoclastic activity of the biomass has been described by Valke and Verstraete (1983). Biomass and pad samples, collected by the methods described previously (section 3.3.3), were transported to the laboratory for immediate analysis. The apparatus used for this method is illustrated in Fig.3.3. The technique consisted of adding increasing amounts of acetate to a series of sludge samples and determining the maximum rate of methane production per litre of mixed liquor. It can be assumed that during a maximum of 24 h the biomass growth is minimal, and that acetate conversion rates obey zero order kinetics and are not affected by substrate concentration between certain limits (Valke and Verstraete, 1983). It is assumed that under the conditions of the test, the sludge attains maximum activity. Based on a survey of

the literature and enrichment studies, the maximun specific activity of acetate cleaving bacteria at 30-35°C can be approximated to 1000 ml CH_4/g VSS day.

Method

From a sample of sludge SS and VSS were determined. A mineral solution containing 2.5 g KH_2PO_4 ; 1.0g/K_2HPO_4 ; 1.0 g NH_4Cl ; 0.1 g $MgCl_2$; 0.2 g yeast extract ; and 0.1 g $Na_2S.7H_2O$ per litre of tap water was prepared (pH 6.7). The sludge sample was diluted with this mineral solution to a VSS conc. of approx. 5 g/l to ensure non-limiting substrate conditions. Samples of this diluted mixed liquor were added to 1 litre bottles and then acclimated at 35°C for 24 h care was taken to minimize contact of the biomass and liquor with the air. After this period different known amounts of acetate(0.3 - 1.0 g HAC/g VSS) were added to a series of these flasks. These flasks were corrected to pH 6.7 with 1 N HCl or 1 N NaOH and flushed with nitrogen for one minute. They were then incubated for 24 h in a heated waterbath at 35°C. Each flask contained a magnetic stirrer which stirred for 5 mins every 30 mins during the course of the experiment. The gas was collected by liquid displacement after passage through 1 N NaOH to remove CO_2. If the maximum CH_4 production rate occurred at the highest sludge load, of 1.0 g HAC/g VSS, it was found necessary to repeat the run with higher acetate concentrations to ensure non-limiting substrate conditions. The percentage of acetogenic bacteria could then be expressed by comparing the ratio of gas produced in the test relative to the theoretical ratio 1000

ml CH_4/g VSSday.

3.4.19 Sample Preparation and Scanning Electron Micrography

Pad samples, removed from the reactor, were lightly blotted to remove excess liquid and were then frozen in liquid nitrogen. After the boiling had subsided the samples were broken into small fragments (4 - 5 mm) and freeze dried at -15 °C and 0.15 torr. These samples were then mounted on microscope stubs with colloidal graphite paint and splutter-coated with gold for 10 minutes at 20 mA. The specimens were then observed using a Hitachi S700 SEM using an accelerating voltage of 20 kV. The method was a modification of that used by Richards and Turner (1984).

3.5 The Investigation of BSM Activity

Radioactively labelled substrates have been succesfully used in the estimation of the heterotrophic activity of bacterial and algal populations in natural waters (Wright and Hobbie, 1966; Vincent and Goldman, 1980). These methods essentially consist of the the incubation of a sample of the water with a labelled substrate and quantifying the uptake of radio-activity over a finite period. The bacterial suspension is removed from the pool of substrate by filtration, often using membrane or glass fibre filter papers (Wright and Hobbie, 1966). This type of study both suffers from restricted methodological approaches and from the erroneous assumption that the uptake of isotopically

labelled substrates can be equated with organic nutrient utilization. Organic compounds may enter a cell but only support the synthesis of a very limited range of compounds (Vincent and Goldman, 1980). McKinley and Vestal (1984) outlined a technique for quantifying the microbial activity in a pile of composting sewage sludge by measuring the incorporation of ^{14}C acetate into the microbial lipids of compost homogenates. They reported that, due to the nature of the assay, the incorportation rates may reflect bacterial rather than fungal activity. Archer (1985) reported the use of ^{32}P to measure the relation between methanogenesis and the growth of *Methanosarcina bakeri*.

Isotope dilution techniques have been attempted by several workers (Jayasuriya and Hungate, 1959; Mackie and Bryant, 1982; Boone, 1982) for the estimation of acetate turnover in anaerobic digester liquors. This method relies on the fact that when a labelled intermediate, usually acetate, is added to the reactor contents, the cold intermediates produced dilute out the label. The loss of this label from the extracellular pool is proportional to both the turnover of the pool and the amount of label in the pool. This technique can only be carried out when the reactor is in steady state operation, i.e. at constant gas production rate, acetate degradation rate and acetate concentration over a finite period. Boone (1984) also reported the use of an isotope dilution technique in the investigation of propionate exchange reactions in methanogenic ecosystems. The diverse populations encountered in digester mixed liquors will often contain other

micro-organisms capable of removing the chosen substrate. Care has to be taken to account for this in the final analysis of experimental data, as it may result in an overestimation of the particular species.

Wimpenny and Parr (1979) described a method in which large bacterial colonies were frozen and sectioned. The sections were disrupted and several oxidative enzymes were identified from the crude unfractionated homogenates. They found that cells nearer the base of the colonies had very low enzyme activities. These methods seem to be of great use providing their limitations are fully appreciated.

3.5.1 Methodology of BSM Activity Tests

In some situations diffusional limitations may affect the transport of substrate to, and the removal of products from, bacteria embedded deeply in a biofilm. Several workers have attempted to prove the existence of a variation of activity within a particle and to utilize this in the formulation of mathematical design models (Williamson and McCarty, 1976; Rittman and McCarty, 1980). This problem may be a disadvantage as inhibitory compounds, and intermediates may accumulate in the biomass aggregate and so reduce substrate removal and hence process performance. The method used in this study was developed in an attempt to support these theories, and it is based on the ability of a colonized biomass support particle to remove acetate from a reaction liquor under defined conditions. The experimental technique was devised to monitor the transport

of acetate through the BSM matrix over a finite period. Acetate was chosen as it is the predominant precursor for methane. The method essentially entailed the incubation of a colonized support particle with labelled acetate for a set period. The supports were removed at fixed times from the reaction vessel and further activity was stopped by freezing the particles "instantaneously" in liquid nitrogen. Each particle was then sectioned whilst still frozen and assayed for radioactivity. Two assays were performed: the total count in each section and the count from the biomass after the free label had been removed. The results were then analysed to give an indication of both the rate of transport through the biomass aggregate and the uptake of biomass at each level.

3.5.2 Method

Three glass bottles, volume 200 ml, were fitted with rubber bungs and a septum were placed in a water bath at 35°C. Each bottle contained a magnetic stirring bar and a fine stainless steel wire for supporting the BSM (Figure 3.4). Pad samples were removed from the digester and stored in a nutrient solution with a small amount of their usual substrate (fruit washing wastewater) in an incubator until required. The BSMs were then transferred to the reaction vessel taking care to ensure minimal contact with air. They were held in suspension with the wire supported from the bottle stopper. The reactor contained the mineral medium (section 3.4.18) and the required concentration of (cold)

acetic acid. The bottles were then incubated for at least 24 hours before addition of the 1.0 ml of 1-^{14}C (the hydroxyl group was labelled $CH_3{}^{14}COOH$) acetate stock solution 20 μCi/ml (Sigma Chemical Co., Poole) using a hypodermic syringe through the septum. The final reaction concentration was 0.1 μCi/ml. Three experiments were run concurrently and at intervals of 0.5 h a pad was immediately removed from the reaction vessel, quenched in liquid nitrogen and stored until the end of the experimental run. The pads were then sliced, while still frozen, into 7 approximately equal sections using a meat slicer. Each section was then resuspended in 5 ml of a solution of cold acetic acid (10 g/l) to surpress further uptake of the label. 0.25 ml of this solution was then dispensed into glass scintillation vials together with 20 ml of scintillation fluid (" Cocktail T ", BDH Chemicals Ltd.) for total counts. The sample for incorprated count (0.25 ml) was filtered through glass fibre filter papers (Whatman GF/F, 20 mm dia.) in a Buchner funnel. The solids were then washed 4 times with acetic acid solution. After drying, the discs were placed in scintillation vials together with 20ml of scintillation fluid. Counts were obtained with a Packard 2000CA scintillation counter corrected for quenching by an external standard method. Counting efficiencies were typically >95%. Experimental runs were performed at three acetate concentrations and two different mixing regimes (stirred continuously at 60 rpm and unstirred). The resuspended samples were assayed for both suspended and volatile suspended solids in each section, and the reaction concentration of acetic acid was determined by GLC. Abiotic

controls were run on the biomass using three different substances to kill the micro-organisms:

(1) acetic acid (conc.), 10 g/l
(2) chloroform 1 g/l
(3) bromoethanesulphonic acid, 1 mM

The third substance is a specific inhibitor of methanogenesis and it was selected to enable an estimation to be made of the non-specific uptake (Boone, 1984). The stages in this experiment are outlined in Figure 3.5.

The results were calculated as follows:

Let DPM_x = activity (total) of sample from pad section
V = sample volume of mixed liqour
$[Ac]_c$ = non-labelled (cold) acetate
$[Ac]_H$ = labelled (hot) acetate

The frozen section was resuspended in 5 ml of acetic acid and 0.25 ml was used for determining the radio-acetate concentration.

actual total count $[DPM]_T = [DPM]_x[5/0.25]$

From theory 0.1 μCi/ml = 2.22×10^5 dpm/ml,
so if the reaction concentration of radiolabelled acetate is 0.1 μCi/ml, then 1mg of cold acetate = 2.22×10^8 $[Ac]_c$ dpm. Therefore mg acetate per section (A) is given by:

$$A = ([DPM]_x \times 20) / (2.22/[Ac]_c \times 10^8)$$

This enabled actual acetate concentration (both incoporated and free label) to be assayed for each section after set time periods. Similarly for the samples filtered through glass fibre filter discs the solids were assayed by scintillation counting.

Let DPM_I = activity of filtered and washed biomass and

$[X]_s$ = solids concentration in sample. Therefore the mg incorporated acetate per section (I) = is given by:

$$I = ([DPM]_I \times 20) / (2.22/[AC]_c \times 10^8)$$

Since the VSS concentration per slice is known (X, g/l), then a sample resuspended in 5 ml of acid:

$$[X]_s = ([X] \times 10^3)/200 \text{ mg VSS/slice}$$

Therefore mg acetate taken up per mgVSS is given by:

$$AC_i = A/I \text{ mgAc/mgVSS}$$

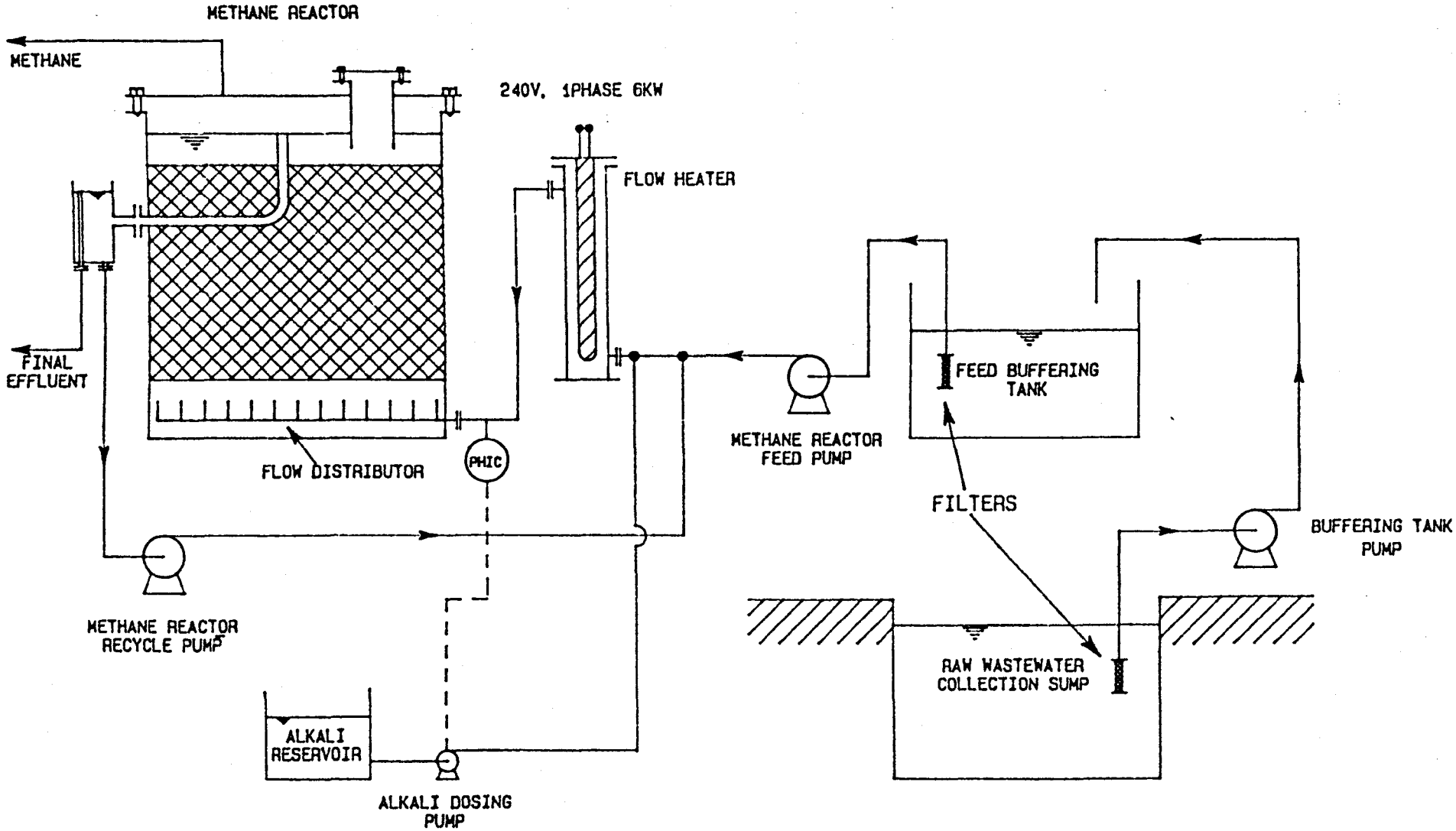


fig. 3.1 process flowsheet for single stage process flowsheet at J. Morleys

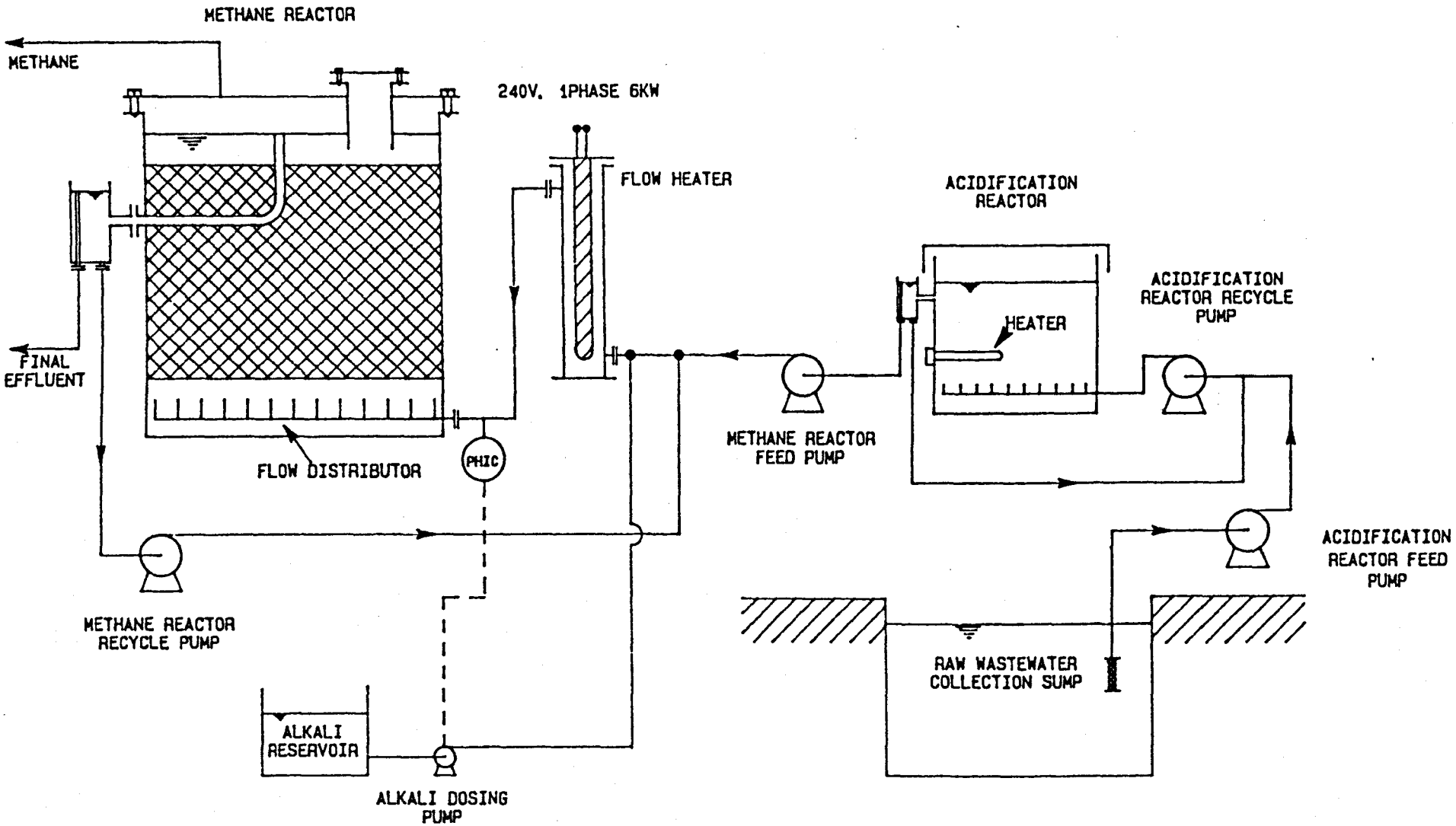


Fig. 3.2 process flowsheet of two stage pilot plant at j. morleys

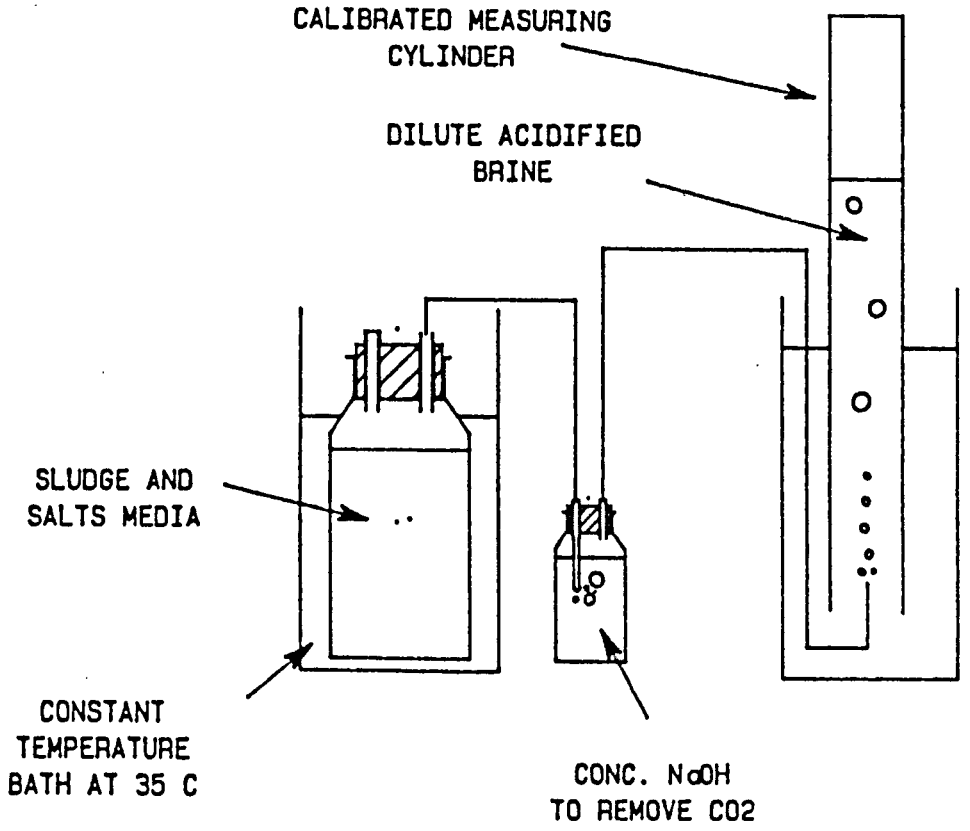


Fig. 3.3 apparatus for sludge activity measurement

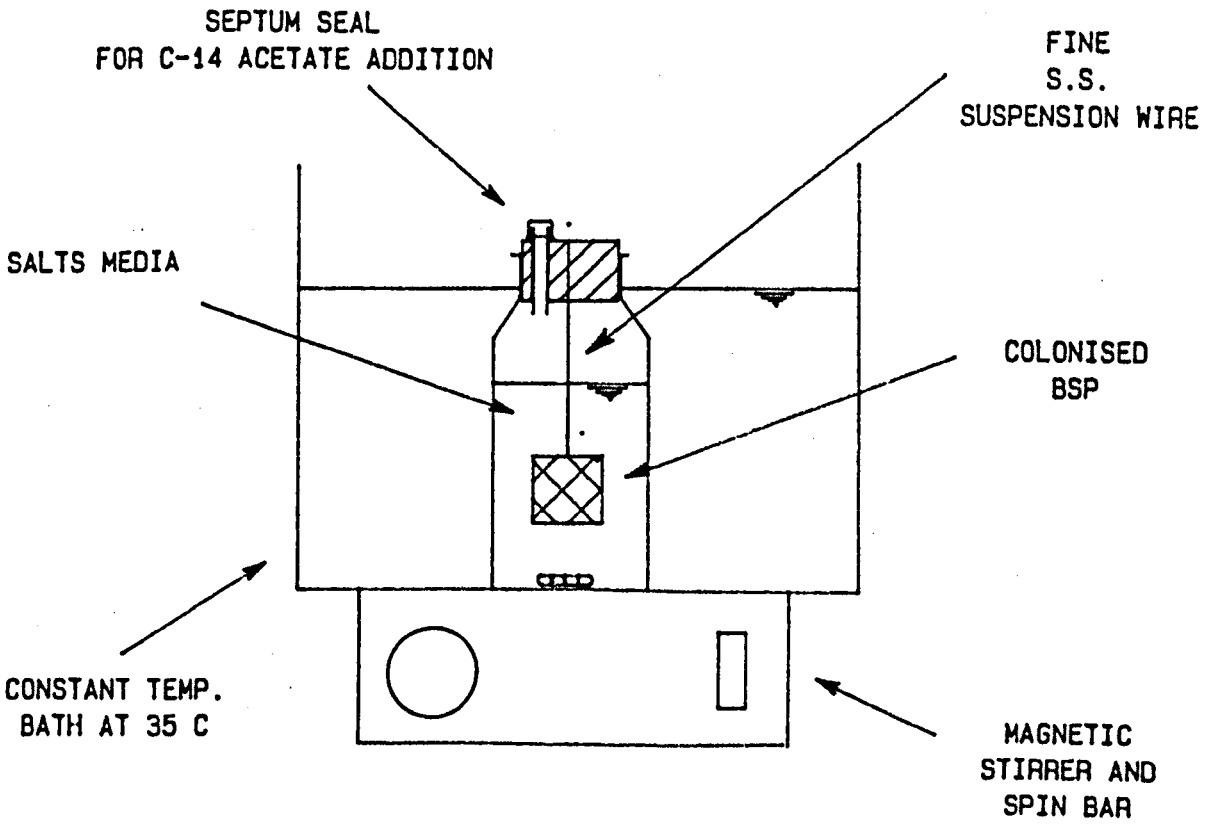


Fig. 3.4 apparatus for radioactive substrate uptake study

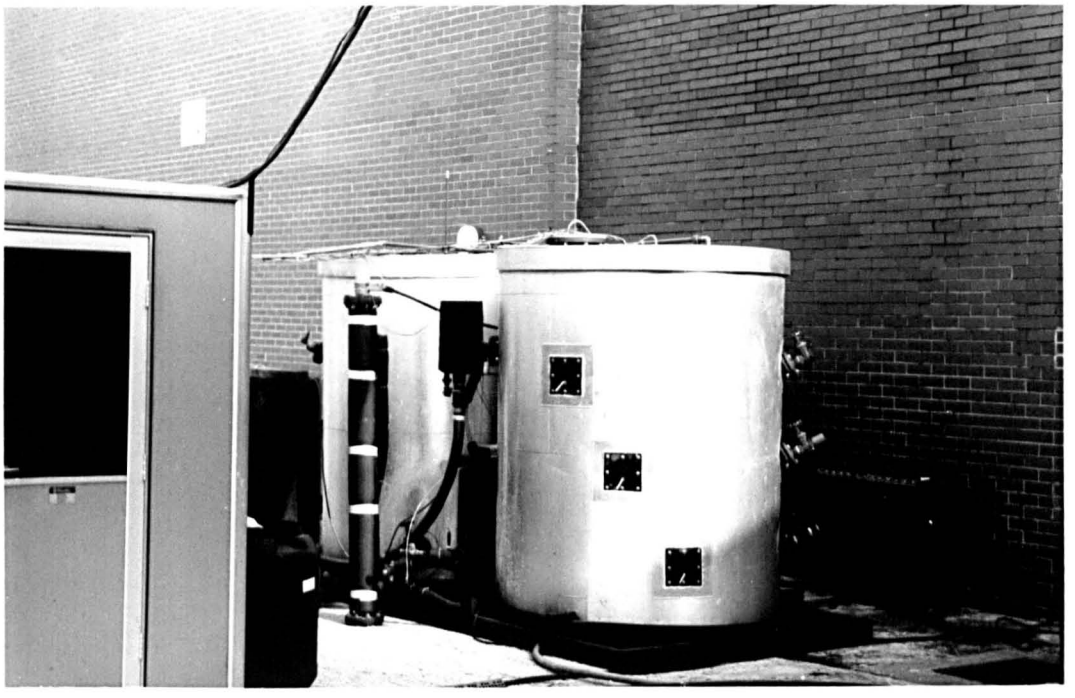


Plate I Pilot Scale Anaerobic Digesters
showing reactors 1 and 2
and flow heater

Plate II Pilot Scale Anaerobic Digesters
showing Two-stage reactor
(acidification reactor at left)

4.0 RESULTS AND DISCUSSION

4.1 Parameter Definition

The reporting of results must be performed in such a way to enable an accurate assessment of experimental data using widely understood parameters. This permits comparison with the results of other workers in the field. To ensure consistency the methods of calculation of derived parameters are presented below to remove the ambiguity sometimes encountered with terms like "loading rate" or "feed rate". The parameters chosen are based on the recommendations from the International Union of Pure and Applied Chemistry (IUPAC) and the International Association of Water Pollution Research and Control (IAWPRC) (Colin *et al.*).

(1) Feed Rate: F, the volume of feed per day (m^3 day^{-1})

(2) Theoretical retention time: Θ_E, the ratio of the empty bed volume V_E (m^3) and the hydraulic volume introduced into the reactor per day F (day)

$$\Theta_E = V_E/F \quad (day)$$

(3) Hydraulic retention time: Θ_V the ratio of the useful reactor volume V_V (m^3) and the hydraulic volume introduced into the reactor each day F (day)

$$\Theta_V = V_V/F$$

(4) Reactor biomass concentration: X_{ML}, the [illegible] biomass concentration within the reactor (kg SS m^{-3})

(5) Support medium biomass concentration: X_{BSM}, concentration of biomass per unit volume of packing (kg SS m^{-3})

(6) Feed and effluent solids concentration: X_F, X_E respectively, the solids concentration per unit volume of feed or effluent, (kg SS m^{-3})

(7) Theoretical solids retention time: Θ_X, defined as:

$$\Theta_X = (X_{ML}/X_e)\Theta_V \text{ days}$$

(8) Chemical oxygen demand removal efficiency: S_f , for filterable COD removal efficiency :

$$S_f = [(s_{FO} - s_{FE})/s_{FO}] \times 100$$

where s_{FO} = filtered COD of the feed mg l^{-1}

s_{FE} = filtered COD Of th effluent mg l^{-1}

and S_t for total COD removal efficiency:

$$S_t = [(s_{TO} - s_{FE})/s_{TO}] \times 100$$

where s_{TO} = total COD of the feed mg l^{-1}

s_{TE} = total COD of the effluent mg l^{-1}

(9) Organic loading rate: B_{LCOD}, the organic load (kgCOD) introduced per unit volume of reactor per day:

$$B_{LCOD} = (S_{TO} \times F)/V_E \quad (kg\ COD\ m^{-3}\ day^{-1})$$

(10) Organic removal rate: B_{RCOD}, the organic load removed per unit volume of reactor per day

$$B_{RCOD} = (S_{TO} \times S_F \times F)/V_E \quad (kg\ COD\ m^{-3}\ day^{-1})$$

(11) Solids loading rate; B_{SS}, the dry weight of solids fed to unit volume of the reactor per day

$$B_{SS} \text{ or } B_{VSS} = (X_F \times F)/V_E \quad (kg\ COD\ m^{-3}\ day^{-1})$$

(12) Gas production rate: r_{vgas}, the volume of gas produced per unit volume of reactor per day ($m^3\ m^{-3}\ day^{-1}$)

(13) Methane composition of the digester gas: X_{meth}, the percentage composition of methane in the biogas by volume.

(14) Reactor superficial upflow velocity: V_{UT}, defined as the upflow velocity in the empty bed ($m\ day^{-1}$)

(15) Methane yield: Y_{meth}, the methane yield per kg COD degraded ($m^3\ CH_4\ kg\ COD^{-1}\ day^{-1}$)

The results refer to pseudo-steady state conditions.

Pseudo-steady state may be defined as a condition in which the concentration of feed, effluent and recycle are relatively constant but the composition and quantity of the biomass is continually changing. The results for the parameters, which were measured daily, were averaged over a five day period. This method is frequently used where there are variations in feed conditions.

4.2 Startup Results

Difficulties have always been reported during the startup of anaerobic digesters (Henze and Harremoes, 1983). In this study an initial period of around one year was neccessary to solve commissioning problems and startup of the reactors before data collection could be attempted.

The composition of the sump effluent was high in both suspended matter and large solids (currants, raisins etc.). It was considered advantageous to attempt to remove this portion of the feed, as hydrolysis is usually rate limiting for a substrate containing high levels of cellulosic material (Eastman and Ferguson, 1981). The use of two filters (described in section 3.2.1) were found satisfactory, provided they were cleaned regularly. Table 4.1 shows the average waste characteristics for the duration of this study.

The raw sump waste analysis shows a reasonable level of suspended matter, a large fraction of which was volatile (90%). Approximately 84% of the COD was soluble. Partial

acidification of the sump contents was apparent with around 8.5% of the total COD occurring as VFA. The approximate retention time in the sump was about 8 hours, depending on the rate of discharge from the factory. During this period some biological activity was apparent from visual observations of the sump contents and its characteristic odour.

The main purpose of filtering and settling the feed was to reduce the solids contribution to the COD. It appears that the increased fermentation in the settling tank actually increased the feed solids content of the effluent and consequently the soluble portion of both the total COD and total carbohydrate were decreased. This may have been due to the position of the takeoff from the sump allowing floating solids to be pumped to the settling tank. The percentage of volatile solids remained almost unchanged (90-91% of the total suspended solids).

Further acidification of the sump effluent in the first stage of the two stage process increased the proportion of soluble COD and carbohydrate from 81 to 86% (significant at the 4 percent level, appendix III) and 75 to 81% (significant at the 9 percent level) respectively. Greater amounts of VFA were generally observed together with depression of the pH. Although not measured, lactic acid and possibly other non-volatile fatty acids were present causing an odour reminiscent of "sour milk". The conditions within the reactor seemed to favour the acetate and propionate type fermentation profile described by Cohen and

Zoetemeyer (1984) and Pipyn and Verstraete (1981).

With respect to other components in the feed (Table 4.2), average values are displayed for the duration of the study. Occasionally nitrogen and phosphorus were added when the COD/N/P ratio exceeded the optimum (section 2.1.8).

Fig. 4.1 shows the time scale for the project. Initial difficulties were experienced in maintaining a stable temperature within the reactor. Increasing the lagging thickness on the associated pipework enabled the temperature to be controlled at 35 $\pm$2°C throughout the reactor, even at high feed rates and low recycle.

The reactors were initially seeded in January/Febuary 1984. After a period of feeding, failure was apparent and the reactors were reseeded in June 1984. The addition of pH control in August 1984 had a highly beneficial effect making the manual addition of large amounts of alkali unnecessary. The startup period still seemed to be of the same order as that reported in the literature, typically 3 months for a filter to reach maturity and around 1-2 months for granulation to occur in a UASB (Lettinga et al.,1982). A definition of what in fact constitutes successful startup is, however, difficult to make.

Screening the feed for possible toxic substances was performed throughout the remainder of 1984. No toxic substances could be identified and further investigations were deemed unnecessary, even though a shock loading of

inhibitory substances could go undetected.

4.2.1 Startup of Reactor 1

In early 1985 a tracer study in reactor 1 (containing 25 mm cubes) indicated severe short-circuiting within the reactor. This prompted the removal of the reactor cover and a solids "survey" of the packed bed. It was discovered that the BSM's had not been effectively retained by the upper mesh and it was thought that large volumes of the bed had been inactive due to short-circuiting of the feed. This theory was confirmed by the fact that the biomass concentration in foam particles near the effluent takeoff point was higher than in those further away. A slight modification was made to the takeoff from the reactor to combat this, and the cover replaced and resealed. A further tracer study showed a vast improvement in liquid distribution in the bed. Fig 4.2 indicates the possible flow regimes both before and after modification.

An additional startup period of around 3 months was initiated and some general results for this period can be seen in Figure 4.3. A gradual increase in loading was accompanied by increased COD removals and low effluent VFA's (less than 1000 mg/l). Effluent solids were very low during startup indicating that some entrapment was occurring. In the initial stages it can be seen that an increase in loading rate produced increased gas production leading to washout of some of the biomass. This resulted in an increase in VFA concentration in the effluent, probably due

to washout of the methanogenic population. Gas yields were of the order of 0.665 m^3/kg COD removed. Analysis of the gas throughout the startup period indicated approximately. 50-60% methane, the remainder being carbon dioxide, with occasionally small amounts of nitrogen (1-3%) usually after some air had been introduced into the reactor. The average gas yields were equivalent to stoichiometric amounts (0.35 m^3 CH_4/kg COD at STP), which led to the conclusion that gas leaks were either not present or insignificant. The presence of nitrogen in the digester gas may have also been due to the stripping of nitrogen from the feed. The minimum hydraulic retention time achieved at this stage was around 7 days.

It seemed that the modifications to reactor 1 were of great benefit and illustrated the need for careful reactor design with packed beds of this type. It was considered that the BSM's was fully colonised after this startup period and that some pseudo-steady state had been reached with regard to biomass growth and washout.

4.2.2 Startup of reactor 2

Investigations with reactor 2, containing the solid blocks of foam, were eventually aborted. Repeated attempts to start this reactor were unsuccessful. Tracer studies were thought to indicate severe short-circuiting and modifications did not improve this. It is possible that the biomass had completely blocked the packing . A solids survey indicated a high solids concentration in the unpacked

section at the base of the reactor. These results are in agreement with those of Huysman *et al.* (1983), who found excessive acidification apparent even with a waste containing no suspended matter and concluded that the diffusion of gas and substrate into the matrix did not occur. No further investigations with reactor 2 were carried out.

4.2.3 Startup Of The Acidification Reactor

The acidification reactor was commissioned in September/October 1985. This was achieved using sludge from the failed reactor No. 2 and raw feed from the sump. Startup was rapid and the results for a typical acidified feed may be compared with those for the unsoured feed in Table 4.1. A crust was often observed on the surface of the acid reactor when the cover was removed. This crust was thick and creamy and presumably resulted from flocculation linked with attached gas bubbles. pH control in the acid reactor was considered to be unjustified as the cost of alkali would have been high and there is no evidence that pH control and recirculation reduce the content of hydrogen in the first phase digester producing a better substrate for the methanogens in the second stage (Joubert *et al.*, 1985). Low pH was favourable in assisting hydrolysis of the solid fraction of the waste to take place.

4.3 Performance Criteria

There are several parameters that can be used to assess

the performance of anaerobic reactors. The following have been adopted here to enable the results of this study to be compared with those of other workers:

(1) hydraulic retention time (HRT);
(2) reactor loading rates;
(3) COD removal; and
(4) gas yields.

All of the results presented in this section are based on the averages of five day periods throughout the total operating period.

4.3.1 Hydraulic Retention Time (HRT)

The significance of HRT is particularly important with respect to immobilised biomass reactors. The ratio of solids retention time (SRT) to HRT can be increased to enable the critical solids retention time to be exceeded. Fig.4.4 displays the performance, with respect to COD removal, for the four different operating regimes tested in this study. This shows a high degree of scatter which is characteristic of the results from pilot scale work and is often caused by variations in feed concentration. Considering the limits of process operation, the vertical lines on the plot indicate that the lower limits of hydraulic retention time were achieved before failure. The criteria used for assessing this point were a sudden irreversible decrease in gas production accompanied by an increase in volatile fatty acid concentration in the effluent. It can be seen that the

system offering the best performance was the two-stage reactor without recycle. Summarised results are displayed in Table 4.3. The shortest retention time achieved in this system was approximately 1.05 day. The results from the single and two stage systems with recycle were similar with shortest retention times of 2.44 and 1.87 days respectively. The worst performance was with the single stage reactor without recycle, 4.25 days. The same pattern is observed for filtered COD. These results are based on the volume of the methane reactor only for all systems.

It is most probable that the reasons for this type of behaviour are related to the substrate available for the methane bacteria. Pre-acidified effluent has been shown to be more readily metabolised by methane bacteria (Cho, 1983) with subsequent high methane yields and COD removal. This is supported by the improved performance exhibited in the two stage systems.

It is important to consider the fact that two-stage systems require a greater total reactor volume and therefore the performance, based on total reactor volume, of the single stage system with recycle is superior to that of the two stage system with recycle.(cf. Table 4.3; 2.94 days compared to 2.57 days, respectively). It has been reported (Mueller and Mancinni 1975) that single stage operation, without recycle, of a substrate that readily acidifies will create a region at the inlet of the reactor of high acidity . The effect of this would be to suppress the methanogenic activity in this area. Conversely, in the case of a recycled

reactor it could be assumed that the dispersed nature of the flow would ensure rapid dilution of toxic metabolites, but the lower substrate flux present would reduce the COD removal rate, especially where substrate removal rates are dependent upon diffusion rates into the biofilm.

4.3.2 Organic Loading Rate and COD Removal Efficiency

Figure 4.5 indicates the observed relation for organic loading rate against COD removal for all four systems (the methane stage in the case of two stage systems). At low loading rates the relation is approximately linear within the ranges of operation, except for the two stage system without recycle. For this configuration, as loading increased, performance became limiting at around 11-12 kg COD/m^3.day^{-1} before the failure point was reached.

Figure 4.6 shows the variation of percentage COD removal with loading rate for the methane reactor. In this type of reactor an accurate assessment of the total biomass inventory precludes the use of the biological loading rate (defined as the COD load per unit mass of biomass) . Loading rates are therefore related to total reactor volume (empty bed). In reality this does not represent the actual situation as large volumes of the bed are occupied by biomass held within the foam BSM. The results of tracer studies (section 4.8) reinforce this statement. COD removal rate falls off most rapidly for the single stage reactor without recycle. Vertical lines on the plots indicate the

maximum loading rates achieved for each process configuration. The pattern for filtered and total COD is similar.

It is interesting to compare the overall performance based on the total reactor volume (acidification and methanogenesis), as well as that based on the methane reactor alone (Table 4.3). The best overall performance was achieved in the two- stage system without recycle (8.3 kg COD $m^{-3}day^{-1}$). The maximum loading rates were almost 2 times greater than those achieved for any other of the systems tested. The maximum loading rates for both the single and the two-stage systems with recycle were 4.29 and 3.07 kg COD $m^{-3}day^{-1}$ respectively, whereas the single stage system without recycle was 3.22 kg COD $m^{-3}day^{-1}$.

It may be concluded that when operating a single stage process with this type of reactor the adoption of recycle is beneficial, whereas for a two stage process the use of recycle is detrimental. This could possibly be due to the detrimental effects of transferring the higher concentrations of VFA, present in the acidified feed, from the lower section, to the sensitive methanogenic population higher in the top section of a packed bed reactor, by high recycle rates. Increased theoretical yields of plug flow reactors compared to completely mixed reactors of the same size have been reported in both theory and practice (Levenspiel, 1972).

Table 4.4 displays the results of various workers, some

treating wastes of this type. Anderson *et al.* (1984) report the use of a fixed film reactor to treat a protein/carbohydrate waste. They attained maximum loading rate of around 14 kg COD $m^{-3}day^{-1}$ (equivalent to an HRT of 6.4 days), which is superior to the loading rates observed here, and they attained far superior COD removal efficiencies of 97%, compared to around less than 50% for this study.

Bull *et al.* (1984) compared the performance of single and two-stage fluidised bed anaerobic reactors in the treatment of a high strength waste. They concluded that two-stage systems gave superior performance for both total and filtered COD removal using a high strength waste, whereas for lower strength wastes the soluble removal efficiencies were similar for both single and two-stage systems. At loading rates greater than 15 kg COD $m^{-3}day^{-1}$ the removal efficiency was around 75%, but this rapidly decreased to 50% with a further increase in loading. A similar pattern was observed with the four systems reported in this study, at around 4-5 kg COD $m^{-3}day^{-1}$.

Cohen *et al.* (1979) investigated the two-stage digestion of a synthetic glucose based substrate. They achieved loading rates approximately 2.7 times higher in a 2 stage process compared to a single stage one (based on methane reactor volume). Their investigations were with completely mixed reactors. This may be compared with the fourfold difference found for the single and two stage systems investigated here.

Dewalle and Chian (1976) conducted an investigation into the performance a completely mixed anaerobic filter, which may be compared to the recycled reactor in this study. Their investigations involved the digestion of landfill leachate of which some 47% of the COD was present as VFA. They concluded that for recycle ratios of the order of 20/1, the system could be considered to be completely mixed. The high recycle was thought to be beneficial in the sense that it enabled the maintenance of a high pH within the reactor and allowed the entire length of the column to be used for substrate removal.

Ghosh's (1984) study of two-stage processes indicated that a loading rate of 3 times that achieved in an equivalent single stage process was possible, and that the hydraulic retention time could consequently be reduced by a factor of two.

Guiot and van den Berg (1985) reported loading rates of 5-25 kg COD $m^{-3}day^{-1}$ at treatment efficiencies of around 96% in terms of COD removal. His reactor was a hybrid of a UASB and a filter. He conluded that the limiting factor for increasing the loading rate appeared to be the activity of the biomass within the reactor.

Jennett and Dennis (1975) investigated the COD removal in the treatment of a high strength waste and reported that higher efficiencies were maintained without solids recycle over the loading range 0.221 to 3.5 kg COD $m^{-3}day^{-1}$ in an

anaerobic filter packed with gravel.

In conclusion , all workers reported improved performance from two-stage systems, and in this study it enabled an increase in the maximum loading of around 4 times (cf. 2.7 times, Cohen *et al.* (1979) and 3 times, Ghosh (1984). Hydraulic retention could be reduced by a factor of four in this study.

The use of recycle at the ratios in this study of approximately 13-10:1 seems only beneficial when treating a readily acidifying substrate. The reasons for the low loading rates in this study are perhaps due to the nature of the waste together with the type of systems with which these reactors were compared. It would seem that performance fell somewhere between that of a filter type and UASB reactor.

4.4.3 Gas Yields

The gas yield from an anaerobic digestion process may be regarded as the most pertinent indication of process performance currently avaliable. In this study the results of gas production are reported as follows;

(1) volumetric yield (m^3 gas m^{-3} reactor volume per day);
(2) total gas yield ; and
(3) methane yield.

All values stated are corrected to STP (0°C and 760 mm

Hg). The volumetric gas yields may be regarded as useful for the comparison of various reactor types, and may also be important in respect of the removal of solids from a retained biomass reactor due to the high gas fluxes causing biomass washout (see section 4.5). Figure 4.7 illustrates the variation of volumetric gas yield with organic loading rate for the operating regimes studied here. Maximum volumetric gas yields of around 3.20 and 2.35 $m^3\ m^{-3}\ day^{-1}$ were attained in the two stage systems with and without recycle systems (2SNORC AND 2S+RC, relative to the methanogenic stage only) respectively. The results for total gas yield per kg of COD degraded are displayed in Fig. 4.8. Maximum total gas yields were attained in the second stage of two stage processes. For single and two stage processes the maximum yield was attained without recycle. Comparisons were not made with combined gas yields as gas production in the first stage reactors was not recorded.

Ghosh's (1984) studies of two-stage digestion indicate an increase in volumetric gas yield to 2.9 day^{-1} compared to 0.4 day^{-1} for a single stage reactor treating the same waste. Guiot and van den Berg (1985) reports a maximum methane production rate of 7 day^{-1}.

The methane yields are displayed in Figure 4.9 When total gas production is reported there is some uncertainty as to how much carbon dioxide is measured. The use of sodium hydroxide for pH correction will increase the measured methane concentration in the biogas by the absorption of carbon dioxide into the digester liquor. This most probably

explains the calculated yields being greater than the theoretical maximum. The effect may have been accentuated in the two-stage processes as the acidified feed being of lower pH would have required proportionately more caustic soda. Table 4.5 displays the results obtained for the average gas yields measured over the duration of the study. Gas yields based on methane are judged against a theoretical yield of 0.35 m^{-3}/kg COD degraded. The sparse nature of the data for methane yields indicates no obvious relationship other than the somewhat lower maximum yields for systems with recycle. The average values obtained from data over the five day operational periods are displayed in Table 4.5, and they indicate that for the two-stage systems methane yields were slightly higher than theoretical. This may have been due to errors caused by the interprolation of weekly methane determinations.

4.4 Operating Observations

Anaerobic degradation of a complex substrate has been shown to proceed in numerous steps (Gujer and Zehnder, 1983). The identification and quantification of intermediate compounds is useful in monitoring the performance of a digester and its response to changes in feed characteristics and variations in loading rate and hydraulic retention time. In this section the results of day-to-day operation are reported with respect to the changes in the nature of the effluent.

4.4.1 Single Stage Without Recycle

During the initial period of study up to day 35 the loading rate did not exceed 2.5 kg COD $m^{-3}day^{-1}$, with corresponding HRT's being greater than six days (Fig. 4.10). At this loading the total COD removal efficiency remained consistently above 95% (Fig. 4.11). On day 39 a loading rate increase produced, almost immediately, a fall in the COD removal efficiency, the filterable to a low value of less than 60%. This fall in efficiency coincided with an increase of VFA in the effluent, from less than 500mg/l to greater than 2000mg/l, for acetic acid with lower values for propionic and N-butyric acid (Figure 4.12). This effect showed that the VFA consuming methane bacteria were stressed to a great extent. The VFA concentrations gradually returned to low values after the loading was reduced. A further increase in loading rate on day 65 (Figure 4.10) produced a similar effect. Influent and effluent pH (Figure 4.13), for this period show that, as expected, low effluent pH corresponds to high effluent VFA concentrations. It may be concluded that at a loading above approximately 2.5 kg COD $m^{-3}day^{-1}$ COD removal efficiency falls. Effluent VFA concentrations increase to values greater than 2000mg/l for acetic acid. Reactor pH was always controlled at 6.5. On many occasions effluent acetic acid levels rose to at least 2000 mg/l and propionic to around 1000 mg/l. The decrease in COD removal efficiency was due mainly to the presence of volatile fatty acids. McCarty and Mckinney (1961) reported that VFA concentrations up to 6000 mg/l can be tolerated with no loss in methane production, provided the pH is maintained above 6.5. It would appear that when effluent pH

was less than this value, indicating the actual pH in the reactor was lower, our findings would agree with those of McCarty and Mckinney are illustrated by the sudden reductions in COD removal efficiency, especially filterable COD removal. In this period of the study the maximum loadings obtained were less than 5 kg COD $m^{-3}day^{-1}$. It appeared as though increases in hydraulic loading were more readily accommodated than increases in organic loading. In all cases the concentration of propionic and n-butyric acids in the effluent mirrored those for acetate, but at a lower level. Effluent pH values showed only small variation.

The adoption of a single stage process without recycle for a wastewater of this type would indicate that, as loading rates increased, the inlet area of the reactor would be subjected to localized low pH values. The preliminary effect of this would be a reduction in the activity of the methane bacteria from this area. Any increase in total gas yield would be due to increased acidogenesis resulting in rapid production of carbon dioxide and hydrogen with a subsequent reduction in the percentage of methane in the gas. To monitor this sucessfully an on-line gas analyser would be necessary.

4.4.2 Single Stage With Recycle

Loading was initiated at approximately 3 kg COD $m^{-3}day^{-1}$ (Figure 4.14). During the initial period, the first 30 days, the loading was maintained at 3.5 kg COD $m^{-3}day^{-1}$. Total COD removal efficiency fluctuated between 65 and 90 % (Figure

4.15). The effluent volatile acids (Figure 4.16) varied around 1250 mg/l for acetic acid (the trough on day 20 cannot be explained by a reduction in loading rate). Attempts to increase loading, typically from day 35 onwards (Figure 4.14), resulted in great reductions in COD removal efficiency, which at times was below 55 percent. These observations were accompanied by a rise in effluent VFA concentration. The depression of effluent pH (day 45, Fig.4.17) coincided with large increases of butyric and propionic acids. The subsequent recovery (assisted by a reduction in loading rate) that followed indicated that the propionic acid consuming populations were severely inhibited. In fact the effluent propionic acid concentration remained high for the remaining period of the study, despite the fact that effluent pH resumed the levels measured before the overload.

During increases in organic loading there was a much higher incidence of fatty acids, other than acetic in the effluent, more so than in the single stage system without recycle. The incidence of high values for propionate indicates that, although in theory completely mixed reactors are able to smooth peaks in feed concentration, greater instability results from the use of recycle and recovery from serious overloads was slower.

4.4.3 Two Stage Without Recycle

This configuration was subjected to the widest variation in loading (on the methane reactor). The loading

rate commenced at around 3.5 kg COD $m^{-3}day^{-1}$ (Figure 4.18) and was increased to approximately 15 kg COD $m^{-3}day^{-1}$. COD removal efficiency was varied, and increases in loading (Figure 4.19) caused corresponding reductions in treatment efficiency. Effluent volatile acids remained below 1000 mg/l for acetic for an initial period, but gradually increased with loading (Figure 4.20). It was observed that, although the feed was corrected to pH 6.5, the effluent pH was frequently depressed to low levels, indicating a reduction in activity of the VFA consuming communities or their washout (Figure 4.21). Generally VFA concentrations varied in step with one another, except during the final stage when the propionic acid concentration rose above 1500 mg/l, and was higher than that of acetic acid. COD removal efficiency showed the greatest variation compared to all other configurations.

4.4.4 Two Stage With Recycle

Loadings in this two-stage regime were not as varied as for the two-stage system without recycle. The loading rate to the methane reactor commenced at around 2.5-3.0 kg COD $m^{-3}day^{-1}$ (Figure 4.22). For this initial period COD removal efficiency was lower (approximately 70%, Figure 4.23), and this lower loading was maintained for 10 days as the COD removal efficiency increased and effluent volatile acids gradually decreased (Figure 4.24). As the loading rate was increased on day 14, COD removal efficiency did not start to fall significantly till around day 25. This may possibly be explained by the fact that the recycle reactor smoothed out

small fluctuations up to the point where the VFA concentrations exceed a threshhold, and performance then reduces significantly. This effect may be observed in this case where COD removal efficiency decreased (Figure 4.23) with a corresponding increase in effluent VFA concentrarion (Figure 4.24). The relative proportions of the different VFA's show that, when a change in loading occurs, concentrations of propionic and butyric acids increase more rapidly, indicating more serious inhibition than overloads and resulting in an increase in acetic acid. For a period of 20 days (Figure 4.24) propionic acid levels remained above 1500 mg/l, almost twice as high as acetic. This is mirrored by a depression in COD removal efficiency over the same period (Figure 4.23). A pH control problem seemed to be responsible for this. Even when pH control was returned to normal and the effluent pH increased to 6.5, the system took a long period to remove the backlog of VFA (Fig. 4.25).

These results show that the type and nature of the wastewater dictates the performance of this type of reactor to a great extent.

Legros *et al.* (1983) indicated that any complex substrate could be characterised by its fermentation profile, which they say is unique and may be defined as either acidic or neutral. Their definition of acid and neutral fermentation profiles is as follows: when an anaerobic complex microbiological ecosystem is allowed to develop spontaneously under septic conditions, the course of the fermentation, as exhibited by the pattern of metabolites

as a function of time, appears reproducible provided that all physico-chemical factors are identical. They classified fermentations that produced a prevalence of lactic acid at a pH below or equal to 4.5 or VFA's with 2 to 5 carbon atoms at pH below or equal to 6.5 as having an acidic profile, and those with a prevalence of lactic acid and VFA's with 2 to 5 carbons together with ethanol and methane, as having a neutral fermentation profile. They would advise the use of a two-stage system for the waste used in this study.

Cohen *et al.* (1984) also distinguished two important fermentation patterns during the digestion of a variety of substrates, and they concluded that acetate, propionate and butyrate are the most important end products of an acidogenic process operating at steady state. They agreed with other workers that lactate formation seemed to be restricted to high dilution rates or shock loadings and was energetically unfavourable at other times.

Boone and Bryant (1980) have shown that the presence of butyric and propionic acids may stimulate the symbiotic association of the H_2 producing and consuming communities. The imposition of high organic loading rates with a substrate of the type used here may encourage spatial rearrangement to encourage inter-species hydrogen transfer. This may be very important in sludge flocs (Wiegant, 1986). The large quantities of hydrogen produced in a single stage process would seriously limit methanogenesis. It has been shown, in this study, that failure of the single stage processes would be a distinct possibility if they were fed

at the same high rate as the two-stage processes.

Noike *et al.*(1985) reported the rate determining step in the degradation of a mixed substrate to be cellulose breakdown. The main products were acetic and propionic acid, but butyric was also detected in large amounts. Starch based wastes produced mainly acetic and N-butyric, and glucose mainly acetic, butyric and caproic acids, the proportions increasing with higher cell residence times.

Eastman and Ferguson (1981) concluded from their studies on the solubilization of particulate material that, during the digestion of primary sewage sludge, the contribution of both acetic and propionic acids were equally important on a COD basis.

It would appear that the main factors affecting methanogenesis were the compositon of the feed and the use of recycle. The performance of the reactors fed with acidified feed was superior in that they were subjected to the widest range of loading rates. The single stage systems were unable to cope so readily with these variations in loading rate. This was most likely due to the reduced hydrogen production in the methane reactor together with the fact that a higher proportion of the substrates was directly available to the bacteria. The use of recycle was detrimental insofar as effluent quality was generally reduced and reactors without recycle recovered more readily than those with. It was most probable that in this instance the transfer of acidic metabolites caused inhibition in

methanogenic populations higher up the bed.

DeWalle and Chian (1976) seemed to find the recycle of effluent in their study beneficial by reducing the amount of buffer required to maintain the reactor pH. Their reactor used a recycle ratio of around 20:1 compared to 6-7:1 to 16:1 in this study. They performed tracer studies and concluded the reactor operated as a completely mixed system.

Fynn and Rankin (1987) noted that methanogenic biomass with potentially high conversion rates for substrate to methane will be inhibited by a metabolically inert substance if the internal substrate concentration rises above an osmotically inhibitory level. The incidence of such compounds in the waste studied here may have reduced COD removal efficiency in this way. The use of recycle would tend to accentuate this by exposing the methanogens to these compounds before they could be degraded in lower sections of the bed.

Joubert *et al.* (1985) studied the effect of high effluent recirculation on the first stage of a two-stage process operated without pH adjustment. They concluded that its effects were beneficial by increasing the internal pH of the reactor to 5.5, so creating an environment in which the metabolic activities of the acidogenic bacteria, and therefore acidogenic degradation, are known to be high (Zoeteymeyer *et al.*, 1982). Both the proportion and amount of hydrogen in the gas from the first phase were reduced, thereby increasing the available substrate. The consequence

of lower hydrogen production in the gas from the first phase may have converted the waste to primarily acetate (Zinder and Mah, 1984) which can be used directly by methanogens.

Pipyn and Verstraete (1981) performed a series of experiments which showed that directing the first stage fermentation of a two stage process to lactate-ethanol production, rather than to volatile fatty acid production, was beneficial in enabling greater substrate concentrations to be made available to the methane bacteria, based on thermodynamic grounds. This fermentation also has a lower overall cell yield, thus enabling lower effluent suspended solids. For this to be feasible very low cell residence times must be used, and this could prove difficult in fixed film reactors or in systems without progressive sludge removal.

Parkin and Speece (1983) reported that, dependent on toxicant concentration and exposure time, the rapid elution of toxic substances exhibited by a plug-flow reactor had a distinct advantage over a completely mixed reactor. Effluent recycle results in a more prolonged toxicant washout rate and therefore potentially longer recovery times. For chronic toxicity attached growth systems provide the solids retention time neccessary to prevent biomass washout at the low HRT's that assist toxicant washout.

Another problem which may have arisen was the inhibition by sodium ions. It has been shown that concentrations greater than 3500 mg l^{-1} (section 2.1.9) can

inhibit anaerobic digestion. On occasions when the influent pH was low, dosing requirements were greater. The use of reactor recycle may have been beneficial in this respect as localized concentrations of alkali would be rapidly diluted below their toxicity threshold, and the recycled effluent would have some buffering capacity of its own.

4.5 Reactor Biomass Concentration

The concept of process intensification in a biological wastewater treatment reactor relies on the ability of the reactor to retain biomass efficiently at concentrations greater than those that could be achieved in a stirred reactor when process throughput exceeds the critical dilution rate. A knowledge of the potential biomass concentrations that can be attained in the reactor should enable more rigorous design procedures to be adopted.

The biomass support medium of the type used in this study has been shown to retain large amounts of biomass (Huysmen *et al.*, 1983; Atkinson, 1984). The retention is thought to be due to physical entrapment rather than chemical attachment. Parameters governing this process are as follows:

(i) the morphology of immobilised species;

(ii) their yield co-efficients;

(iii) the substrate concentration;

(iv) the prescence of other adherent species;

(v) the upflow velocity in the reactor; and

(vi) the relative proportions of fixed and suspended biomass.

The accurate estimation of the total biomass inventory in a reactor, together with the effluent solids concentration, can be used to determine the solids retention time (SRT). The results presented here refer to the volatile and total suspended solids and, although these may be an indication of the ability of a particular support medium or reactor to retain biomass, they do not offer a reliable estimate of the biomass activity. This is discussed later (section 4.6).

4.5.1 Biofilm Structure

Biofilm reactors do not seem to be dominated by homogeneous biofilms (Henze and Harremoes, 1983), and fixed beds often have a loosely attached film, responsible for a significant part of the activity. The nature of the support material is believed to influence greatly the type of film formed, but other factors play an important role, in particular the liquid upflow characteristics (Pol *et al.*,1982) or rising gas bubbles (Young and Dahab, 1983). The type of feed will dictate the biofilm composition and, in the case of high concentrations of influent solids, damage

may be caused to the biofilm structures in UASB and expanded bed reactors and problems with clogging could be encountered in fixed beds. If the influent solids contain a high proportion of inert material, this may be incorporated into the biomass and would result in reduced efficiency after a period. This is more likely to occur in a reactor containing a biomass support. Henze and Harremoes (1983) discussed the effects of gas bubbles and concluded that they may adhere to flocs or bed paricles causing them to rise and be washed out of the reactor. They also commented that rising gas bubbles may cause channelling and short-ciruiting within the reactor. Visual observations of pellets from a sludge blanket reactor using SEM have shown that clearly defined passages do exist in the structure, presumably caused by the release gaseous products from within the aggregate (Wiegant and de Mann, 1985). The effects of sudden variations in hydraulic and organic loading rate may well have different results. High hydraulic loading may often cause severe washout in many types of reactor, but the use of an efficient packing which retains the biomass means that normal process operation can be resumed when the throughput is returned to normal. An organic overload often affects the pellet or biomass structure due to the sudden increase in gas production. Fixed film reactors are superior in this respect to more conventional systems (Henze and Harremoes, 1983).

The actual estimation of biomass within fixed film reactors is often difficult. In this study a method was developed to attempt to measure the non-entrapped biomass

together with the biomass entrapped in the support particles. The methods described (section.3.3.3) could be subject to error, but were considered to be the best available and caused minimal disturbance to the reactor configuration during operation. Estimation of both interstitial solids (i.e. those suspended between the support particles) and BSM solids was attempted at the end of each period of process operation. Although interstitial solids were withdrawn from various depths in the bed, no satisfactory method could be found for withdrawal of pad samples from all depths. For this reason the deepest possible samples were taken at a maximum depth of approximately 0.75 m and they may or may not have been indicative of the unsampled part of the bed. It is probable that these samples are reasonably representative as there was little variation in the various samples taken from the highest to the deepest sample at 0.75 m.

4.5.2 Pad Solids

Table 4.6 indicates the sampling positions and the solid concentrations at the respective points in reactor 1, after initial commissioning but before the effluent takeoff was modified. Although there seemed to be little variation in SS and VSS concentration from the highest to the lowest samples, the variation from one place to another would seem to indicate a higher solids concentration, higher growth and substrate availibility where short-circuiting flow existed. It could be argued that areas of high fluid flow should contain less solids due to localized increases in hydraulic

loading, but this seems unlikely considering that the higher solids concentrations occurred near the effluent takeoff. Further pad solids surveys of this scale were not attempted as this necessitated removal of the reactor lid and involved the associated problems of resealing. Tracer studies of the bed before and after the outlet was modified indicated that short-circuiting was drastically reduced, and it was therefore considered that biomass concentrations in the different areas were, to all intents and purposes, the same.

Table 4.7 shows the pad solids concentrations determined at the end of the periods of each stage of the study. It can be seen that the highest pad solids concentrations were achieved in the two stage processes. This is probably due to the fact that the substrates from the acidification reactor enabled greater biomass growth in the support particles. It may also be possible that the higher upflow velocities, in the two stage systems helped to washout the light, flocculent, less dense biomass from the particles.

The pad biomass concentrations in single stage processes were 0.389 and 0.320 g VSS/l, for systems with and without recycle respectively and the volatile solids components were 85 and 75 % for systems with and without recycle respectively. Higher biomass concentrations were measured in the two stage processes, 0.469 and 0.560 g VSS/l for two stage systems with and without recycle, respectively, their volatile percentages were both 77%.

4.5.3 Interstitial Solids

The withdrawal of the interstitial solids from the void space between the pads was performed carefully with a sampling tube to ensure that the solids within the pads were not included. It is difficult to say what quantity of the pad solids were included, but it was found that gentle washing of the colonised BSP's did not cause a significant amount of the pad solids to be washed out. It was necessary to squeeze the pads by hand to remove these solids. Table 4.8 shows the total and volatile suspended solids and the levels of abstraction. Values for these solids before and after initial comissioning show that a large amount of solids existed beneath the packed section, equivalent to around 10 g/l, although the concentration throughout the bed voidage was much smaller, typically around 2 g/l. During the first phase of process operation (single stage no recycle), concentrations both beneath the bed and in the voids showed a great increase with values of SS as high as 15 g/l beneath the bed and around 1.3 g/l in the voids. The distribution of volatile solids remained of the same order as the initial values, varying from 64% VSS in the unpacked section to 84% within the packing.

After a period of operation using recycle the most noticeable change was the variation of solids concentration in the unpacked section. It had been reduced to the same order as those in the bed voids between the packing, typically 0.8-2.0 g VSS/l, with the percentage of VSS in the

interstitial solids remaining about the same.

A similar pattern was observed in the two-stage processes. The interstitial solids concentration was around half the values observed in the single stage processes and was thought to be due to the washing out of biomass by the higher liquid and gas upflow velocities. The addition of recycle resulted in the same effect with single stage processes in that it reduced the solids concentration beneath the bed to the same order as between the BSM's. The process configuration in which no recycle was used resulted in an extremely large concentration beneath the bed of around 17 g VSS/l. The proportion of VSS was higher in the upper section of the bed in the two stage systems than in their single stage counterparts. However, for the final phase of the study the percentage of VSS beneath the bed fell to around 48%, indicating an accumulation of inerts from the feed over the total period of operation or possibly some precipitation.

The most significant conclusion seems to be that single and two stage processes with recycle have lower solids concentrations both in the pads and intertitices, and without recycle large amounts of solids are retained beneath the packed section.

Table 4.9 shows biomass concentration and the percentage of suspended and entraped solids, in each system as a percentage of the total for results from the literature. This is compared with values from this study.

The percentage of fixed and suspended solids cannot be reported as there was a variation throughout the bed. Total concentrations are of the same order as for other reactor types reported in the literature.

4.5.4 Influent and Effluent Solids

The presence or absence of solids in the feed or effluent will affect the the overall solids retention time in the system. Generally a high SRT should favour the selection of methanogenic and acetogenic bacteria and it has been shown earlier (sec.2.1.3-5) that their production is rate limiting. There are not many reports of the affects of solids concentrations in the feed on the process performance of fixed film reactors. It is possible that they may have the following affects dependent on their composition.

Volatile solids that can be readily degraded will usually have the same affect as an organic overload, namely, a sudden increase in gas production followed by a washout of biomass and a decrease in general process efficiency. Inert solids, although not necessarily affecting process performance in the short term, may accumulate in the biofilm so reducing the space availiable for colonization by biomass. This is to be avoided as in the long term it can seriously limit process performance necessitating enforced solids removal. van den Berg and Lentz (1980) found that higher inert loadings may be permitted if the inert material is soluble or if it is not absorbed into the biofilm. Henze and Harremoes (1983) considered theoretically the maximum

possible inert loading rate. They concluded that in the case of the acid reactor loadings may be 4-6 times higher than a reactor for methane production, primarily due to the lower solids retention times required for the acidogenic bacteria. They also commented on the ability of the various types of reactor to absorb selectively suspended solids. They draw analogies with the two-layer film commonly found in nitrifying fixed beds, concluding that in many cases the biomass aggregate will contain an inner layer of active organisms with an outer layer of lightly attached biofilm that is easily sloughed off.

Figures 4.26 - 4.33 display the results of influent and effluent solids for the duration of this study together with the various parameters thought to influence washout. The following parameters are discussed in relation to their effect on the effluent solids concentration:

(i) hydraulic retention time and superficial upflow velocity;
(ii) increases in gas production;
(iii) influent solids concentration.

Hydraulic retention time

Examination of the effluent concentrations of suspended solids indicated that the single stage systems, operated at the highest HRT's and lowest loads, produced an effluent which was consistently around 250 mg SS/l and seemed to be independent of the recycle rate. This indicates that even at

superficial upflow velocities of around 7.5 m/day (0.3 m/h) the BSM's efficiently retain biomass. Two stage systems, although operated at similar maximum superficial upflow velocities compared to single stage systems, generally produced higher effluent suspended solids concentrations.

Gas production

Comparing gas production and biomass washout it seems that the two-stage systems, producing far higher volumetric gas yields than the single stage processes, have the highest washout of solids. It appears that when gas production peaks a period of high effluent biomass concentration follows and then falls off rapidly. This would suggest entrapment of the two layer type. The two-stage systems with recycle seem less susceptible to this effect.

Feed solids

There was no obvious correlation between influent and effluent solids concentrations other than the increase in loading rate and subsequent biomass washout exhibited when the feed contained readily degradable solids. After periods when effluent solids concentrations are high it is possible that more sites become available for entrapment of influent solids and this continues until the capacity of the bed reaches a maximum.

The most likely reason for washout was considered to be the scouring effect produced by gas bubble formation. Visual

observations of the surface of a single piece of colonised support medium, with the naked eye, revealed several small "pinholes" which were most likely channels for the transport of gaseous products produced within the BSM.

There are limitations to the results presented here as the actual biomass concentrations in the effluent are constantly varying. At the end of the period of study the single stage reactor pad biomass concentration was lower than in the two stage systems. This may indicate that the limit of potential maximum entrapment had not been reached, possibly due to mass transfer limitations. It was not possible to extend the periods of operation any longer due to the time scale of the project.

Considering the results of other workers, Martensson and Frostell (1983) described the performance of a carrier-assisted sludge bed reactor (CASB) in which a carrier material (5-25 μm) was used to provide a nucleus for pellet formation in the sludge bed and increase the density of the flocs. Using two different wastes, one containing a large portion of unhydrolysed material and the other almost completely hydrolysed, they found that the maximum loads for the two were 5 and 24 kg COD m^{-3} day^{-1} respectively. The feed with the largely unhydrolysed component produced a more bulky sludge, allowing a maximum biomass concentration of around 8-9 kg m^{-3} compared to 15-20 kg m^{-3} for the completely hydrolysed waste. This would tend to indicate that the hydrolysed waste produced larger quantities of sludge, similar to the situation observed in this study.

Microscopic examination of the sludge flocs from the partially unhydrolysed waste showed they had a very bright fluffy exterior and a black interior. A similar effect was observed with the BSM's in this study on occasions of continued overloading.

Noike *et al.* (1985) noted a shift in dominance between bacilli and sarcinae at SRT's of 6.5-9.5 days. This was accompanied by a change in the colour of the mixed liquor from light brown, in which bacilli were dominant, to black, in which sarcinae were dominant. The occurrence of a light fluffy outer layer with a black interior would tend to indicate that the SRT of the outer layer may be less than the inner core.

Fynn and Whitmore (1984) concluded that the use of foam BSM's permitted a five-fold increase in culture density in the reactor at high substrate concentrations, composed predominantly of *Methanobacteria formicium*. They subjected the freely suspended particles to a dilution rate of twice the critical dilution rate and found that the freely suspended biomass in the system remained relatively constant. Subjecting the BSM's to a large hydraulic shock resulted in complete removal of entrapped solids, irrespective of the particle porosity, in one hour at a linear flow rate of approximately 2.2 m day^{-1} compared with 8.2 m day^{-1} would seem to indicate that the colonization by mixed cultures is far more resilient to washout.

According to the work of Atkinson *et al.* (1984), the

particle biomass hold-up depends solely on the substrate concentration. A point is reached at which the rate of attrition and the rate of biomass growth attain equilibrium. They also reported that the density of a colonised particle was slightly greater than that of water. In this study although the BSM's was retained in a fixed bed, its low density would reduce deformation of pads at the bottom of the bed from the weight of particles above it. This however assumes that the bouyancy effect of adherent gas bubbles is negligible.

Fiebig and Dellweg (1985) described a series of experiments with a sludge bed reactor with a packed section above it. They recorded biomass concentrations in the sludge bed of the order of 22 g/l and 29 g/l in the packed section. The waste was synthetic and based on acetic acid. Effluent solids were seen to rise from 200-300 mg/l at a gas production of 30 vol/vol reactor.day to 400-1000 mg/l at 40 vol/vol reactor/day.

Guiot *et al.* (1985) reported similar observations with a sugar-based waste. They attained approximately 20 and 28 g/l in their system, and they found that little COD reduction was achieved across the filter section indicating that it only served to maintain a large quantity of biomass in the reactor. This is to be expected as the packing was only non-porous plastic rings. Maximum liquid velocities of around 1-4 m h^{-1} were used compared to 0.34 m h^{-1} in this work.

Other workers have reported high biomass concentrations in their reactors. The anaerobic attached film expanded bed reactor (AAFEB) process, developed by Jewell *et al.* (1984) accumulated large quantities of biomass of the order of 30-40 kg VSS m^{-3}. Stephenson and Lester (1986) evaluated four configurations of anaerobic processes: a single stage CSTR, a single stage AFBR and two two stage systems. They attained biomass concentrations from 1.15-5.65 kg VSS m^{-3} of expanded bed. Tesch *et al.* (1983) reported effluent VSS concentrations of around 2000 mg/l at 17 h HRT in a packed bed of clay particles. They did not comment on the solids concentrations in the bed. Young and McCartys' (1969) classic study on the anaerobic filter indicated that for this type of reactor, biomass accumulation was between 1.3-6 g VSS/l. Effluent solids concentrations were of the order of 7-90 mg SS/l.

Investigations have been conducted with various media in an anaerobic fixed bed reactor (Young and Dahab, 1983). It was concluded that the majority of the waste treatment took place in the lower levels in the filter and was associated to a large extent with biological solids that were loosely held within the voids between the matrix. For this reason the ability of the media to retain high concentrations of biological solids, either as suspended, attached or entrapped growth, is an important design consideration. Selection of media for use at full scale should permit the withdrawal of biological solids to prevent accumulations that can contribute to short-circuiting and loss of treatment efficiency.

4.6 Biomass Activity

The efficient conversion of substrate to methane relies essentially on the activity of the biomass within an anaerobic digester. Although a reactor may contain large amounts of biomass, as has shown to be the case in this study, the activity and mass transfer effects will govern the overall substrate removal. This section reports the investigation into biomass activity for the different operating regimes used. As the most important source of methane has been shown to be acetate, the method employed by Valke and Verstraete (1983) for estimating acetoclastic activity was thought to be the most suitable. This was linked to a new method developed for establishing activity gradients within individual biomass support particles (sec 4.7). The activity of biomass, both freely suspended and colonized on support particles, was measured.

4.6.1 Acetoclastic Activity

Biomass and pad samples were withdrawn from the reactor during the final periods of operation of each phase of the study. Samples were generally abstracted from the top 0.5 m of the reactor. This may not be fully representative of the situation throughout the reactor, but it was considered the best method availiable and suitable for comparison of the relative activity of biomass from this one region in the reactor. During the experiments on colonized BSM's, they were transferred to the apparatus intact rather than

expelling the biomass from the BSM into the vessel of media solution. The reason for this was that on occasions when the biomass was expelled, the gas production was severely reduced or ceased completely. This may have been due to some inhibitory compound within the matrix or the disruptive influence of expelling the biomass into the media.

The activities presented for the freely suspended biomass used solids removed using the sampling rod and a siphon arrangement described earlier (sec 3.3.3). BSM samples were withdrawn by hand. Care was taken to ensure minimal contact with the air and the salts media used for the determination was warmed to approximately 35°C to reduce temperature shock.

Table 4.10 displays the results from these studies, based on gas production. It is important to realize that this method can only estimate the acetate-cleaving bacteria and not the methane produced by other communities. The percentage of acetoclastic methanogens was estimated based on studies from enrichment cultures which may also influence the results. However they are useful in determining the relative activities of the biomass in various modes of operation.

The results indicate that the freely suspended biomass was of a higher activity than that immobilised in the pads in the systems without recycle. The method does not take into account any variation in activity throughout the cross

section of the pad. The biomass at the core of the BSM, which may be relatively inactive, is included in the VSS determination. It is possible that the BSM may have higher activity in the outer layer with mass transfer effects reducing substrate accessibility to the core. In the single stage systems with recycle there is no significant difference in activity of suspended and fixed biomass, indicating that the recycle enables better mixing and therefore better substrate avaliability for the attached biomass.

Considering the two-stage systems, far greater activity was observed in the two stage reactor without recycle than in any other system, with the freely suspended biomass showing higher activity than the attached growth. This greater activity was most likely due to feed composition. In systems with recycle the transfer of acidic products from the acidogenically active region may cause inhibition of acetoclastic activity in freely suspended biomass more than fixed biomass if mass transfer is limiting.

Valke and Verstraete (1983) reported the activity of biomass from a UASB reactor by a similar method and found that the activity of the acetoclastic biomass increased from 10 to 33% after 2 months of operation. This is much greater than the maximum value of 7% found in this study. It is quite possible that continued operation in the two-stage without recycle regime may have resulted in an increase in activity or perhaps the measured activity was reduced due to inhibition or localised reduction in pH during the course of

the experiment.

The fact that when sludge was removed from the pads their gas production was severely inhibited or reduced to zero was suprising as it would seem likely that mass transfer effects, if present, would be substantially reduced. As no determinations were attempted on the activity of the biomass from the bottom section of the bed, there is no comparison with the activities from this area. Binot *et al.* (1983) reported activities of the biomass in their investigations with a citric acid waste of 0.32 and 0.27 l CH_4 /g VSS day^{-1}, for an immobilised cell reactor and stirred tank reactor respectively. These results compare favourably with those of Valke and Verstraete (1983).

Activities in the present system (0.024 to 0.07 l CH_4/g VSS day^{-1}) were significantly lower than similar reported systems. The actual retention times, much less than those based on the V/Q retention time, were greatly reduced and so the dilution rates were much closer to the critical retention time at which washout of the slow-growing methanogenic bacteria would occur. This would tend to cause the activity of freely suspended biomass to decrease. It was perhaps an oversight that solids samples were not removed from the space beneath the bed for activity measurements since appreciable amounts of acetate could have been removed in this region.

4.7 BSM Activity Gradients

It has been shown in the previous section that a difference in activity existed between the freely suspended biomass in the voids between the colonised BSM's and the immobilised biomass itself. The series of experiments reported in this section were designed to identify whether a difference in activity was observed at different points in a fully colonized BSM. The experiments were based on the principle of inoculating an actively digesting BSM with a radioactively labelled substrate. The reactions were stopped instantaneously at various time intervals by freezing with liquid nitrogen. The sectioned BSM was then assayed for total radioactivity in each section and also for incorporated acetate. The rationale being that the concentration of free label (i.e. the labelled substrate that was not taken up by the cells) would indicate the mass transfer of substrate through the particle and the concentration of incorporated acetate would indicate acetoclastic activity. The experiment was performed at three different bulk acetate concentrations, both with and without stirring. These regimes were chosen as they were thought to represent the range of conditions encountered in normal process operation. The consistency of the results was probably affected by:

(i) lack of biofilm homogeneity, possibly due to gas bubbles and voids in the colonised medium; and

(ii) variation of activity in different support particles taken from the reactor,

Ideally a "core" should have been removed from the BSM to "follow" a gradient, but this was considered unsatisfactory as reduced levels of label would have made the counts unreliable.

Fig. 4.34 to 4.36 show the total acetate concentrations (free label and incorporated) at various levels for all the experiments. The observed concentration ranged from low values to the high value equivalent to the bulk liquid concentration. The results must be intepereted with care as the total count will have had contributions from both incorporated and free label. It can be seen that high bulk liquid concentrations give higher acetate concentrations at the centre of the pad. Generally the activities at the centre will have been lower than those shown on the graphs due to the fact that core sections were not taken. Even after 1.5-2.0 h there was a noticeable difference in activity in all cases.

More important are the actual rates of uptake as these are more indicative of the rates of use by methanogens. Figures 4.37 to 4.39 illustrate these results. Uptake progressed at a higher rate when the bulk acetate concentration was lower. This is most likely due to the increasingly inhibitory effect of VFA on acetate utilization. Localised depression of pH could accentuate this. In all of the systems examined the amount of acetate in the outer sections was at least twice that of the observed concentration in the inner sections. Slight discrepancies may be explained by variation in slice

thickness. It does seem as though there was little active biomass at the centre of the particle. The concentration of incorporated acetate was generally 1000 times less than that measured as free label. Uptake for the bulk acetate concentration of 200 mg/l was far higher (x10) than for any other system.

Figures 4.40 and 4.41 show the values for incorporated and free acetate (found by difference) concentration for the complete BSM. Values of the free label were seen to increase up to 3-4 times in magnitude in one hour. The effects of the stirred and unstirred vessels are more noticeable. It can be seen that stirring increased the transfer of substrate through the biofilm and in doing so a reduction in substrate uptake was apparent at high concentrations. Variations in the solids content of each section may have been responsible for some differences.

A further experiment was attempted to assess the uptake of acetate by other species present and involved the use of specific inhibitors of methanogenesis. The results, displayed in table 4.11 indicate that uptake was negligible.

The intepretation of the results of radioactively labelled biomass may only be used qualitatively, rather than quantitatively, due to variations in slice thickness and original activity in the pad. The results for the movement of acetate through the pad are good, indicating that mild agitation is beneficial for increasing mass transfer rates, but may be detrimental if bulk substrate concentrations are

high. The bacteria deep in the biofilm may be protected by a stagnant liquid layer which is destroyed by agitation. The actual concentrations, both measured and taken up, are much less than those in the bulk solution; this indicates that mass transfer is limiting and so reduces activity greatly. This poses an important question with regard to the design of media in that at depths below the outer layer (3.5-4 mm, assuming a homogeneous biofilm) acetate penetration and activity in the biofilm are negligible. The optimum size for a foam support particle, based on these observations and in this type of reactor, should have a maximum biofilm depth of no larger than twice the maximum penetration depth of 3.5-4.0mm.

The results from acetate uptake were not sufficient to enable reaction kinetics to be calculated. Henze and Harremoes (1983) considered the significance of diffusional limitations in fixed bed reactors. They indicated that diffusional resistance does not become significant until the biofilm thickness reaches 1 mm (for K_s = 0.2 kg COD.m^{-3}) for conditions in an anaerobic fixed film reactor.

4.8 Reactor Hydraulics and Mixing Studies

The use of tracers has received much attention for the investigation of fluid flow and residence time distributions in chemical and biochemical reactors (Levenspiel, 1972). Data obtained using these techniques may be analysed to determine the actual retention time and the dispersion number, and they can be used to show the

presence of stagnant zones and short-circuiting within the reactor. There have been several models used to classify the performance of a real reactor with respect to the ideal extremes of completely mixed and plug flow. Those used in this study were the dispersion model and the tanks in series Model (Levenspiel, 1972). Other more complex multiparameter models have been reported by Cholette and Cloutier (1959), and were used to estimate the relative proportions of plug and mixed flow where appropriate. The use of such models was selected based on the observed shape of the tracer curves and knowledge of the reactor geometry. The objectives of this section of the work were to identify the nature of the flow within the methane reactor and to assess the effect of recycle on this hydraulic regime. Tracer studies were performed at the end of each period of study. The following results were then reported for each system (calculated using computer programme described in appendix II);

(i) hydraulic retention time, actual and theoretical;
(ii) variance of normalised data;
(iii) dispersion number, calculated by curve matching;
(iv) value of N for the tanks in series model (where N is the number of equal sized completely mixed tanks which best represents the observed response);
(v) volume of reactor dead space; and
(vi) proportion of mixed and plug flow;

Fig. 4.42 illustrates the normalized tracer curve for reactor 1 during the period of operation of single stage

without recycle. It can be seen that there is an initial lag period before the curve peaks, due to the plug flow regime in the packed section. The curve then peaks and is followed by a gradual decrease due to mixed flow regions and stagnant regions in the reactor in which the tracer was retained. Parallel paths through the reactor may be the cause of small secondary peaks. Levenspiel (1972) described the effect of mixed sections before and after a plug flow reactor. They also cause the curve to depart from the symmetrical Gaussian curve characteristic of ideal plug flow systems by changing the assumption that the boundary conditions of the vessel are closed.

Fig. 4.43 displays the tracer curve for the single stage system with recycle. The recycle (recycle ratio 13:1) can be seen to cause the response to approach that of a completely mixed reactor. This has been reported in cases of plug flow reactors operated at high recycle ratios (Samson *et al.*, 1985; DeWalle and Chian, 1977). There is little evidence of short-circuiting, the occasional secondary peaks during the study being due to the recycle.

The second stage of two-stage systems was generally operated at a higher throughput and the recycle ratio was therefore slightly smaller (10:1) for the same recycle rate. Fig. 4.44 shows the result of this tracer study. The flow regime showed less dispersion than single stage reactors for this reason.

The tracer response for the two stage system without

recycle (Fig. 4.45) had the same general shape as the system with recycle apart from a small secondary peak possibly indicating some internal recirculation.

Table 4.12 shows the different models fitted to the observed tracer curves together with their schematic representation. The models were fitted to the decay portion of the experimental curves using a least squares regression routine (SAS, Statistical Analysis System, Cary, North Carolina). Estimates were made using fitting parameters A1 and B1 (defined in Table 4.12). A mass balance on the tracer was not attempted since previous experiments on the absorption of this tracer onto biological solids showed less than 1-2% absorption and the reactor dead volume was therefore calculated based on the difference of observed and theoretical retention time.

The results of all the tracer experiments are summarised in Table 4.12. As would be expected systems with recycle show a higher degree of dispersion. However there may be several reasons for this type of flow regime. The operation with recycle in the bottom section of the reactor could cause backmixing which would result in a more disperse tracer curve. The production of gas at various sites in the digester will also cause dispersion, the effect being accentuated at high gas production rates.

It is convienient to consider the tanks-in-series model (Levenspiel, 1972), which can also be used to characterise the degree of plug or mixed flow in a reactor. The reactor

most closely approximating to mixed flow, based on the results of the tanks-in-series model, appears to be the single stage system without recycle, with a value N = 1.28. This was not in agreement with the dispersion model which indicated that the single stage without recycle reactor has the most plug flow characteristics and it would be expected that the addition of recycle would result in the value of N being reduced, not increased to 1.67. This may be due to some error in the tracer study or, more likely that the shape of the tracer curve departed from the ideal extremes of plug and mixed flow and was therefore outside the range of application of the dispersion model. The most striking fact about all the systems tested was the large difference in the theoretical retention time (based on an estimated working volume of reactor of 2.5 m^3) and that based on the results of tracer studies. Prior to the modification of the effluent off-take, it was expected that a large amount of short-circuiting occurred which would appear on the tracer curves as secondary peaks. It is more likely that the biomass volume reduces the actual liquid volume of the reactor available for fluid flow. An example may illustrate this. If we consider that the reactor contains 100,000 biomass support particles of size 25 mm sq., then the total volume of the pads is 1.56 m^3 (assuming the volume of the plastic is negligible, as they are 97 percent void). Table 4.12 shows tabulated values for the dead space (V_{DEAD}). It appears that the dead volume in the two stage systems is not greatly different from the total volume of the BSP's. (1.6 and 1.7 m^3 as compared with 1.56 m^3). The high superficial upflow velocities would tend to washout the interstitial

solids, so causing the total biomass holdup to approach the amount retained in the biomass support particles.

Results of Multiparameter Model Analysis

The completely mixed model was employed using data from all the tracer studies. It can be seen (Table 4.12) that the estimated values for the mixed volume (V_M), are close to the actual values, (V_A), measured in the tracer study for all systems. The results from the two-compartment model fitted to all the systems, show that the model predicts the volumes of plug and mixed flow for systems without recycle with the most accuracy. Its use is grossly in error for systems with recycle. This may be due to the increased gas production observed during this experiment and the departure of the observed tracer curves from ideal.

Monteith and Stephenson (1981) reported on the mixing efficiencies of full-scale digesters and concluded that short-circuiting and dead zones accounted for as much as 77% of the theoretical volume availiable for active mixing. Samson and Van den Berg (1985) reported that in their studies of downflow fixed film reactors, dead space in one reactor exceeded 55%. They reported that little or no short-circuiting was observed even at these values for dead space. They concluded that this was due to the accumulation of biomass and the effect this has on restricting the flow through the packing. The volume of dead space in the reactors of this study varied between 66 and 83%. It is not possible to say whether gas production in this study

affected the dispersion to as great an extent as the recycle rate or superficial upflow velocity. Stevens *et al.* (1986) reported the effect of tracer diffusion into biofilms and the effect on the residence time distribution (RTD). They explained the long tails on their tracer curves as a result of diffusion into and out of the biofilm resulting in an increase in the measured HRT if the characteristic retention time of the tracer in the biofilm exceeded that in the bulk liquor. Judging from the present results it is difficult to say whether or not this happened as the effect may have been obscured by other parameters, such as small variations in feed rate etc.. Their use of a fluidized bed reactor provided a good solution to the problem of reducing the dead volume in the reactor, due to the superior mixing characteristics found in fluidized beds.

Harremoes and Riemer, (1980) presented a mathematical model for the RTD in a submerged denitrification filter, but concluded that variability in the biofilm thickness together with the effect of gas bubbles in the filter meant that the models reviewed were not sufficient to explain the tailing observed in their experiments. The magnitude of gas hold-up in this study was not evaluated.

In conclusion, Figures 4.46 (model 1) and 4.47 (model 2) show the results of model fitting to the decay portion of the observed tracer curves for the two models. Results of the multiparameter model analysis indicate that the mixed reactor model provides a resonable estimate of reactor volume, in all cases.

The results for the series plug-mixed model (model 2) do seem a little more varied. The reason for the low plug flow volume in the first tracer study is most probably due to the fact that at low feed rates the mixed flow charcteristics in the bottom zone beneath the packed section cause dispersion higher up the bed. All the models tested indicated that the system with the greatest degree of plug flow is the two-stage reactor without recycle which showed the best COD removal performance.

4.9 Steady State Modelling

The use of mathematical models to express the behaviour of anaerobic reactors has been well researched as they provide a useful tool for process design and simulation. This enables us to identify parameters which contribute to improved process performance. Difficulties occur due to the fact that the process is complex biologically and in a dynamic state.

Most types of reactor contain both fixed and suspended biomass each of which is subject to different environments due to diffusional and kinetic effects. The problem may be classified into two sections, firstly the hydraulic behaviour of the reactor and secondly the kinetics of substrate removal. Dynamic models will predict the performance of a reactor over a time period taking into account such factors as biomass growth and death, and steady state models will predict conversion assuming constant

substrate and biomass concentrations. The latter are considered here.

4.9.1 Reactor Hydraulics

In some cases full scale reactors may be made to approach either completely mixed or plug flow regimes, and this will be dependent on factors such as reactor geometry, feed and recycle rates together with the nature of the biomass within the system. The reactor may also be considered as compartmented with regions of mixed and plug flow joined together. Van der Meer and Heertjes (1983) described a steady state model for a UASB reactor consisting of two or three mixed sections for the sludge bed and blanket and a plug flow section for the settler. This model adequately expressed experimental results in terms of Monod kinetics. Jennings *et al.* (1976) used a plug flow model for a submerged filter. Tracer studies may be useful in this respect that they will indicate possible flow regimes within a reactor. The tanks in series model may also be of use in developing models (James, 1982). Mueller and Mancini (1975) reported the modelling of an anaerobic filter as a series of completely mixed tanks for both the gas and liquid phases. All these models provided an adequate procedure for design, provided kinetic considerations were definable. Most reactors seem to operate on a dispersed plug flow basis.

4.9.2 Removal Kinetics

Various types of removal kinetics have been used to

express the overall performance of anaerobic systems, including zero order, half order, first order (Henze and Harremoes, 1983) and variable order, together with Monod kinetics.

Various models have been proposed which account for the inhibition of anaerobic systems the most well known of which is that due to Andrews (1968). The flow regime in the reactor can have an effect on the kinetics by making the observed substrate removal appear to obey different rate relationships. The various models usually select a rate limiting step which controls the overall kinetics of removal.

Atkinson *et al.* (1984) reported that the overall rate of substrate (glucose) uptake of a foam BSM, similar to the type used in this study, containing a mixed microbial culture was dependent on substrate concentration. The overall removal rate obeyed half order kinetics, but liquid phase diffusion limitations could be neglected in the fluidised bed reactor as the upflow velocities were high and the system contained little suspended biomass.

Mueller and Mancini (1975) reported studies on the kinetics and application of an anaerobic filter using two types of substrate removal kinetics, Michelis-Menten and first order. They concluded that, although the more complex Michelis-Menten kinetics, employing an inhibition function but neglecting solids transport and biofilm diffusion, adequately simulated steady state data, the first order

model simulated filter performance equally well and was adequate for design purposes.

Duarte and Anderson (1982) found that Monod type kinetics could not be used to predict the effect of pH and that its use in anaerobic digestion modelling must be limited to situations where the pH is constant.

Lindengren (1983) presented two mathmatical models for the anaerobic filter process: one used a relatively complex Monod based model with a pH inhibition function, and the other with first and zero order kinetics, which he found adequately simulated steady state data. Forster and Wase (1983) attempted to predict removal kinetics for a UASB reactor treating a confectionery waste, and their results indicated that first order removal kinetics and plug flow offered the best results and that the Monod model was unsatisfactory. More complex models have been described which include liquid layer mass transport, molecular diffusion and Monod kinetics (Rittman and McCarty, 1978; Suidan and Wang,1985)

It seems as though the bulk substrate concentration in a fixed film reactor will affect the removal kinetics. Dewalle and Chian (1976) studied the kinetics of substrate removal in a completely mixed anaerobic filter. They found that if the bulk substrate concentration was much greater than K_s (Monod saturation constant) the removal approached zero order kinetics. Whereas when the substrate concentration was much less than K_s, first order kinetics

were approached. These results may be compared to those of Harremoes (1976) who, from his work on denitrification, concluded that removal kinetics may be divided into three categories: (1) zero order heterogeneous reactions in a pore which is fully penetrated by the substrate (leading to bulk zero order kinetics); (2) partially penetrated pores with zero order kinetics (leading to bulk half order kinetics); and (3) first order reactions in a partially penetrated pore (leading to bulk first order removal kinetics at a reduced rate).

Due to the variability of process configurations investigated in this study. The observed experimental data were fitted with various hydraulic models and first order kinetics using a least squares regression technique. The intention was to assess which model provided the best representation of the observed data. Models analysed were as follows:

(i) plug flow first order;
(ii) completely mixed first order;
(iii) Young and McCarty's (1969) empirical model; and
(iv) tanks in Series

Expressions were derived relating COD removal efficiency to hydraulic retention time (based on empty bed volume) (appendix IV). The results of the regression analysis were compared for all different reactor configurations, and those with the lowest mean square residuals were considered the best fit for the experimental

data. The results are displayed in Table 4.13 and Figs 4.48 to 4.51. They indicate that for the single stage reactor with recycle the best fits are the empirical model of Young and McCarty based on total COD removal efficiency and the complete mixed first order based on soluble COD. For the two stage reactor with recycle the best fit is obtained with the tanks-in-series model for both total and soluble COD. For systems without recycle, the tanks-in-series model and the plug flow model represent best the total COD removal, while the tanks-in-series model is best for soluble COD. The two stage reactor without recycle is best represented by the plug flow model for total COD and tanks in series for soluble COD.

Performance modelling was considered doubtful due to the disperse nature of the data and the fact that variability in feed conditions would have greatly affected model fitting. These results are only valid in the regions investigated in this study but there is resonable evidence that they may be extrapolated from the results of other workers using similar reactors.

None of the models take into account inhibition due to volatile fatty acids but the pH in all experiments was controlled at 6.5. It has been demonstrated that the proportion of un-ionized VFA, thought to be responsible for inhibition, is low at this pH (Andrews 1978). The assumption that first order kinetics apply has been shown when bulk substrate concentrations are low compared to K_s values.

The results from the model fitting are somewhat limited due to the fact that conditions in the reactor were not set up specifically to determine operational kinetics. The operating points covered a restricted portion of the full operating range of the systems.

Another consideration from the model fitting includes the number of tanks in the tanks in series model. These results are displayed in Table 4.13 and may be compared with those from the tracer studies (section 4.8). The results did not reinforce one another very well, except for the two stage system without recycle in which the value of N based on tracer studies was 2.99 compared with the value of 2.72 from the tanks-in-series model based on soluble COD removal.

4.10 Examination of Colonized BSM by SEM and TEM

Scanning electron microscopy has been a useful technique in the investigation of the nature of biofilms in biological reactors (Richards and Wilson, 1983 ; Richards and Turner, 1984). There have been many reports of artefact formation due to sample preparation and fixation. The BSM samples from this study were withdrawn during the final phase of operation of the two-stage operation without recycle. (Sample preparation was described earlier in section 3.4.19). Plate III shows a fragment of biomass and the support matrix indicating some attached and suspended biomasss. In comparison with other workers it seems as though the majority of the biomass is composed of a fibrillar network (Plate IV and V). Several workers

(including Richards and Turner, 1984) have reported that sample preparation by fixation in gluteraldehyde and subsequent critical point or air drying can remove the slime layer and expose the underlying bacteria. They concluded that the sputter-cryo technique was the preferred method for preserving the true state of the biofilm.

Alibhai and Forster, (1986) observed that the surface of UASB granuals in their digester, treating spent fermentation liquor, was composed of a wide range of methanogenic species including rods, cocci, and sarcinae. *Methanothrix soehngenii* did not appear to be a component of their compact granules.

Robinson *et al.* (1984) investigated the biofilm in a fixed bed anaerobic reactor with SEM and observed that *Methanothrix* as present on the surface of the biofilm with *Methanosarcina* embedded deeper in the film. Most of the bacteria were surrounded by an exopolysacchride matrix which was very dense towards the base of the biofilm. An extensive network of "volcano-like" channels were observed throughout the matrix which may have facilitated gas and nutrient exchange to the lower regions of the biofilm. This phenomenon was also observed by Wiegant and de Man (1986) in SEMs of UASB pellets. The BSMs from this study showed a fibrillar matrix similar to that observed by Richards and Turner (1984). The results were such that no definite conclusions could be drawn from the SEMs and TEMs of the BSMs. Plates VI and VII display some TEMs from a fragment of a biomass support particle. Long filaments were common and

were approximately 0.65 um in diameter but of indeterminant length. Plate VI shows one of the morphological forms of a non-cyst-forming microbe. No cyst forms were identified in this particle but have been observed in abundance elsewhere (Robinson *et al.*,1984). Plate VII shows sections through a filament; the two layer cell wall and ragged outer layer were similar to those reported by Robinson.

The "volcano-like" passages observed by other workers could be seen with the naked eye on some of the particles withdrawn from the reactor. This would tend to suggest that pore diffusion effects may influence reaction kinetics in some circumstances.

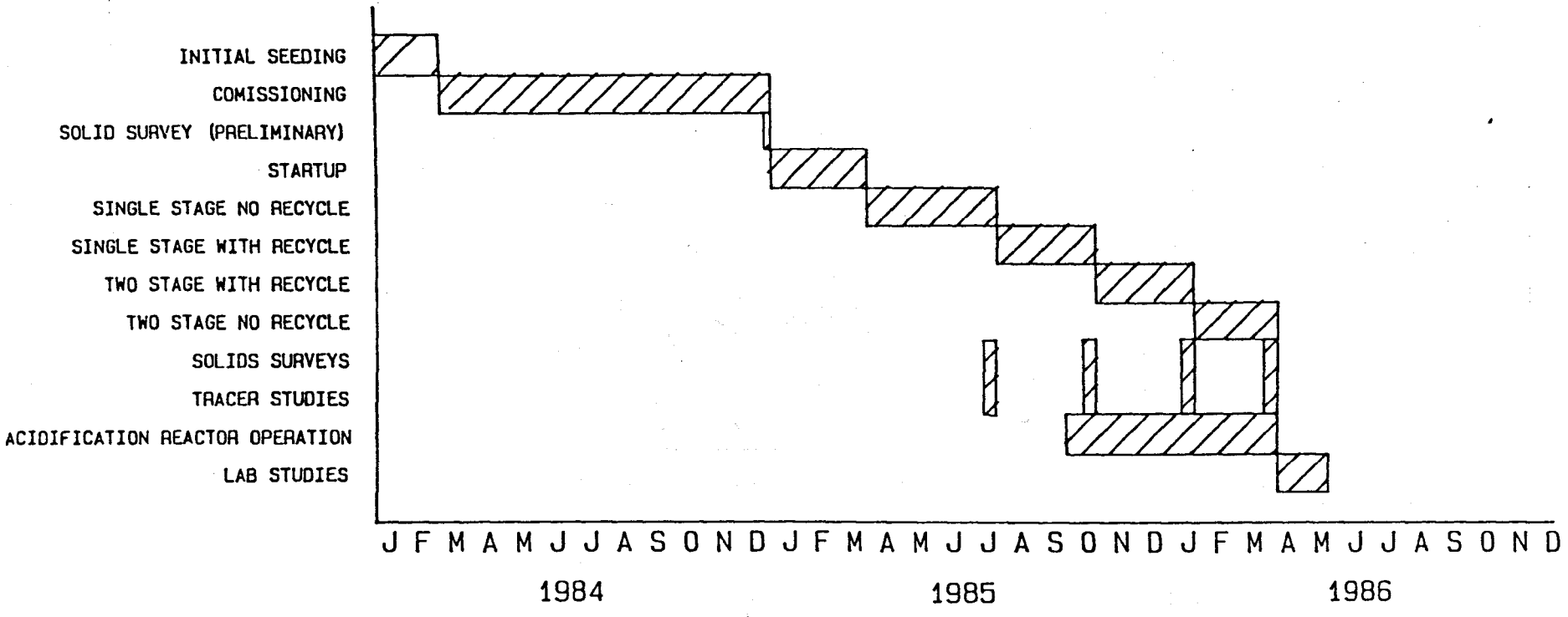


Fig. 4.1 Programme of this study

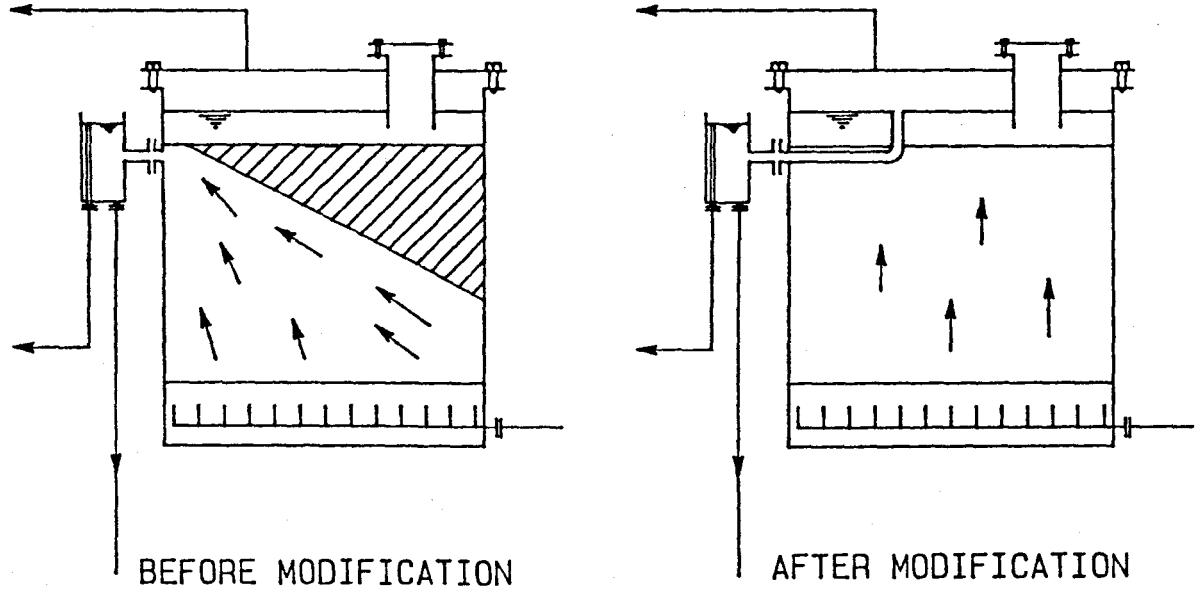


Fig. 4.2 The effect of offtake modification in reactor 1 to the flow regime

(SHADED AREA SHOWS POSSIBLE DEAD-VOLUME)

FIG. 4.3 STARTUP RESULTS FOR REACTOR 1

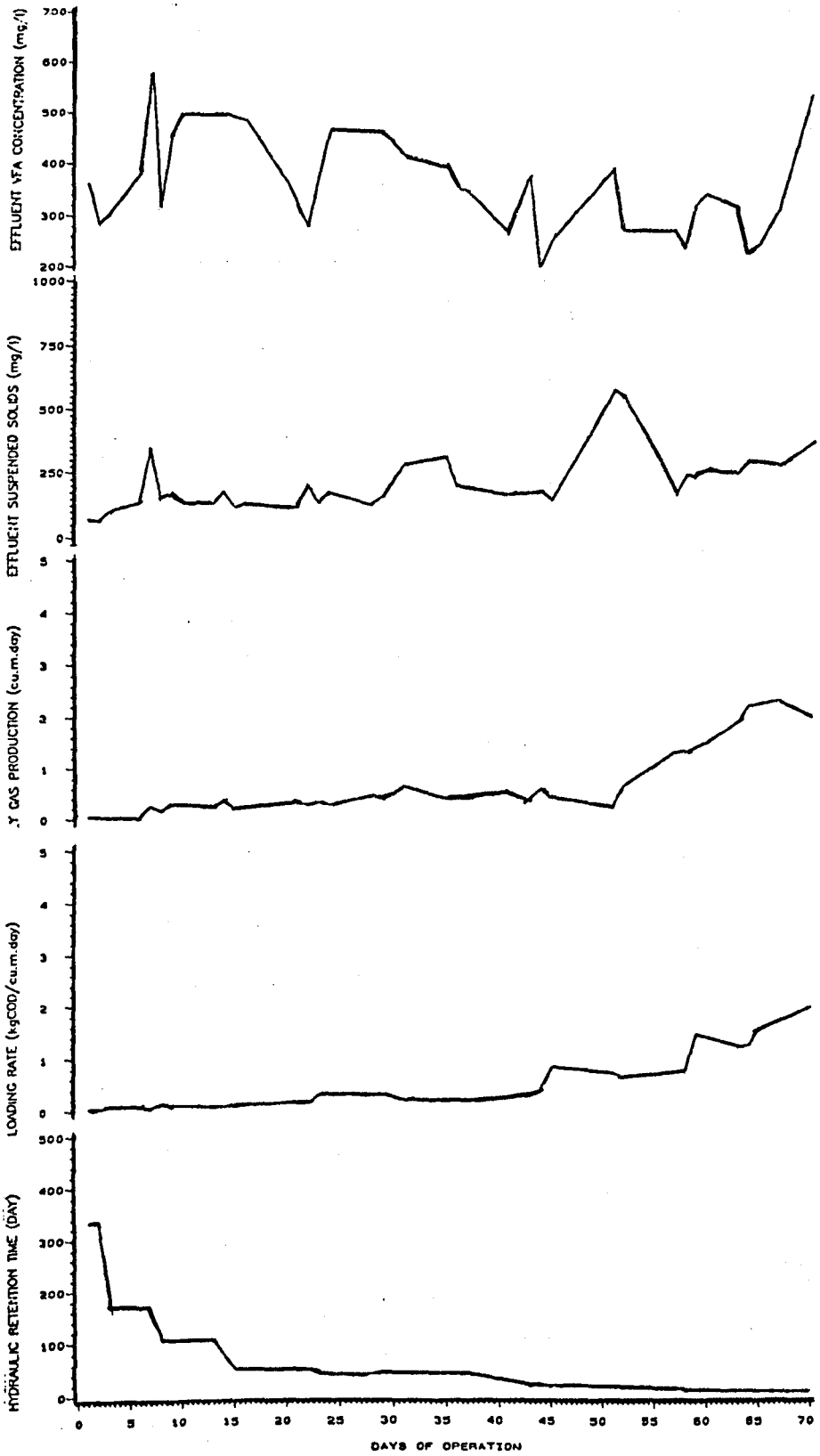


Fig. 4.4 Total COD Removal vs. Hydraulic Retention Time

(Lines indicate limits of minimum HRT attained before failure in this study)

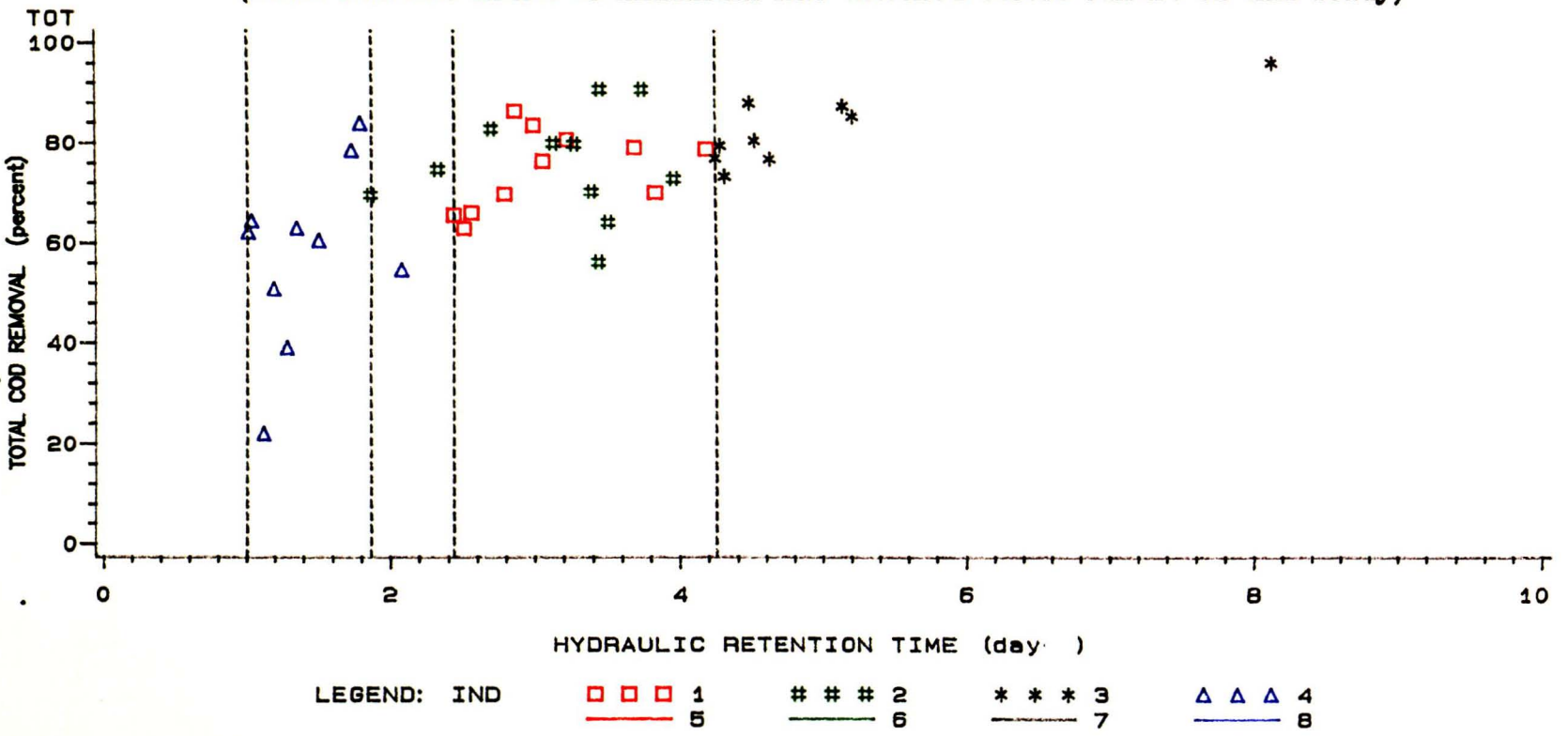


Fig. 4.5 Organic Loading Rate vs. Organic Removal Rate

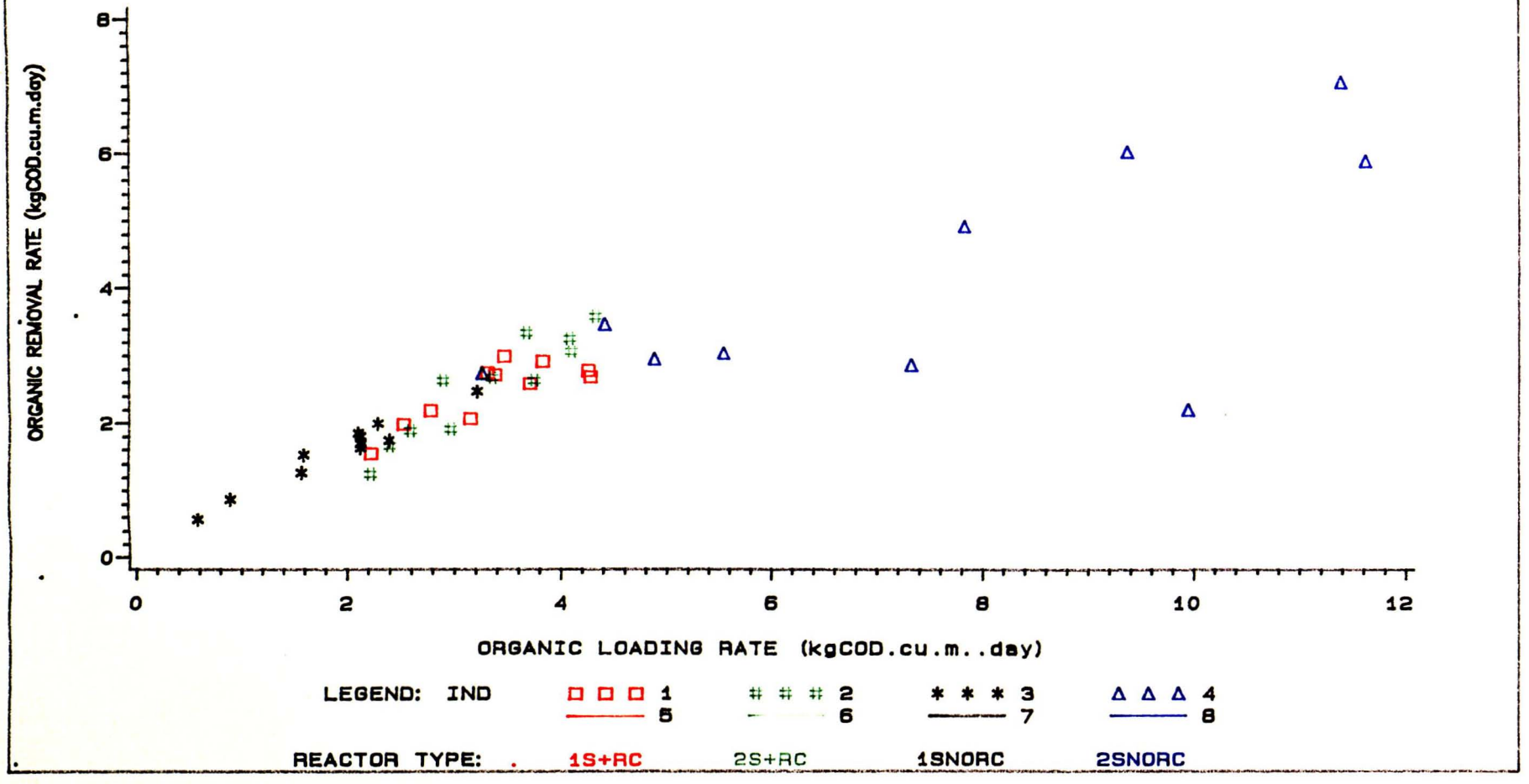


Fig. 4.6 Total COD Removal vs. Organic Loading Rate

(lines indicate maximum loading rates attained)

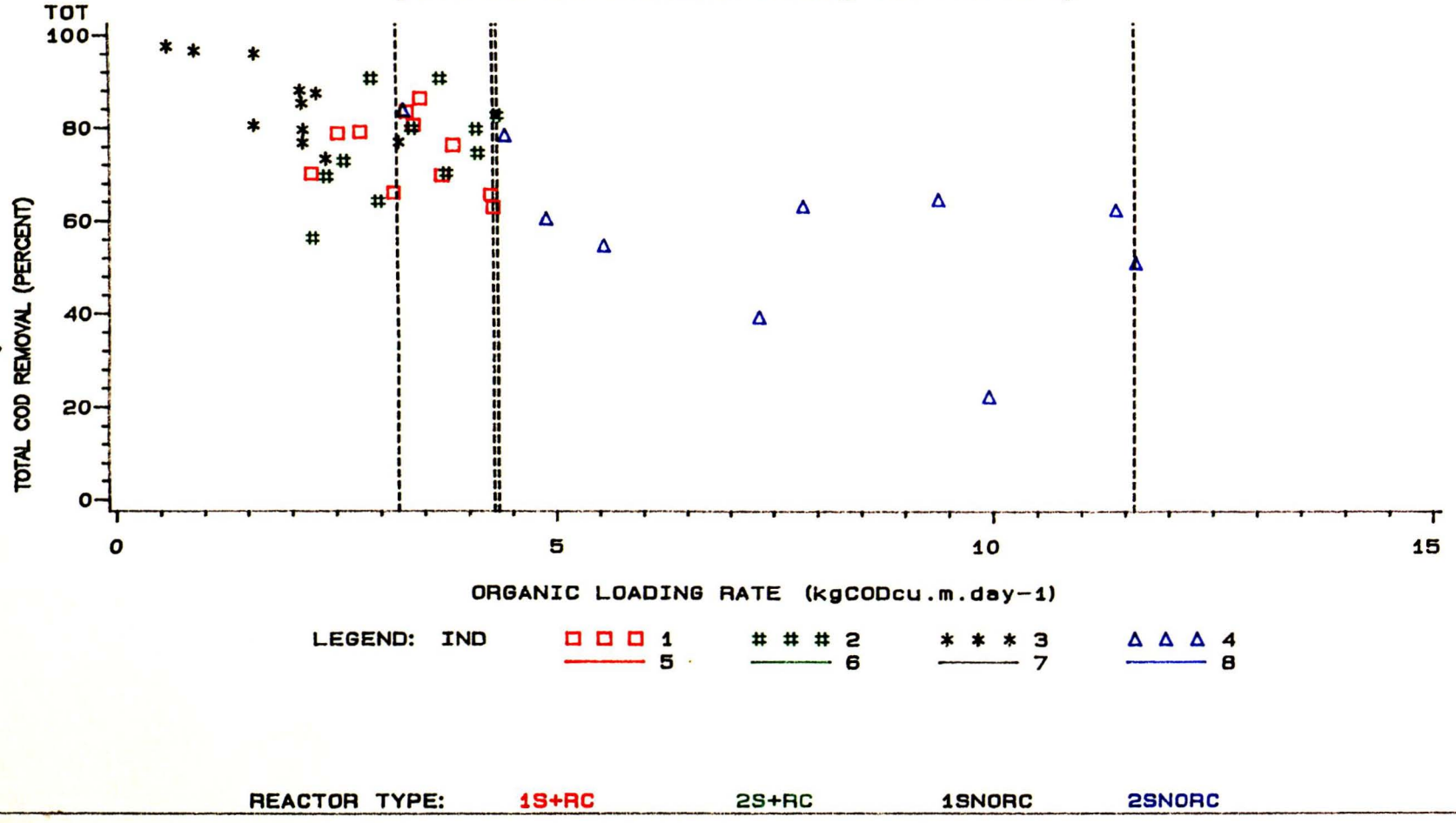


Fig. 4.7 Volumetric Gas Yield vs. Organic Removal Rate

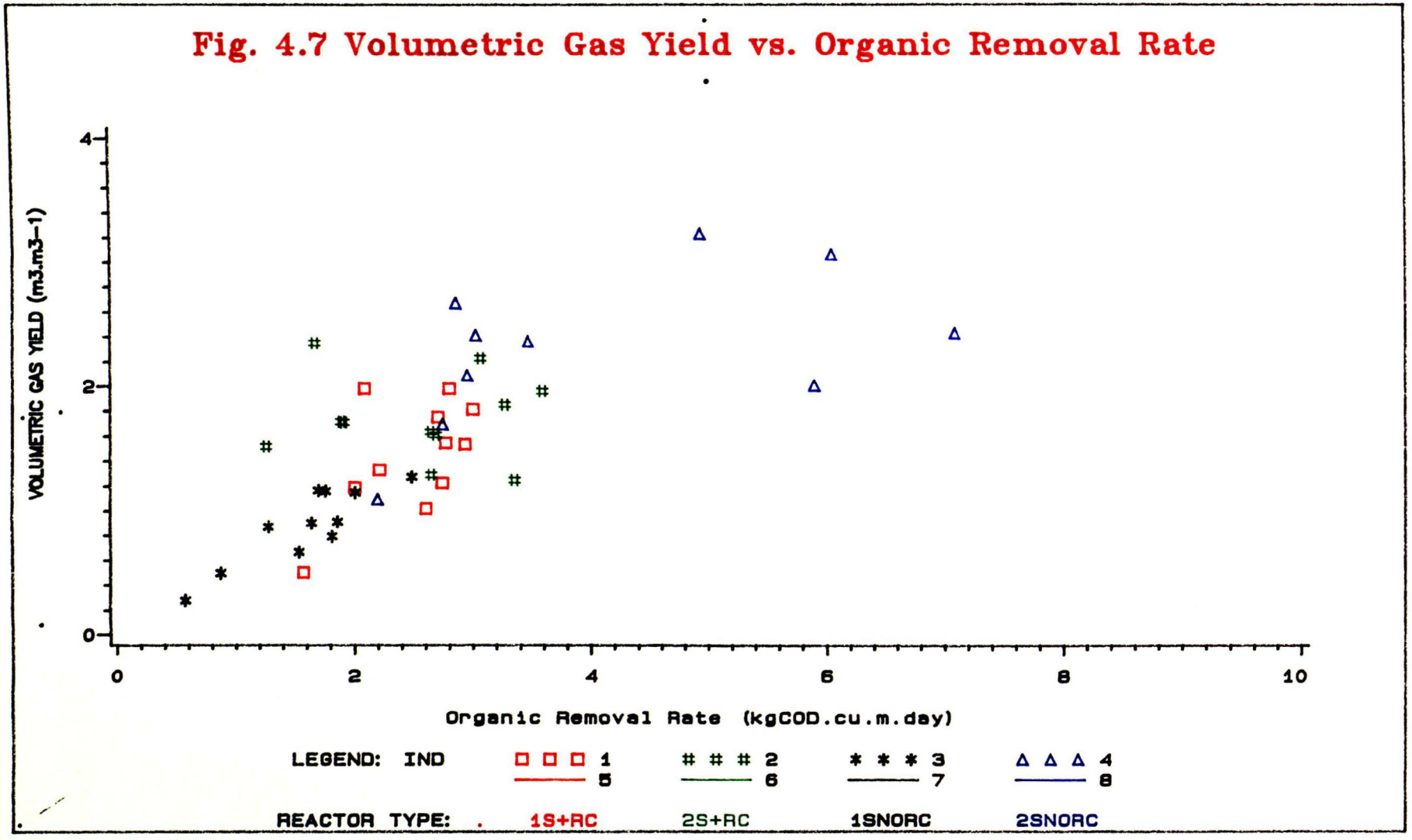


Fig. 4.8 Total Gas Yield vs. Organic Removal rate

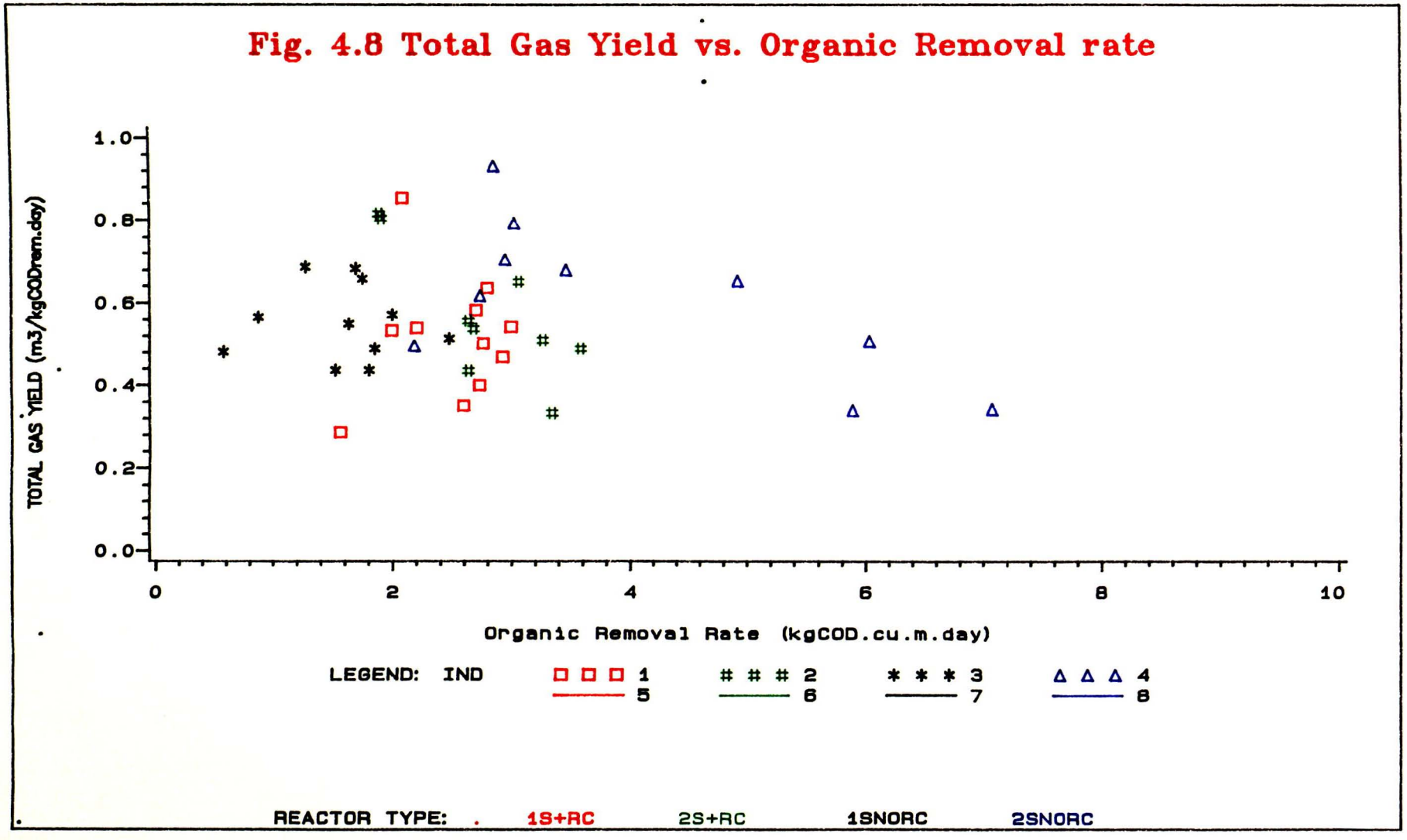


Fig. 4.9 Total Methane Yield vs. Organic Removal rate

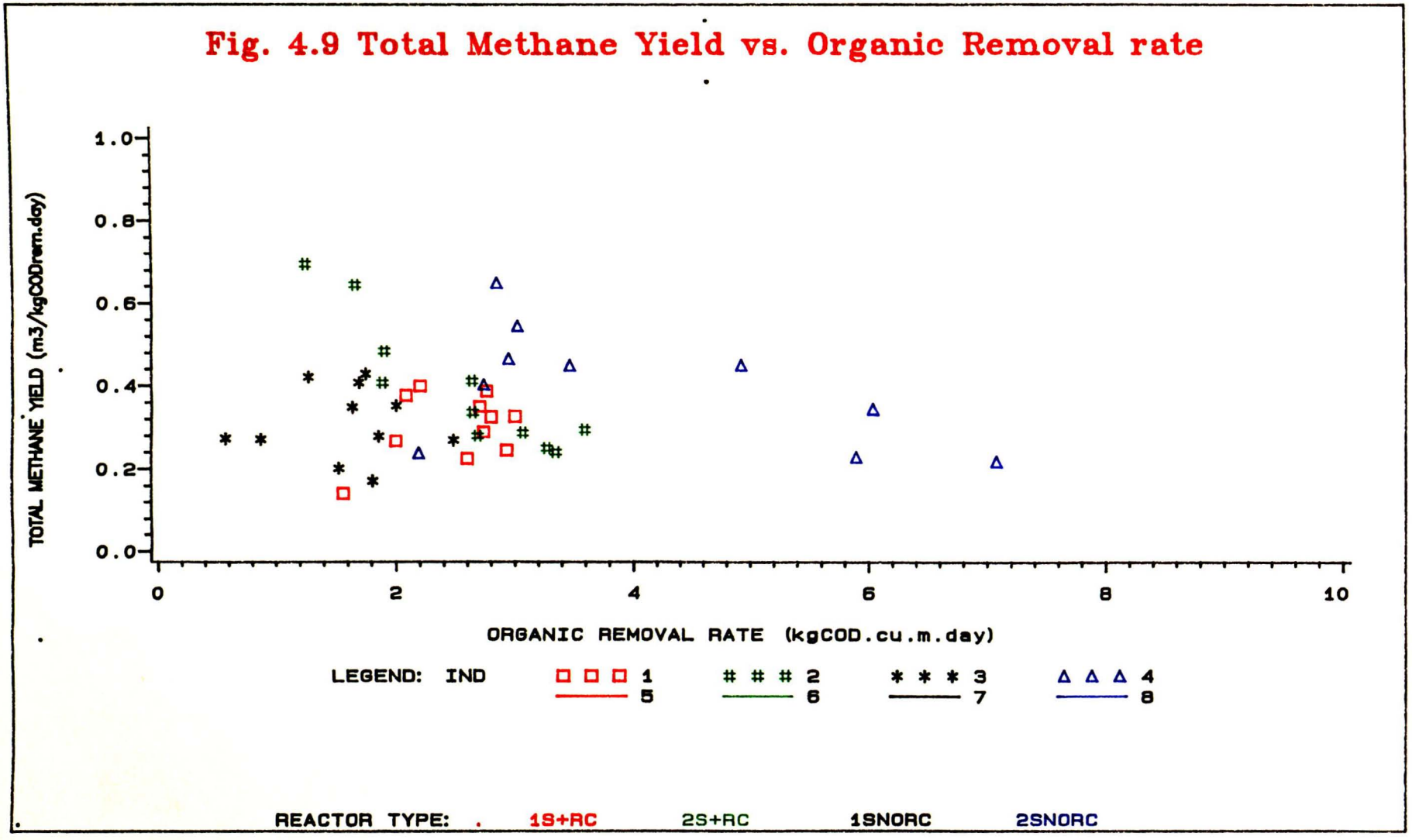


Fig.4.10 Days of Operation vs. Loading Rate and Hydraulic Retention Time

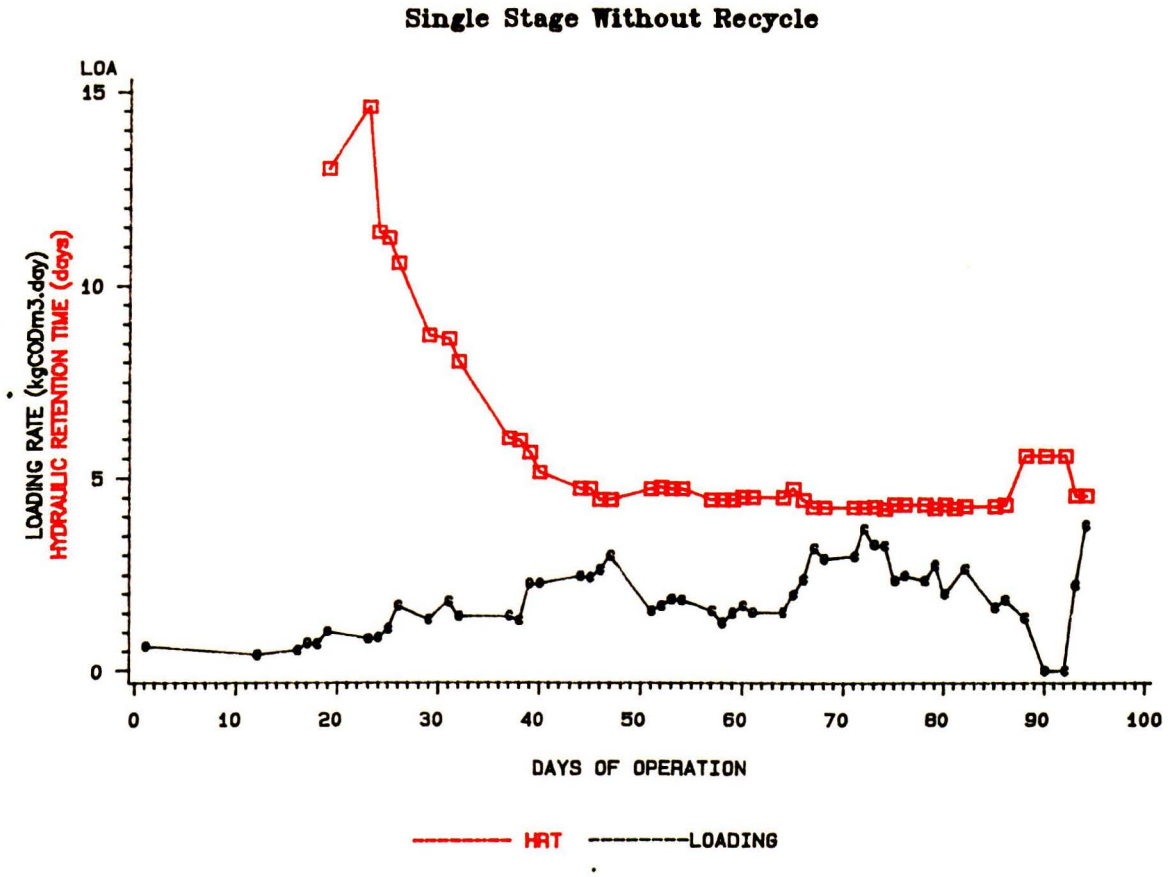


Fig. 4.11 Days of Operation vs. COD Removal Efficiency

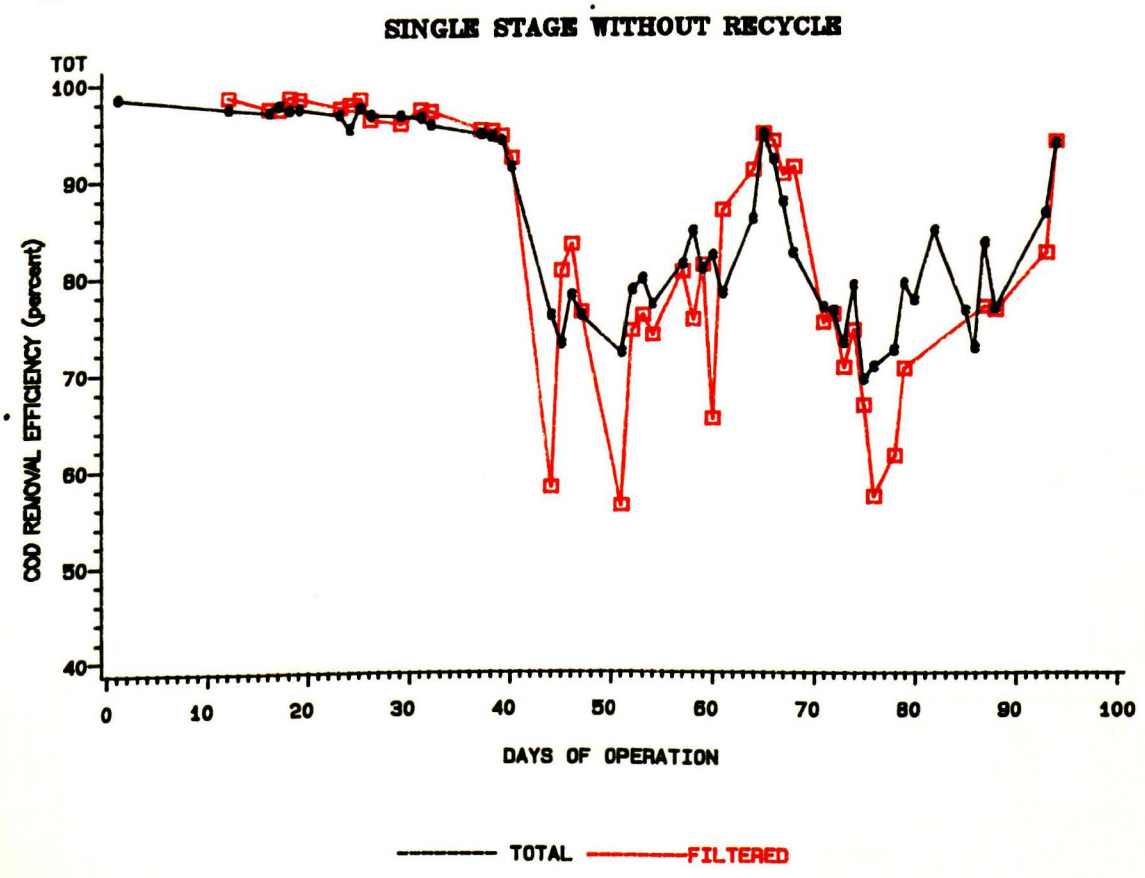


Fig. 4.12 Days of Operation Vs. Effluent VFA Concentration

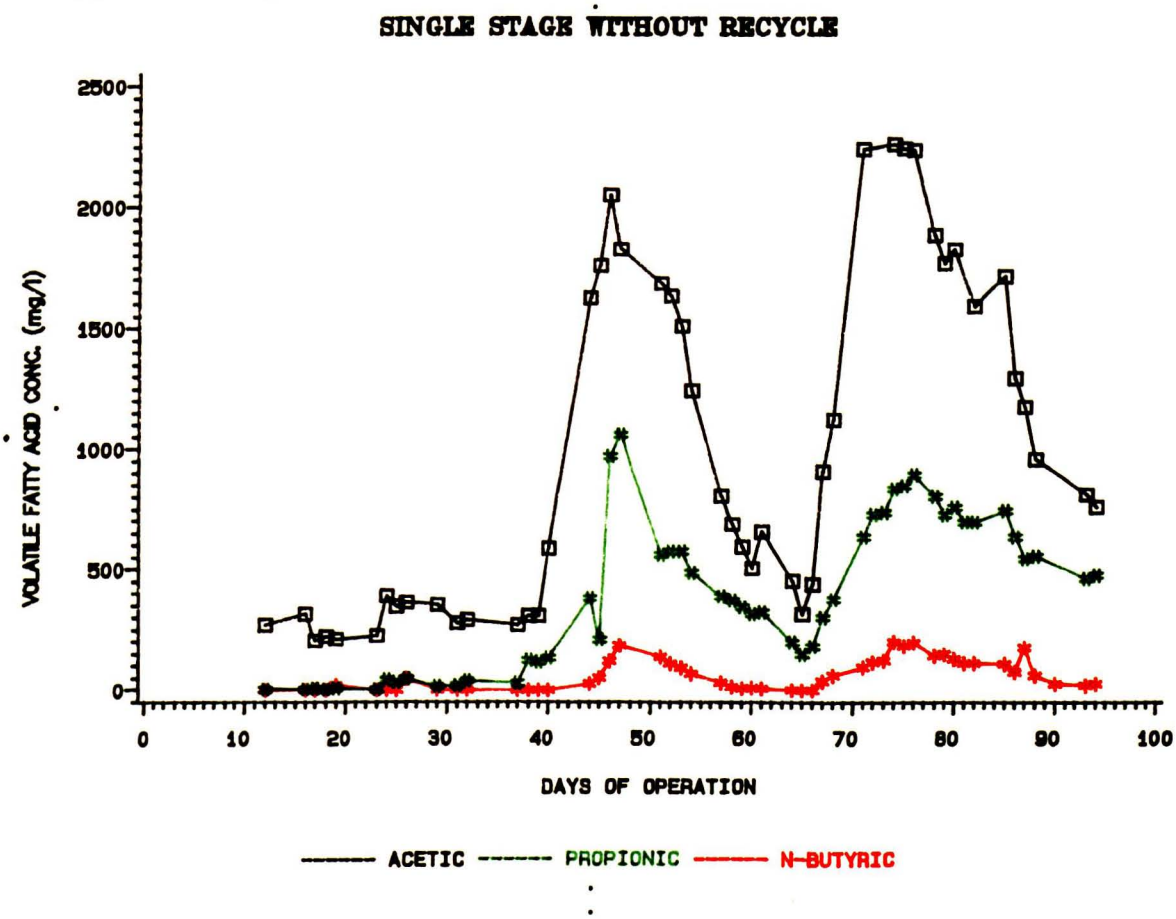


Fig. 4.13 Days of Operation vs. Influent and Effluent pH

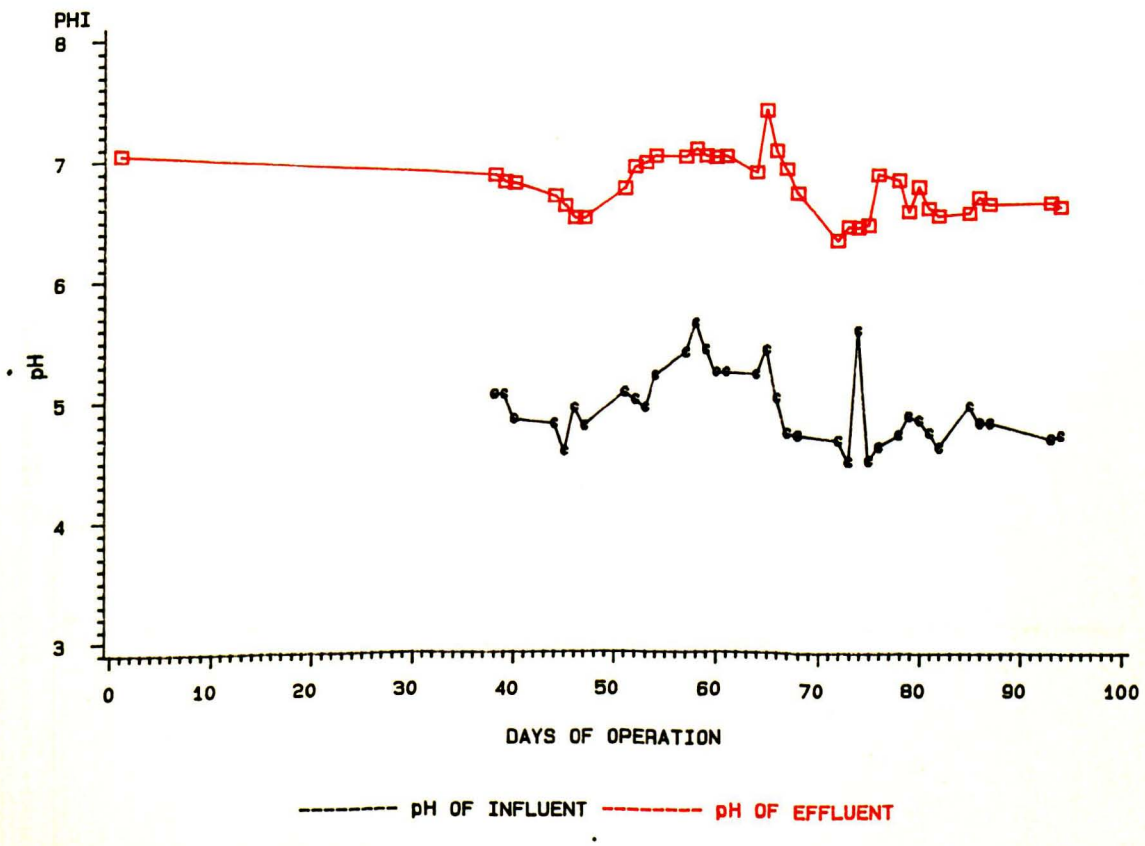


Fig.4.14 Days of Operation vs. Loading Rate and Hydraulic Retention Time

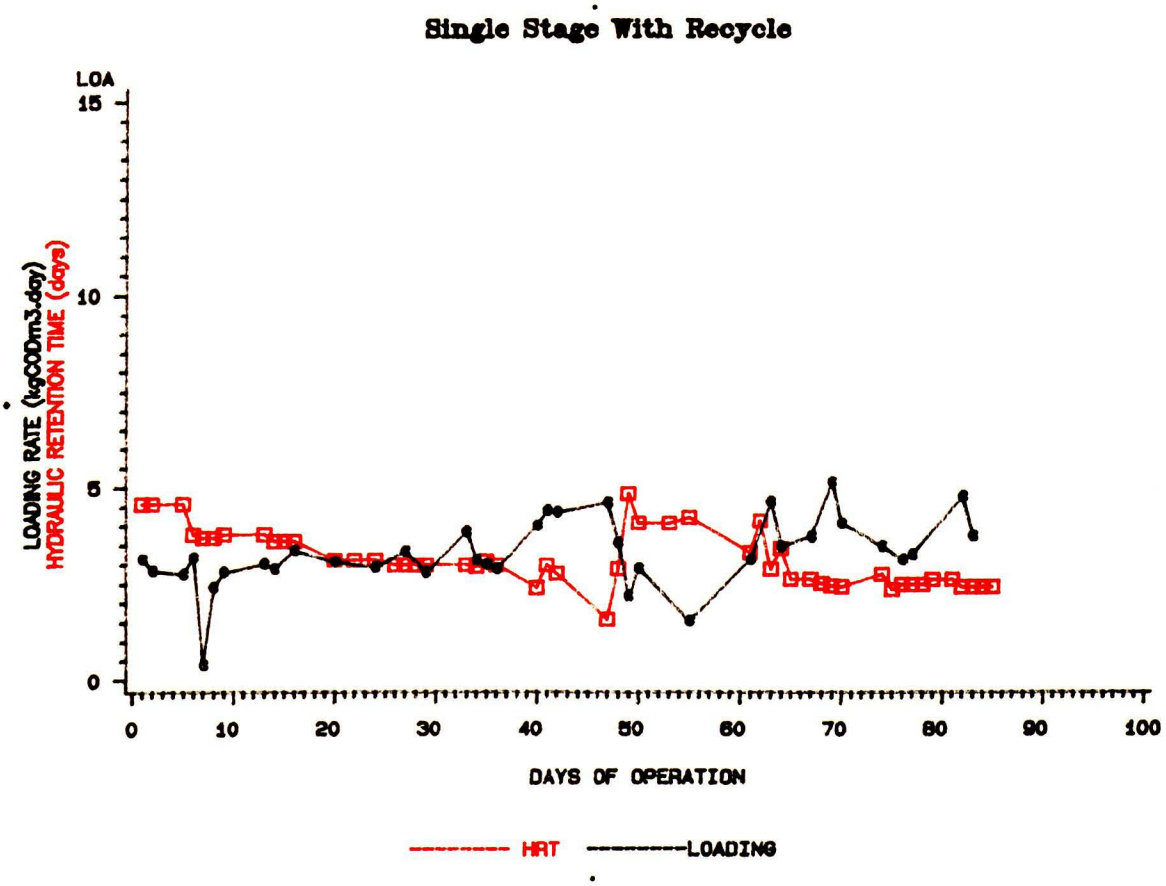


Fig. 4.15 Days of Operation vs. COD Removal Efficiency

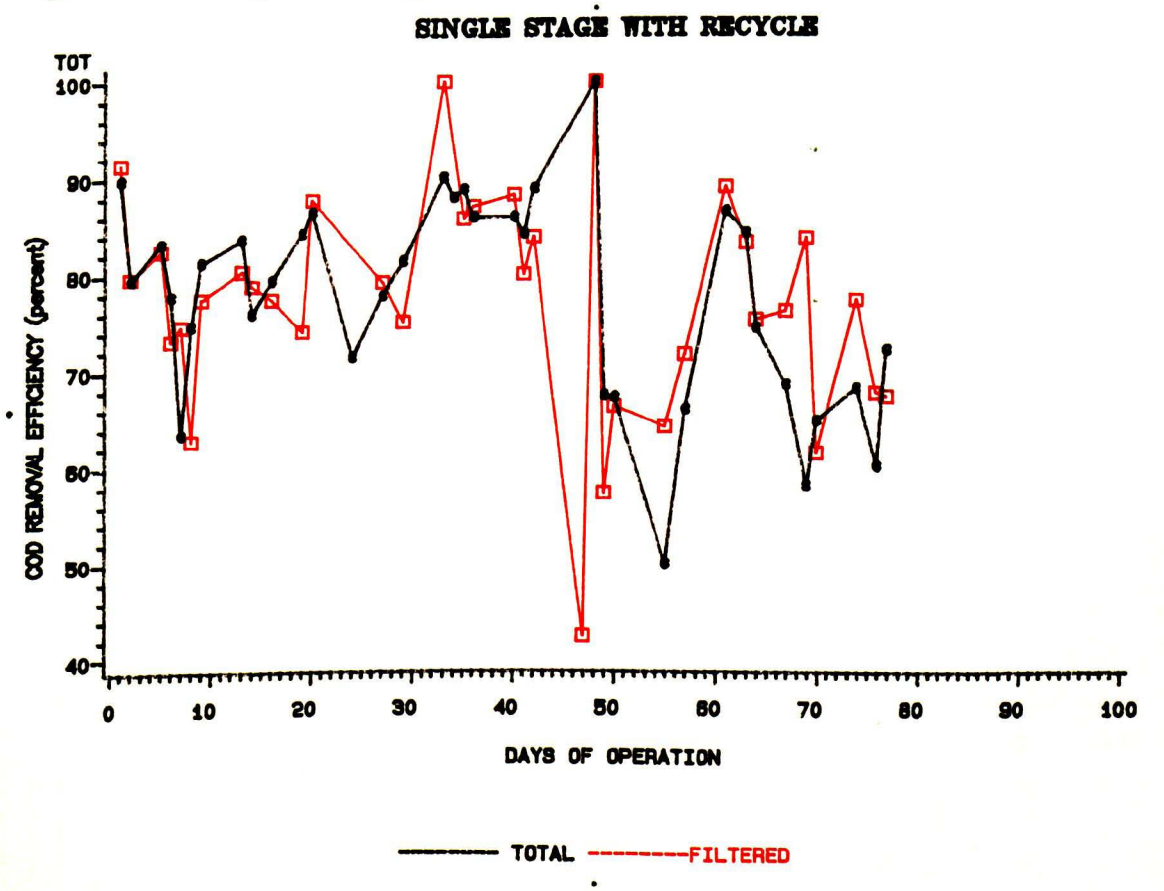


Fig. 4.16 Days of Operation Vs. Effluent VFA Concentration

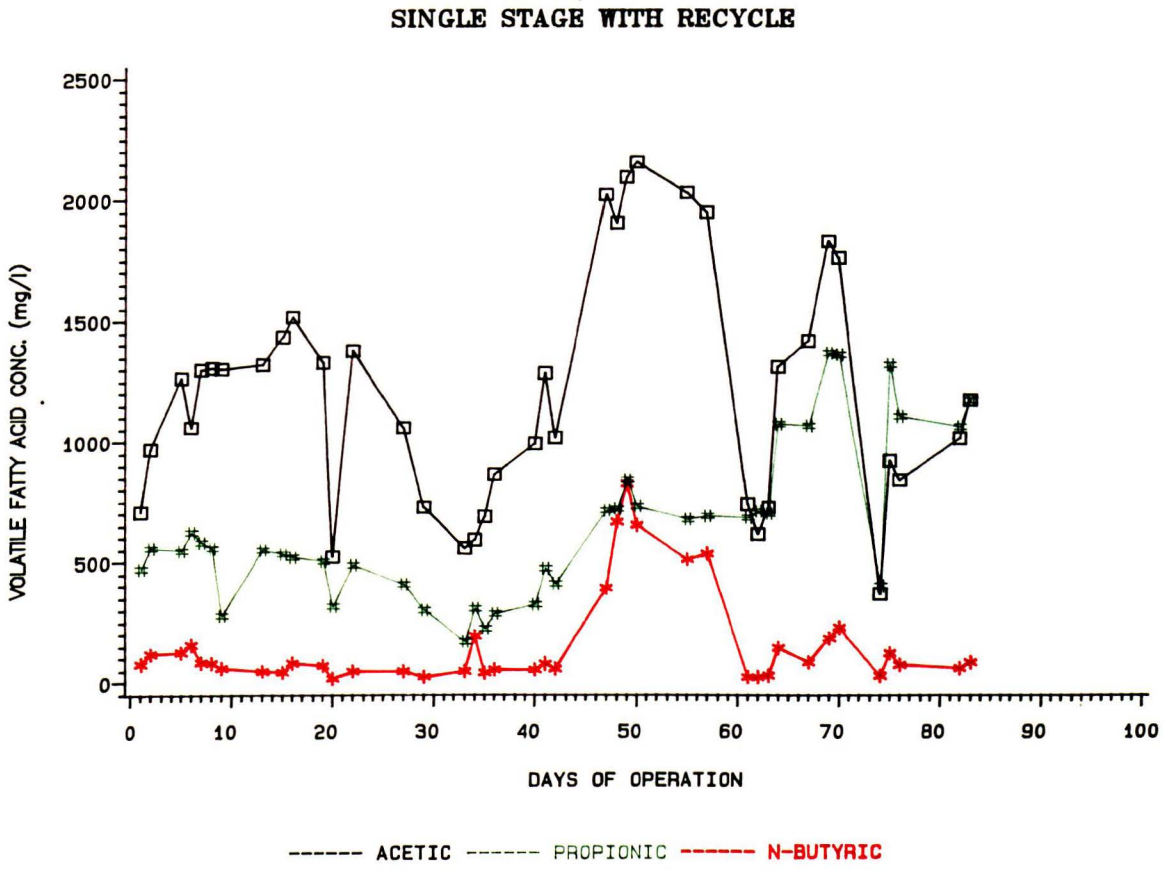


Fig. 4.17 Days of Operation vs. Influent and Effluent pH

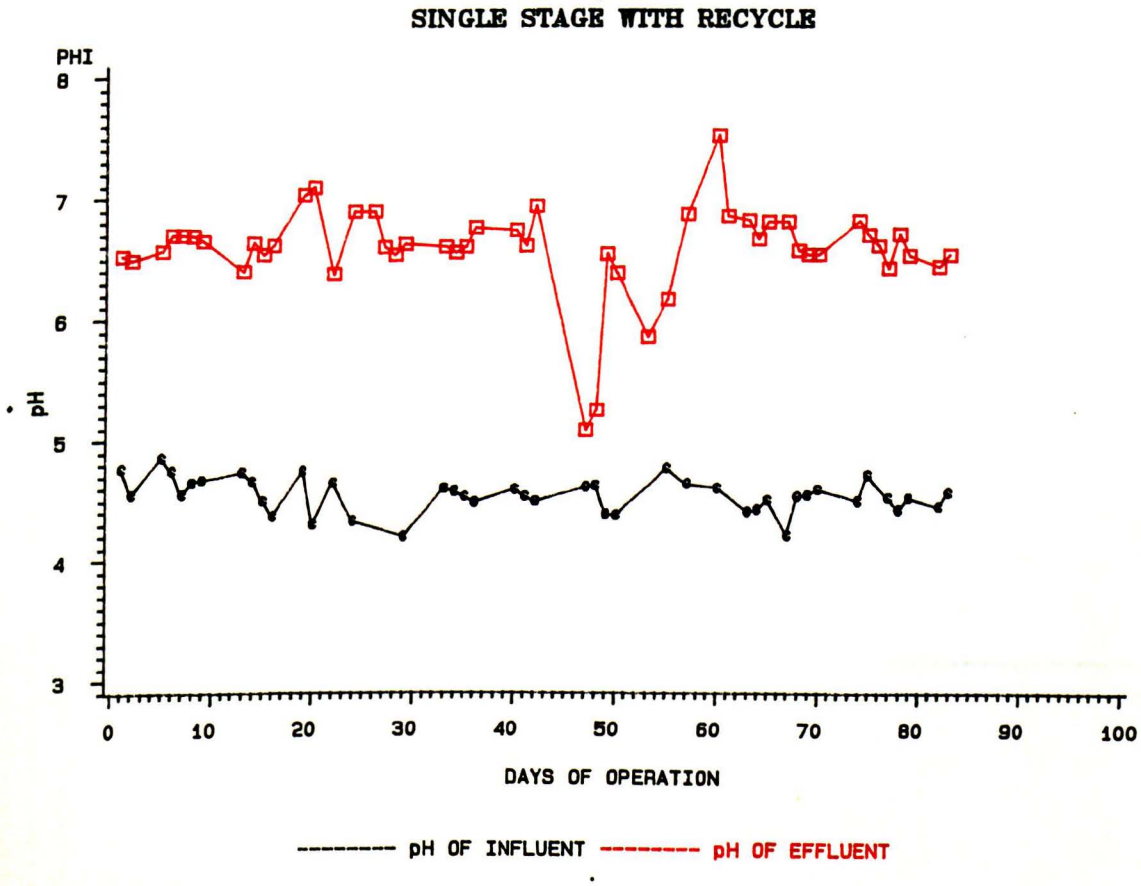


Fig.4.18 Days of Operation vs. Loading Rate and Hydraulic Retention Time

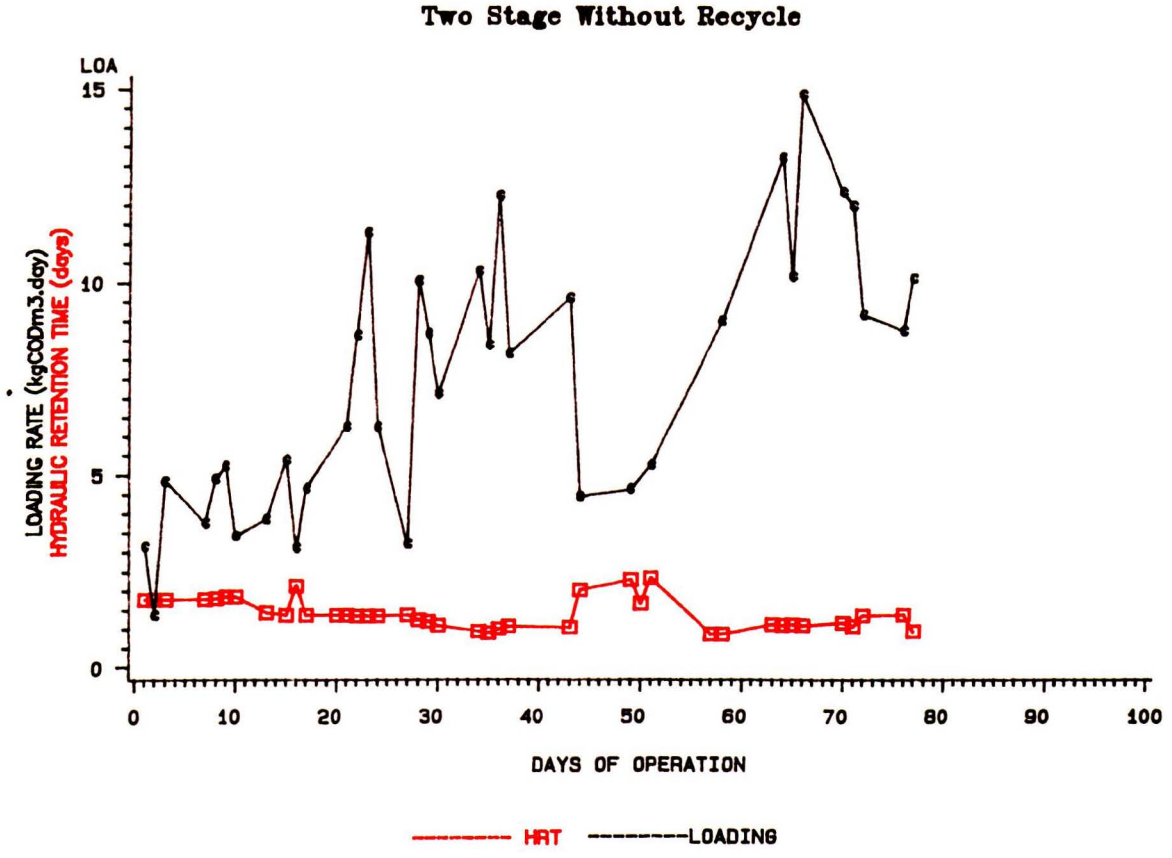


Fig. 4.19 Days of Operation vs. COD Removal Efficiency

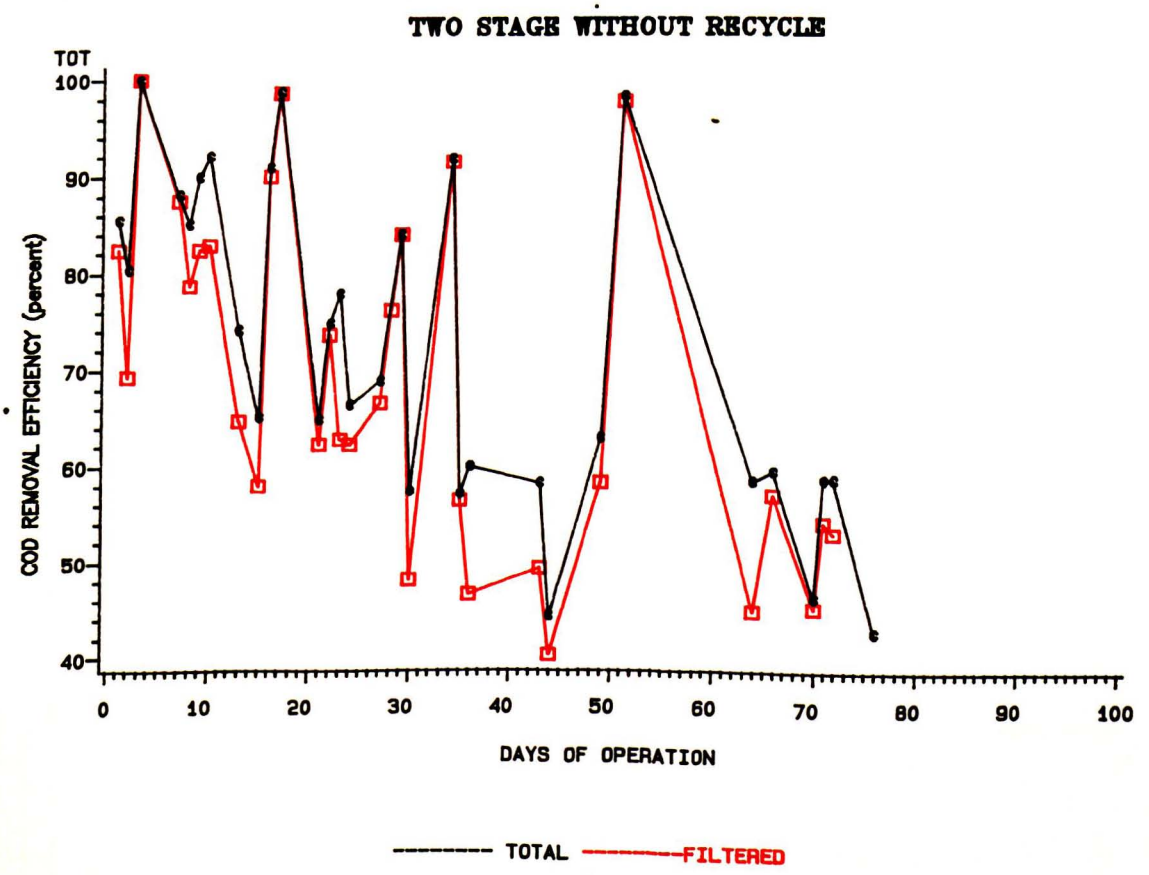


Fig. 4.20 Days of Operation Vs. Effluent VFA Concentration

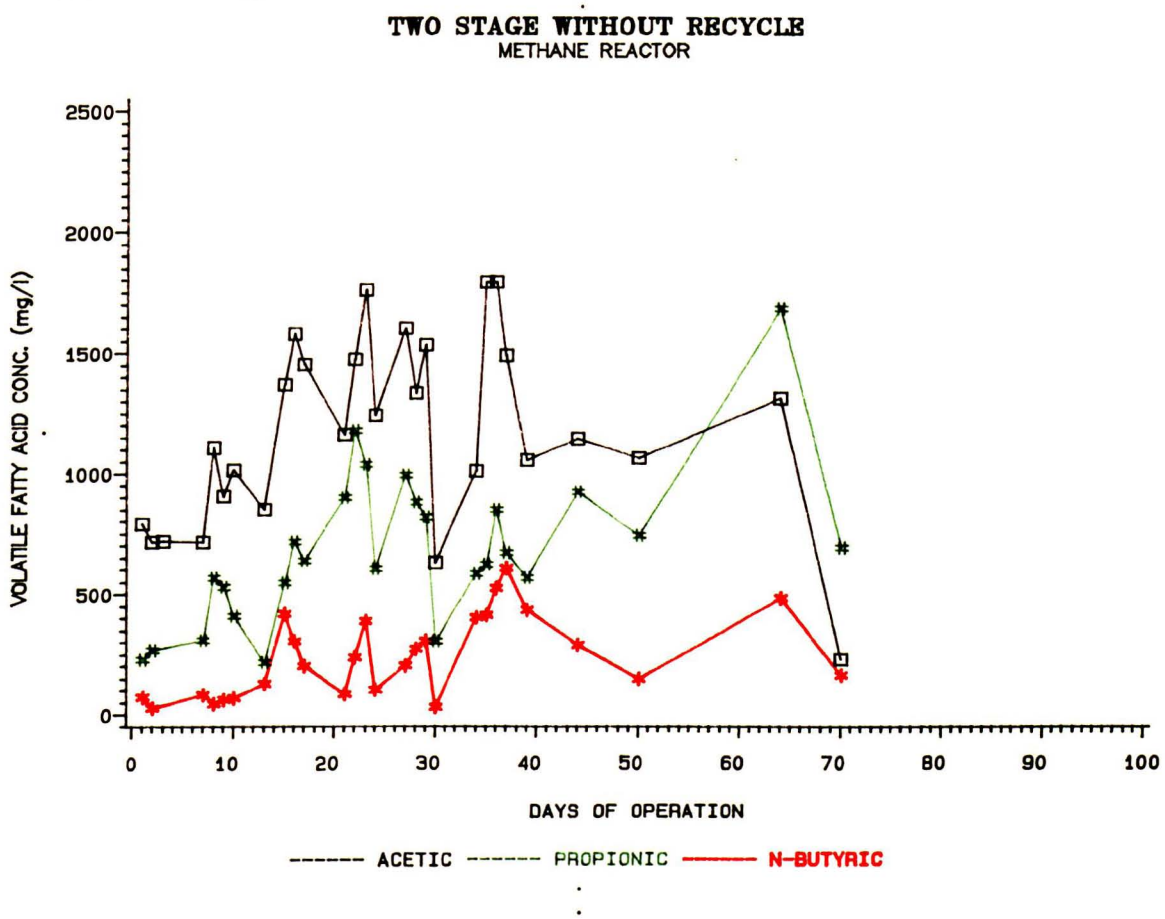


Fig. 4.21 Days of Operation vs. Influent and Effluent pH

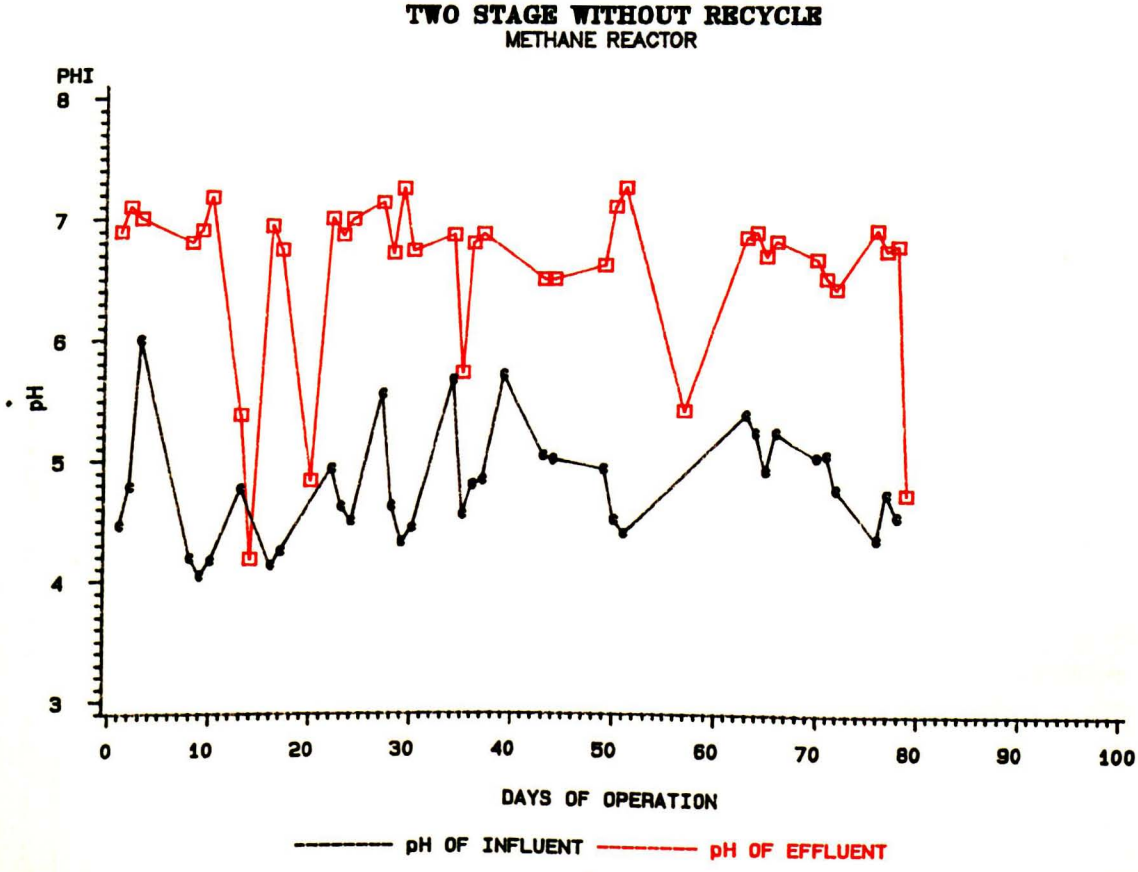


Fig.4.22 Days of Operation vs. Loading Rate and Hydraulic Retention Time

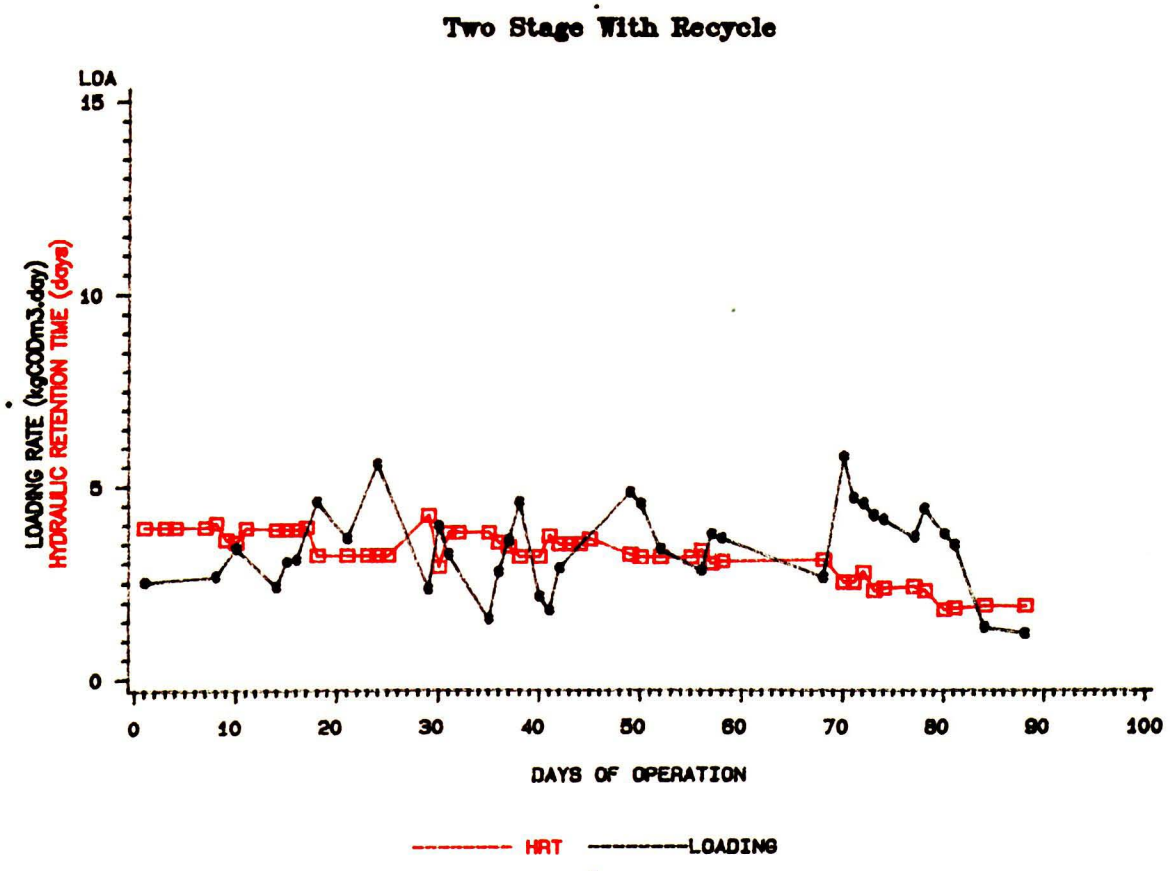


Fig. 4.23 Days of Operation vs. COD Removal Efficiency

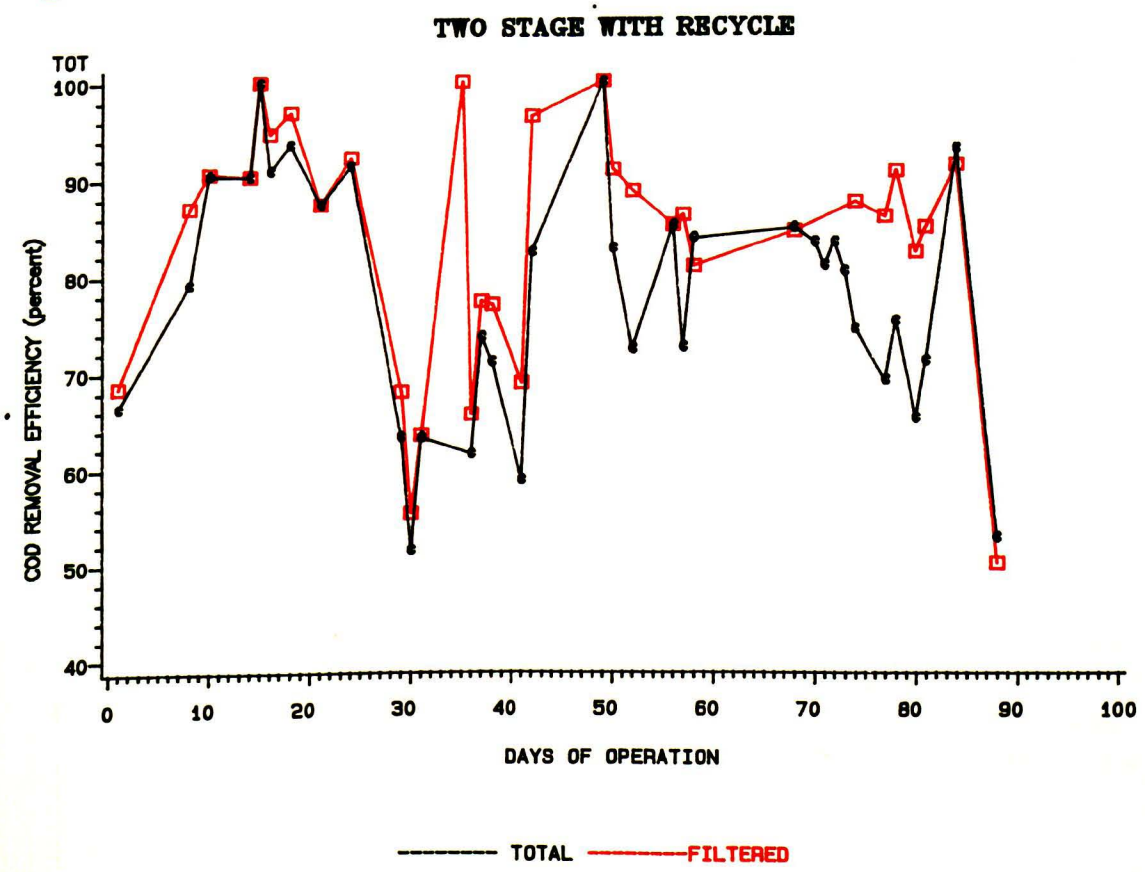


Fig. 4.24 Days of Operation Vs. Effluent VFA Concentration

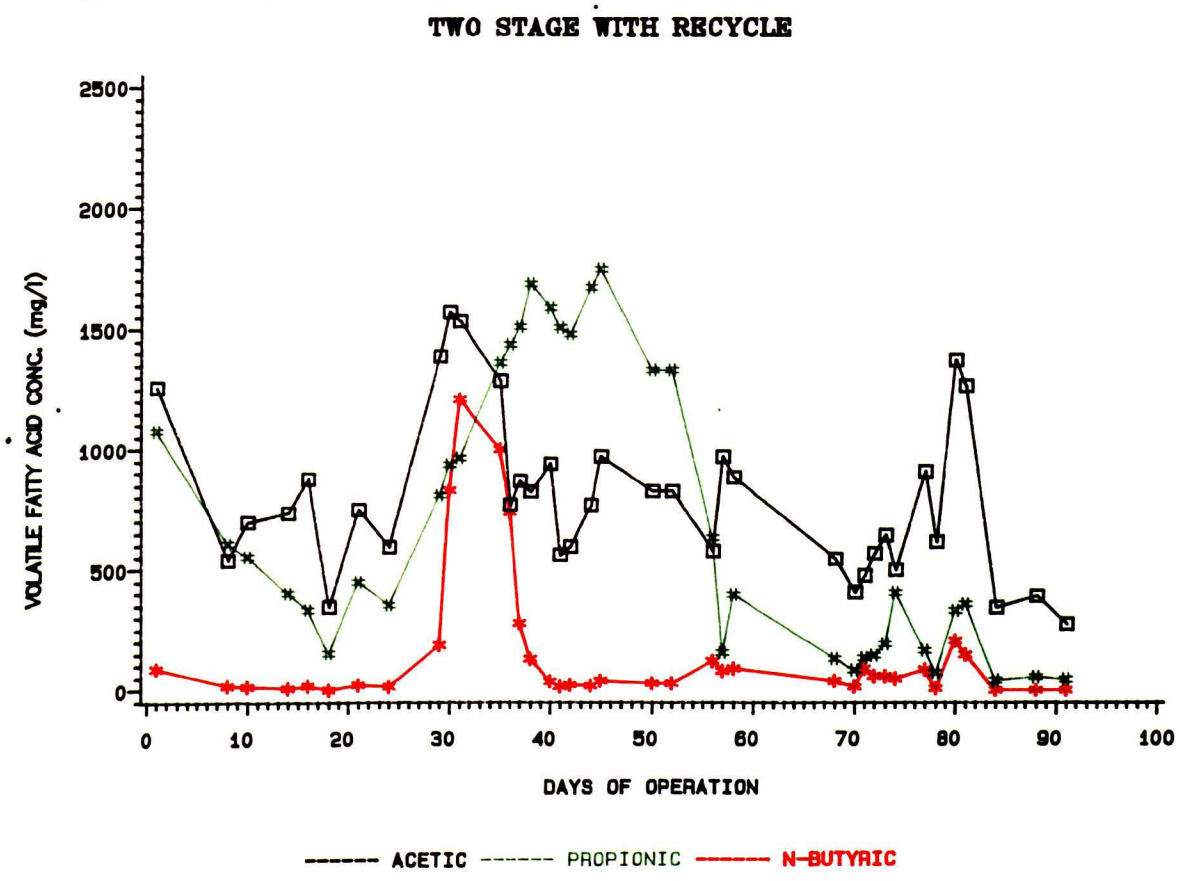


Fig. 4.25 Days of Operation vs. Influent and Effluent pH

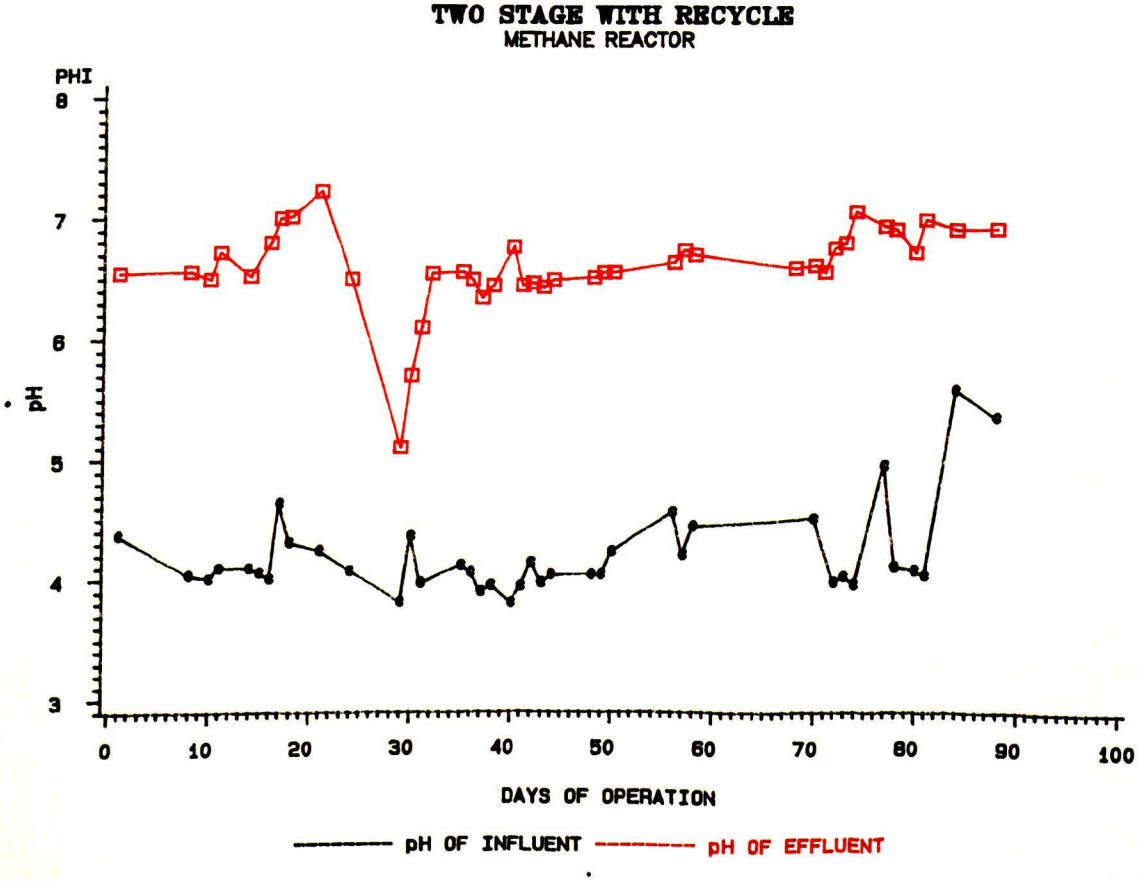


Fig.4.26 Days of Operation Vs. Solids Concentration

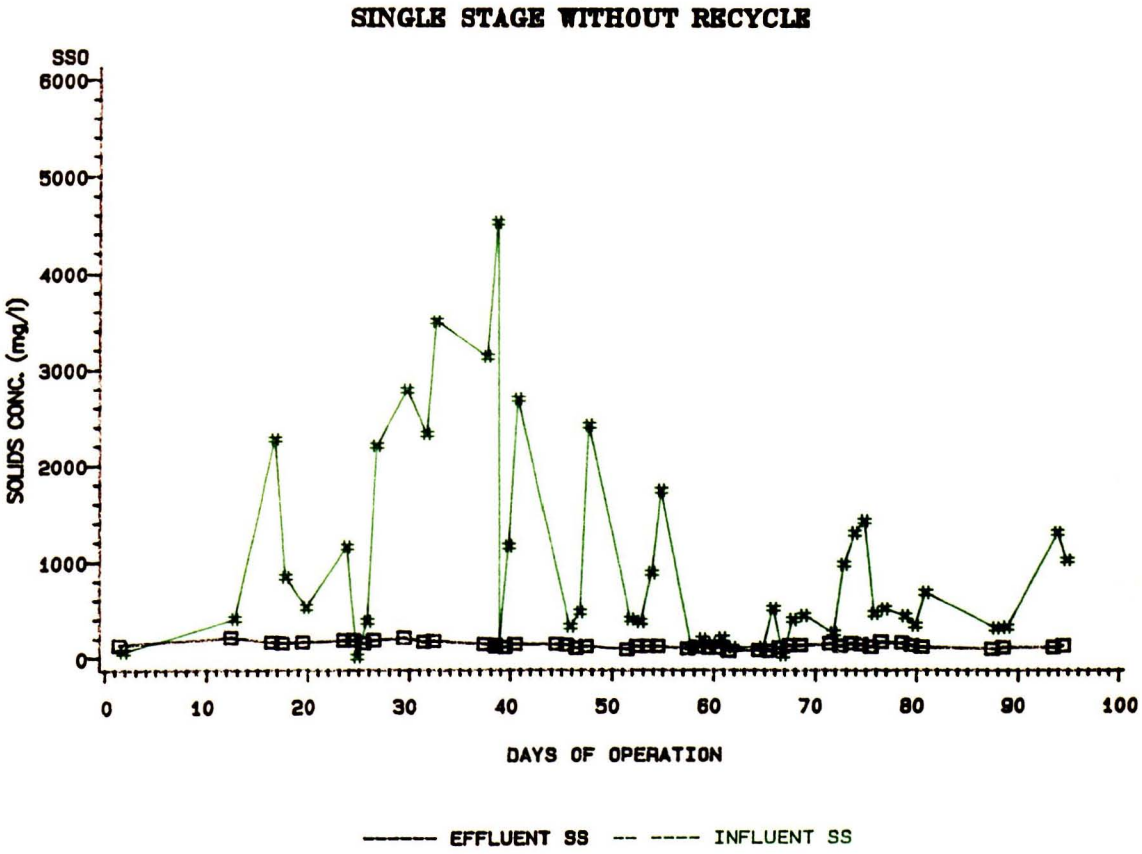


Fig. 4.27 Biomass Washout Parameters

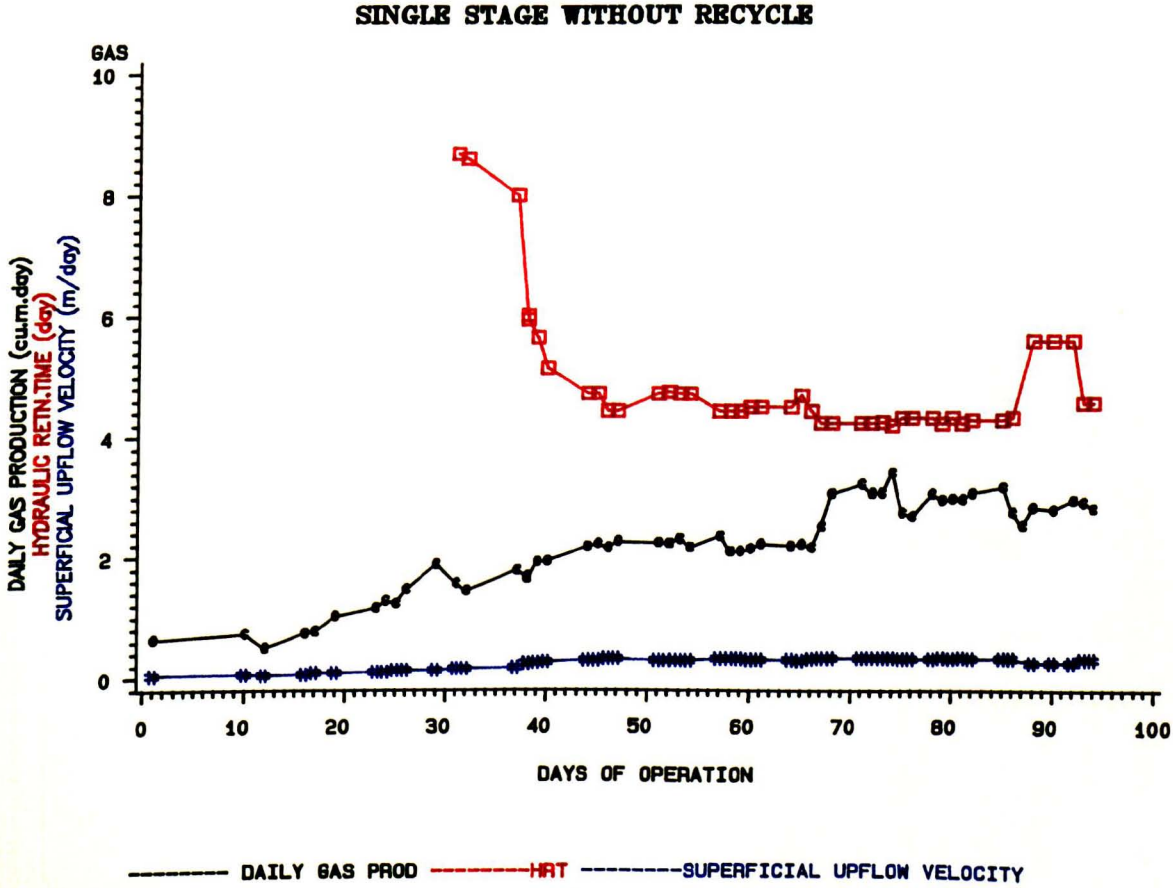


Fig.4.28 Days of Operation Vs. Solids Concentration

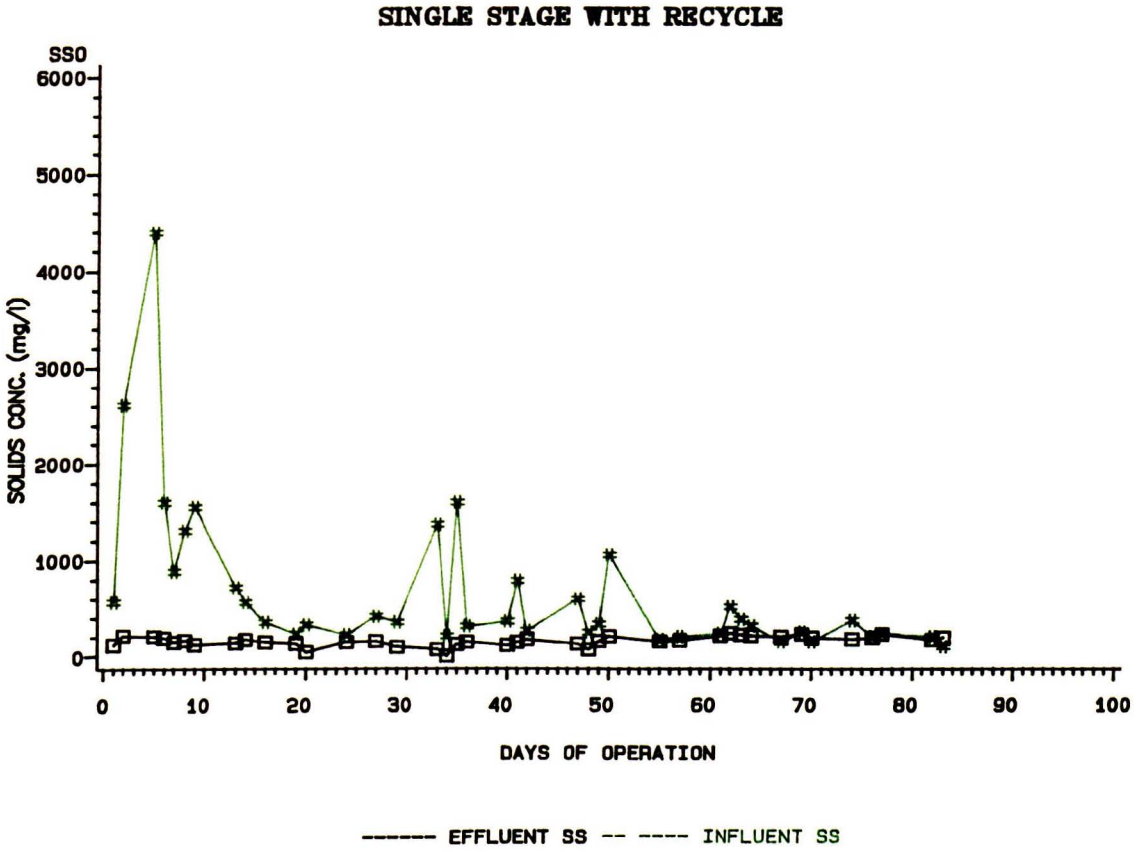


Fig. 4.29 Biomass Washout Parameters

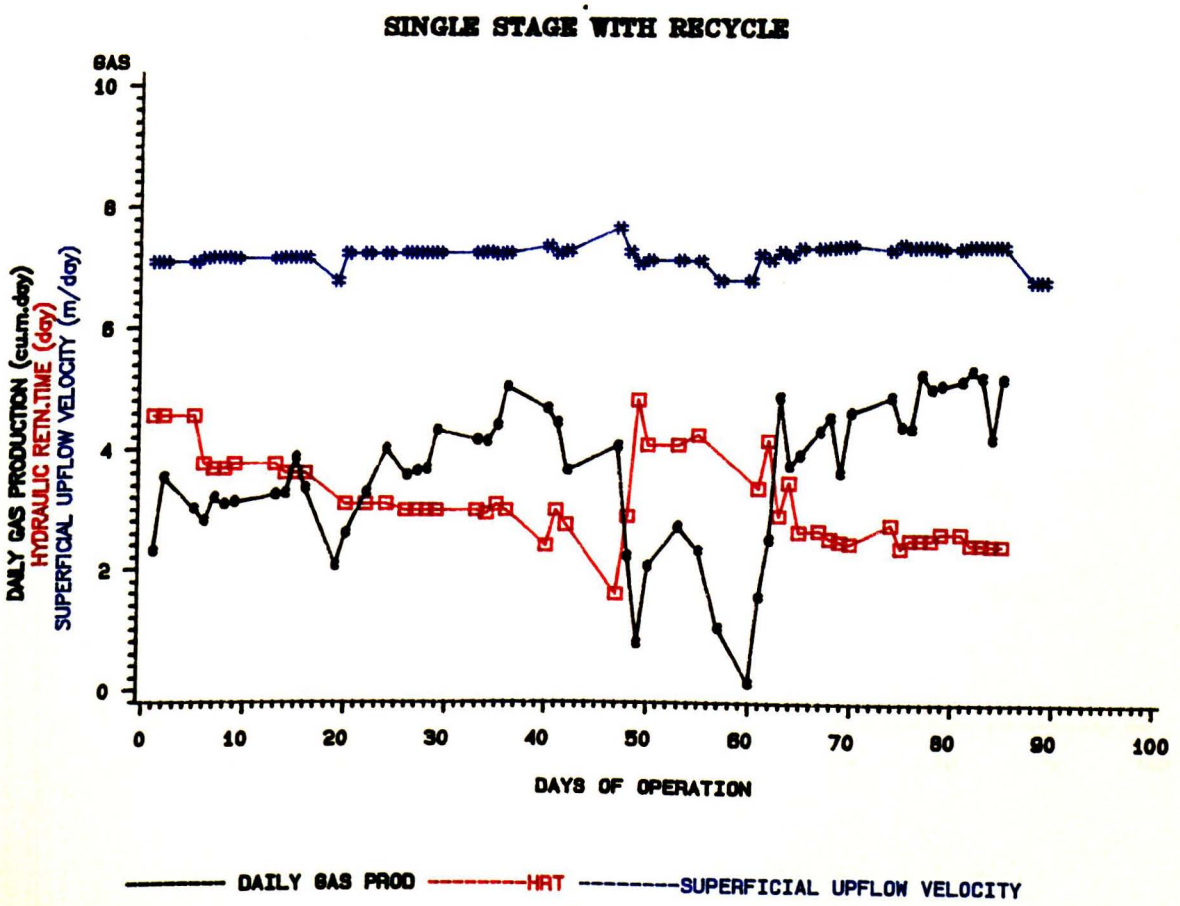


Fig. 4.30 Days of Operation Vs. Solids Concentration

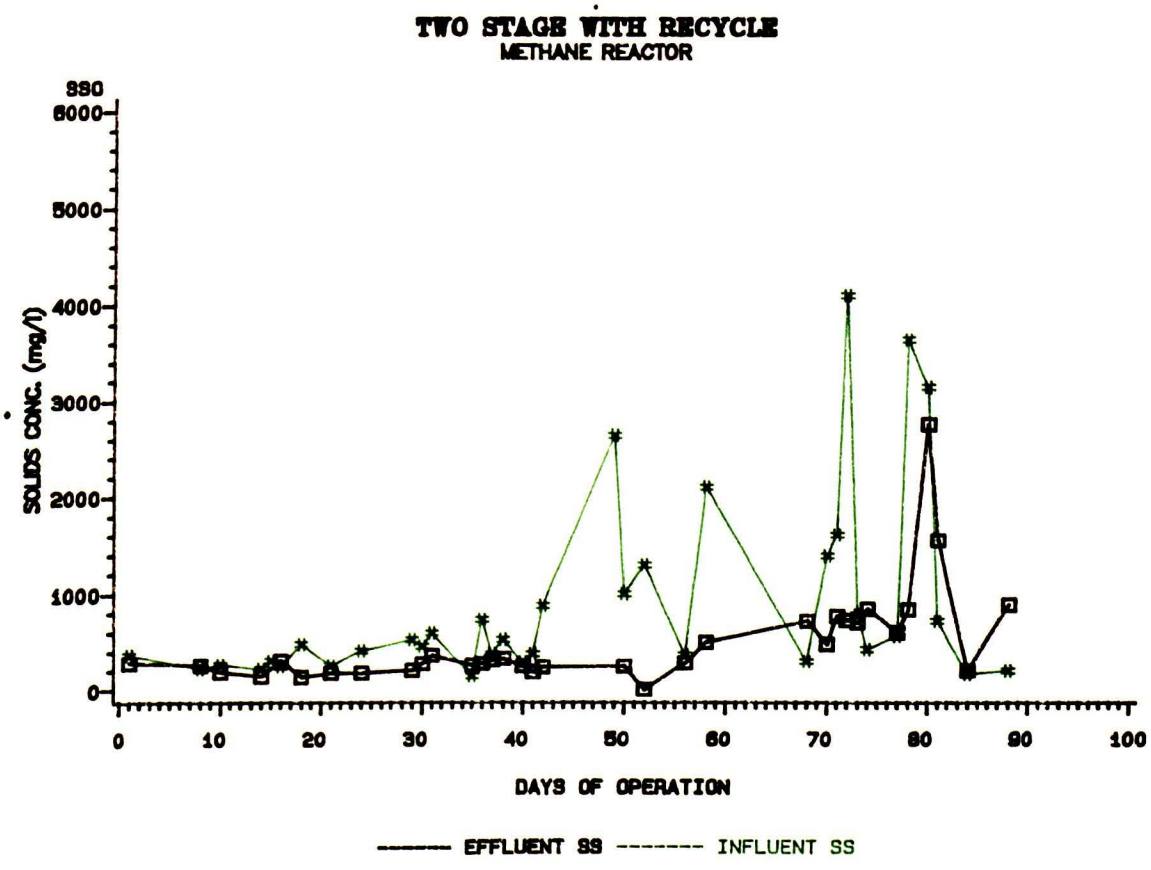


Fig. 4.31 Biomass Washout Parameters

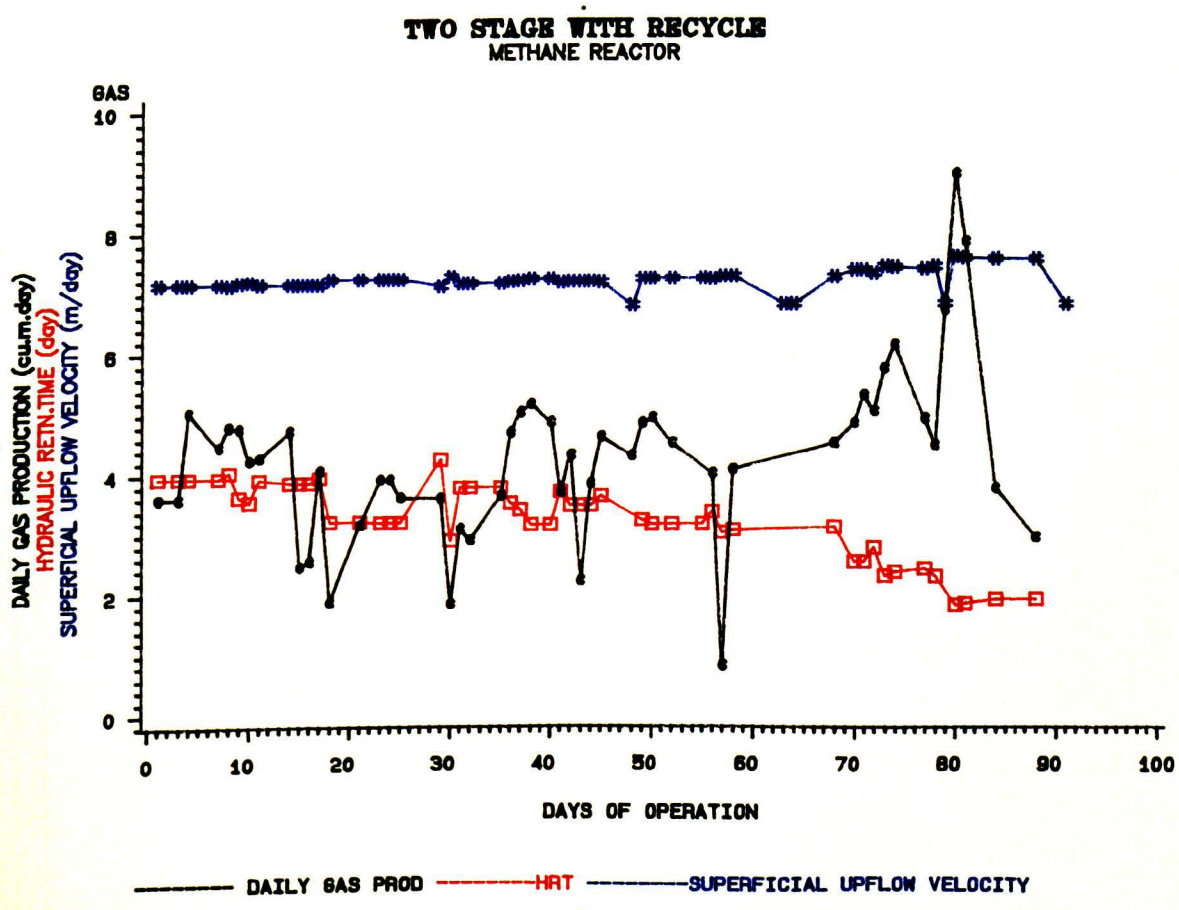


Fig. 4.32 Days of Operation Vs. Solids Concentration

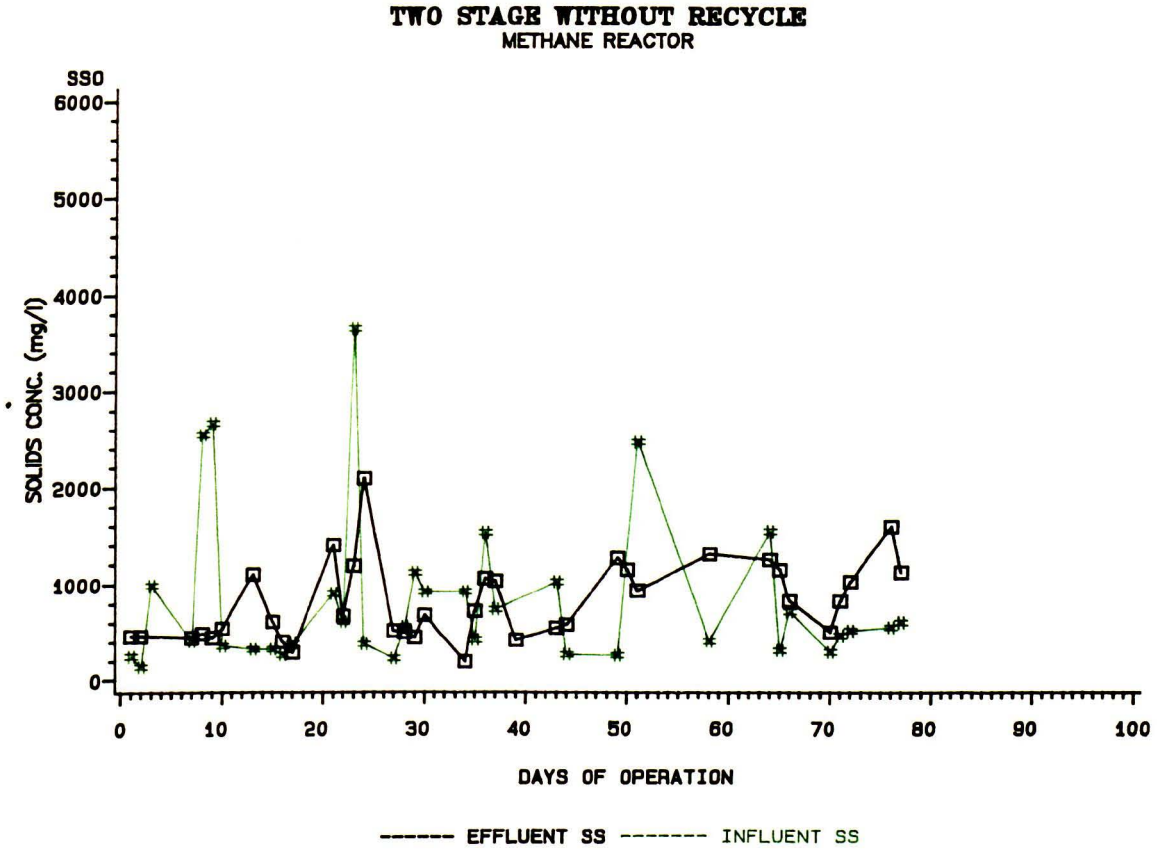


Fig. 4.33 Biomass Washout Parameters

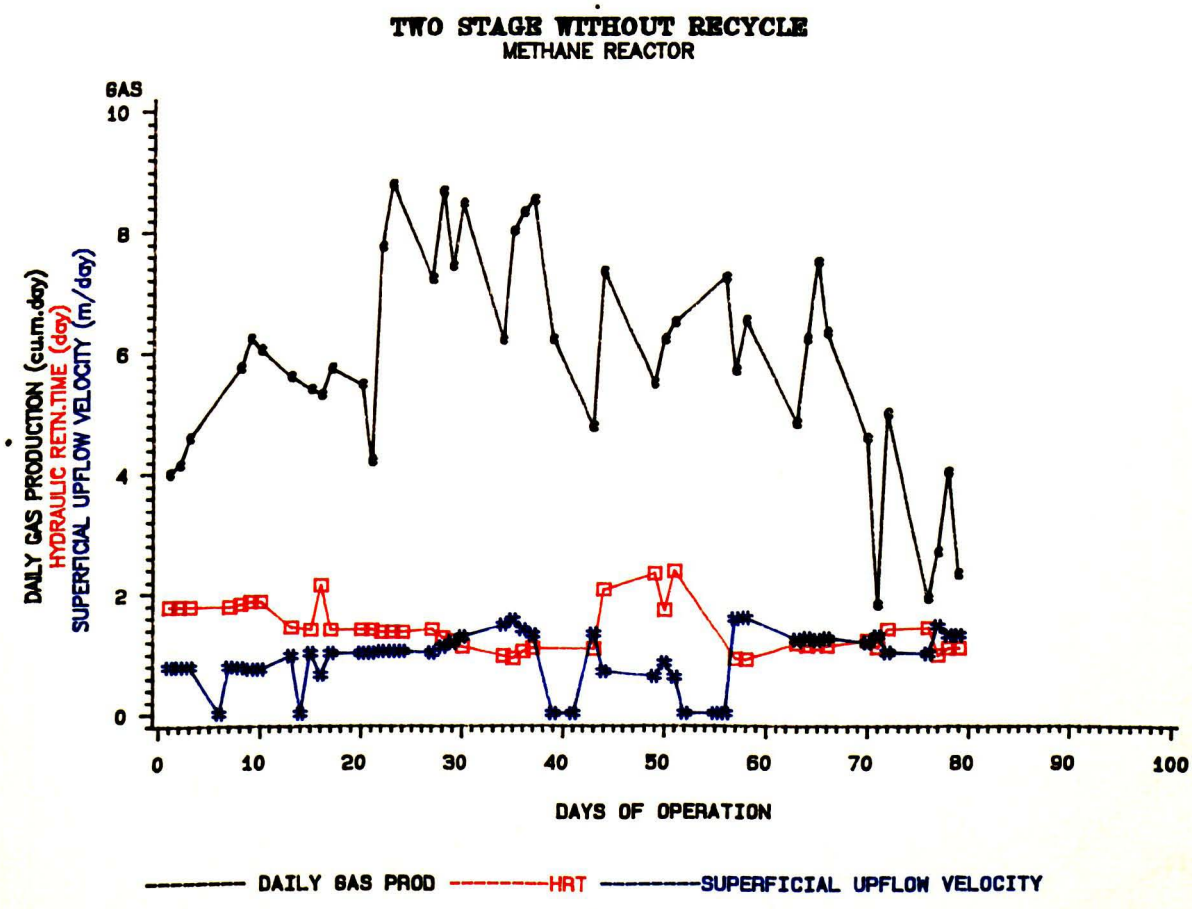
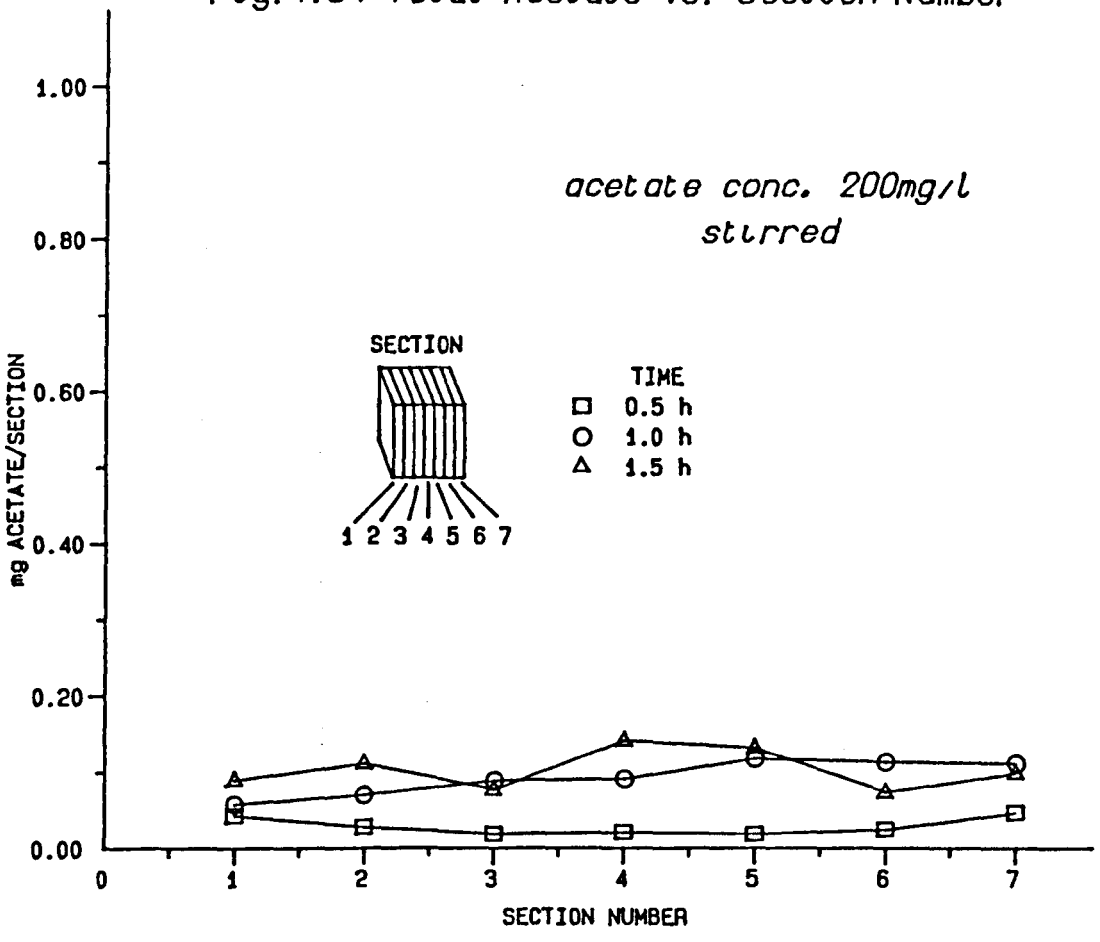


Fig.4.34 Total Acetate vs. Section Number



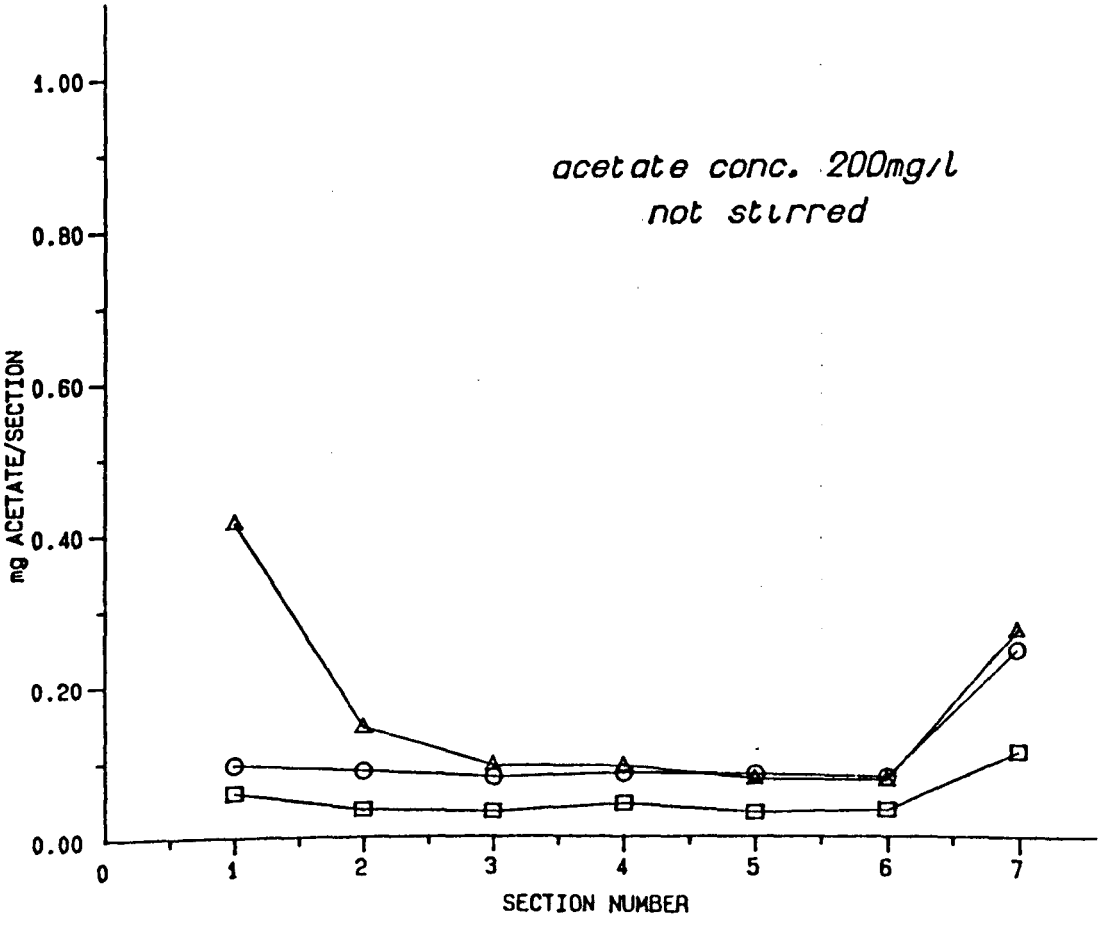
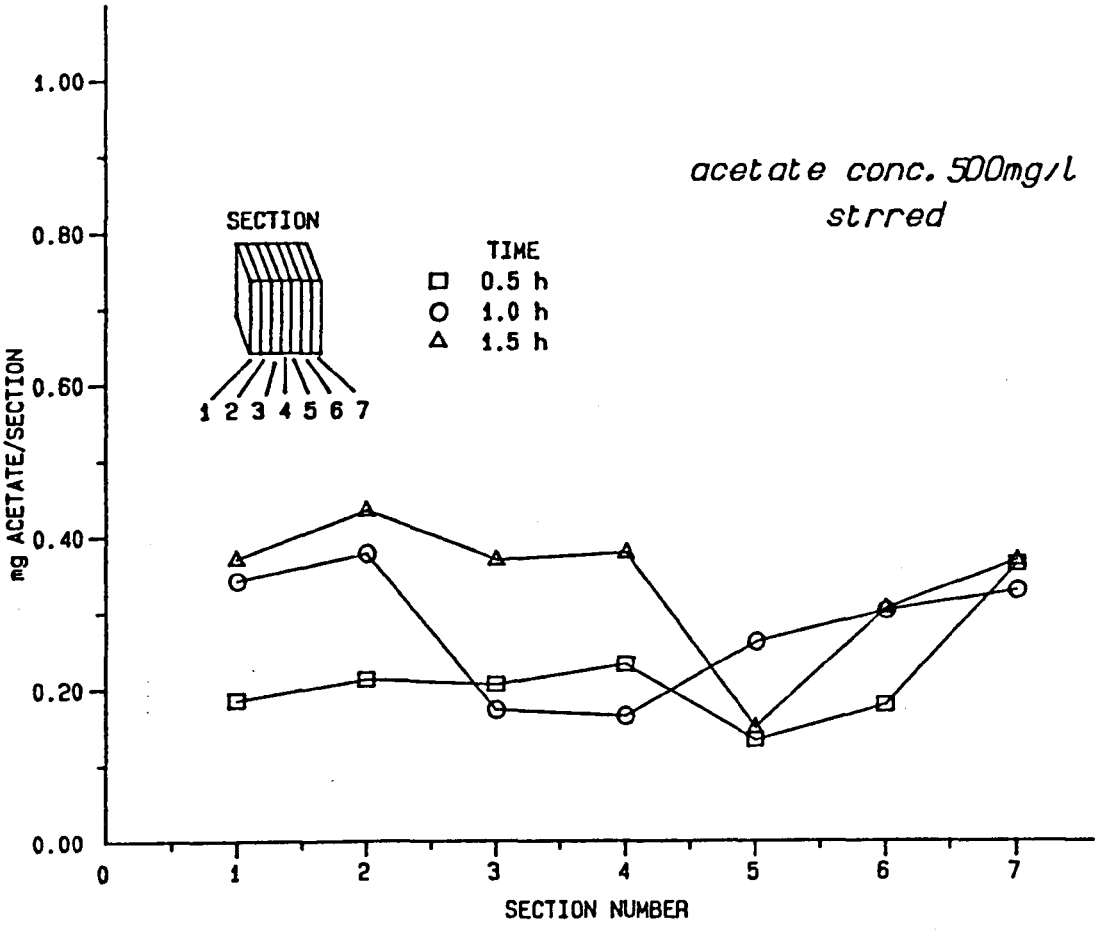


Fig.4.35 Total Acetate vs. Section Number



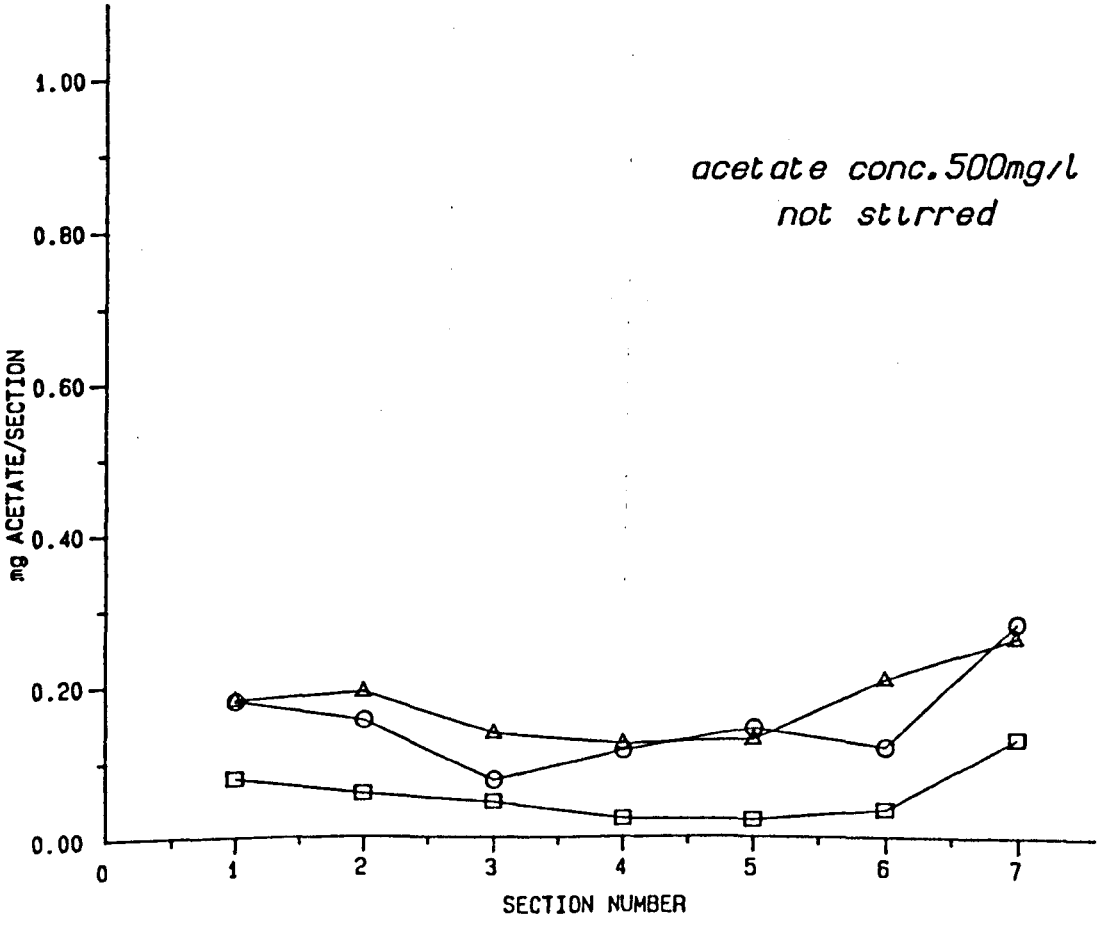
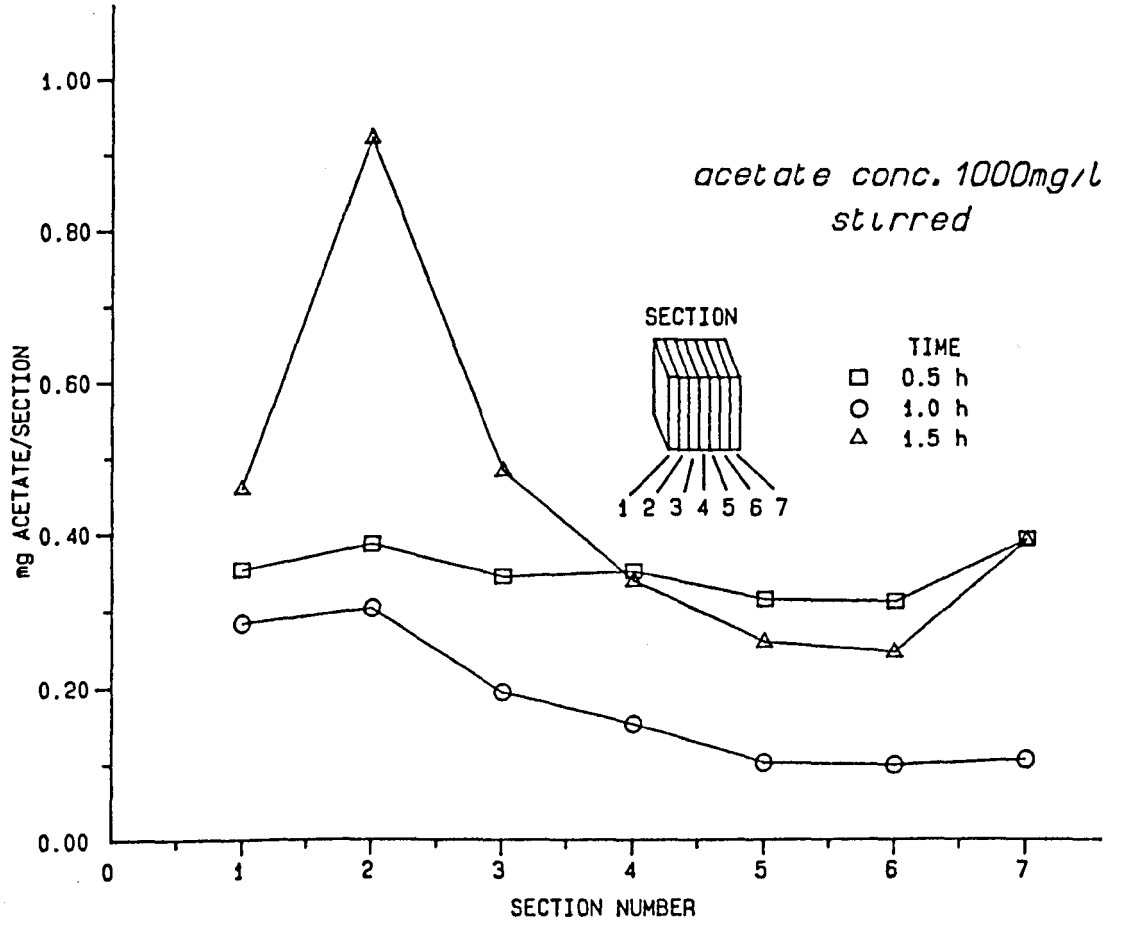


Fig.4.36 Total Acetate vs. Section Number



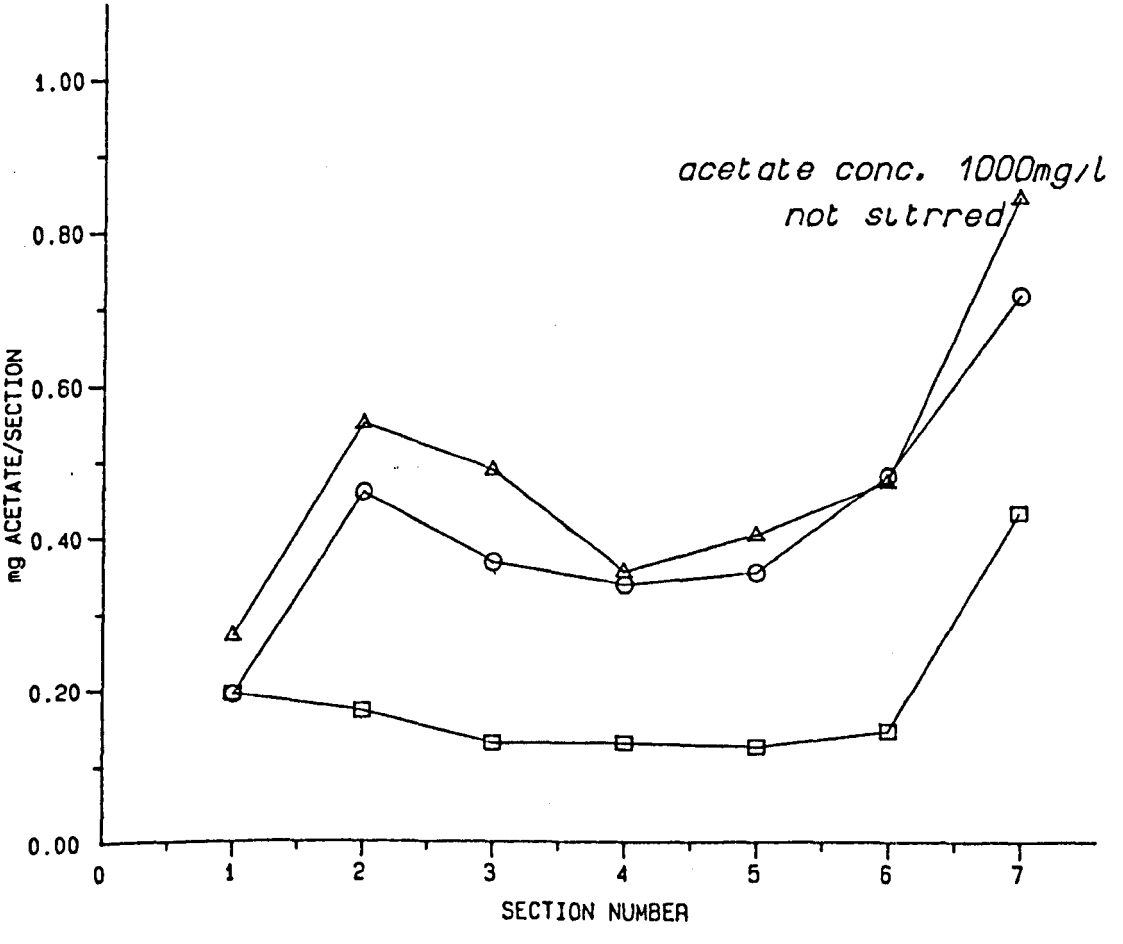
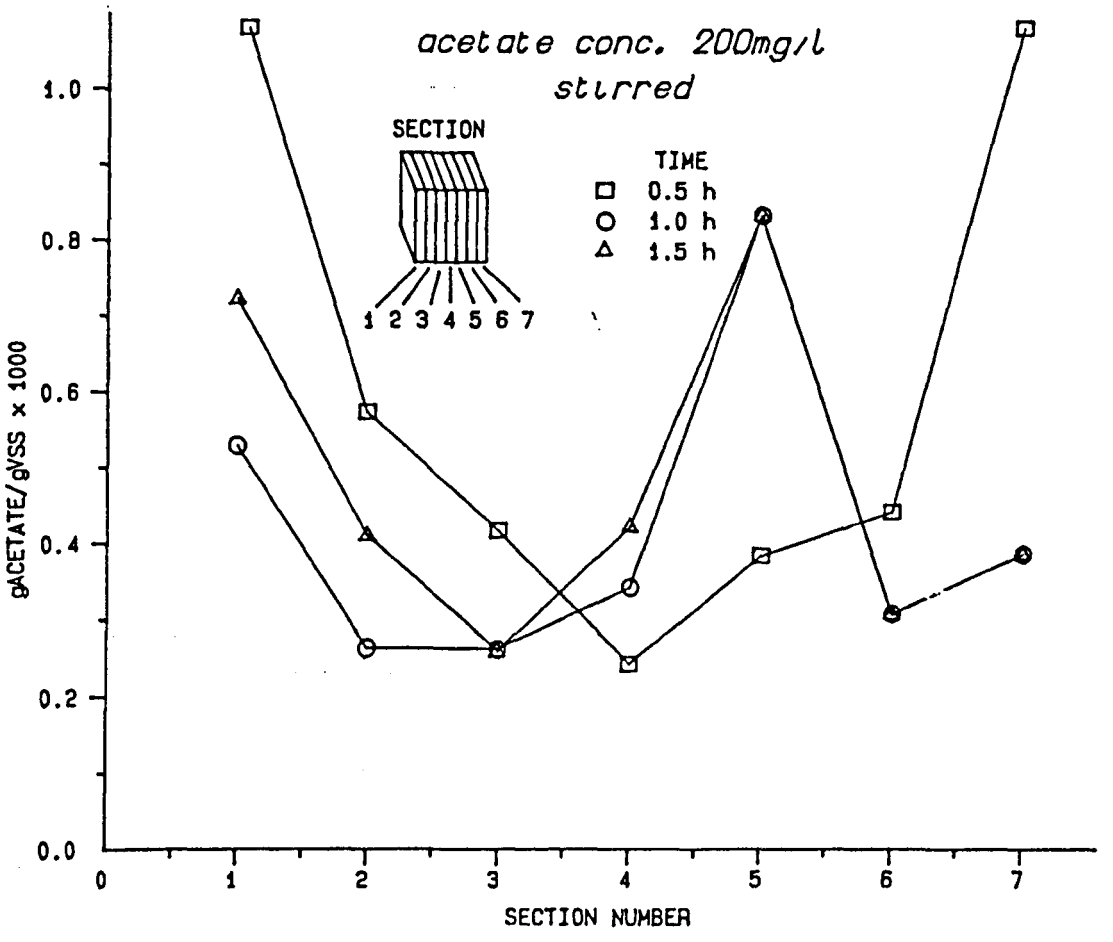


Fig.4.37 Incoporated Acetate vs. Section Number



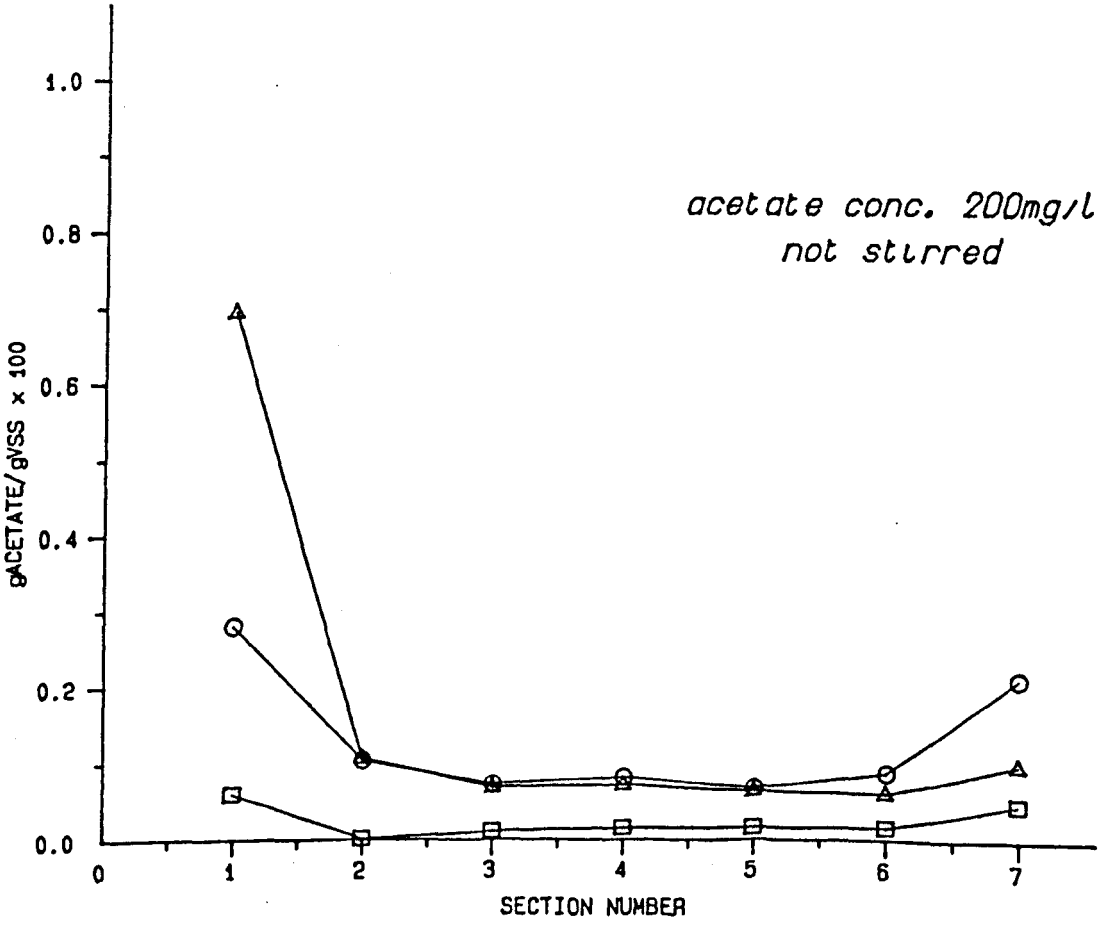
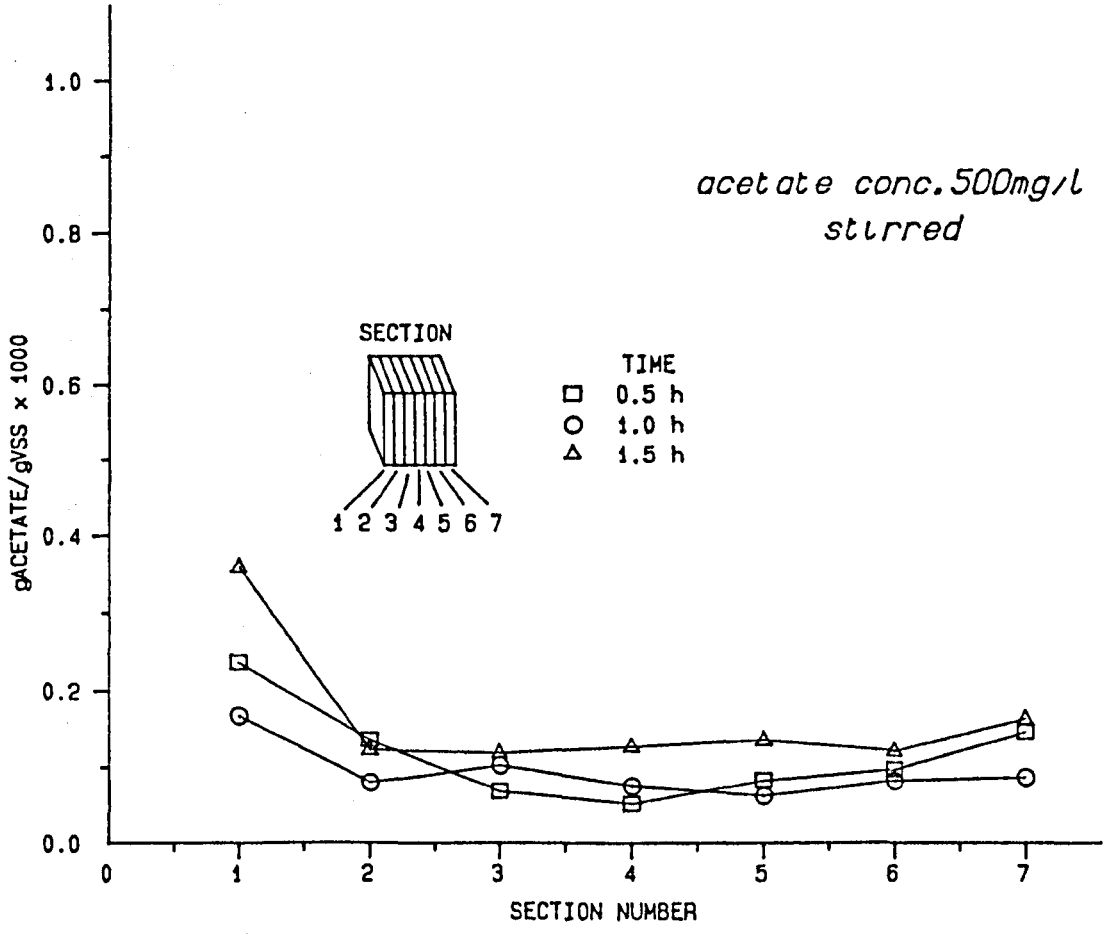


Fig. 4.38 Incorporated Acetate vs. Section Number



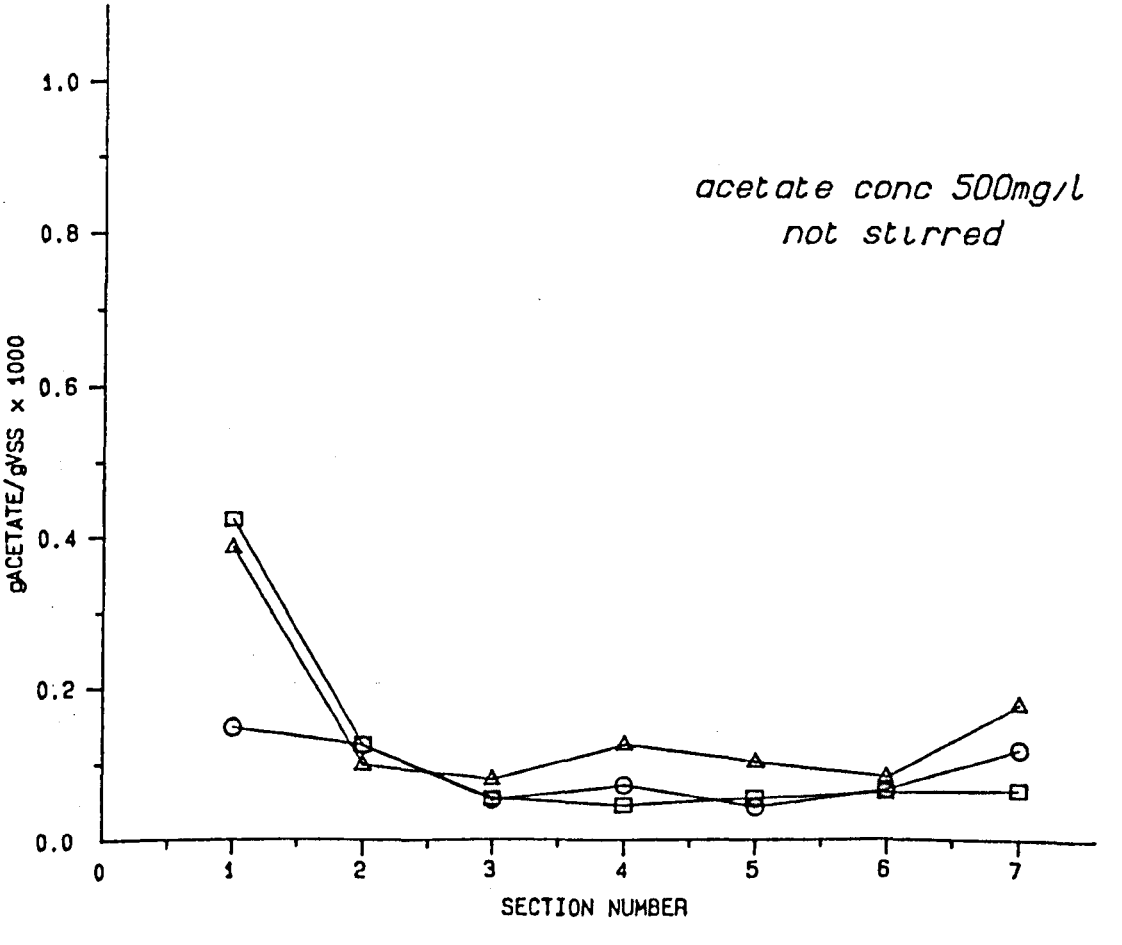
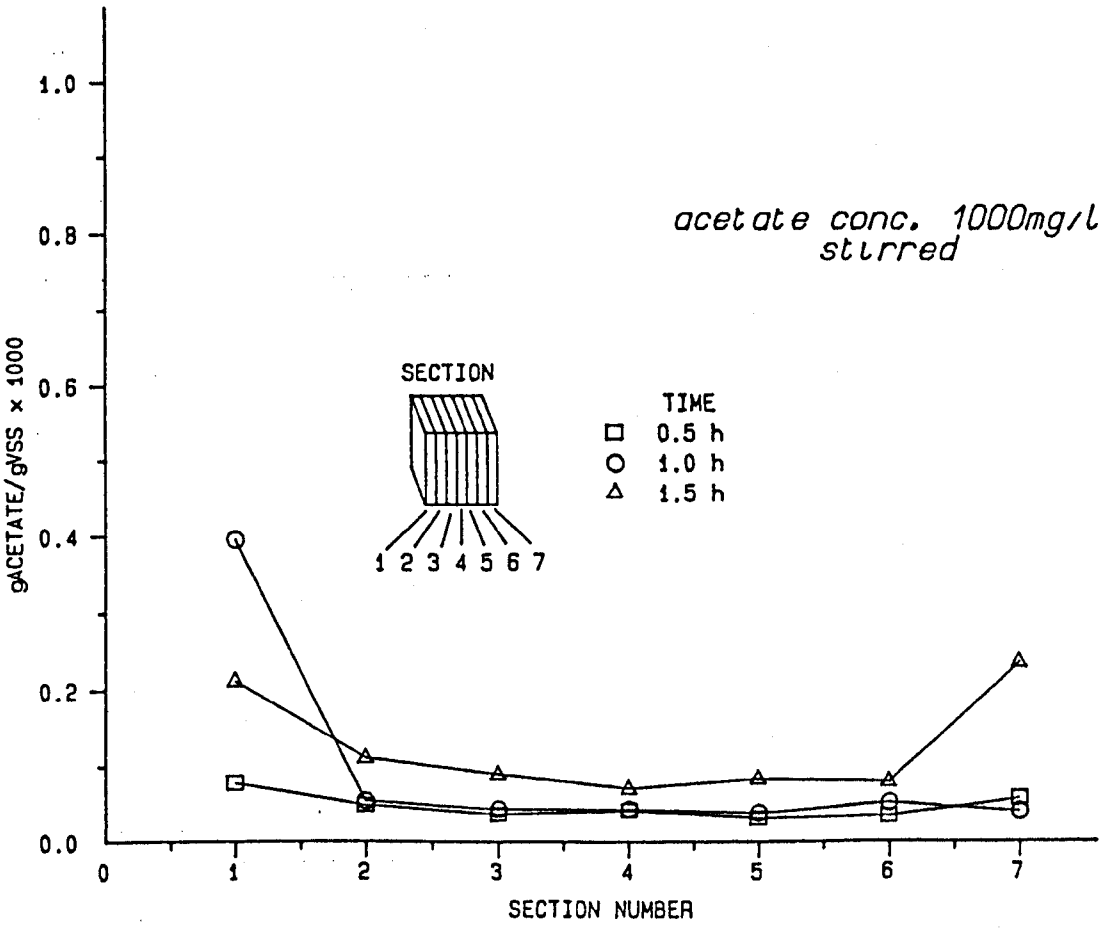
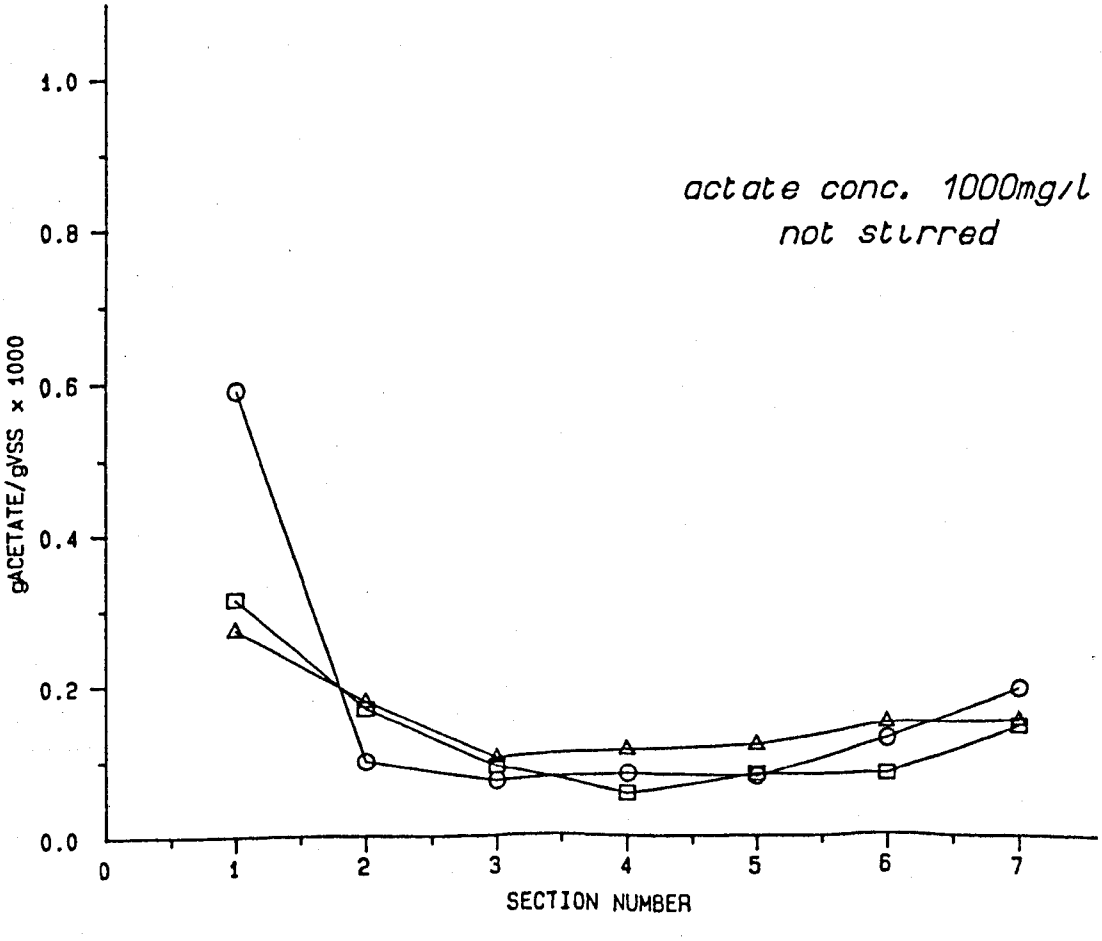
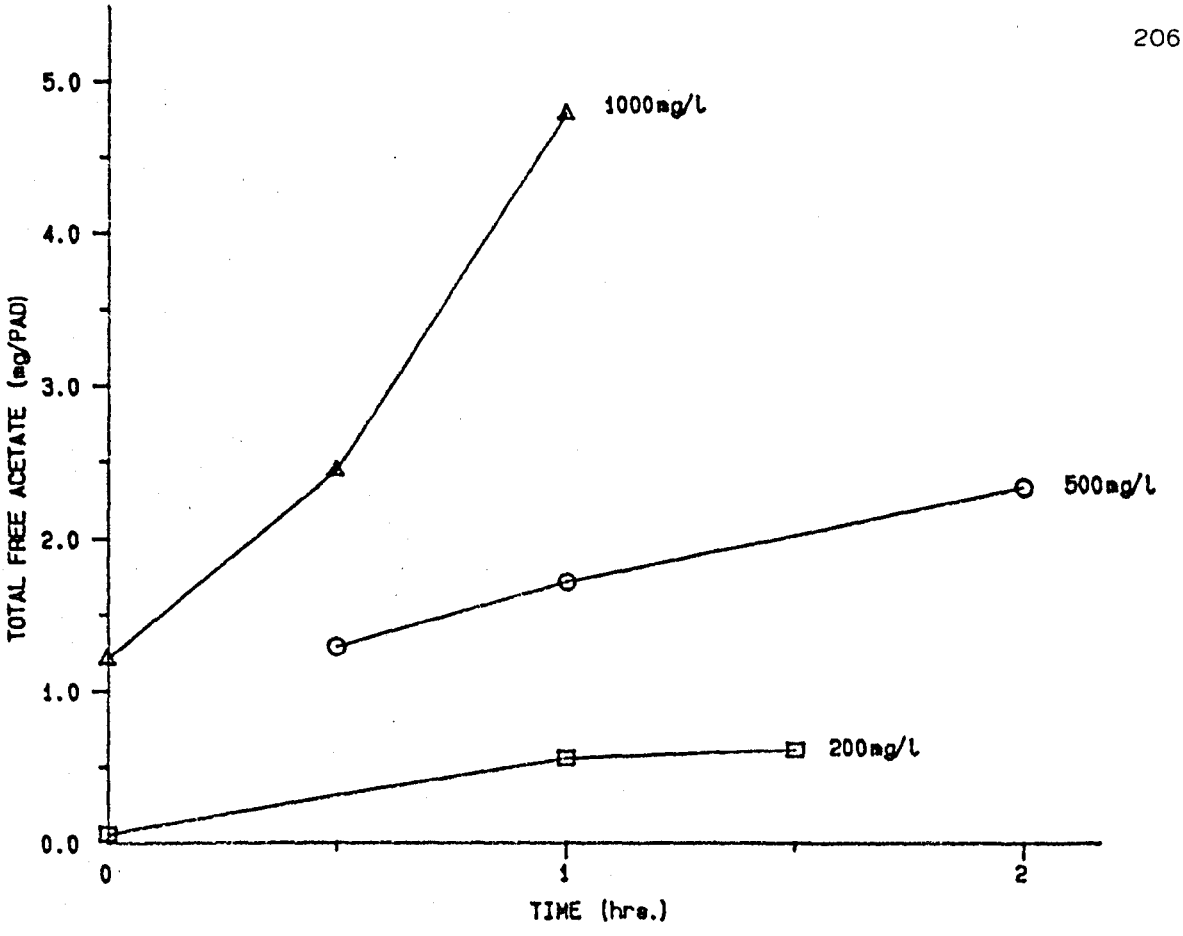


Fig.4.39 Incorporated Acetate vs. Section Number





(stirred reactor)



(unstirred reactor)

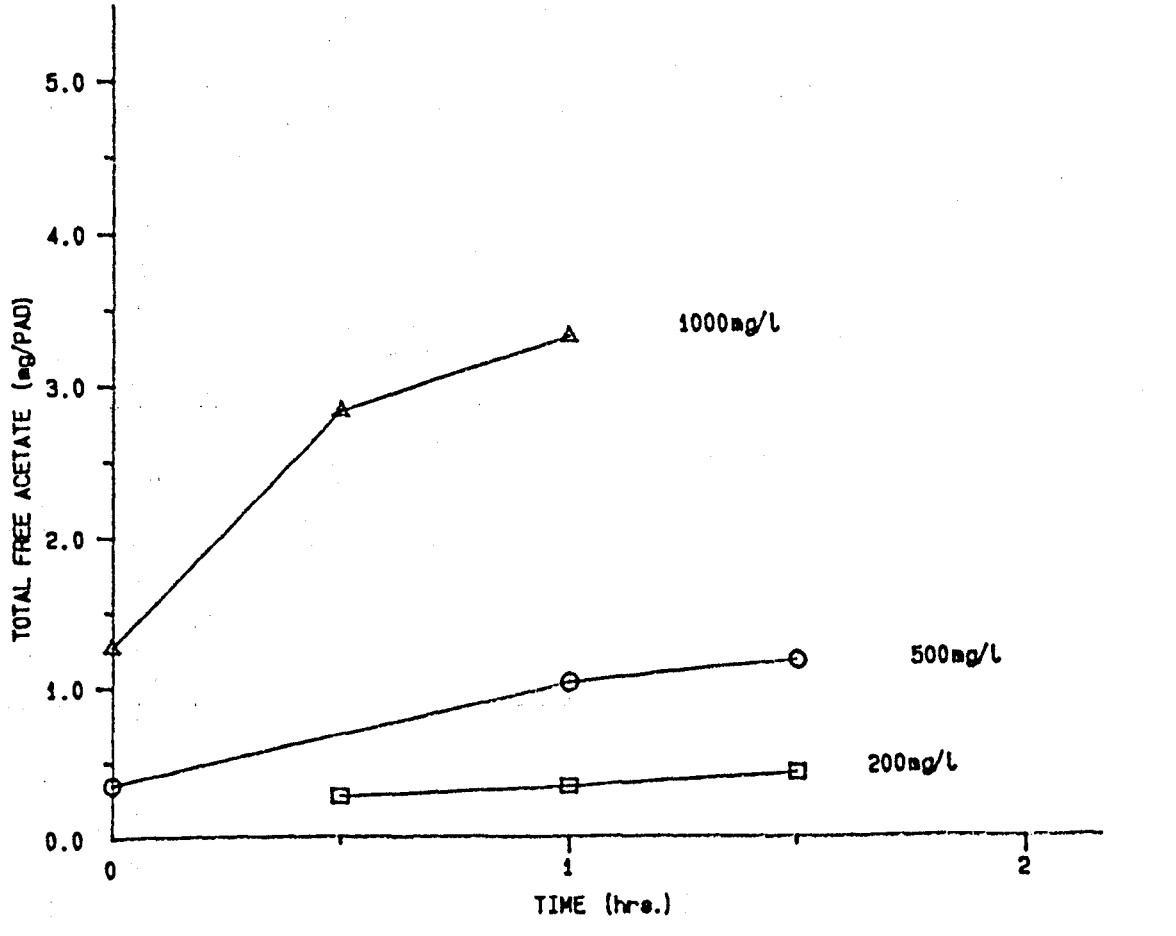


FIG.4.40 FREE LABEL CONCENTRATION VS. TIME

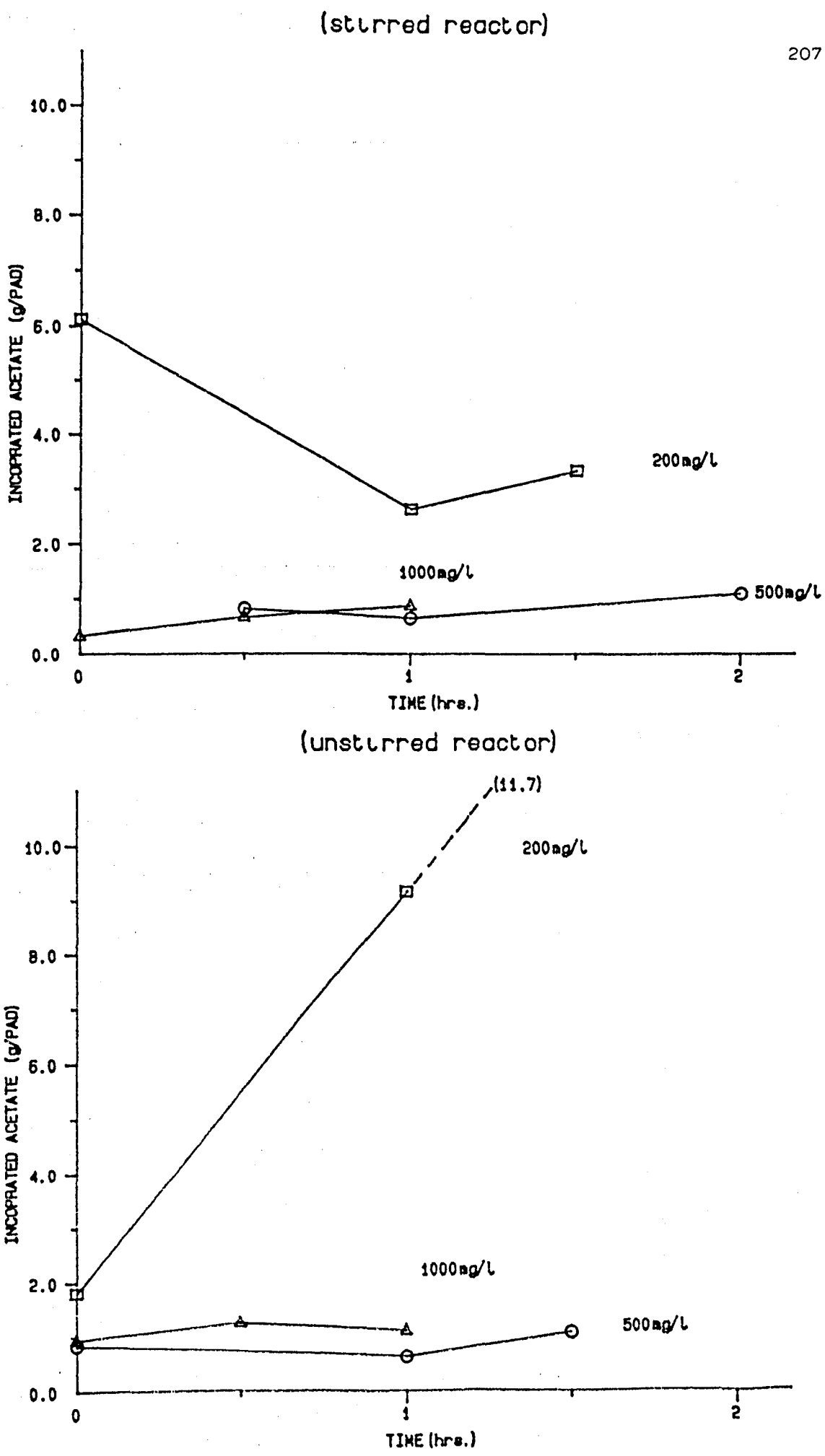
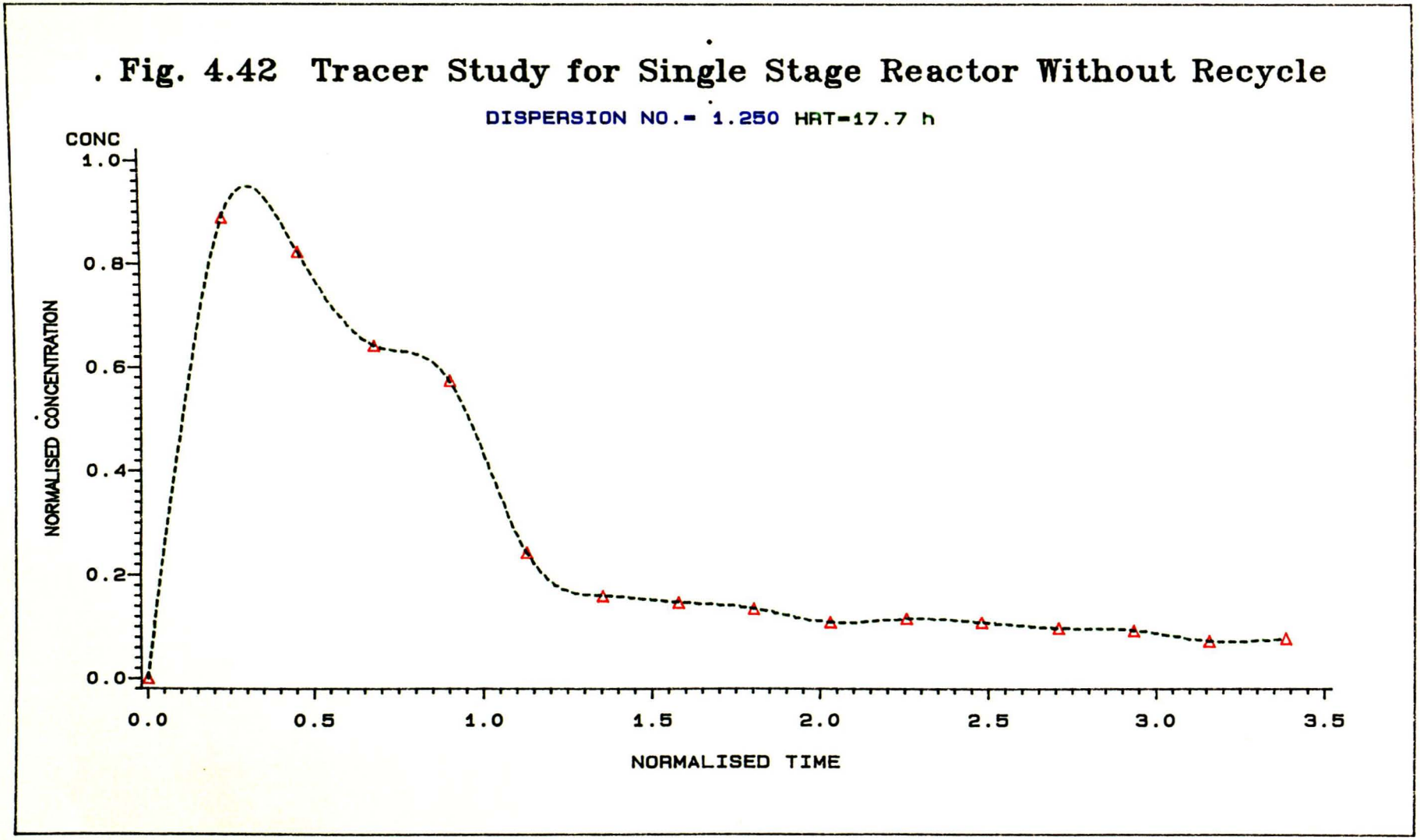
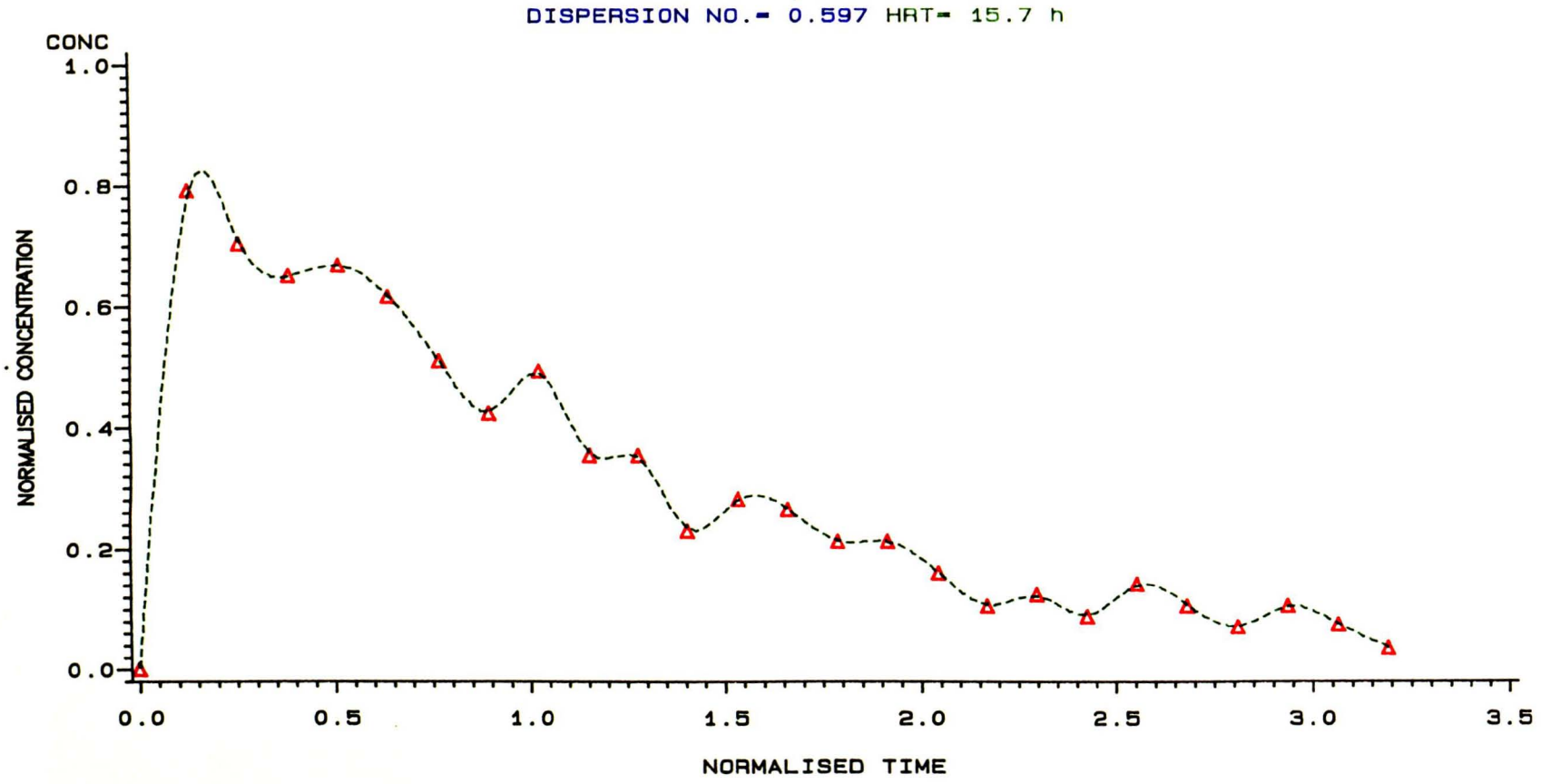


FIG.4.41 INCORPORATED ACETATE CONC. VS. TIME

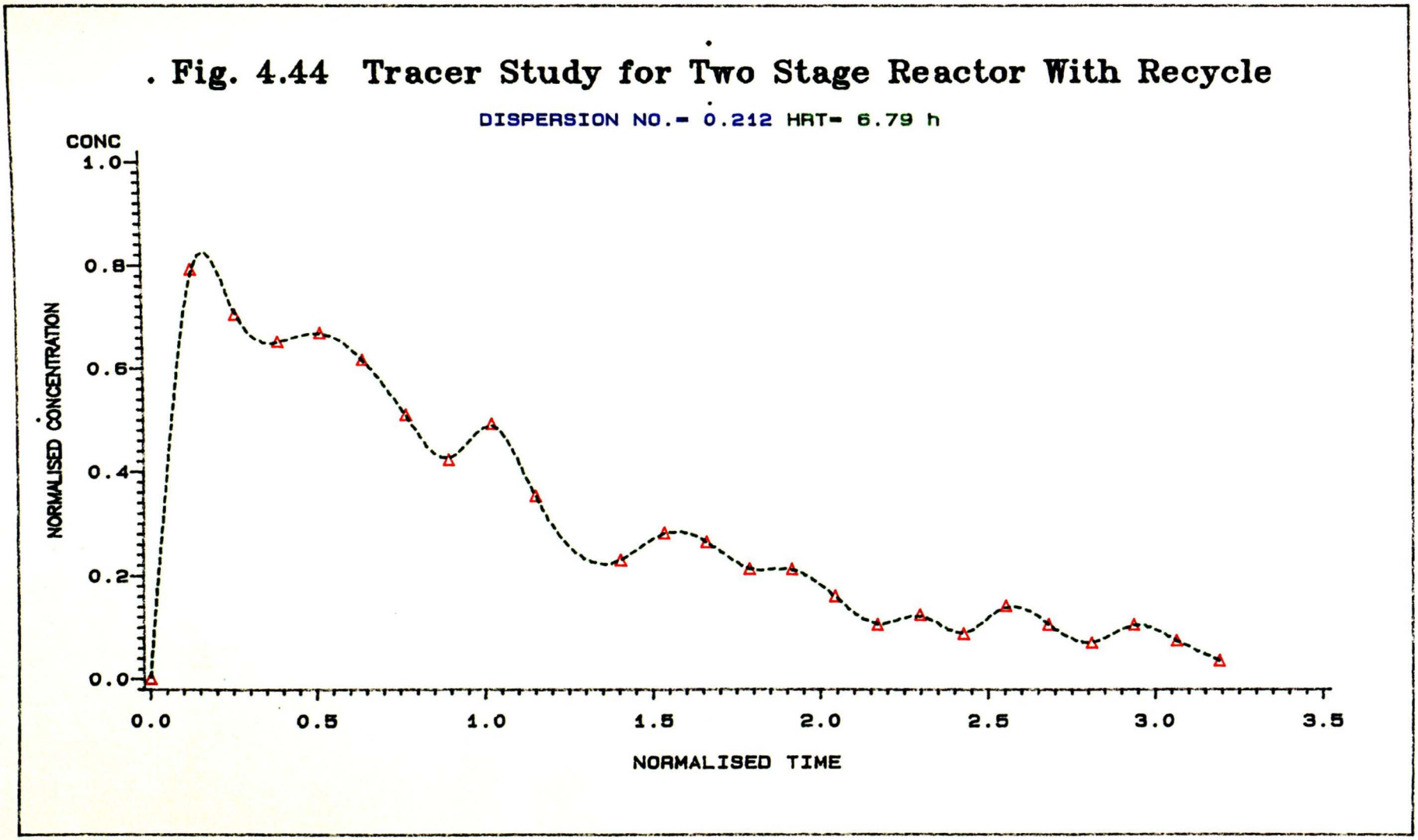
. Fig. 4.42 Tracer Study for Single Stage Reactor Without Recycle



. Fig. 4.43 Tracer Study for Single Stage Reactor With Recycle



. Fig. 4.44 Tracer Study for Two Stage Reactor With Recycle



. Fig. 4.45 Tracer Study for Two Stage Reactor With Recycle

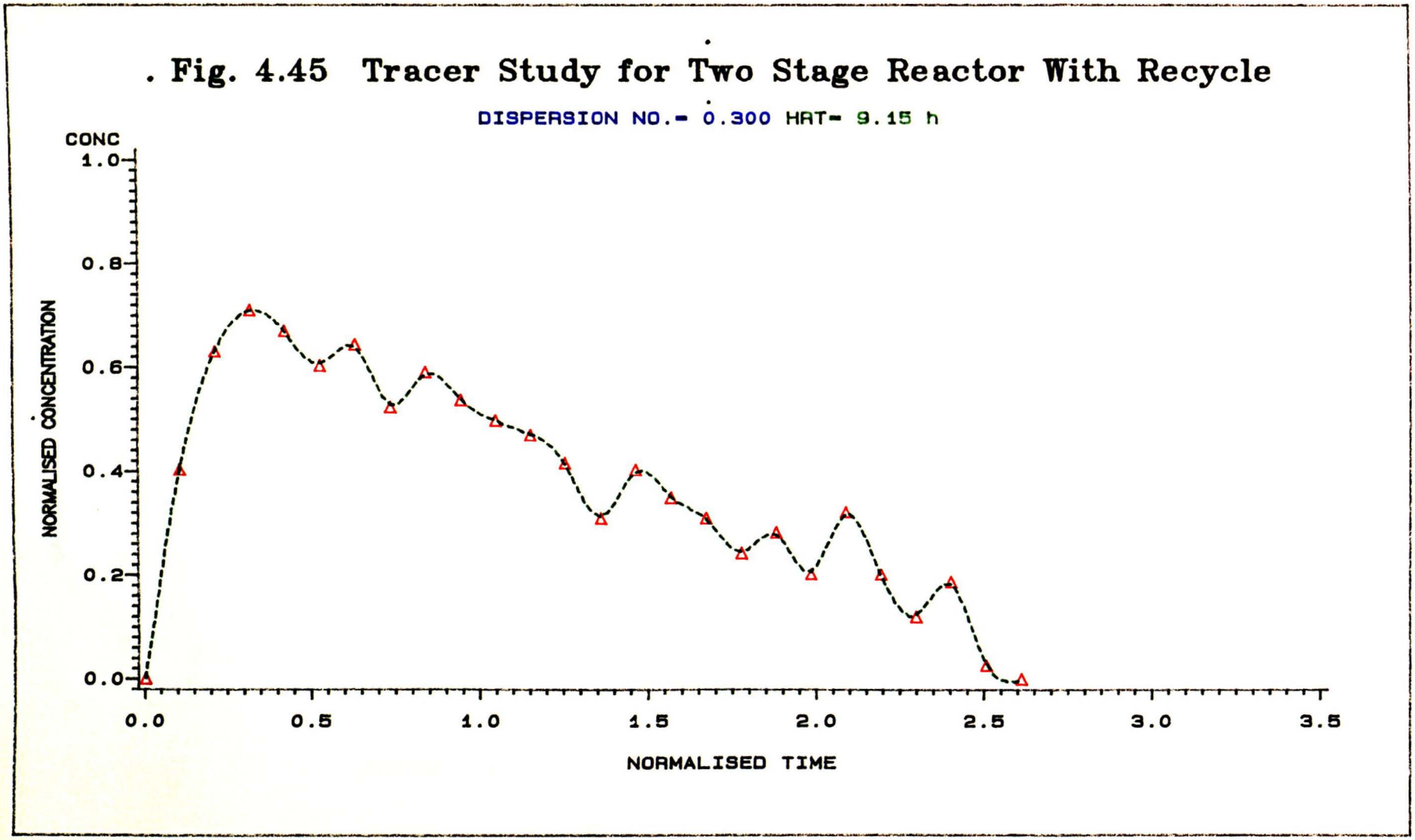


Fig. 4.46 Normalised Tracer Concentration Vs. Normalised Time.

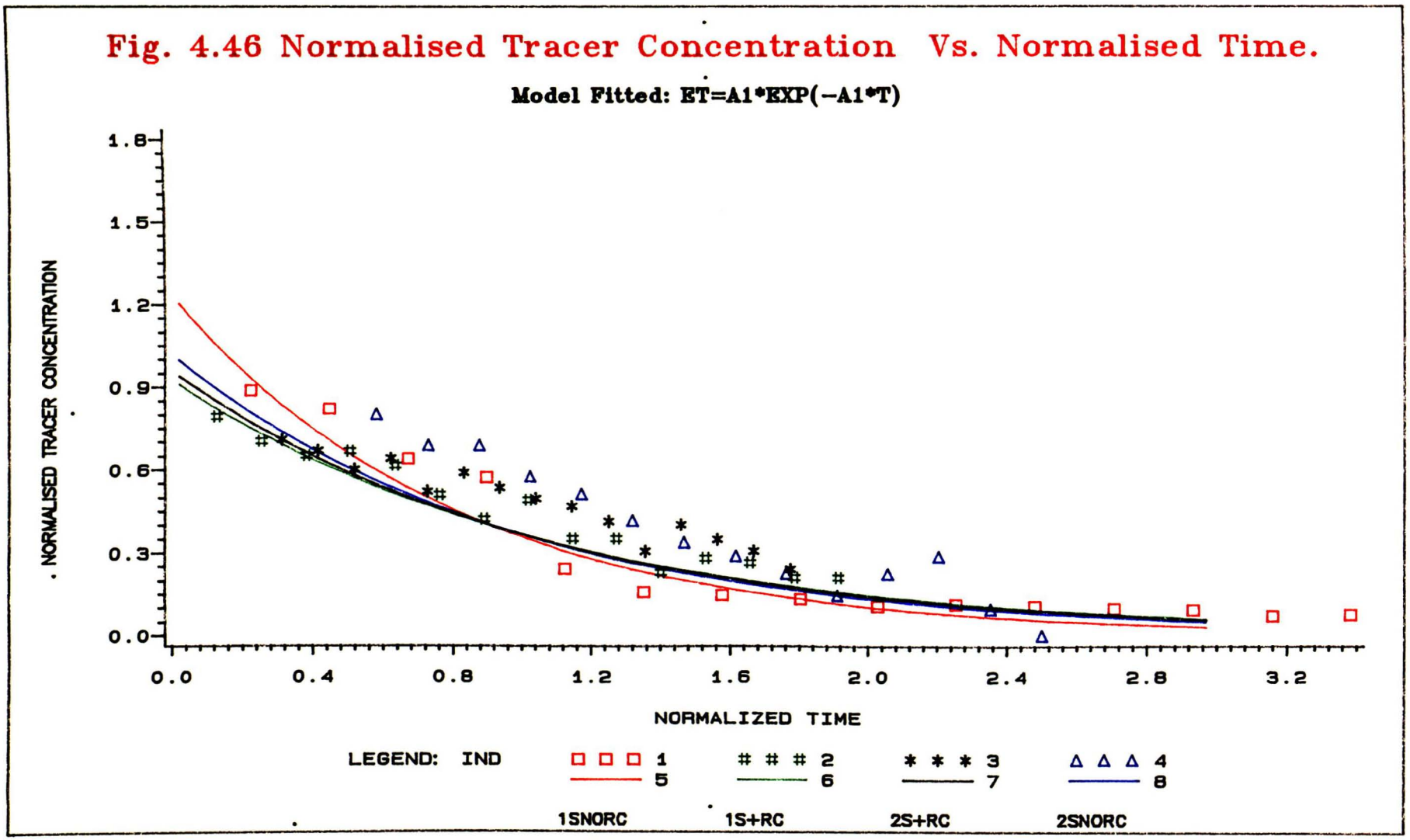


Fig. 4.47 Normalised Tracer Concentration Vs. Normalised Time.

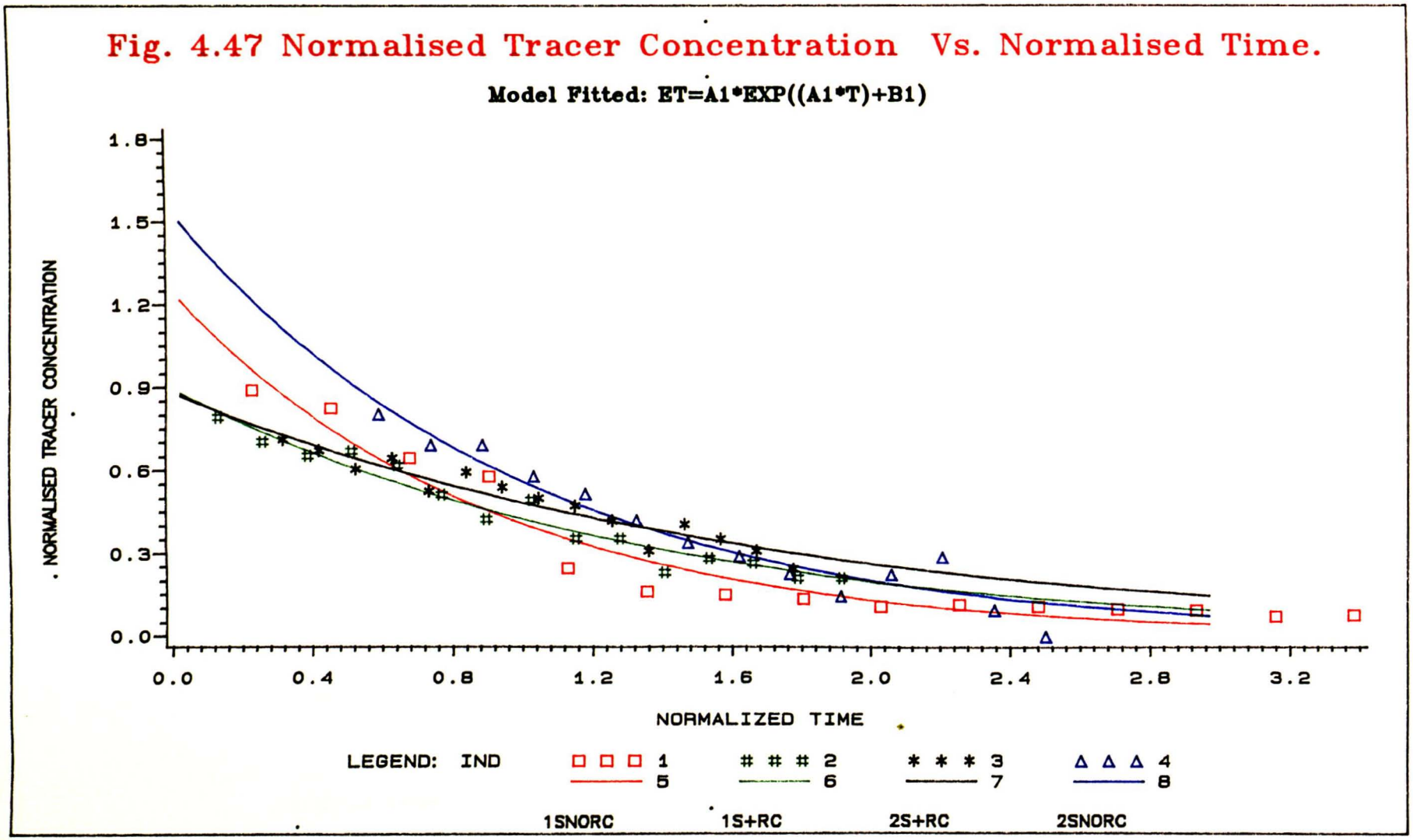


Fig. 4.48 Total COD Removal vs. Hydraulic Retention Time

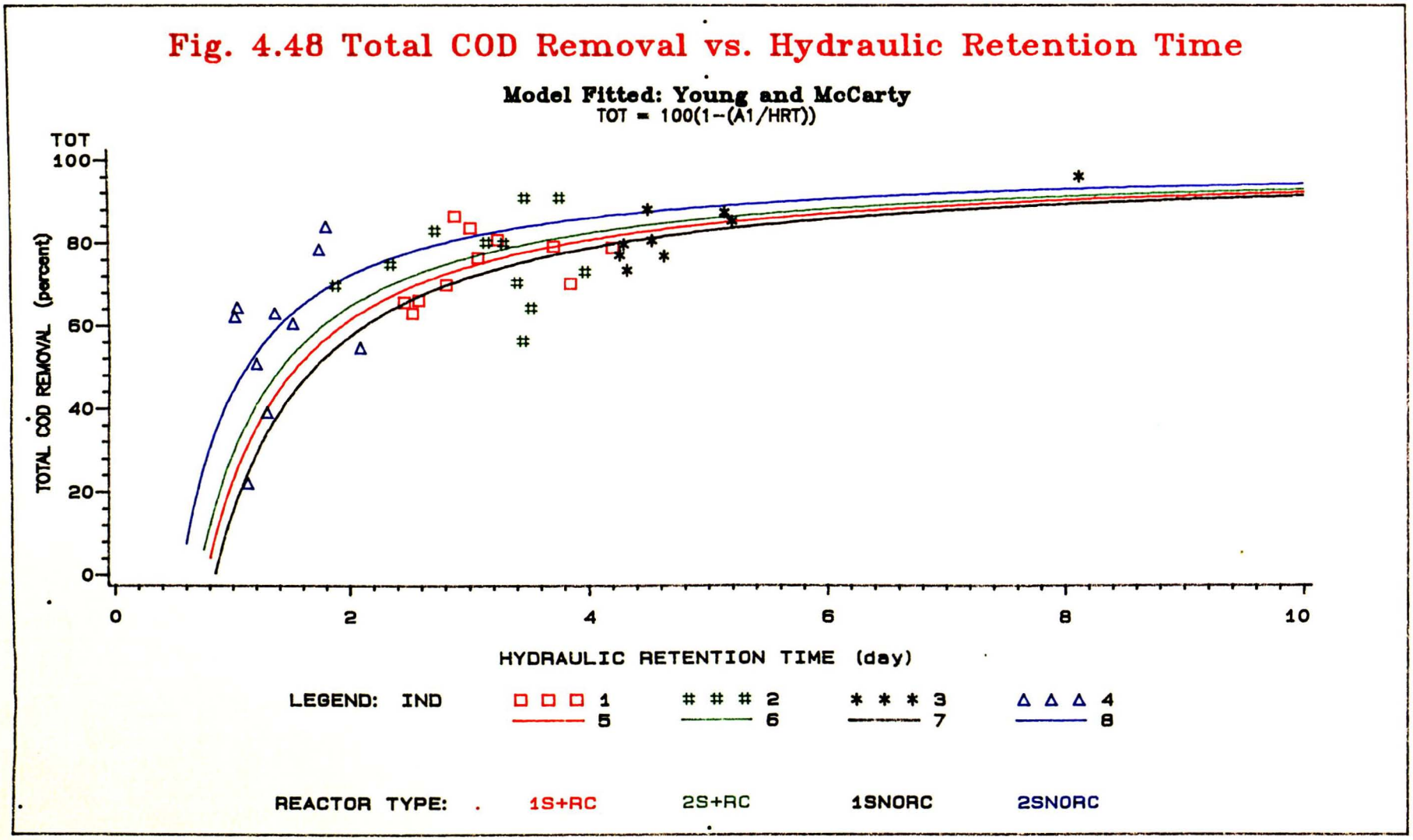


Fig. 4.49 Total COD Removal vs. Hydraulic Retention Time

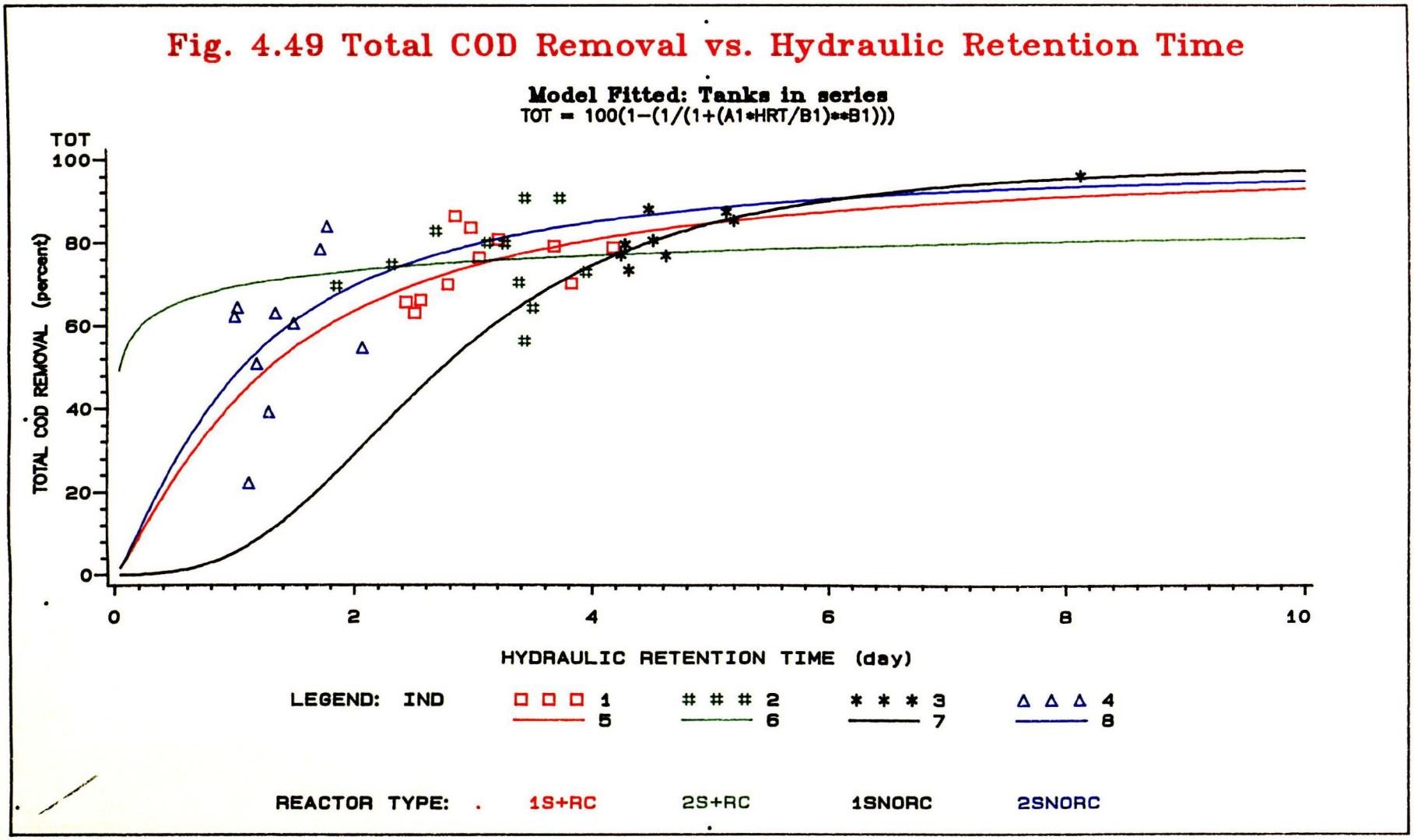


Fig. 4.50 Total COD Removal vs. Hydraulic Retention Time

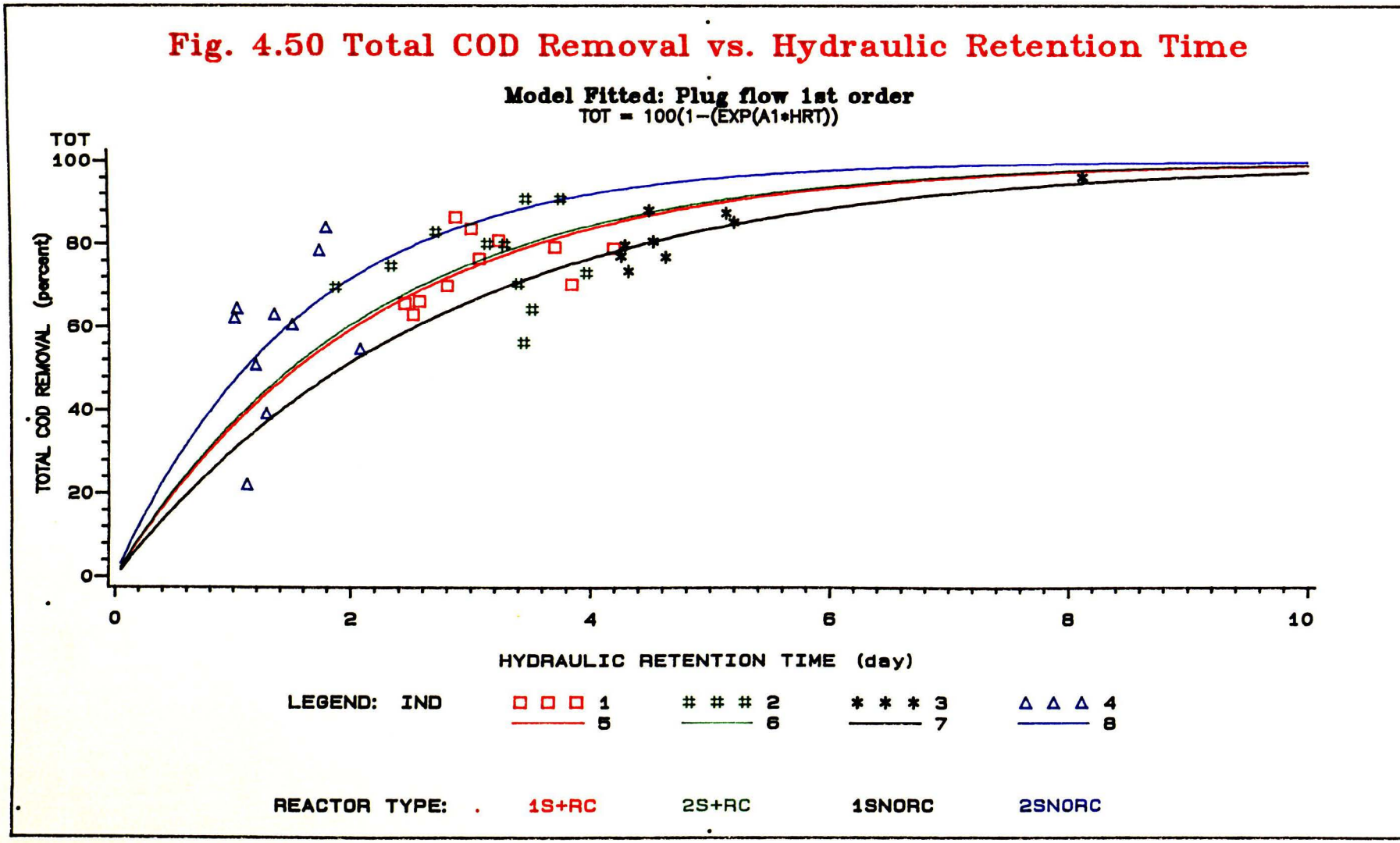


Fig. 4.51 Total COD Removal vs. Hydraulic Retention Time

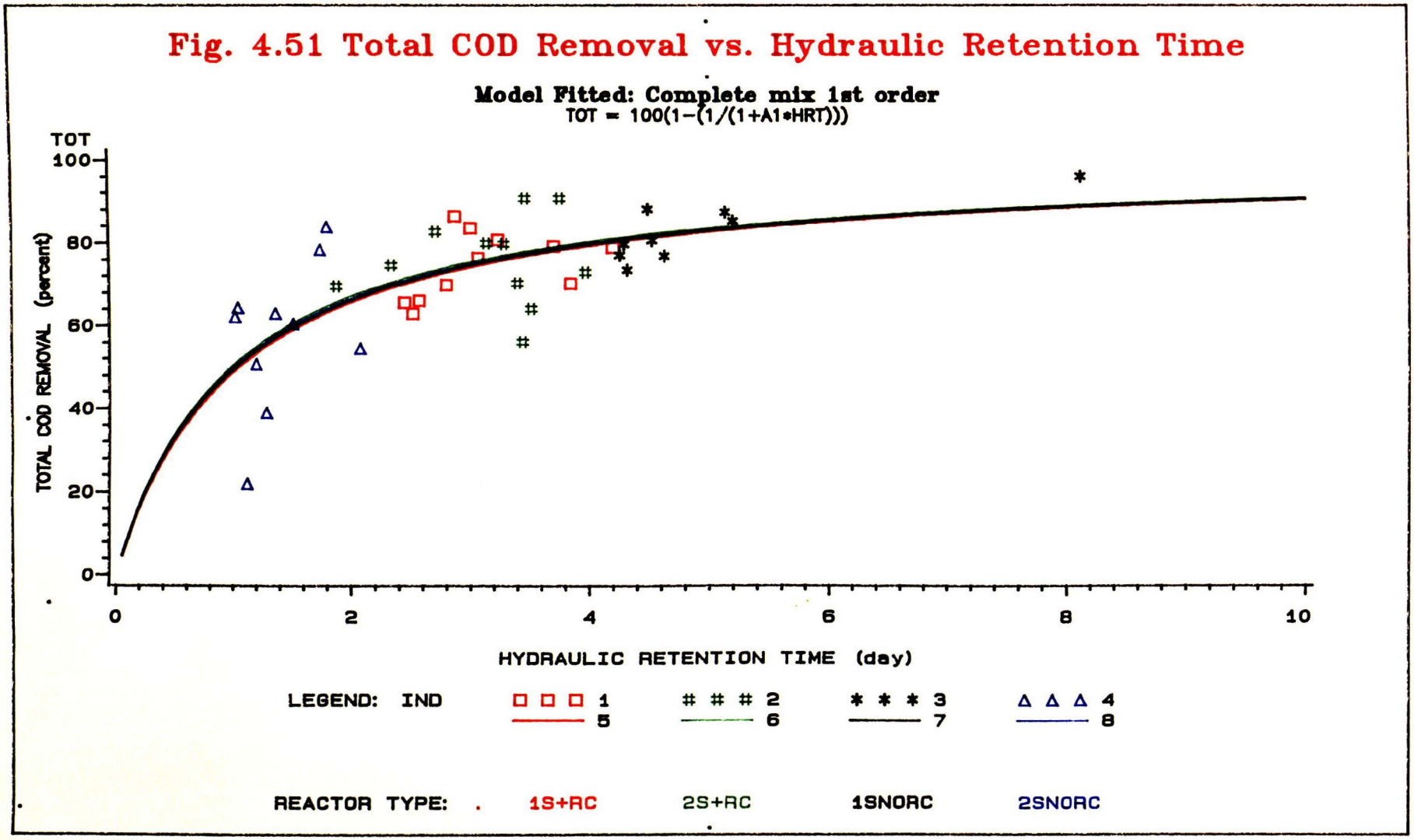


Table 4.1 Characteristics of the different feedstocks used in this study
(all parameters, except pH in mg/l)
* (numbers in brackets are average soluble as a % of total)

WASTE / PARAMETER	SUMP EFFLUENT (straight from factory sump)			FILTERED AND SETTLED (after 2 filters and settlement in holding tank)			ACIDIFIED (after acidification in 1st stage)		
	MAX	MIN	AVERAGE	MAX	MIN	AVERAGE	MAX	MIN	AVERAGE
TOTAL COD	18300	2780	10943 *	16028	3861	10465 *	17240	2340	10035 *
SOLUBLE COD	16440	5614	9773 (84)	15246	3494	8758 (81)	16740	1300	8617 (86)
TOTAL CARB.	9750	750	4829	9158	27	2235	9575	550	3264
SOLUBLE CARB	8225	613	3441 (84)	7575	16	1896 (75)	8200	504	2984 (81)
INFLUENT SS	3088	242	780	8003	60	936	2380	147	869
INFLUENT VSS	2850	149	722 (90)	7485	60	926 (91)	2522	111	725 (86)
VFA									
C1	809	240	441	715	255	449	1566	280	528
C2	1005	16	207	711	91	381	680	171	335
C3	50	0	9	153	0	20	30	0	8
C4	118	0	28	118	0	38	725	8	88
C5	27	0	1	60	0	4	96	0	4
C6	177	0	23	312	0	40	278	0	44
C7	53	0	5	222	0	16	102	0	10
C8	36	0	1	102	0	9	155	0	5
TOTAL AS ACETIC			844			1217			1296
PH	5.62	3.90	5.11	5.25	4.34	4.74	5.62	3.92	4.46

Table 4.2 Additional waste characteristics of sump effluent (all in mg/litre)

all parameters are average values

NH3-N	97		TOTAL SOLIDS	7.014 g/l	CALCIUM	87.3	
ORGANIC N	264		TOTAL VOLATILE SOLIDS	4.90 g/l	LIGNIN	20	
P	19		LEAD	0.244	ZINC	64.0	
ALKALINITY	236	as CaCO3	NICKEL	1.088			
SULPHATE	102		IRON	1.727			
SULPHITE	2.2		COPPER	0.119			
SULPHIDE	21		MANGNESIUM	133.0			

Table 4.3 summary o performance for dufferent reactors un thus study

REACTOR TYPE	MAX. LOADING RATE BASED ON METHANE REACTOR VOL. kgCOD/m3.day	MAX. LOADING RATE BASED ON TOTAL REACTOR VOL. kgCOD/m3.day	MAX. REMOVAL RATE BASED ON METHANE REACTOR VOL. kgCOD/m3.day	MAX. REMOVAL RATE BASED ON TOTAL REACTOR VOL. kgCOD/m3.day	MINIMUM HRT (DAY) * 1	* 2	RECYCLE RATIO	COD % REMOVAL RANGE
1S+RC	4.29	4.29	2.94	2.94	2.44	2.44	13:1	63-86
2S+RC	4.34	3.07	3.59	2.57	1.87	2.62	10:1	56-91
1SNORC	3.22	3.22	2.48	2.48	4.25	4.25	-	73-98
2SNORC	11.62	8.30	7.07	5.05	1.05	1.47	-	39-84

1S+RC = SINGLE STAGE WITH RECYCLE 2S+RC = TWO STAGE WITH RECYCLE

1SNORC = SINGLE STAGE NO RECYCLE 2SNORC = TWO STAGE NO RECYCLE

* (1) based on methane reactor volume (2) based on total reactor volume
loading and removal rates based on methane reactor only

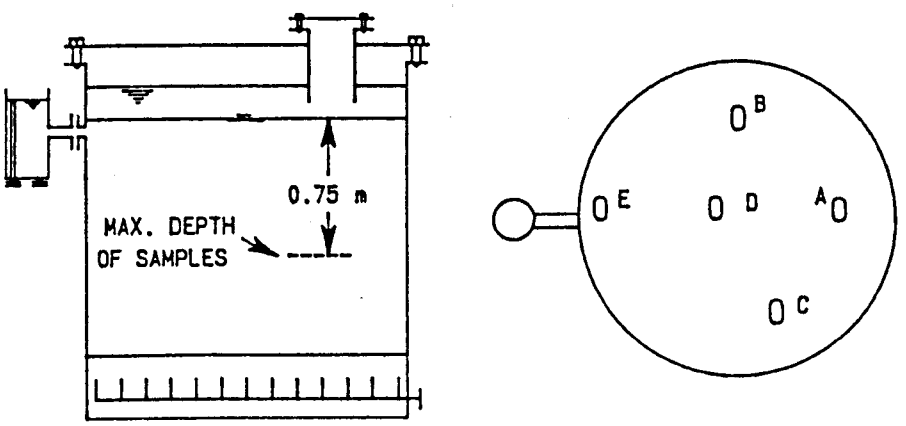
Table 4.4 Performance data for anaerobic reactors treating carbohydrate waste

PROCESS reactor size	WASTE TYPE	WASTE STRENGTH mgCOD/l	LOADING RATE kgCOD/m3.day	HYDRAULIC RETENTION TIME	TEMP °C	GAS YIELD m3CH4/kgCOD	COD REMOVAL %	REFERENCE
FILTER 28.5l	FOOD PROCESSING CARBOHYDRATE	8475	1.6-10.3	0.54-3.45 (day)	35		30-94	PLUMMER et al (1968)
FILTER 10L	CONFECTIONERY WASTE	5128-10249	5-10.3	24 (HRS.)	35	0.5-0.7	15-39	WHEATLEY et al (1985)
FILTER 28.5L	VOLATILE ACIDS	1500-6000	0.4-3.4	4.5-72 (HRS.)	25		68-98	YOUNG AND McCARTY (1969)
FILTER 12.2 L	SUGAR REFINERY	6000-13000	6	27 (HRS.)	35	3.6m3/m3.day	75	TESCH et al (1983)
FLUIDISED BED 600m3	SWEET WHEY	5000-20000	8.2-29.1	15-35 (HRS.)	35		58.9-92.3	SWITZENBAUM (1983) AND DANSKIN
UASB 6m3	SUGAR LIQUID	4-6000	20-25	4 (HRS)	28-30		92-95	LETTINGA (1980)
DOWNFLOW SFF 22.5L	SYNTHETIC SUGAR LIQUID	500-2000	4.5-11.6	-	35		56-79	VAN DEN BERG (1985)
FILTER	HIGH STRENGTH CARBOHYDRATE	58000	14	6.4 (day)	35	0.34-0.36	99.4	ANDERSON et al (1981)
FLUIDISED BED	SOFT DRINK BOTTLING	6000	4-18.5	-	35			HICKEY AND OWENS (1981)
FILTER	PROTEIN CARBOHYDRATE		3.2-27.2	3-24 (HRS.)	35		50-90	MULLER AND MANCINNI (1975)
FILTER/UASB	SOLUBLE SUGAR	2500	5-51		27	7 vol/vol.day	<40->93	GUIOT AND VAN DEN BERG (1985)
FLUID BED 4L	GLUCOSE	6000-120000	1.5-18		35		<50-75	BULL LESTER AND STERRITT (1983)
SOFT DRINK	SOFT DRINK	26-38±1000	6.1		35	0.4-2.V/V.DAY	84-96	GHOST et al (1985)
1S+RC			4.29	2.44d			50-90	
2S+RC	FRUIT WASHING	10250	4.34	1.87d	35		51-93	
1SNORC			3.22	4.3d			68-98	THIS STUDY
2SNORC			11.62	1.00d			48-98	

Table 4.5 Average Gas Yields For Each Scheme[+]

Reactor TYpe	Maximum volumetric Gas Yield m3/m3.day	Average Total Gas YIeld m3/kgCOD/m3.day	Average Methane Yield m3CH4/kgCOD.day
1S+RC	1.99	0.519	0.303
2S+RC	2.34	0.681	0.394
1SNORC	1.20	0.555	0.307
2SNORC	3.201	0.608	0.399

(+ based on methane reactor volume)
average methane yields are based on duration of each study



sample area	sample depth	SS (gram/pad)	VSS (gram/pad)
A	0.15	0.13360	0.09520
	0.30	0.13140	0.09140
	0.45	0.13480	0.09160
	0.60	0.15440	0.10900
	0.75	0.14520	0.10120
B	0.15	0.15660	0.11260
	0.30	0.18100	0.12880
	0.45	0.22060	0.15000
	0.60	0.24980	0.17440
	.	.	.
C	0.15	0.11140	0.07660
	0.30	0.09720	0.06840
	0.45	0.09360	0.06000
	0.60	0.06560	0.05360
	0.75	0.10200	0.05240
	.	.	.
D	0.15	0.18020	0.12540
	0.30	0.22900	0.15700
	0.45	0.15540	0.10020
	0.60	0.19920	0.13620
	0.75	0.14640	0.09860
	.	.	.
E	0.15	0.2300	0.14760
	0.30	0.22800	0.14120
	0.45	0.18280	0.15300
	0.60	0.19140	0.12000

TABLE 4.6 REACTOR PAD SOLIDS BEFORE MODIFICATION
OF EFFLUENT OFF-TAKE
(single stage without recycle)

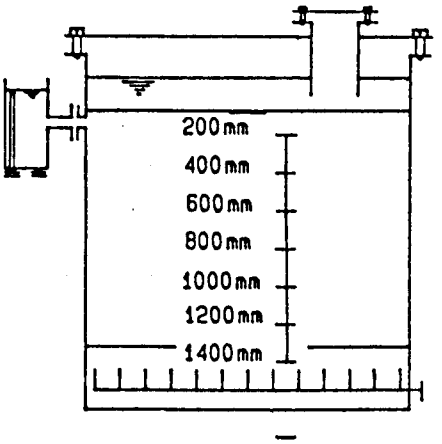
Table 4.7 Pad Solids Concentration at end of each period of study
(all samples taken from approx. 0.5m deep from bed)

scheme	samples	mean SS/pad g/l	mean VSS/pad g/l	mean VSS/pad %	gSS/litre *	gVSS/litre *
2SNORC	12	0.560	0.430	77	28	22
2S+RC	12	0.469	0.362	77	23	18
1S+RC	9	0.389	0.329	85	19	16
1SNORC	9	0.320	0.240	75	16	10

* based on 50 BSP's/litre of size 25mm sq.

1S+RC = SINGLE STAGE WITH RECYCLE 1SNORC = SINGLE STAGE WITHOUT RECYCLE
2S+RC = TWO STAGE WITH RECYCLE 2SNORC = TWO STAGE WITHOUT RECYCLE

Table 4.12 Results of Tracer Studies



	after seeding			end of commissioning		
depth	SSg/l	VSSg/l	%VSS	SSg/l	VSSg/l	%VSS
200	0.223	0.188	84	0.453	0.372	82
400	0.299	0.256	86	0.982	0.792	81
600	0.471	0.368	78	1.464	1.104	75
800	0.553	0.402	73	1.270	0.994	78
1000	0.445	0.316	71	0.774	0.678	88
1200	0.385	0.295	77	2.952	2.168	73
1400	9.554	5.696	60	9.700	6.204	64

	1S+RC			1SNORC		
depth	SSg/l	VSSg/l	%VSS	SSg/l	VSSg/l	%VSS
200	0.886	0.710	80	0.626	0.524	84
400	0.907	0.771	85	0.721	0.627	87
600	0.913	0.712	78	0.907	0.717	79
800	1.160	0.864	74	1.386	1.054	76
1000	1.371	1.042	76	1.278	0.973	76
1200	1.382	0.995	72	1.410	1.086	77
1400	2.172	1.492	69	14.80	10.13	68

	2S+RC			2SNORC		
depth	SSg/l	VSSg/l	%VSS	SSg/l	VSSg/l	%VSS
200	0.398	0.346	87	0.378	0.350	93
400	0.417	0.359	86	0.407	0.354	87
600	0.642	0.526	82	0.839	0.663	79
800	0.834	0.617	74	1.201	0.894	74
1000	0.924	0.651	70	0.987	0.740	75
1200	1.441	1.100	76	1.240	0.972	78
1400	1.151	0.829	72	17.276	8.372	48

Table 4.8 Interstitial Solids for Reactor 1 showing sampling positions

	FIXED BED	MOVING BED	EXPANDED BED	FLUIDIZED BED	RECYCLED BED	UASB
BIOMASS CONCENTRATION (kgSS/m3)	5-15	5-15	10-30	10-20	5-15	5-15
ATTACHED (% OF TOTAL)	20-80 * 50-90	50-80	90-100	95-100	0	60-80
SUSPENDED (% OF TOTAL)	20-80 * 10-50	20-50	0-10	0-5	100	20-40

* denotes upflow reactor (other is for downflow)

		1S+RC	2S+RC	1SNORC	2SNORC
BIOMASS CONCENTRATION (kgSS/m3)	ENTRAPPED	17	20	15	28
	SUSPENDED	0.9-2.17	0.40-1.15	0.6-14.8	0.38-17.3

Table 4.9 Biomass concentration and nature for various reactors (Henze and Harremoes, 1983) and for this study

REACTOR SCHEME		SOLIDS CONC. gVSS/l	GAS VOLUME V ml gas/day	V ml gas/gVSS.day	acetoclasts % of total	average acetoclasts % of total
1SNORC	PADS	0.493	13	26	1.5	1.5
		0.474	13	27	1.5	
		0.532	-	-	-	
	FREE	1.056	32	64	3.2	3.7
		1.022	36	-	4	
		0.815	38	-	4	
1S+RC	PADS	0.982	-	-	-	3.5
		1.045	48	46	4.5	
		0.949	23	24	2.5	
	FREE	0.712	28	39	4	3.5
		1.340	36	27	3	
		-	-	-	-	
2S+RC	PADS	1.493	46	31	3	3.3
		1.370	62	45	5	
		1.232	30	24	2	
	FREE	1.513	44	29	3	3.3
		1.404	37	26	3	
		1.534	54	35	4	
2SNORC	PADS	1.462	85	58	6	5
		1.366	63	46	5	
		1.388	48	34	3	
	FREE	1.423	99	70	7	7
		1.350	88	65	7	
		1.352	-		-	

Table 4.10 Reactor 1 Biomass Acetoclastic Activity

TIME (HRS)	Abiotic control reagents 1	2	3	4
0.25	1219	846	287	2134
0.5	1244	801	324	5008
1.0	1209	1330	747	9851
1.5	1962	1718	1519	11912
2.0	1507	2028	1569	20764
2.5	1405	1523	1050	21605
3.5	1482	1306	1528	27353

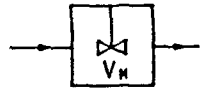
values in table are in DPM for a 4 ml sample

1 = addition of specific inhibitor to methanogenesis
2 = addition of chloroform
3 = addition of excess acetate
4 = no addition

Table 4.11 Non-specific uptake control of radioactively labelled substrate

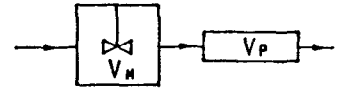
REACTOR TYPE	FEED l/day	RECYCLE RATIO	HRT # hrs.	HRT @ hrs.	% ACTIVE volume	D/uL	VARIANCE	n	GAS PROD. m3/day
1SNORC	583	-	17.7	103	17	1.250	0.779	1.28	3.00
1S+RC	691	13	15.7	86.8	18	0.557	0.597	1.67	2.30
2S+RC	2160	10	9.6	28	34	0.278	0.405	2.47	5.03
2SNORC	2534	-	6.8	24	28	0.212	0.334	2.99	4.00

MODEL1



$ET = A1 \pm EXP(-A1 \pm T)$

MODEL2



$ET = A1 \pm EXP((-A1 \pm T) + B1)$

TERM DEFINITION

$A1 = V/V_M$

$B1 = V_P/V_M$

V_M = MIXED VOLUME (litres)

V_P = PLUG VOLUME (litres)

MODEL1			MODEL2						ACTUAL	
REACTOR TYPE	A1	V_M	REACTOR TYPE	A1	B1	V_M	V_P	$\%V_P$	V_T	V_A	V DEAD
1SNORC	1.23	349	1SNORC	1.128	0.097	381	37	9	418	430	2070
1S+RC	0.925	488	1S+RC	0.753	0.173	600	104	15	704	452	2048
2S+RC	0.959	901	2S+RC	0.605	0.300	1428	428	23	1856	864	1636
2SNORC	1.018	697	2SNORC	1.012	0.415	701	291	29	992	718	1782

Table 4.12 Modelling of reactor hydraulics based on tracer studies

actual retention time from tracer data

@ theoretical retention time based on 2.5 m3 reactor vol.

Table 4.13 Results of Regression analysis for steady state data

(VALUES IN TABLE ARE THE MEAN SQUARE RESIDUALS FOR THE FITTING OF INDICATED MODELS TO EXPERIMENTAL DATA)

based on total COD

MODEL	1S+RC	2S+RC	1SNORC	2SNORC
CSTR	48	121	24	265
PLUG	52	156	14	263
Y+M	46	134	21	273
T IN S	52	123	14	295

based on soluble COD

MODEL	1S+RC	2S+RC	1SNORC	2SNORC
CSTR	70	78	56	269
PLUG	83	116	37	225
Y+M	72	83	50	220
T IN S	77	77	36	215

CSTR = COMPLETELY MIXED FIRST ORDER MODEL

PLUG = PLUG FLOW FIRST ORDER MODEL

Y+M = YOUNG AND McCARTY EMPIRICAL MODEL

T IN S = TANKS IN SERIES MODEL

values of N for the tanks in series model

MODEL	1S+RC	2S+RC	1SNORC	2SNORC
total	1.27	0.28 *	2.84	1.32 *
soluble	0.67	0.37	3.75	2.72

* (FOR THE METHANE STAGE ONLY IN TWO STAGE SYSTEMS)

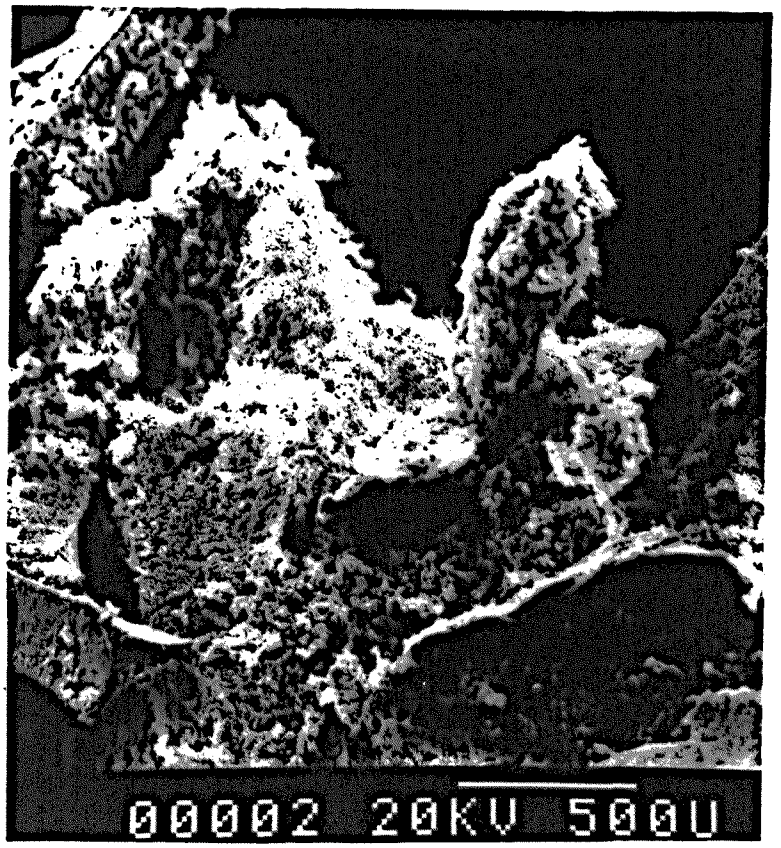


Plate III Scanning electron micrograph of BSM fragment

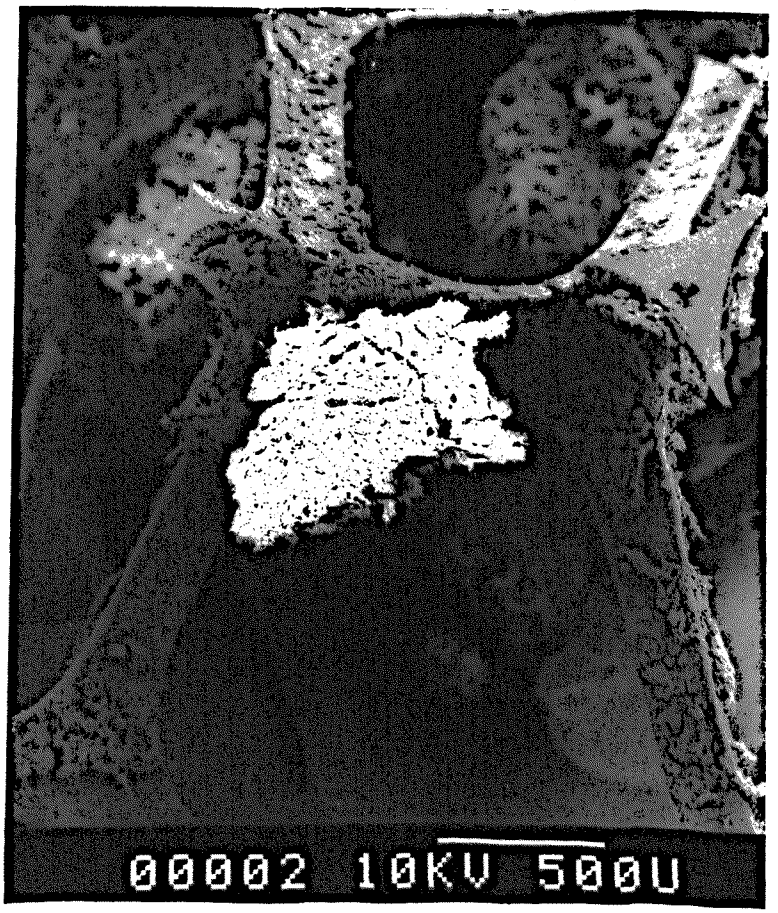


Plate IV Scanning electron micrograph of of BSM fragment

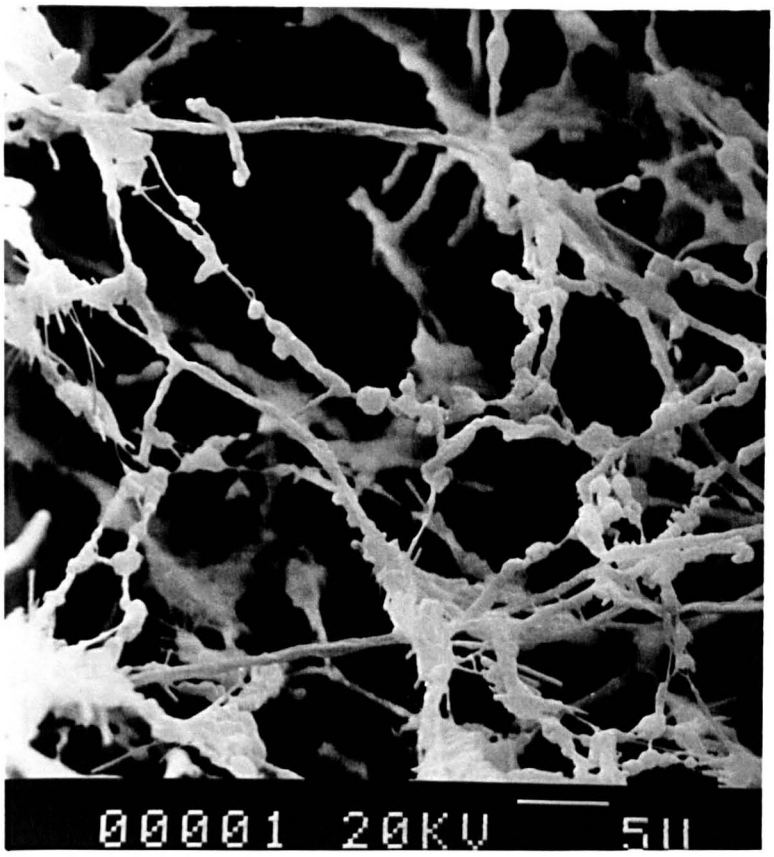


Plate V Scanning electron micrograph
of BSM fragment

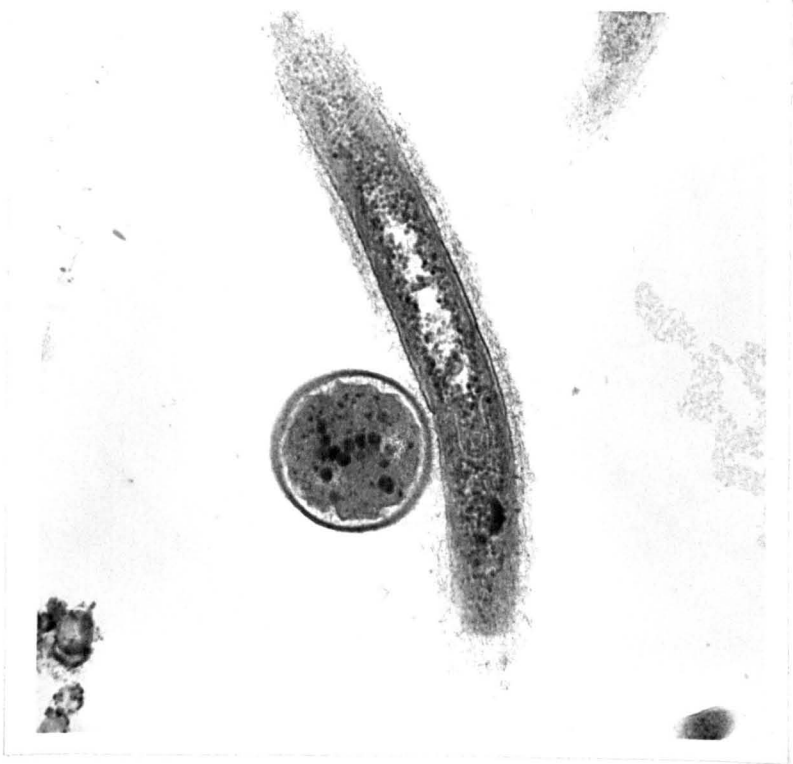


Plate VI Transmission electron micrograph
of of BSM fragment

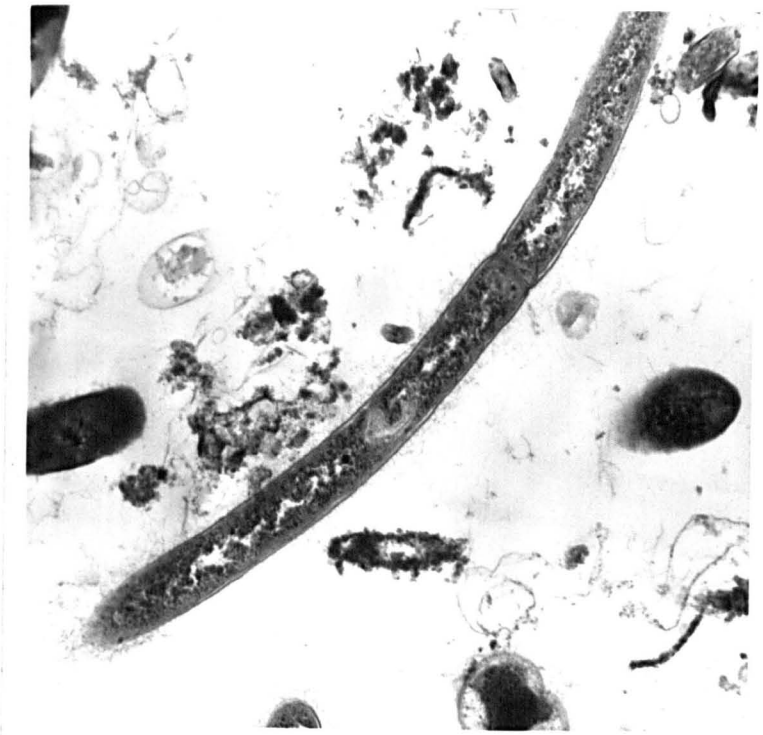


Plate VII Transmission electron micrograph
of of BSM fragment

CHAPTER FIVE: CONCLUSIONS

The following conclusions can be drawn from the results of this study on the anaerobic treatment of an industrial wastewater using a reticulated foam biomass support:

(1) Reactor Startup

Difficulties were experienced during the initial stages of startup and were related to fluid short-circuiting in the reactor bed and inadequate pH control. These faults were successfully rectified by a modification of the effluent offtake and the addition of automatic pH control, the latter being considered essential in the digestion of an acidic wastewater of this type. The use of an acidification reactor in the two-stage systems enabled the percentage of soluble COD and carbohydrate to be increased from 81 to 86 percent and from 75 to 81 percent respectively at an HRT of 0.5 day^{-1}.

Startup of the reactor containing a solid block of foam was aborted as continued failure was experienced despite the addition of pH control. This was thought to be due to the transfer of substrate through the media or the clogging of foam pores from the initial seed of digested sewage sludge. Difficuties were also experienced by Huysmen (1983) in this respect. These results highlight the need for careful process design.

(2) Perfomance Criteria

Performance criteria were assessed for the four systems based on shortest achievable hydraulic retention time, maximum loading rate, and highest gas production rate attained before failure. With respect to hydraulic retention time it was found that the two-stage system without recycle performed best overall based on methane reactor volume:

Reactor	2SNORC	<	2S+RC	<	1S+RC	<	1SNORC
min. HRT (day)	1.05		1.87		2.44		4.25

whereas when based on total reactor volume:

Reactor	2SNORC	<	2S+RC	<	1S+RC	<	1SNORC
min. HRT (day)	1.47		2.62		2.44		4.25

It can be concluded that the use of recycle in single stage processes is beneficial whereas it is deterimental for two-stage processes when treating this type of reactor.

Maximum gas yields were attained in the two stage processes of 3.2 and 2.35 $m^3 m^{-3}$ for the 2SNORC and 2S+RC respectively. Average methane yields were slightly higher than theoretical for two-stage processes and slightly lower than theoretical for single stage processes. These observations could have been due to the fact that the methane determinations were made once a week and used to reflect the performance over the whole week.

(3) Operating Observations

The single stage process without recycle generally showed a significant decrease in COD removal efficiency at loadings above 2.5 kg COD m^{-3} day^{-1}. Periods of increased loading caused an increase in effluent VFA such that the relative proportions were always in the order: acetic > propionic > butyric. For the single stage system with recycle higher levels of VFA were recorded, indicating more severe inhibition and instability. The two-stage without recycle system, which was fed at the highest loading rate, exhibited the lowest effluent VFA concentrations compared to all the other systems tested. For the two stage system with recycle, variations in feed concentrations were buffered to a certain extent but the system showed serious inhibition when overloaded.

Recycle, in both single and two stage systems, generally caused greater instability and slower recovery from serious overload. This was indicated by high propionic and butyric acid concentrations in the effluent. The cause was thought to be the rapid transfer of reactor liquors, containing high concentrations of VFA, from the inlet region of the reactor to regions higher in the reactor reducing the activity of biomass in this area.

(4) Reactor Biomass

It was observed that at lower superficial upflow velocities (i.e. systems without recycle through the bed) large amounts of biomass were retained in the unpacked

region at the bottom of the reactor. Concentrations of up to 17 g VSS/l were recorded in the two stage process without recycle and 10-15 g VSS/l in the single stage system without recycle. For systems with recycle, solids concentrations beneath the packing were of the same order as interstitial solids. Pad solids concentrations were higher in two stage than in single stage processes and were close to values reported in the literature from laboratory studies (Huysmen, 1983). In all systems tested the percentage of volatile solids in the bottom section of the reactor was less than the interstitial value.

Observations of the factors that were thought to influence the washout of biomass indicated that at superficial upflow velocities of 7.5 m day^{-1} (0.087 m sec^{-1}) the biomass support particles effectively retained biomass, and that this was much higher than values reported in the literature for pure cultures (Fynn and Whitmore, 1982; 1984). High gas production was thought to influence biomass washout and was most likely due to the scouring effect of rising gas bubbles or the generation of gas from within the biomass aggregate, so lifting the surface layers. There was no obvious correlation of feed solids and effluent solids.

(5) Biomass Activity

The investigation of biomass activity showed that freely suspended biomass was of a higher activity than that immobilised in the BSM's in systems without recycle. For the two stage system without recycle activity was much higher

than in any other system. Comparison of the results with those of other workers showed that the maximum percentage of acetoclastic methanogens in this study was approximately 7 percent compared to values as high as 33 percent in a UASB reactor (Valke and Verstraete, 1983). This may have been due to the nature of the wastewater and the tendency to produce a greater proportion of acidogenic biomass. There is a possibility that, if operation of the reactor was continued in the two stage without recycle mode, the activity of the reactor biomass would have increased and permitted further increases in loading.

(6) Activity Gradients

The results of a series of experiments on activity measurements within individual biomass support particles must be interpreted with care due to the method of sectioning the pads. In all cases there was a difference in activity between the inside and outside of the pads. Uptake of the radioactively labelled substrate proceeded at a higher rate when the bulk substrate concentration was lower. For bulk liquid acetate concentrations of 200mg/l the uptake was of the order of 10 times that for the higher concentrations tested. Stirring of the bulk liqour increased the rate of substrate transport through the biomass agregate at all concentrations, but not necessarily the rate of uptake.

The most significant result was that in this study all the activity appeared to be confined to the outer layers

of the support particle indicating that the optimum size of the pad should be of the order of twice the maximum penetration depth (3-4 mm), i.e. 8-10mm square. This does not take into account the cost of the foam, a large proportion of which is to cut the foam to size, and other economic factors including the changes in hydraulic behaviour of the bed. It also may be advantageous to increase the unpacked section beneath the bed as it has been shown that large amounts of biomass were retained in this area.

(7) Reactor Hydraulics and Mixing

The use of bromophenol blue as a tracer in this type of reactor was most useful as there was little absorption of the dye onto organic material. Tracer studies were used to ascertain the flow regime within a particular reactor system and, consistent with theory, showed that the adoption of a high recycle rate caused the flow regime to approach completely mixed flow. Further analysis of tracer data indicated the true retention time for each reactor system tested and enabled the dead volume to be calculated. Active volume varied between 17-34 percent of the total methane reactor volume. In the two-stage systems the dead volume was not greatly different from the total colonized pad volume. Fitting the observed tracer curves to two multiparameter models showed that modelling as a single mixed tank provides a reasonable estimate of the reactor volume. A plug-mixed series model did not predict the volumes quite as accurately, but predicted the system with the highest amount

of plug-flow characteristics. The use of tracer data may be useful in determining when a reactor of this type requires biomass removal.

(9) Steady State Modelling

Attempts were made to fit treatment efficiency data to various simple reactor models. Statistical analysis indicated that, due to the disperse nature of the data and the difficulty in finding any consistent removal kinetics, the results were of little use in the development of a complex mathematical model apart from their agreement with the various hydraulic regimes in the reactor.

(10) Microscopic observations

Observations with scanning and transmission electron micrographs indicate that attached and suspended growth were present in the reactor and the majority of the biomass seemed to be composed of a dense fibrillar network. Further identification of the micrographs were doubtful as artefact formation may have been caused by sample preparation.

CHAPTER SIX: SUGGESTIONS FOR FUTURE RESEARCH

Much of this work has enabled a greater understanding of the behaviour of this type of biomass support medium and the hydraulic regimes that exist in fixed beds of this type. It is considered that research in the following areas would be most useful:

(1) Further refinement of the BSM activity gradient experiments, for example the use of a core section, could enable the results to be used to derive an expression for substrate removal. The method may be adapted to test for the utilization of other substrates, for example glucose, and therefore establish the existence of a difference in activity for acidogenic bacterial populations within a biomass aggregate. The assay of co-enzymes could also be attempted.

(2) Laboratory investigations using ideal substrates would be advantageous in elucidating the observed kinetic coefficients at steady state.

(3) Preliminary investigations indicated that the size of the BSM particle used in this study was larger than optimum and an evaluation of performance with other sizes and geometries of BSM would be advantageous.

(4) An investigation of the maximum superficial upflow velocity, beyond that in this study, would provide valuable information on biomass holdup when treating low strength

wastes.

(5) There are other ways in which foam BSM's could be used in an anaerobic reactor, including in downflow fixed film or fluidised bed mode. These systems would consist of mainly entrapped growth.

(6) There were obviously limitations in monitoring the composition of gas on a weekly basis. The use of an online gas analyser would enable better interpretation of results during periods of reactor instability.

(7) Fixed film reactors have demonstrated the ability to tolerate inhibition from toxic and shock loads, more than suspended growth systems. An evaluation of the effect of recycle in reducing this could be an asset in the treatment of industrial wastes in situations where waste strength increases during the day.

(8) Operation of this type of reactor in batch mode may reduce the requirement for balancing sumps or storage tanks.

(9) Measurement of the effects of gas holdup and the deformation of the BSM's during process operation in a fixed bed would be advantageous for process scale-up.

Appendix I

Model Fitting

The procedure used in this study for fitting the experimental data to theoretical models was the non-linear regression package, PROC NLIN, (Statistical Analysis System, SAS). This routine was used to produce least squares estimates of the non-linear model fitting parameters. The procedure used the secant method which is similar to the Gauss-Newton method except that the derivatives are estimated from the history of iteration rather than being supplied analytically. This has been described elsewhere (SAS User's Guide, 1982).

Reactor Models

The four reactor models fitted to experimental data are briefly described here. Derivation of these models is described elsewhere (Levenspiel, 1972).

(i) Young and McCarty empirical filter model

$$TOT = 100(1 - A1/HRT)$$

where TOT = percentage COD removal efficiecy (%)

A1 = empirical constant (day)

HRT = hydraulic retention time (day)

(ii) Tanks in series model

$$TOT = 100(1-(1/(1+(A1*HRT/B1))^{**}B1)))$$

where TOT = percentage COD removal efficiency (%)

A1 = constant

B1 = number of completely mixed equal sized tanks

HRT = hydraulic retention time (day)

(iii) Plug-flow first order model

$$TOT = 100(1-EXP(A1*HRT))$$

where TOT = percentage COD removal efficiency (%)

A1 = first order rate constant (day^{-1})

HRT = hydraulic retention time (day)

(iv) Completely mixed first order model

$$TOT = 100(1-1/(1+A1*HRT))$$

where TOT = percentage COD removal efficiency (%)

A1 = first order rate constant (day^{-1})

HRT = hydraulic retention time (day)

Appendix II

Computation Of HRT And Dispersion Number From Tracer Data

Computation of the actual hydrualic retention time and dispersion number was performed using the Fortran program listed below (Fig.A2). The program assigns storage for the input and output data files and reads in time and concentration data (ITIME and FTEE respectively). It then calls subroutine SIMIN which uses Simpson's rule to perform successive intergrations and return values of normalised time and concentration (THETA and ETHETA respectively) to the main program. After output of the results the variance of the tracer curve is calculated. This value is then used to iteratively solve for the dispersion number, using the bisection method to refine successive guesses. This loop continues until the dispersion number reaches the desired accuracy or the solution is not reached after fifty iterations. The dispersion number is then written to the output file and the terminal.

```
      PROGRAM GRAHAM
      DIMENSION ITIME(50),FTEE(50),ETEE(50),TETEE(50),TSQET(50)
     +,THETA(50),ETHETA(50)
      CHARACTER*10 IFIL1,IFIL2
      WRITE(6,140)
  140 FORMAT(' ENTER INPUT/OUTPUT DATAFILE NAMES  WITH QUOTES! ')
      READ(5,*)IFIL1,IFIL2
      OPEN(1,FILE=IFIL1)
      OPEN(8,FILE=IFIL2)
      READ(1,*)NDIM,H
      READ(1,*)(ITIME(I),FTEE(I),I=1,NDIM)
      NSTEP=NDIM-1
      CALL SIMIN(FTEE,NDIM,NSTEP,H,SINT)
      I=1
   20 ETEE(I)=FTEE(I)/SINT
      TETEE(I)=ITIME(I)*ETEE(I)
      TSQET(I)=(ITIME(I)**2)*ETEE(I)
      I=I+1
      IF(I.LT.NDIM)GOTO 20
      CALL SIMIN(TETEE,NDIM,NSTEP,H,TMEAN1)
      CALL SIMIN(TSQET,NDIM,NSTEP,H,TMEAN2)
      I=1
   30 THETA(I)=ITIME(I)/TMEAN1
      ETHETA(I)=ETEE(I)*TMEAN1
      I=I+1
      IF(I.LT.NDIM)GOTO 30
      WRITE(6,40)
   40 FORMAT(8H    ITIME,3X,4HF(T),3X,4HE(T),4X,5HTE(T),4X,6HT2E(T),4X,
     +1H@,5X,2HE@)
      WRITE(6,45)(ITIME(I),FTEE(I),ETEE(I),TETEE(I),TSQET(I),THETA(I),
     +ETHETA(I),I=1,NDIM)
   45 FORMAT(I8,6F8.4)
      SIGSQ=TMEAN2-TMEAN1**2
      TMSQ=TMEAN1**2
      VAR=SIGSQ/TMSQ
      WRITE(6,50)TMEAN1,TMEAN2,VAR
   50 FORMAT(/28H  THE SYSTEM MOMENTS ARE M1=,F9.4,5H.HRS./
     +,20X,11H AND M2=    ,F8.4,5H.hrs ,/,22H THE SYSTEM VARIANCE= ,F8.4,
     +/,F8.4,5H.HRS.)
      CLOSE(1)
      D1=1E-5
      D2=1000.0
      NCOUNT=0
   55 D3=(D1+D2)/2.0
      CALL DISPF(D1,ANV1,VAR)
      CALL DISPF(D2,ANV2,VAR)
      CALL DISPF(D3,ANV3,VAR)
      IF (ANV1*ANV3.LT.0.0)THEN
        D2=D3
      ELSE
        D1=D3
      ENDIF
      NCOUNT=NCOUNT+1
      IF (NCOUNT.GT.50)THEN
        WRITE(6,65)
   65   FORMAT(' FAILED TO CONVERGE IN 50 ITERATIONS !')
        WRITE(8,110)
  110   FORMAT(' FAILED TO CONVERGE IN 50 ITERATIONS !')
        CLOSE(1)
        CLOSE(8)
        STOP
      ENDIF
      IF (ABS(ANV3).GT.1.0E-4)GOTO 55
      WRITE(6,70)D3,NCOUNT
   70 FORMAT(' Dispersion number = ',F8.4,' after ',I5,' iterations')
      WRITE(8,80)
   80 FORMAT(8H    ITIME,3X,4HF(T),3X,4HE(T),4X,5HTE(T),4X,6HT2E(T),4X,
     +1H@,5X,2HE@)
      WRITE(8,90)(ITIME(I),FTEE(I),ETEE(I),TETEE(I),TSQET(I),THETA(I),
     +ETHETA(I),I=1,NDIM)
   90 FORMAT(I8,6F8.4)
      WRITE(8,100)TMEAN1,TMEAN2,VAR
  100 FORMAT(/28H  THE SYSTEM MOMENTS ARE M1=,F9.4,5H.HRS./
     +,20X,11H AND M2=    ,F8.4,5H.hrs ,/,22H THE SYSTEM VARIANCE= ,F8.4,
     +/,F8.4,5H.HRS.)
      WRITE(8,120)D3,NCOUNT
  120 FORMAT(' Dispersion number = ',F8.4,' after ',I5,' iterations')
      CLOSE(1)
      CLOSE(8)
      STOP
      END
      SUBROUTINE DISPF(DISP,ANV,VAR)
      ANV=(2.0*DISP)-(2.0*DISP**2)*(1.0-EXP(-1.0/DISP))-VAR
      RETURN
      END
      SUBROUTINE SIMIN(F,NDIM,NSTEP,H,SINT)
      DIMENSION F(50)
      IF(MOD(NSTEP,2).EQ.0) GOTO 1
      PRINT *,'EXECUTION TERMINATED IN SIMIN BY ODD NSTEP'
      STOP
    1 SINT=0
      DO 2 J=2,NSTEP,2
    2 SINT=SINT+F(J-1)+4.0*F(J)+F(J+1)
      SINT=SINT*H/3.
      RETURN
      END
```

Fig. A2 Fortran program to calculate retention time and dispersion number

Appendix III

The Kruskal-Wallis test may be used to compare the averages of independent samples. It is used to test the null hypothesis H_0 that k independent samples are from identical populations. The test is an alternative nonparametric procedure to the F testing for the equality of means in the one-factor analysis of variance when the experimenter wishes to avoid the assumption that the samples were selected from normal populations. The test is performed as follows:

(1) All observations are ranked together, assigning ties the average rank;

(2) All ranks are summed for each sample; and

(3) The test statistic H is computed where:

$$H = \frac{12}{N(N+1)} \sum_{i}^{k} \frac{R_i^2}{n_i} - 3(N+1)$$

where n_i = number of observations of the i^{th} sample

R_i = sum of the ranks for the i^{th} sample

N = total number of observations.

It may be shown that provided the sample sizes are not very small that

$$H \simeq X^2_{k-1} \qquad \text{(chi-squared distribution)}$$

If H falls in the critical region $H > X^2_{1-\alpha}$ with $v = k-1$ degrees of freedom, reject H_0 at the level of significance; otherwise, accept H_0.

REFERENCES

Alibhai, K.R.K. and Forster, C.F., (1986) "An examination of the granulation process in UASB reactors", Envir. Tech. Lett., Vol. 7, pp105-112.

Anderson, G.K., Donelly, T. and Letten, D.J., (1980) "Anaerobic Treatment of high-strength industrial wastewaters" in: Curi, K. (ed.), Treatment and disposal of liquid and solid industrial wastes, Permagon Press, Oxford, U.K.

Anderson, G.K., Donelly, T. and McKeown, K.J., (1982) "Identification and control of inhibition in the anaerobic treatment of industrial wastewaters" Proc. Biochem. Vol. 17, pp28 to 31,

Anderson, G.K. and Donelly, T., (1984) "The application of anaerobic packed-bed reactors to industrial waste-water treatment", Wat. Poll. Cont., Vol. 83, No. 4, pp491-496.

Anderson, G.K. and Saw, C.B., (1986) "Comparison of industrial processes for waste treatment" paper presented at PIRA conference,Cost effective treatment of paper mill effluent using anaerobic technologies, Leatherhead, U.K., Jan 15/16

Anderson, G.K., Saw, C.B. and Fernandes, M.I.A.P, (1986), "Application of porous membranes for biomass retention in biological wastewater treatment processes", Proc. Biochem., Vol. 21, pp174-182.

Andrews, J.F., (1969), "Dynamic model of the anaerobic digestion process", Jour. San. Eng. Div. ASCE, Vol. 95 (SA1), pp95.

Andrews, J.F., (1978), "The development of a dynamic model and control strategies for the anaerobic digestion process": in James, A. (ed.), Mathematical models in water pollution control , Chichester, J. Wiley, chpt. 13

Antonie, R.L., (1974) "Evaluation of a rotating disc wastewater treatment plant", Water Poll. Cont., Vol. 46, pp498.

Archer, D.B., (1984), "Detection of quantitation of methanogens by Enzyme-Linked Immunosorbent Assay", Appl. and Environ. Microb., Vol. 48, No. 4, pp797-801.

Archer, D.B., (1985), "Uncoupling of methanogens from growth of *Methanosarcina barkeri* by phosphate limitation", Appl. and Environ. Microb., Vol. 50, No.5, p1233-1237.

Archer, D.B., (1986), "Hydrogen as a process control index in a pilot scale anaerobic digester", Biotechnol. Lett., Vol. 8, no. 3, pp197-202.

Atkinson, B., (1974), "Immobilised cells: their application and potential", paper presented at: Process engineering aspects of immobilised cell systems, 28-29 March, University

of Manchester Institute of Science and Technology, Manchester, U.K.

Atkinson, B., Black, G.M., Lewis, P.J.S. and Pinches, A., (1979), "Biological particles of given size, shape and density for use in biological reactors", Biotech. and Bioeng., Vol. 21, p193.

Atkinson, B., Cunningham, J.D. and Pinches, A., (1984), "Biomass holdups and overall rates of substrate (glucose) uptake of support particles containing a mixed microbial culture", Chem. Eng. Res. Des., Vol. 62, pp155-164.

Bachmann, A., Beard, V.L., and McCarty, P.L., (1985), "Perfomance characteristics of the anaerobic baffled reactor", Water Res., Vol. 19, no. 1, pp99-106.

Balch, W.E., Fox, G.E., Magrum, K.J., Woese, C.R. and Wolfe, R.S., (1979), "Methanogens: reevaluation of a unique biological group", Microbiol. Rev., vol 43, pp260.

Banfield, F.S., Meek, D.M. and Lowden, G.F. (1978), "Manual and automated gas-chromatographic procedures for the determination of volatile fatty acids", WRC technical report, TR76.

Benjamin, M.M., Ferguson, J.F. and Buggins, M.E., (1981), "Treatment of sulphite evaporator condensate with an anaerobic reactor", in: TAPPI environmental conference, April 29, New Orleans, pp1-10.

Benjamin, M.M., Woods, S.L. and Ferguson, J,F., (1982), "Toxicity and biodegradability of pulp mill waste constituents to anaerobic bacteria", in: paper presented at IAWPR-specialised seminar on anaerobic treatment, June, Copenhagen, Denmark.

Binot, R.A., Bol, H.P., Naveau, H.P. and Nyns, E.J., (1983) "Biomethanation of immobilised fluidised cells", Wat. Sci. Tech., Vol. 15, Nos. 8/9, pp103-116.

Bolle, W.L., van Breugel, J, van Eybergen, G.C., Kossen, N.W.F. and van Gils, W., (1986), "Kinetics of anaerobic purification of industrial wastewater", Biotech. and Bioeng., Vol. 28, pp542-548.

Boone, D.R., (1982) "Terminal reactions in the anaerobic digestion of animal waste", Appl. and Environ. Microb., vol 43, no.1, p57-64.

Boone, D.R., (1984), "Propionate exchange reactions in methanogenic ecosystems", Appl. and Environ. Microb., Vol. 48, No. 4, pp863-864,

Boone, D.R. and Bryant, M.P., (1980), "Propionate degrading bacterium *Syntrophobacter wolinii* sp. nov. gen. nov., from methanogenic ecosystems", Appl. Environ. Microb., Vol. 40, pp626-632.

Brown, L., Rhead, M.M. and Hill, D., (1984), "The use of

bromophenol blue as a tracer in sewage works", Wat. Res., Vol. 18, No. 9, pp1983-1087.

Bryant, M.P., (1979), "Microbial methane production theoretical aspects", Journal of animal science, Vol. 48, no.1, pp193

Bryant, M.P., (1976), "The microbiology of anaerobic degradation and methanogenesis with special reference to sewage", in: schlegel H.G. (ed.), Microbial Energy Conversion, Permagon Press, Oxford, U.K., p107-117.

Bryant, M.P., Wolin, E.A., Wolin, N.J. and Wolfe, R.S., (1967), "*Methanobacillus omelianskii*, a symbiotic association of two species of bacteria", Arch. Microbiol., Vol. 59, pp20.

Bull, M.A., Sterritt, R.M., Lester, J.N., (1984), "An evaluation of single and separated phase anaerobic industrial wastewater treatment in fluidized bed reactors", Biotech. and Bioeng., Vol. 26, pp1054-1065.

Bull, M.A., Sterritt, R.M. and Lester, J.N., (1982), "The effect of organic loading on the performance of anaerobic fluidised beds treating high-strength wastewater", Trans. I. Chem. Eng., Vol. 60, pp373-376.

Butler, G.A., (1984), "Anaerobic digestion of starch process effluents at Tenstar Products Ltd.",in paper presented at BABA workshop on anaerobic digestion, Dept. of Chemical Engineering, Univ. of Birmingham, 24th may.

Calzada, J.F., de Arriola, M.C. and Castaneda, N.O., (1984), "Methane from coffee pulp juice: experiments using polyurethane foam reactors", Biotech. Lett., Vol. 6, no. 6, pp385-388.

Camilleri, C., (1984), " La methanisation a cellules fixees", Eau, Industrie, Nuisances, Vol. 84, pp35-38.

Carrondo, M.J.T., Silva, J.M.C., Figueira, M.I.I., Ganho, R.M.B. and Olivera, J.F.S., (1983) " Anaerobic filter treatment of molasses fermentation wastewater", Water Sci. and Tech., Vol. 15, No.8/9 pp117-126.

Chain, E.S.K. and DeWalle, F.B., (1977), "Treatment of high strength acidic wastewater with a completely mixed anaerobic filter", Water Res., Vol. 11, pp295-304.

Chang, J., Noike, T., Matsumoto, J., (1983), " Effect of pH on methanogenesis in anaerobic digestion", Proc. Japan. Soc. of Civil Eng., no.333, pp101-108.

Cho, Y.K., (1983), "Performance of a two-stage methane digester from alcohol stillage derived from sugar cane molasses", Biotech. Letts., Vol. 5, pp555-560.

Cheung, M.Y., Oakley, D.L. and Forster, C.F., (1986), Paper submitted to Environ. Tech. Lett.

Cholette, A and Cloutier, L., (1959), "Mixing efficiency determination for continuous flow systems", Can. J. Chem. Eng., Vol. 37, No. 3, pp105-112.

Civit, E., Duran de Bazua, C., Englemann, G., Gonzalez, S. and Hartman, L., (1984), Environ. Tech. Lett., Vol. 5, pp89-96.

Cillie, G.G., Henzen, M.R., Stander, G.J. and Baillie, R.D., (1969) "Anaerobic digestion IV The application of the process in waste purification", Water Res., Vol. 3, pp623-643.

Cohen, A., Zoetemeyer, R.J., van Deursen, A. and van Andel, (1979) "Anaerobic digestion of glucose with separated acid production and methane formation", Water Res., Vol. 13, pp571-580.

Cohen, A., van Gemert, J.M., Zoeteymeyer, R.J. and Breure, A.M., (1984), "Main characteristics and stoichiometric aspects of acidogenesis of soluble carbohydrate containing wastewaters", Proc. Biochem., Vol. 19, pp228-232.

Colin, F., Ferrero, G.L., Gerletti, M., Hobson, P., L'Hermite, P., Naveau, H.P. and Nyns, E.J., (1983), "Proposal for the definition of parameters and analytical measurements applicable to anaerobic digestion", Agricultural Wastes, Vol. 7, pp183-193.

Corpe, W.A., (1980), "Microbial surface components involved in adsorbtion of microorganism onto surfaces" in: Bitton, G. and Marshell, K.C., (eds.), Adsorbtion of microorganisms onto surfaces, pp105-173, John Wiley, New York, 1980

De Vocht, M., van Meenen, P., van Assche, P., Verstraete, W., (1983), "Influence of sedimentation and adhesion on selection of methanogenic associations", Proc. Biochem. Vol.18, pp31-34.

Dewalle, F.B., Chian, E.S.K., (1976), "Kinetics of substrate removal in a completely mixed anaerobic filter", Biotech. and Bioeng., Vol. 18, p1275.

Delafontaine, J., Naveau, H.P. and Nyns, E.J., (1979), "Fluorimetric monitoring in anaerobic digesters", Biotechnol. Lett., Vol. 1, part 2, pp71-74.

Doddema, H.J. and Vogels, G., (1978), "Improved identification of methanogenic bacteria by fluorescence microscopy", Appl. and Environ. Microb., Vol. 36, No. 5, pp752-754.

Dolfing, J., and Mulder, J.W., (1986), "Comparison of methane production rate and coenzyme F_{420} content of methanogenic consortia in anaerobic granular sludge", Appl. and Environ. Microb., Vol. 49, No. 5, pp1142-1145.

Donelly, T., (1986), "Anaerobic digestion, developed for the industrialist", paper presented at PIRA conf., Cost effective treatment of papermill effluents using anaerobic

technologies, 14-15 Jan., Leatherhead.

Duarte, A.C. and Anderson, G.K., (1982), "Inhibition modelling in anaerobic digestion", Wat. Sci. Tech., Vol. 14, pp749-763.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F., (1956), "A colorimetric method for the determination of sugars and related substances", Anal. Chem., Vol. 28, pp350-356.

Eastman, J.A. and Ferguson, J.F., (1981), "Solubilization of particulate organic carbon during the acid phase of anaerobic digestion",J.W.P.C.F., vol 53, no. 3, pp352-366.

Ferguson, J.F., Eis, B.J. and Benjamin, M.M., (1984), "Neutralization in anaerobic treatment of an acidic waste", Water res., vol. 18, no. 5, pp573-580.

Ferguson, J.F., Eis, B.J. and Bemjamin, M.M., (1982), "Neutralization in anaerobic treatment of an acidic waste", presented at IAWPR specialised seminar on anaerobic treatment, June, Copenhagen, denmark

Fernandes, X., Cantwell, A.D. and Mosey, F., (1985), "Anaerobic biological treatment of sewage", Water Poll. Cont., Vol. 84, No. 1, pp99-110.

Fiebig, R., and Dellweg, H., (1985), "Comparison between the process performance of an UASB reactor and a UASB-fixed film combination with an acetic acid enrichment culture", Biotechnol. Lett., Vol. 7, No. 7, pp487-498.

Fletcher, M. and Floodgate, G.D., (1973), "An electron-microscopic determination of an acidic polysaccharide involved in the adhesion of a marine bacteria to solid surfaces", J. Gen. Microb., Vol. 74, pp325-324,

Forster, C.F. and Wase, D.A.J., (1983), "Anaerobic treatment of dilute wastewaters using an upflow sludge blanket reactor", Environ. Poll. (series A), Vol. 31, pp57-66.

Freeman, R.R., (1961), "Production of concentrated cell suspensions: The sponge fermenter", Journal of Bio. and Microb. Tech. and Eng., Vol. 3, No. 4, pp339-350.

Fynn, G.K., and Whitmore, T.N., (1982), "Colonization of polyurethane reticulated foam biomass support particles by methanogen species", Biotech. Lett., Vol. 4, No. 9, PP557-582.

Fynn, G.K. and Whitmore, T.N., (1984), "Retention of methanogens in colonized biomass support particle", Biotechnol. Lett., Vol. 6, No. 2, pp81-86

Fynn, G.K. and Rankin, (1987), "Methanogenesis by methanogen species immobilised on reticulated foam biomass support particles. Inhibition of methane production at high substrate concentrations", Biotechnol. Letts., Vol. 9, No. 1, pp67-70.

Ghosh, S., (1984), "Feasibilty of two phase anaerobic stabilization of industrial waste with simultaneous production of energy", Environ. Tech. Letts., Vol. 5, pp373-382.

Ghosh, S., Ombregt, J.P. and Pipyn, P., (1985), "Methane production from industrial wastes by two phase anaerobic digestion", Water Res., Vol. 19, No. 9, pp1083-1088.

Ghosh, S. and Pohland, F.G., (1974), "Kinetics of substrate assimilation and product formation in anaerobic digestion", .J.W.P.C.F., vol. 46, pp748-759.

Guiot, S.R. and van der Berg, L., (1984), "Performance and biomass retention of an upflow anaerobic reactor combining a sludge blanket and filter", Biotechnol. Letts., Vol. 6, No. 3 pp161-164, 1984

Guiot, S.R. and van den Berg, L., (1985), "Performance of an upflow anaerobic reactor combining a sludge blanket and filter treating sugar waste", Biotech. and Bioeng., Vol. 27, pp800-806.

Gujer,W. and Zehnder, A.J.B., (1983),"Conversion Processes in anaerobic digestion", Water Sci. and Tech., Vol. 15, No. 8/9, pp127-168.

Hanaki, K., Matsuo, T. and Nagase, M., (1981), "Mechanism of inhibition caused by long chain fatty acids in anaerobic digestion process", Biotech. and Bioeng., vol. 23, pp1591-1610.

Harper, S.R. and Pohland, F.G., (1986), "Recent developments in hydrogen management during anaerobic biological wastewater treatment", Biotech. Bioeng., Vol. 28, pp585-602.

Harremoes, P., (1976), "The significance of pore diffusion to filter denitrification", Journ. Water Poll. Control Fed., Vol. 48, No. 2, pp377-388.

Harremoes, P. and Reimer, J. (1975), "Pilot-plant experiments on downflow filter denitrification", paper presented at: Nitrogen as a water polutant, conference, Copenhagen, Denmark, May 18-20, 1975.

Henze, M., and Harremoes, P., (1983), "Anaerobic treatment of wastewater in fixed film reactors- a literature review", Water Sci. and Tech., Vol. 15, No. 8/9, pp1-102.

Heyes, R.H., and Hall, R.J., (1981), "Anaerobic digestion modelling- the role of H_2", Biotechnol. Lett., vol. 3 No. 8, pp431-436.

Hickey, R.F. and Owens, R.W., (1981), "Methane generation from high-strength industrial wastes with the anaerobic biological fluidised bed" in: paper presented at The 3rd symposium on Biotechnology in energy production and conservation, May 12-15, Gatlinburg, Tennessee

Hill, D.T. and Barth, C.L., (1977), "A dynamic model for the simulation of animal waste digestion", Jour. Water Poll. Control Fed., Vol. 49, pp2129-2143.

Hobson, P.N., (1984), "Anaerobic digestion of agriculutral wastes", Water Pol. control., vol 83, no. 4, pp507-513.

Hobson, P.N., Boulsfield, S. and Summers, R., (1974), "Anaerobic digestion of organic matter", Crit. Rev. Environ. Control, vol. 4, p131-191.

Holm-Hansen, O, (1973) "Determination of total microbial biomass by measurement of adenosine triphosphate", Limnol. Oceanogr., Vol. 14, pp740-747.

Hungate, R.E., (1969), "A roll tube method for the cultivation of strict anaerobes", in: Norris, J.R., (eds.) Methods in Microbiology, New York, Academic press Inc., Vol. 3b, pp117-132.

Hungate, R.E., (1966) in: The rumen and its Microbes, New York Academic Press Inc., New York, p245.

Huss, L. (1982), "Applications of the anamet process for waste water treatment" in: Hughes, D.E. *et al.* (ed.), Anaerobic digestion 1981, Elsevier Biomedical press, Amsterdam, pp137-150.

Huysman, P., van Meenen, P., van Assche, P. and Verstraete, W., (1983), "Factors affecting the colonization of non-porous and porous packing materials in model upflow methane reactors", Biotechnol. Lett., Vol. 5, No. 9, pp643-648.

Iwugo, K.O. and Winnicki, R., (1976), "A study of mixing characteristics of sewage stabilization ponds with radioactive tracers", Publ. Hlth. Eng., Vol. 4, pp138-142.

James, A., (1982) "The modelling of anaerobic processes used in wastewater treatment", in: James, A., An introduction to water quality modelling, Wiley-Interscience, Chichester, U.K., chpt. 13

James, A.T. and Martin, A.J.P., (1952), "Gas-liquid partition chromatography: the separation and micro-estimation of volatile fatty acids from formic to dodecanoic acid", Biochem. J., Vol. 50, pp679-690.

Jayasuriya, G.C.N, and Hungate, R.E., (1959), "Lactate conversions in the bovine rumen", Arch. Biochem. Biophys., Vol. 82, pp274-287.

Jennett, J.C. and Dennis, N.D., (1975), "Anaerobic filter treatment of pharmaceutical waste", J. Water Poll. Control Fed., Vol. 47, No. 1, pp104- 1975

Jennings, P.A., Snoeyink, V.L. and Chian, E.S.K., (1976), "Theoretical model for a submerged biological filter", Biotech. and Bioeng., Vol. 18, pp1249-1273.

Jeris, J.S., and McCarty, P.L., (1965), "The biochemistry of methane fermenttion using ^{14}C tracers", J. Water Poll. Control Fed., vol. 37, no. 2, p178.

Jewell, W.J. and Morris, J.W., (1982), " Influence of varying temperature, flowrate and substrate concentration on the anaerobic attached film expanded bed process" In: Proc. of 36th ind. waste conf. Purdue University, Lafayette, Indiana., May 1981, Ann Arbor Sci.

Jopson, R.N., Webb, L.J. and Board, N.P., (1986), "Effect of paper making additives on anaerobic effluent treatment" paper presented at PIRA conf. Jan 1986.

Joubert, W.A., Britz, T.J. and Lategan, P.M., (1985), "The effect of effluent recirculation on the performance of a two stage anaerobic process", Biotechnol. Letts., Vol. 7, No. 11, pp853-858.

Kaspar, H.F. and Wuhrmann, (1978a), K.,"Kinetic parameters and relative turnovers of some important catabolic reactions in digesting sludge", Appl. Environ. Microb., vol. 36, No.1, pp1-7.

Kaspar, H.F. and Wuhrmann, K., (1978b) "Product Inhibition in anaerobic sludge", Microbial Ecol., Vol. 4, p241.

Keffer, C.E. and Urtes, H.G., (1963),"Digestion of volatile acids", J. Water Poll. Control Fed., Vol. 35, pp334-343.

Kite, O.A. and Stringer, P.R., (1986), "The use of oxygen for sulphide control" paper presented at PIRA conference Jan. 1986.

Kristjansson, J.K., Sconbeit, P. and Thauer, R.K., (1982), "Different K_s values for hydrogen of methanogenic and sulphate reducing bacteria; an explaination for the apparent inhibition of methanogenesis by sulphate" Arch. Micrbiol., Vol. 131, p278-282.

Lawrence, A.W. and McCarty, P.L., (1965), " The role of sulphide in preventing heavy metal toxicity in anaerobic treatment", J. Water Poll. Fed., vol. 37, no. 3, p392.

Lawrence, A.W. and McCarty, P.L., (1969), "Kinetics of methane Fermentation in anaerobic Treatment", J. Water Poll. Control Fed., vol. 14, no.2(ii), R1-17.

Legros, A., Asinari, C.M., Naveau, H.P. and Nyns, E.J., (1983), "Fermentation profiles in bioconversions", Biotechnol. Letts., Vol. 5, No. 1, pp7-12.

Lettinga, G., Velsen, A.F.M., Hobma, S.W., de Zeeuw and Klapwijk, A., (1980), "Use of the upflow sludge blanket reactor concept for biological treatment, especially for anaerobic treatment", Biotechnol. Bioeng., Vol. 22, pp699-744.

Lettinga, G., (1982), "Design operation and economy of

anaerobic treatment", paper presented at: IAWPR-specialised seminar on anaerobic treatment, June, Copenhagen, Denmark.

Lettinga, G., (1984), "The prospects of anaerobic waste water treatment" in: Ferrero, G.L., Ferranti, M.P. and Naveau, H. (eds.), Anaerobic digestion and carbohydrate hydrolysis of waste, England, Elsevier Applied science Publishers Ltd, pp262-273.

Lettinga, G., van Velsen, A.F.M., de Zeew, W., Hobma, S.W., (1979), "Feasibility of the upflow anaerobic sludge blanket (UASB) process", Proceedings from the National conference on environmental engineering, ASCE, San Fransisco, July 9-11, pp

Lettinga, G., (1986), "Future prospects for anaerobic treatment at papermills", paper presented at PIRA Conference, Cost effective treatment of papermill wastes using anaerobic technologies, January 14-15, 1986, Leatherhead , U.K.

Levenspiel, O., (1972), Chemical Reaction Engineering, 2nd edition, John Wiley, Chichester, U.K.

Li, A and Corrado, J.J., (1985), in: Proc. of the 40th industrial waste conference, Purdue University, Lafayette, Indiana.

Li, A., Kothari, D., Corrado, J.J., (1984), in: Proc. of the 39th industrial waste conference, Purdue University, Lafayette, Indiana, p627.

Lindgren, M., (1983), "Mathematical modelling of the anaerobic filter process", Wat. Sci. and Tech., Vol. 15, Nos. 8/9, pp197-208.

Mackie, R.I. and Bryant, M.P., (1982), "Metabolic activity of fatty acid propionate, butyrate and CO_2 to methanogenesis in cattle waste at 40 and 60°C", Appl. Environ. Microb., Vol. 41, pp1363-1373.

Mah, R.A., (1978), "Studies on an acetate fermenting strain of *Methanosarcina*." Appl. Environ. Microbiol., vol.35, p1174.

McCabe B.J., and Eckenfelder, W.W. Jr., (1958), in: Biological Treatment of sewage and industrial wastes, Vol. 2, Reinhold, New York.

McCarty, P.L. and McKinney, R.E., (1961), "Volatile acid toxicity in anaerobic digestion", J. Wat. Poll. Control Fed., Vol. 33, No. 3, pp223.

McCarty, P.L., (1982), "One hundred years of anaerobic treatment", in: Hughes *et al.*, (eds.), Anaerobic Digestion 1981, Elsevier Biomedical Press, Amsterdam, pp3-22.

McInerney, M.J., Bryant, M.P. and Stafford, D.A., (1971), " " in: Anaerobic Digestion. Proc. of the 1st int. symp. on anaerobic digestion, Applied Science Publishers, London

McKinley, V.L. and Vestal, J.R., (1984), "Biokinetic analyses of adaptation and succession: microbial activity in composting municiple sewage sludge", Appl. Environ. Microb., Vol. 47, No. 5, pp933-941.

Melchoir, J., Binot, R., Perez, I.A., Narveau, H. and Nyms, E.J., (1982), "Biomethanation; its future development and influence of physiology of methanogenesis" , J. Chem. tech. Biotechnol., Vol. 32, pp189-197.

Messing, R.A., (1982), "Imobilized microbes and a high rate continuous anaerobic waste processer", paper presented at 6th Symposium, Energy from biomass and wastes, Lake Buena Vista, Florida, Jan. 25-29, pp

Meynell, P.J., (1982), Methane: planning a digester, 2nd edition, Prism press Ltd., Dorchester, U.K., 1982

Montgomery, H.A.C., Dymock, J.F. and Thom, N.S., (1962), "The rapid colorimetric determination of oranic acids and their salts in sewage-sludge liquor", Analyst, Lond., Vol. 87, pp949-955,

Monteith, H.D. and Stephenson, J.P., (1981), "Mixing efficiencies in full scale anaerobic digesters by tracer methods", J. Water Poll. Control Fed., Vol. 53, No.1, pp78-84.

Mosey, F.E. and Hughes, D.A., (1975), " The toxicity of heavy metal ions to anaerobic digestion" Water Poll. Con.,. Vol. 74, No.1, pp18-35.

Mosey, F.E., Foulkes, M. and Jago, D.A., (1978), "Chromatographic analysis of digester gases", Water Research Centre technical report TR85, July

Mosey, F.E. and Foulkes, M., (1983), "Control of the nanerobic digestion process", paper presented at: Stabilization and Disinfection of sewage sludge conference, UMIST/WRC, April 1983.

Mosey, F.E, (1985), "Redox potentials in wastewater treatment", Chemical Engineer, may 1985, pp21-24.

Mueller, J.A. and Mancinni, J.L. (1975), "Anaerobic filter kinetics and application", paper presented at: Proc. 30th industrial waste conference, Purdue University, Lafayette, Indiana.

Murray, W.D. and van den Berg, L. (1981), "Effect of support material on the development of microbial fixed films converting acetic acid to methane" J. App. Bacteriol., vol.51, pp257-265.

Noike, T., Endo, E., Chang., J., Yaguchi, J. and Matsumoto, J., (1985), "Characteristics of carbohydrate degradation and the rate limiting step in anaerobic digestion", Biotech. Bioeng., Vol. 27, pp1482-1481.

Noone, G.P., (1982), "Process design of anaerobic sludge digestion",Effluent and water treatment journal, May 1982.

Oakley, D.L., Forster, C.F. and Wase, D.A.J., (1985), "Bacterial attachment in relation to the operation of an anaerobic expanded bed reactor", paper presented at: Advances in fermentation II, conference, Kings College, London, 23-25 september, 1985, pp20-33.

Owen, W.F., Stuckey, D.C. and Healy, J.B., (1979), "Bioassay for monitoring biochemical methane potential and anaerobicf toxicity", Water Res., Vol. 13, pp485-492.

Parkin, G.F. and Owen, W.F. (1986), "Fundamentals of anaerobic digestion of wastewater sludges", J. of Environ. Eng. ASCE, Vol. 111, No. 5, pp867-920.

Parkin, G.F. and Speece, R.E., (1983), "Attached verses suspended growth anaerobic reactors: response to toxic substances", Wat. Sci. and Tech., Vol. 15, No. 8/9, pp261-290.

Pipyn, P. and Verstraete, W., (1981), "Lactate and ethanol as intermidiates in two phase anaerobic digestion", Biotechnol. Bioeng., Vol. 23, pp1145-1154.

Plummer, A.H., Malina, J.F. and Eckenfelder, W.W., (1968), "Stabilization of a low solids carbohydrate waste by an anaerobic submerged filter", Proc. 23rd ind. waste conf. Purdue University, Vol. 132, p462...

Pol, H.L.W., de Zeew, W.J., Velzeboer, C.T.M., Lettinga, G., (1983), "Granulation in UASB reactors", Wat. Sci. and Tech., Vol. 15, pp291-304.

Richards, S.R. and Turner, R.J., (1984), "A comparative study of techniques for the examination of biofilms by scanning electron microscope", Wat. Res., Vol. 18, No. 6, pp767-773.

Richards, S.R. and Wilson, A.J., (1983), "Rapid SEM techniques to investigate colonisation of BSP's in the Captor process", Environ. Tech. Lett., Vol. 4, pp183-188.

Rinzema, A., (1986), "Application of the UASB process for anaerobic treatment of food processing wastes", paper presented at the ARFC subject day, 24 th June, 1986

Ripley, L.E., Boyle, W.C. and Converse, J.C., (1986), "Improved alkalimetric monitoring for anaerobic digestion of high-strength wastes", J. Water Poll. Control Fed., Vol. 58, No. 5, pp406-411.

Rittmann, B.E. and McCarty, P.L., (1978), "Variable order model of bacterial film kinetics", ASCE J. Envir. Poll. Div., EE5.

Rittmann, B.E. and McCarty, P.L., (1980), "Model of steady state biofilm kinetics", Biotech. Bioeng., Vol. 22, pp2243-2357.

Robinson, R.W., Akin, D.E., Nordstedt, R.A., Thomas, M.V. and Henry, C.A., (1984), "Light and electron microscopic examinations of methane producing biofilms from anaerobic fixed bed reactors", Appl. Environ. Microb., Vol. 48, No. 1, pp127-136. 1, pp127-136.

Sahm, H., (1984), "Anaerobic wastewater treatment " in: Fiechter, A., Advances in Biochemical engineering Biotechnology, Sperring Verlag, Germany.

Salkinoja-Salonen, M.S., Hakulinen, R., Valo.R, and Apajalahti, J., (1983a), "Biodegradation of recalcitrant organochlorine compounds in fixed film reactors", Water Sci. Tech., vol. 15, nos. 8/9 pp309-320.

Salkinoja-Salonen, M.S., Nyns, E.J., Sutton, P.M., van dan Berg, L. and Wheatley, A.D, (1983b), "Starting up of an anaerobic fixed film reactor", Water Sci. and Tech, vol.15, nos. 8/9, pp305-308.

Samson, R., van den Berg, L. and Kennedy, K.J., (1985), "Mixing characteristics and startup of anaerobic downflow stationery fixed film reactors (DSFF)", Biotech. and Bioeng., Vol. 27, pp10-19.

SAS users guide, 1981, SAS Institute Inc., Box 8000, Cary North Carolina.

Sawyer, C.N. and McCarty, P.L., (1967), Chemistry for sanitary engineers, McGraw-Hill Book Co., pp

Scheifinger, C.C. and Wolin, M.J., (1973), "Propionate formation from cellulose and soluble sugars by combined cultures of *Bacteriodes succinogenes* and *Selenomonas ruminintium*", Appl. Microb., Vol.26, p789.

Schlegel, S. and Kalbskopf, K.H., (1982) "Treatment of liquors from heat-treated sludge using anaerobic contact process", In: Hughes, D.E. *et al.*, Anaerobic Digestion 1981, Amsterdam Elsevier Biomedical Press, Amsterdam, pp169-184.

Shaw, W.H.R., (1954), "Toxicity of cations towards living systems" Science, vol. 120, p361.

Shimp, R.J. and Pfaender,P., (1982), "Effects of sample area and flowrate on marine bacterial growth in activated carbon columns", Appl. Environ. Microb., Vol. 44, No. 2, pp471-477.

Shore, M., Broughton, N.W. and Bumstead, N., (1984), "Anaerobic treatment of wastewater in the beet sugar industry", Water Poll. Cont., Vol. 83, pp499-506.

Shieh, W.L. and Mulcahy, L.T., (1983), "Fluidized bed biofilm reactor (FFBR) kinetics -a rational design and optimization approach", Wat. Sci. and Tech., Vol. 15, No. 8/9, pp321-332.

Smart, P.L. and Laidlaw, I.M.S., (1977), "An evaluation of some fluorescent dyes for water tracing", Water Resourc. Res., Vol. 13, pp15-33.

Smith, P.H., (1970), "Methanogenesis in sludge digestion", Abstract of papers American Chem. Soc., watr 49, Febuary

Speece, R.E., and McCarty, P.L., (1964), "Nutrient requirements and biological solids accumulation in anaerobic digestion", in: Advances in water Pollution research, Vol. 2, pp305-322, proc. of int. conf. Sept , Permagon Press, Oxford, U.K.

Standard methods for the examination of water and wastewater, (1980), APHA, Fifteenth edition.

Stafford, D.A., Spensley, R.A. (1986), "The control and optimisation of anaerobic and aerobic treatment systems", paper presented at; I Chem. E. symp., 15-17 April, Bradford University, Bradford, U.K.

Stander, G.J., (1950) "Effluents from fermentation industries: I a review of methods of disposal and utilisation ", J. Inst. sewage purification, vol.4, p438.

Stephenson, T. and Lester, J.N., (1986), "Evaluation of startup operation of four anaerobic processes treating a synthetic meat waste", Biotech. and Bioeng., Vol. 28, pp373-380.

Stevens, D.K., Berthouex, P.M. and Chapman, J.W., (1986), "The effect of tracer diffusion in biofilm on residence time distributions", Wat. Res., Vol. 20, No. 3, pp369-375.

Suidan, M.T. and Wang, Y.T., (1985), "Unified analysis of biofilm kinetics", ASCE, J. Env. Eng., Vol. 111, No. 5, pp

Switzenbaum, M.S. and Danskin, S.C., (1982), "Anaerobic expanded bed treatment of whey", paper presented at 36th Ind. waste Conf., 1981, Purdue University, Lafayette, Indiana.

Tait, S.J. and Friedmann, A.A, (1980), "Anaerobic rotating biological contactor for carbonaceous wastewaters", J. Poll. Control Fed., Vol. 52, pp2257-2269.

Tesch, W., Schneider, K. and Bachofen, R., (1983), "Performance Of an anaerobic filter for purification and methane production from waste water of a sugar refinery", Proc. Biochem., Vol. 18, pp34-37.

Thauer, R.K. and Jungermann, K., (1977), "Energy conservation in chemotrophic anaerobic bacteria", Bacteriol. Rev., Vol.41, No. 1, p100-180.

Thauer, R.K., (1981), "Biochemistry and energetics", paper presented at: Second international symposium on anaerobic digestion, 6-11 September, Travemunde, Germany.

Thiel, P.G., (1969), "The effect of methane analogues on methanogenesis in anaerobic digestion", Wat. Res., vol.3, p215.

Toerien, D.F., (1967), "Population description of the non-methanogenic phase of anaerobic digestion. I. isolation, characterization and identification of numerically important bacteria.", Wat. Res., Vol. 4, pp129-148.

Valke, D. and Verstraete, W., (1983), "A practical method to estimate the acetoclastic biomass in anaerobic sludges", J. Water Poll. Control Fed., Vol. 55, No. 9, p1191-1195.

van Beelen, P., Geerts, W.J., Pol, A. and Vogels, G.D., (1983), "Quantification of coenzymes and related compounds from methanogenic bacteria by HPLC", Anal. Biochem., Vol. 131, pp285-290.

van den Berg, L., Lentz, C.P., Athey, R.J. and Brook, E.A., (1974), "Assesment of methanogenic activity in anaerobic digestion: apparatus and method", Biotech. and Bioegn., Vol. 16, pp1459-1469.

van den Berg, L., (1977), "Effect of temperture on growth and activity of a methanogenic culture utilizing acetate", Canadian Journal of Microb., vol. 23, pp898-902.

van den Berg, L. and Lentz, C.P., (1977), "Food processing waste treatment by anaerobic digestion", in: Proceedings of the 32nd Industrial waste conference, May, 1977, Purdue University, Lafayette, Indiana, Ann Arbor Sci., Ann ARbor, Mich., pp252-258.

van den Berg, L. and Lentz, C.P., (1980), "Comparison between up and downflow anaerobic fixed film reactors of varying surface to volume ratios for the treatment of bean blanching waste", In: Proc. of 34th ind. waste conf. may 8,9,10 1979, Purdue University, Layfayette, Indiana, Ann Arbor Sci. Ann Arbor Mich., p319-325.

van den Berg, L., Kennedy, K.J. and Sampson, R,. (1985), "Anaerobic downflow stationery fixed film reactors: performance under steady state and non-steady state loading" Wat. Sci. Tech., Vol. 17, No.1, pp89-102.

van der Meer, R.R. and Heertjes, P.M. (1983), "Mathematical description of anaerobic treatment of wastewater in upflow reactors", Biotech. and Bioeng., Vol. 25, pp2531-2556.

Vincent, W.F. and Goldman, C.R., (1980), "Evidence for algal heterotrophy in Lake Tahoe, California-Nevada", Limon. Oceanogr., Vol. 25, No. 1, pp89-99.

Vogels, G.D., Keltjens, J.T., Hutten, T.J. and van der Drift, C., (1982), "Co-enzymes of methanogenic bacteria" Zbl. Bact. Hyg. I. Abt. Orig., Vol. C3, pp258-264.

Walker, I., and Austin, E.P., (1981), "The use of plastic, porous biomass supports in a pseudo fluidized bed for effluent treatment" in: Cooper, P.F. and Atkinson, B. (eds.), Biological fluidized bed treatment of water and wastewater, Chichester, published for the WRC by Ellis Harwood Ltd, pp272-283.

Walker, I. Cooper, P.F., Crabtree, H.E. and Aldred, R.P., (1984), "Evaluation of the "Captor" process for uprating an overloaded sewage works", paper presented at: symposium "Process engineering aspects of imobilized cell systems", UMIST, Manchester, March 28-29.

Webb, L.J., (1986), "Characteristics of mill effluents suitable for anaerobic treatment", paper presented at Cost effective treatment of papermill effluents using anaerobic technologies, PIRA, January 1986.

Wheatley, A.D. and Cassell, L., (1985), "Effluent treatment by anaerobic biofiltration", Wat. Poll. Cont., Vol.84, No. 1, pp14-19. pp10-11.

Whitmore, T.N., Lazzari, M. and LLoyd, D, (1985), "Comparative studies of methanogenesis in thermophilic and mesophilic anaerobic digesters using membrane inlet mass spectrometry", Biotechnol. Lett., Vol. 7, No. 4, pp283-288.

Whitmore, T.N., and LLoyd, D.,(1986) "mass spectrometric control of the thermophilic anaerobi digestion process based on the levels of dissolved", Biotechnol. Lett., Vol. 8, No.3, pp203-208.

Wiegant, W. M. and de Man, G., (1986), "Granulation of biomass in thermophilic upflow anaerobic sludge blanket reactors treating acidified wastewaters", Biotech and Bioeng., Vol.28, pp718-727.

Williamson, K. and McCarty, P.L., (1976), "Steady state biofilm modelling", J. Wat. Poll. Control, Fed., Vol. 48, No. 2, pp281.

Wimpenny, J.W.T. and Parr, J.A., (1979), "Biochemical differentiation in large colonies of *Entrobacter cloacae*", J. of Gen. Microb., Vol. 114, pp487-489.

Wright, R.T. and Hobbie, J.E., (1966), "Use of glucose and acetate by bacteria and algae in aquatic ecosystems", Ecology, Vol. 47, No. 3, pp447-464.

Young, J.C. and Dahab, M.F., (1983) "Effect of media design on the performance of fixed bed anaerobic reactors", Water Sci. and Tech., vol. 15, no. 8/9, pp369-384.

Young, J.C. and McCarty, P.L., (1969), "The anaerobic filter for waste treatment", J Water Poll. Control Fed., vol.41, No.5, part 2, R160.

Zeikus, J.G., (1981), "Microbial intermediary metabolism in anaerobic digestion" in Stafford, D.A., Wheatley, B.I. and Hughes, D.E., (eds.), Anaerobic Digestion 1981, London, Applied Science publishers Ltd., pp61-89.

Zehnder, A.J.B. and Whurmann, K., (1977), "Physiology of *Methanobacterium* strain AZ", Arch. Microbiol., Vol. 111, pp199-205.

Zehnder, A.J.B., (1978), "Ecology of methane formation", in: Mitchell, R. (ed.), Water Pollution Microbiology, Vol. 2, John Wiley and sons., New York, Chpt. 13, pp349-376.

Zehnder, A.J.B., Ingvorsen, K. and Marti, T., (1981), "Microbiology of methane bacteria", paper presented at the second international symposium on anaerobic digestion, Travemunde, Germany, 6-11 september 1981

Zoetemeyer, R.J., van den Heuvel, J.C. and Cohen, A., (1982), "pH influence on acodogenic dissimilation of glucose in an anaerobic digester", Wat. Res., Vol. 16, pp303-311.