CONTROL OF BIOMASS IN ANAEROBIC REACTORS USING ULTRAFILTRATION MEMBRANES

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Thesis submitted to the
University of Newcastle upon Tyne
for the degree of
Doctor of Philosophy
December, 1993

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To my parents

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to Dr. G.K. Anderson for supervising this research and his critical review during the preparation of this thesis. I am also indepted to Professor M.B. Pescod for kindly allowing me to use the facilities of this Department, and other academic staffs for their assistance and advice. The patient guidance of Dr. A. James and Mrs L.M. Evison during the initial stages of this research is deeply appriciated.

Special thanks are offered to the Technical staff of this Department, in particular, Mr. John. Hamilton, Mrs. Pat Johnston, Mr. Les Heeple, Mrs. Joan Baugh, Mr. John Mooree, Mr. Keith Scholefield and Mr. John Allan , whose friendship and support are most appreciated. Together with Miss Amanda Allen, Mr. Ufuk Alkan, Mr. Darren Draper and Mr. Forbes McDoughall ,they all made the Environmental Engineering Laboratory a better place to work in.

The words are not enough to express my deep gratitude to Bahar Kasapgil but at least I could say thanks God I found her and she made everything very easy for me during the time I spent here. I hope I do too for her.

I would like to dedicate this work to my parents, Mr. Hamdi Ince and Mrs. Munevver Ince in order to express my utmost gratification for all that they have sacrificed and the continuous supports they offer. I would also like to thank to my sisters' Mrs.Sukufe Can, Mrs.Sule Akmirza and Miss.Nese Ince and my brother and his wife' Ahmet Ince and Rabia Ince for their encouragement and love. Without their moral support this study would not have been finished.

ABSTRACT

Anaerobic processing has become recognized as a simple and energy-efficient means of treating and stabilizing many high strength organic industrial wastes and is also not subjected to the operating limitations of aerobic processes. The literature review presented in this thesis outlines the advances made in the understanding of the microbiology and biochemistry of the process and the considerable advances in reactor configurations in achieving high SRT/HRT ratios.

The efficacy of the anaerobic process is dependent on maintaning a high biomass concentration in the reactor, which in turn is dependent on the performance of the solids separator. The anaerobic contact process employs gravity settling for solid-liquid separation but the poor settleability of the anaerobic sludge may result in a poor performance of the contact process. By employing a much more efficient separation process such as ultrafiltration (UF), the performance of the anaerobic system can be significantly improved. In this study, therefore, a new method of operating a completely mixed anaerobic digester using a crossflow UF membrane technique was investigated in order to control the concentration of active biomass in the reactor and to determine the extent of any other advantages that can be gained over other reactor configurations .

The study was carried out in four stages. In the first stage the performance of an anaerobic contact digester using a crossflow UF membrane technique was investigated for brewery wastewater treatment. The results obtained from this stage showed that under

steady-state conditions, at an influent substrate concentration of approximately 120 g COD/l (100 g BOD/l) with a hydraulic retention time of 4.2 days giving an organic loading rate of 28.5 kg COD/m^3.d , overall COD and BOD removal efficiencies of 99% and almost 100% respectively were achieved and the system had not reached its maximum loading capacity. Throughout the operation, HRT was maintained in the range of 2.5-4.2 days, largely determined by the flux rate. Microbiological analyses including Microscopic Count, Plate Count, Most Probable Number and Specific Methanogenic Activity test confirmed that there was almost no biomass loss through the membrane which, in turn, resulted in the maintenance of a high stability of the system under load changes. The UF membrane showed a remarkable consistency throughout the study, retaining a high concentration of active biomass in the digester and demonstrating that fouling by anaerobic biomass will reach a limiting level.

In the second stage of the study the effect of Mixed Liquor Suspended Solids (MLSS) on the kinetics of the membrane reactor was investigated. The results showed that the kinetic coefficients estimated from the four steady-state runs had slight variations from each other but which could be mainly due to the changes in the numbers and the dominant species throughout the operation of the system. The increase in the MLSS concentrations did not significantly affect the kinetics of the system,

In the third stage of the study the Specific Methanogenic Activity (SMA) technique was used to determine the methane production capacity of the membrane reactor , thus allowing suitable OLRs to be applied and to assess the effects of MLSS concentration on the activity of acetoclastic methanogenic bacteria in the digester. The results showed that any deterioration in acetoclastic methanogenic capacity of the system can be improved by increasing the sludge wastage rate. Ratios of the actual methane production rate to the potential methane production rate of less than 0.7 were found to be satisfactory in order to run the system efficiently in terms of COD removal and methane yield.

In the final stage of the study the possible effects that membrane systems may have on the microbial population in the reactor was investigated. Therefore,microscopic examinations have frequently been carried out in order to determine the effects of the new configuration on any variation in the morphology or on the properties of methanogens as well as any change in the number of non-methanogens throughout the operation of the membrane reactor. This investigation showed that the membrane system configuration did have an apparent effect on the dominant methanogenic species throughout operation of the membrane reactor. For example *Methanococcus* species were the most dominant group at the beginning of the start-up period, becoming the third most dominant group at the end of the study. As a result, studying the changes in the number of viable methanogens and the dominant species may help to determine a reason for the deterioration in performance of a digester.

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ABBREVIATIONS AND SYMBOLS

AF	Anaerobic filter
ATP	Adenosine triphosphate
BOD	Biochemical oxygen demand
COD	Chemical oxygen demand
CUMAR	Crossflow ultrafiltration membrane anaerobic reactor
EMP	Embden-Meyerhof pathway
F/M	Food-to-microorganism ratio
HRT	Hydraulic retention tiime
MLSS	Mixed liquor suspended solids
MLVSS	Mixed liquor volatile suspended solids
MWCO	Molecular weight cut-off
NAD	Nicotinamide adenine dinucleotide or pyridine nucleotide
NRB	Nitrate reducing bacteria
OHPA	Obligate hydrogen producing acetogenic bacteria (also OPRA)
OLR	Organic loading rate
OPRA	Obligate proton-reducing acetogenic bacteria (also OHPA)
SRB	Sulphate reducing bacteria
SRT	Sludge retention time
SS	Suspended solids
TSAD	Two-stage anaerobic filter
VFA	Volatile fatty acids
VSS	Volatile suspended solids

A	Total membrane area required
C_b	Bulk concentration
C_g	Gel formation concentration
C_w	Solution concentration at membrane surface
I_o	Cake thickness having a resistance equal to the resistance of the filter medium
J	Average flux of the membrane, (flow rate per unit membrane area)
k	Back diffusion mass transfer coefficient
k_d	Decay constant, ($time^{-1}$)
K_s	Half velocity constant , substrate concentration at one half the maximum growth rate, (mass/unit volume)
k_{3M}	Multiple substrate degradation coefficient for soluble microbial products
N	Number of organisms per unit volume
Q	Flow rate, (volume/time)
r_d	Decay rate, (i.e., rate of biomass loss)
r_g	Rate of bacterial growth, (mass/unit valume.time)
r_s	Rate of substrate utilization, (mass/unit volume.time)
R_m	Hydraulic resistance of the membrane
S_o	Influent substrate concentration
S	Concentration of growth limiting substrate in solution, (mass/unit volume)
S_{po}	Total soluble microbial product concentration (mass/unit)
S_p	Soluble microbial product concentration (mass/unit)
V_r	Reactor volume
W	Wastage rate, (mass/time)
X_0	Concentration of microorganisms in the influent, (mass VSS/unit volume)

X	Concentration of microorganisms in the reactor, (mass VSS/unit volume)
X_A	Active biomass concentration (mass/unit)
X_e	Concentration of microorganisms in the effluent, (mass VSS/unit volume)
Y	Maximum yield coefficient, (mass/mass), (defined as the ratio of the mass of cells formed to the mass of substrate consumed, measured during any finite period of logarithmic growth)
Y_g	Observed yield coefficient (mass/mass)
a	Growth associated product formation coefficient
μ_{max}	Maximum specific growth rate, ($time^{-1}$)
α	Growth associated product formation coefficient
ν	Net specific formation rate (defined as difference between the specific product formation rate and the product consumption rate)
θ	Hydraulic retention time (mass/time)
ΔP	Pressure drop across the cake

CHAPTER 1

INTRODUCTION

Escalating energy costs and a growing awareness that there is a limit to the availability of economically recoverable fossil fuels has lead to an increasing interest in the use of microbial systems for energy production. Anaerobic biological treatment systems afford many industries the opportunity to dispose of their wastewaters while achieving resource recovery in the form of methane, the primary component of natural gas. The production of biogas is generally in excess of the energy required to operate an anaerobic treatment system, and it can be utilized to generate heat and/or power for other on-site services. There are also many other advantages of anaerobic processes which are possibly less obvious than being self-sufficient in energy. In many ways these may be more attractive to the industrialist who is considering its use (Anderson and Donnelly, 1977) and these include:

(i) low sludge production,

(ii) ability of biomass to lie dormant for several months and then be fully operational within 2-3 days (of great value when seasonal wastewaters are to be treated),

(iii) capability of operation on a stop/start basis,

(iv) limited environmental nuisance, since the process is totally enclosed and all the exhaust gas is either burned in a gas utility or an automatically controlled flarestack,

(v) low nutrient requirement,

(vi) high organic throughput,

(vii) automatic biological overload control facility , and

(viii) measurable and identifiable intermediate products which can offer additional control potential.

The most fundamental requirement in the design and operation of an anaerobic system for the treatment of industrial wastewaters is that the essential anaerobic bacteria should not be washed out of the system at a greater rate than they can reproduce.

The simplest method of achieving the required solids retention time (SRT) is to use a completely mixed, straight through reactor without recycle, in which the SRT is equal to the hydraulic retention time (HRT), however, this would lead to reactors of very large volume, and hence would be unsuitable for most industrial wastewater. It can be used for the digestion of concentrated wastes, particularly those in which the organic matter is predominantly in the form of settleable solids which can be more economically concentrated before anaerobic treatment rather than afterwards, for eample sewage sludges. It has limited value for the treatment of liquid effluents because the " washout " of the microorganisms will pose a serious problem, particularly if high loading rates were to be obtained. Logically, for the technology to be acceptable in the industrial field the SRT needs to be controlled independent of the HRT.

It is the provision of a long, mean SRT which has led to the development of a wide range of anaerobic treatment processes, differing essentially only in their method of retaining the microorganisms. Each of the different design configurations has implications for the ratio of solid retention time/hydraulic retention time (SRT/HRT). A high SRT is desirable for process stability and minimal sludge production. A short HRT minimises the reactor volume and hence reduces capital costs and land requirements. Consequently a design configuration which is capable of maintaining a long SRT at a relatively short HRT will allow the system to be operated at high volumetric loading rates.

In order to overcome the above problems the anaerobic contact digester was developed which differs from a conventional anaerobic digester system in that a settlement tank is used to concentrate and recycle the biomass to the digester. Anaerobic treatment processes with solids recycle allow the system to have a longer SRT to obtain a high treatment efficiency, low effluent substrate concentration with a short HRT, and consequently a smaller digester. Incorporation of solids recyling increases the SRT/HRT ratio, thereby allowing higher hydraulic loadings than are otherwise possible, while still retaining a long SRT.

The major problem in the practical application of the anaerobic contact process has always been the separation of the biomass from the effluent for recycle to the digester. Formation of gas tends to continue in the settlement tank and the gas bubbles so formed buoy up the solids, thus preventing efficient sedimentation. Improvements in the performance of the separator have been achieved through the use of chemical coagulants and through process and component design modifications such as the incorporation of a spiral baffle as part of the settler design; vacuum degasification; flocculation followed by the use of a lamella clarifier; and the application of a cold, thermal shock to the biomass prior to sedimentation in order to arrest gasification in the settling tank and encourage flocculation of the solids. The anaerobic contact process has been used for the treatment of a wide variety of wastewaters although settlement problems have restricted its application in the treatment of high strength industrial wastewaters for economic reasons. In other words the loading rates attainable in an anaerobic waste treatment process are primarily dictated by the biomass retention in the reactor and loss of biomass will affect the digester performance in terms of gas yield and will also result in a poor quality effluent due to the high solids content. By employing a much more efficient separation process such as ultrafiltration, the performance of the anaerobic system can be drastically improved.

Ultrafiltration is a pressure-driven membrane separation process. Unlike reverse osmosis it employs a more open membrane and uses a lower pressure. The process usually employs a pump to supply the pressure and the flow across and through the membrane. The ultrafiltration membranes can reject solutes with a size ranging from 1 nm to 1000 nm (1 micron) therefore by incorporating ultrafiltration as the separation step for the suspended growth anaerobic reactor, the biological solids in the reactor can be completely retained and a high reactor biomass concentration achieved at almost any desired level. In addition the following advantages are also expected for this coupled system:

(i) a shorter start-up time is possible since seed material will be retained by the membrane once it is added to the reactor,

(ii) influent particulate organics can be degraded since they will also be held in the system by the ultrafiltration process, and

(iii) a high quality effluent can be achieved since little or no particulate material will be present in the effluent.

This study was undertaken to comprehensively examine certain important properties of the crossflow ultrafiltration membrane anaerobic reactor (CUMAR) system with respect to the feasibility of retaining an adequate amount of active biomass. However, very little work with the CUMAR system has been found in the literature except with respect to its performance. Therefore, an approach which examines the feasibility of retaining highly active biomass in the CUMAR system from different perspectives was adopted such as changes in the dominant species throughout the operation of the system , optimizing the acetoclastic methanogenic activity of the digester sludge, determining the kinetics of the membrane system at high biomass levels and its overall performance.

CHAPTER 2

LITERATURE REVIEW

2.1. Fundamentals of Anaerobic Digestion

2.1.1. Microbiology and Biochemistry

The microbiology and biochemistry of anaerobic digestion have been investigated by a number of researchers (McCarty, 1964b; Toerien *et al.*.,1967; Bryant *et al.*., 1967; Mah et al.,1977; Zeikus, 1977; Patel and Roth,1978; Kaspar and Wuhrmann, 1978a,1978b; McInerney *et al.*., 1981,1979; Gujer and Zehnder,1983; Boone, 1985). The anaerobic degradation of complex particulate organic material has been described as a multi-step process of both series and parallel reactions. First, complex polymeric material such as polysaccharides, proteins and lipids (fat and grease) are hydrolyzed by extracellular enzymes to soluble products of small enough size to allow their transport across the cell membrane. These relatively simple, soluble compounds are fermented or anaerobically oxidized to short-chain fatty acids, alcohols, carbon dioxide, hydrogen and ammonia. The short-chain fatty acids (other than acetate) are converted to acetate, hydrogen gas and carbon dioxide. Lastly, methanogenesis occurs from carbon dioxide reduction by hydrogen and from acetate.

The major groupings of bacteria and the reactions they mediate are as follows (Zinder 1984):

(i) fermentative bacteria,

(ii) hydrogen-producing acetogenic bacteria,

(iii) hyrogen-consuming acetogenic bacteria,

(iv) carbon dioxide-reducing methanogens, and

(v) acetoclastic methanogens.

Harper and Pohland (1986) established a nine step model in which each of the steps is linked by bacterial product and their individual substrate specifications shown in Figure 2.1. In this chapter the discussion will be based mainly upon the steps proposed by these authors.

2.1.1.1. Hydrolysis

Complex wastes containing macromolecular biopolymers such as lipids, proteins and carbohydrates are required to be broken down or hydrolyzed as a first step before they can be taken up by the microorganisms. Hydrolysis of these macromolecules is carried out by specific extracellular enzymes, the reaction rates of which are influenced by pH, cell residence time and the waste constituents in the digester produced by hydrolytic bacteria namely, the cellulytic, proteoytic, lipolytic and aminolytic bacteria (Hungate 1982; Payton and Haddock, 1986). During catabolic metabolism, energy is released in the form of adenosine triphosphate (ATP) which is used for internal repairs, movement, maintenance of pH and salinity gradients (Mosey,1981), and for the synthesis of cellular biopolymers. The conversion process is illustrated in Figure 2.2. Eastman and Ferguson (1981) demonstrated that the hydrolytic bacteria may also ferment the intermediate products to simple volatile fatty acids, carbon dioxide, hydrogen, ethanol as final products.

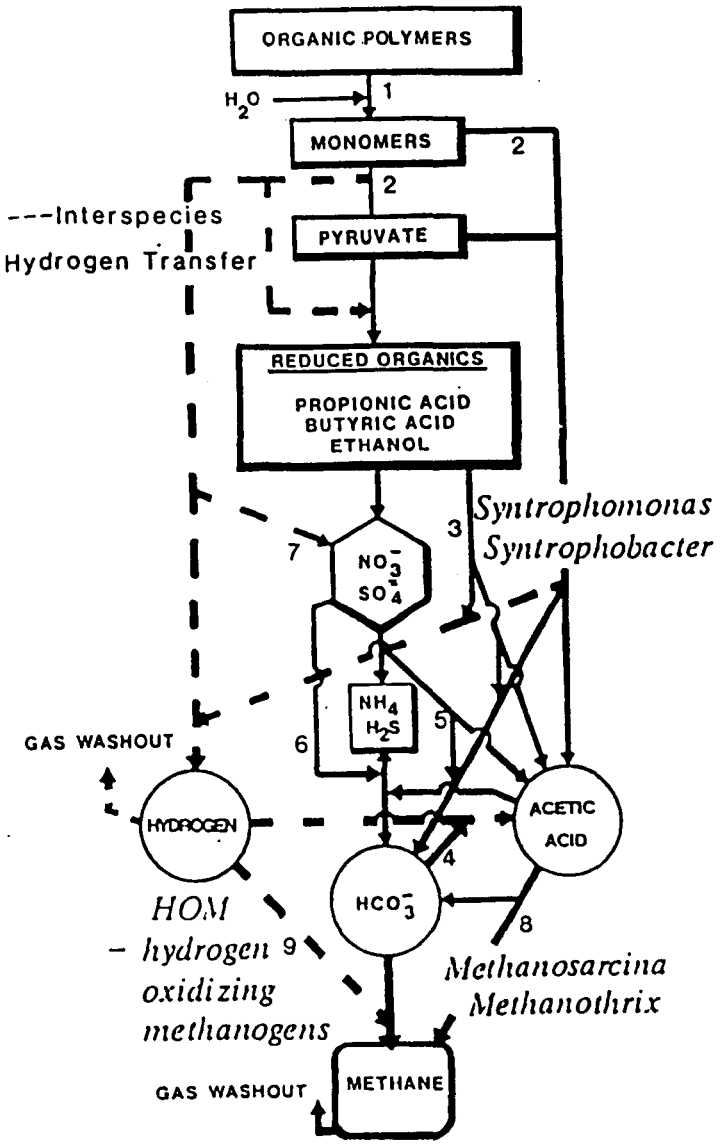
Stronach *et al.*. (1986) pointed out that in an anaerobic digestion process, the hydrolysing

bacteria and their enzymes are of paramount importance because their activity produces the simpler substrates for the succeeding step in the degradation sequence.

2.1.1.2. Acidogenesis and Acetogenesis

The breakdown products such as amino acids, sugars and long chain fatty acids of the hydrolysis phase of biopolymers are now ready to be fermented by acid forming bacteria to the intermediary products carbon dioxide, acetate and hydrogen (Figure 2.2.). Sorensen et al. (1981) pointed out that acetate is the most important compound produced in the fermentation of organic substrates by the bacterial population, with propionate production of secondary consequence.

Acid producing bacteria can be categorized into two groups. The first group are known as acidogens or fermentative bacteria and metabolize amino acids and sugars to the intermediary products, acetate or hydrogen (Figure 2.2). Acidic end-products and ammonia are formed from the amino or amide groups of amino acids. Temperature, pH and the composition and nutrient quality of the influent feed are crucial to end-product formation.The catabolizm of these organic compounds is mediated by a large number of both obligatory and facultatively anaerobic microorganisms and the process utilizes single amino acids, pairs of amino acids or a single amino acid in conjunction with a non-nitrogenous compound. The conversion of single amino acids is carried out under anaerobic conditions by clostridia, mycoplasmas and streptococci. The amino acid arginine is metabolized to ammonia, carbon dioxide and ATP, or nithine to acetate, propionate, valerate and butyrate, and lysine mainly to acetate and butyrate. Butanol, butyric acid, acetone and iso-propanol are generally produced by the bacteria of the genera *Clostridum* and *Butyribacterium* , for example *Cl.butyricum* produces butyrate, Cl. acetobutylicum mainly acetone and butanol and *Cl. butylicum* produces butanol in



Legend:

1) hydrolysis of organic polymers
2) fermentation of organic monomers
3) oxidation of propionic and butyric acids and alcohols by OPRA
4) acetogenic respiration of bicarbonate
5) oxidation of propionic and butyric acids and alcohols by SRB and NRB
6) oxidation of acetic acid by SRB and NRB
7) oxidation of hydrogen by SRB and NRB
8) acetoclastic methane formation
9) methanogenic respiration of bicarbonate

Figure 2.1 Substrate conversion patterns associated with the anaerobic treatment of wastewaters (Source: Harper and Pohland, 1986)

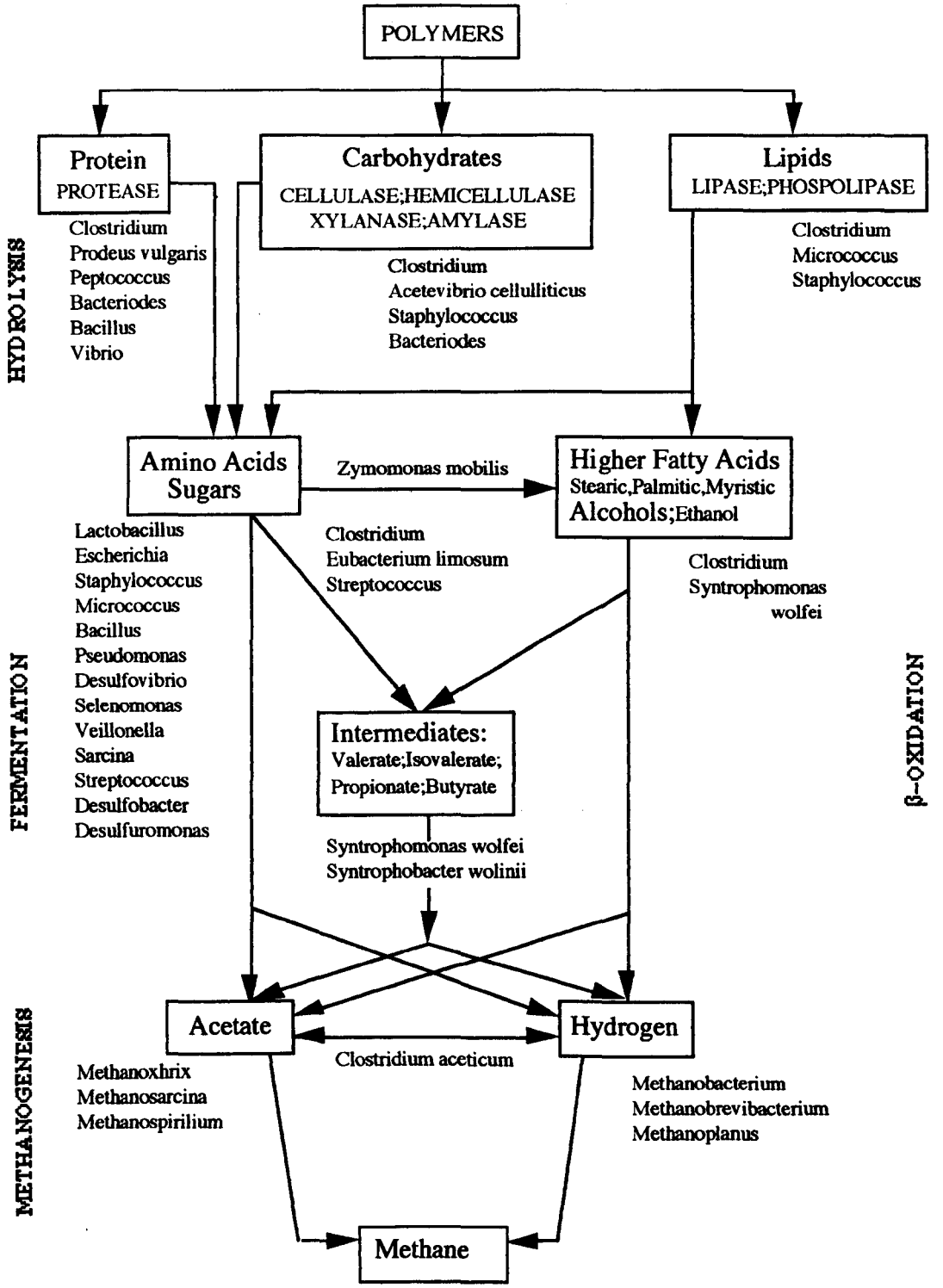


Figure 2.2 The breakdown of organic polymers (Source: Stronach et al., 1986)

addition to hydrogen, carbon dioxide and iso-propanol. The conversion of sugars to pyruvate via the Embden Meyerhof-Parnas (EMP) pathway initiates butyric acid fermentation (Stronach *et al.*, 1986).

The end products of this phase are determined by the fate of pyruvate. Doelle (1981) described the acetate production pathway as shown in Figure 2.3, from which it can be seen that pyruvate is decarboxylated to acetyl-CoA, carbon dioxide and acety-CoA is maintained in equilibrium with acetyl phosphate by the enzyme phosphoacetyl transferase. The enzyme acetokinase then converts the acetyl posphate to acetate, with the production of one mole of ATP (Chung, 1976). One mole of glucose produces,therefore, 2 moles of acetate,with 2 moles of carbon dioxide and hydrogen evolved during pyruvate degradation. Two clostridal strains are known to have the ability to convert one mole of

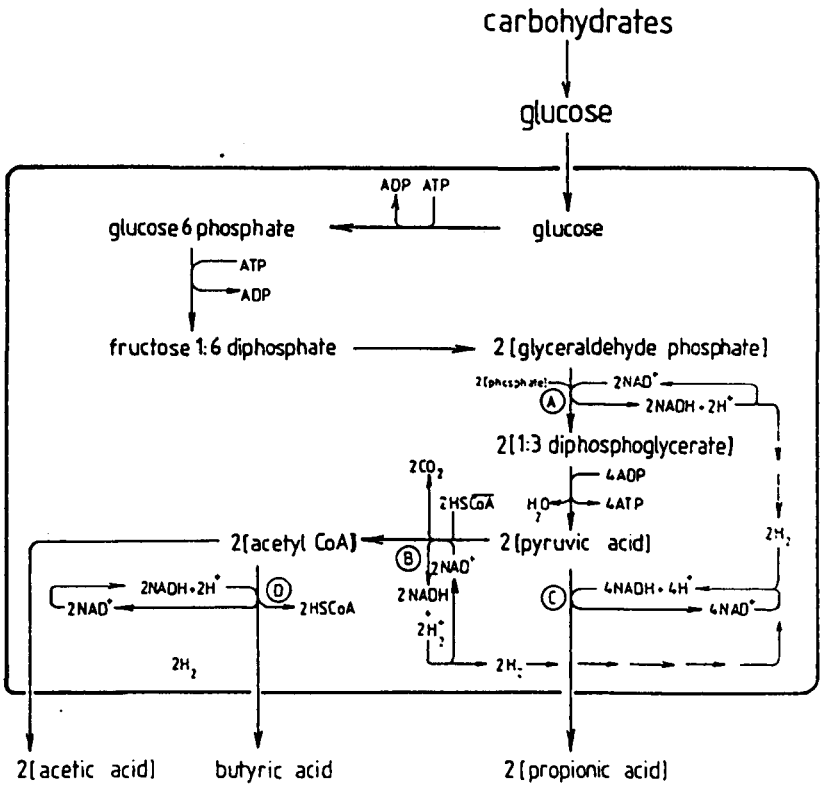


Figure 2.3 Catabolic pathways possible for anaerobic conversion of glucose (Source: Mosey, 1983)

glucose into 3 moles of acetate. For example, *Cl.thermoaceticum* and *Cl. formicoaceticum* do not release carbon dioxide and hydrogen and produce the third mole of acetate from pyruvate reaction.

Under balanced operating conditions, hydrogen is rapidly removed by the methanogenic bacteria, which results in the preferential production of acetate from pyruvate. Unstable operations cause the accumulation of hydrogen which results in the formation of butyric and propionic acid (Mosey,1982), thereby removing the excess hydrogen and allowing the acidogenic bacteria to regenerate their NAD.

Lactate is also a common end-product of bacterial fermentation. The lactic acid bacteria, categorized as the hemo- and heterofermentative group, produce lactate as their major end-product.

The second group of acid forming bacteria are known as obligate hyrogen producing acetogenic bacteria (OHPA) and produce acetic acid, carbon dioxide and hydrogen from propionate, butyrate and other higher fatty acids by the ß-oxidation process (Figure 2.2). This process removes an acetate (C_2-unit) molecule from fatty acids at each reaction until all fatty acids are converted to acetate molecules.

A syntrophic association exists between the OHPA and the hydrogen consuming bacteria. The only OHPAs that have been isolated exist as co-cultures with a hydrogen utilizing bacteria (McInerney and Byrant,1979). The major biochemical interactions for the conversion of the principal precursor substrates by these bacteria are defined in Table 2.1 in terms of associated redox half-reactions and biochemical standard free enegy levels (Harper and Pohland, 1986). Accordingly, when a particular metabolic pathway dominates a particular substrate conversion sequence, it is frequently regulated by the intensity of hydrogen (or formate) production and its potential for accumulation to

Table 2.1 Some redox half-reactions responsible for anaerobic microbial conversion of selected substrates.

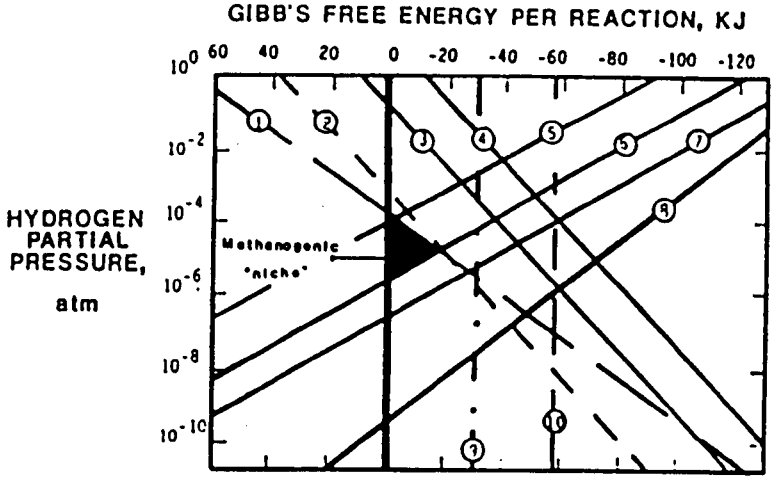
Electron donating reactions		$\Delta G^0(KJ)$ /reaction
Pr*opionate* → *Acetate*	$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + H^+ + HCO_3^- + 3H_2$	+ 76.1
Butyrate → *Acetate*	$CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + H^+ + 2H_2$	+ 48.1
Ethanol → *Acetate*	$CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + H^+ + 2H_2$	+ 9.6
Lactate → *Acetate*	$CH_3CHOHCOO^- + 2H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 2H_2$	- 4.2
Acetate → *Methane*	$CH_3COO^- + H_2O \rightarrow HCO_3^- + CH_4$	- 31
Electron accepting reactions		$\Delta G^0(KJ)$ /reaction
HCO_3^- → *Acetate*	$2HCO_3^- + H^+ + 4H_2 \rightarrow CH_3COO^- + 4H_2O$	- 104.6
HCO_3^- → *Methane*	$HCO_3^- + H^+ + 4H_2 \rightarrow CH_4 + 3H_2O$	- 135.6
Sulfate → *Sulfide*	$SO_4^= + H^+ + 4H_2 \rightarrow HS^- + 4H_2O$ $CH_3COO^- + SO_4^= + H^+ \rightarrow 2HCO_3^- + H_2S$	- 151.9 - 59.9
Nitrate → *Ammonia*	$NO_3^- + 2H^+ + 4H_2 \rightarrow NH_4^+ + 3H_2O$ $CH_3COO^- + NO_3^- + H^+ + H_2O \rightarrow 2HCO_3 + NH_4^+$	- 599.6 - 511.4
Nitrate → N_2	$2NO_3^- + 2H^+ + 5H_2 \rightarrow N_2 + 6H_2O$	- 1120.5

Source: Harper and Pohland (1986).

inhibiting levels. Therefore, a lack of syntrophy between the hydrogen-producing acidogens and the hydrogen-consuming methanogens, sulphate-reducing bacteria (SRB) or nitrate-reducing bacteria (NRB) can result in excessive accumulation of hydrogen or intermediate conversion products unless other hydrogen sinks (Fe(III), Mn(IV), oxygen,

unsaturated compounds, etc.) are available. In the case of the potential inhibition of hydrogen, this usually requires both the ultimate cleavage of acetate and the reduction of carbon dioxide. Conversion of organic fatty acid homologues (butyric and propionic acids) to acetate and hydrogen is accomplished by organisms that grow only when hydrogen is used by the hydrogenotrophs, a process termed "interspecies hydrogen transfer" (Iannotti *et al.*, 1973; Wolin, 1977). In the case of obligate hydrogen from the acids and its utilization by methanogens it is necessary to permit reactions that yield energy for the growth of both species. Hence, a common characteristic of such syntrophic associations is the "thermodynamic barrier" to the reduction of protons to hydrogen, a barrier that can be overcome by coupling the formation of hydrogen to the reduction of carbon dioxide to methane.

As indicated in Table 2.1, the lowering of the free enegy of a reaction to a negative and, hence, thermodynamically favorable level by syntrophic association of the species constituting the microbial consortium can be used to suggest possible process configurations as well as control options. If the typical reactions indicated in Table 2.1 are plotted against hydrogen partial pressure, a methanogenic "niche' can be established, indicating that propionic acid oxidation to acetate becomes favorable only at hydrogen partial pressures below 10^{-4} atmosphere, that for butyric acid oxidation below 10^{-3} atmosphere, and that for ethanol and lactate oxidation below about one atmosphere (Figure 2.4). This approach can be extended similarly as indicated in Figure 2.4 to illustrate the favorability of bicarbonate respiration over acetic acid cleavage at hydrogen partial pressures above 10^{-4} atmosphere. Also evident in Figure 2.4 is the favorability of sulphate reduction over bicarbonate respiration and the favorability of acetate cleavage by sulphate-reducing bacteria (SRBs) over cleavage by methanogens. In addition, sulphate reduction by hydrogen is favored over acetate cleavage by sulphate-reducing bacteria (SRBs) at hydrogen partial pressures above 10^{-4} atmosphere (Malina and Pohland, 1992).



Legend:

1) propionic acid oxidation to acetic acid
2) butyric acid oxidation to acetic acid
3) ethanol to acetic acid
4) lactic acid to acetic acid
5) acetogenic respiration of bicarbonate
6) methanogenic respiration of bicarbonate
7) respiration of sulphate to sulphide
8) respiration of sulphite to sulphide
9) methanogenic cleavage of acetic acid
10) SRB mediated cleavage of acetic acid

Basis:
Acetic acid, 25mM; propionic, butyric, lactic acids and ethanol, 10mM; sulphate and sulphite, 5mM; bicarbonate, 20mM; and methane, 0.7 atm.

Figure 2.4 Graphical representation of hydrogen dependent thermodynamic favorability of anaerobic reactions (Source: Harper and Pohland, 1986)

2.1.1.3. Methanogenesis

The end-products of the non-methanogenic phase are finally converted into methane and carbon dioxide (Figure 2.2) by a morphologically diverse, but physiologically coherent group of bacteria called methanogens. Table 2.2 summarize the main methanogenic reactions and the corresponding free energy changes under standard conditions.

Methane is mainly produced by two mechanisms, firstly by the decarboxylation of acetate

and secondly by the reduction of carbon dioxide. The acetate to methane conversion has figured prominently in several investigations (Bott and Thauer, 1989; Fischer and Thauer, 1989; van de Wijngaard *et al.*, 1988; Terlesky and Ferry, 1988b; Terlesky *et al.*, 1987; Pine and Barker, 1956; Stadtman and Barker, 1949). Jeris and McCarty (1965) made a detailed study of methane production from medium-length, even-carbon-chain, fatty acids, carbohydrates and leucine. They found that acetate was the major volatile acid produced, from which around 70% of the methane was derived. The remaining approximately 30% was produced from the reduction of carbon dioxide.

Table 2.2 Stoichiometry and free energy changes of methanogenic reactions under standard and practical conditions.

Reaction	ΔG^o (kJ/CH_4) at pH 7
$HCOO^- + 3H_2 + H^+ \rightarrow CH_4 + 2H_2O$	- 134.3
$4CO + 2H_2O \rightarrow CH_4 + 3CO_2$	- 185.1
$4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O$	- 102.5
$CH_3OH + H_2 \rightarrow CH_4 + H_2O$	- 121.1
$4CH_3NH_2 + 2H_2O + 4H^+ \rightarrow 3CH_4 + CO_2 + 4NH_4^+$	- 101.6
$2(CH_3)_2NH + 2H_2O + 2H^+ \rightarrow 3CH_4 + CO_2 + 2NH_4^+$	- 86.3
$4(CH_3)_3N + 6H_2O + 4H^+ \rightarrow 9CH_4 + 3CO_2 + 4NH_4^+$	- 80.2
$2CH_3CH_2 - N(CH_3)_2 + 2H_2O \rightarrow 3CH_4 + CO_2 + 2CH_3CH_2NH_2$	- 70.0
$CH_3COO^- + H_2O \Rightarrow CH_4 + HCO_3^-$	- 28.2
$4H_2 + CO_2 \Rightarrow CH_4 + 2H_2O$	- 139.2
$4HCOO^- + 2H^+ \Rightarrow CH_4 + 2HCO_3^- + CO_2$	- 126.8

Source: Oremland (1988) and Thauer *et al.*, (1977).

The methane formation pathway from carbon dioxide, including the possible role of coenzyme M (CoM), as shown in Figure 2.5 has been extensively reviewed (Allmansberger *et al.*, 1989; Weil et al., 1989; Rouviere *et al.*, 1988; Bobik and Wolfe,

1988; Kengen *et al.*, 1988; Rouviere and Wolfe, 1988; Jones *et al.*, 1987; Gunsalus *et.al.*, 1976). CoM is the smallest enzyme known, exceptional in its high sulphur content and acidity, and is required by methylcoenzyme M reductase, an enzyme universal in methanogens and active in the final steps of carbon dioxide reduction. ATP operates as the activator in the reaction and CoM the carrier.

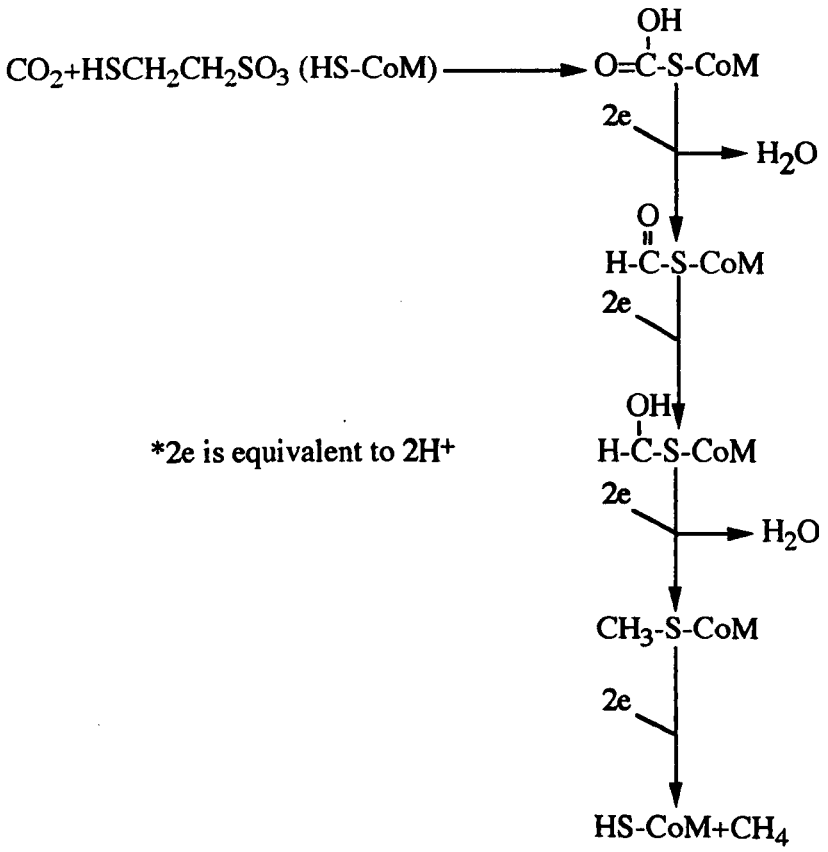


Figure 2.5 Schematic presentation of the possible role of Coenzyme M in the reduction of carbon dioxide (Source: Gunsalus, 1976).

Methane production is generally considered a slow process which is often the rate limiting step in anaerobic digestion (Henze and Harremoes,1982). As effective COD removal from the liquid phase takes place in this phase, the performance of methanogens is therefore really important in anaerobic digestion in order to achieve high substrate removal and determines the quality of the effluent.

The effluent quality of an anaerobic treatment process in terms of COD removal, methane production and methane yield depends on growth kinetics of methanagenic bacteria. Many studies have been carried out to determine kinetic coefficients in pure cultures of methanogens, some of which are summarized in Table 2.3. The data from pure and mixed cultures as well as information generated from mathematical models, a group of kinetic constants of anaerobic bacteria produced by Henze and Harremoes (1982) are also given in Table 2.4.

The classification of the methanogens reported in the literature defines nineteen genera and more than 50 species. The current phylogenetic tree supports the classification of the methanogens into three major groups that correspond to the orders proposed by Balch *et*

Table 2.3 Kinetic coefficient of methanogens in mesophilic phase

Substrate	Species	Kinetic Coefficient			Reference
		K_S mM/l	Y kg VSS/kgCOD	μmax 1/d	
Acetate	Methanothrix sohngenii	0.4-0.6	0.023	0.11	Huster *et al.*, 1982
	Methanosarcina barkeri	4-5	0.024	0.21	Wandrey, Aivasidis,1983
	Methanobacterium sp.	0.17	0.01	0.26	Cappenberg, 1975
	Methanococcus mazei	-	-	0.53	de Zeeuw, 1984
H_2/CO_2	Methanospirillum hungatei	0.002	0.021	0.05	Robinson & Tiedje,1984
	Methanobrevibacter snithii	0.001	0.045	4.02	Pavlostathis *et al.*, 1991
	Methanobacterium formicicum	0.002	0.051	0.29	Schonheit *et al.*, 1980
	Methanosarcina barkeri	-	0.087	3.02	Weimer, Zeikus,1978a,b

Table 2.4 Kinetic coefficients of mixed cultures of anaerobic bacteria in digesters

Bacterial Group	Kinetic Coefficient			
	μ_{max}	Y	K_S	K
	1/d	kg VSS/kg COD	mg COD/l	kg COD/kg VSS.d
Acetate Producing Bacteria	2.0	0.15	200	13
Methane Producing Bacteria	0.4	0.03	50	13
Overall	0.4	0.18	-	2

Source: Henze and Harremoes (1982).

al. (1979). The orders have been further divided into six families. At present, three orders and six families are recognized within the methanogens. Table 2.5 summarizes a recent attempt to organize them but new species are still being described and this is therefore not exhaustive.

Balch *et al.* (1979) suggested a new approach to the systematics of methanogens based on their 16S ribosomal RNA (ribonucleic acid). This scheme was deeply criticized by Zeikus (1983) who stated that it did not fully represent the DNA complement of a methanogen and therefore did not express many of its characteristics. It is known that a number of other features distinguish them from typical bacteria. Methanogens have no peptidoglycan in their cell walls, but possess unusual membrane lipids and unique tRNA (Transfer Ribonucleic Acid) and 5S rRNA (Ribosomal Ribonucleic Acid). All methanogens, with the possible exception of *Methanothrixsoehngenii* (Zehnder *et.al.*, 1980), exhibit the property of autofluoresecence as a consequence of the fluorescent factors F_{420} and F_{342}. This enables their distinction and possible identification within anaerobic sludges using fluorescence microscopy (Mink and

Table 2.5 Methanogen classification

ORDER	FAMILY	GENUS	SPECIES	GRAM REACTION	MORPHOLOGY	SUBSTRATE
Methanobacteriales	Methanobacteriaceae	Methanobacterium	M.formcicum[1]	+	Long rods, filaments	H_2,CO_2,formate
			M.bryanti[2]	+	Short long rods	H_2,CO_2,formate
			M.termoautotrophicum[3]	+	Long rods,filamants	H_2,CO_2,formate
			M.wolfei[24]	+	Rods	H_2,CO_2
			M.alcaliphilum[24]	+	Rods	H_2,CO_2
			M.uliginosum[24]	-	Rods	H_2,CO_2
			M.thermoformicicum[24]	+	Rods	H_2,CO_2,formate
		Methanobrevibacter	M.arbophilius[4]	+	Short rods	H_2,CO_2,formate
			M.ruminantium[5]	+	and short	H_2,CO_2,formate
			M.smithii[6]	+	chains	H_2,CO_2,formate
	Methanothermaceae	Methanothermus	M.fervidus[7]		Short rods	H_2,CO_2
			M.sociabilis		Rods	H_2,CO_2
Methanococcales	Methanococcaceae	Methanococcus	M.vannielli[9]	-	Irregular	H_2,CO_2,formate
			M.voltae[6]	-	cocci	H_2,CO_2,formate
			M.maripaludis[8]	-	single or pairs	H_2,CO_2,formate
			M.thermolithotrophicus[10]	-		H_2,CO_2,formate
			M.halophilus[11]	-		Methanol, methylamines
			M.jannaschi(*)[8]	-	Irregular cocci	H_2,CO_2,formate
			M.deltae(*)[15]	-		H_2,CO_2,formate
			M.frisisus	-	Irregular cocci	H_2,CO_2
Methanomicrobiales	Methanomicrobiaceae	Methanomicribium	M.mobile[12]	-	Short rods single	H_2,CO_2,formate
			M.paynter(*)[13]	-	Short rods single	H_2,CO_2
		Methanogenium	M.cariaci[14]	-	Irregular	H_2,CO_2,formate

Table 2.5 Methanogen classification (continued)

ORDER	FAMILY	GENUS	SPECIES	GRAM REACTION	MORPHOLOGY	SUBSTRATE
			M.marisnigri[14]	-	cocci, single	H_2,CO_2,formate
			M.olentangyi(*)[15]	ND	or pairs	H_2,CO_2,formate
			M.thermophilicum[16]	+	Irregular cocci	H_2,CO_2
			M.aggregands[24]	+	Irregular cocci	H_2,CO_2,formate
			M.bourgense[24]	+	Irregular cocci	H_2,CO_2,formate
			M.tationis[24]	ND	Irregular cocci	H_2,CO_2,formate
		Methanospirillum	M.hungatei[17]	-	Spirillum,regular rods and filaments	H_2,CO_2,formate
	Methanoplanaceae	Methanoplanus				
	Methanosarcinaceae	Methanosarcina	M.limicola[19]	-	Plated shaped	H_2,CO_2,formate
			M.barkeri[20]	+	Pseudosarcina	H_2,CO_2,formate
			Mmazei[18]	+	irregular cocci in large aggregates	
			M.thermophila[24]	ND	Pseudosarcina	Acetate
			M.acetivorans[24]	+	Pseudosarcina,coccoid	methylamines
			M.vacuolate24	ND	Pseudosarcina	
		Methanococcoides	M.methylutents(*)[22]	+	Irregular cocci	Methanol, methylamines
		Methanoxrix (@)	M.shoengenii[21]	+	Irregular cocci sheath forming long filament	Acetate
			M.concilli[24]	ND	Sheated rod	Acetate
		Methanolobus (@)	M.tindarius[23]	+	Irregular cocci single or loose	Metanol methylamines

(1) Bryant et al., 1967; (2) Schnell,1974; (3) Zeikus & Wolfe,1972; (4) Zeikus & Ward,1974; (5) Smith & Hungate, 1958; (6) Balch et al.,1979; (7) Stetter et al.,1981; (8) Jones et al.,1983); (9) Stadtman & Barker,1951; (10) Huber et al.,1982; (11) Zhilina,1983); (12) Paynter & Hungate,1968; (13) Rivard,1983; (14) Romesser et al.,1979; (15) Corder,1983; (16) Rivard & Smith,1982; (17) Ferry et al.,1974; (18) Mah,1980; (19) Wildgruber et al.,1982; (20) Schnellen,1947; (21) Zehnder et al.,1980; (22) Sowers & Ferry,1983; (23) Konig & Stetter,1982; (24) Boone & Whitman,1987. (*) Species not yet named ; (@) Family not yet assigned

Dugan,1977). The factors isolated from methanogenic bacteria such as F_{420}, F_{430}, and F_{342} can be described as follows:

F_{420} is a low molecular weight coenzyme (8-oh-5-deazaflavin) which has a blue fluorescence under ultra-violet light and a maximum absorption at a wavelength of 420 nm. This specification of methanogenic bacteria therefore has led to the use of epifluorescent optics in order to count methanogenic bacteria (Keltjens and van der Drift, 1986).

F_{430} is a yellow compound with a tetrapyrrole structure which contains nickel (Pfaltz *et al.*, 1987; Diekert *et al.*, 1981: Whitman and Wolfe, 1980). This information explains the nickel requirement, peculiar to methanogenic bacteria. Furthermore, F_{430} is not fluorescent and has two maximum absorption peaks at 430nm and 275 nm. Unlike F_{420} and F_{342}, the maximum absorption peak of F_{430} does not shift as a result of changes in the oxidation / reduction state of the medium.

F_{342} is another fluorescent coenzyme which is liable to changes in the oxidation /reduction state as in the case of F_{420} (Gunsalus and Wolfe, 1978). As yet little is known about this compound.

2.1.2. Environmental Factors

Anaerobic digestion can be influenced by environmental factors such as pH, temperature, avaliability of nutrients and toxic substances. These factors can affect the system either by enhancing or inhibiting parameters including substrate removal, biogas production. decay rate, specific growth rate, start-up and responses to changes in input. In this section these factors will be discussed.

2.1.2.1. pH

Near neutral pH is desirable for operation of most anaerobic digestion processes. Deviations from this optimum may cause excess production and accumulation of volatile fatty acids and ammonia. In addition, the intensity of pH will affect the solubility and reaction behavior of other potentially influencing substances, including both organic and inorganic species.

Pohland *et al.* (1987) pointed out that low pH and excessive acid production and accumulation, which destroys the bicarbonate buffer system, are more inhibitory to methanogens than fermentative bacteria. Acidogenic bacteria can also continue producing fatty acids despite low pH , therefore aggravating the environmental condition further. However, methanogenic bacteria such as *Methanosarcina barkeri* and *Methanosarcina vacuolata* can grow well at low pH with an optimum pH of 5 when cultured on hydrogen and methanol (Maestrojuan *et.al.*, 1991). Similarly, hydrogen - oxidizing and methylotrophic methanogens have been found at very alkaline pH values (Boone,1991). Therefore, it can be derived that some biochemical interactions and degradation pathways may be influenced by pH, including possible inhibition of hydrogen production.

2.1.2.2. Temperature

Methanogenesis has been shown to be strongly temperature-dependent, with reaction rates generally increasing with temperature up to 60 °C although with a trough frequently occuring somewhere between 40 and 50 °C. There are three temperature ranges defined in anaerobic digestion but only mesophilic (near 35 °C) and thermophilic (50-60 °C) are known to be optimum. Deviations from this will decrease the methanogenic rates. The significance of temperature on the rate of anaerobic digestion would suggest that

the final operating reactor temperature be considered as one of the principal design parameters. Rapid alterations in reactor temperature of even a few degrees can result in a marked upset in microbial metabolism and necessitate several days' recovery.

2.1.2.3. Nutrients

In addition to the fundamental requirements for macronutrients such as carbon and nitrogen, the inability of many anaerobes to synthesize some essential vitamins or amino acids often necessitates supplementation of the culture medium with specific nutrients for growth and metabolism. Generally, the gross level of essential nutrients can be evaluated if the biomass is known thus the C:N ratio is frequently utilized to describe this micronutrient requirement. Henze and Harremoes (1983) reported that this ratio will be affected by substrate specificity, but if measured as COD, COD:N ratios of about 400:7 and 1000:7 have been estimated as required at high and low substrate loadings respectively. Similarly, a N:P ratio of approximately 7:1 has been reported as required (Stronach *et al.*, 1986). In addition to nitrogen and phosporus, several other materials have been identified as micronutrients for methanogens (Speece *et al.*, 1983). The most important of these are iron, nickel, magnesium, calcium, sodium, barium, tungstate, molybdate, selenium and cobalt. A summary of the available information is compiled in Table 2.6. In the case of selenium, tungsten and nickel, these elements are implicated in the enzyme systems of acetogenic and methanogenic bacteria.

2.1.2.4. Toxicity and Inhibition

Inhibition of the anaerobic digestion process can be mediated to varying degrees by toxic materials present in the system. These substances may be components of the influent waste stream, or byproducts of the metabolic activities of the digester bacteria. Inhibitory toxic compounds include sulphides, volatile acids, ammonia, alkali and alkaline earth

metals and heavy metals in the systems. Duarte and Anderson (1983) and Swanwich *et al.*, (1969) pointed out that the latter have been considered as the most common and major factors governing reactor failure.

Table 2.6 Micro-nutrients which improve anaerobic process performance

Elements or Compounds	Effects	Benifical Concentration (mg/l)	Reference
K^+	Increase in activity	200-400	Kugelman & McCarty, 1965
Na^+	Increase in activity	100-200	Kugelman & McCarty, 1965
Ca^{++}	Flocculation	100-200	Kugelman & McCarty , 1965
Mg^{++}	Flocculation	75-150	Kugelman & McCarty, 1965
Fe^{++}	Increase in activity and precipitation of sulphise	0.2	Henze & Harremoes 1982 ; Speece 1983
Ba^{++}	Flocculation	0.01-0.1	Stronach *et al.*, 1986
Nickel	Carbon monoxide dehyogenase, Hhyrogenesa nicotinic acid hydroxyase and dedyogenase dependent	0.01	Stronach *et al.*, 1986; Henze & Harremoes 1982
Cobalt	Vitamin B_{12} dependent	20	Stronach *et al.*, 1986; Henze & Harremoes 1982
Tungsten	Formate dehydrogenase dependent	-	Stronach *et al.*, 1986
Selenium	Formate dehyogenase, glycine reductase, hydroxylase and dehydrogenase dependent	0.8	*Stronach et al.*, 1986
Sulhide	Sulphur source of cell synthesis	0.1-10	Stronach *et al.*, 1986; Henze & Harremoes 1982

Anaerobic reactor instability is generally manifested by a marked and rapid increase in VFA concentrations. This is frequently indicative of the failure of the methanogenic population due to other environmental distruptions such as shock loading, nutrient depletion or infiltration of inhibitory substances. Ianotti and Fischer (1984) suggested that acetate was the least toxic of the VFAs, whilst McCarty *et al.* (1963) and Hobson and Shaw (1976) stated that propionate was a major cause of digester failure. Ianotti and Fischer (1984) observed that microbial growth was significantly inhibited at 35 g/l acetic acid and in excess of 3 g/l propionic acid concentrations .

According to Andrews (1969), conversion-rate inhibition by VFAs at acidic pH values can be attributed to the existence of unionized VFAs in significant quantities in the system. These unionized acids are present in amount dependent upon the total concentration of VFAs in solution.

A pH-dependent equilibrium exists between the ionized and unionized components of VFAs:

$$CH_3COOH \Rightarrow CH_3COO^- + H^+ \qquad (2.1)$$

As the pH value decreases, the equilibrium shifts to the left, resulting in an increase in the concentration of unionized VFAs. Krocker et al. (1979) found that digester failure becomes increasingly more likely as the concentration of the unionized acid rises to above 10 mg/l.

Anderson *et al.* (1986) pointed out that sulphate in the influent of an anaerobic digester could inhibit methanogenesis due to both the competition for acetate and hydrogen by SRBs and the production of sulphide from sulphate reduction by SRBs. Sulphide toxicity has been observed at concentrations ranging from 200 to 1500 mg/l until acclimatization

occurred or the sulphide concentration could be reduced by precipitation or release into the gas phase. Therefore, the potential toxic effects of sulphide, normally present in solution as a weak acid, would be a function of pH as well as resulting from the presence of precipitants such as most heavy metals (Stronach *et al.*, 1986).

Although ammonia is an important buffer in anaerobic digestion processes, high ammonia concentrations can be a major cause of operational failure. The inhibitory effects of ammonia, as far as is known, influence only the methanogenesis phase in an anaerobic reactor. Ammonia-nitrogen concentrations in the range 1500-3000 mg/l were observed to cause inhibition of methanogenic microorganisms at alkaline pH and, at concentrations in excess of 3000 mg/l, the ammonium ion was claimed to be toxic irrespective of pH, whilst free ammonia caused toxicity at concentrations greater than 150 mg/l (Hobson and Shaw, 1976).

Heavy metal toxicity has often been implicated as being the cause of failure of anaerobic microbial conversion processes, as influenced by the oxidation-reduction potential, pH and ionic strength and the resultant speciation of the metals or metal complexes. The toxicity limits reported in the literature vary widely from study to study. The order of decreasing heavy metal toxicity of most frequent concern has been recorded by Mosey and Hughes (1975) as Zn = Cu = Cd > Cr(VI) = Cr(III) >> Fe whereas Ni > Cu > Pb > Cr > Zn by Theis and Hayes (1978). One of the most effective methods proposed by Lawrence and McCarty (1965) for heavy metal control would be by precipitating the metals except chromium, as insoluble salts by the addition of equivalent concentrations of sulphide or sulphide precursors if they are not already present in the waste stream.

McCarty and McKinney (1961a,1961b) pointed out that the toxicity of salts in anaerobic digestion is associated with the cation portion of the salts. Table 2.7 shows the concentrations of strongly and moderately inhibitory cations.

Table 2.7 Inhibition concentration of alkali and alkaline-earth cations

Cation	Inhibitory Concentration (mg/l)	
	Moderately	Strongly
Na^+	3500 - 5500	8000
K^+	· 2500 - 4500	12000
Ca^{++}	2500 - 4500	8000
Mg^{++}	1000 - 1500	3000

Source: McCarty and Mckinney (1961a,1961b)

2.2 Process Monitoring and Control

Much research have been carried out on the microbial, biochemical, and physical characterization of the various process configurations and their operational sensitivies. However, a universally accepted menu of indicator parameters, sufficient to allow feedback control for the variety of process types and configurations used, has not been established. As a result, monitoring and control is largely reactionary, without a sufficient linkage between fundamental principles and applications of the technology in practice. These issues have been widely discussed by Hickey *et al.*, (1991) and Weiland and Rozzi (1991).

Monitoring could be implemented in either the liquid/slurry phase or in the gas phase, with the former more frequently involving measurements of pH, total and individual volatile acids, alkalinity, and COD, TOC, VSS, or other indicator parameter changes and the latter more frequently involving gas production and quality, mainly including analyses for methane and carbon dioxide. Depending upon process circumtances, these analyses may be augmented by the measurement of specific inorganic and organic compounds, microbial/biomass characterization, and gas constituents such as hydrogen, carbon monoxide, hydrogen sulphide, ammonia, and trace volatile compounds.

pH, alkalinity and volatile fatty acids are an integral expression of the acid-base conditions of anaerobic microbial treatment processes. Monitoring of pH, either internal or external to the anaerobic microbial treatment process can be used as a control technique, and methods are available to provide appropriate pH adjustments or buffer capacity as required (Loewenthal *et al.*, 1991). Alkalinity or buffer requirements for pH adjustment can generally be estimated on the basis of neutralization of excess volatile acids and dissolved carbon dioxide (Li and Sutton , 1984). The results obtained from recent studies (Anderson and Yang, 1992; Rozzi *et al.*, 1985) have shown that the bicarbonate concentration is a more sensitive state parameter than both alkalinity and pH for anaerobic digester control. The bicarbonate concentration in an anaerobic digester can be calculated according to the different methods developed by Anderson and Yang (1992), Powel and Archer (1989), Rozzi and Bunetii (1981).

Renard *et al.* (1990) pointed out that monitoring individual volatile acids, particularly propionic acid, can also be used to direct loading adjustments and to prevent substrate overloads. Alkali consumption for pH control has been used as a process variable and an indirect measure of total volatile acids (Denac *et al.*, 1990, 1988).

Gas-phase monitoring is a frequently applied technique for assessing the efficiency and state of anaerobic microbial stabilization processes. A decrease in the methane content of the gas phase normally signals a concomitant decrease in treatment efficiency of continuous-flow systems. Unfortunately, such gas quality changes usually occur after a stress is imposed, and thereby reflect an effect rather than a warning of impending problems. Nevertheless, there has been and continues to be increasing operational interest in utilizing automated gas quality measurements in terms of, not only methane, but hydrogen (Harper and Pohland, 1986; Mosey, 1983a,1983b; Mosey and Fernandes, 1989), carbon dioxide (Denac *et al.*, 1988; Cayless *et al.*, 1989; Moletta, 1989), carbon monoxide (Hickey *et al.*, 1991; Hickey *et al.*, 1987) as well. Mosey and Fernandes

(1989) pointed out that the concentration of hydrogen is more suitable for use as an alarm indicator than a steady state parameter for daily analysis due to the transient nature of the response of hydrogen to variations in loadings. In addition, Hickey *et al.* (1989) stated that carbon monoxide may be used as an indicator for the detection of metal induced inhibition in anaerobic digesters.

Recent studies (Ince *et al.*, 1992; Monteggia 1991; James *et al.*, 1990) showed that monitoring any changes in the numbers or activities of the methanogenic bacteria in the reactor using such available techniques as Microscopic Count, Most Probable Number (MPN), Coenzyme F_{420}, ATP, Dehydrogenic Activity and Specific Methanogenic Activity (SMA) can be used as a control parameter. The latter technique is more reliable and makes the determination of the most appropriate organic loading rate (OLR) possible rather than the use of conventional parameters such as pH, alkalinity, chemical oxygen demand (COD), volatile fatty acids (VFA) and gas yield which only provide information concerning the current conditions inside the reactor. In addition the SMA test provides a safe guideline for potential further increases in OLR. Various methods have been developed by van der Berg et al. (1974), Owen et. al. (1979), Valcke and Verstraete (1983), Rozzi *et al.* (1983), de Zeeuv (1984), Dolfing and Bloemen (1985), James *et.al.* (1990) and Monteggia (1991) to determine the methanogenic activity of anaerobic sludges.

The technique described by Monteggia (1991), determines the acetoclastic methanogenic activity of the sludge. The reason for using this technique is that approximately 70 % of the methane formed during the anaerobic digestion of a complex substrate results from acetic acid (McCarty, 1964).

The SMA test can also be used for the determination of the optimum operating conditions of anaerobic reactors. Three fundamental operating conditions were defined by

Monteggia (1991) in a study of laboratory-scale upflow anaerobic sludge blanket reactors. Operating condition one corresponded to an actual methane production (AMP) in the digester of 60% of the potential methane production (PMP) of the sludge using the SMA test, thus resulting in high operating stability and an excellent COD removal. Operating condition two was identified as being from approximately 60% to 100% of the PMP, resulting in a lower COD removal and a stability dependent on the available alkalinity. Operating condition three took place at excessive organic loading rates (i.e. where the AMP in the digester is greater than the PMP) resulting in an irreversible imbalance in the sequential stages of anaerobic biodegredation.

The identification of methanogenic species using a microscopic count and the MPN technique as a control parameter of a digester is also becoming increasingly attractive. For instance, any deterioration in the performance of a digester may have been due to the change in the dominant species or to the species composition.

2.3. Kinetics and Mathematical Modelling

The substrate conversion in anaerobic digestion can be decribed on the basis of a continuous culture theory and associated process kinetics. Many mathematical models, including microbial reaction mechanisms, together with the regulating environmental factors affecting process viability and substrate utilization efficiency have resulted in very complicated expressions which describe the full range of process taking place. Because of their complexity and the uncertainties in selection and measurement of input and output parameters, modelling studies are generally based upon selected fundamental principles and are generalized in order to enhance applications for process and design control. McCarty and Mosey (1991) stated that the kinetic expressions, rate constants, mass balances and conversion coefficients are the tools of the mathematical modeler to describe a process.

Normally, operational models are developed to describe the effect of " growth limiting " substrates, essential nutrients, and/or environmental conditions on microbial growth. Accordingly, examination of the kinetics of the hydrolysis of complex organic polymers, requiring solubilization of carbohydrates, lipids and proteins (Figure 2.1), has received considerable attention, usually employing first-order kinetics either for batch or continuous-flow applications whereas the Monod model and its variations (Table 2.8) are widely used to simulate growth on already soluble substrates.

Table 2.8 Kinetic models used in anaerobic microbial treatment processes.

First Order	$\mu = \frac{kS}{S_o - S} - k_d$	$-\frac{dS}{dt} = kS$	$S = \frac{S_o}{w + k\theta_c}$
Monod	$\mu = \frac{\mu_{max} S}{K_s + S} - k_d$	$-\frac{dS}{dt} = \frac{\mu_{max} SX}{Y(K_s + S)}$	$S = \frac{K_s(1 + k_d\theta_c)}{\theta_c(\mu_{max} - k_d) - 1}$
Grau	$\mu = \frac{\mu_{max} S}{S_o} - k_d$	$-\frac{dS}{dt} = \frac{\mu_{max} SX}{YS_o}$	$S = \frac{S_o(1 + k_d\theta_c)}{\theta_c\mu_{max}}$
Contois	$\mu = \frac{U_m S}{BX + S} - k_d$	$-\frac{dS}{dt} = \frac{U_m SX}{Y(BX + S)}$	$S = \frac{BYS_o(1 + k_d\theta_c)}{BY(1 + k_d\theta_c) + \theta_c(U_m - k_d - 1}$
Chen & Hashimoto	$\mu = \frac{\mu_{max} S}{KS_o + (1 - K)S} - k_d$	$-\frac{dS}{dt} = \frac{\mu_{max} SX}{KX + YS}$	$S = \frac{KS_o(1 + k_d\theta_c)}{(K - 1)(1 + k_d\theta_c) + \theta_c\mu_{max}}$

Source: Pavlostathis and Giraldo-Gomez (1991).

2.3.1 Cell Growth

Biological growth kinetics are based on two fundamental relationships : growth rate and substrate utilization rate. The rate of growth of bacterial cells is given by a first order expression in terms of bacterial mass:

$$r_g = \frac{dX}{dt} = \mu X \qquad (2.2)$$

where r_g = rate of bacterial growth, (mass/unit volume.time)

X = concentration of microorganism, (mass per unit volume)

μ = specific growth rate, (time^{-1})

t = time

2.3.2. Substrate Limited Growth

The effect of the growth limiting substrate (i.e., the essential nutrient) concentration on the rate of microbial growth has been described by various mathematical models which are given in Table 2.8. However, the most widely used kinetic model is that of Monod (1949):

$$\mu = \mu_m \frac{S}{K_s + S} \qquad (2.3)$$

where μ_{max} = maximum specific growth rate, (time^{-1})

S = concentration of growth limiting substrate in solution, (mass/unit volume)

Ks = half velocity constant, substrate concentration at one half maximum growth rate, (mass/unit volume)

2.3.3. Cell Growth and Substrate Utilization

New cells, together with organic and inorganic end products, are produced by the utilization of substrate in continuous-growth culture systems. The following expression can be given to describe the relationship between cell growth rate and substrate utilization rate:

$$r_g = -Yr_s \quad \text{-->} \quad \frac{dX}{dt} = -Y\frac{dS}{dt} \tag{2.4}$$

where r_S = rate of substrate utilization, (mass/unit volume.time)

Y = maximum yield coefficient, (mass/mass), (defined as ratio of mass of cells formed mass of substrate consumed, measured during any finite period of logarithmic growth)

S = concentration of growth limiting substrate in solution, (mass/unit volume)

When the specific growth rate is given by Monod kinetics (Eq. 2.3) the final equation can be written as follows:

$$-\frac{dS}{dt} = \frac{1}{Y}\mu_m \frac{S}{K_s + S} \tag{2.5}$$

2.3.4. Endogenous Respiration.

Endogenous respiration, commonly defined as the self-destruction of biomass, cell maintenance, predation, and cell death and lysis are processes leading to a decrease in cell mass. These processes are important in waste treatment systems, especially anaerobic systems, since they usually operate at low specific growth rates. To account for the effect of these processes on the net growth rate, a microorganism decay rate is usually used for the modification of the growth rate.

The loss of biomass is assumed to be linear during endogenous respiration:

$$r_d = -\frac{dX}{dt} = k_d X \tag{2.6}$$

where r_d = decay rate, (i.e., rate of biomass loss)

k_d = decay constant, (time^{-1})

The net rate of growth is therefore :

$$r_{gn} = r_g - r_d = (\mu - k_d)X \tag{2.7}$$

The effect of endogenous respiration on the net bacterial yield are accounted for by defining an observed yield as follows:

$$Y_{obs} = -\frac{r_{gn}}{r_s} \tag{2.8}$$

2.3.5. Steady-state Models

In this chapter, steady-state models for the new membrane anaerobic contact reactor system will be developed since this process has been used throughout the study. The structure of the model will be similar to that used in the anaerobic contact process (Anderson and Donnelly, 1978; Donnelly, 1984), where a settling tank was used for biomass recyle. The two main parameters used to produce the necessary equations and to develop the model are substrate and microorganism concentrations and are obtained by generating a mass balance across the process shown in Figure 2.6.

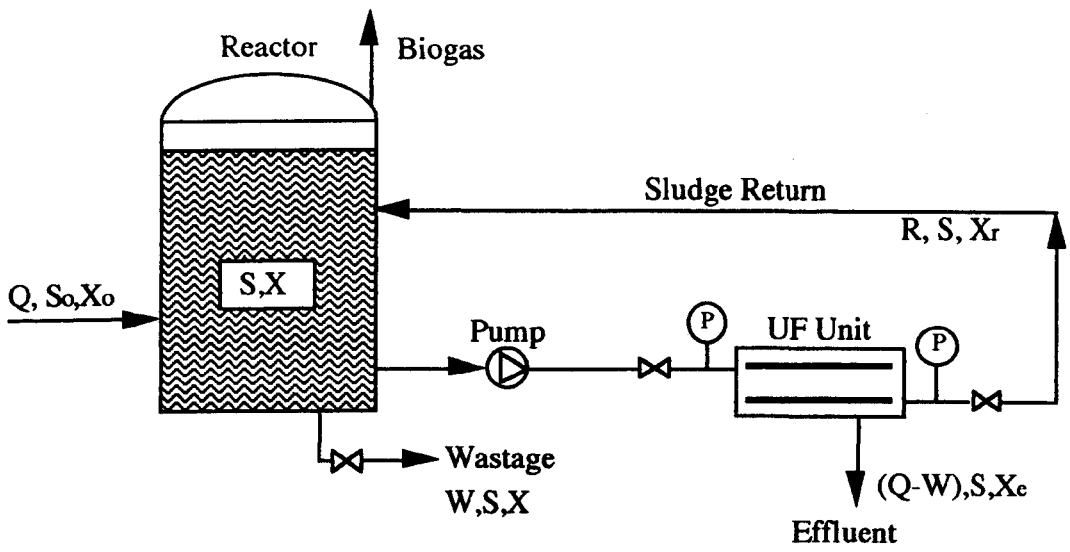


Figure 2.6 Schematic diagram of combined anaerobic contact reactor-ultrafiltration (UF) membrane system showing mass balance

Microorganism Mass Balance:

The rate of accumulation of microorganisms in the process can be determined by the sum of the rate of microorganisms entering the process, the rate of increase due to growth rate, the loss due to endogenous respiration, the rate of loss due to deliberate wastage and the rate of loss in the effluent.

Expressed mathematically this becomes :

$$\frac{dX}{dt}V_r = QX_o + \mu XV_r - k_d XV_r - WX - (Q - W)X_e \qquad (2.9)$$

where dX/dt = rate of change of microorganism concentration in the reactor measured in terms of mass (volitile suspended solids), (mass VSS/unit volume.time)

V_r = reactor volume

Q = flow rate, (volume/time)

X_0 = concentration of microorganisms in the influent, (mass VSS/unit volume)

X = concentration of microorganisms in the reactor , (mass VSS/unit volume)

X_e = concentration of microorganisms in the effluent, (mass VSS/unit volume)

W = wastage rate, (mass/time)

If it is assumed that the concentration of microorganisms in the influent and loss of biomass in the effluent can be neglected, Equation 2.9 can be rewritten as follows:

$$\frac{dX}{dt} = (\mu - k_d)X - \frac{WX}{V} \tag{2.10}$$

At steady-state (dX/dt = 0), the following Equation can be written:

$$\mu = k_d + \frac{W}{V} \tag{2.11}$$

The mean cell residence time, SRT, is usually defined as the mass of organisms in the reactor divided by the mass of organisms removed from the system each day but for the UF-membraned process, since there is no biomass loss (negligible) in the permeate (effluent) and the concentration of biomass in both reactor and the deliberate wastage are the same, SRT is expressed as follows:

$$SRT_{mean} = \frac{V}{W} \tag{2.12}$$

Therefore from Equations 2.11 and 2.12:

$$\mu = (\frac{1}{SRT}) + k_d \tag{2.13}$$

This shows that the growth rate can be controlled by varying the cell residence time.

Substrate Mass Balance:

The rate of accumulation of substrate in the process can be detemined by the sum of the rate of raw waste entering process, the rate of removal by microorganisms, the overall washout rate and the substrate loss due to deliberate wastage.

Expressed mathematically this becomes:

$$(\frac{dS}{dt})V_r = QS_o - (\frac{\mu XV_r}{Y}) - (Q - W)S - WS \tag{2.14}$$

where S_o = influent substrate concentration

S = reactor substrate concentration

Substituting Equation 2.13 into Equation 2.14 gives;

$$\frac{dS}{dt} = \frac{Q(S_o - S)}{V_r} - \frac{X}{Y}(\frac{1}{SRT} + k_d) \tag{2.15}$$

Substituting Equation 2.3 into Equation 2.10 gives:

$$\frac{dX}{dt} = X(\frac{\mu_{max} S}{K_s + S} - k_d - \frac{1}{SRT}) \tag{2.16}$$

At steady state , the effluent substrate and reactor biomass concentrations are found to be given as:

$$S = \frac{K_s(1/SRT + k_d)}{\mu_{max} - (1/SRT + k_d)} \tag{2.17}$$

and

$$X = \frac{Y(S_o - S)}{(1/SRT + k_d)HRT} \tag{2.18}$$

Equations 2.3, 2.9 and 2.14 form the basis of the model. If the kinetic coefficients are known, Equations 2.17 and 2.18 can be used to predict microorganism and effluent substrate concentrations under steady-state conditions.

2.3.6. Determination of Kinetic Constants

In order to determine the growth constants, the process should be run over a range of SRTs and effluent substrate concentrations by operating the process at various OLRs and wasting a specific quantity of sludge from the reactor at steady-state. The following equations are applied to the data collected at steady-states in order to estimate the constants:

$$\mu = \frac{\mu_{max} S}{K_s + S} = (k_d + \frac{1}{SRT}) \tag{2.19}$$

$$\text{i.e. } (\frac{1}{S})(\frac{K_s}{\mu_{max}}) + \frac{1}{\mu_{max}} = SRT/(1 + k_d SRT) \tag{2.20}$$

At steady-state, Equation 2.15 gives;

$$(\frac{1}{SRT})(\frac{1}{Y}) + \frac{k_d}{Y} = \frac{(S_o - S)}{XHRT} \tag{2.21}$$

Plots of Equations 2.20 and 2.21 will yield the kinetic constants; μ_{max} ,K_S, k_d and Y from the gradients and intercepts of the two straight lines.

2.4. Microbial Product Formation in Anaerobic Digestiom

Many studies have been carried out on microbial product formation in biological processes and reported in the literature by Germirli (1990), Artan *et al.* (1990), Orhon *et al.* (1989), Artan (1987), Hejzlar and Chudoba (1986a, 1986b), Chudoba (1985a,1967), Gaudy and Blachly (1985), Rittmann *et al.* (1987), Sykes (1981), Daigger and Grady (1977), Eckhoff and Jenkins (1967). The results obtained from these studies showed that the treated wastewaters had a certain amount of organics which are not biodegradable and it was not possible to remove these residual organics even after extended contact periods. Some other studies were also carried out under ideal growth conditions with totally biodegradable substrates on pure cultures and the results confirmed that these residual organics are the products produced by microorganisms.

The experimental studies on the oxidation of glucose in batch reactors (McWhorter and Heukelekian, 1964; Tenney and Stumm, 1965) showed that the residual COD was found to be in the range 1-15% of the initial COD.

The results obtained from the study of aerobic batch reactors (Chudoba, 1967) showed that a certain relationship exists between the quantity of the residual organics and the initial concentration of the substrate and an increase in the initial COD results in a linear increase in the residual COD.

Chudoba (1985a) pointed out that the soluble organics in treated wastewaters may contain some degradable compounds from the raw wastewater, degradable compounds produced by the process microorganisms, non-biodegradable compounds from the raw wastewater and non-biodegradable compounds produced by the microorganisms. The latter can be classified into three catogories: compounds excreted by microorganisms into the environment, compounds produced as a result of substrate metabolism and bacterial

growth, and compounds released during the lysis and degradation of microorganisms. Figure 2.7 shows the components of soluble COD in a biological reactor effluent.

It has been reported (Ekama *et al.*, 1986) that raw wastewaters may consist of biodegradable and nonbiodegradable organic compounds. The biodegradable organic compounds can be divided into two parts; one are soluble, readily biodegradable organics while the other part are particulate, slowly biodegradable organics. Nonbiodegradable organics can also be subdivided into two parts; soluble inert and particulate inert organics which can be seen in Figure 2.8.

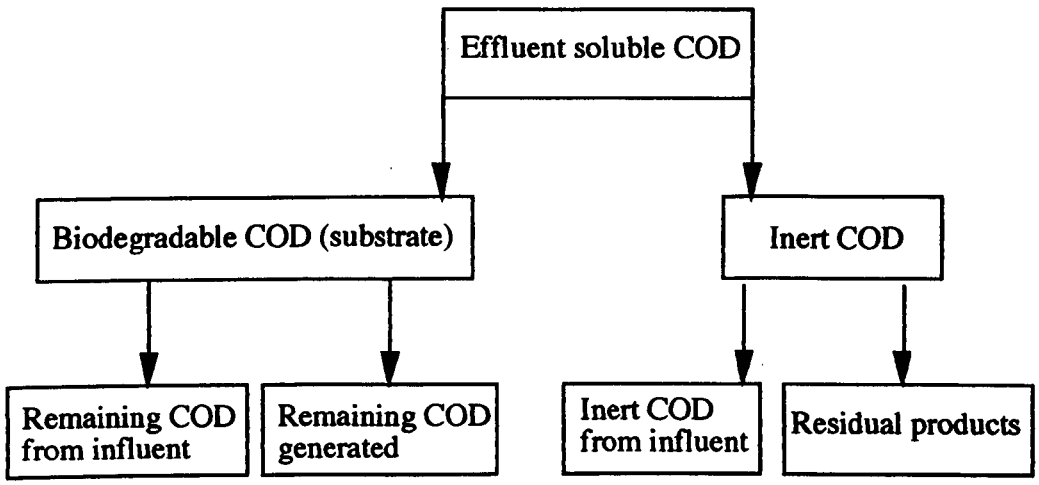


Figure 2.7 Components of soluble COD in biological reactor effluent

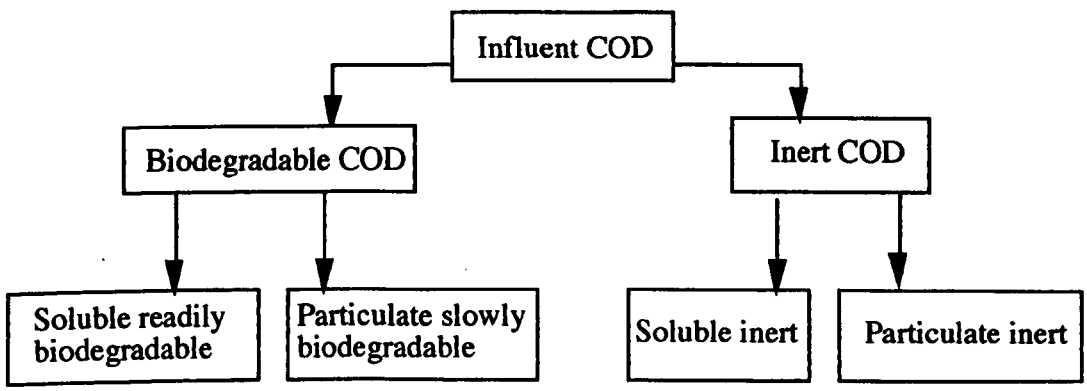


Figure 2.8 Influent COD fractions

Grady and Lim (1980) pointed out that the concentration of nonbiodegradable organics can be estimated by plotting the specific substrate utilization rate against the concentration of soluble organics, in which the curve will either pass through the origin if all the substrate is biodegradable or have a positive abscissa intercept equal in magnitude to the nonbiodegradable organic concentration. This estimation is based on the viable cell concentration.

Microbial products can also be estimated if the inert organic fraction of the feed is determined. Many studies have been carried out by Germirli (1990), Orhon *et al.* (1989), Henze *et al.* (1987), Ekama *et al.* (1986) in order to develop some techniques for the determination of the inert COD fraction of wastewaters. Two methods which are called the comparison method and the incremental method were reported from their works.

The models developed for conventional suspended growth systems are based on the fact that the soluble organics in the effluent have the same characteristics as those in the influent, however recent studies have shown that this is not necessarily the case since inert soluble organics are produced by microorganisms within the biological systems. Consequently, such mathematical models have been rearranged considering the microbial product formation concept.

Eckhoff and Jenkins (1967) developed the first model incorporating the concept of microbial product formation in which the effluent soluble COD at steady state consisted of residual substrate,S_S, and non-biodegradable metabolic end products,S_r ;

$$S_T = S_S + S_r \qquad (2.22)$$

S_r may be derived from the mass balance equation:

$$S_r = \alpha Y(S_{so}-S_s) \quad (2.23)$$

where S_{so} = influent substrate concentration (mass/unit)

α = growth associated product formation coefficient

Y = yield coefficient (mass/mass)

Daigger and Grady (1977) proposed a model which was based on the fact that the majority of the soluble organics in the effluent is not the original substrate but the products of microbial metabolism. This model assumes that all of the effluent COD is due to the microbial products. The product concentration, S_p, is given by the following expression:

$$S_p = \nu X\theta \quad (2.24)$$

where ν = net specific formation rate (defined as difference between the specific product formation rate and the product consumption rate)

X = biomass concentration (mass/unit)

θ = hydraulic retention time (mass/time)

Sykes (1981) introduced a model which is applicable only to treatment plants with high COD removal efficiencies. The total concentration of organic, S_T , in the effluent is mostly microbial excreta and is expressed as follows:

$$S_T = aY_gS_{TO} \quad (2.25)$$

where S_{TO} = influent COD concentration (mass/unit)

Y_g = observed yield coefficient (mass/mass)

a = growth associated product formation coefficient

The above equation shows that the effluent concentration is a function of sludge age and influent soluble COD concentration.

Rittmann et al. (1987) pointed out that the soluble microbial products are formed not only at a rate proportional to the rate of substrate utilization but also at a rate proportional to the concentration of biomass. The model may be shown as follows:

$$S_p = S_{PO}/(1+(k_{3M}X_A\theta/S_{PO}) \quad (2.26)$$

where S_{PO} = total soluble microbial product concentration (mass/unit)

S_p = soluble microbial product concentration (mass/unit)

k_{3M} = multiple substrate degradation coefficient for soluble microbial products

X_A = active biomass concentration (mass/unit)

It is also stated that sludge age is the fundamental parameter which controls S_p, because the product $X_A\theta$ is determined by the sludge age, θ_c.

Artan (1987) developed a model incorporating the soluble microbial product formation concept by modifying the model proposed by the IAQPRC Task Group. According to Artan's model, microbial residual products are generated in two different ways. One alternative is that a fraction of the slowly degradable particulate matter is hydrolysed to yield soluble microbial residual products while the remainder generates readily biodegradable substrate. The other alternative suggests that a fraction of the original substrate is directly converted into soluble microbial residual products by the growth mechanisms. The soluble inert product generation model is shown schematically in Figure 2.9. The dotted lines in Figure 2.9 indicate the growth dependent, soluble inert product formation alternative.

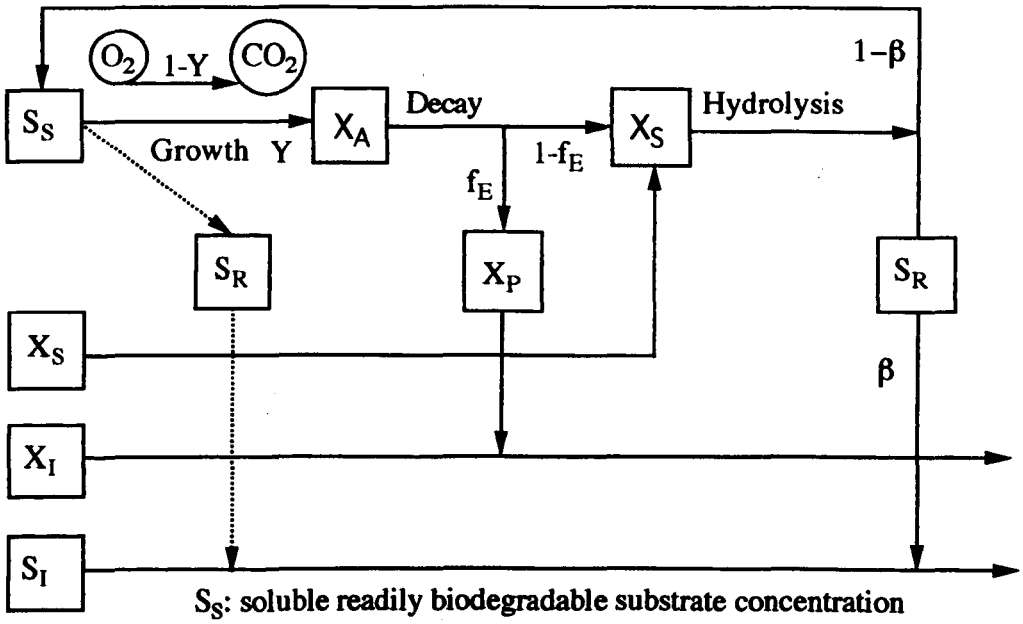


Figure 2.9 Process scheme for the soluble inert product generation

As mentioned above, there have been many studies carried out on both aerobic batch and aerobic continuous systems in order to determine the inert soluble COD production produced by biomass and many mathematical models incorporationg the microbial product formation concept have been developed. On the other hand there have not been any works reported on microbial product formation in anaeobic systems.

2.5. Crossflow Membrane Filtration

2.5.1. Crossflow Filtration

Crossflow filtration is a relatively new technique, and until now applied mainly to solute separation processes. This technique is also now used for particulate separation.

In the traditional field of separation of particulate matter, so called 'dead-end' filtration is predominant. Such filtration techniques generally caused medium blockage problems which were usually circumvented by relatively expensive and not always totally efficient methods (if applicable), such as the addition of filter aids or flocculants.

Generally, dead-end filtrations is not appropriate for filtration of very fine suspensions, nor for the production of a very pure filtrate. Suspensions entailing the formation of a compressible impermeable cake are a real problem. This is why two costly processes are usually applied for these purposes: the use of filter aids and the use of flocculants. Both these methods have also other disadvantages apart from the costs since they complicate the filtration process, they require space for storage and, as far as filter aids are concerned, they pose problems of disposal and contaminate the solids recovered.

Crossflow filtration is a complementary technique suitable in those fields of application where dead-end filtration is not appropriate i.e. for the filtration of very fine and very dilute suspensions without the addition of flocculants and filter aids, in cases of very exacting demand for purity of the filtrate, and when solids recovery is of secondary importance. The process is quasi-continuous, and the inherent properties of the filter medium are more relevant to filtration than in the dead-end process. Furthermore, crossflow filtration has opened new fields of application: separation of colloids, molecules and ions, as well as stable emulsions, depending on the nature of the filtering barrier.

'Crossflow' is perhaps not a sufficiently illustrative term. It should describe a process where the liquid to be treated flows parallel to the filter medium (Figure 2.10). Perhaps a better term would have been 'parallel filtration'. This method is basically a cake-free method (or is intended to be such). Its purpose is to prevent the formation of the cake. Particles deposited on the filter medium are swept away by the feed flow and the self-cleansing ability of the flowing liquid increases with its velocity.

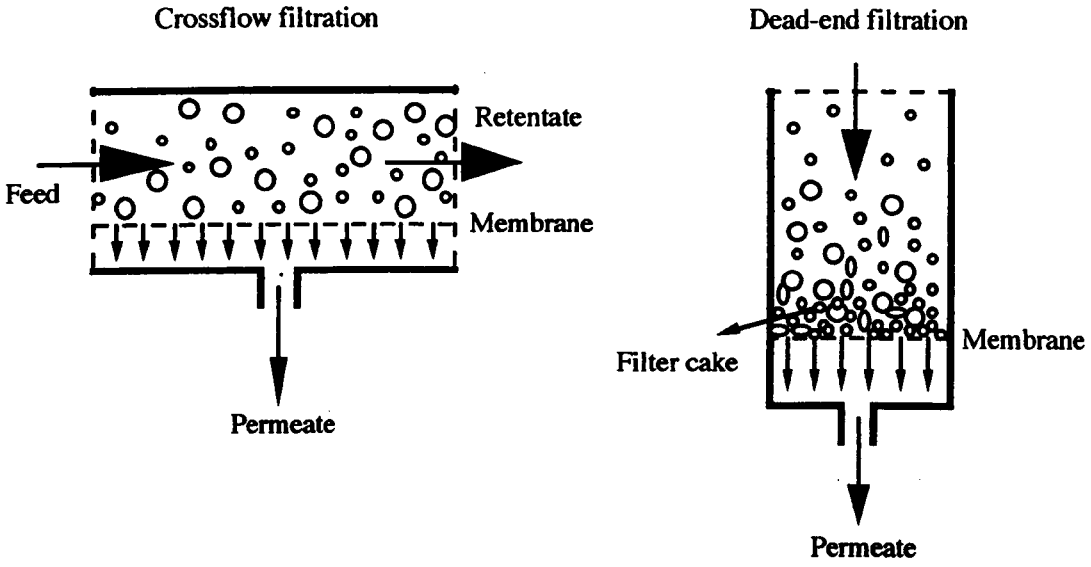


Figure 2.10 Principles of crossflow filtration

Crossflow filtration and membrane filtration have become nearly synonymous (Murkes, 1986). In principle they are not, since the term 'membrane filtration' refers to the type of filter medium and does not presuppose anything about the flow pattern. The term 'crossflow' refers, on the other hand, to the flow pattern and does not presuppose anything about the type of medium. In practice, however, crossflow filtration overlaps almost completely, with a few marginal exceptions, membrane filtration. In other words, membrane filtration is almost always carried out by crossflow, whereas crossflow can basically be carried out with any filter medium, but this would not be very practical.

2.5.2 Membrane Processes

A membrane is a selective barrier that permits some components to pass through it while at the same time preventing the passage of others. In particular, membranes may have the ability to retain large molecule compounds while at the same time allowing the solvent to pass through under the influence of a driving force.

It is the ability to reject particles of molecular dimensions that principally distinguishes a membrane from an ordinary filter. Another distinguishing feature is the fact that the driving force for a filter is always the pressure difference across the filter, which forces the water through while the suspended particles are retained. In the case of membranes there are a number of driving forces in addition to pressure including gradients in concentrations, electrical potential and temperature.

Figure 2.11 shows the pore size of three principal membrane separation processes used in water and wastewater treatment. The removal or rejection characteristics of a membrane are usually rated on the basis of the nominal pore size or the molecular-weight cutoff (MWCO) of the membrane. Microfiltration membranes (MF) are generally rated by pore size and are capable of removing micron sized (10^{-6}m) materials from liquids. Ultrafiltration membranes (UF) remove materials that are of the order of nanometer in size (10^{-9} m) or larger. Reverse osmosis (RO), or "hyperfiltration", membranes are capable of removing ion-sized materials such as sodium, chloride, calcium, and sulfate, as well as small non-polar organic molecules. Other membrane processes such as electrodialysis, pervaporation, and membrane distillation employ an electrical potential,

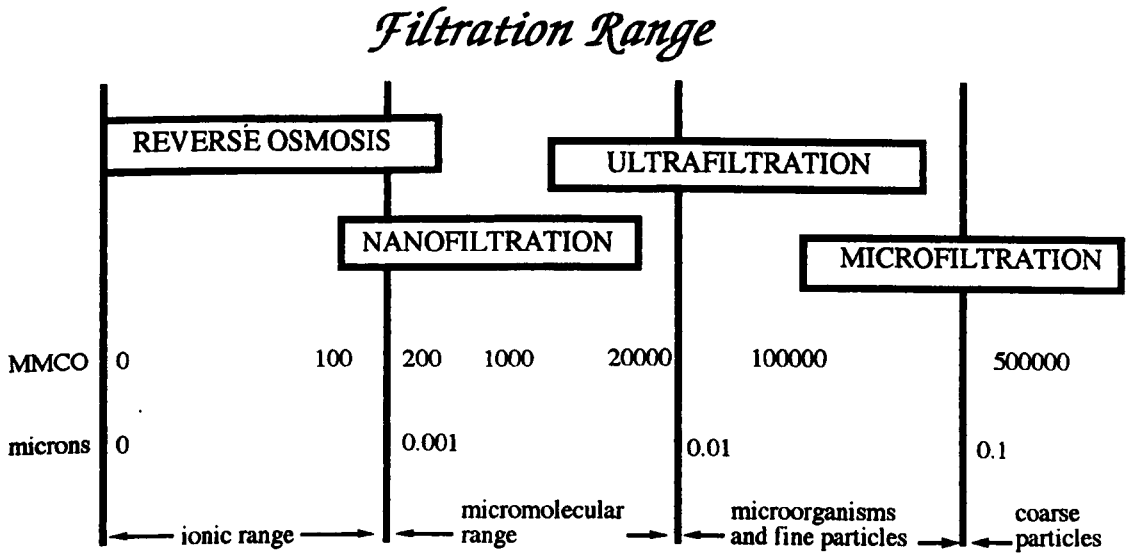


Figure 2.11 Molecular weight distribution of membrane processes

concentration, or temperature gradient, respectively, as the driving force compared to RO, UF, MF which are pressure driven processes of barrier separation. Comparisons of membrane processes are given in Table 2.9.

Table 2.9 Comparison of membrane processes

Ultrafiltration	Reverse osmosis	Microfiltration
Operates on difficult colloidal water	Requires extensive pre-treatment of colloids	Rapidly fouled by colloids giving high replacement costs
Low pressure operation (2-6 bar)	High pressure (10-30 bar)	Low pressure (2-4 bar)
Low energy consumption	High energy	Low energy
High recovery (up to 95 %)	Low recovery (50-80 %)	100% recovery
Chemical tolerance pH 1-13	pH 2-11	pH 1-13
High temperature operation possible 80 °C	45 °C max.	High temperatures possible
High resistance to oxidizing agents	Limited resistance to oxidizing agents	High resistance to oxidizing agents
Stream sterilizable membranes available	Stream sterilization not possible	Stream sterilization possible
Hygienic module designs available	Modules not as hygienic	Hygienic designs available

2.5.3. Membrane Configurations

Plate and frame membranes

The earliest membrane seperators manufactured were of the plate and frame type taking advantage of the flat sheet membrane material available. This configuration has been

refined over the years and is still successfully used today, although it is usually confined to difficult process applications where its advantages outweigh the relatively high capital cost involved (Figure 2.12).

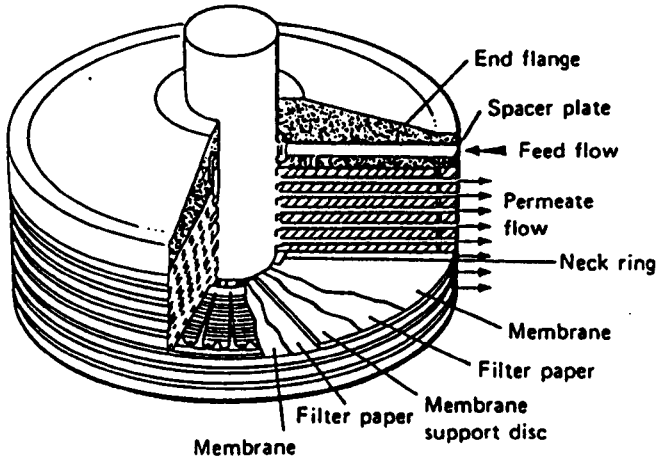


Figure 2.12 Plate and frame membrane

Spiral wound membranes

The spiral wound membrane element was developed to overcome the high cost of plate and frame systems and fits into a tubular pressure vessel. This system also offers savings in energy and space. The assembly is known as a module. Spiral wound membranes are made in standard sizes based on 100 mm, 150 mm, and 200 mm internal diameter vessels (Figure 2.13).

Tubular membranes

Systems were developed early in the history of UF and have found applications in a number of areas where ability to tolerate high levels of suspended solids is important. Polymeric tubular membranes are cast onto porous support media such as non-woven fabric or glass-reinforced plastic. Inorganic tubular membranes, however, are usually self-supporting due to the greater strength of the membrane material (Figure 2.14).

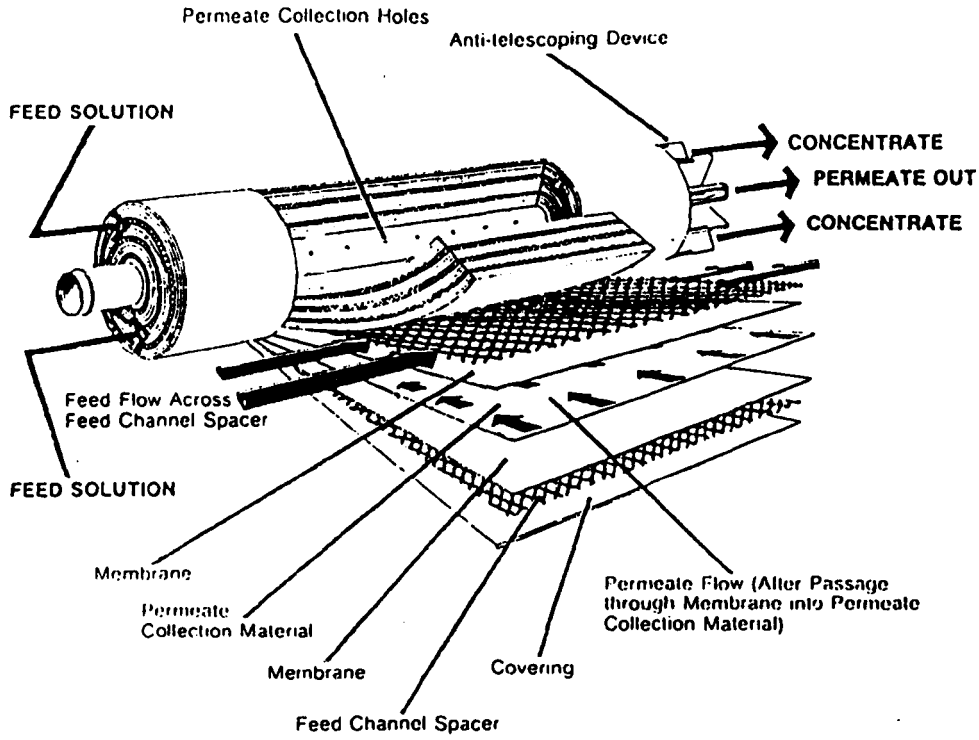


Figure 2.13 Spiral wound membrane

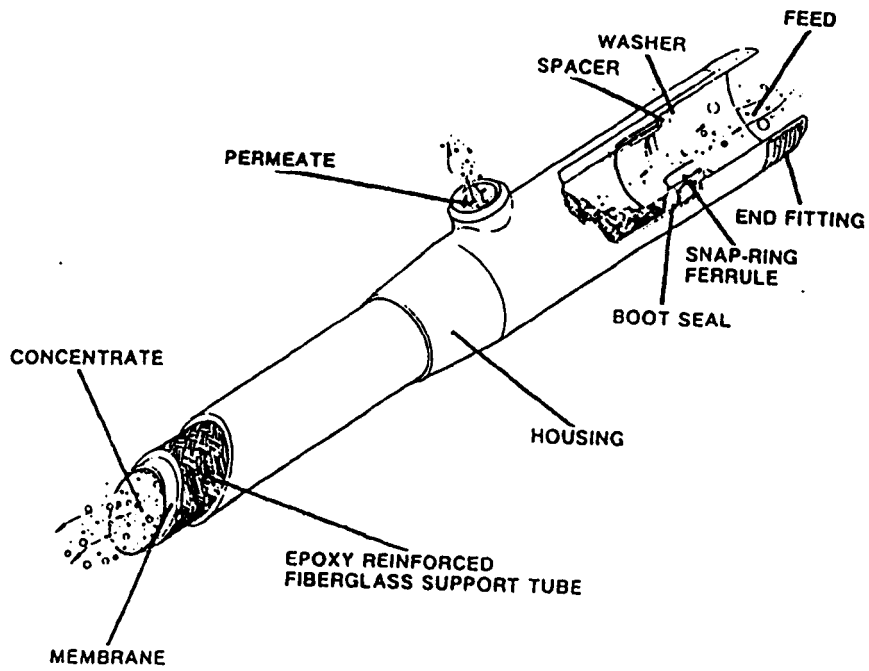


Figure 2.14 Tubular membrane

Hollow fibre membranes

These membranes were developed to increase the filtration area per unit volume. The structure is anisotropic, similar to flat sheet construction with the active membrane surface on the inside of the hollow fibre (Figure 2.15). Membranes are also available with a membrane layer on both the inside and the outside surface of the hollow fibre. The hollow fibres are self-supporting, enabling the use of backflushing for cleaning. This is the preferred technique in water and wastewater treatment and their low overall cost has led to a large number of installations.

2.5.4. Ultrafiltration Membranes

2.5.4.1. Process and its description

Ultrafiltration is a low pressure membrane separation process capable of removing high molecular weight dissolved materials, colloids, microorganisms and suspended solids from liquids.

Ultrafiltration membranes are characterized by a cross-flow of liquid over the membrane surface as opposed to the perpendicular flow of conventional filtration. This cross-flow prevents the built-up of a filtercake at the membrane surface and, therefore, helps to maintain the filtration rate (Figure 2.16).

2.5.4.2. Membrane Properties

The breakthrough in membrane preparation by Loeb and Sourirajen in the late 1950s paved the way for making ultrafiltration a practical process. In contrast to reverse osmosis, where cellulose acetate has occupied a predominant position, a variety of

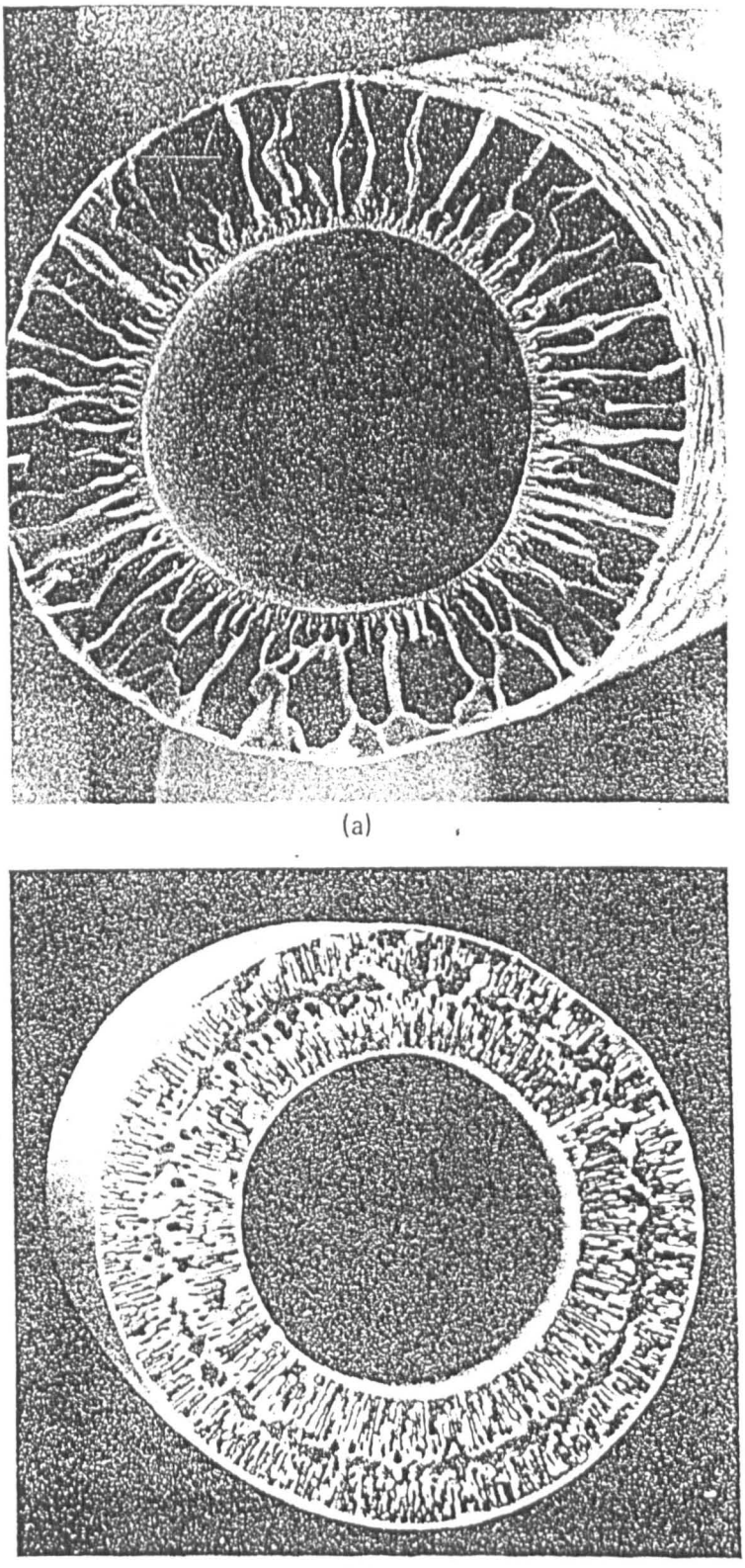


Figure 2.15 Photomicrograph of hollow fibre membrane (a) membrane layer on internal surface (b) membrane layers on internal and external surface

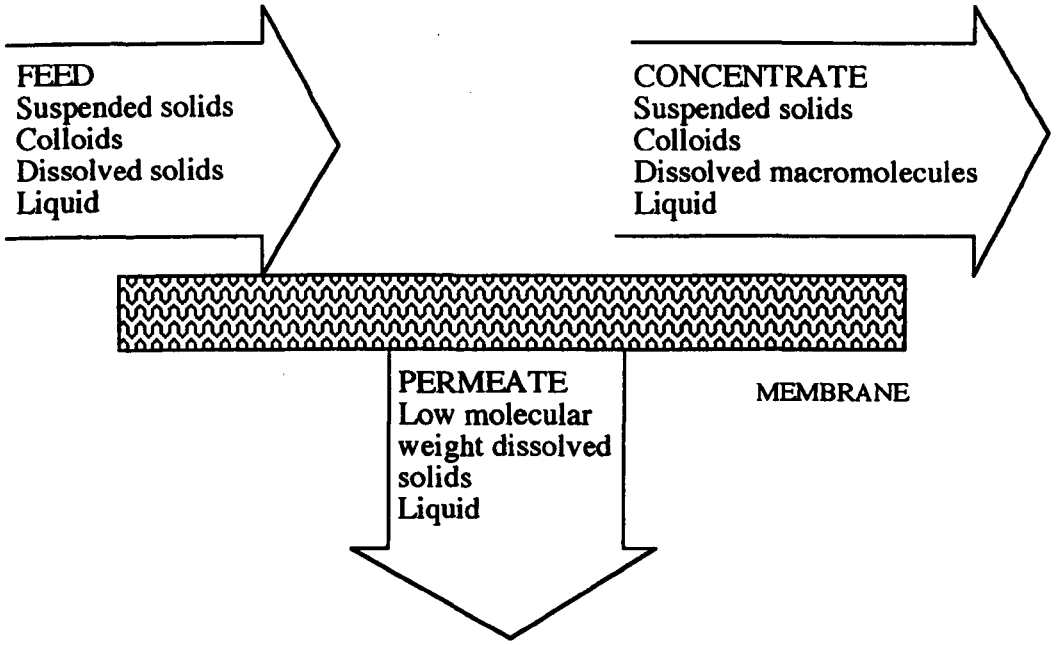


Figure 2.16 Diagram of ultrafiltration process

synthetic polymers have been employed for ultrafiltration membranes. Many of these membranes can be handled dry, have superior organic solvent resistance, and are less sensitive to temperature and pH. Membranes made from organic polymers such as polysulfone, cellulose acetate, polyamide, or polycarbonate are most common and offer the greatest degree of flexibility with respect to rejection characteristics and module design. The same polymer can be used, and the preparation can be varied, to produce membranes of different porosity. The different types of membrane constuction along with their materials and molecular weight cut-offs (MWCOs) are given in Table 2.10. Table 2.11 indicates some properties of typical commercial ultrafiltration membranes. MWCO is used as a measure of rejection. However, shape, size ,and flexibility are also important parameters. For a given MWCO, more rigid molecules are better rejected than flexible ones. Ionic strength and pH often help determine the shape and rigidness of large molecules.

Table 2.10 Some of UF membrane constructions along with their materials and molecular weight cut-offs (MWCOs)

Construction	Materials	MWCO
Hollow fibre	Polysulphone	6 000
Hollow fibre	Polysulphone	10 000
Hollow fibre	Polysulphone	20 000
Hollow fibre	Polysulphone	100 000
Hollow fibre	Polyacrylonitrile	13 000
Hollow fibre	Polyacrylonitrile	80 000
Hollow fibre	Polyamide	10 000
Hollow fibre	Polyamide	50 000
Hollow fibre	Fluoropolymer	200 000
Spiral wound	Polyacrylonitrile	10 000
Spiral wound	Polyacrylonitrile	20 000
Spiral wound	Polysulphone	3 000 - 100 000
Spiral wound	Polymeric organic	-
Spiral wound	Thin film composite	1 000 - 15 000
Spiral wound	Polyether sulphone	4 000 - 200 000
Spiral wound	Polyolefine	20 000
Spiral wound	Composite	20 000
Spiral wound	Fluoropolymer	10 000 - 200 000
Spiral wound	Polyvinylidene fluoride	10 000 - 200 000
Plate & frame	Polyvinylidene fluoride	4 000 - 200 000
Plate & frame	Polyether sulphone	4 000 - 200 000
Plate & frame	Polysulphone	3 000 - 200 000
Plate & frame	Polyacrylonitrile	4 000 - 200 000
Plate & frame	Cellulose triacetate	20 000
Tubular	Polysulphone	4 000 - 200 000
Tubular	Polyolefine	20 000
Tubular	Polyether sulphone	4 000 - 200 000
Tubular	Composite	20 000
Tubular	Zirconium oxide	10 000 - 80 000
Tubular	Polyvinylidene fluoride	4 000 - 200 000
Tubular	Polyacrylonitrile	4 000 - 400 000

Table 2.11 Some properties of typical commercial ultrafiltration membranes

Material	pH	Maximum Pressure (bar)	Maximum Temp. (°C)
Polysulphone	2-12	15	70
Polyarcylonitrile	2-10	10	60
Cellulose Acetate	3-6	25	30
Polyethersulfone	2-12	30	70
Fluoropolymer	2-12	10	60
Polyvinylidene fluoride	2-12	10	70
Polyvinylchloride	2-12	10	50

2.5.4.3. Concentration Polarization

The membrane flux varies with the type of membranes and the operating conditions such as crossflow velocity, transmembrane pressure, and particularly the degree of concentration polarization. As the permeate passes through the membrane, a concentration gradient is established between the membrane surface and the bulk solution. The concentration polarization results in a layer of highly concentrated solution of retained solute on the surface of the membrane. This gel layer retards the flow of ultrafiltration. The mechanism can be explained as follows.

Due to the convective flux through the membrane a concentration of the solution at the surface C_w increases and eventually reaches a gel formation concentration C_g (see Figure 2.17). The concentration decreases away from the membrane surface and its value in the bulk of solution is C_b. The flux through the membrane F_w^o depends on a concentration according to the relationship:

$$F_w^{\,o} = k.\ln\frac{C_w}{C_b} \qquad (2.27)$$

where k = back diffusion mass transfer coefficient

C_w = solution concentration at membrane surface

C_b = bulk concentration

C_g = gel formation concentration

As C_w becomes equal to C_g and therefore constant, the flux J will become the function of solution concentration only and will vary in proportion to ln C_b.

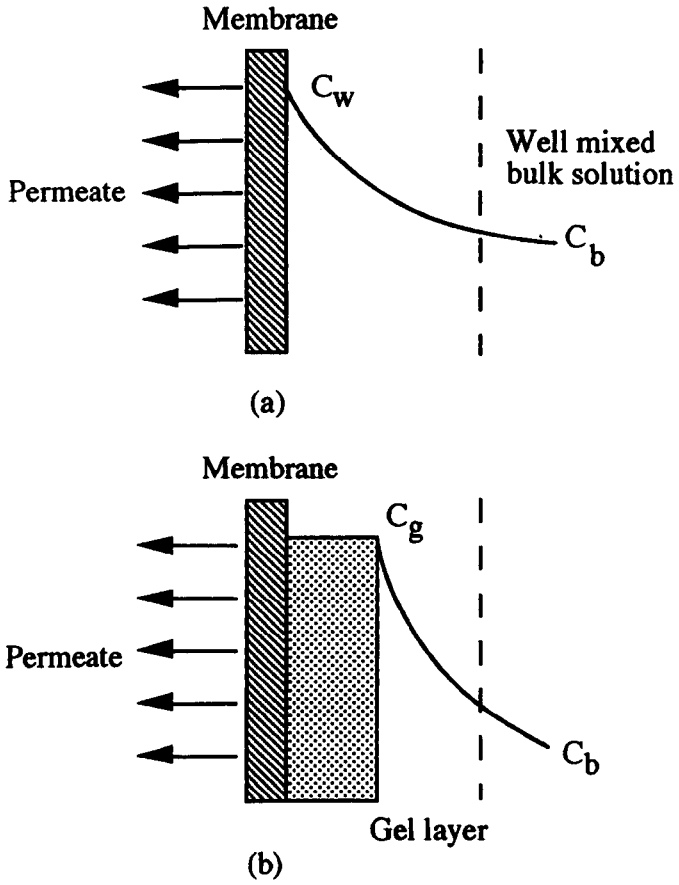


Figure 2.17 Schematic representation of (a) concentration polarization (b) gel layer formation

There are a number of correlations for the mass transfer coefficient, the most widely used being the Dittus-Boelter relationships, namely:

$$k=aV^{0.33} \quad \text{for laminar flow} \tag{2.28}$$

$$k=bV^{0.80} \quad \text{for turbulent flow} \tag{2.29}$$

where a,b are system constants and V is feed velocity.

The flux is, on the other hand, related to the pressure. Figure 2.18 illustrates the effect of increasing the pressure differential across the membrane upon the flux.

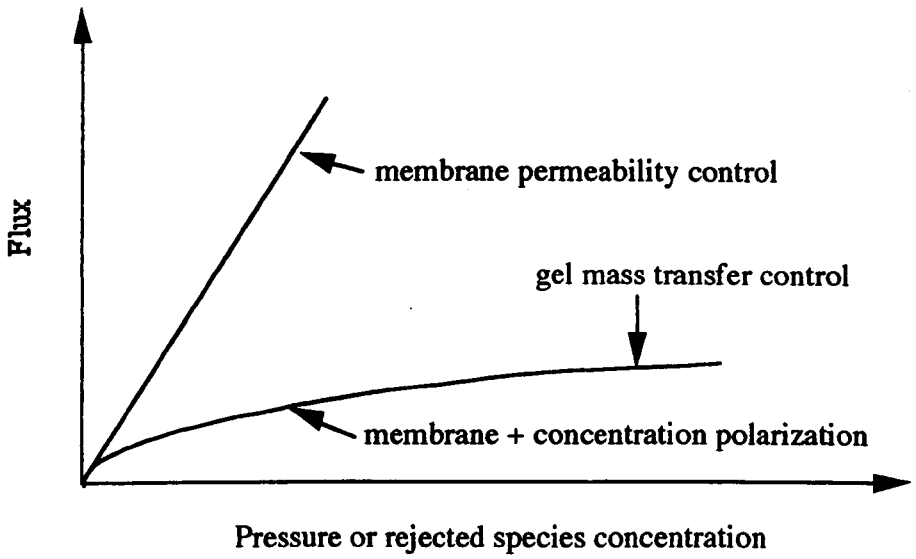


Figure 2.18 Effect of pressure differential upon membrane flux in ultrafiltration

Initially, the flux increases with the pressure drop then the solids start to build up adjacent to the membrane surface; and the rate of flux increase with ΔP begins to level off. When the concentration next to the membrane exceeds a critical value, a gel forms. The flux is then determined by two flow resistances in series:

$$F_w^{\,o} = \frac{\Delta P}{R_g + R_m} \tag{2.30}$$

where R_g = hydraulic resistance of the polarization layer

R_m = hydraulic resistance of the membrane

R_g, is primarily a function of the rate at which gel material can back-diffuse into the mainstream of flow. Since this is not influenced by the pressure drop across the membrane, membrane flux reaches a plateau. Often operation at too high a pressure will consolidate the gel layer and the flux will actually be lower than it would be at lower pressure. By combining Eqs. 2.27 and 2.30 the following relationship can be obtained:

$$\ln \frac{C_w}{C_b} = \frac{\Delta P}{(R_g + R_m)k} \tag{2.31}$$

As long as concentration C_w is less than C_g, C_w will increase with pressure, but the moment C_w equals C_g an increase in ΔP brings about an increase of the layer resistance R_g, and the flux will no longer vary with pressure. The R_g increase will be brought about by either layer compaction or the growth in its thickness, or both.

Concentration polarization is usually minimized in ultrafiltration by operating at high feed velocities parallel to the membrane surface and by utilizing thin channel designs. High temperature operation reduces the solvent viscosity, usually increases the back-diffusion rate of gelled material, and increases the critical gel concentration.

2.5.4.4. Membrane Fouling and Control

Membrane separation is a relatively new science, and many of the processes that take place at the membrane surface and inside the pore structure during filtration are still very obscure. The phenomenon of membrane fouling in particular has been the subject of many investigations (Le, 1984 ;Suki, 1983 ; Hiddink, 1980 and Hayes, 1974). Generally, fouling occurs with all types of feed, but proteins and colloids are the most notorious foulants (Le, 1984). Biological suspensions on the other hand are exremely complex mixtures which make the task of characterising their fouling behaviour almost out of the question.

In RO, UF are MF membranes, foulants are carried by convection to the surface of the membrane. A number of mechanisms have been suggested by which these materials may be transported away from the membrane as a function of the foulant's particle size (or molecular weight). If transport to the membrane is greater than back-transport, an accumulation of foulants near the surface of the membrane may result. This phenomenon, referred to as concentration polarization, may increase the resistance to flow across the membrane and reduce the permeate flux.

Reductions in flux caused by concentration polarization are typically reversible through hydrodynamic perturbations such as flow reversal (back flushing) and pulsing. The term membrane fouling is primarily reserved for the description of "irreversible" losses in the transmembrane flux that cannot be recuperated hydrodynamically or chemically (Figure 2.19).

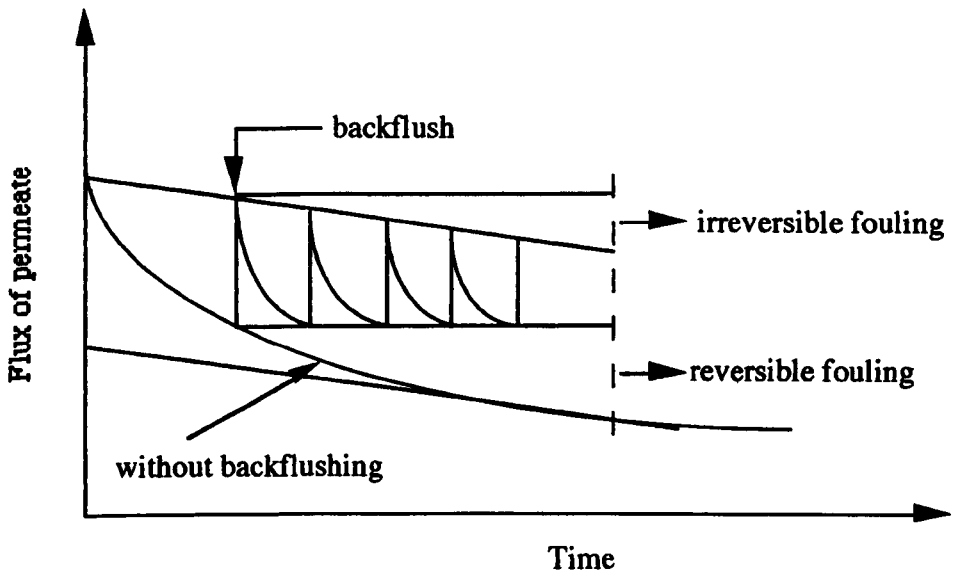


Figure 2.19 Permeate flux versus time indicating losses in permeate flux due to fouling

Materials that enter membrane pores, become strongly sorbed to the membrane surface, or both, may reduce the effective number or diameter of membrane pores. For cases in which this occurs, a long-term trend of decreasing flux as a result of fouling may be

observed. Fouling will be affected by the degree of concentration polarization and in turn the size of foulant in the feed water.

Microorganisms can also contribute to fouling. The deposition and growth of microorganisms on membranes can result in a layer of material that increases resistance to permeate flux. In addition to the resistance caused by the bacteria alone, the microbial "cake" may be an effective filter for retaining smaller colloidal materials on the membrane surface and further reducing permeate flux. Microorganisms also release organic solutes that are large enough to be retained by the membrane and form a gel layer. If these layers are easily removed by backwashing or simple chemical treatment, the reduction in flux is operationally reversible. The soluble products released by microbes may, however, adsorb on to membrane surfaces. Depending on the strength of the adsorbtive interaction and the location at which adsorption occurs, it may not be possible to recuperate losses in flux. In addition, cellulosic membranes may be degraded by bacteria resulting in a decrease in the rejection characteristics of the membrane and a poorer quality of product.

Many investigations have been carried out in order to find an efficient method for dealing with these difficulties. The methods investigated have intended the use of foam balls to clean tubular membranes, vibration, flow pulsation, rubbing the surface by adding particulate material to the washing liquid, washing with different detergents, backwashing with pure water or with filtrate and osmotic backwashing.

All these depolarization and cleaning procedures complicate the plant lay out, and bring about a higher consumption of water, energy and chemicals. They also significantly reduce the net operation time. Worst of all, they are seldom sufficiently efficient. It is thus easily understood that developing more productive membranes with inherently higher flux is of little practical importance as long as the fouling problem cannot be solved adequately.

It appears that the most efficient and natural way to combat deposits and concentration polarization is to increase the flow velocity in order to enhance the shear force. In practice, however, the velocity increase is limited to a value which is not high enough. Higher flow velocities bring about higher pressure drops through the membrane module, which in turn entails lower pressure available for filtration and often makes it impossible to connect modules in series. Furthermore, higher flow velocities necessitate bigger and more expensive circulation pumps, higher energy consumption and lower recovery of filtrate (ratio filtrate/feed flow).

There are basically three methods that can be applied separately or, if feasible, jointly to solve these serious problems.

(i) prevention of deposit formation by carrying out filtration in an electrical field,

(ii) using so-called dynamic or secondary membranes, and

(iii) generating sufficiently high-shear forces.

2.5.4.5. Design Considerations

To minimize capital investment for membranes, as well as the operating cost of membrane replacement, it is obviously desirable to operate at as high a membrane flux as possible. In other words, the main criterion used in the design of any crossflow membrane filtration process is based on an overall minimum energy input/volume of permeate (flux) pruduced.The desirable features are;

(i) high flux,

(ii) back-flushable,

(iii) high membrane area / volume ratio allowing low pressure drop whilst

maintaining high tangential velocities,

(iv) easy installation and continuous operation with minimum supervision,

(v) low operating pressure,

(vi) easy maintenence and simple membrane replacement,

(vii) low energy consumption, and

(viii) relatively low capital costs.

The process design for an ultrafiltration membrane unit essentially involves finding the total membrane area required for treating a wastewater flow. This requires the determination of the membrane flux (J), or permeate flow rate. The membrane area can then be calculated from the relation:

$$A = \frac{Q}{J} \tag{2.32}$$

where A = total membrane area required,

J = average flux of the membrane, (flow rate per unit membrane area),

Q = influent flow rate, (volume per time),

Design computations for a variety of systems show that no single equipment and process design is optimum for all ultrafiltration applications. Rather, it is necessary to obtain pilot-plant information on flux and retention for the specific separation of interest to provide the basis for determining the optimum process design.

2.5.4.6. Factors Affecting Crossflow UF Membrane Filtration

Flow Velocity and Membrane Flux

Flow velocity is of fundamental importance for the performance of any crossflow membrane filtration. Should the flow velocity become zero, the crossflow stops and

dead-end filtration starts. The cake formed on the membrane at zero velocity becomes thinner when the flow velocity, parallel to the membrane, increases. The thickness of the cake layer in a circular channel with diameter d is determined by shear force γ generated by the liquid flowing with a velocity v :

$$\gamma = \frac{8v}{d} \tag{2.33}$$

Higher velocities entail a thinner deposit layer, lower hydraulic resistance and therefore higher filtrate flux. These phenomena are well known in the field of ultrafiltration.

An infinite velocity would ideally be expected to give a zero layer thickness and correspondingly constant flux, which would depend on the inherent resistance of the medium only. In practice the flux is never really constant, but above a certain velocity of flow the flux improvement is small or virtually non-existent. An economic velocity optimum should be assessed in every individual case. Exceeding this optimum value would entail an unnecessary energy expenditure. The optimum velocity varies very much depending on the liquid filtered: sometimes it is quite low, sometimes very high. The flux decline occurs at any velocity of flow, mainly because of the internal plugging of the pores. This plugging accounts for the flux decline even if the medium is totally clean on the surface.

Plugging of Membrane and Membrane Flux

Larger pores facilitate the penetration of small particles inside the pores and, therefore, they promote the internal clogging. Murkes and Carlsson (1988) pointed out that from this point of view it is in most preferable to use micropore media with a pore size below 1 micron. This fact explains the paradoxical behaviour of media during crossflow membrane filtration. Opposite to that which is normally valid in traditional filtration, more

open media usually yield, after a certain initial time, a lower filtrate flux owing to a higher degree of internal clogging. On the other hand, too large pores can not retain the very small particles and the filtrate may then not be absolutely particle free.

It has been reported that in many cases the skinned semipermeable membranes for ultrafiltration give just as high or still higher flux than the much open microporous media, in spite of their intrinsically much lower hydraulic resistance. The reason is that the semipermeable membranes cannot, obviously, be penetrated by particles as are the microporous media.

In spite of the fact that filter media with rather large pore size can be used in crossflow membrane filtration, especially in combination with an intentionally deposited layer of filter aids. Specific advantages of this technique are obtained when the filtration objective is to get a totally clear filtrate with the highest possible stable flux. This can be done by means of sufficiently large shear forces generated near the filter medium surface so that the thickness of the deposit on the membrane is minimized.

Thus, the main factors which govern the performance of crossflow UF membranes may be summarized as follows:

(i) pore size, or tightness of the medium,

(ii) generated shear force at the surface of the medium, and

(iii) features of the deposited layer and control of its formation.

Formation of a Deposit Layer

It would be expected that at equilibrium the hydraulic resistance of the deposited layer would at least theoretically be constant, but this is, however, seldom the case in practice. The flux is almost always declining at a very slow rate, even when high shear forces are generated.

Barker et al. (1985) investigated this phenomenon in crossflow membrane filtration of finely dispersed $TiO_2.2H_2O$ powder in water. Ceramic microporous tubes were used with a pore size of 0.2 micron and the crossflow velocity was 3 m/s, which is rather a high figure for this type of filter. The specific resistance of the deposited particle layer increases with time entailing a flux decline. This fact was found to be a result of a continuous enrichment of the cake layer with fine particle fractions. Thus, it is understandable that the flux decline occurs in spite of the constancy of the cake thickness. Other research workers have corroborated these findings. Fischer *et al.* (1986) found that there occurs a selective deposition of particles on the surface of a crossflow channel. The particles constituting the layer were finer than in the original suspension which means that there is a fourth factor of importance in crossflow membrane filtration namely the particle size distribution in suspension. Especially important are the colloids in the feed to the filter because of their detrimental influence on flux and on flux decline (Mietton 1984).

Inlet Concentration

Some authors report experimental findings indicating the validity of a logarithmic relationship between the inlet concentration of the dispersed phase and the magnitude of the flux (the relationship is given in section 2.5.4.3). Other authors doubt it since, in crossflow membrane filtration, the mass transfer does not occur, or not only, through diffusion. Whatever the case, it can be said generally that crossflow membrane filtration is relatively poorly sensitive to concentration. The curve of flux versus concentration is individual and differs for various applications. For separation of oil emulsions, for instance, the flux decline is rather slow up to quite high concentration values of around 10-15% oil. For higher concentrations the flux decline is much faster.

Temperature and Pressure

As far as other relevant parameters are concerned, there is no difference from other filtration methods. Higher temperatures entail lower viscosities and are, of course, beneficial provided they do not harm the product. Crossflow UF membranes are not more temperature-resistant and can not be operated at relatively more elevated temperature than microfiltration membranes.

Pressure is also an important parameter in order to achieve a higher flux. An increase in pressure does not entail a proportinally higher flux. After a certain pressure has been exceeded (usually between 1 and 5 bar) the opposite may occur: a further pressure increase may bring about a compaction of the secondary layer and, thus no increase in flux.

Some Simplified Qualitative Relationships

Murkes and Carlsson (1988) referred to a few qualitative relationships which were corroborated experimentally. These are given below.

- Flux J as a function of flow velocoty v :

$$J = \text{conct.}\, v^{3/2} \qquad \text{for } v \to 0 \tag{2.34}$$

and

$$J = \frac{B\Delta P}{\mu I_o} \qquad \text{for } v \to \infty \tag{2.35}$$

These relationship are shown in Figure 2.20.

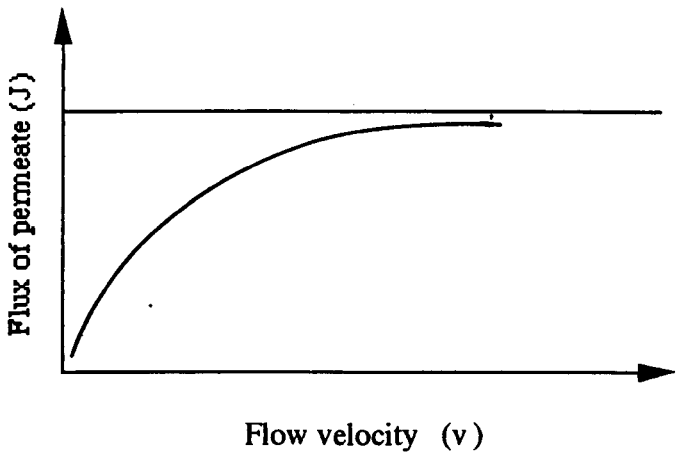


Figure 2.20 Permeate flux versus flow velocity in crossflow ultrafiltration

- Flux J as a function of the hydraulic diameter of the channel D:

$$J = \frac{k_1 \Delta P}{k_2 \Delta P D + I_o} \tag{2.36}$$

$I = f(D)$ is illustrated in Figure 2.21.

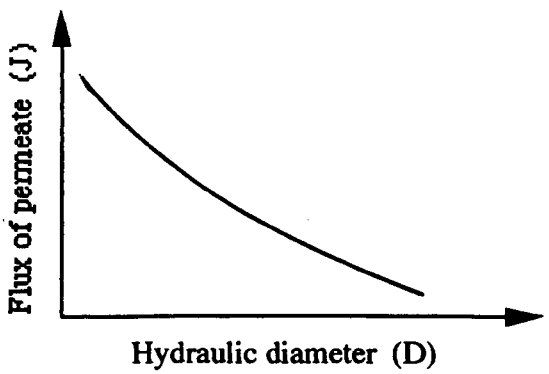


Figure 2.21 Permeate flux versus hydraulic diameter in crossflow ultrafiltration

- Deposit thickness I as a function of flow velocity v :

$$I = const.\Delta P / v^{1.5} \quad \text{for} \quad v \to 0 \tag{2.37}$$

$$I = const.\Delta P / v^{2} \quad \text{for} \quad v \to \infty \tag{2.38}$$

$l = f(v)$ is shown grafically in Figure 2.22.

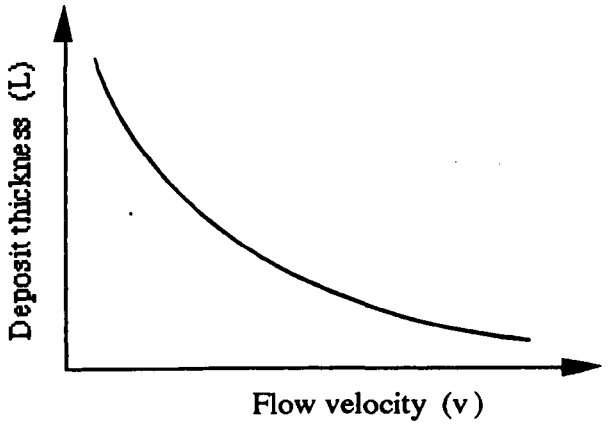


Figure 2.22 Deposit thickness versus flow velocity in crossflow ultrafiltration

In these formulae I_0 is the cake thickness having a resistance equal to the resistance of the filter medium and B is the permeability of the equivalent cake. ΔP is the pressure drop across the cake and μ is the liquid viscosity.

2.5.5. Applications of Membrane Technologies in Wastewater Treatment

Membrane separation processes and membrane reactors are today a well established technological area characterized by several separation processes already operating in a wider variety of cases than any other existing technique. The integration of membrane operations with traditional technologies or the design of new productive cycles based mainly on membrane operations is becoming an attractive area of engineering research.

Potentially, ultrafiltration is one of the most promising membrane applications among the other membrane processes such as reverse osmosis, microfiltration and electrodialysis, because apart from its role in the water field it also has wide potential application for in industry.

The applications of membrane technologies in pilot- and full-scale systems for treatment of wastewaters deriving from maize processing, electroplating, oil-field brines, brewery, wool scouring, textiles, abattoir, mining, pulp and paper, power generation, pig farming, sugar industry, domestic use, night-soil, starch fermentations, fertilizer manufacturing and water works sludges were widely studied by Anderson *et al.* (1986), Bindoff *et al.* (1987), Buckley (1992), Calabro *et al.* (1990), Chaufer and Deratani (1988), Chiemchaisri *et al.* (1992), Choate (1983), Drioli and Molinari (1990), Ekengren *et al.* (1990), Fane *et al.* (1992), Gibson *et al.* (1981), Le (1987), Molinari and Drioli (1988), Plessis and Swartz (1992), Rose *et al.* (1992), Ross *et al.* (1992), Ross *et al.* (1990), Ross *et al.* (1988), Saw *et al.* (1985), Schoeman *et al.* (1992), Squires (1992), Strohwald and Jasobs (1992), Strohwald and Ross (1992), Strohwald (1991) Townsend (1991), Zaidi *et al.* (1992), Zaidi *et al.* (1991).

2.6 Combined Anaerobic Contact Reactor - Crossfow Ultrafiltration Membrane System

2.6.1 Anaerobic Contact Reactor and Its Limitations

Various anaerobic digester designs have been advocated for the full-scale treatment of industrial effluents with a view to obtaining high biomass levels, longer sludge retention times and shorter hydraulic retention times, which are the key economic factors. The classification of digester configurations according to the prevailing principle of solids-liquid separation and biomass retention is summarized in Table 2.12. Among them the anaerobic contact digester was the first advanced anaerobic treatment technology.

The anaerobic contact reactor comprises a continuously-fed, completely mixed reactor stage followed by solids/liquid separation (see Figure 2.23). A degasification step is

frequently included in the system design. The effluent is discharged from the settling device and the settled biomass returned to the digester vessel where it is mixed with the incoming feed.

Table 2.12 Classification of full-scale digester configurations according to the prevailing principle of biomass retention

Principle of Biomass Retention	Digester Design	References
External settling and return of suspended sludge	CONTACT DIGESTER	Schroepfer et al., 1955 Steffen and Becker, 1961
Internal settling of suspended and granular sludge	CLARIGESTER PROCESS	Hemens et al., 1962 Stander, 1966
Internal settling of granular sludge	UASB PROCESS	Letinga et al., 1979
Immobilisation of biofilm on stationary packing media	UPFLOW FILTER PROCESS	Young and McCarty, 1969 Taylor, 1972
Immobilisation of biofilm on stationary packing media	DOWNFLOW FILTER PROCESS	Van den Berg et al., 1979
Immobilisation of biofilm on carrier-assisted non-stationary surface	FLUIDIZED BED PROCESS	Jeris et al., 1977
Immobilisation of biofilm on carrier-assisted non-stationary surface	EXPANDED BED PROCESS	Switzenbaum and Jewel, 1980
Separation and recycle of bacteria by ultrafiltration membranes	MEMBRANE ASSISTED PROCESS	Choate et al., 1983 Ross et al., 1990

The bacteria in an anaerobic contact reactor occur as suspended flocs and the system is maintained in suspension by mechanical stirring, gas sparging or recycle. Inert particles in the feedstock may act as media to convert the reactor to the carrier-assisted contact process, but in general the bacteria must form flocs to remain in the system. Separation of

flocs and treated wastewater occurs in a separator assembly such as a sedimentation tank (see Figure 2.23) from which the suspended settled flocs are recycled to the reactor at moderate rates to prevent shear forces from disrupting the floc structure. This separation of solids and liquids is a crucial operation in the anaerobic contact digester and the removal of gas-producing particles is difficult. Gas-stripping or cooling of the effluent en route to the separator may counteract the problem: a shock temperature reduction from 35-15 °C arrests gas production in the settler and enhances the flocculation of solids. The latter can also be achieved by the use of cougulants such as sodium hydroxide, followed by ferrous chloride. Lane (1984) detailed problems in the separation of solids from discharged mixed liquor, because of the continuation of gas production in the settler due to high ambient temperatures. The application of a vacuum to the settling tank feed will degasify the liquid, however, and reduce the problem. The sludge concentration in the anaerobic contact reactor rarely exceeds 5-10 g/l VSS, and the superficial liquid velocity in the settler should not be greater than about 1 m/h to allow for sufficient settling of flocculated sludge.

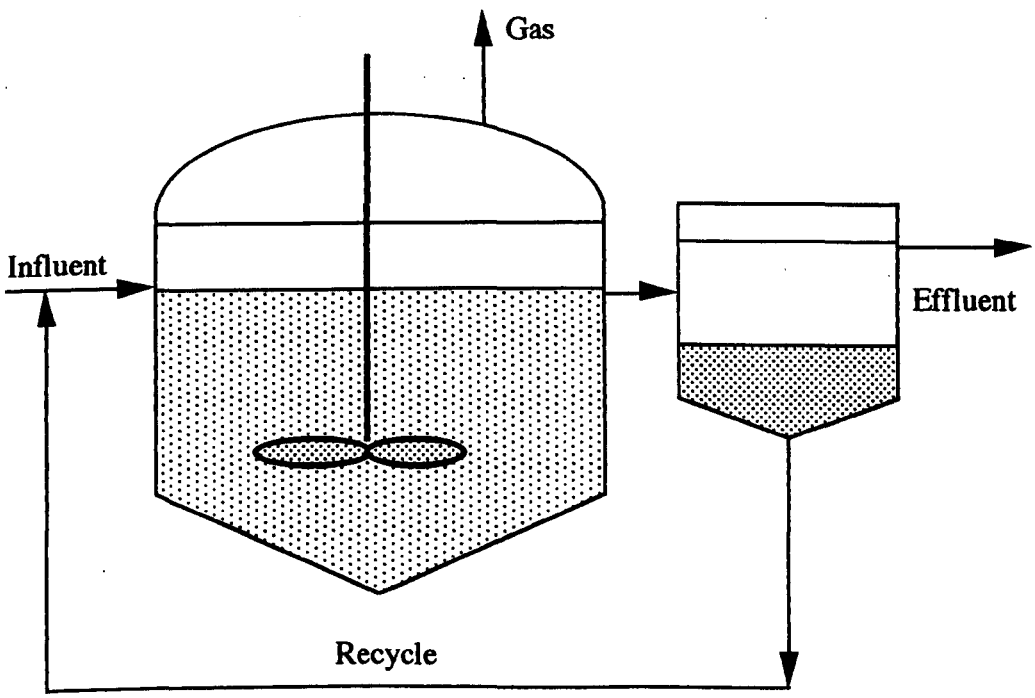


Figure 2.23 Schematic drawing of anaerobic contact reactor system

The anaerobic contact process was initially developed to treat meat packing waste and has subsequently been used for food-processing and other wastes. The process was one of the first of many anaerobic digestion systems to incorporate the retention of microorganisms in the digester independent of HRT. Instability in the contact process can be attributed to inactivation of the digester biomass, waste composition and also indirectly to the retention time of solids materials. The fruit and vegetable processing industries, for example, produce wastes rich in carbohydrates; and the resulting fluctuations of pH have detrimental effects on the slower-growing methanogenic reactor population.

A pilot plant anaerobic contact system designed by Lane (1984) employed a tank of working capacity 23 m^3, with a floating gas holder similar to those in use with conventional continuously stirred tank reactor (CSTR) systems, of capacity 10.5 m^3. The gas produced in the anaerobic digestion process was used, at a rate of 20 l/min, to agitate the digester contents by passage through a draught tube apparatus. The latter was provided with a jacket through which water was circulated at 12 l/min, to maintain the reactor temperature at 36 ± 2 °C. Discharge of the mixed digesting sludge from the digester to the settling tank occured by gravity flow. The disadvantages of the design included the tendency of the sludge to float, leading to difficulties in solids recycle: this was the result of continuous gas evolution in the settler unit, with gas bubbles adhering to flocs and causing them to rise in the vessel. The thermophilic contact process employed by Schlegel and Kalbskopf (1981) also manifested sludge flotation, but at the elevated temperatures of the system poor sedimentation was ascribed to the lack of flocculation of the digester biomass and low biomass production. Sludge settling characteristics tend to deteriorate at sludge loading rates in excess of 0.25 kg COD/kg VSS.d, and biomass separation from the medium becomes more difficult above a mixed liquor VSS concentration of 18 g/l ; gas production in the sedimentation vessel enhances these limitations (Mosey , 1981).

The contact process has been succesfully employed at small scale for the treatment of animal wastes (Stafford, 1983) although minimal HRTs of 12-15 days were nesessary for the particular feedstock type. COD reductions of between 90 and 95% can be achieved in wastewaters with COD values in the range 2-10 g/l when high volumetric loadings are applied (Mosey, 1981). BOD_5 loadings of 0.44-2.5 kg /m^3.d are frequently found with HRTs of between 0.5 and 5 days, and 70-98% BOD removals have been reported although lower operational efficiency in the treatment of domestic sewage was observed. The anaerobic contact process is reported generally for the treatment of high strength wastewaters, as the protracted retention times necessary for the conversion of dilute wastes renders the system impractical: secondary treatment of effluent may be necessary, and problems in sludge separation have been recorded. However, a total organic carbon (TOC) removal efficiency of 90% at organic loadings of 2 kg TOC/m^3.d of dilute wastewater (1 g/l) was reported at HRTs of 3-6 h in an anaerobic contact digester, the optimum loading rate being 1.7 kg TOC/m^3.d (Heertjes and Meer, 1979).

Relatively high loading rates of 10 kg/m^3.d of complex wastes such as rum stillage have been applied to contact digesters; these contained little readily biodegradable carbohydrates. Low loading rates of around 2 kg/m^3.d were found to be necessary for the effective treatment of carbohydrate-rich potato wastes, and frequent reinoculation of bacteria into the system was required (van den Berg and Lentz, 1980). COD removal efficiencies of 70-90% were claimed for all the high strength wastes tested; these were found to depend upon settling efficiency but not upon the SS concentration of the waste. Low settleability caused a high SS content and therefore high COD values in the effluent.

Effluents from starch manufacturing processes, wine distillation and yeast production, at COD values of 10, 22, and 45 g/l respectively, were treated by a reversed-flow type clarigester. Loading rates of 2.4 kg COD/m^3.d (24 °C), 3.2 kg COD/m^3.d (33 °C) and 4

kg COD/m^3.d (35 °C) respectively were applied to the system and COD reductions of 97% and 80% achieved. The reactor was limited by the control of sludge return, as the solids in the digester were required to settle back into the reaction vessel under the influence of gravity. Sludge-wasting was considered unnecessary as solids-loss in the effluent was balanced by synthesis of biomass.

2.6.2. Development of Membrane Anaerobic Contact Reactor System and Its Advantages

The application of membrane technologies to anaerobic wastewater treatment systems arose from the need for the retention of an adequate concentration of active biomass in a digester since this is one of the major problems encountered in many anaerobic systems.

A higher biomass concentration will give, not only a good digester performance, but also a better quality effluent, while a lower biomass concentration will result in a longer solids retention time (SRT), thus requiring a larger volume of reactor and consequent higher capital costs.

As discussed above various methods of biomass retention have been developed for a range of reactor configurations with the anaerobic contact process being the first of many advanced anaerobic digestion technologies. The major difficulty encountered in the full-scale application of this process was the settlement of biomass in the sedimentation tank, especially where the mixed liquor suspended solids (MLSS) concentration was greater than 10 g/l. This has restricted its application in the treatment of high strength industrial wastewaters for economic reasons.

The application of membrane technologies makes it possible to eliminate final settling tanks, thus minimizing biomass separation problems, especially for the treatment of high strength industrial wastewaters and for high rate treatment systems.

The use of membranes as biomass separators in anaerobic digester systems treating industrial effluents was pioneered in the early eighties by Epstein and Korchin, (1981) and Choate et al., (1983) resulting in the development of ultrafiltration membranes in combination with anaerobic digesters (by Dorr-Oliver) known as the MARS process, but this process has never been employed in full-scale installations. Independent pilot-scale research into the use of locally manufactured UF membranes and modules (Strohwald, 1988) for solids-liquid separation in the anaerobic treatment of industrial effluents was begun in 1987 (Ross et al., 1988). Significant departures from overseas practice in the form of differences in ultrafiltration membrane design, the use of unsupported tubular UF membrane (MEMTUR) modules (Strohwald, 1991a) at low inlet pressures and integration with the degister system led to the development of what has come to be known as the anaerobic digestion ultrafiltration process (ADUF) for the treatment of organic industrial effluents (see Figure 2.6). The design comprises two main unit processes; an anaerobic digester and an external UF unit. In the ADUF process the permeate is the final effluent while the sludge concentrate containing the bacteria, is rapidly recycled back to the digester, enhancing its performance. This process has many advantages compared to other systems, some of which are summarized below:

i) it enables the retention of a high concentration of active biomass thus minimizing the required reactor volume,

ii) it prevents biomass loss in the effluent resulting in greater stability under load changes and variations in influent characteristics,

iii) it provides an effluent almost free of suspended solids,

iv) it eliminates the need for a sedimentation tank thus minimizing the biomass separation problems caused by system overloads which in

turn are a major problem inherent in suspended growth processes, and

v) it enables positive control of solid retention time (SRT) and hydraulic retention time (HRT).

2.7 Summary

In this chapter, many significant microbiological ,biochemical and technological advances made in understanding and implimentation of anaerobic digestion processes have been pointed out with respect to industrial wastewater treatment. Although advances in the development of a wide range of reactor configurations of completely mixed anaerobic processes for the separation of biomass from final effluents have been made, in full-scale applications these have been limited by the settling problems of biomass particularly at high concentrations which have restricted its application in the treatment of high strength industrial wastewaters for economic reasons. In order to overcome the above problems the membrane anaerobic reactor system has been developed which has many advantages compared to other systems, some of which have been widely discussed in this chapter. Comparative studies showed that the membrane anaerobic reactor system has been found to be a superior process in its loading capacity, COD removal efficiency and resilience to transient conditions. However, data concerning optimization of the microbial population in a reactor, its maximum loading capacity for treatment of a variety of industrial wastewaters and operating conditions of membrane reactor is scarce.

CHAPTER 3

OBJECTIVES OF RESEARCH

It has been pointed out in the previous chapter that there is a need to investigate the potential of applying membrane separation to the anaerobic contact process for industrial wastewater treatment. In an attempt to fulfil this need, the objectives outlined below were considered to be necessary for the pilot-scale investigation. These were:

(i) to assess the effect of MLSS concentration on the microbial kinetics (growth rate, K_s, k_d) of the crossflow ultrafiltration membrane anaerobic reactor system,

(ii) to assess the effect of MLSS concentration on specific methanogenic activity (SMA) in the reactor and to determine the optimum process conditions for maximising SMA,

(iii) to assess the effect of MLSS concentration on the dominant species and their numbers present in the reactor,

(iv) to assess the treatment capacity of the new reactor configuration system, particularly at high biomass concentrations,

(v) to assess the capability of the ultrafiltration membrane to retain a high concentration of active biomass in the reactor, and

(vi) to evaluate the dependency of membrane flux on the concentration of biomass in the reactor.

It is hoped that this newly developed membrane anaerobic reactor will eventually solve the problem of retaining an adequate amount of active biomass in the reactor and thus lead to further improvements of the anaerobic contact reactor system. For instance higher organic loading rates and smaller reactor volumes could be achieved, thus increasing the SRT/HRT ratio and reducing the capital cost.

CHAPTER 4

EQUIPMENT, MATERIALS AND METHODS

4.1. Description of Crossflow Ultrafiltration Membrane Anaerobic Reactor (CUMAR) System

Figure 4.1 shows a schematic drawing of the experimental system used in this study. It consisted of a crossflow membrane unit and a 120 l completely mixed, suspended growth anaerobic reactor. An overall view of the CUMAR system can be seen in Plate 4.1. The design specifications of the anaerobic reactor are given in Appendix 4.1. The reactor pH and temperature were automatically maintained within the ranges of 6.9 - 7.2 and 36 °C +/-1°C. The reactor was fed at the same rate as the permeate rate by using a level controller placed within the reactor and controlling the reactor feed pump, hence the active volume of the digester remained constant throughout the study. A variable speed pump was used to recirculate the reactor contents through the membranes which were themselves operated in parallel. The crossflow velocity and operating pressure were controlled by adjusting the flow and pressure regulators which are shown in Plate 4.2.

The crossflow membrane unit consisted of two independent but identical cells with two cylindrical channels, each 12 mm diameter and 320 mm long. Each cell held an ultrafiltration membrane (UF) of 0.024 m^2 total surface area. Plate 4.3 shows a close-up view of the assembled filtration unit used. The UF membrane used in this study (supplied by Paterson Candy International) was manufuctured from fluoropolymer with a molecular weight cut-off (MWCO) of approximately 200 000.

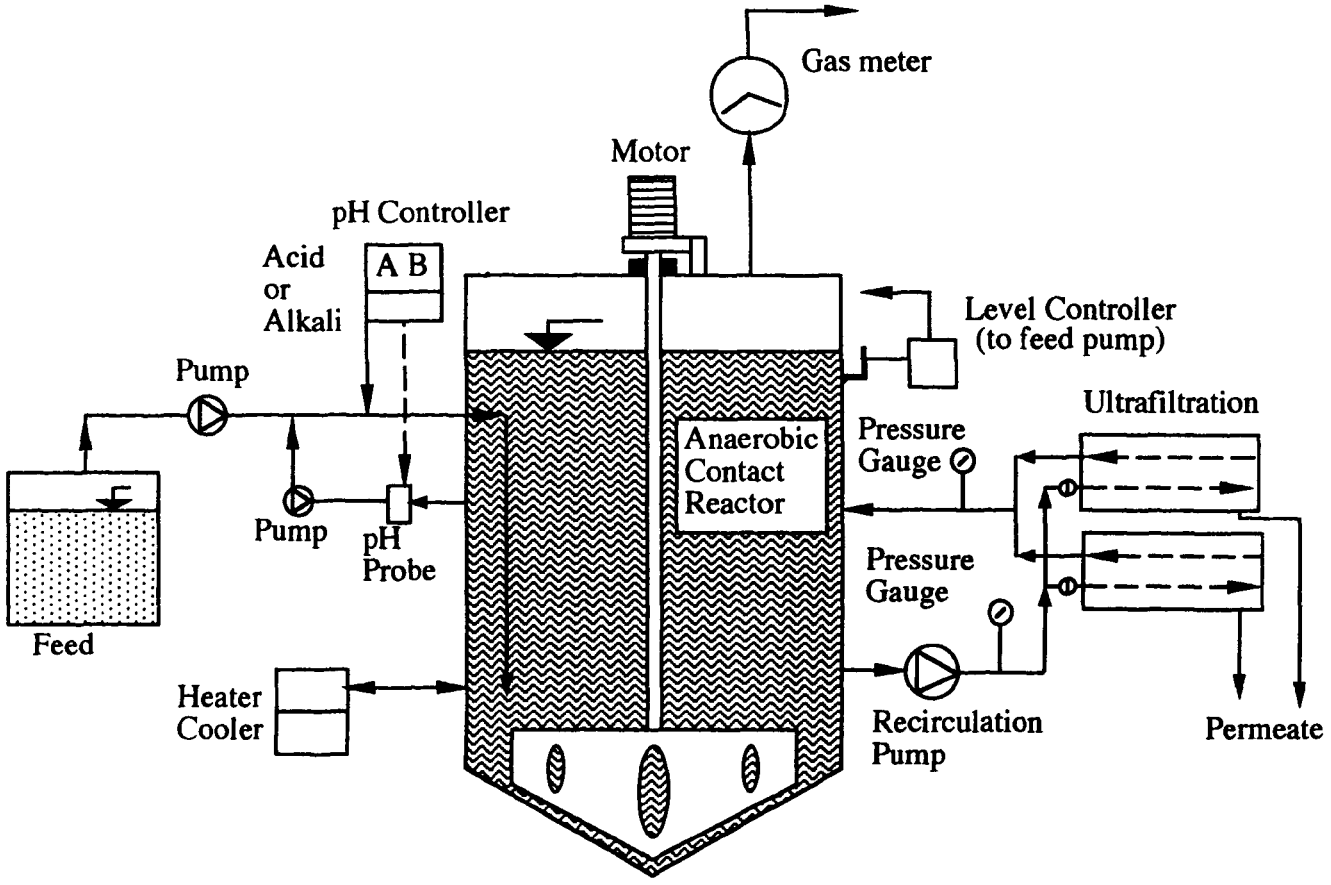


Figure 4.1 Schematic diagram of CUMAR system

Plate 4.1 An overall view of CUMAR system

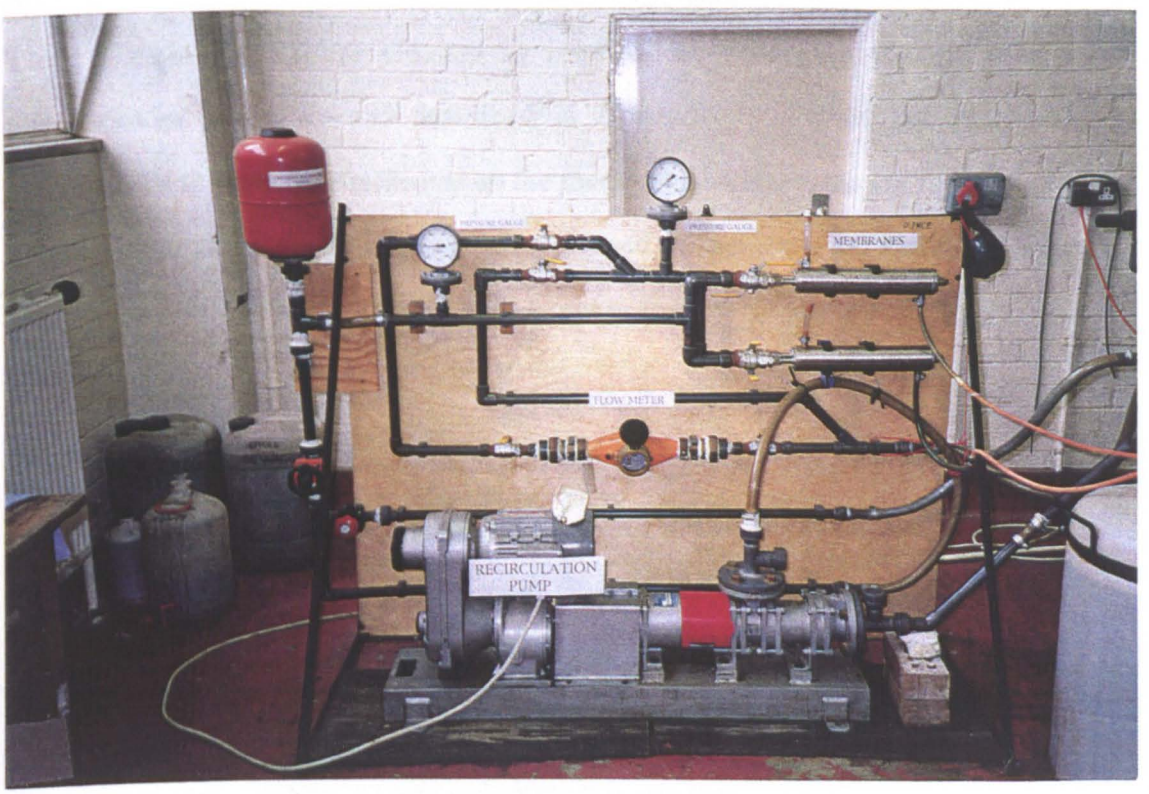


Plate 4.2 Pressure and flow regulators

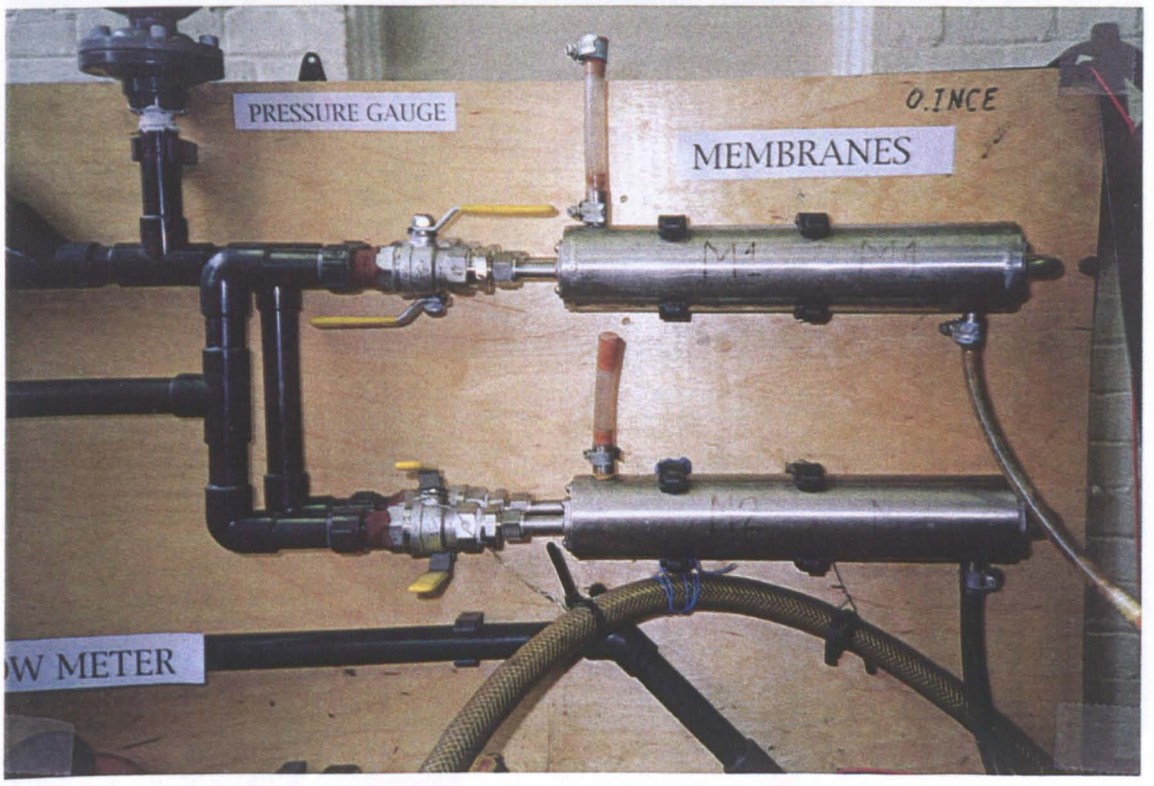


Plate 4.3 A close-up view of the assembled filtration unit

The UF membranes were cleaned as required according to the manufacturer's recommended procedure and described in Appendix 4.2. When the flux rate was too low , due to biomass attachment on the membrane surfaces, the UF membranes were removed so that the membranes could be cleaned or replaced (see Plate 4.4). Plate 4.5 shows a comparison of membranes with biomass attachment and cleaned ones ready for reuse.

4.2. Analytical Methods

Throughout the operation period, routine analyses were carried out daily to check steady-state conditions with the monitoring schedule and analytical methods and instrumentation used in this study being listed in Tables 4.1 and 4.2.

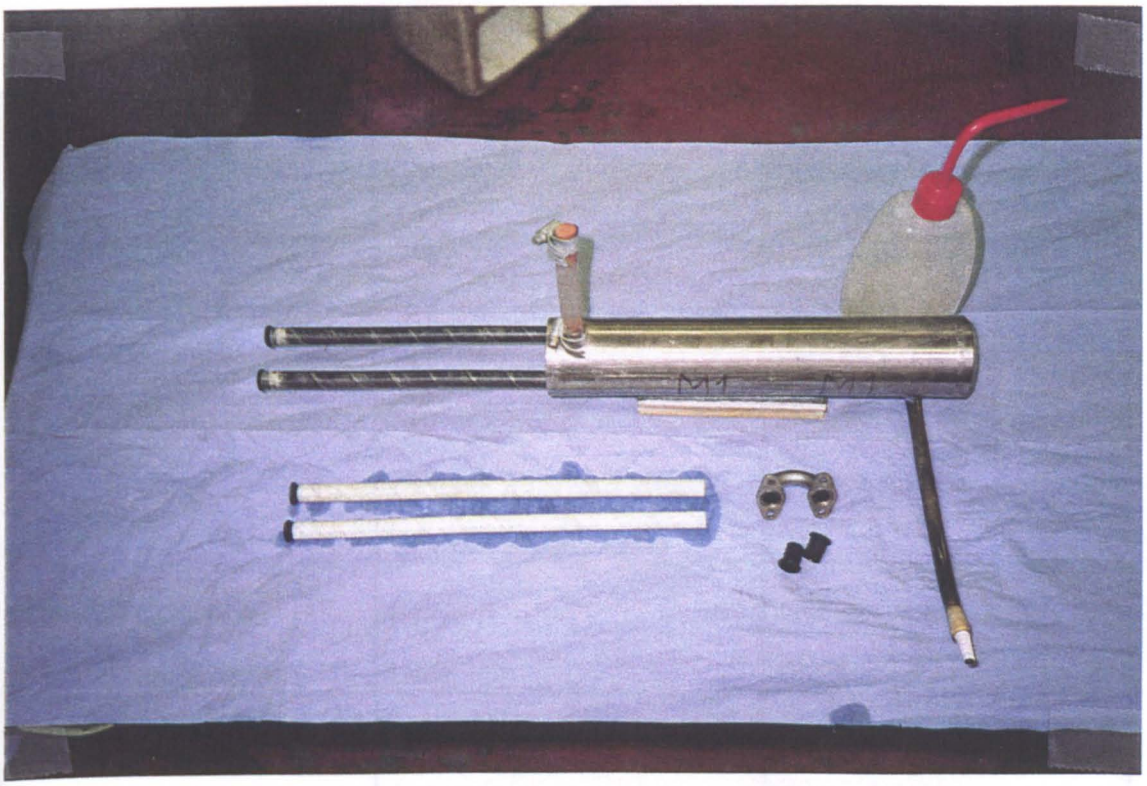


Plate 4.4 Ultrafiltration membranes

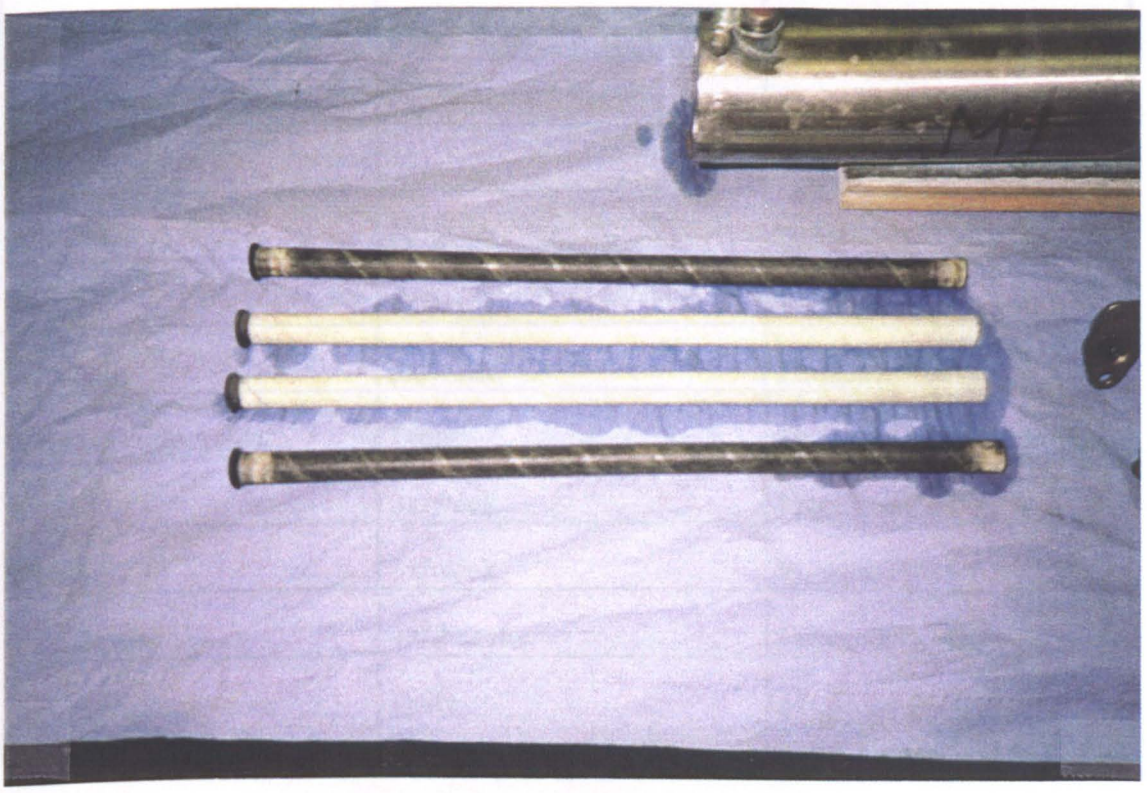


Plate 4.5 Comparison of membranes with biomass attachment and cleaned ones ready for reuse

Table 4.1 Monitoring schedule

Parameter	Frequency	Sampling Location
Influent rate	Daily	Pump setting
COD : Influent	3x/week	Feed line
Effluent	3x/week	Permeate
Reactor	1x/week (unsteady-state)	Sampling point
	3x/week (steady state)	
Gas : Production	Daily	Wet gas meter
Composition	Daily	Gas line
Solids : Feed SS	1x/week	Feed line
Effluent SS/VSS	1x/week	Permeate
Digester SS/VSS	1x/week (unsteady state)	Sampling point
	3x/week (steady state)	
Temperature	Continuous	Temperature probe port
TKN	1x/week	Permeate/feed
NH_3-N	1x/week	Permeate
PO_4-P	1x/week	Permeate/feed
Alkalinity	3x/week	Permeate/feed
Volatile Fatty Acids	Daily	Permeate/feed
Turbidity	3x/week	Permeate/feed
Colour	3x/week	Permeate/feed
Particle size	1x/2weeks	Permeate
pH	Daily	pH probe port

Table 4.2 Analytical methods and instrumentation

Parameter	Method	Instrument/Reference
Influent rate	Feed pump setting	Peristaltic Watson Marlow (S170)
COD	Dichromate closed reflux	Standard Methods (1985)
Gas : Production Composition	Gas meter Gas chromatography	Wet gas meter Pye Unicam 304
Suspended Solids Volatile Suspended Solids	Gravimetric Gravimetric	Standard Methods (1985) Standard Methods (1985)
Temperature	Probe/Indicator Heater controller Cooler	RS components Churchill Thermo circulator Grand FC15 cooler
TKN	Distillation and titration	Standard Methods (1985)
NH_3-N	Distillation and titration	Standard Methods (1985)
PO_4-P	Ascorbic acid	Standard Methods (1985)
Alkalinity	Titration	Standard Methods (1985)
Volatile Fatty Acids	Gas-liquid chromatography	Becker 403 with Pye Unicam autojector and integrator
pH	pH meter	Kent EIL 9143
Particle size	Counter	Coulter Counter Electronics
Crossflow velocity	Pump setting and flow meter	Mono Merlin pump CAB12H1R4/H1
Turbidity	Turbidity meter	HACH model 2100 A
Colour	Lovibond discs	BDH, Lovibond Nesslerisep

4.3. Enumeration of Anaerobic Bacteria

4.3.1. Sample Preparation

All enumeration studies were completed immediately after sampling. A method based on that described by Pike et.al. (1972) was used to homogenize the sludge. Homogenization of the sample (10 ml) with sterile Ballotina Grade 2 glass beads (dia 0.2 mm) was carried out using a " Whirlimix " test-tube shaker at 2500 rpm for 10 minutes after which, samples were ready for microscopic examination.

The required dilutions were made using Balch Media 3 (see Appendix 4.3) except for the plate count (Ringers solution, see Appendix 4.4). 9 ml of the media was transferred to screw-capped tubes fitted with butyl rubber septa (Hungate tubes, Bellco Glass Inc.). Transfer of the media between the tubes was carried out using gassed 1 ml sterile plastic syringes fitted with 24 x 0.5 mm hypodermic needles. All operations with the samples were carried out inside a Microflow Anaerobic Cabinet (see Plate 4.6). Strict anaerobic techniques, based on those described by Hungate (1969) and modified by Bryant (1972), were observed throughout all media preparations and sample handling.

4.3.2. Direct Microscopic Count

Enumeration of the total bacteria and total fluorescent methanogenic bacteria populations in the samples was made using a Zeiss D-7082 Epiflourescence Microscope fitted with a 50 W high pressure mercury lamp. The samples were diluted and homogenized to give counts of between 100 and 400 per field of view and counted using a Neubauer Chamber (see Plate 4.7). This had a depth of 0.1 mm and an area of 1 mm^2. Zeiss x 63 water immersion lenses were used with a x 10 eyepiece, i.e. a magnification of 630.

Plate 4.6 Microflow anaerobic cabinet

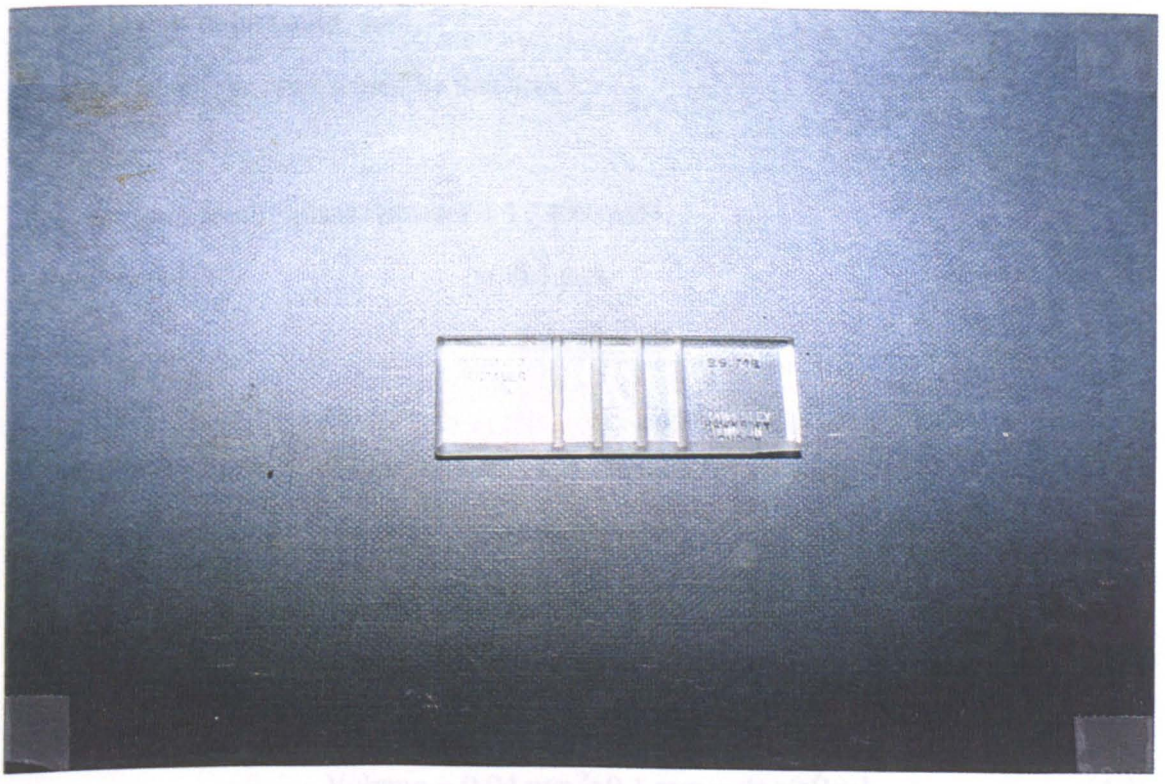


Plate 4.7 A neubauer chamber

A minute drop of sample was placed on to the Improved Neubauer Chamber which has ridges portioned off into regular, cubical chambers of a precisely known volume (2.10^{-5} ml). The total number of methanogenic and non-methanogenic bacteria in 80 of these small chambers was counted and an average taken. Clumps of bacteria were counted as one organism and, to avoid errors, only bacteria on the top and the left graduation lines were counted in each small cube.In order to count only the number of methanogens, ultra-violet light was used since these bacteria fluoresce under such conditions.

The calculations for obtaining the methanogenic and non-methanogenic number of bacteria per mililitre were as follows:

$$N = YxD / V \tag{4.1}$$

where N = number of organisms per unit volume,

Y = mean count per square,

D = dilution factor, and

V = volume represented by that area

Area for each small square chamber = $1 / 400\ mm^2$

Chamber depth = 0.1 mm

Volume = $2.5x10^{-7}$ ml

Considering the area of one field of view utilized for the methanogenic count to be equivalent to 16 times the area of one small chamber,

$$\text{Area} = 16x1 / 400\ mm^2 = 0.04\ mm^2 \tag{4.2}$$

therefore ,

$$\text{Volume} = 0.04\ mm^2 x 0.1\ mm = 4x10^{-6}\ ml \tag{4.3}$$

The number of non-methanogens was obtained by substracting the number of methanogens from the total count.

Changes in the morphology of the fluorescent methanogenic population, which were subdivided into 6 distinct groups, (namely small rods (0.2 to 0.5 by 3 μm), medium rods (0.3 to 0.6 by 6 μm), long rods (0.3 to 0.6 by 10 μm), cocci, sarcina and filaments) were recorded throughout the study.

4.3.3. Counts of Viable Methanogens

The Most Probable Number (MPN) technique was used to count viable methanogenic bacteria in the sludge samples as described by Siebert and Hattingh (1987) and Zehender *et al.* (1980) using Media 3 (Balch *et al.*, 1979) which contains those constituents shown in Appendix 4.3.

Tenfold dilutions with five replicates at each dilution were made within the range of 10^{-5} - 10^{-14} into previously prepared screw-capped Hungate tubes using gassed plastic syringes. This took place inside an anaerobic cabinet. The inoculated media were then statically incubated, at 35 ^{o}C, for 4 - 6 weeks. The growth was recorded as the number of positive tubes at each dilution by detection of methane in the head space using gas chromotography (GC). A Becker Model 403 GC with a thermal steel column (1.5x4 mm) operating at 55 ^{o}C using helium as the carrier gas (flow rate of 50 ml/min) was used to detect the methane in a 1 ml sample of gas. All positive tubes were examined under epifluoresence microscopy to confirm the MPN results.

The numbers of positive tubes at different dilutions were used to obtain the most probable numbers of methanogenic bacteria in the samples from the probability tables described by Greenberg *et al.* (1985).

4.3.4 Plate Count

0.1 ml of samples or diluted samples using Ringer solution (see Appendix 4.4) were spread, using a glass spreader, onto the plates which contained a 15-20 ml of Reinforced Clostridial Agar (see Appendix 4.5). The plates were then statically incubated in an anaerobic cabinet, at 35 ^{o}C , for 24 to 48 hours. The colony counter (Gallenkamp) was used to count the numbers of anaerobic bacteria.

4.4. Specific Methanogenic Activity (SMA) Test

4.4.1 Experimental Equipment

The SMA test equipment used in this study was originally used by Monteggia (1991), a schematic diagram being shown in Figure 4.2. The system consisted of eight, 1-litre digestion flasks submerged in a water bath which had a temperature controller. Continuous mixing of the sludge in the digestion flasks was maintained using magnetic stirrers during the test period.

The gas metering system is shown in Figure 4.3 and consisted of a three-way solenoid valve controlled by a pressure measurement device (manometer or pressure transducer), a gas bulb for temporary storage of the gasses and the necessary tubing for interconnection of the anaerobic reactor and the units of the system. The solenoid valve was set so that the two normally open ports (1 and 2) communicate with the pressure measurement device and the gas bulb. When the third port was closed, the pressure in the reactor and in the bulb increased progressively. As the pressure inside the system reached a set value, the control system sent an electric signal to a control interface that activated the three-way solenoid valve, simultaneously closing the second port (to maintain the pressure inside

the reactor) and opened the third port to the atmosphere. This made the connection of the bulb to the atmosphere, releasing excess gas accumulated during the build-up in pressure. The valve was deactivated after an interval of time (3 second for the complete release of the gases) and a new cycle was initiated.

A microcomputer Amstrad Model 1620 connected to the gas metering system by using an 8 channel analog input board Model DAS-4 supplied by Metrabyte Corporation was used to simultaneously monitor the gas production of the eight independent digesters.

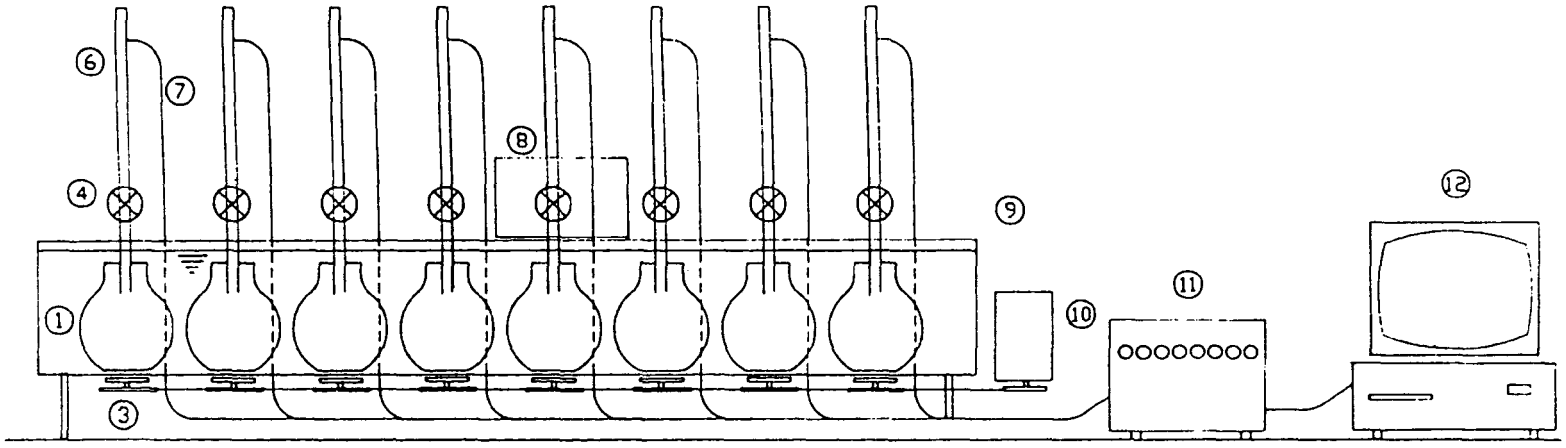
The device used for calibration of the eight digesters with their respective gas flow meters is shown in Figure 4.4 and was described by Monteggia (1991), the eight digesters and the respective gas flow meters were individually calibrated by injecting a known volume of gas.

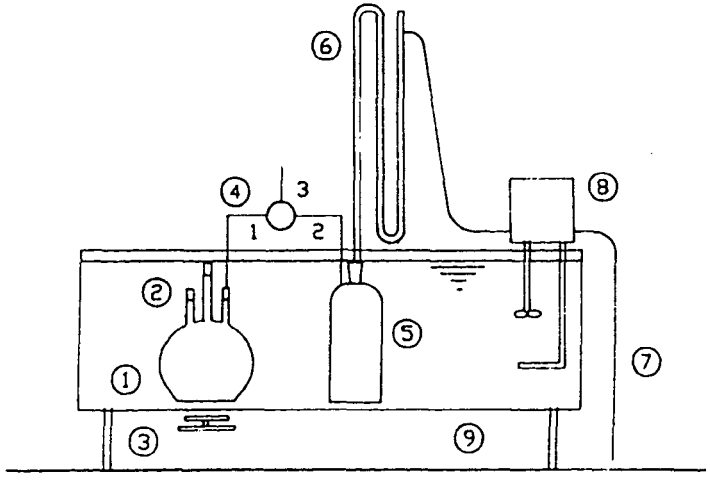
4.4.2 Laboratory Routine for the SMA Test

The procedure given below has been adapted for its application in this study and is a combination of two separate methods for the measurement of SMA as outlined by James *et al.* (1990) and Monteggia (1991):

i) the volatile suspended solids content of the sludge was determined before the test was started,

ii) the required volume of sludge was added to the flasks and the samples were diluted with a mineral stock solution (Table 4.3) in order to obtain the pre-determined concentration of volatile suspended solids for





1- DIGESTION FLASKS V=1000 ml
2- PORT WITH SEPTUM FOR GAS SAMPLING
3- MAGNETIC STIRRER
4- THREE-WAY SOLENOID VALVE
5- GAS BULB V=80 ml
6- MANOMETER WITH ELECTRIC CONTACT POINTS
7- WIRING
8- WATER BATH HEATER
9- WATER BATH
10- STIRRER MOTOR
11- SOLENOID VALVE CONTROLLER BOARD
12- MICRO-COMPUTER WITH DATA AQUISITION INTERFACE

Figure 4.2 Illustration of experimental set-up for Specific Methanogenic Activity test

(Source: Monteggia, 1991)

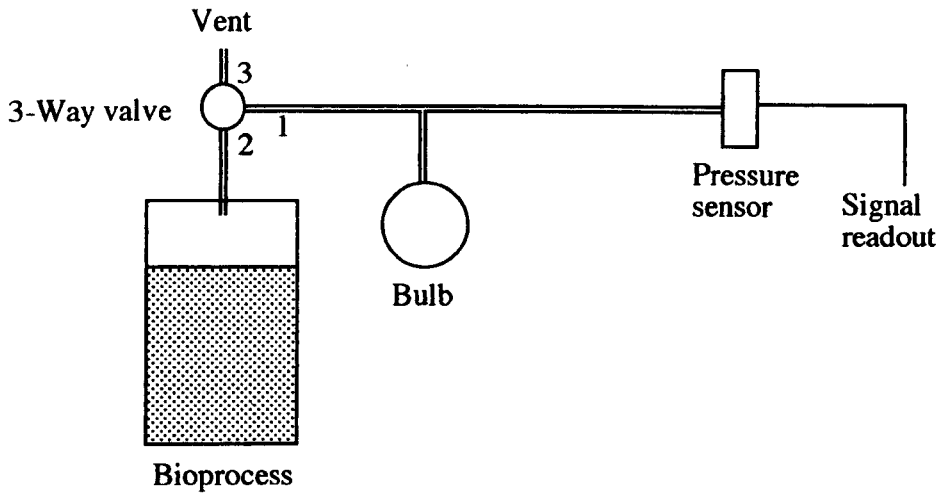


Figure 4.3 Schematic description of gas flow metering system (Source:Monteggia, 1991)

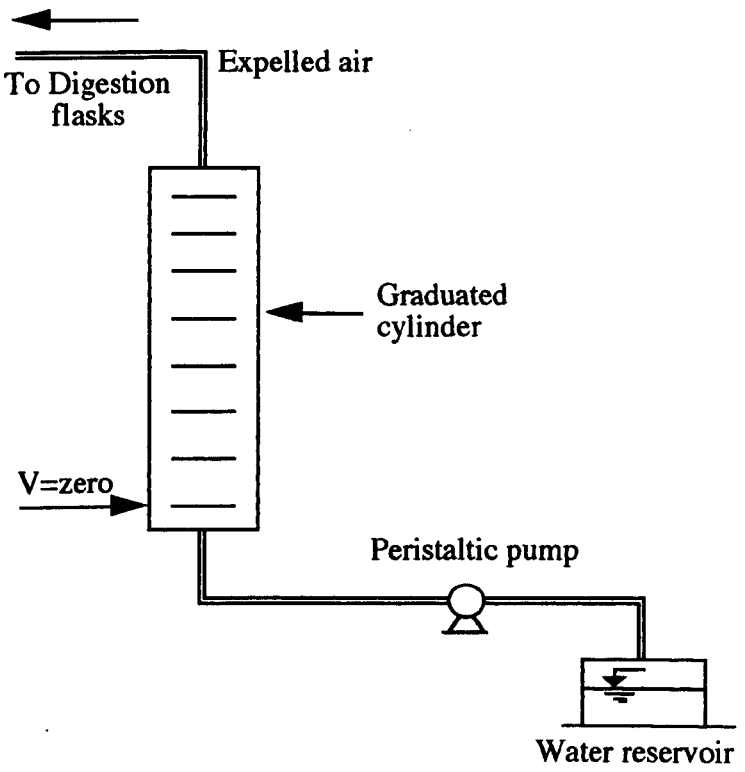


Figure 4.4 Device used for calibration of gas flow meters (Source:Monteggia, 1991)

each SMA test. The total volume of mixed liquor in each digestion flask was 900 ml,

iii) the water level inside the manometers was adjusted at the level at which the respirometer was calibrated,

iv) the mixed liqour was flushed with helium gas for a period of approximately 10 minutes at a pressure of (35-70 kN/m^2) to remove traces of oxygen from the mixed liqour and the headspace of the flasks. After flushing each digestion flask, the central stopper was immediately replaced to avoid recontamination with atmospheric oxygen and a final check was made to detect the occurence of some leakage through the glass connections,

v) the temperature of the water bath was set to 35 °C by using an electric water heater with a mechanical circulator to obtain a homogeneous temperature throughout the water bath,

vi) a further 12-16 hours was allowed for acclimatization of the microorganisms with the dilution water and the test temperature of 35 °C. Although gas production during this period was negligible (since no feed was available) the microcomputer with data acquisition system was switched on in order to observe the behaviour of the gas measurement system and to allow for the correction of some eventual malfunctioning of the system,

vii) the substrate (acetic acid) was injected through the latex septum installed on the side arm of the digestion flask,

viii) the mixing system was switched on and the data acquisition system

was reset in order to store the number of cycles generated by the gas production from the moment that the substrate had been injected and mixed with the microorganisms. The number of cycles observed per hour for each digestion flask was automatically transferred to the hard disk of the computer for safe storage and later calculations,

ix) a 0.4 ml sample of the headspace gas of each digestion flask was collected at regular intervals, using a 1 ml volume syringe for analysis of the methane concentration,

x) the test was concluded when a sharp decrease in the rate of gas production due to the complete consumption of the injected substrate was observed, and

xi) the volume of methane produced per unit of time was calculated and the specific methanogenic activity was determined using the formulae given below:

$$G = A \times B \times C \times D \times 24 \quad (4.4)$$

$$S = G / (E \times F) \quad (4.5)$$

where A = volume of gas released per cycle (ml/cycle)

B = number of cycles per hour

C = percentage of methane measured at specific interval of time (%)

D = flask constant

E = concentration of volatile suspended solids (gram/litre)

F = volume of liquid in the digestion flask (litre)

G = gas volume (ml CH_4/day)

S = specific methanogenic activity (ml CH_4 / g VSS.d)

Methane analyses using gas chromotography were carried out at intervals of time according to the expected changes in composition of the gases and the total duration of the test. It was noted that significant changes in gas composition occured at the beginning of the test, therefore , gas sampling every 2 hours during the first 8 hours of the test was generally used. A maximum 8 to 10 hours gas sampling interval was used towards the end of the tests since slight changes in gas composition were observed during that period. In this study, the hourly values of methane concentration were obtained by graphic interpolation.

Table 4.3 Mineral stock solution

Chemical Compositions	Concentration (mg/l)
KH_2PO_4	2500
K_2HPO_4	1000
NH_4Cl	1000
$MgCl_2$	100
$Na_2S.7H_2O$	100
Yeast extract	200

The pH of the solution should be adjusted to 6.8
Source: Valcke and Verstraete (1983).

4.5 Experimental Set-up for Determination of Microbial Products in Anaerobic Reactor

Batch reactors were set-up for the determination of the inert soluble COD fraction of the brewery wastewater using glucose as an additional substrate with no inert fraction of COD.

4.5.1. Description of Anaerobic Batch Reactors

A bank of five, bench-scale completely mixed anaerobic digesters was employed, using 5-litre pyrex aspirator bottles with multi-socket lids (see Plate 4.8). One was run as a stock reactor whilst the rest of the others were used for the determination of inert soluble COD. The lids had four openings, the centre one provided for a glass rod stirrer with rubber attachments to enhance mixing, sealed with a quick-fit waterseal, and driven at 90 rev./min. One housed the feed line via a glass pipe, another was a gas outlet with a syringe cap for gas analysis with a further connection to an aspirator for gas collection, the volume of which was measured by water displacement while the fourth one was used for sampling. The digesters were contained in a water bath, in which the water was maintained at 35 ^{o}C by means of a recycle and heat exchange system. Nitrogen gas was used to give anaerobic conditions before the start-up of the reactors .

4.5.2. Description of Aerobic Batch Reactors

A set of five, bench-scale reactors using 5-litre pyrex aspirator bottles was employed (see Plate 4.9). One was operated as a stock reactor whilst the rest of the others were used for determination of inert soluble COD. During the operation, excess aeration was supplied to create a good mixing in batch reactors. Nitrogen and phosphate were added as indicated in Standard Methods (APHA, 1985) for the BOD test.

4.6. Inert COD Determination Methods

The methods described below were used for the determination of the inert soluble COD fraction of the wastewater used. The biomass in both anaerobic and aerobic stoct reactors

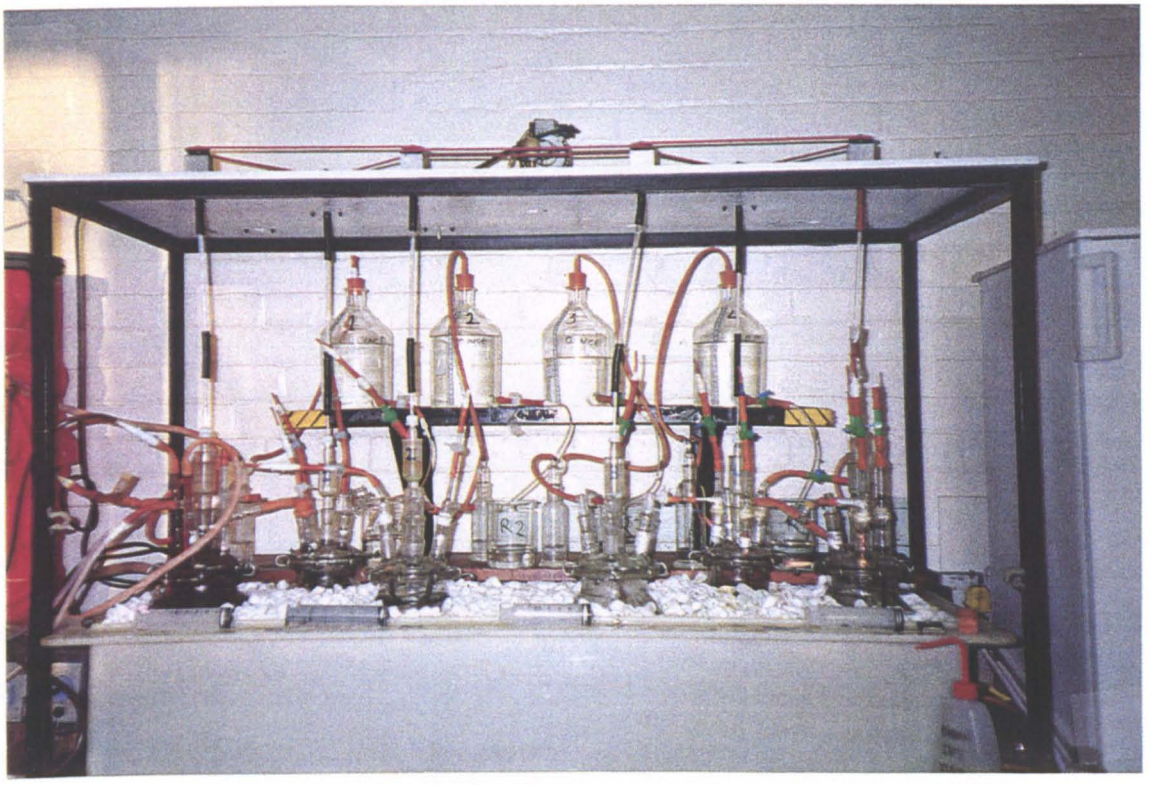


Plate 4.8 Anaerobic batch reactors

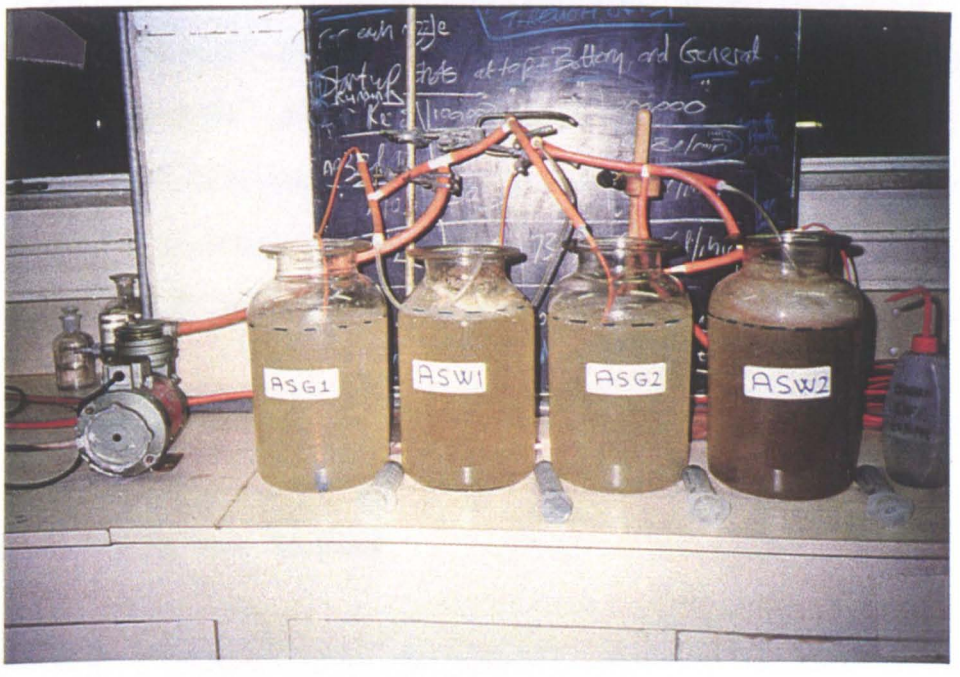


Plate 4.9 Aerobic batch reactors

was fed with a mixture of 50% glucose and 50% wastewater for a period of 21 days before first seeding the other reactors.

4.6.1. Incremental Method

According to Germirli (1990), the incremental method yields a linear relationship between the concentration of initial soluble COD and the minimum levels of the COD profiles obtained from a set of experiments performed with increasing soluble initial COD values.

The experimental procedure described by Germirli (1990) was used in this study. According to the procedure, all reactors were fed with the same concentration of diluted beer wastewater and added glucose in appropriate increments to the 2nd, 3rd and 4th reactors in order to give the desired relationship with an intercept equal to the inert COD of the wastewater tested.

After the anaerobic and aerobic reactors were lightly seeded with acclimatized biomass from stock reactors, soluble COD analyses were carried out periodically using GF/C filter paper until the COD profile reaches a plateau (variations of +/-5% in COD were taken as an indication that the minimum COD was attained) (Figure 4.5) . The intercepts of the plots obtained from the ultimate soluble COD values at the plateau versus the initial soluble COD were taken as the initial soluble inert COD fraction of the wastewater (Figure 4.6).

4.6.2. Comparison Method

According to Germirli (1990), the method consisted of two batch reactors running parallel to each other with the same initial COD, one with the wastewater tested, the other with glucose. In this study four batch reactors were run in parallel so that two

different concentrations could be tested in each run. The soluble COD values of the periodic samples were collected and the difference between the ultimate soluble COD values at the plateau (variations of +/-5% in COD were taken as an indication that the minimum COD was attained) for the wastewater and the corresponding glucose dilution was considered to be the inert initial soluble COD fraction of the wastewater tested (Figure 4.7).

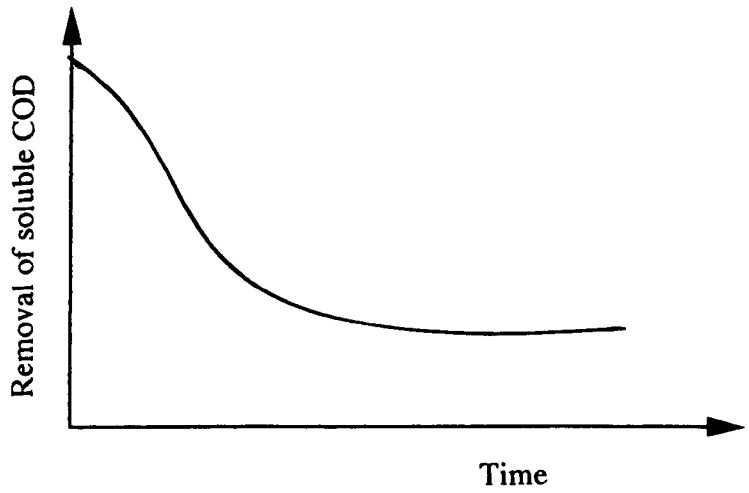


Figure 4.5 Typical soluble COD removal curve for a batch reactor

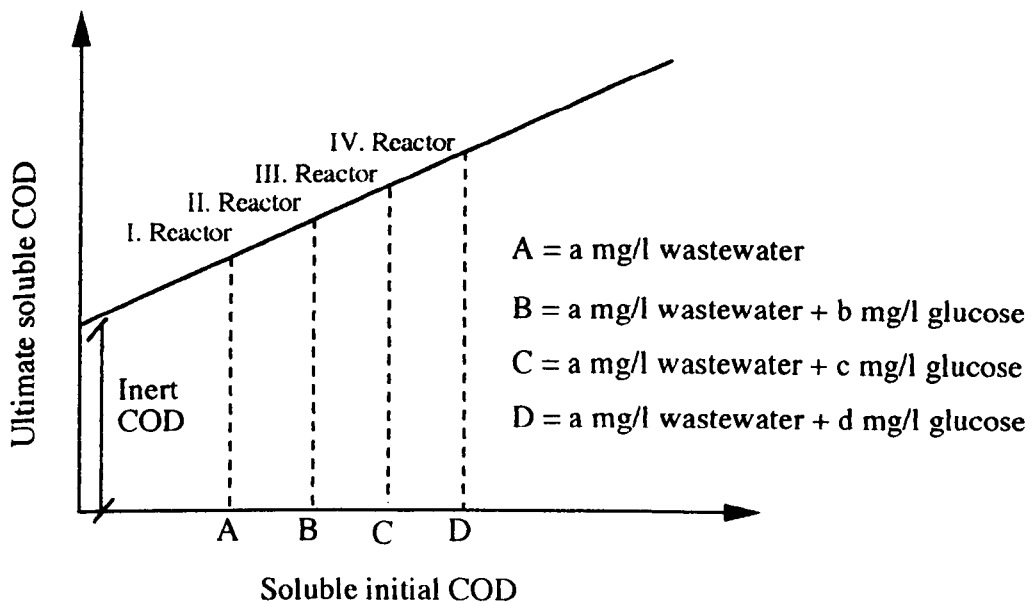


Figure 4.6 Incremental method for determination of initial inert soluble COD

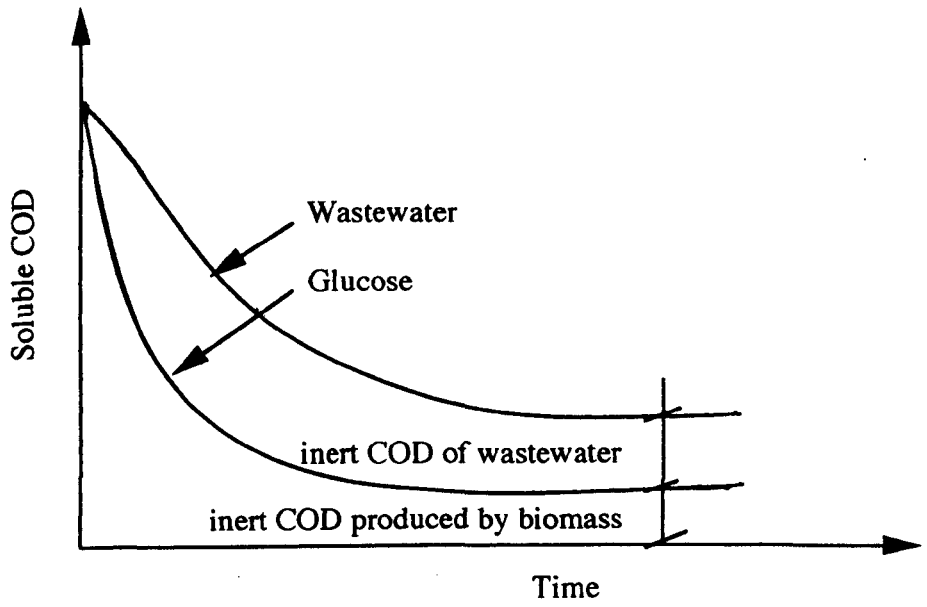


Figure 4.7 Comparison method for determination of initial inert soluble COD

4.7. Wastewater Characteristics

The wastewater used throughout the study was collected from a local brewery and the characteristics are given in Table 4.4. Although the wastewater had a high COD concentration, glucose was added to increase the strength of the feed after an OLR of 20 kg COD/m^3.d had been reached since the HRT of the CUMAR system was largely determined by the flux rate of the membrane filter.

The raw wastewater had a COD:N:P ratio of 400: 0.7:0.4. Throughout the operation of the CUMAR system , the COD:N:P ratio was maintained in the influent at a ratio of 400:5:1 by adding urea and KH_2PO_4 in order to supplement nitrogen and phosphorus respectively. No trace metals were added to the feed since their concentrations were found to be sufficient compared to the minimum requirements reported by Takashima and Speece (1989).

The pH of the feed was adjusted by adding $NaHCO_3$ to a level close to neutral during the start-up period of the CUMAR system and maintained an alkalinity in the range 1000-2000 mg/l as $CaCO_3$. After that, the amount of $NaHCO_3$ added to the feed was gradually decreased to a point after which there was no need to add alkalinity (after an OLR of 7 kg COD/m^3.d had been reached) due to the quantity of alkalinity produced in the digester.

Table 4.4. Characteristics of brewery wastewater and composition of trace nutrients in brewery wastewater

Parameter	Concentration (mg/l)	Compound	Concentration (mg/l)
COD	80000-90000	$MgSO_4.7H_2O$	0.005
BOD_5	65000-80000	$FeCl_3$	0.005
TKN	110-210	$CaCl_2$	0.005
PO_4-P	90-100	KCl	0.005
Suspended Solids	100-150	$CoCl_2$	0.001
pH (units)	3.5-4.5	NiCl	0.001

4.8 Seed Sludge

100 l seed sludge was collected from a local municipal wastewater treatment plant for inoculating the CUMAR system. The sludge, after screening, was allowed to stand at room temperature for 24 hours before being introduced into the digester. The concentration of volatile suspended solids in the sludge was found to be 11000 mg/l which was 75% of the total suspended solids.

For the anaerobic batch reactors, the seed sludge inoculum was taken from the CUMAR system after being operated 150 days at which point it had a very active anaerobic biomass as determined by the SMA test. For the aerobic batch reactors, the inoculation sludge was obtained from an advanced activated sludge pilot plant system running in the Department of Civil Engineering, University of Newcastle upon Tyne.

4.9 Methodology of Steady-state Operation

The methodology adopted for the steady-state operation of the CUMAR was based on limiting substrate source (measured as COD in this study). The peak in the growth curve usually coincides with the low point of the substrate remaining curve at which point maximum COD removal occurs as shown in Figure 4.8. After reaching the maximum concentration of biomass, biomass could be counterbalanced by the loss of cells from the reactor and the increase in biomass due to the inflowing substrate , i.e. biomass would attain some constant level resulting in dX/dt approaching zero. The conditions wherein dX/dt=0 can be defined as a steady-state with respect to biomass. Since there is no biomass loss in the effluent of the CUMAR system steady-state conditions were achieved when the COD removal efficiency reached maximum. At which point dX/dt=0 was maintained by deliberate sludge wastage.

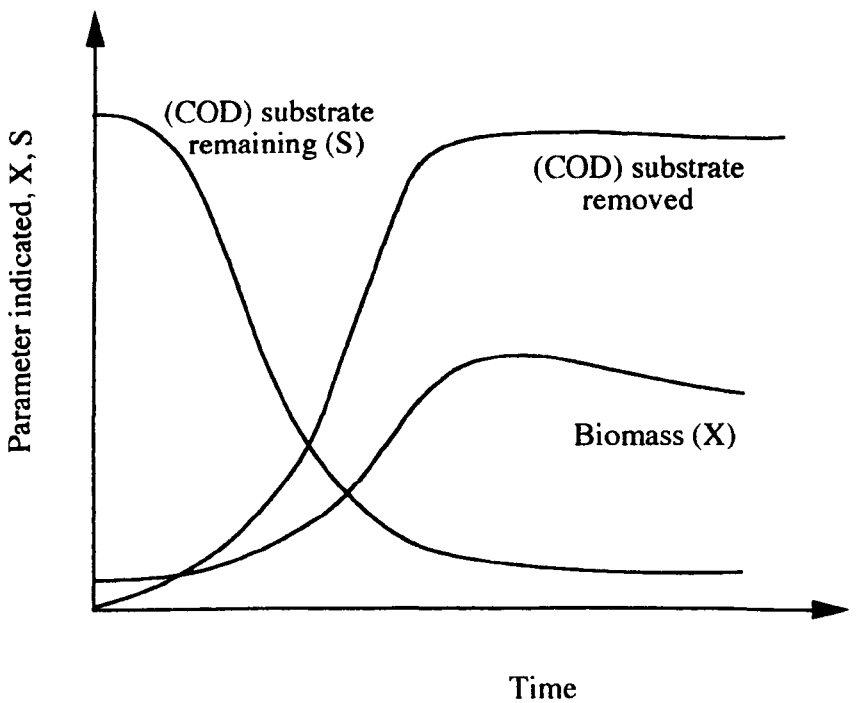


Figure 4.8. Typical drawing growth and substrate removal for heterogeneous population

CHAPTER 5

PERFORMANCE OF MEMBRANE REACTOR

5.1. Performance of Anaerobic Contact Reactor

5.1.1. Start-up Procedure and Initial Loading Conditions

One hundred litres of digesting sludge, taken from a municipal wastewater treatment plant, was first sieved through a mesh with a diameter of 0.1 mm in order to remove waste materials which could cause pump failure. Secondly, the sludge was left at room temperature for 24 hours so that the biomass settled, after which 80 litres of settled sludge was then drawn from the bottom of the tank and introduced into the reactor through the overflow line. The rest of the reactor was filled with tap water to a level of 120 litres. Following this, the reactor contents were flushed with nitrogen for 30 minutes so that anaerobic conditions could be established. Finally the temperature of the reactor content was gradually increased from room temperature to 36 °C over a period of 48 hours without feeding.

The anaerobic contact reactor was initially fed with brewery wastewater (see Chapter 4 Section 4.7) at a strength of 2.5 g/l to give an organic loading rate (OLR) of approximetely 1 kg COD/m^3.d with a hydraulic retention time (HRT) of 2.5 days.

The VFA concentration in the digester immediately increased to about 1600 mg/l in the first week of the operation which resulted in a high VFA/alkalinity ratio of 0.7 and a

COD removal efficiency of 14%. The OLR was therefore reduced to 0.7 kg COD/m^3.d for the following 3 weeks during which the COD removal efficiency improved to about 80%. Figure 5.1 shows the COD removal efficiency and VFA/alkalinity ratio of the system during start-up.

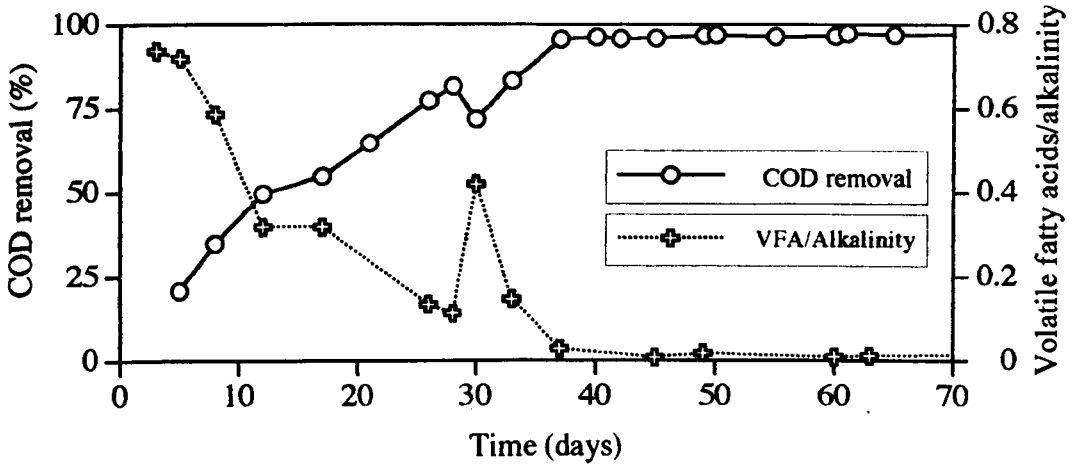


Figure 5.1 Plot of COD removal efficiency and volatile fatty acid / alkalinity ratio against operating time of CUMAR systeem

Acclimatization of the digester sludge was completed after 40 days operation followed by exponential increases in OLR as shown in Figure 5.2 while the MLVSS concentration in the digester increased from approximately 8500 mg/l to over 10000 mg/l which in turn resulted in an increase in the MLVSS/MLSS ratio of 5%.

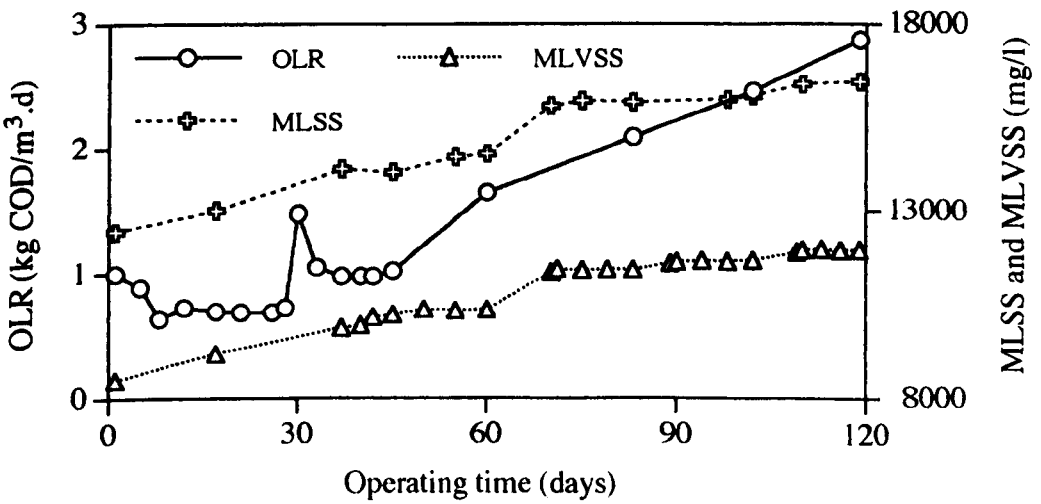


Figure 5.2 Changes in organic loading rate (OLR) and solids in CUMAR system

5.1.2. Steady-state Operation and Results

After the start-up period, the CUMAR system was subjected to a programme of steady-state operation over a wide range of hydraulic retention times and organic loading rates in order to evaluate its treatment efficiency. The COD concentration of the wastewater used in this study was in the range of 80-90 g/l. Increases, in OLR at loadings greater than 20 kg COD/m^3.d , were achieved by supplementing the wastewater with glucose since the HRT was largely determined by the flux rate. Throughout the operation increases in total volatile fatty acids concentration and reductions in gas production were taken as indicators of any impending failure.

Steady-states were obtained over four ranges of MLVSS, i.e. 10-15 g/l , 20-25 g/l , 30-35 g/l and 40-50 g/l. For each range of MLVSS , at least six steady-states were achieved by varying the OLR and by deliberate wastage of sludge from the reactor.Table 5.1 shows the steady-state results of the system. The sludge age was calculated as described in Chapter 2 Equation 2.12. Over a 16 month operating period, a maximum OLR of 28.5 kg COD/m^3.d at an F/M ratio of 0.55 kg COD/kg VSS.d was achieved, at which point the system performed very well , i.e. 97% COD and almost 100 % BOD removal efficiencies. Throughout the study, the HRT was maintained in the range of 2.5-4.2 days. The total volatile fatty acids in the permeate was found to be below 200 mg/l during the steady-state operation of the CUMAR system. The methane content of the biogas produced in the digester decreased from 80 % to 65% towards the end of the operation which resulted in a methane yield of 0.28 m^3 CH_4/kg $COD_{removed}$ which is probably due to the high OLRs being applied to the digester causing a change in methanogenic species

Although the COD:N:P ratio was maintained in the influent at a ratio of 400:5:1 by adding urea and KH_2PO_4 it was noted that the COD:N:P ratio consumed in the digester by the biomass was 400:2.3:0.2. During the operating period, the MLVSS increased from 8 g/l to over 50 g/l and the overall performance of the system is shown in Figures 5.3a-c.

Table 5.1 Steady-state results of CUMAR system

Influent COD g/l	Effluent COD mg/l	Effluent VFA mg/l	Reactor COD mg/l	Methane Content %	Methane Yield m^3 CH_4/ kg COD	MLSS g/l	MLVSS g/l	Sludge Age days
4.2	50	10	145	78	0.38	14.5	10.4	480
5.3	65	30	170	79	0.39	15.9	11.4	320
6.6	80	30	190	81	0.38	16.1	11.7	232
8.6	90	20	260	78	0.40	16.4	12.0	195
10.1	100	20	310	80	0.40	16.90	12.5	177
11.7	120	15	350	79	0.40	17.8	13.2	160
12.7	150	10	370	79	0.39	18.8	14.0	135
14.6	180	25	390	81	0.41	20.0	15.0	122
26.5	220	30	660	79	0.39	26.7	19.9	103
28.6	260	40	700	79	0.37	26.9	20.1	98
31.6	270	30	860	78	0.36	27.3	20.9	91
33.5	310	25	900	77	0.35	27.5	21.2	89
35.2	360	40	950	77	0.35	28.3	21.8	86
38.1	390	30	1000	76	0.34	28.8	22.8	84
41.5	430	40	1070	75	0.33	29.9	23.6	81
43.0	450	60	1100	73	0.31	30.2	24.6	80
46.0	460	50	1130	74	0.34	31.2	25.0	78
48.3	490	35	1140	75	0.33	37.3	30.0	77
51.5	500	50	1180	74	0.34	37.8	30.8	76
56.5	530	70	1250	73	0.32	38.9	32.1	76
60.2	600	70	1350	73	0.33	39.6	33.2	74
64.3	640	80	1500	71	0.32	41.0	34.10	72
66.3	650	80	1570	73	0.33	41.3	34.90	70
68.2	670	60	1630	71	0.31	41.3	35.20	69
81.5	780	80	1820	71	0.29	45.9	39.20	66
88.0	820	90	2100	69	0.30	48.7	42.3	64
95.2	860	90	2400	70	0.30	49.8	44.0	63
101.2	940	100	2710	67	0.28	50.6	44.6	61
108.6	990	160	2960	63	0.28	54.2	48.4	60
119.0	1150	210	3350	62	0.28	58.1	51.0	58

Table 5.1 Steady-state results of CUMAR system (continued)

HRT days	Organic Loading Rate kg COD/m^3.d	Biological Loading Rate g COD/g VSS.d	Specific Substrate Utilization Rate g COD/g VSS.d	COD Removal (%) in Reactor	COD Removal (%) in Effluent
2.5	1.7	0.16	0.15	96.5	98.8
2.6	2.1	0.18	0.17	96.8	98.8
2.7	2.5	0.21	0.20	97.2	98.8
3.0	3.0	0.24	0.23	97.0	99.0
3.2	3.2	0.25	0.24	97.0	99.0
3.4	3.4	0.26	0.25	97.0	99.0
3.0	4.3	0.30	0.29	97.0	98.7
3.1	4.7	0.31	0.30	97.3	98.8
3.6	7.4	0.37	0.36	97.5	99.2
3.6	7.8	0.39	0.38	97.5	99.0
3.7	8.5	0.41	0.40	97.3	99.2
3.8	8.8	0.42	0.41	97.3	99.0
3.8	9.2	0.42	0.41	97.3	99.0
3.9	9.8	0.43	0.42	97.4	99.0
3.9	10.5	0.44	0.43	97.4	99.0
3.8	11.2	0.46	0.44	97.5	99.0
4.0	11.6	0.46	0.45	97.5	99.0
3.6	13.5	0.45	0.44	97.6	99.0
3.7	14.0	0.46	0.45	97.7	99.0
3.8	15.0	0.47	0.46	97.8	99.0
3.9	15.6	0.47	0.46	97.7	99.0
3.9	16.5	0.48	0.47	97.7	99.0
3.9	17.2	0.49	0.48	97.6	99.0
3.9	17.4	0.49	0.48	97.6	99.0
4.1	19.8	0.51	0.49	97.8	99.0
4.0	21.8	0.52	0.50	97.6	99.0
4.1	23.0	0.53	0.51	97.5	99.0
4.2	23.8	0.53	0.52	97.3	99.0
4.1	26.7	0.55	0.54	97.3	99.0
4.2	28.5	0.56	0.54	97.2	99.0

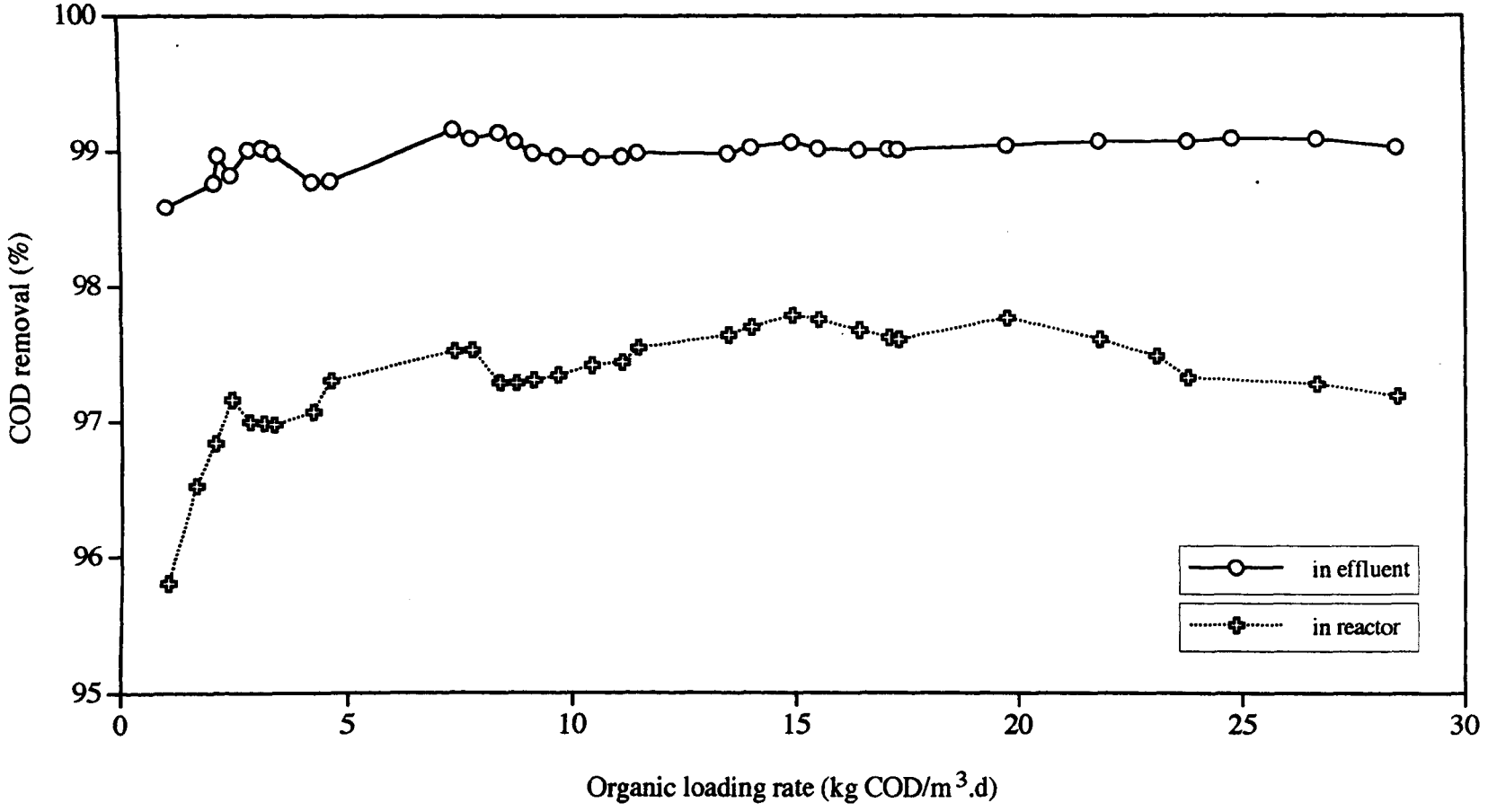


Figure 5.3a COD removal efficiency of CUMAR system during steady-state operation

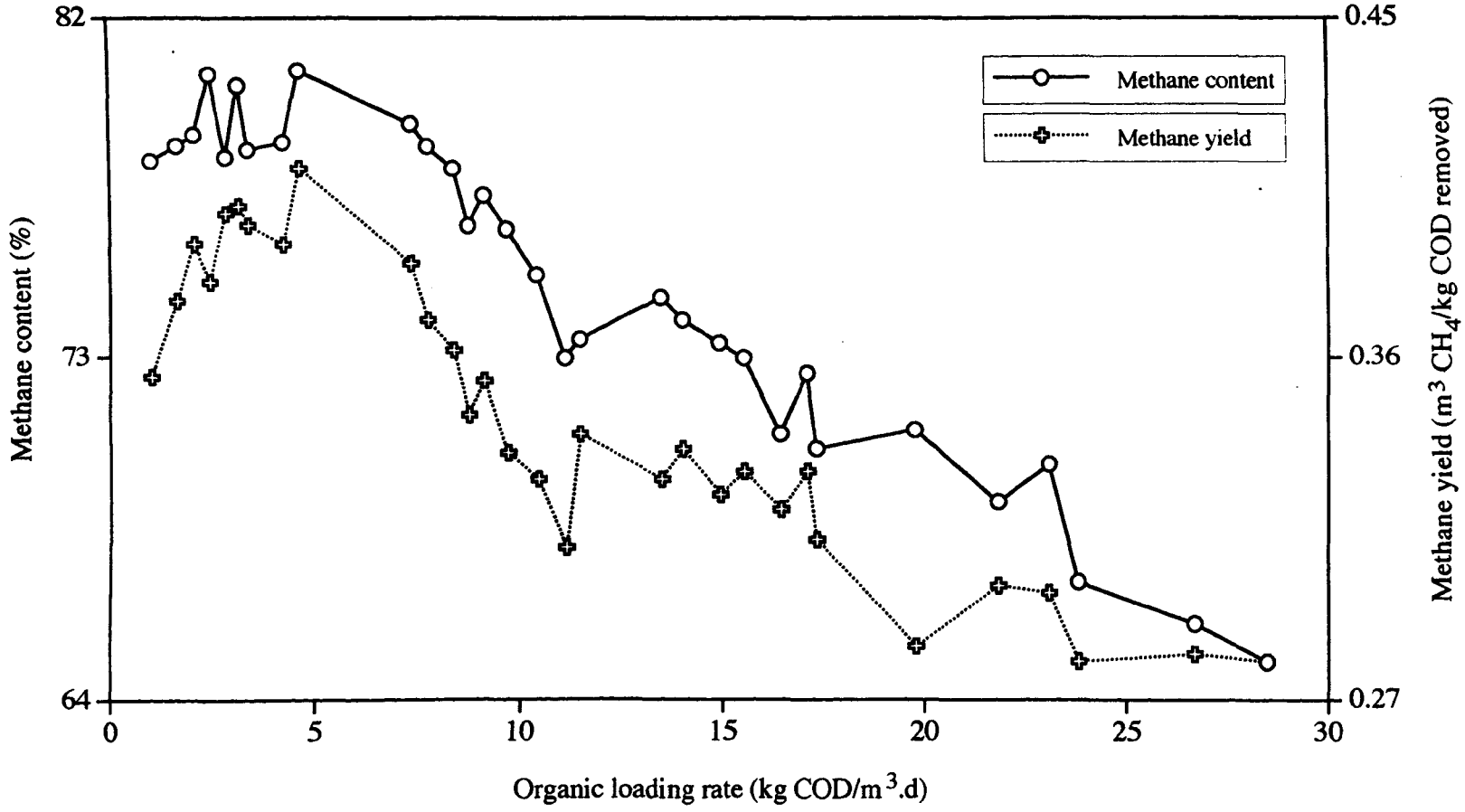


Figure 5.3b Methane content and methane yield of CUMAR system during steady-state operation

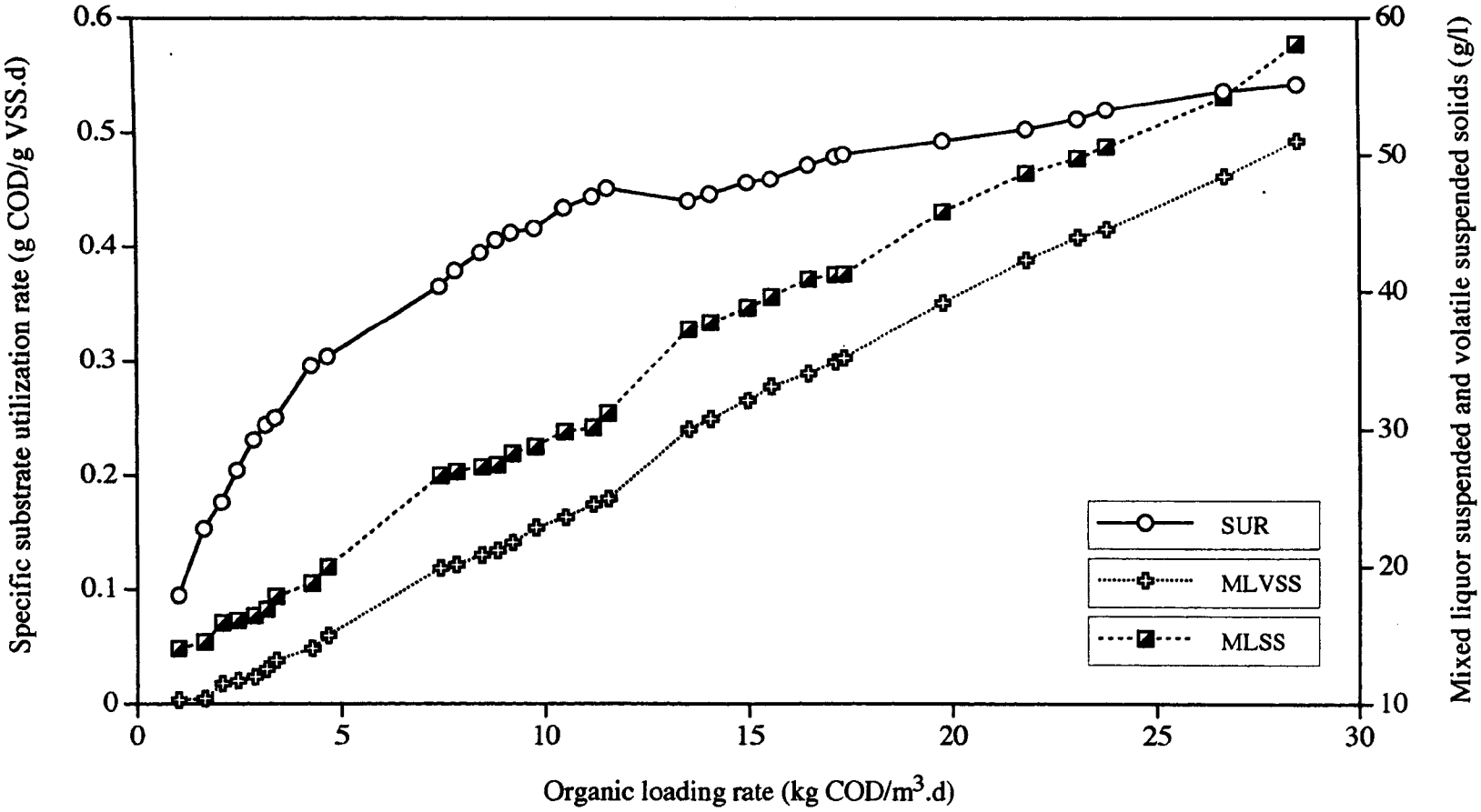


Figure 5.3c Specific substrate utilization rate (SSUR) against organic loading rate of CUMAR system during steady-state operation

5.2. Performance of Crossflow Ultrafiltration Membrane Unit

5.2.1. Crossflow Ultrafiltration Membrane Flux

The flux rate through the membrane throughout the study varied with the performance of the digester and the operating conditions. During the operation, different ranges of crossflow velocity and pressure were used to adjust the HRT.

In the early stage of the operation, a crossflow velocity of 2.4 m/sec and an average pressure of 240 kN/m^2 were maintained over a period of 87 days. As can be seen in Figure 5.4a the biomass concentration in the digester increased from 4 g/l to 13 g/l during that period, resulting in a decrease in the flux rate, i.e. from 115 l/m^2.h to 77.5 l/m^2.h.

Figures 5.4a-d show the flux rates at different crossflow velocity and pressure versus operating time during the remainder of the operation. The limiting MLSS concentrations are given in Table 5.2 from which it may be seen that the digester could be operated at any biomass concentration less than those given in Table 5.2 without a separation problem, provided that the sludge can be recirculated.

Table 5.2 Limiting MLSS concentrations in digester

MLSS (g/l)	Average Pressure (kN/m^2)	Crossflow Velocity (m/s)
212	240	2.4
176	171	2.4
240	206	2.9
279	206	3.2

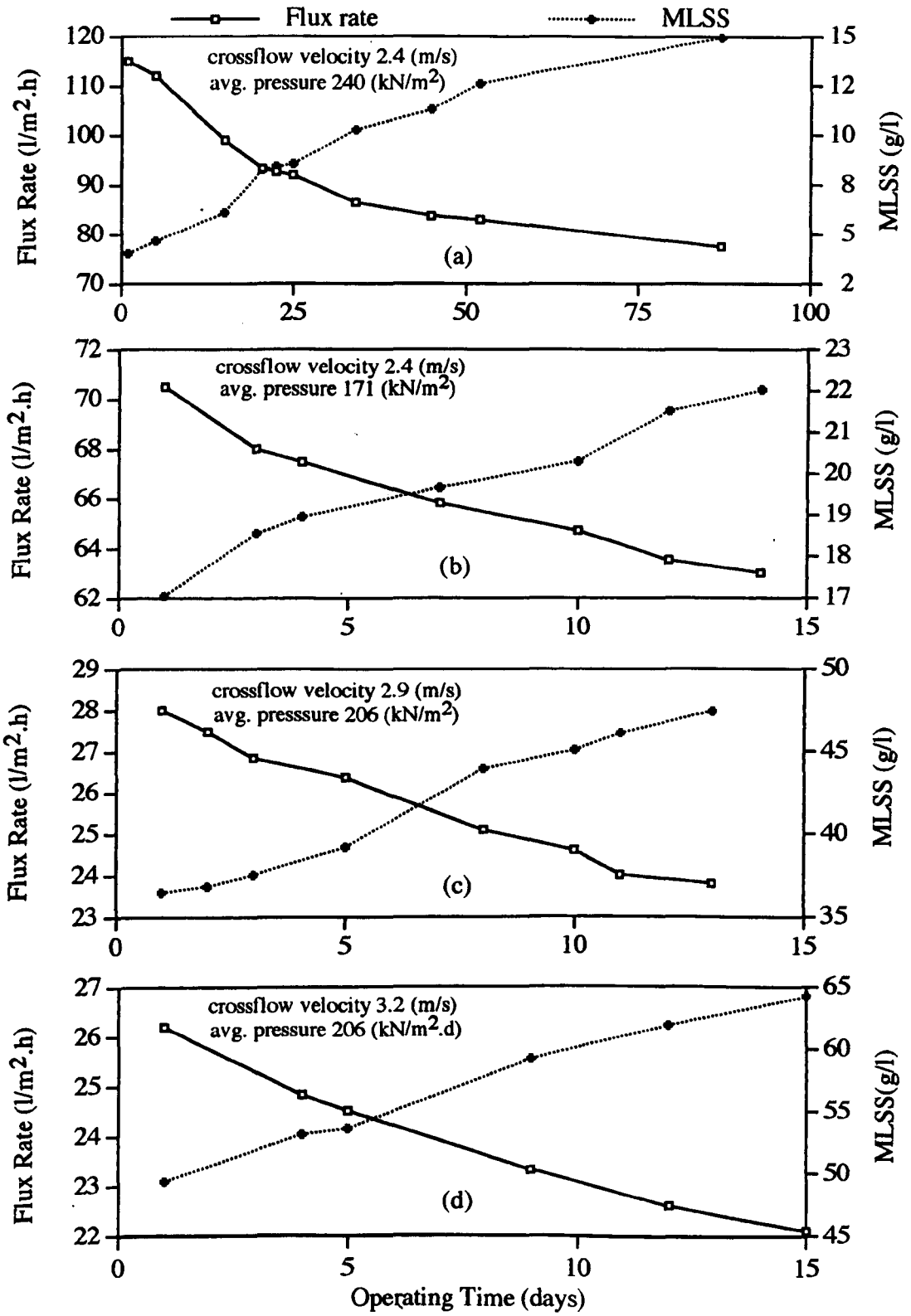


Figure 5.4a-d Flux rates of the CUMAR system against operating conditions

5.2.2. Colour, Turbidity and Suspended Solids

The true colour of the wastewater fed to the digester was in the range 2400-3500 ° Hazen and as can be seen from Figure 5.5, approximately 50-70% colour removal was achieved through the membrane.

The turbidity of the permeate was found to be 0.2-0.6 NTU, shown in Figure 5.6, while the suspended solids (SS) and volatile suspended solids (VSS) were in the range of 0.5-2.0 mg/l and 0.5-1.0 mg/l respectively indicating almost 100% biomass separation by the membrane unit. In addition to this, particle sizes which passed through the ultrafiltration membrane were measured by Coulter Counter with no particles with a diameter greater than 0.4 micron being found in the permeate.

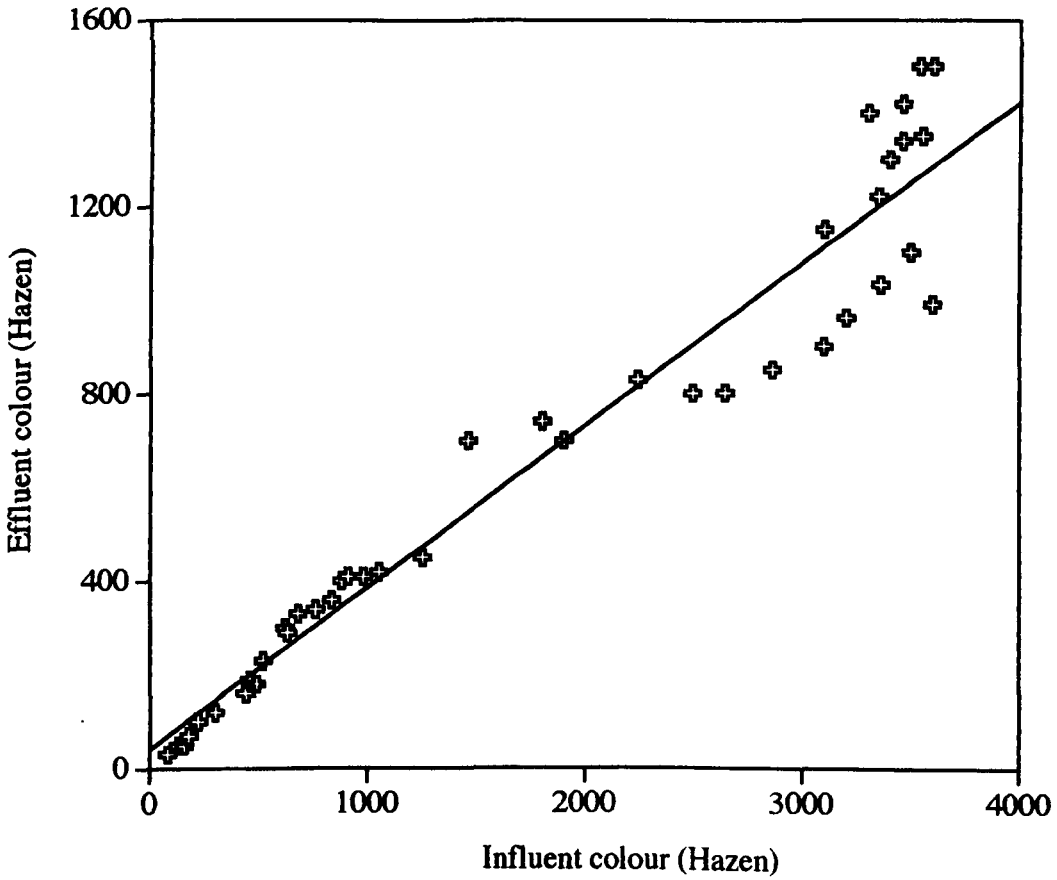


Figure 5.5 Colour removal of CUMAR system by the UF membranes

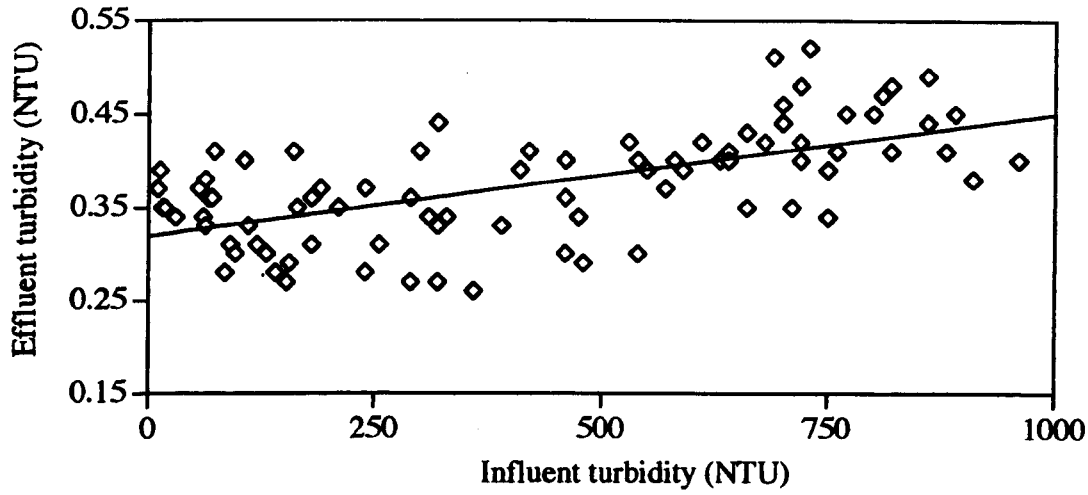


Figure 5.6 Turbidity in permeate throughout operation of CUMAR system

5.2.3. Specific Methanogenic Activity Test (SMA), Most Probable Numbers (MPN) and Microscopic Count

Throughout the operation of the system , specific methanogenic activity (SMA) tests, most probable number (MPN) and microscopic counts were carried out and the results obtained from SMA tests showed that almost no methanogenic activity was found in the permeate. This was also confirmed by the results of MPN, microscopic count and plate count shown in Figure 5.7.

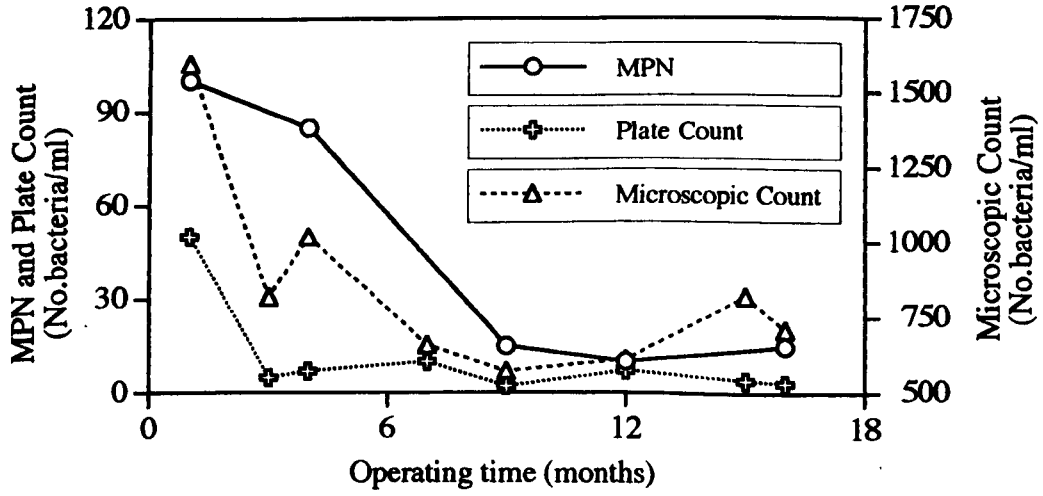


Figure 5.7 Microbiological analyses in permeate of CUMAR system

5.3 Analyses of Reactor and Effluent COD

Throughout the operation, the overall BOD removal efficiency of the CUMAR system was found to be almost 100 % resulting in less than 200 mg/l BOD in the effluent at steady-state conditions, whilst COD removal efficiencies of 97% and 99% were achieved in the reactor and in the permeate respectively. It is therefore assumed that the residual COD in the permeate and in the reactor might have contained some organic matter which was not biologically degradable. In other words, some non-biodegradable organic compounds may have been produced by the microorganisms within the system.

The CUMAR system was then operated further for some time after reaching steady-state in order to determine whether or not the persistent residual organic compounds were non-biodegradable. The results obtained from three different steady-states were plotted in Figures 5.8-10. where it can be seen that significant removal of the residual organics was not possible even with extended contact times.

The implication of the results was that these organics may be regarded as non-biodegradable microbial products provided the wastewater has no inert COD fraction.

Once the inert soluble COD fraction of wastewater has been determined the amount of microbial products produced within the system can be estimated. The incremental and the comparison methods were employed and a relationship between the specific substrate utilization rates and the effluent soluble COD concentrations of the CUMAR system was determined in order to estimate the inert soluble COD fraction of the brewery wastewater. For comparison purposes the wastewater was subjected to the two methods in both anaerobic and aerobic batch reactors.

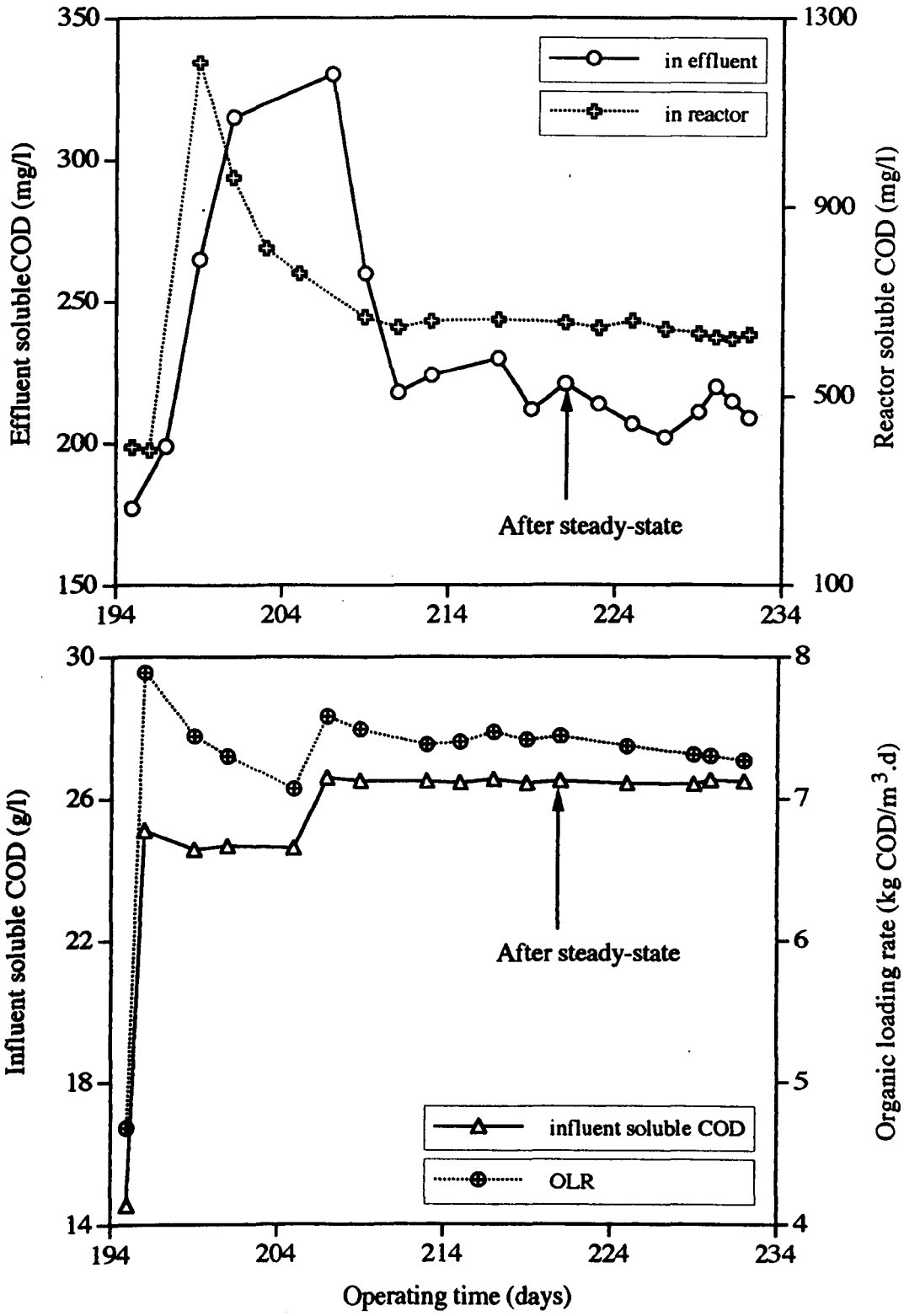


Figure 5.8 Monitoring changes in soluble COD in effluent and in reactor after a steady-state

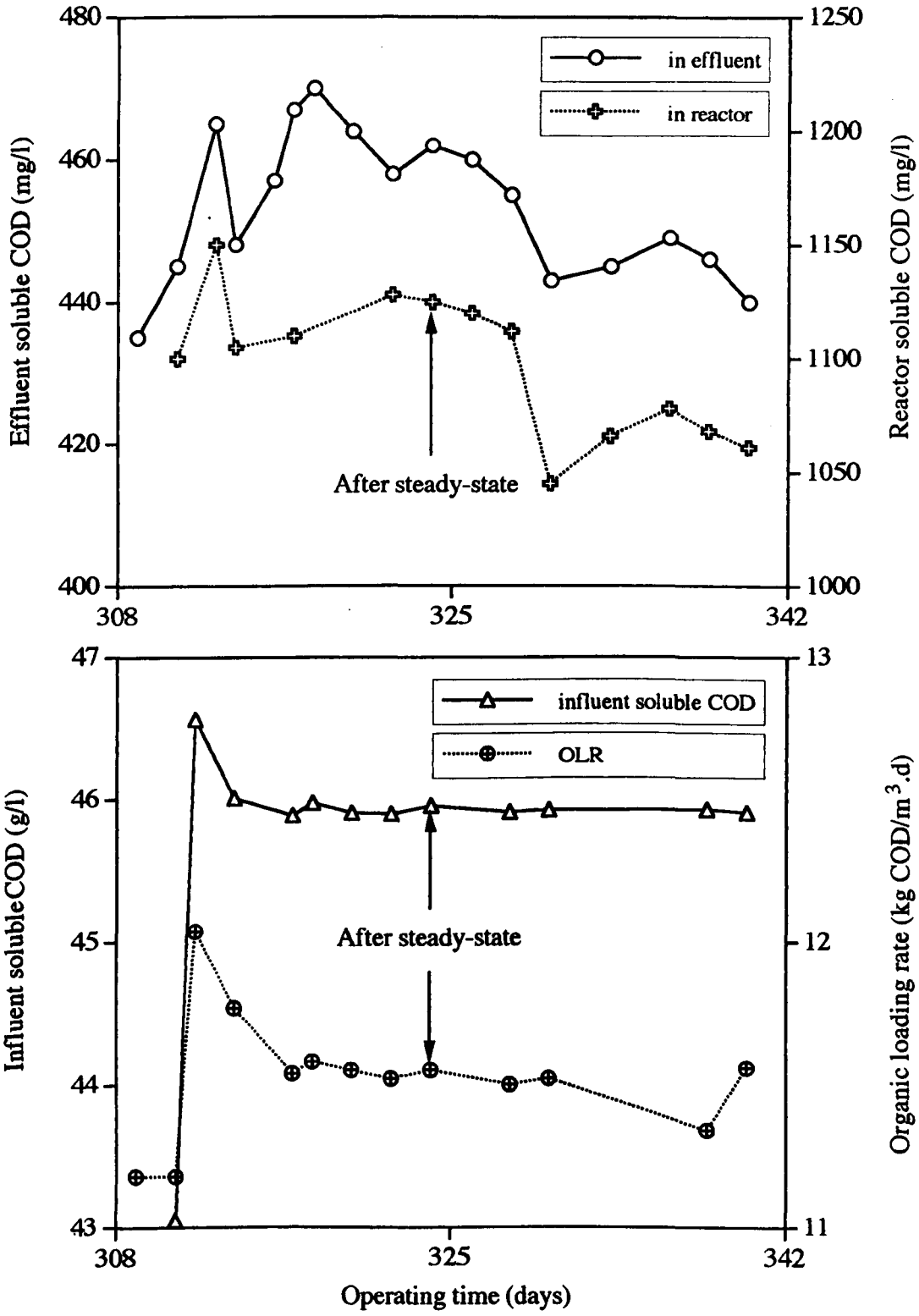


Figure 5.9 Monitoring changes in soluble COD in effluent and in reactor after a steady-state

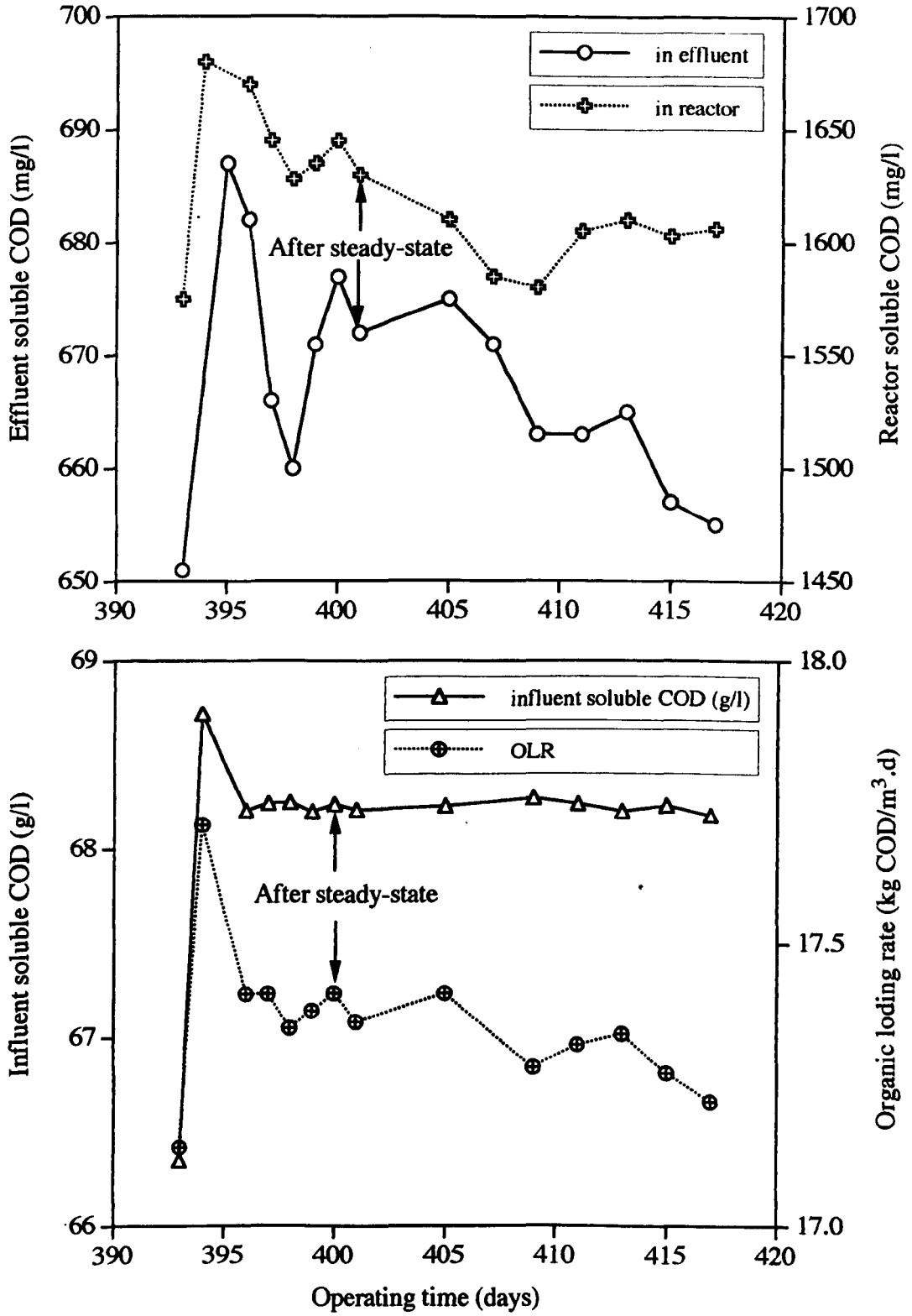


Figure 5.10 Monitoring changes in soluble COD in effluent and in reactor after a steady-state

5.3.1. Incremental Method

A set of four anaerobic and aerobic batch reactors, each having a volume of 5 litre was used. The first one was fed with the sample diluted to an initial COD of 880 mg/l and which was increased in the next three reactors to 1200 mg/l, 1500 mg/l and 1800 mg/l respectively using incremental glucose additions for the anaerobic batch reactors whilst an initial COD of 1060 mg/l of the diluted sample was maintained in all aerobic batch reactors with incremental addition of glucose being carried out in the 2nd, 3rd and 4th reactors in order to have 1350, 1700 and 2050 mg/l COD respectively. All the anaerobic and aerobic batch reactors were then seeded with the acclimatized biomass with an initial concentration of 30 mg/l VSS. A portion of the mixed liquor was periodically removed for the COD measurement at 588 hours for the anaerobic batch reactors and 373 hours for the aerobic reactors.

The results obtained from this study are given in Tables 5.3-4 and plotted in Figures 5.11-12. It can be seen that the COD profiles show a similar trend in which the soluble COD concentration is first reduced to a minimum, then increases and is reduced again to a final plateau which is lower than the previously achieved minimum level. The TVFAs in all reactors had been degraded by the end of test period, as shown in Figure 5.11.

The observed values of the first mimimum and ultimate soluble COD levels for the four anaerobic and four aerobic reactors are given in Table 5.5 and Table 5.6. The linear plots of these data, as shown in Figures 5.13-14, were analyzed with the least square method and the following expressions were obtained:

for the first mimimum COD concentrations:

$$S = 0.043S_O + 9.4 \qquad \text{for the anaerobic tests} \qquad (5.1)$$

and

$$S = 0.047S_O + 2.5 \qquad \text{for the aerobic tests} \qquad (5.2)$$

Table 5.3 Results of incremental method from anaerobic batch reactors

Time (hours)	R1 COD mg/l	R2 COD mg/l	R3 COD mg/l	R4 COD mg/l	R1 TVFA mg/l	R2 TVFA mg/l	R3 TVFA mg/l	R4 TVFA mg/l
0	880	1200	1500	1800	10	12	10	5
23	830	1020	1400	1610	110	170	215	320
33	810	980	1320	1540	220	390	325	480
55	730	820	1200	1500	400	660	410	850
75	620	760	1150	1500	415	550	815	1120
92	540	665	1070	1475	460	530	790	1200
115	480	600	1030	1410	315	380	745	1060
132	315	385	840	1270	200	225	585	975
180	160	200	690	1165	90	110	420	820
252	90	110	460	850	4	70	330	730
295	75	110	435	720	5	20	275	600
315	65	75	290	410	2	7	122	370
334	50	57	195	390	1	2	42	212
351	52	68	105	270	0	5	36	100
364	60	60	80	210	1	2	12	50
388	60	70	70	130	1	0	4	35
397	50	75	75	105	0	1	0	22
417	43	70	87	100	0	1	2	7
432	45	60	90	95	1	1	2	4
449	43	54	80	95		1	0	5
465	43	55	73	90		0	0	3
483		53	70	93			1	1
501		55	68	97			1	2
514			64	110			1	0
528			65	100				2
541			64	85				1
554			65	80				0
565				83				0
577				83				1
588				80				

Table 5.4 Results of incremental method from aerobic batch reactors

Time (hours)	G1 (mg/l COD)	G2 (mg/l COD)	G3 (mg/l COD)	G4 (mg/l COD)
0	1060	1350	1700	2050
17	540	600	680	950
32	60	70	90	96
48	52	63	86	100
66	67	72	95	120
78	62	83	100	145
101	57	67	90	120
121	54	62	85	110
154	50	62	85	100
177	47	63	83	98
212	46	65	85	94
230	48	62	82	95
241	46	62	82	90
262	48	60	85	90
274		63	80	95
285		60	82	90
298		60	82	88
304		63	80	90
316			80	90
324			80	88
341			80	92
356				90
373				88

For anaerobic reactors

R1 = First reactor (880 mg COD/l wastewater)

R2 = Second reactor (880 mg COD/l wastewater + 320 mg COD/l glucose)

R3 = Third reactor (880 mg COD/l wastewater + 620 mg COD/l glucose)

R4 = Fourth reactor (880 mg COD/l wastewater + 920 mg COD/l glucose)

R1 TVFA = Total volatile fatty acids in the first reactor
R2 TVFA = Total volatile fatty acids in the second reactor
R3 TVFA = Total volatile fatty acids in the third reactor
R4 TVFA = Total volatile fatty acids in the fourth reactor

For aerobic reactors

G1 = First reactor (1050 mg COD/l wastewater)
G2 = Second reactor (1050 mg COD/l wastewater + 300 mg COD/l glucose)
G3 = Third reactor (1050 mg COD/l wastewater + 650 mg COD/l glucose)
G4 = Fourth reactor (1050 mg COD/l wastewater + 1000 mg COD/l glucose)

S = Residual COD concentration

S_o= Influent COD concentration

for the ultimate COD concentrations:

$$S = 0.040S_O + 6.3 \qquad \text{for the anaerobic tests} \qquad (5.3)$$

and

$$S = 0.047S_O + 2.0 \qquad \text{for the aerobic tests} \qquad (5.4)$$

It can be seen from Equations 5.1-5.4 the intercepts of Equations 5.1 and 5.3 are greater than that of those in Equations 5.2 and 5.4 while the slopes are less. This could be explained as being due to the different sludge ages at which the experiments were run.

According to the above equations, inert soluble COD concentrations of 9 mg/l and 3 mg/l from the first minimum values and 6 mg/l and 2 mg/l from the ultimate COD values from both anaerobic and aerobic batch reactors respectively were estimated for this wastewater. It was concluded that the brewery wastewater has comparatively little inert soluble COD.

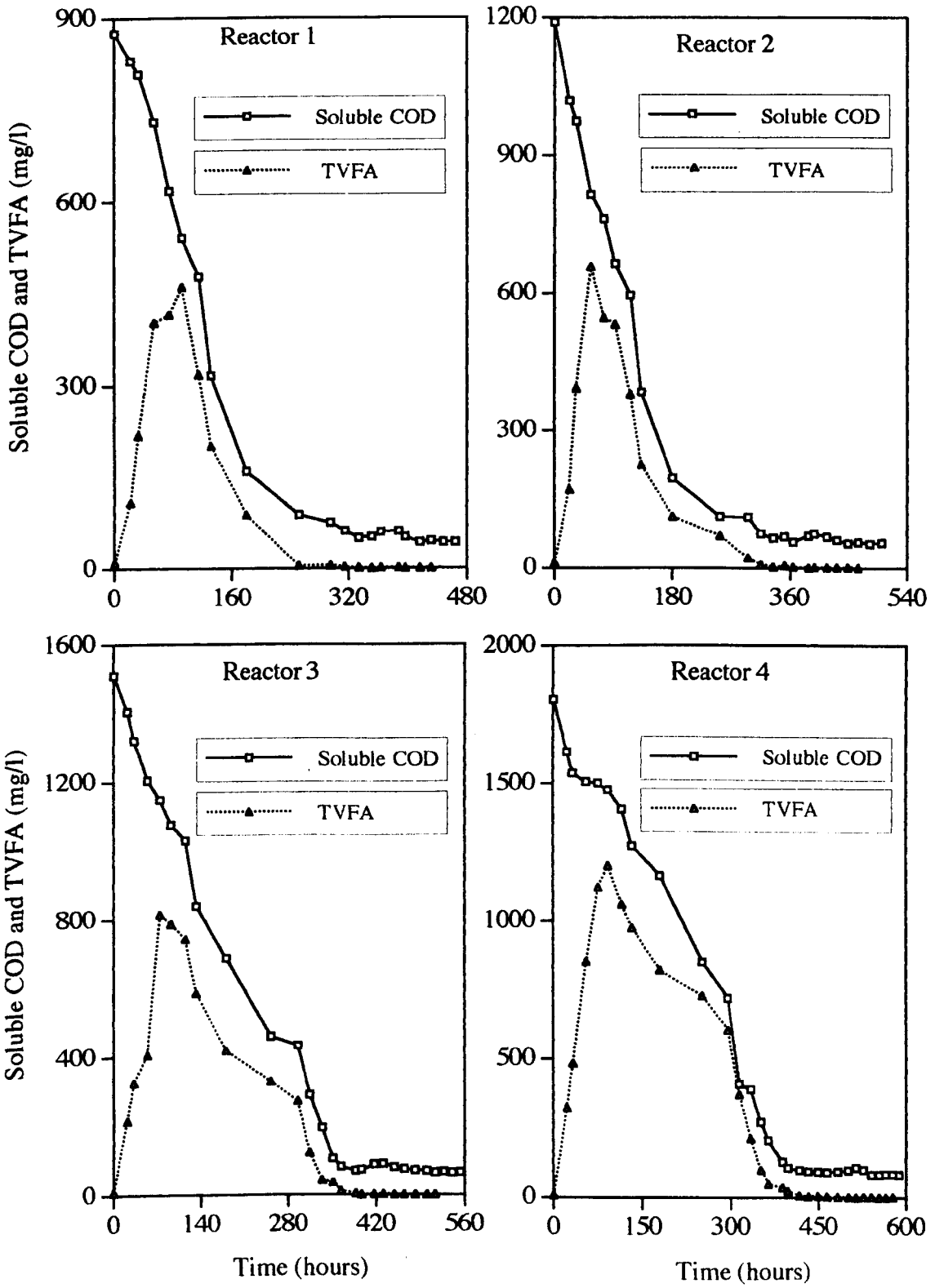


Figure 5.11 Results of incremental method from anaerobic batch reactors

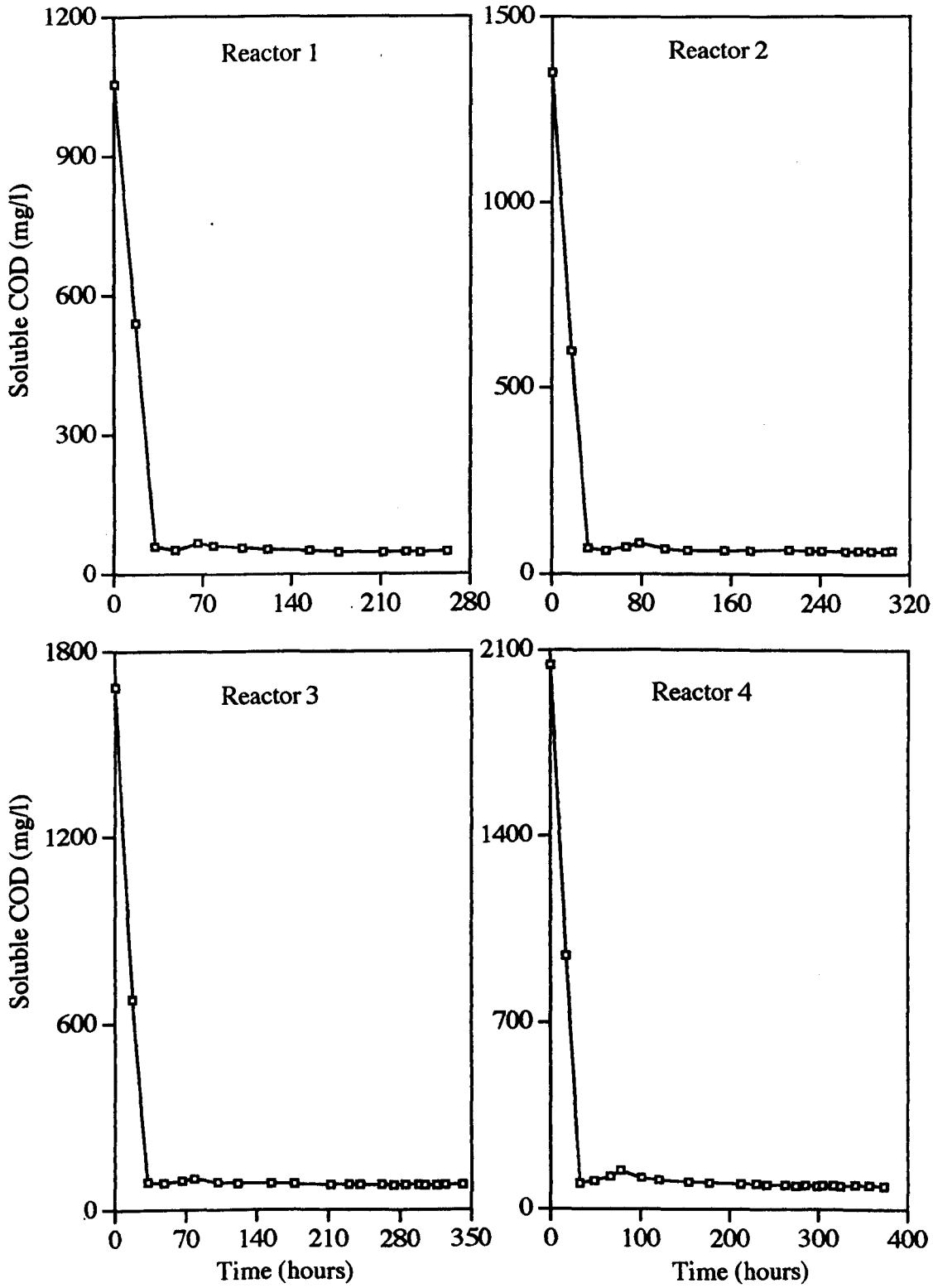


Figure 5.12 Results of incremental method from aerobic batch reactors

Table 5.5 Observed first minimum and ultimate COD values of incremental method from anaerobic batch reactors (see Table 5.3)

Run	Initial Soluble COD mg/l	First Minimum Soluble COD mg/l	Ultimate Soluble COD mg/l
1	880	50	43
2	1200	57	53
3	1500	70	64
4	1800	90	80

Run 1 = First reactor (880 mg COD/l wastewater)

Run 2 = Second reactor (880 mg COD/l wastewater + 320 mg COD/l glucose)

Run 3 = Third reactor (880 mg COD/l wastewater + 620 mg COD/l glucose)

Run 4 = Fourth reactor (880 mg COD/l wastewater + 920 mg COD/l glucose)

Table 5.6 Observed first minimum and ultimate COD values of incremental method from aerobic batch reactors (see Table 5.4)

Run	Initial Soluble COD mg/l	First Minimum Soluble COD mg/l	Ultimate Soluble COD mg/l
1	1050	52	46
2	1350	63	60
3	1700	86	80
4	2050	96	88

Run 1 = First reactor (1050 mg COD/l wastewater)

Run 2 = Second reactor (1050 mg COD/l wastewater + 300 mg COD/l glucose)

Run 3 = Third reactor (1050 mg COD/l wastewater + 650 mg COD/l glucose)

Run 4 = Fourth reactor (1050 mg COD/l wastewater + 1000 mg COD/l glucose)

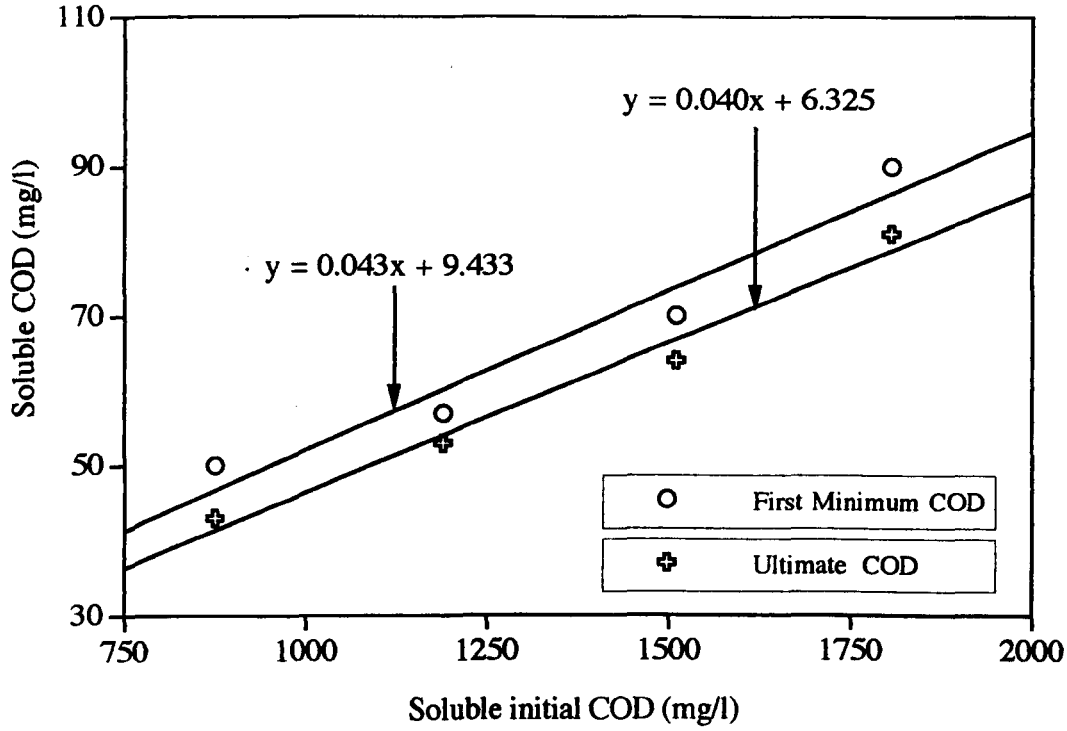


Figure 5.13 Plots of first minimum and ultimate COD of incremental method from anaerobic batch reactors

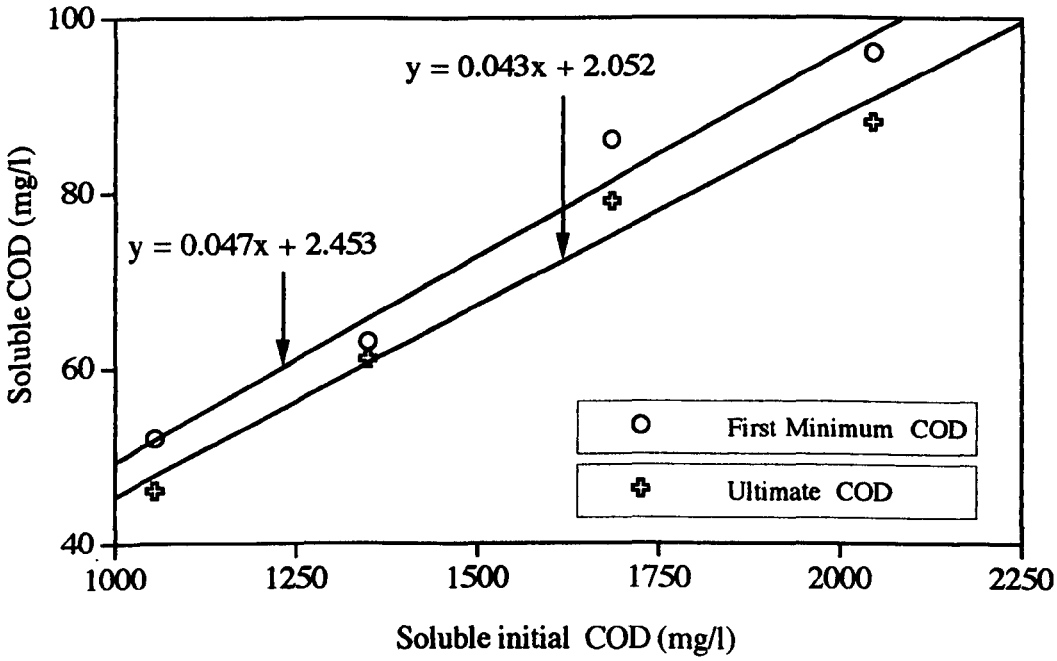


Figure 5.14 Plots of first minimum and ultimate COD of incremental method from aerobic batch reactors

5.3.2. Comparison Method

Two different dilutions of 1030 mg/l and 1980 mg/l were added to the anaerobic batch reactors with the acclimatized biomass having an initial concentration of 40 mg/l VSS whilst 980 mg/l and 1960 mg/l were prepared for aerobic batch tests at an initial VSS concentration of 30 mg/l. The data were periodically collected for 518 hours from the anaerobic reactors and for 384 hours from the aerobic reactors and are given in Tables 5.7-8 showing a reduction to a minimum level, then an increase and a subsequent decrease to a plateau which remains practically unchanged with time. Figures 5.15a-c and Figures 5.16a-b show the results obtained from the anaerobic and the aerobic reactors, from which it can be seen that the brewery wastewater has almost no inert soluble COD.

5.3.3. Specific Substrate Utilization Rate and Effluent COD

The concentration of non-biodegradable COD in the brewery wastewater used in this study was estimated by plotting specific substrate utilization rate (SSUR) against the effluent and the reactor soluble COD concentrations. If a curve had a positive abscissa intercept it may be considered that the wastewater contained non-biodegradable soluble COD and the SSUR would be zero when a concentration of biodegradable soluble COD is zero.

The SSURs calculated from the data collected throughout the operation of the CUMAR system were plotted against the effluent soluble COD concentrations (Figure 5.17) yielding the following expression:

$$SSUR = 0.301 Log(S_{effluent}) - 0.356 \quad (5.5)$$

where $S_{effluent}$= effluent COD concentration.

Table 5.7 Results of comparison method from anaerobic batch reactors

Time (hours)	R1 COD mg/l	R2 COD mg/l	R3 COD mg/l	R4 COD mg/l	R1 TVFA mg/l	R2 TVFA mg/l	R3 TVFA mg/l	R4 TVFA mg/l
0	1060	1030	2020	1980	15	20	30	27
6	755	980	1600	1900	330	140	415	160
18	650	860	1560	1830	420	230	675	240
30	620	720	1500	1820	480	540	800	315
42	590	640	1380	1740	470	600	980	360
66	510	545	1160	1380	400	480	1090	910
92	390	340	1080	1180	230	220	890	1060
113	252	190	1030	1130	135	95	765	950
136	190	180	910	970	85	90	590	790
170	165	135	470	580	35	30	200	230
210	100	100	365	300	10	10	180	125
252	85	95	340	280	8	5	165	110
280	66	80	280	210	4	2	45	67
298	82	62	255	180	6	1	110	130
312	95	75	250	160	4	4	90	30
334	70	85	230	145	3	3	67	5
356	62	90	165	130	2	3	25	3
371	63	90	147	120	1	4	10	2
394	64	80	130	130	1	2	1	2
413	62	70	130	150	1	2	1	1
431	63	60	145	155		1	3	1
442		58	130	140		1	4	1
455		58	120	125		1	2	1
469			115	115			2	1
481			120	110			1	1
492			115	110				1
505			115	115				1
518			115	110				1

Table 5.8 Results of comparison method from aerobic batch reactors

Time (hours)	G1 (mg/l COD)	G2 (mg/l COD)	G3 (mg/l COD)	G4 (mg/l COD)
0	1040	990	2030	1960
16	880	700	1890	1580
28	95	100	190	120
42	86	65	260	115
60	120	67	230	88
81	140	70	270	95
104	125	80	250	110
127	110	72	240	115
152	75	75	210	120
172	65	70	160	100
194	67	65	115	97
211	66	60	100	90
232	62	57	100	87
258	63	60	93	88
281	63	60	90	90
304		59	92	87
317			90	85
330			90	85
351				87
370				85
384				84

For anaerobic reactors

R1 = First reactor (1060 mg COD/l glucose)

R2 = Second reactor (1030 mg COD/l wastewater)

R3 = Third reactor (2020 mg COD/l glucose)

R4 = Fourth reactor (1980 mg COD/l wastewater)

R1 TVFA = Total volatile fatty acids in the first reactor

R2 TVFA = Total volatile fatty acids in the second reactor

R3 TVFA = Total volatile fatty acids in the third reactor

R4 TVFA = Total volatile fatty acids in the fourth reactor

For aerobic reactors

G1 = First reactor (1040 mg COD/l glucose)
G2 = Second reactor (990 mg COD/l wastewater)
G3 = Third reactor (2030 mg COD/l glucose)
G4 = Fourth reactor (1960 mg COD/l wastewater).

From this expression the inert soluble COD of the brewery wastewater was estimated to be approximately 15 mg/l.

On the other hand the plot of SSURs against the reactor soluble COD concentrations shown in Figure 5.18 yielded the following equation:

$$SSUR = 0.317Log(S_{reactor})-0.531 \quad (5.6)$$

where $S_{reactor}$ = reactor COD concentration.

The above equation gives a concentration of approximately 45 mg/l inert COD which the brewery wastewater may have.

Both results are very close to each other and yield S/S_o ratios of 0.00015 and 0.0005 respectively when compared to the concentration of the brewery wastewater.

The results obtained from the above three methods confirm that the brewery wastewater contains comparatively little inert soluble COD. The soluble COD in the effluent may therefore be regarded as microbial products. However, the soluble COD in the effluent could also be from sources other than microbial products, i.e. reduced inorganics (sulphides), therefore, additional studies should be carried out.

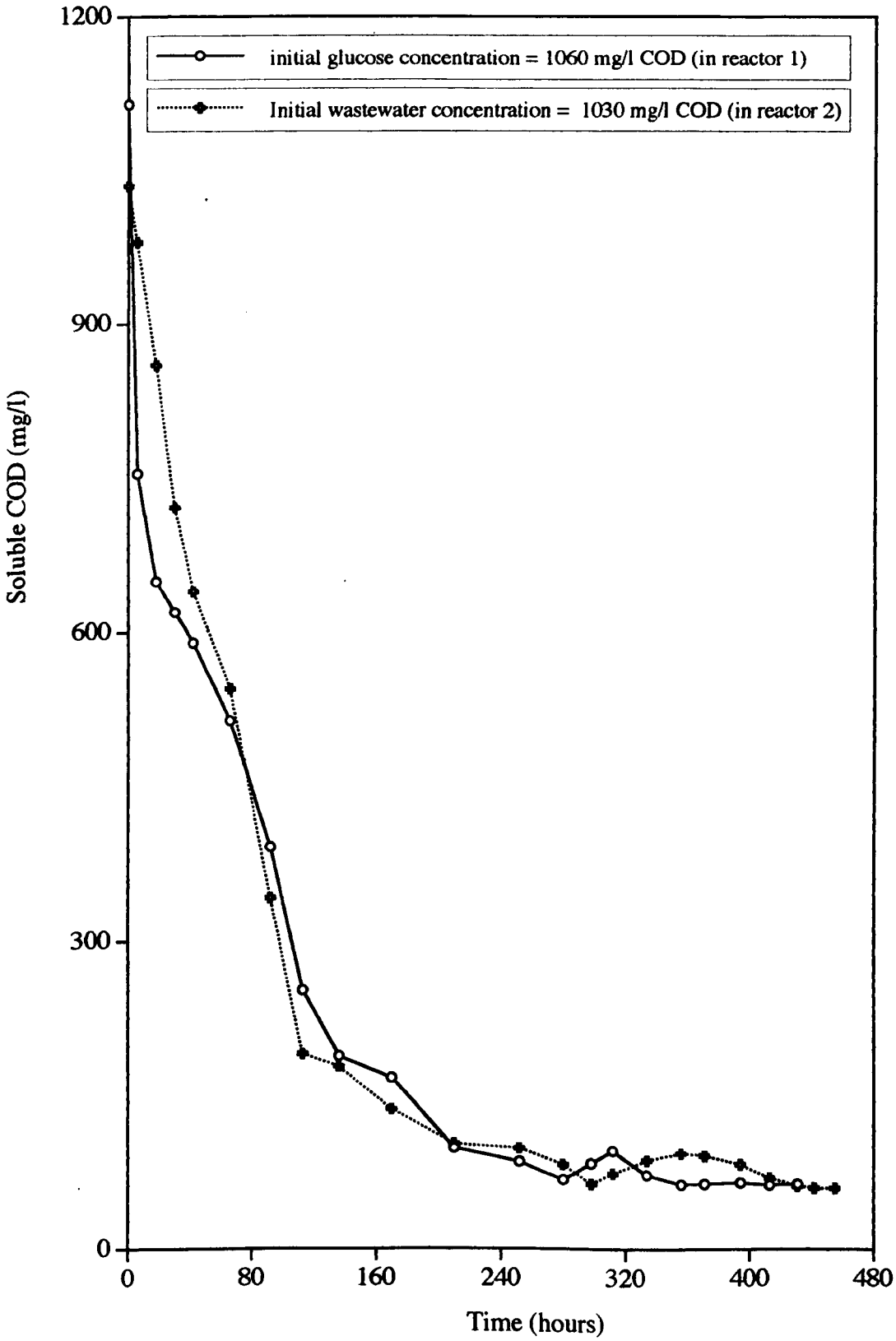


Figure 5.15a Plots of comparison method results from anaerobic batch reactors

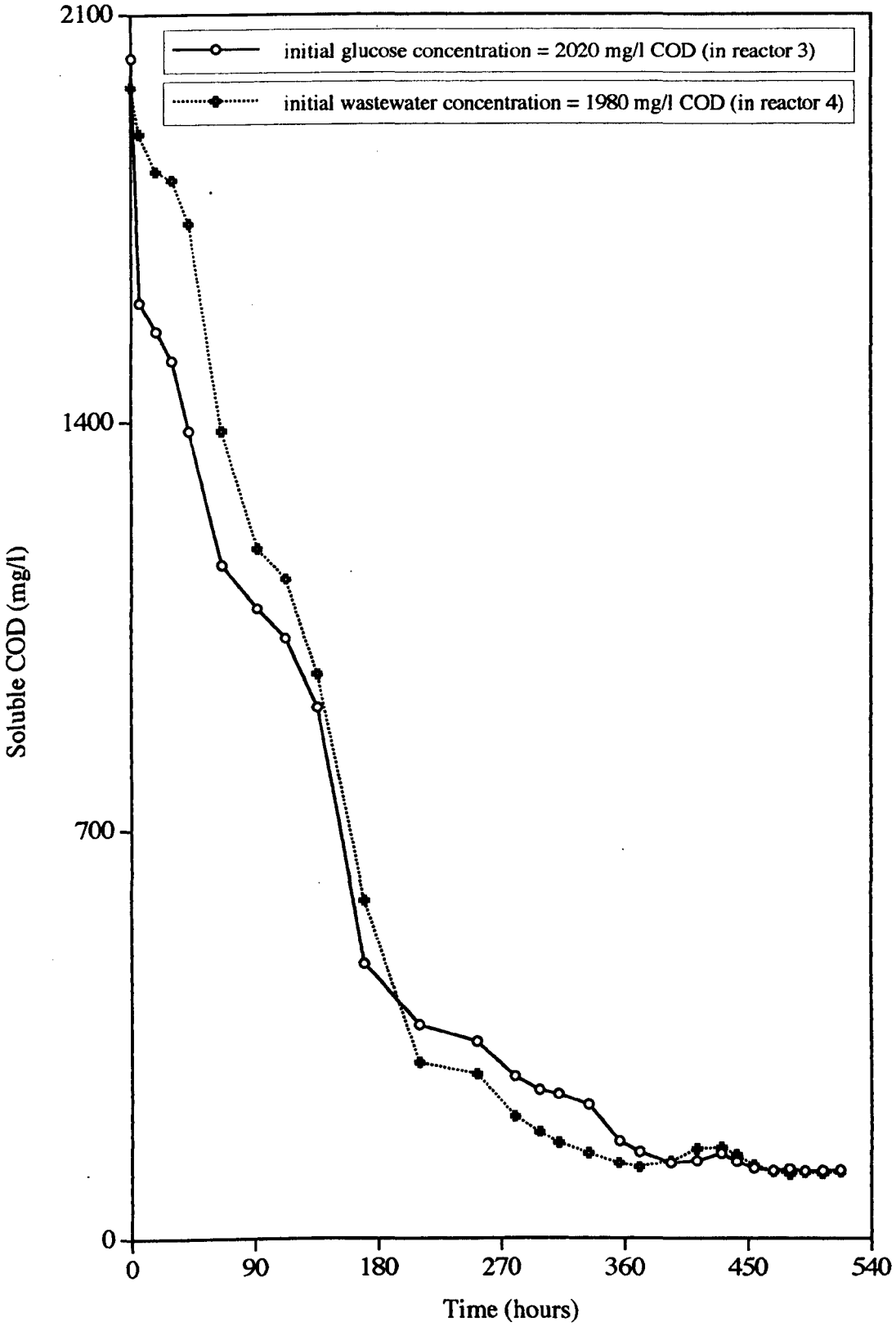


Figure 5.15b Plots of comparison method results from anaerobic batch reactors

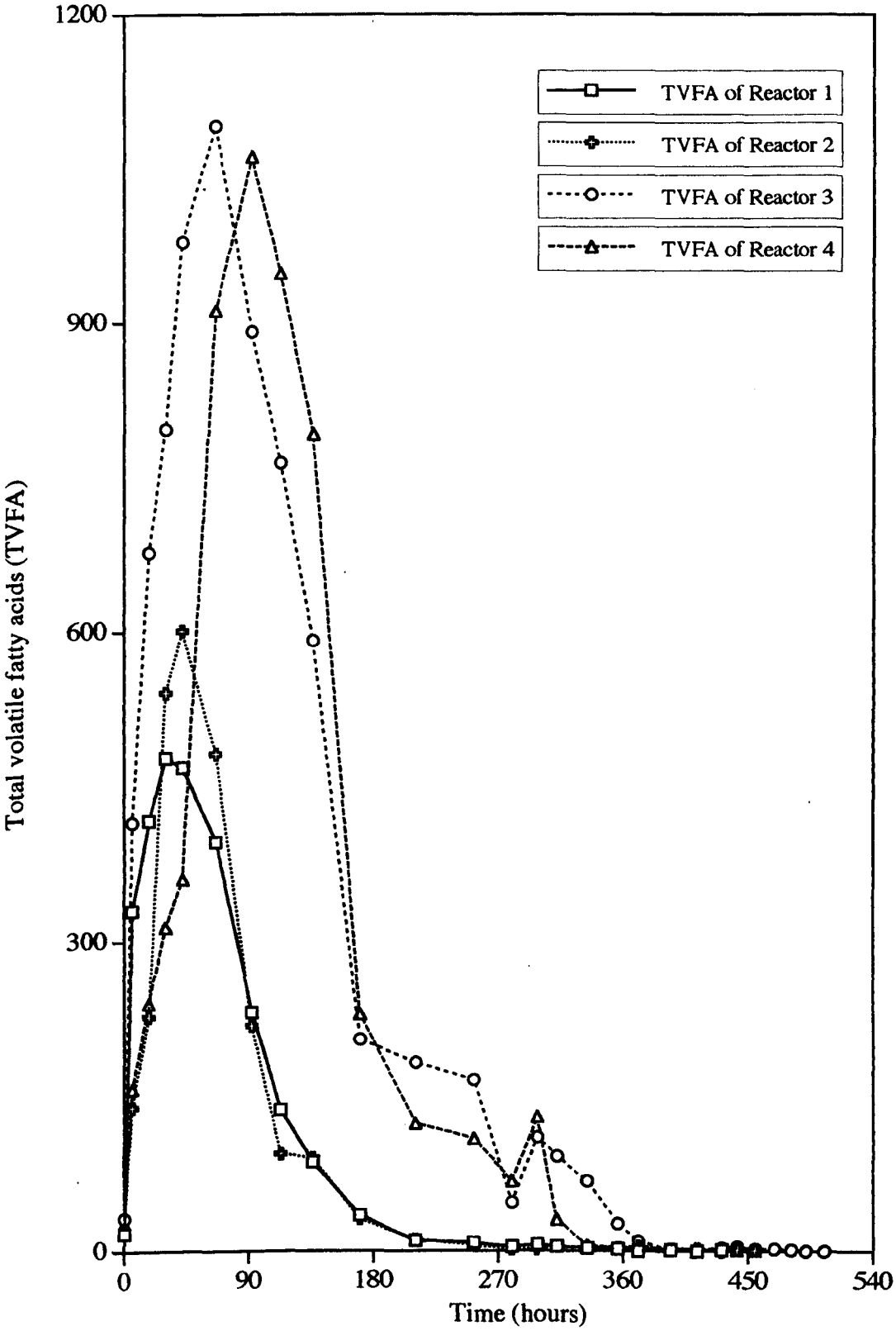


Figure 5.15c Plots of comparison method results from anaerobic batch reactors

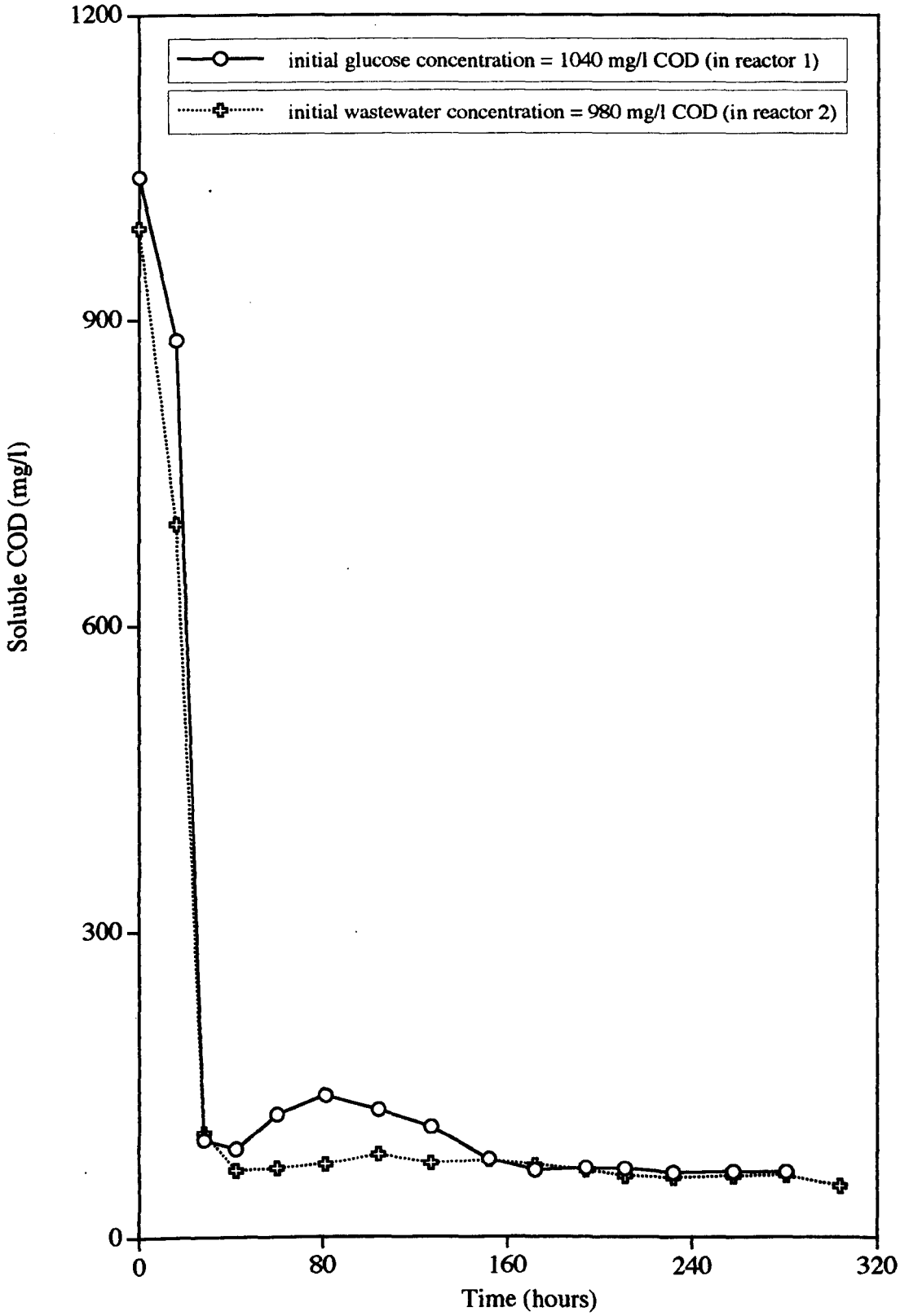


Figure 5.16a Plots of comparison method results from aerobic batch reactors

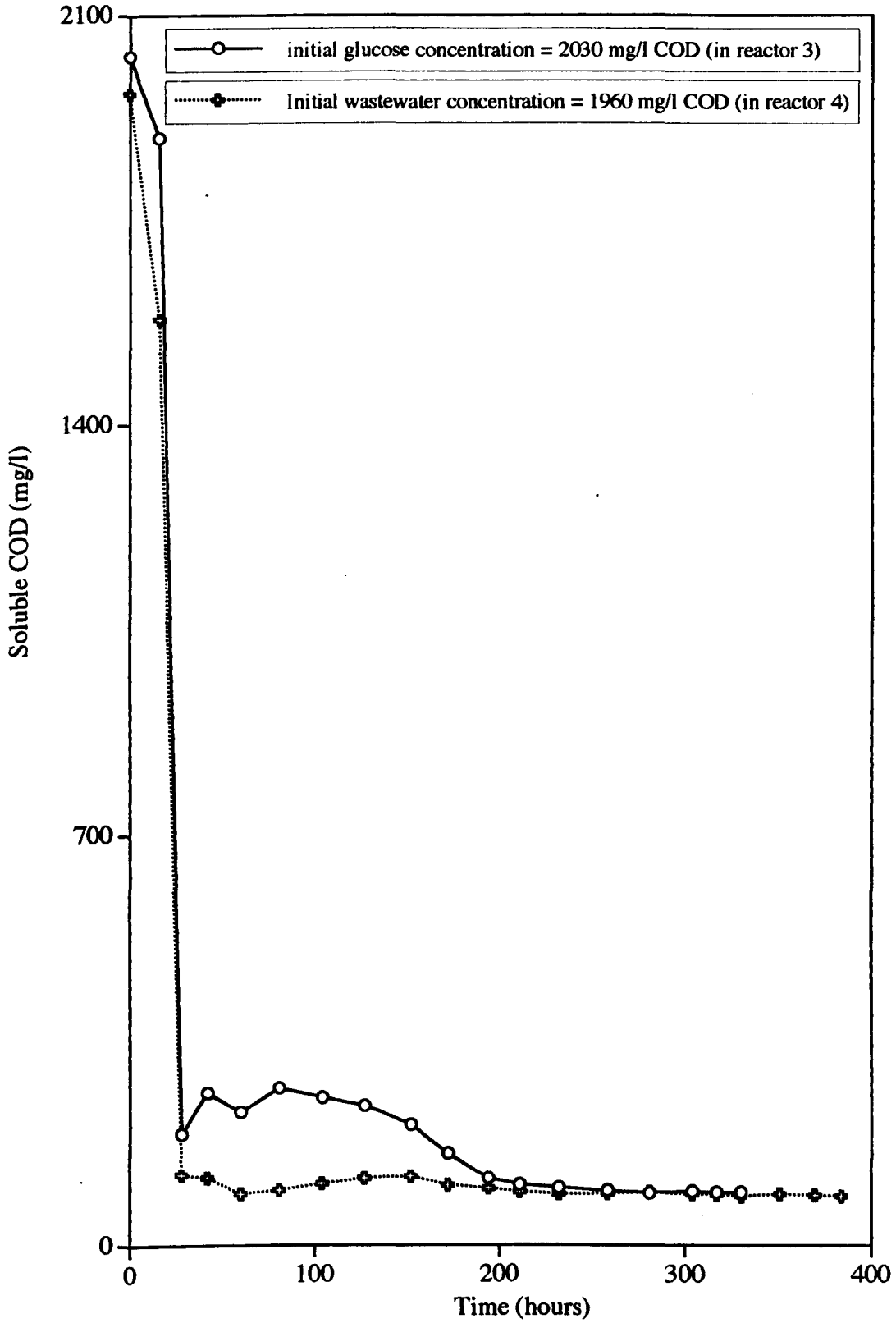


Figure 5.16b Plots of comparison method results from aerobic batch reactors

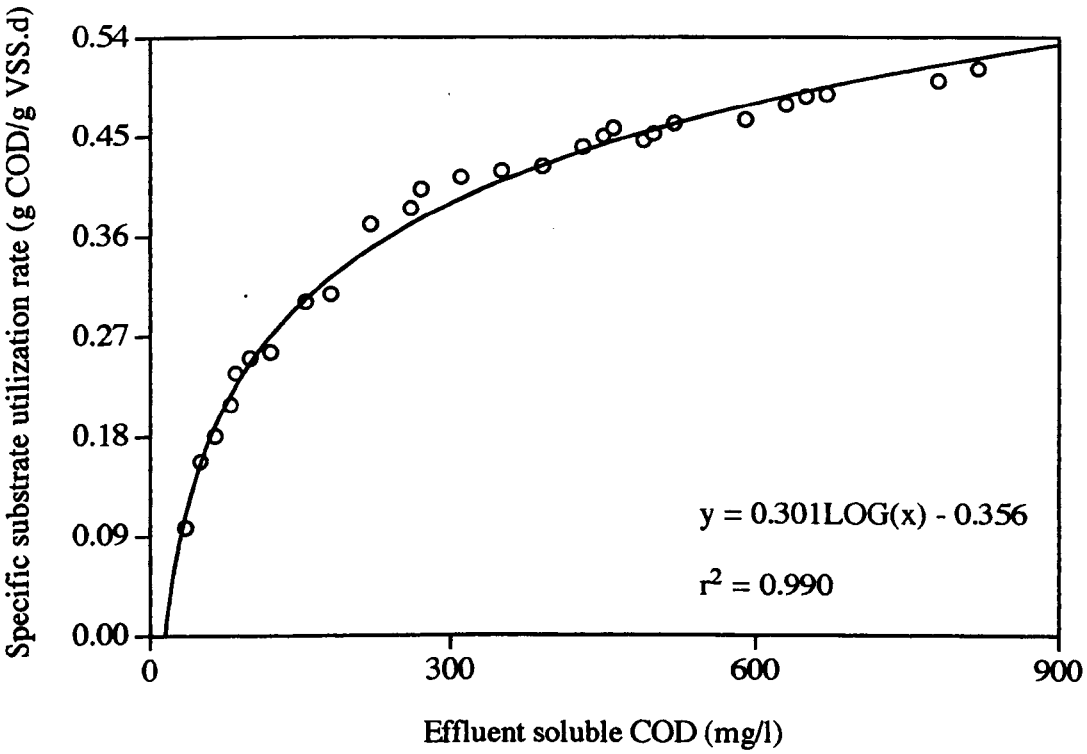


Figure 5.17 Plots of specific substrate utilization rate against effluent soluble COD

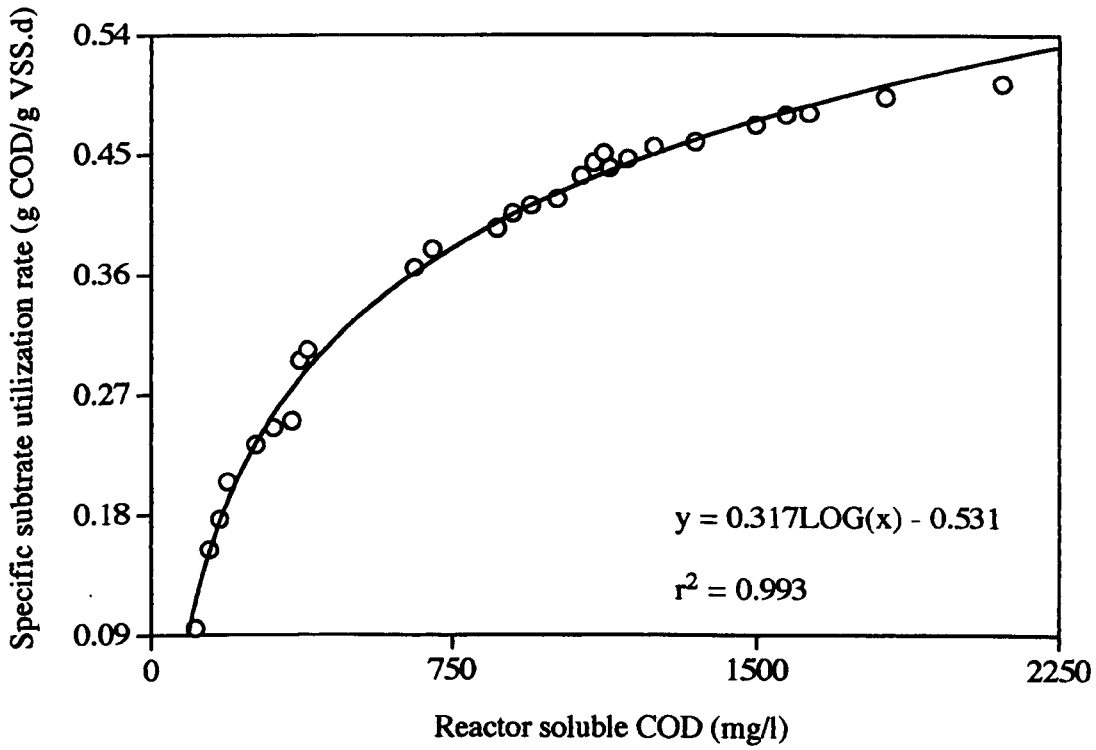


Figure 5.18 Plots of specific substrate utilization rate against reactor soluble COD

The following equation (Sykes, 1981) which is applicable only to treatment plants with high COD removal efficiencies will be used here in order to establish a relationship between the influent and the effluent soluble COD since the COD removal efficiency of the CUMAR system was over 99% in the effluent during the steady-state operation:

$$S_{effluent} = aY_g S_{influent} \quad (5.7)$$

where a = coefficient of proportionality, g COD/g VSS

Y_g = observed yield, g VSS/g COD

The plot of the effluent soluble COD concentrations against the influent soluble COD concentrations is given in Figure 5.19 yielding the following equation:

$$S_{effluent} = 0.009S_{influent} + 13 \quad (5.8)$$

The overall biomass yield of the CUMAR system was estimated (in Chapter 5.6) to be 0.038 g VSS/g $COD_{removed}$. Therefore "a" can be derived from the above equation and is approximately 0.24. This means that the amount of biomass produced in the digester will produce "a" fraction of its production as a non-biodegradable soluble COD.

A plot of SRT against effluent soluble COD concentration is given in Figure 5.20 showing microbial product formation. For example, an increase in biomass production (a decrease in SRT) will yield an increase in effluent soluble COD concentration.

From the above results, it may be concluded that the effluent soluble COD concentration is not independent from the influent soluble COD concentration and may be expressed as not only a function of influent soluble COD but also a function of SRT.

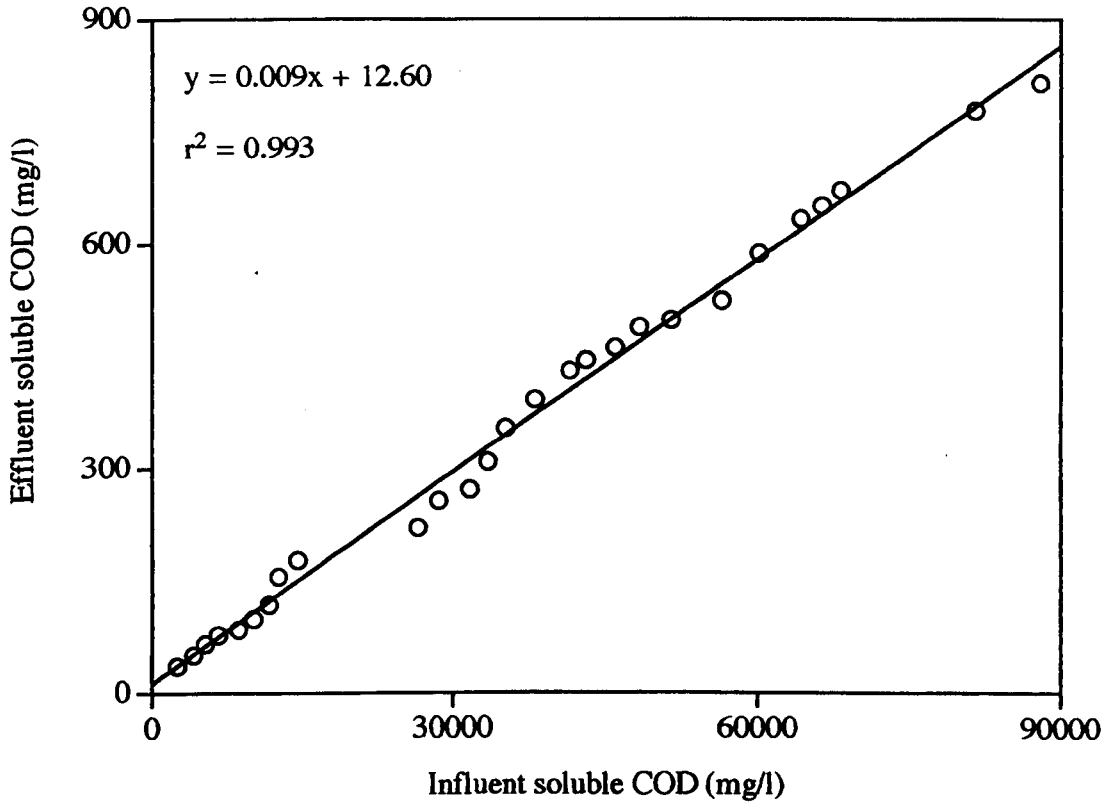


Figure 5.19 Effluent soluble COD against influent soluble COD

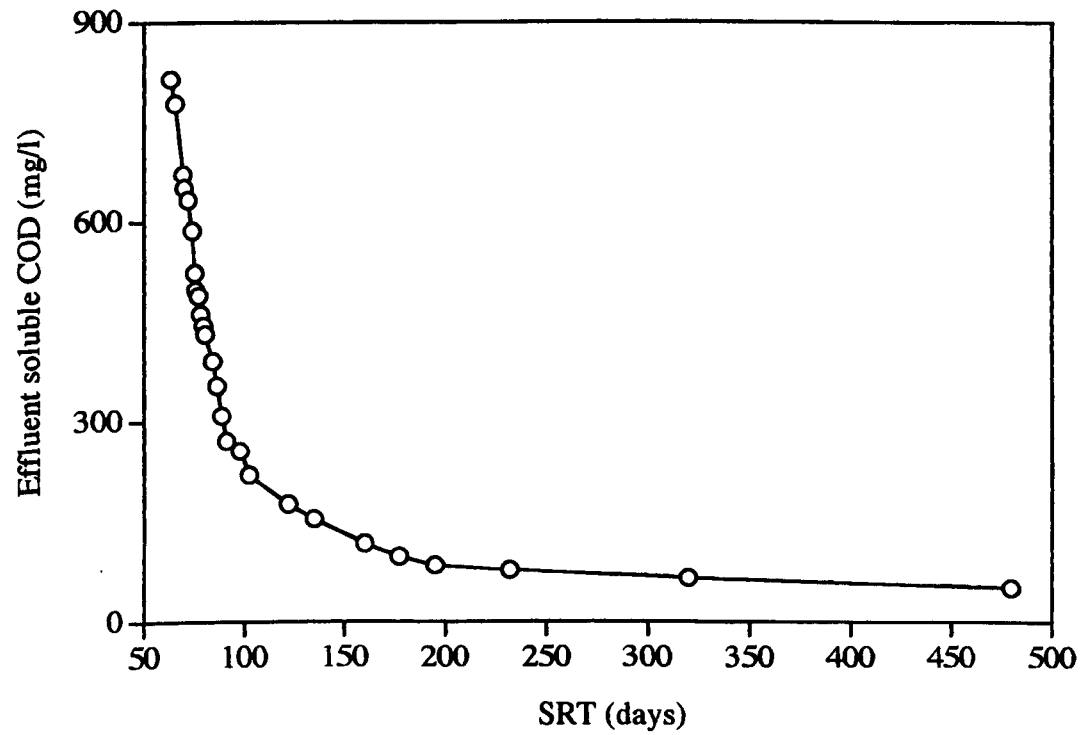


Figure 5.20 Solid retention time against effluent soluble COD

5.4 Discussion

Organic loading rates can be applied efficiently at up to 6 kg COD/m^3.d in full-scale applications of conventional anaerobic contact digesters. Table 5.9 shows a comparison of organic loading rates of a range of anaerobic bioreactors. Further increases in OLR are restricted by an inadequate amount of biomass retained in the anaerobic contact digesters. In this study, therefore, a new method of operating a completely mixed digester using a crossflow ultrafiltration membrane technique for the retention of active biomass and for the determination of the extent of any other advantageous that can be gained over other reactor configurations (Table 5.9) was studied. Retaining an adequate amount of active biomass in the digester (determined by MLVSS measurement and SMA tests) using a membrane separation technique resulted in excellent organic matter removal throughout the study.

The activity of the initiate biomass inoculum affects the rate of start-up. Therefore the seed sludge was taken from a fully-operational local digester treating municipal wastewater. An OLR of 1 kg COD/m^3.d, corresponding 0.1 kg COD/kg VSS.d was decided to be the initial loading to the digester since the loading rate during the initial phase of start-up of an anaerobic reactor was reported (Stronach et al., 1986) to be 0.1 kg COD/kg VSS.d which corresponds to 1.2 kg COD/m^3.d for a system with a concentration of biomass of 10-20 g/l VSS. However, the first weeks results showed that COD removal and methane yield were very low, the OLR was therefore maintained at approximately 0.5 kg COD/m^3.d for the following four week operation since a period of acclimatization to the waste would be necessary before an increase in COD loading was attempted. The performance of the system might also be affected due to high recirculation of the reactor contents and the high pressure applied to the biomass while passing through the membranes.

Table 5.9 Comparison of reactor loading rates of anaerobic bioreactors

Anaerobic Reactor Type	Operational Temperature °C	Loading Rates (kg Units/m^3.d)	Units	COD Removal (%)
Conventional				
(a) Standard Rate	Ambient	0.32-0.8	VS	N.R
(b) High Rate	35	3.2	VS	N.R
Anaerobic Contact Reactor	35	1-6	COD	80-95
Upflow Anaerobic Sludge Blanket Reactor	35	5-30	COD	80-95
Two-stage System	25-50*	10-60	COD	-
	30-40**	8-25	COD	-
Anaerobic Filter	35	2-16		80-95
Expended Bed Reactor	35	1-20	COD	-
Fludised Bed Reactor	35	1-20	COD	80-87

Source: Donnelly (1984), Anderson and Saw (1984) and Speece (1983)

N.R. = not reported

* Acidogenic Reactor

** Methanogenic Reactor

Although it has been reported (Stronach *et al.*, 1986) that methanogenic bacteria have relatively long generation times of 0.5-2 days and that 4-8 months may be required for the attainment of microbial steady-state in suspended biomass systems, the acclimatization period of both the acetogenic and methanogenic population in the CUMAR system was completed after 40 days operation. This could be explained by the retention of a sufficient quantity of active biomass using the membrane process in this study.

Despite a specific quantitiy of nutrients being required for growth and maintenance of the cell, Stronach *et al.* (1986) pointed out that the effect of excessive levels of nutrients on start-up of the digester process must be taken into consideration and excess concentrations of many nutrients are inhibitory rather than stimulatory to anaerobic degradation. Consequently, the concentrations of micro- and macro-nutrients in the brewery wastewater were determined before the system was run. It was found that nitrogen and phosphate were insufficient in the wastewater. Hence a COD:N:P ratio of 400:5:1 was generally maintained in the feed but steady state results showed that only a COD:N:P ratio of 400:2.3:0.2 was consumed by the biomass throughout the operation.

During the start-up period the VFA/alkalinity ratio was generally maintained at less than 0.4 in the effluent by adding $NaHCO_3$ to the feed and controlled by using a pH controller. At the beginning of start-up, the amount of alkalinity added to the feed (approximately 2000 mg/l as $CaCO_3$) was not sufficient to maintain the pH near neutral, hence a pH controller was employed in order to continuously monitor and control the pH, adding sodium hydroxide if necessary. After the start-up period, the alkalinity requirement of the system decreased proportionally as the OLR increased. Neither alkalinity addition to the feed nor automatic addition of NaOH by pH meter and controller to the digester was necessary after an OLR of 7 kg COD/m^3.d had been reached. This stability of pH in the digester could be explained by the buffering capacity of bicarbonate alkalinity which was formed from CO_2(aq) in the digester.

The percentage of COD removal after the start-up period was generally over 99% in the permeate with no sudden increases in volatile fatty acids (VFA) concentrations with increasing OLRs being seen, confirming that the digester was performing very well. The substrate utilization rate increased proportionally with increasing OLR with a corresponding increase in biomass concentration (Figure 5.3c). This indicated that the loading rate applied was probably the rate limiting factor for biomass production.

The results obtained from this study were most encouraging with respect to biomass retention and the treatment efficiency of the new anaerobic system. Clearly, the CUMAR system was capable of higher OLRs and had not reached its maximum treatment capacity. During the operation period, the maximum OLR applied was 28.5 kg COD/m^3.d. at which point COD removal efficiencies of approximately 99% in the permeate and over 97% in the digester were achieved (Figure 5.3a). The excellent COD removal efficiencies of the digester throughout the operation can be explained by the separation process which was employed in this study.

The inert COD studies showed that the brewery wastewater had almost no inert COD fraction and the soluble COD in the effluent may have been produced within the system by the biomass. These microbial products could not be removed from the effluent even with extended contact times.

The methane content of the biogas produced in the digester ranged from 80% to 65% and the methane yield ranged from 0.38 to 0.28 m^3 CH_4/kg $COD_{removed}$ over the experimental period. As can be seen in Figure 5.3b the methane yield and methane percentage were reasonably constant up to an OLR of 9 kg COD/m^3.d after which a decrease in both methane percentage and methane yield was observed, corresponding to the applied OLRs. This might be explained by the high OLRs which favour an increase in growth rate for acidogenic bacteria over methanogenic bacteria. Even under these conditions, the digester performed well which indicated that a new equilibrium must have been established between the bacterial populations in the digester, resulting in the process continuing with no sign of an impending failure.

As mentioned in Chapter 5.1.2. the OLR was increased, after 20 kg COD/m^3.d , by adding glucose, however the OLR could also be increased either by increasing the flux rates, employing a higher membrane area or by increasing the crossflow velocity or

increasing dramatically the deliberate sludge wastage rate which would reduce SRT significantly. The latter was not applied since it might have had a negative affect on the performance of the digester. On the other hand it would have been very expensive to buy a membrane module simply to increase the surface area and flux rate . Therefore, only the crossflow velocity and transmembrane pressure were increased in order to increase the flux. A crossflow velocity of 3.2 m/s was applied towards the end of study. A crossflow velocity greater than this was not used since it caused continuous sludge leaking from the recirculation pump which was not easy to control.

The results showed that flux rate is partly dependent upon the concentration of biomass. During wastage of sludge under steady-state conditions the flux rates remained reasonably constant whereas no biomass was wasted at unsteady-state conditions, during which period the flux rates decreased as the biomass concentration increased at all applied crossflow velocities and pressures.

The maximum reduction in flux rate was experienced at a crossflow velocity of 2.4 m/s and an average pressure of 171 kN/m^2 within the range 17-22 g/l MLSS whereas the minimum reduction in flux rate was observed at a crossflow velocity of 3.2 m/s and at an average pressure of 206 kN/m^2 within a range of 49-64 g/l MLSS. This implies that the flux rate is not only dependent on the concentration of biomass, but is also a function of crossflow velocity and applied pressure.

It is worth noting that the membranes were not cleaned during each set of flow velocities and pressures. This did not cause biomass separation problems and a consistent flux rate was maintained. After each set was completed a chemical solution, recommended by the UF membrane manufacturer, Paterson Candy International, (i.e. 2 g NaOH pellets + 5 ml sodium hypochloride to a litre) was used to clean the membranes.

The results demonstrated that the UF membrane had an ability to remove colour, turbidity and retain almost 100% biomass in the digester. This can be seen (Figures 5.5-7) from the results of SS, VSS, particle size measurements, total counts, microscopic counts, plate counts, SMA and MPN tests.

CHAPTER 6

KINETICS OF MEMBRANE REACTOR

Process kinetics play a central role in the development and operation of anaerobic treatment systems. Based on the biochemistry and microbiology of the anaerobic process, kinetics provide a rational basis for process analysis, control and design. In addition to the quantitative description of the rates of waste utilization, process kinetics also deal with operational and environmental factors affecting these rates. A sound knowledge of kinetics allows for the optimization of performance, a more stable operation as well as better control of the process.

Most of the early attempts to kinetically describe the anaerobic treatment process relied upon the so-called rate-limiting step approach. Generally speaking, when a process is composed of a sequence of reactions, one step is usually very much slower than the other steps. In this situation the rate of product formation may depend upon the rates of all the steps preceding the slowest step, but will not depend upon the rates of any of the subsequent, more rapid steps. The slowest step in a sequence of reactions has been called the rate-controlling, rate-limiting or rate-determining step (Hill, 1977). Lawrence (1971) proposed that in anaerobic digestion processes the rate limiting step be defined as that step which causes process failure to occur under imposed conditions of kinetic stress. In the context of a continuous culture, kinetic stress refers to the imposition of a continually reducing value of the solids retention time until it is lower than its limiting value and results in washout of the microorganisms. In anaerobic treatment, washout-type failure leads to near cessation of methane production, decreased COD removal and a build-up in the concentration of long- and sort-chain fatty acids.

In anaerobic digestion, the rate-limiting step is related to the nature of the substrate, process configuration, temperature, and loading rate (Speece, 1983). The effect of the growth limiting substrate concentration on the rate of microbial growth has been described by various mathematical models, however, the most widely used kinetic model is that of Monod.

According to the Monod model, the rate limiting substrate concentration is related to the specific growth rate in the absence of any inhibitory conditions. The kinetic coefficients, maximum specific growth rate, half saturation constant, growth yield and decay rate can then be estimated for the particular substrate used in the study.

The CUMAR system was operated over four ranges of biomass concentration in order to evaluate the effect of mixed liqour suspended solids (MLSS) concentration on the kinetics of the system. At least six steady-state data were collected in each range of MLSS by operating the system at different solids retendion times (SRTs) and effluent substrate concentrations. The kinetic coefficients of the system were determined graphically from the steady-state data by plotting Equation 2.20 and Equation 2.21 which yield two straight lines enabling the relevant kinetic coefficients to be calculated. Mixed liquor volatile suspended solids (MLVSS) and soluble COD concentrations were used as the measure of biomass and substrate concentrations throughout the study.

6.1. Kinetic Coefficients from the First Steady-state Run

A set of eight steady-state data was collected in this run by varying the organic loading rates and the solids wastage rates while the MLVSS concentration was maintained in the digester within a range of 10000 -15000 mg/l.

Table 6.1 shows the derived data for the graphical determination of kinetic coefficients of the first steady-state run. Figures 6.1 and 6.2 were plotted according to the model derived in Chapter 2, Section 2.3, using the data given in Table 6.1. The kinetic coefficients of the homogeneous bacterial populations within the digester were estimated from Figures 6.1 and 6.2 using the least square method and the results given in Table 6.2.

Table 6.1 Data derived from first range of steady - states for the graphical determination of Monod kinetic coefficients

SRT (d)	1/SRT (1/d)	(S_i-S_r) / HRT*X (mg COD/mg VSS.d)	$1/S_r$ (1/mg COD/l)	SRT/(SRT*k_d+1) (d)
480	0.0021	0.154	0.00689	166.4
320	0.0031	0.177	0.00592	142.6
232	0.0043	0.205	0.00532	121.9
195	0.0051	0.233	0.00387	110.9
177	0.0056	0.245	0.00328	104.8
160	0.0063	0.251	0.00284	98.6
135	0.0074	0.296	0.00270	88.5
122	0.0082	0.304	0.00255	82.7

Table 6.2 Results of first steady-state run

MLVSS range (mg/l)	Maximum specific growth rate (μ_{max}) (1/d)	Growth yield (Y) (g VSS / g COD)	Decay rate (k_d) (1/d)	Half saturation constant (K_s) (mg COD/l)
10000 -15000	0.0223	0.0392	0.00388	375

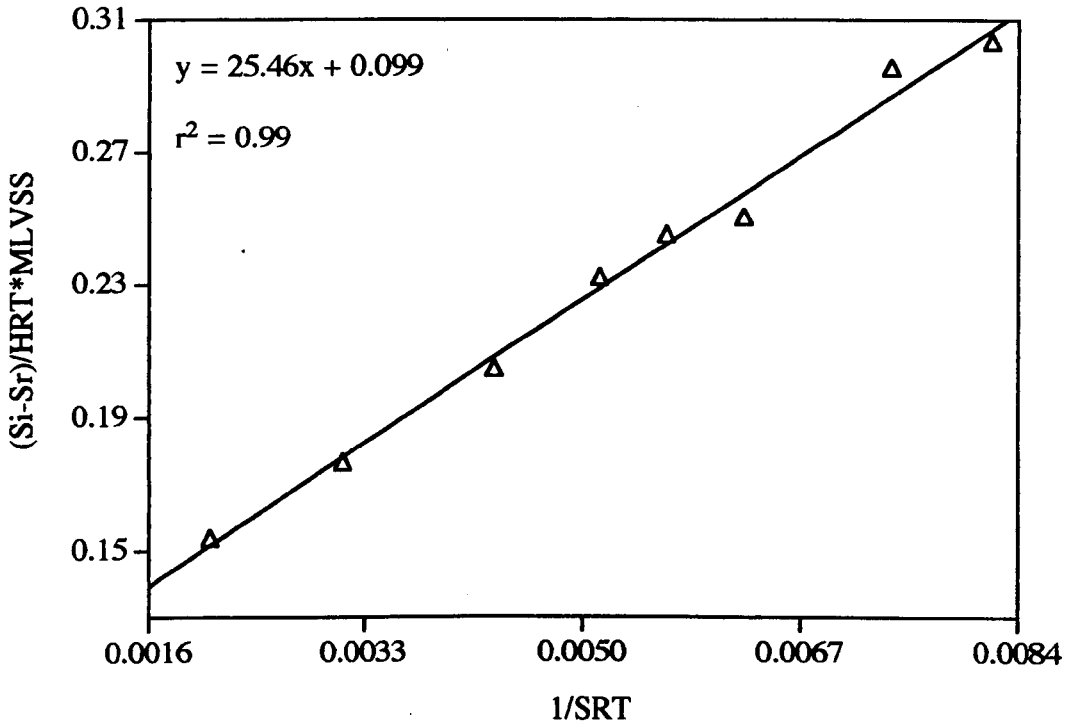


Figure 6.1 Determination of growth yield and decay rate of first run

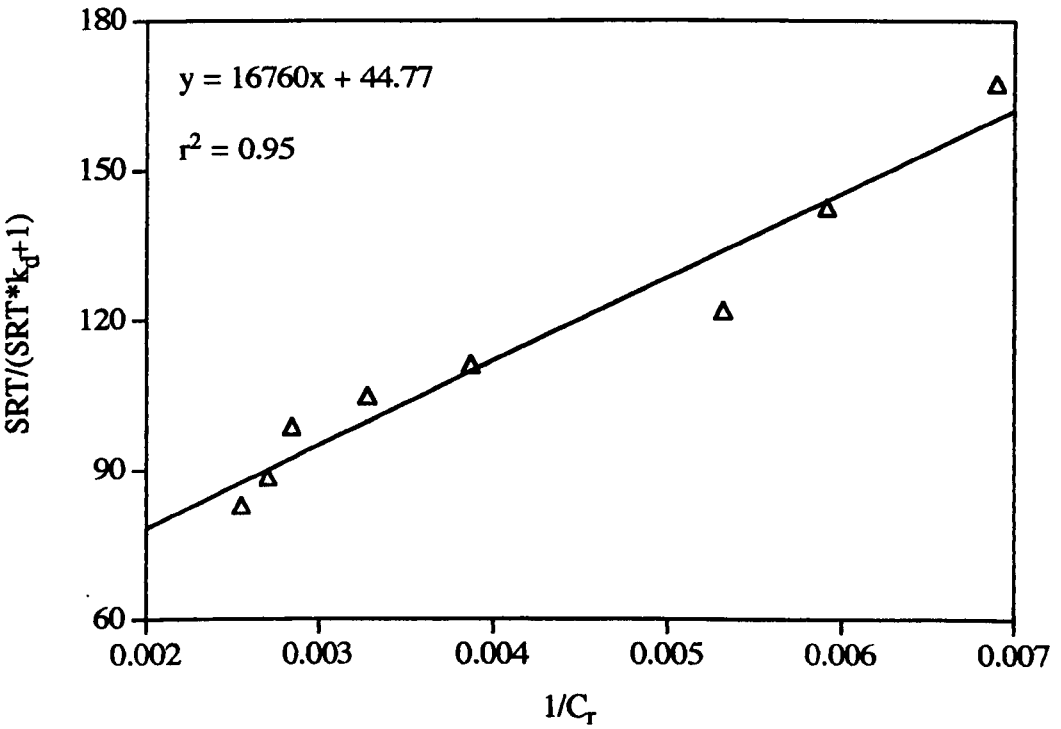


Figure 6.2 Determination of maximum specific growth rate and half saturation constant of first run

6.2. Kinetic Coefficients from the Second Steady-state Run

Nine steady-state sets of data were collected during the second steady-state run, maintaining a MLVSS range between 20000 mg/l and 25000 mg/l. The derived data given in Table 6.3 were plotted as shown in Figures 6.3 and 6.4 and analyzed using the same method given in Section 6.1. The estimated kinetic coefficients of the system are given in Table 6.4.

Table 6.3 Data derived from second range of steady - states for the graphical determination of Monod kinetic coefficients

SRT (d)	1/SRT (1/d)	(S_i-S_r) / HRT*X (mg COD/mg VSS.d)	$1/S_r$ (1/mg COD/l)	SRT/(SRT*k_d+1) (d)
103	0.0097	0.365	0.00152	75.2
98	0.0102	0.379	0.00142	72.7
91	0.0109	0.394	0.00116	68.8
89	0.0113	0.406	0.00110	67.4
86	0.0116	0.412	0.00106	66.1
84	0.0119	0.416	0.00099	64.7
81	0.0124	0.434	0.00093	62.6
80	0.0125	0.443	0.00091	62.1
78	0.0128	0.451	0.00088	61.3

Table 6.4 Results of second steady-state run

MLVSS range (mg/l)	Maximum specific growth rate (μ_{max}) (1/d)	Growth yield (Y) (g VSS/g COD)	Decay rate (k_d) (1/d)	Half saturation constant (K_s) (mg COD/l)
20000 - 25000	0.0230	0.0366	0.00355	480

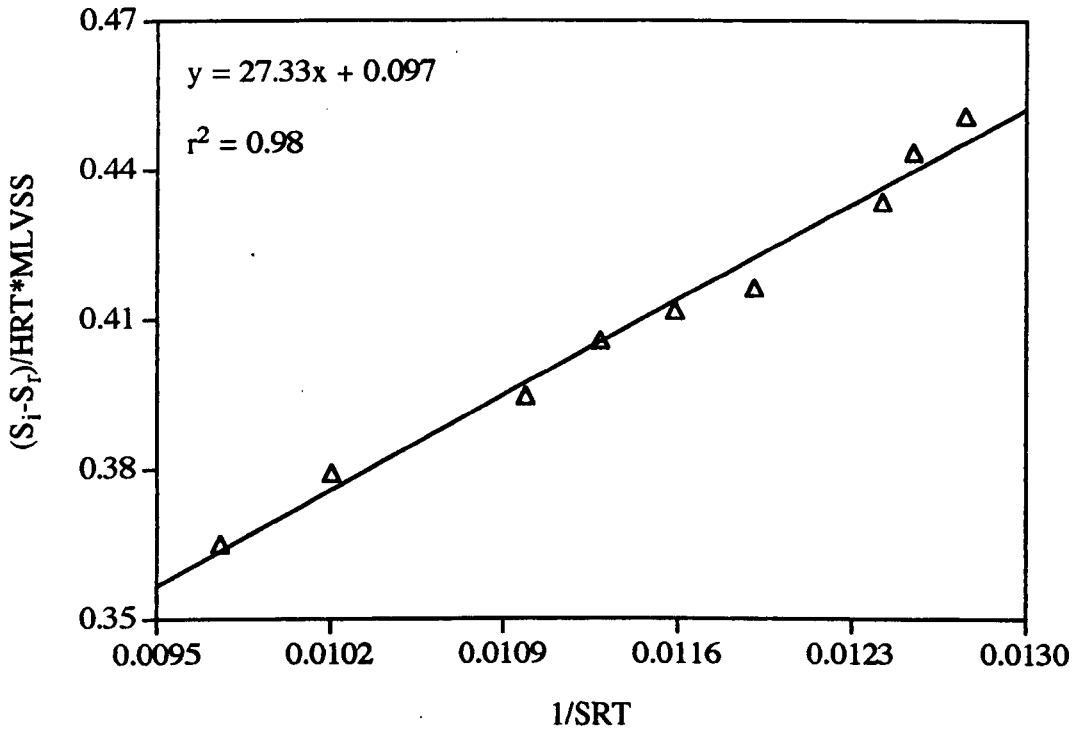


Figure 6.3. Determination of growth yield and decay rate of second run

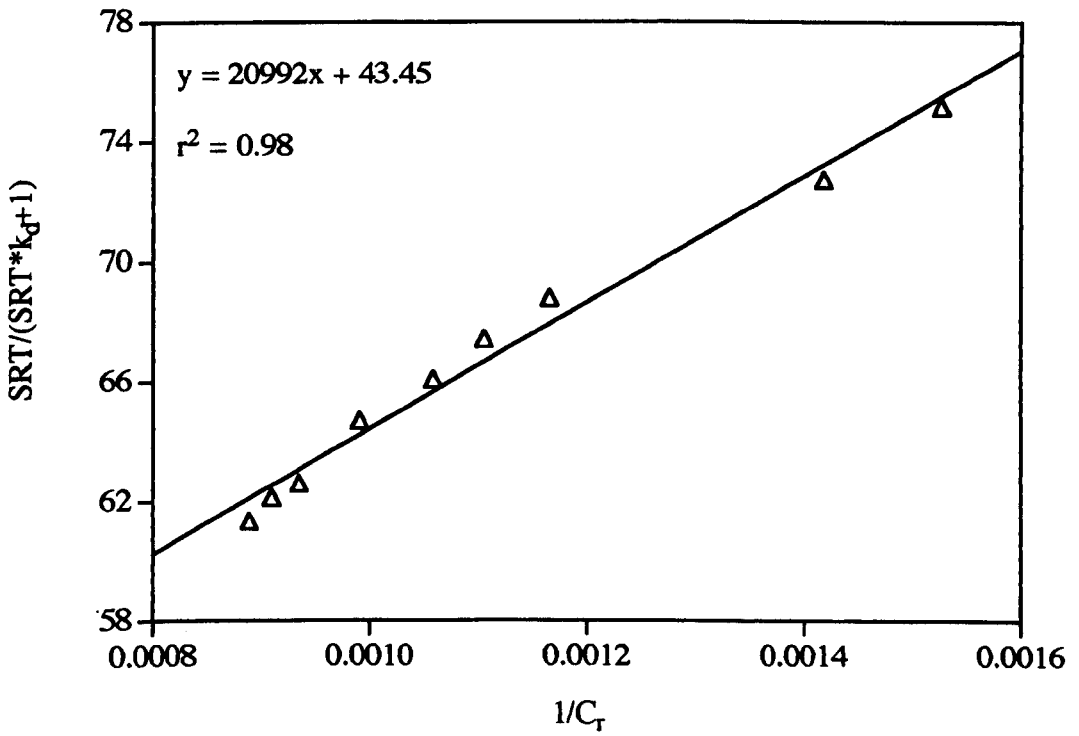


Figure 6.4 Determination of maximum specific growth rate and half saturation constant of second run

6.3. Kinetic Coefficients from the Third Steady-state Run

The MLVSS was increased from 30000 mg/l to 35000 mg/l in the digester during which period seven steady-state data were collected. Figures 6.5 and 6.6 were plotted from the derived data given in Table 6.5 in order to determine the kinetic coefficients of the system in this run. From Figures 6.5 and 6.6, kinetic coefficients, representing the overall characteristics of the homogeneous bacterial populations within the digester, were estimated and are given in Table 6.6.

Table 6.5 Data derived from third range of steady - states for the graphical determination of Monod kinetic coefficients

SRT (d)	1/SRT (1/d)	(S_i-S_r) / HRT*X (mg COD/mg VSS.d)	$1/S_r$ (1/mg COD/l)	SRT/(SRT*k_d+1) (d)
77	0.0129	0.439	0.00087	63.4
76	0.0131	0.446	0.00084	62.6
75	0.0133	0.456	0.00080	62.3
74	0.0135	0.459	0.00074	61.3
72	0.0138	0.472	0.00067	59.9
70	0.0142	0.479	0.00064	58.6
69	0.0144	0.481	0.00061	58.2

Table 6.6 Results of third steady-state run

MLVSS range (mg/l)	Maximum specific growth rate (μ_{max}) (1/d)	Growth yield (Y) (g VSS/g COD)	Decay rate (k_d) (1/d)	Half saturation constant (K_s) (mg COD/l)
30000 - 35000	0.0215	0.0355	0.00280	420

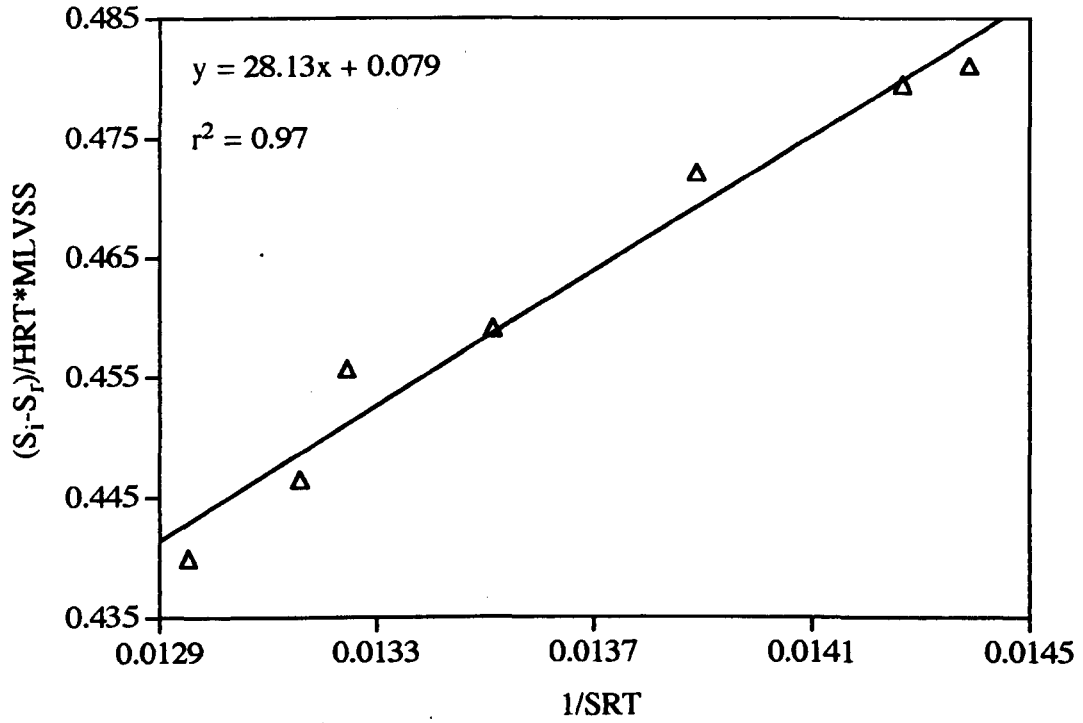


Figure 6.5. Determination of growth yield and decay rate of third run

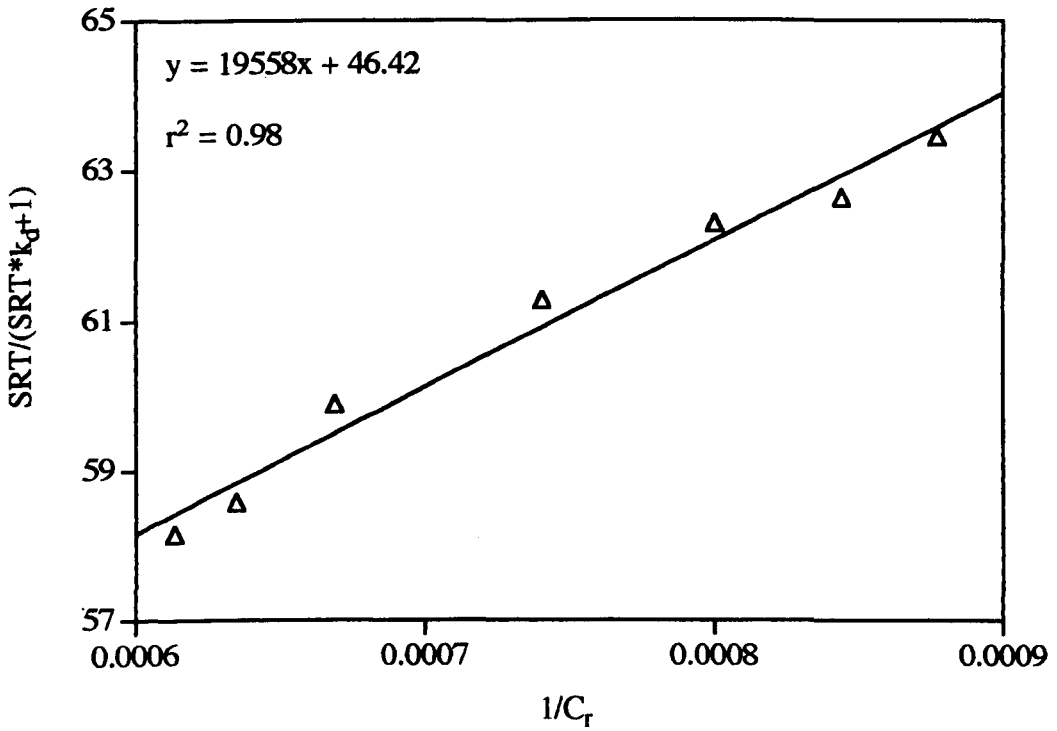


Figure 6.6. Determination of maximum specific growth rate and half saturation constant of third run

6.4. Kinetic Coefficients from the Fourth Steady-state Run

Six steady-states reached in the range of 39000 mg/l - 51000 mg/l MLVSS concentrations were used to determine the kinetic coefficients in the last run. Figures 6.7 and 6.8 were plotted using the derived data given in Table 6.7 and from which the kinetic coefficients of the system were estimated. The results are given in Table 6.8.

Table 6.7 Data derived from fourth range of steady - states for the graphical determination of Monod kinetic coefficients

SRT (d)	1/SRT (1/d)	(S_i-S_r) / HRT*X (mg COD/mg VSS.d)	$1/S_r$ (1/mg COD/l)	$SRT/(SRT*k_d+1)$ (d)
66	0.0152	0.493	0.00054	55.1
64	0.0157	0.504	0.00047	53.6
63	0.0160	0.512	0.00042	52.9
61	0.0164	0.520	0.00037	51.8
60	0.0168	0.536	0.00034	50.8
58	0.0171	0.542	0.00030	49.9

Table 6.8 Results of fourth steady-state run

MLVSS range (mg/l)	Maximum specific growth rate (μ_{max}) (1/d)	Growth yield (Y) (g VSS/g COD)	Decay rate (k_d) (1/d)	Half saturation constant (K_s) (mg COD/l)
39000 - 51000	0.0227	0.037	0.00292	460

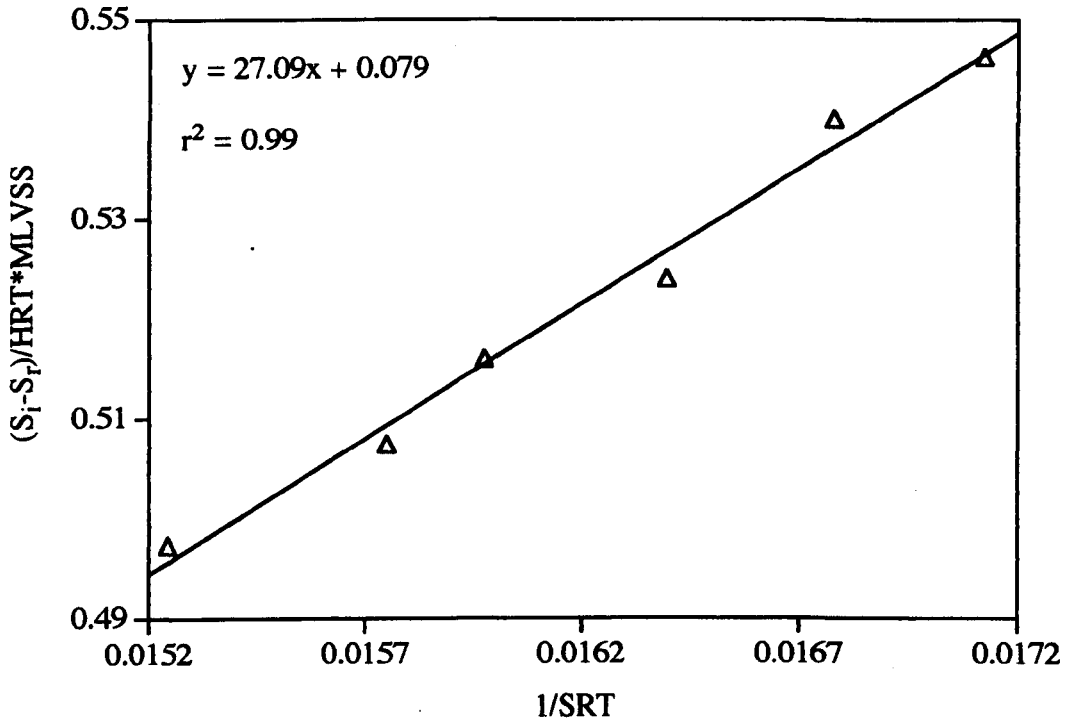


Figure 6.7. Determination of growth yield and decay rate of fourth run

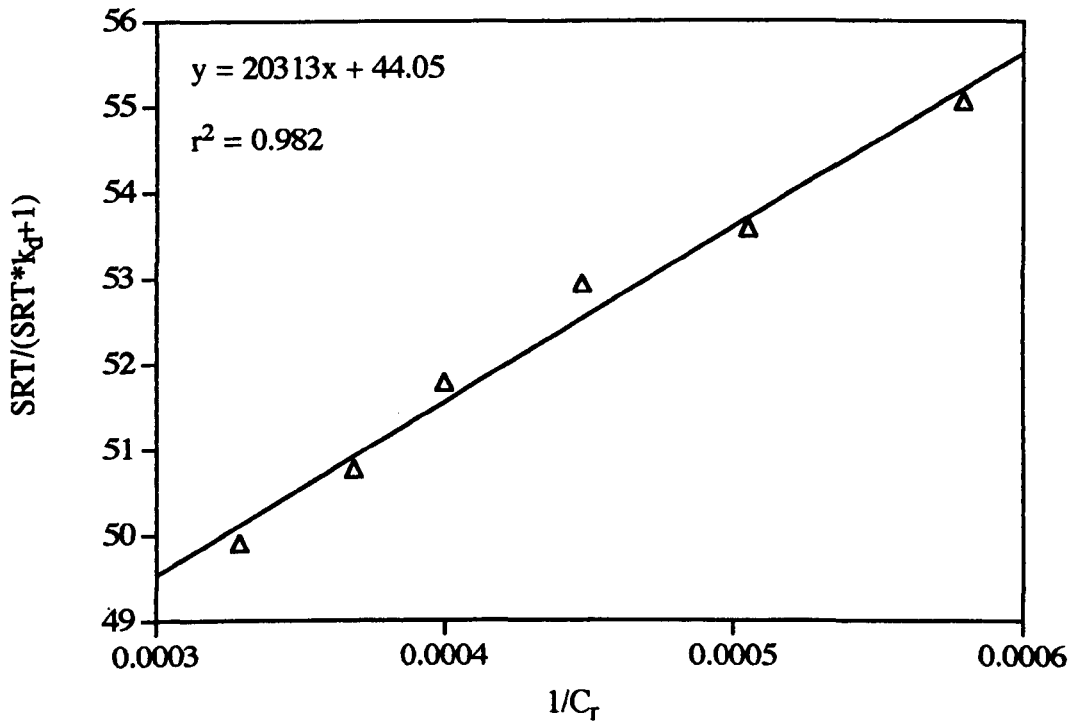


Figure 6.8. Determination of maximum specific growth rate and half saturation constant of fourth run

6.5. Overall Kinetics

All the derived data from the four range of steady-state runs were plotted as shown in Figures 6.9 and 6.10 in order to determine the overall kinetic coefficients of the CUMAR system. The estimated values are given in Table 6.9. For comparison a summary of all the kinetic coefficients obtained from the four range of steady-state runs and overall operation of the system are also given in Table 6.10.

Table 6.9 Results of overall system

MLVSS range (mg/l)	Maximum specific growth rate (μ_{max}) (1/d)	Growth yield (Y) (g VSS/g COD)	Decay rate (k_d) (1/d)	Half saturation constant (K_s) (mg COD/l)
10000 - 51000	0.0222	0.0378	0.0037	390

Table 6.10 Comparison of kinetic coefficients of the system at different operation ranges

Operation	MLVSS range (mg/l)	Maximum specific growth rate (μ_{max}) (1/d)	Growth yield (Y) (g VSS/g COD)	Decay rate (k_d) (1/d)	Half saturation constant (K_s) (mg COD/l)
Run 1	10000 - 15000	0.0223	0.0392	0.00388	375
Run 2	20000 - 25000	0.0230	0.0366	0.00355	480
Run 3	30000 - 35000	0.0215	0.0355	0.00281	420
Run 4	39000 - 51000	0.0227	0.0370	0.00292	460
Overall	10000 - 51000	0.0222	0.0378	0.00370	390

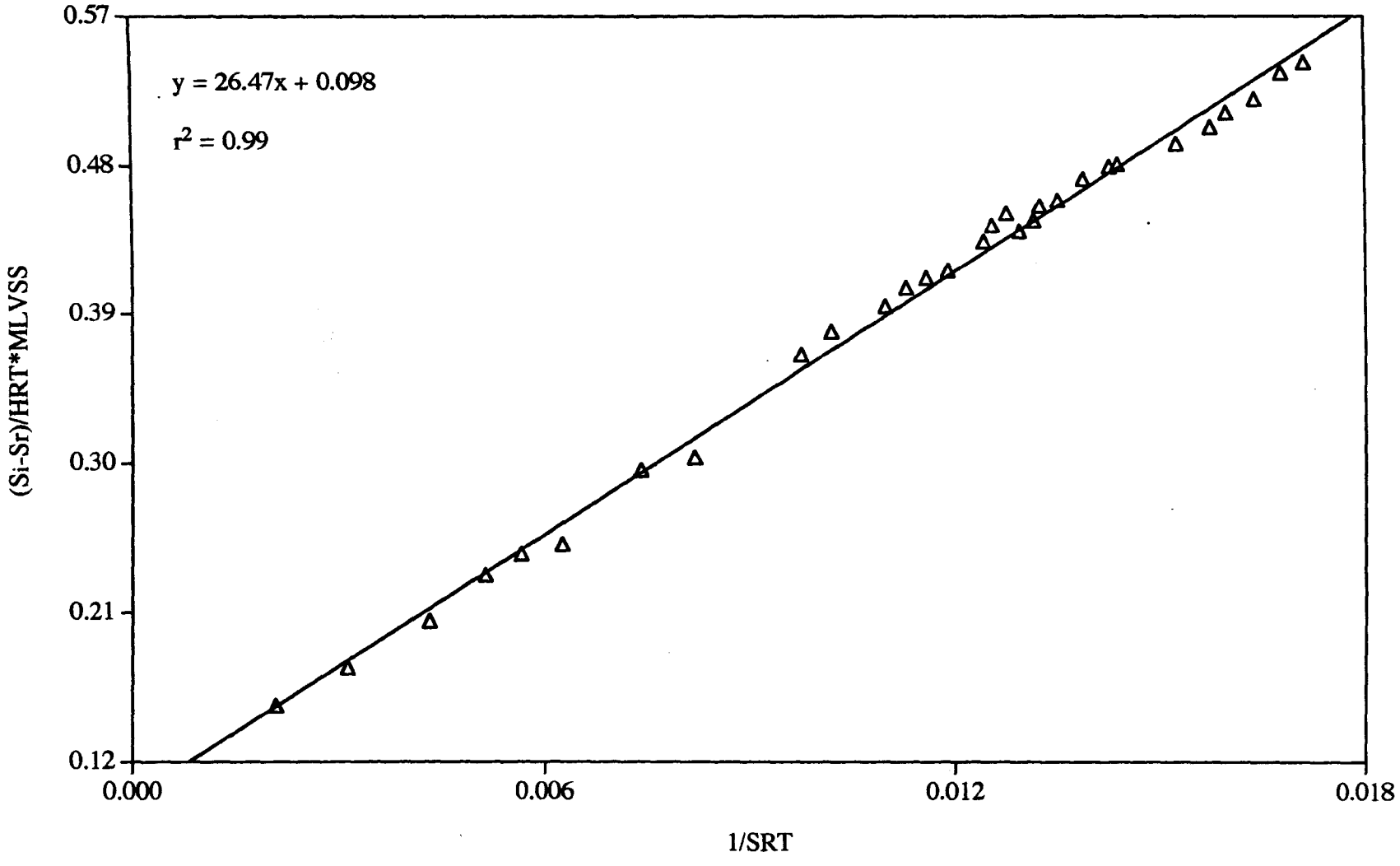


Figure 6.9. Determination of growth yield and decay rate of overall system

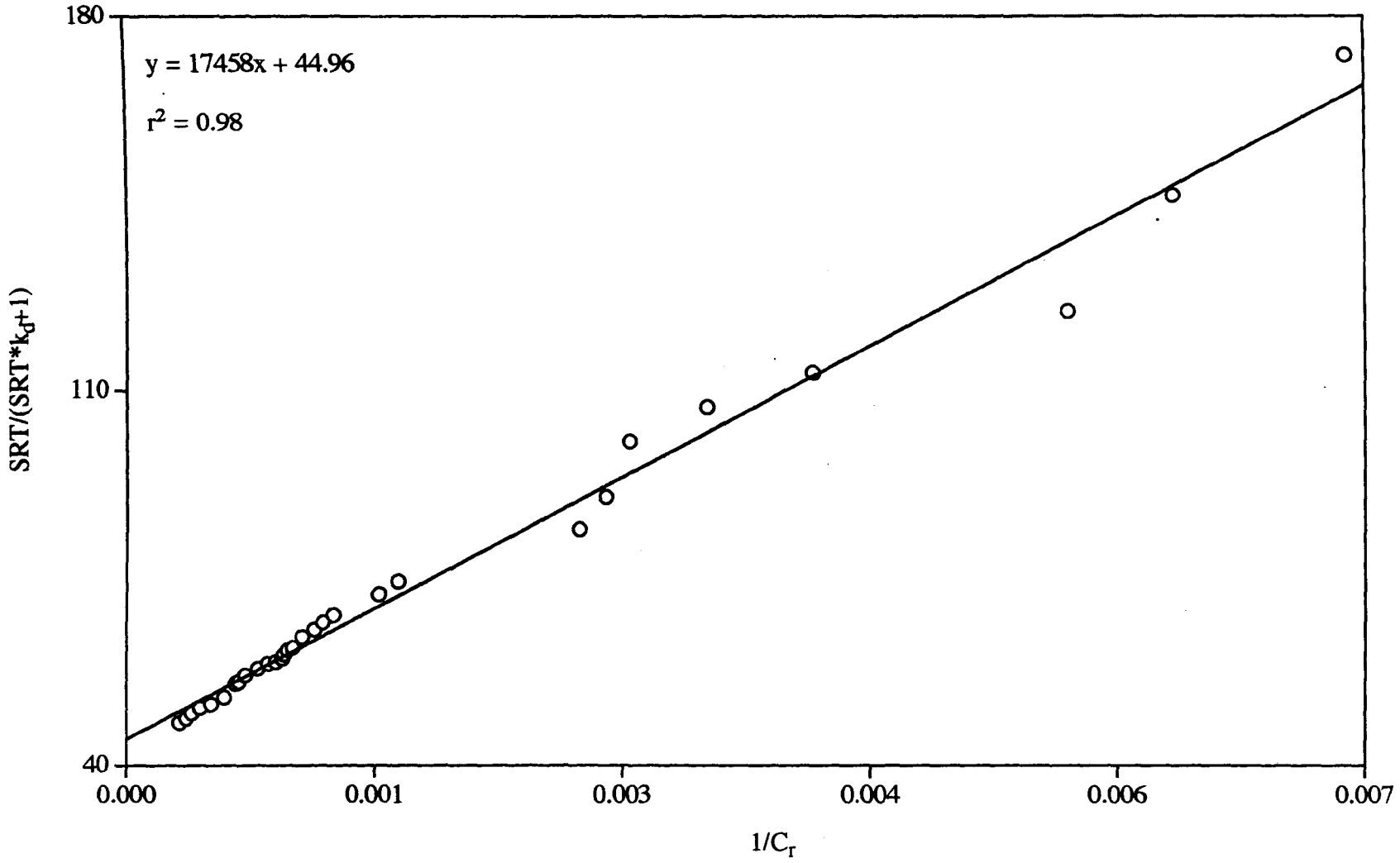


Figure 6.10. Determination of maximum growth rate and half saturation constant of overall system

6.6. Discussion

Most of the kinetic values reported in the literature have been estimated by the use of enrichment, mixed cultures or pure cultures in laboratory-scale digesters. Pavlostathis (1990) stated that these studies have yielded not particularly useful information as far as the biochemistry and microbiology of anaerobic processes and that they were also limited in terms of kinetics. Since a need definitely exists for pilot-scale experimentation for the more accurate delineation of the kinetics of anaerobic processes, kinetic studies were carried out on the pilot-scale CUMAR system.

In the first steady-state run, the maximum specific growth rate was found to be low whilst the decay rate showed higher value compared to those reported by Fernandes (1986) and Henze and Harremous (1983). This might have been due to the low sludge wastage rate (high SRTs) and high biomass concentrations in the digester during the increase in MLVSS concentrations. High crossflow velocities and high transmembrane pressures were applied in this run in order to achieve higher flux rates. This resulted in high recirculations of the reactor contents through the membranes which might have also contributed to an increase in the death rate of the cells. The half saturation constant was found to be in good agreement with the results reported by Fernandes (1986) but the growth yield was higher. These differences may be explained by the use of different substrate and seed composition.

In the second steady-state run, the growth yield was found to be lower than those obtained in the first steady-state run while the half saturation constant showed a higher value. A slight increase in maximum growth rate and decrease in the decay rate were observed, probably due to the increase in the sludge wastage rate. It was also assumed that the variations in the kinetic coefficients might have been due to the changes in the numbers and composition of the microbial population in the digester. Therefore the

numbers and the composition of the microbial population in the digester were monitored throughout the operation of the CUMAR system using the microscopic count technique.

The microscopic examination of the inoculation sludge showed that a wide range of methanogens was present namely cocci shaped species being the dominant group followed by sarcina, short rods, medium rods, long rods, and filaments. A significant increase in the numbers and composition of methanogenic species was recorded during the first steady-state run compared to the initial results. The numbers of both methanogenic and non-methanogenic bacteria increased significantly by approximately 50% and 12% of their initial values respectively. The dominant species were found to be the same as in the inoculation sludge but the second dominant species ,*Methanosarcina* , in the inoculation sludge were found to be the third dominant group in this run. After the second range of steady-states had been reached, some changes in the species composition and in their numbers were recorded. The first dominant species, *Methanococcus* , were found to be the second dominant group while short rods became the first dominant group. These changes might have affected the growth constants of the anaerobic system since each species has a different growth rate from each other.

The third range of steady-state results showed a slight change in the half saturation constant and in the maximum specific growth rate while growth yield remained almost the same compared to those obtained in the second run but the decay rate value significantly decreased. As can be seen from the results of the 1 st , 2nd and 3rd runs, the decay rate value of the system decreased from 0.0039 to 0.0028, probably due to the increase in the sludge wastege rate (lower SRTs). The changes in the numbers and the composition of both methanogenic and acidogenic population in the digester were also observed in this run. A detailed discussion on the microbiol composition of the system throughout the operation has been made in Chapter 8.

The last range steady-state results showed a slight increase in all kinetic coefficients of the system. This might have been due to the use of glucose as an additional substrate to increase the organic loading rate to above 20 kg COD/m^3.d.

The variations in the kinetic coefficients of the system throughout the operation were assumed to be mainly due to:

(i) the changes in the dominant species in the digester (the applied organic loading rates which might have caused the change in the numbers and the composition of the microbial population),

(ii) the increase in MLSS concentration from approximately 12000 mg/l to over 60000 mg/l,

(iii) the use of MLVSS as the measure of viable population density,

(iv) the changes in the feed composition (glucose was used in the last run as additional substrate), and

(v) the applied transmembrane pressures and the crossflow velocities (high recirculation of the reactor content).

It can ,however, be said that the changes in the kinetic coefficients were unduly significant throughout the whole study indicating that the system had an ability to cope with high substrate and biomass concentrations.

The results also showed that the CUMAR system can accommodate higher substrate and biomass levels than the maximum carried out in this study.

In order to compare the kinetic coefficients of the CUMAR system, Table 6.11 was prepared together with the data reported by a number of researchers. As can be seen in Table 6.11, the maximum specific growth rate and the decay rate of the CUMAR system showed lower values than the others while the growth yield and the half saturation coefficients were different from some of the reported values. The growth yield of the system had a higher value than those reported by Robinson and Tiedge (1984), Gujer and Zehnder (1983) and Cappenberg (1973). The half saturation coefficient of the system showed higher than those values reported by Robinson and Tiedge (1984), Gates (1967) while it was lower than those reported by Donnelly (1984), Hansford (1974), Andrews *et all.* (1969) and Stewart *et all.* (1959).

The specific growth rate (μ) has been defined by Equation 2.2, and a relationship between the rate of substrate removal and the growth of biomass (Y) has been established (Equation 2.4). It has been shown that the specific growth rate (μ) may be constant for a time during growth (exponential phase), but eventually it decreases until it becomes zero at the top of the growth curve. If the carbon source has been removed when growth stops and the experimental environment is not deficient in anything but the carbon source, it may be concluded with some confidence that the decreasing growth rate was related to , or in any event may be correlated with, the decreasing concentration of the carbon source. Thus, we could conclude that, at higher concentrations of substrate, the specific growth rate may not be related to the decreasing concentration of the carbon source, i.e. substrates could be present at concentrations in excess of those that will affect the specific growth rate, and only at lower concentrations is specific growth rate dependent on carbon source. The carbon source remaining in the effluent from a biological treatment process may consist of small amounts of many different compounds. Some may be metabolic intermediates and/or end products. Some of those may be fragments of cellular structural components, nucleotides, peptides, and other contents from dead or

dying cells. Thus, the COD removed makes no distinction as to whether the residual COD is due to non metabolizable organic matter in the original sample or to organic by-products of the purification mechanism. In this study, therefore, inert COD studies were carried out in order to determine whether the residual COD in the effluent of the CUMAR system was due to inert COD fraction of the brewery wastewater or produced by biomass within the system. The results obtained from this study (discussed widely in pages 116-140) showed that the brewery wastewater contained considerably little inert COD. The residual COD in the effluent of the system was therefore considered to be mostly non-biodegradable microbial products. In order to determine whether the persistent residual organic compounds were non-biodegradable the CUMAR system was operated for further some time at three different steady-states (Figures 5.8-5.10). The results showed that a significant removal of the residual organics was not possible even with extended contact times (approximately 15 days). It was concluded that the effluent COD of the CUMAR system was mostly non-biodegradable. As stated by Gaudy and Gaudy (1980), a low specific growth rate (μ) may be correlated to a low level of biodegradable substrate in the effluent. This can explain the low cell yield of the CUMAR system since the specific growth rate (μ) approaches the decay rate (k_d).

The maximum growth rate (μ_{max}) was calculated according to Equations 2.19 and 2.20. As can be seen from Equations 2.19 and 2.20 μ_{max} is mainly a function of sludge age and effluent substrate concentration. The low μ_{max} values of the CUMAR system obtained at different stages could be due to the effluent COD concentrations of the system which was found to be mostly non-biodegradable microbial products.

The rate of endogenous respiration can change because of changes in environmental conditions, such as temperature, and in natural populations because of changes in the predominance of specific species. Also, the factors which affect the endogenous

respiration constant (k_d) may in fact vary depending upon the specific growth rate (Gaudy and Gaudy, 1980). During the operation of the CUMAR system the temperature was kept approximately constant. However, changes in the numbers and composition of the dominant species in the digester occured. For example, the most dominant species in the inoculum sludge was *Methanococcus* followed by *Methanosarcina* , short, medium, filamentous and long rod shaped species. In the first steady-state run, the most equally dominant groups were *Methanococcus* and short rod species followed by medium, *Methanosarcina* , filamentous and long rod species. Short rod species showed a significant increase in their numbers (approximately 2 times) during this period while the numbers of long rod species were found to have decreased (approximately 2 times).

Moreover, different reactor operation and reactor configuration and feed composition might have had an effect on having a rise to a low k_d. For example high recirculation of the reactor content caused disintegration of the flocs which was observed during microscopic examinations. This could further have resulted in breaking down of species' cells. Another factor could be no sludge wastage during start-up period and operating the CUMAR system at long sludge ages.

Nelson and Lawrence (1980) pointed out that the use of MLVSS for the determination of the kinetic coefficients of any biological systems will yield different values. They made a comparison between the values of kinetic coefficients using the viable microbial mass and the total biomass measurements. The results showed that growth yield and maximum specific growth rate were not significantly affected by using either MLVSS or viable biomass but decay rate and half saturation constant values were significantly affected by such corrections. High half saturation constant values were obtained when MLVSS was used as biomass values. It was also stated that different kinetic coefficient values from

experiments at the same sludge retention times can be obtained but in different reactor configurations.

Table 6.11 Summary of values of kinetic coefficients for various substrates utilized in mesophilic anaerobic treatment processes

Type of Feed	K_s (mg COD/l)	μ_{max} (1/d)	Y (g VSS/g COD)	k_d (1/d)
Brewey wastewater*	388	0.0222	0.0378	0.0037
Milk based synthetic waste	24	0.14	0.37	0.07
Yeast waste	3100	0.084	0.073	0.005
Dextrose, Tryptone and Beef Extract	4200 5660	0.186 0.067	0.14 0.18	0.1 0.0247
Rum slops	12270	0.129	0.225	0.0667
Glucose	22.5-630	0.161-30	0.14-0.17	0.021-6.1
Long-chain fatty acids	105-3180	0.085-0.55	0.04-0.11	0.01-0.015
Sort-chain fatty acids	12-500	0.13-1.2	0.025-0.047	0.01-0.027
Acetate	11-421	0.08-0.7	0.01-0.054	0.004-0.037
H_2 / CO_2	0.016-0.6	0.05-4.07	0.017-0.045	0.088
Glucose+Peptone+ Lab Lemco Powder	2269	0.251	0.179	0.021

* This investigation

Note the data for Table 6.11 was compiled from the following sources; Kissalita *et al.* (1989), Ahring and Westermann 1987, Lin *et al.* (1986), Noike *et al.* (1985), Donnelly

(1984), Robinson and Tiedge (1984), Haung (1983), Heyes and Hall (1983), Gujer and Zehnder (1983), Zoetemeyer *et al.* (1982), Massey and Pohland (1978), Ghosh and Pohland (1974), Peterson (1975), Hansford and Richter (1974), Cappenberg (1973), Kugelman and Chin (1971), Lawrance and McCarty (1969), Gates *et al.* (1967), Stewerd *et al.* (1959).

CHAPTER 7

CHANGES IN METHANOGENIC ACTIVITY

Anaerobic digestion is a microbial process requiring careful design and control. In practice, engineers and plant operators base their design generally on loading rate, expressed in terms of reactor volume without reference to the quality or quantity of either the seed sludge or the active biomass developed within the reactor during operation. It is common practice to use volatile suspended solids (VSS) or total suspended solids (TSS) to determine the biomass concentration in the reactor and sludge wastage but these parameters do not distinguish between microbial biomass and any other particulate organic material which may be present in the reactor, nor does it give any indication of the potential methanogenic activity of the microbial biomass present (Reynolds, 1986).

In order to determine methanogenic activity, different techniques have been developed by a number of researchers (Monteggia 1991, James *et al.* 1990, Concennon *et al.* 1988a,b; Reynolds 1986, Dolfing and Bloemen 1985, Shelton and Tiedje 1984, Valcke and Verstraete 1983, Owen *et al.* 1979, Van den Berg *et al.* 1974). In this study the technique developed by Monteggia (1991) was used for the purposes given below:

(i) to determine the changes in methanogenic activity in the digester and the permeate,

(ii) to asses the effect of mixed liqour suspended solid (MLSS) concentration on the activity of methanogens in the digester, and

(iii) to optimize the methanogenic activity in the digester.

7.1. Methanogenic Activity Changes During Start-up Period

The CUMAR system was initially fed with diluted brewery wastewater at an organic loading rate (OLR) of 1 kg COD/m^3.d corresponding to an F/M ratio of approximately 0.11 kg COD/kg VSS.d. A specific methanogenic activity (SMA) test was immediately carried out and the results obtained from both the reactor and the SMA tests, as shown in Figure 7.1, indicated that the actual methane production (AMP) rate of the reactor sludge was very low, i.e. 1 ml CH_4/g VSS.d, but was 90% of the potential methane production (PMP) rate at this OLR. The total volatile fatty acids (TVFA) in the effluent immediately increased to over 1600 mg/l after 3 days operation. The soluble COD removal efficiencies were found to be 14% in the overflow from the reactor and 21% in the membrane permeate respectively whilst the methane content of biogas produced in the digester reached only 10% by the end of first weeks' operation (see Chapter 5, Figures 5.1 and 5.2).

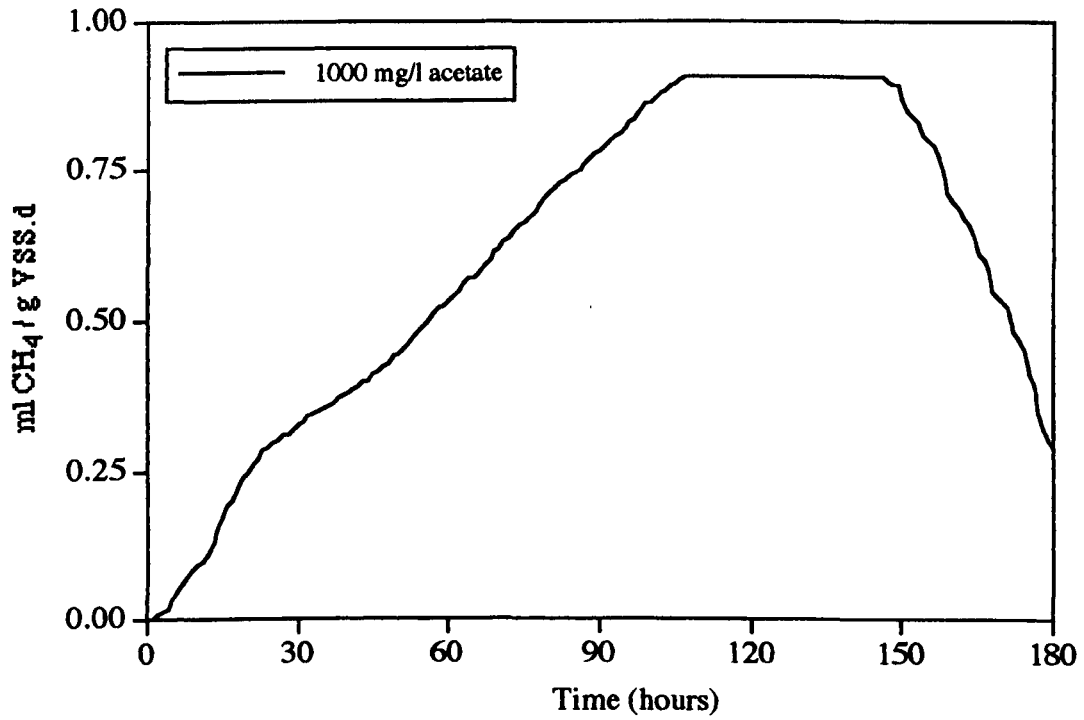


Figure 7.1 Plot of specific methanogenic activity at an organic loading rate of 1kg COD/m^3.d (on day 3)

The OLR was therefore maintained at approximately 0.7 kg COD/m^3.d in the second week of operation since an acclimatization period was necessary for the biomass in the digester for the new wastewater and the different reactor configuration. On day 12 the results showed that both the AMP and the PMP rates noticeably increased to approximately 6 ml CH_4/g VSS.d and 7.5 ml CH_4/g VSS.d respectively yielding an AMP/PMP ratio of 0.8. The TVFAs decreased to 700 mg/l while COD removal effficiencies of 50% in the reactor and 65% in the permeate were achieved at this OLR. The methane content of biogas produced in the digester increased to 15%. The second week SMA test results are given in Figure 7.2.

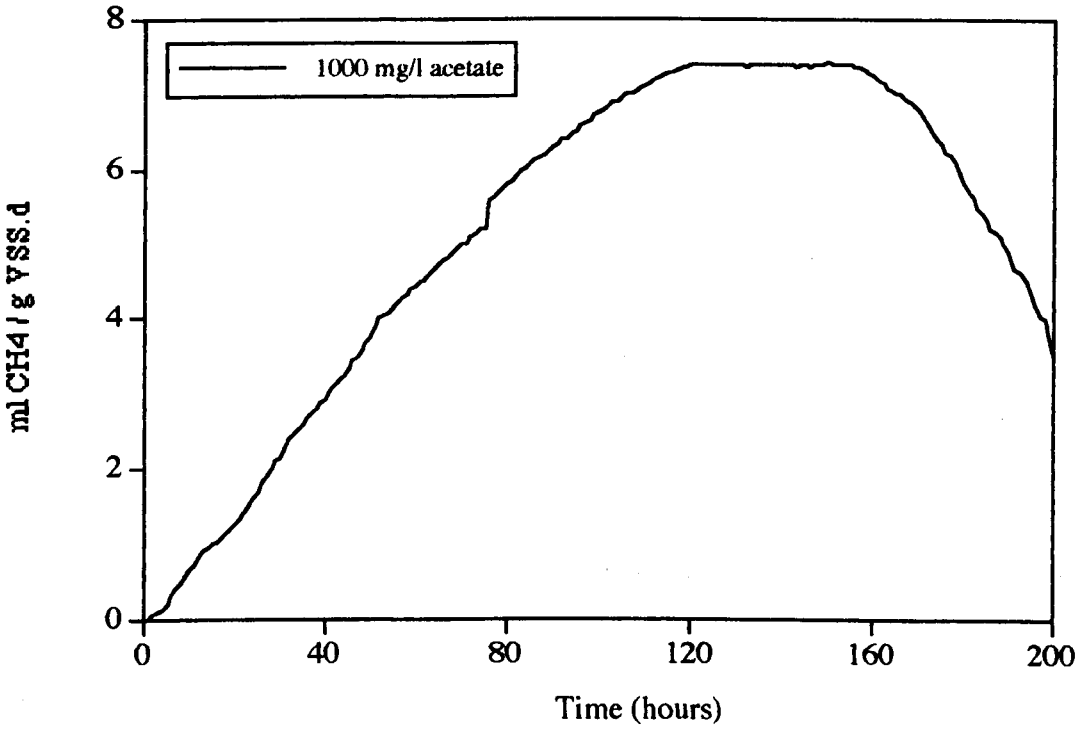


Figure 7.2 Plot of specific methanogenic activity at an organic loading rate of 0.7 kg COD/m^3.d (on day 12)

The same OLR was maintained during the third week operation due to the high TVFAs in the permeate and the low COD removal efficiency of the system. The third week results, as shown in Figure 7.3 (on day 21), showed that the AMP rate of the digester sludge was doubled while the PMP rate obtained from the SMA test was found to have

increased approximately 2.5 times its previous value (on day 12). This yielded an AMP/PMP ratio of 0.6. The TVFAs in the permeate decreased to 400 mg/l while the COD removal efficiency of the system improved to over 65% in the reactor and 77% in the permeate towards the end of three weeks operation.

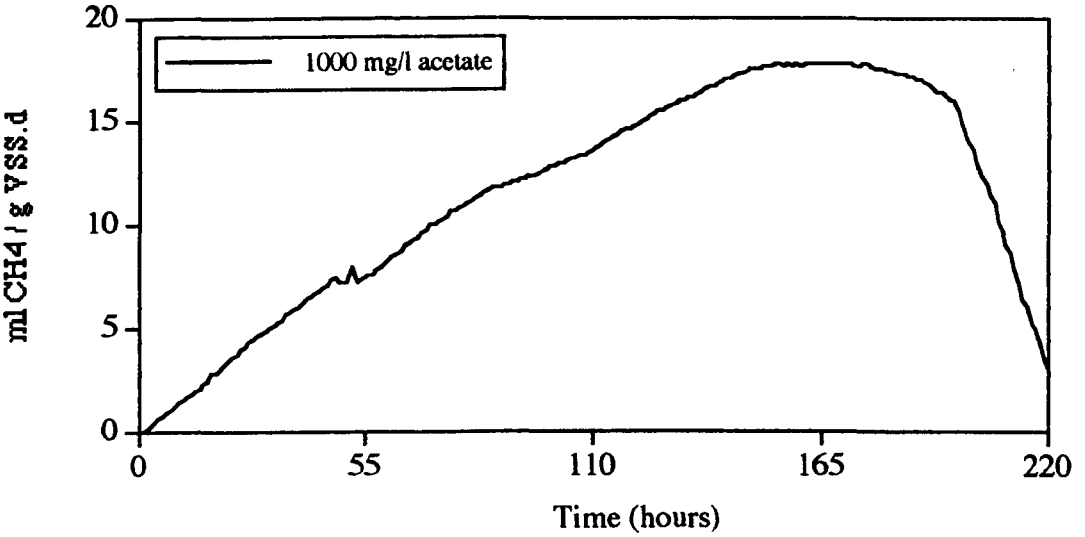


Figure 7.3 Plot of specific methanogenic activity at an organic loading rate of 0.7 kg COD/m^3.d (on day 21)

According to the results obtained in the third week it was assumed that the CUMAR system could be loaded at higher OLRs. It was then decided to double the OLR to 1.5 kg COD/m^3.d on day 30. Both the AMP and the PMP rates were significantly increased to 24 ml CH_4/g VSS.d and 32 ml CH_4/g VSS.d respectively at this OLR yielding an AMP/PMP ratio of 0.76. However the TVFAs and the COD concentrations in the reactor showed a sharp increase to 860 mg/l and 1360 mg/l respectively. This was thought to be due to the increase in AMP/PMP ratio indicating that the system was operating near its maximum load (76%) leading to the conclusion that further increasing the OLR would probably have resulted in a sharp increase in TVFAs in the digester causing a significant decrease in the COD removal efficiency of the system or even system failure. In other words the amount of acetoclastic methanogenic bacteria in the digester was not sufficient to consume the COD load. The SMA results obtained on day 32 are shown in Figure 7.4.

On day 32 the OLR was therefore decreased to 1 kg COD/m^3.d and maintained at the same level for the next two weeks (until day 46) after which the system performed very well , i.e. a COD removal efficiency of 96% in the digester and 98% in the permeate. The TVFAs in the permeate were found to be less than 15 mg/l while the methane content of the biogas produced in the digester reached 79% with a methane yield of approximately 0.36 m^3 CH_4/kg $COD_{removed}$ at this OLR. The AMP rate of the digester sludge had not changed significantly but the increase in the quantity of biomass in the digester had increased the COD removal efficiency of the system. The PMP rate, as shown in Figure 7.5, significantly increased to 50 ml CH_4/g VSS.d yielding an AMP/PMP rate of 0.45. From this ratio it may be seen that only 45% of the acetoclastic methanogenic capacity of the digester sludge was used towards the end of the start-up period, in other words a high acetoclastic methanogenic capacity had been established in the digester. The overall performance of the CUMAR system can be seen in Chapter 5, in Figures 5.1 and 5.2

During the start-up period the MLVSS concentration in the digester increased from approximately 8500 mg/l to over 10000 mg/l which resulted in an increase in the MLVSS/MLSS ratio of 5%. The alkalinity added (as $NaHCO_3$) to the feed was proportionally decreased from 3000 mg/l (as $CaCO_3$) to 2000 mg/l as the COD removal efficiency of the system increased throughout the start-up period. However, the alkali requirement was controlled automatically, based on continuous measurements of the pH of the digester sludge using a pH meter and controller in order to ensure satisfactory environmental conditions for the methanogenic bacteria and to maintain the same pH range in the digester.

The above results show that higher OLRs can be applied to the CUMAR system without having any adverse effects on its performance provided an SMA test is carried out before an increase in OLR applied since the additional permissible OLR can be determined by this technique.

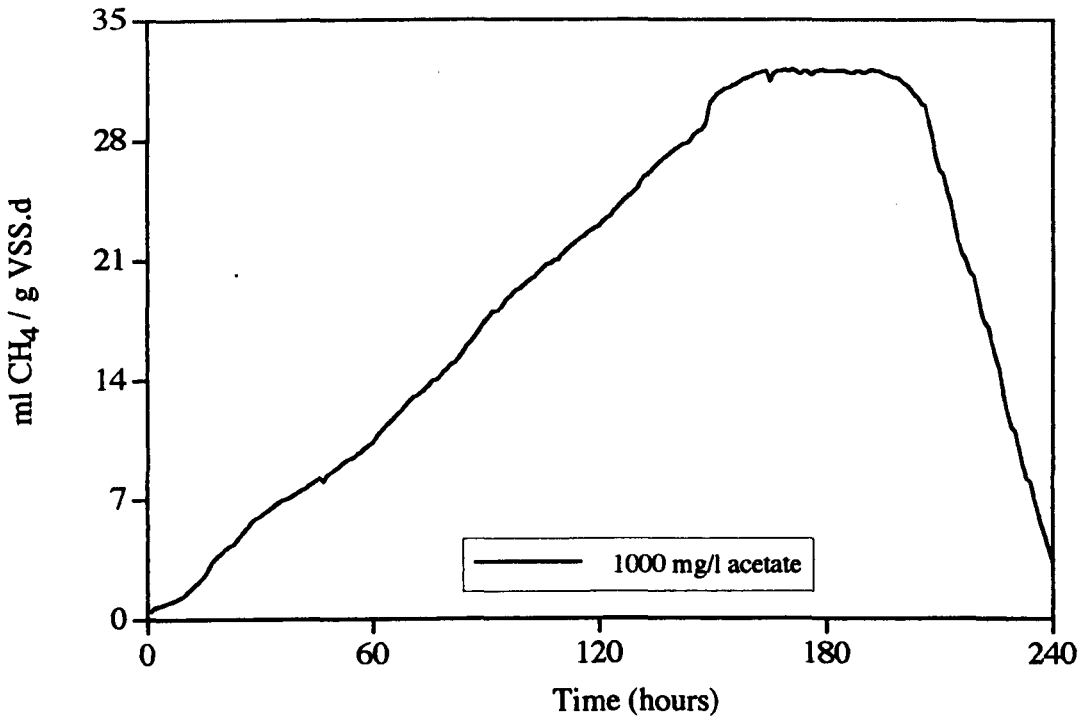


Figure 7.4 Plot of specific methanogenic activity at an organic loading rate of 1.5 kg COD/m^3.d (on day 32)

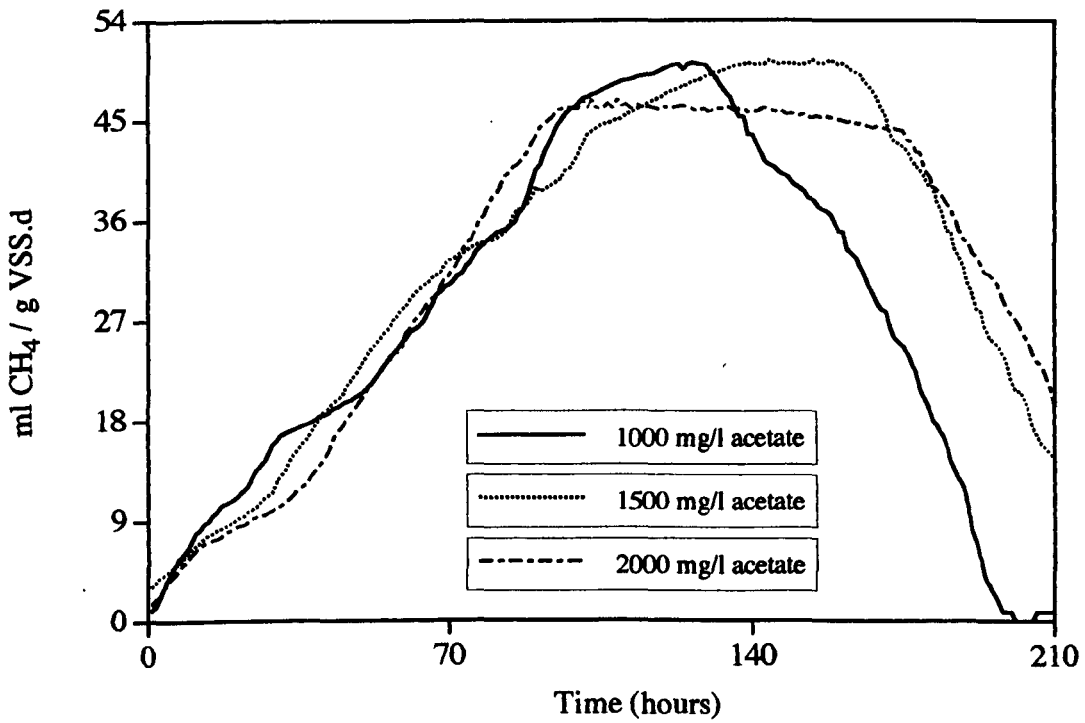


Figure 7.5 Plot of specific methanogenic activity at an organic loading rate of 1 kg COD/m^3.d (on day 42)

7.2. Changes in Methanogenic Activity During Steady-state Operation

After the start-up period exponential increases in OLRs were applied. The SMA tests were generally carried out after the system had reached steady-states. The PMP rates for the digester sludge obtained from the SMA tests at different OLRs are shown in Figures 7.6-7.19. Figures 7.20-7.22 were also plotted in order to compare the changes in both the AMP and the PMP rates and to evaluate the effect of MLVSS concentration on the activity of the methanogenic bacteria in the digester during the operation of the system.

The OLR was first increased to approximately 1.7 kg COD/m^3.d after the start-up period and the results obtained on day 55 showed that both the AMP and the PMP rates increased by 45% and 30% respectively from their previous values achieved on day 42 at the OLR of 1 kg COD/m^3.d. This yielded an AMP/PMP ratio of 0.57. The system performed very well at this OLR achieving a COD removal efficiency of 97% in the digester and 99% in the permeate. The TVFAs in the permeate did not significantly increased after an increase in OLR and was found to be less than 20 mg/l at the steady-state. As can be seen in Figure 7.20 the AMP rate of the system sharply increased up to an OLR of 5 kg COD/m^3.d.at which point the AMP rate was found to be 90 ml CH_4/g VSS.d while maintaining approximately the same COD removal efficiencies as achieved at an OLR of 1.7 kg COD/m^3.d. After that, slight increases in the AMP rates were observed reaching 112 ml CH_4/g VSS.d during increases in OLRs up to 16 kg COD/m^3.d. Further increase in OLR resulted in a slight decrease in the AMP rate which was found to be 105 ml CH_4/g VSS.d at an OLR of 17.5 kg COD/m^3.d. However, it reached the maximum value obtained in this study even after an increase in OLR had been applied. The maximum AMP rate of 118 ml CH_4/g VSS.d was reached at an OLR of approximately 23 kg COD/m^3.d with an overall COD removal efficiency of 99%. The AMP rate of the system did not show any significant changes during the remainder of the operation and was maintained at approximately the same level which was achieved at an OLR of 23 kg COD/m^3.d.

The PMP rates, as can be seen in Figure 7.20, showed noticeable increases up to an OLR of 9 kg COD/m^3.d, at which point the PMP rate was found to be 170 ml CH_4/g VSS.d which was the maximum value obtained in this study. The COD removal efficiency of the system was never found to be less than 99% in the permeate during the increases in OLRs. After an OLR of 9 kg COD/m^3.d, slight decreases in the PMP rates were observed up to an OLR of 17.5 kg COD/m^3.d at which point the PMP rate was found to be 140 ml CH_4/g VSS.d but this did not adversely affect the performance of the system. Further increases in OLRs resulted in slight increases in the PMP rate reaching approximately the same level achieved at an OLR of 15.5 kg COD/m^3.d. The TVFAs in the permeate showed slight increases, to approximately 200 mg/l, towards the end of the operation. Although the methane content of the biogas produced in the digester considerably decreased to 65% with a methane yield of 0.28 m^3 CH_4/kg $COD_{removed}$ the system performed very well having an overall COD removal efficiency of no lower than 99%. at the maximum applied OLR of 28.5 kg COD/m^3.d. The overall performance of the CUMAR system during steady-state operation can be seen in Chapter 5, in Figures 5.3a-c.

Throughout the steady-state operations of the CUMAR system the AMP/PMP ratios were maintained in the range 0.55-0.75 and are presented in Table 7.1. This resulted in a very high COD removal efficiency, stable performance and decreased the time which was required to reach steady-state after an increase in OLR.

As can be seen in Figure 7.22 increases in the sludge wastage rates (decreases in SRTs) resulted in sharp increases in both the AMP and the PMP rates of the system. However, the AMP and the PMP rates did not proportionally increase with the increases in MLVSS concentration in the digester which can be seen in Figure 7.22 although a relationship was observed between OLR and MLVSS.

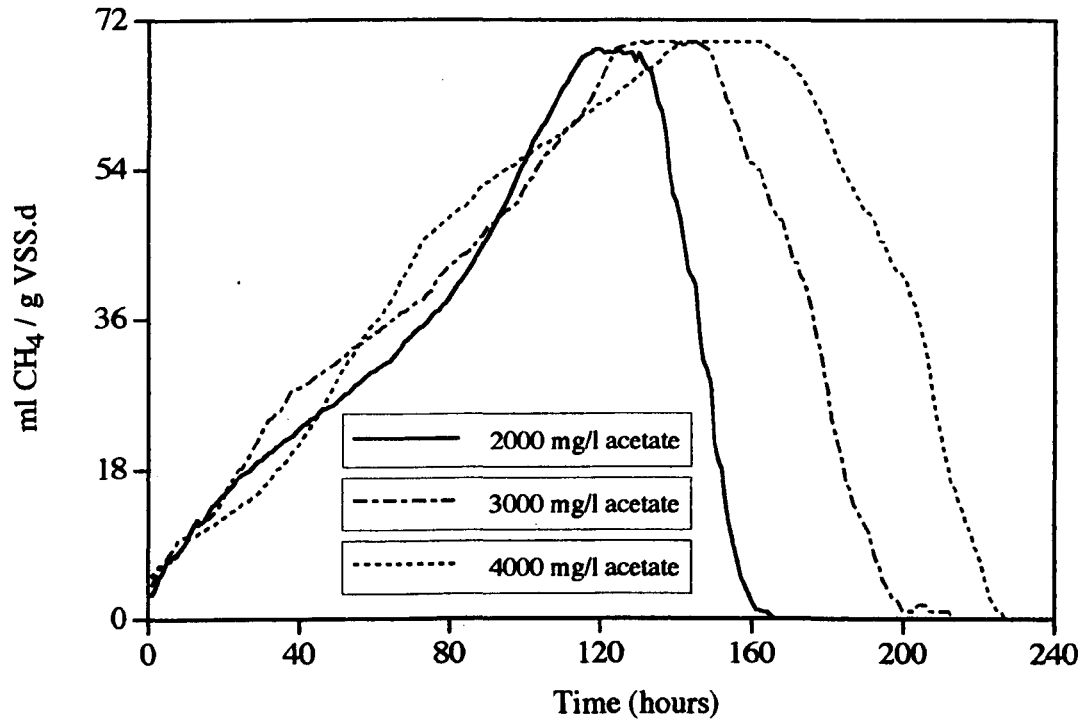


Figure 7.6 Plot of specific methanogenic activity at an organic loading rate of 1.7 kg COD/m^3.d (on day 55)

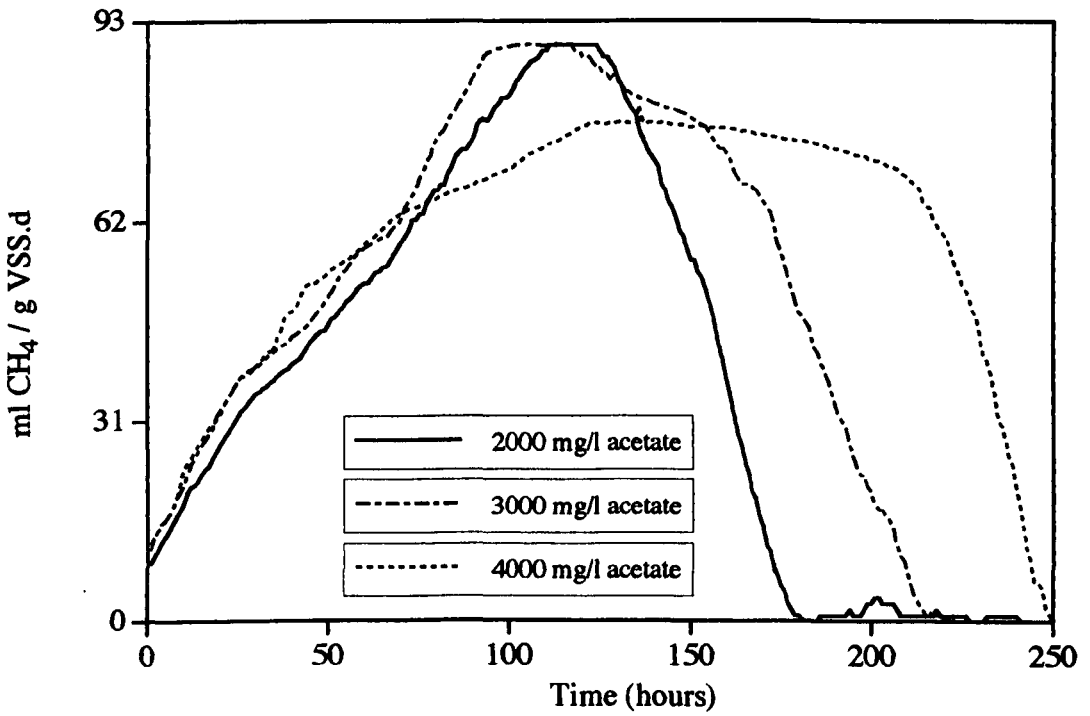


Figure 7.7 Plot of specific methanogenic activity at an organic loading rate of 2 kg COD/m^3.d (on day 75)

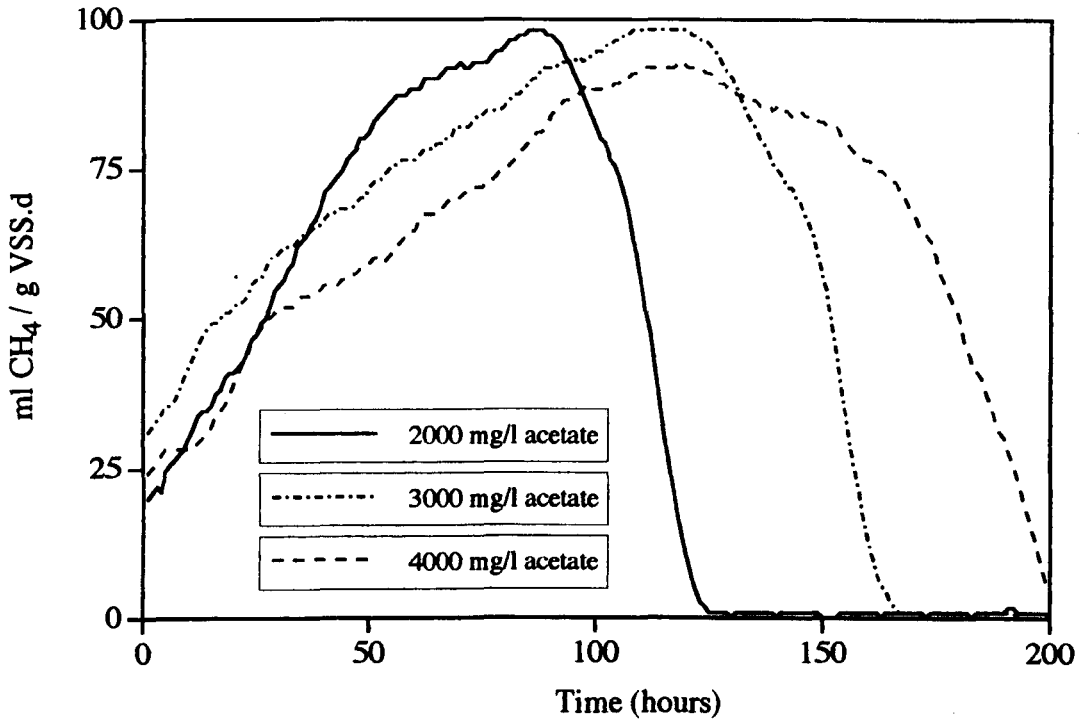


Figure 7.8 Plot of specific methanogenic activity at an organic loading rate of 2.5 kg COD/m^3.d (on day 98)

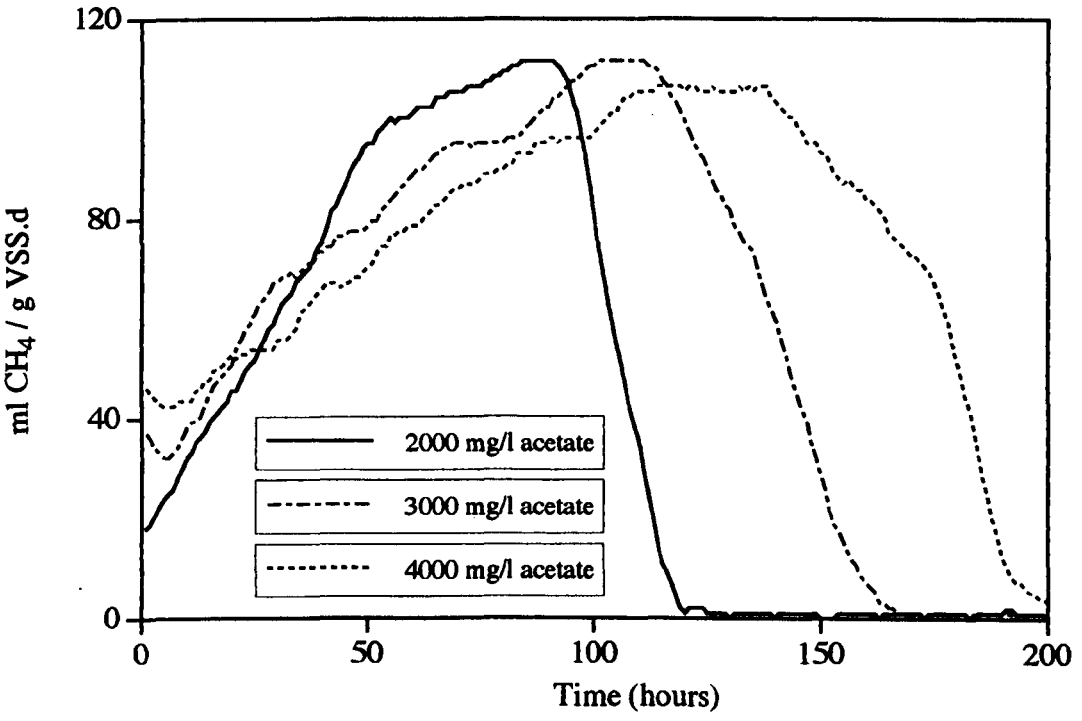


Figure 7.9 Plot of specific methanogenic activity at an organic loading rate of 3 kg COD/m^3.d (on day 119)

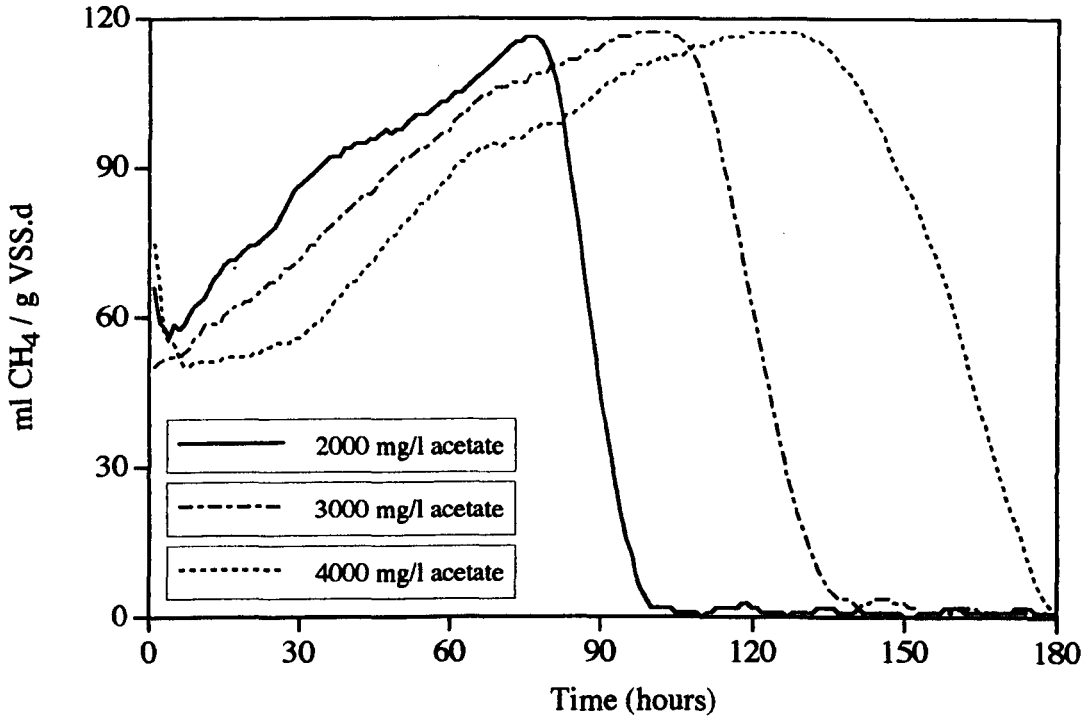


Figure 7.10 Plot of specific methanogenic activity at an organic loading rate of 3.6 kg COD/m^3.d (on day 143)

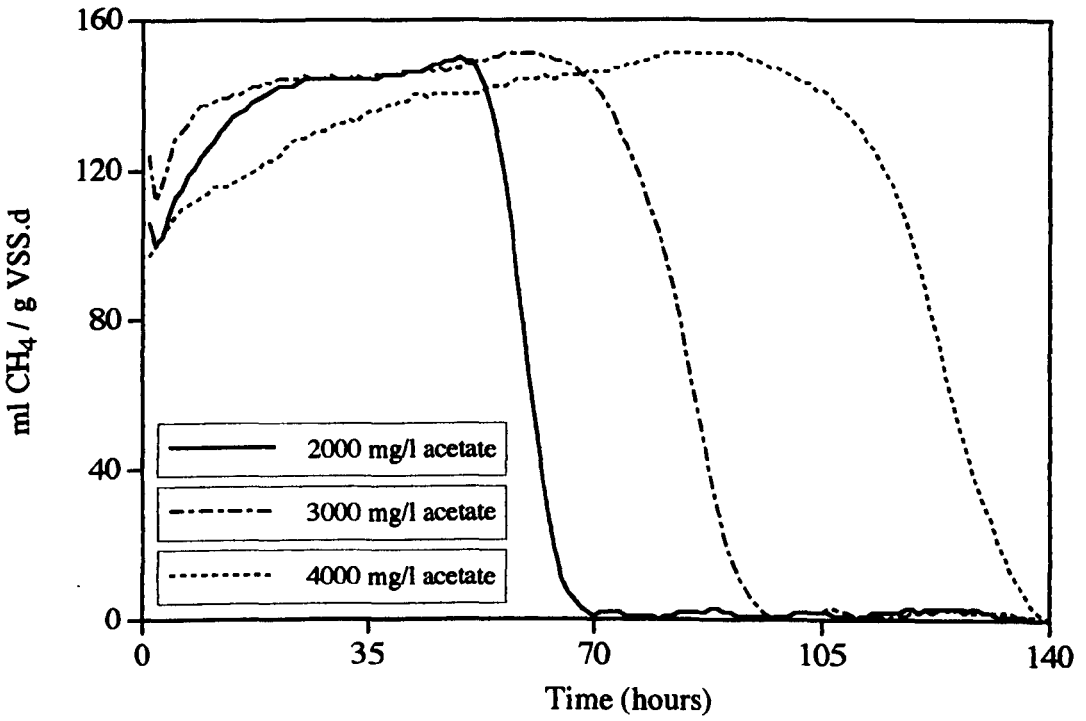


Figure 7.11 Plot of specific methanogenic activity at an organic loading rate of 4.7 kg COD/m^3.d (on day 184)

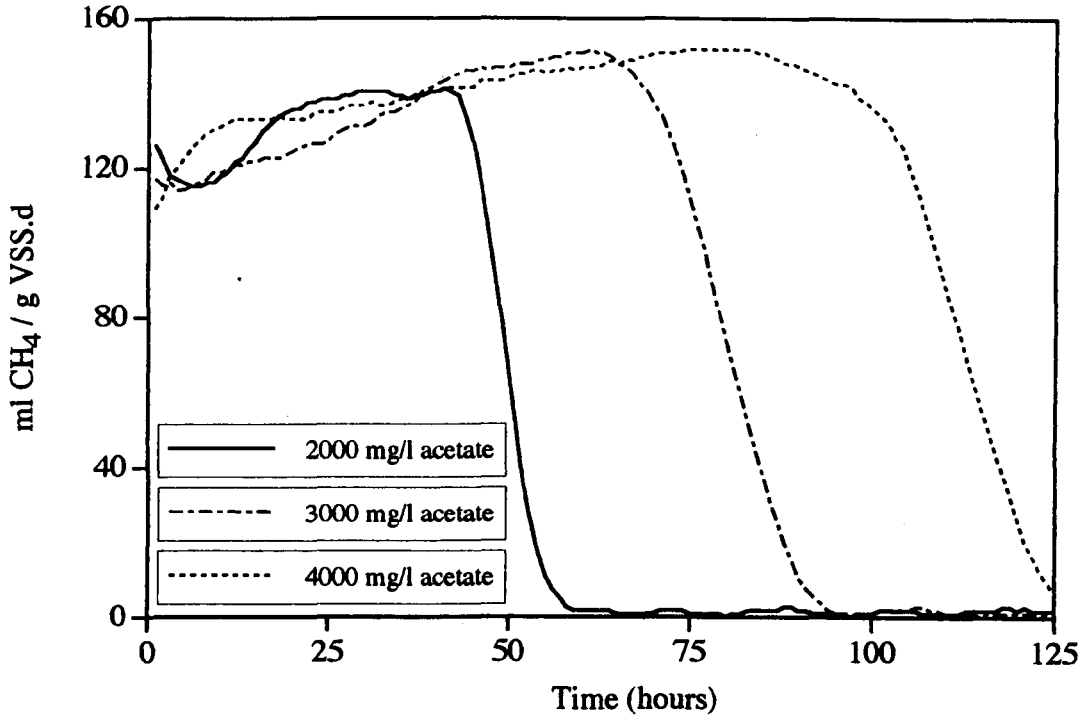


Figure 7.12 Plot of specific methanogenic activity at an organic loading rate of 7.4 kg COD/m^3.d (on day 213)

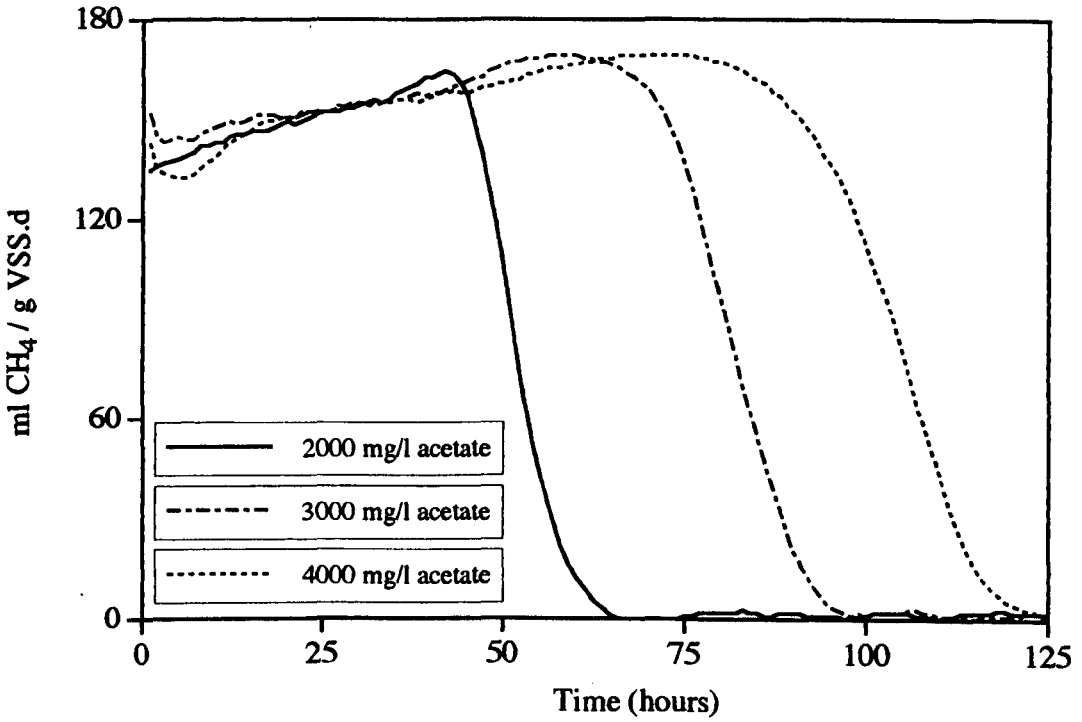


Figure 7.13 Plot of specific methanogenic activity at an organic loading rate of 8.8 kg COD/m^3.d (on day 259)

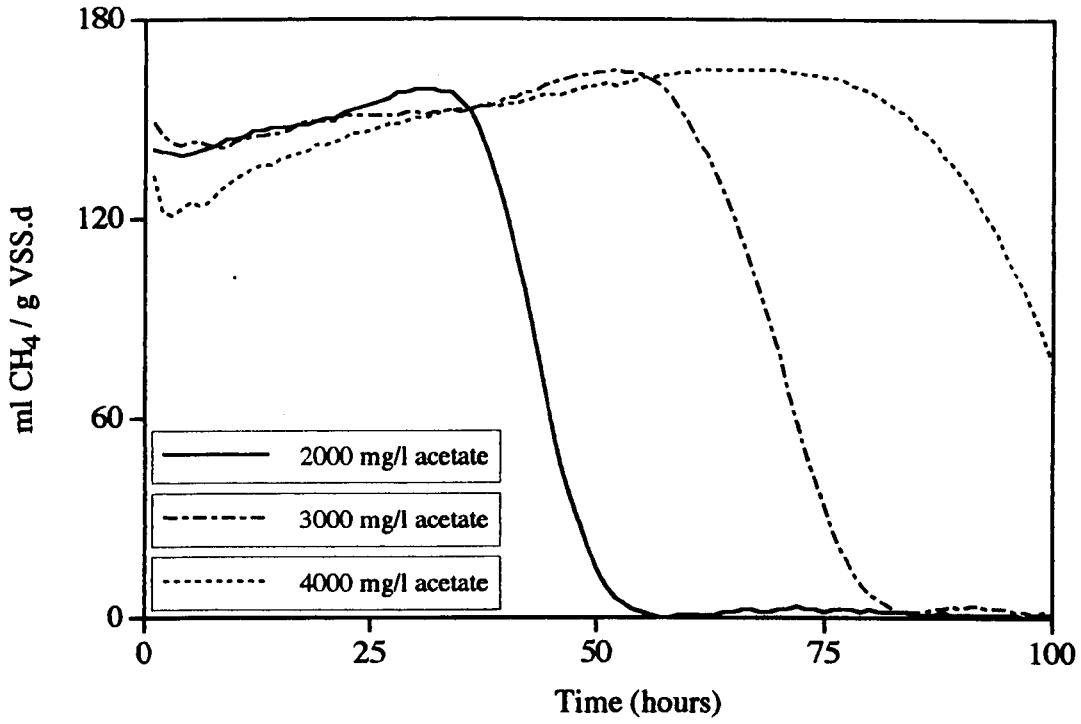


Figure 7.14 Plot of specific methanogenic activity at an organic loading rate of 11.5 kg COD/m^3.d (on day 324)

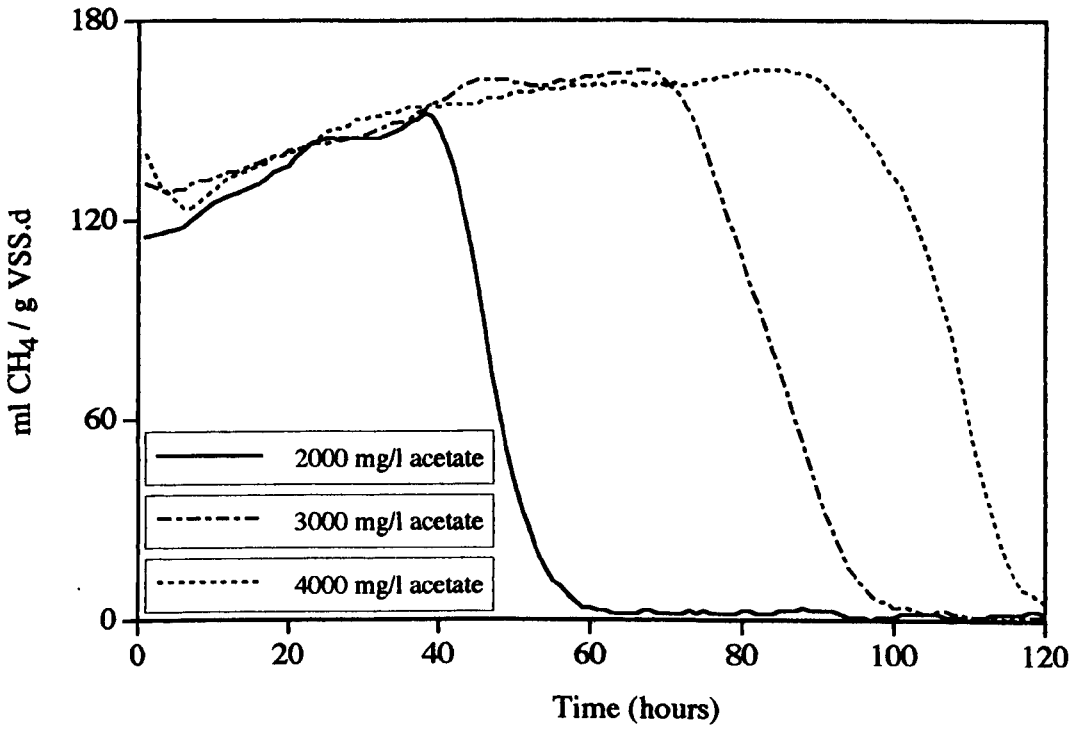


Figure 7.15 Plot of specific methanogenic activity at an organic loading rate of 14 kg COD/m^3.d (on day 352)

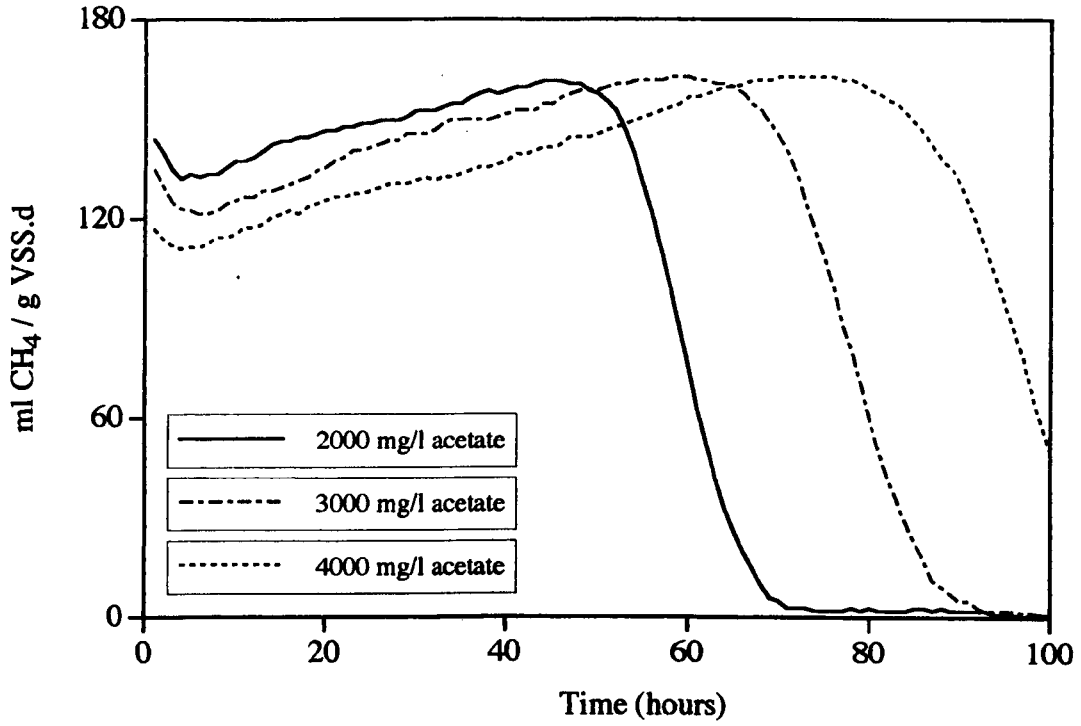


Figure 7.16 Plot of specific methanogenic activity at an organic loading rate of 15.5 kg COD/m^3.d (on day 372)

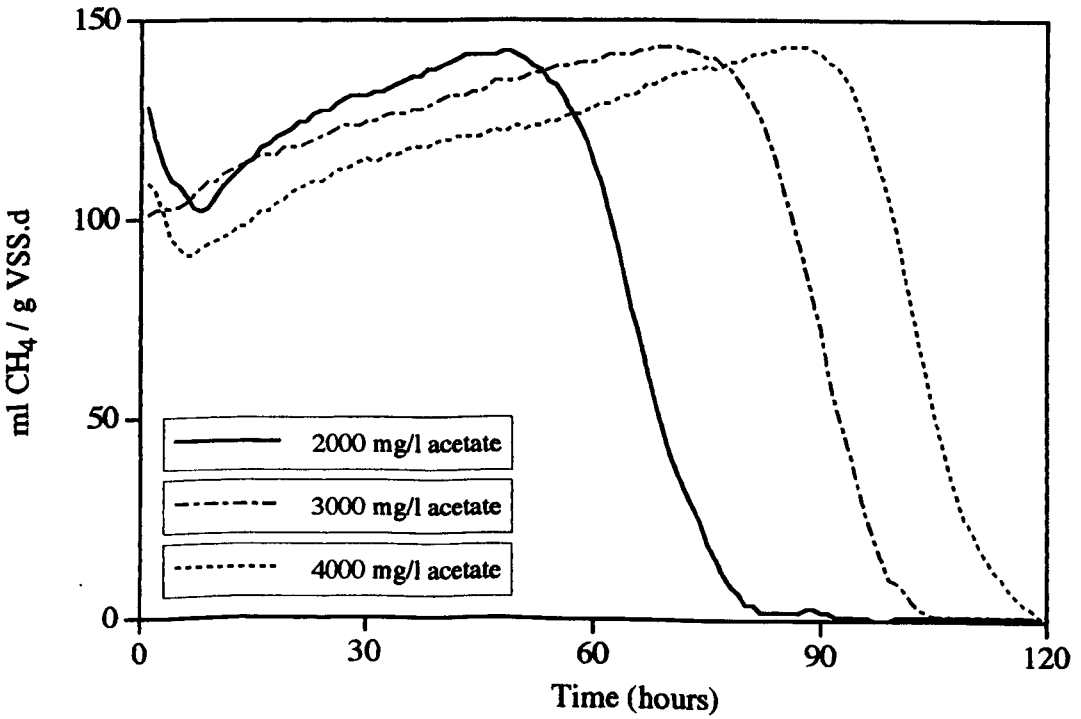


Figure 7.17 Plot of specific methanogenic activity at an organic loading rate of 17.5 kg COD/m^3.d (on day 405)

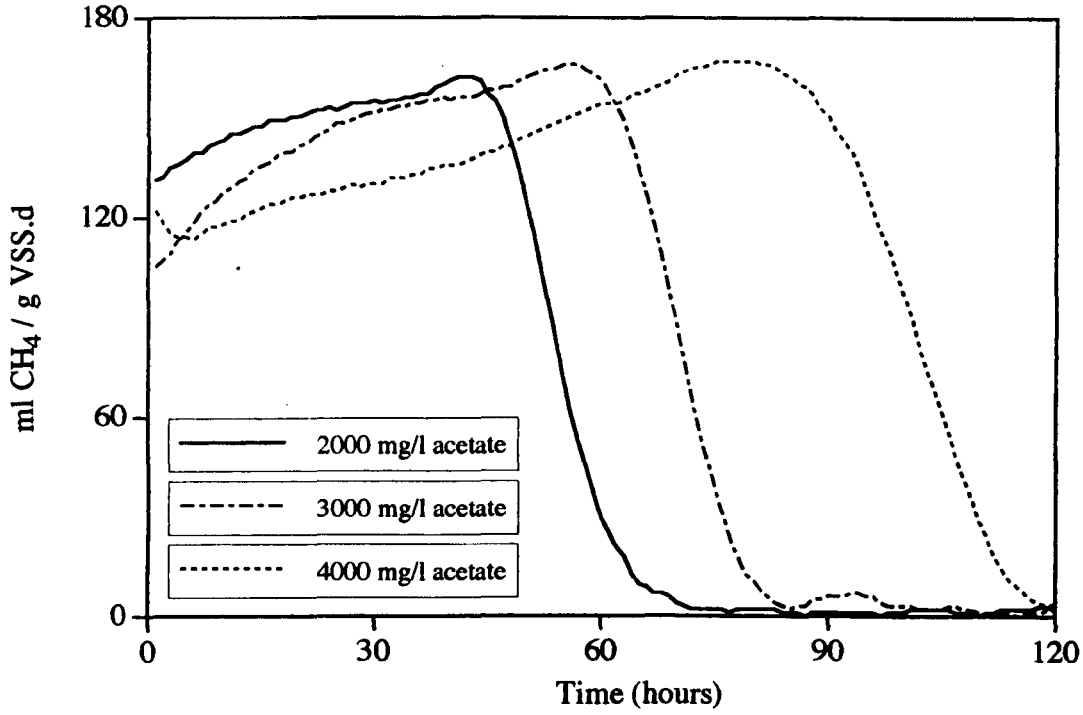


Figure 7.18 Plot of specific methanogenic activity at an organic loading rate of 23 kg COD/m^3.d (on day 447)

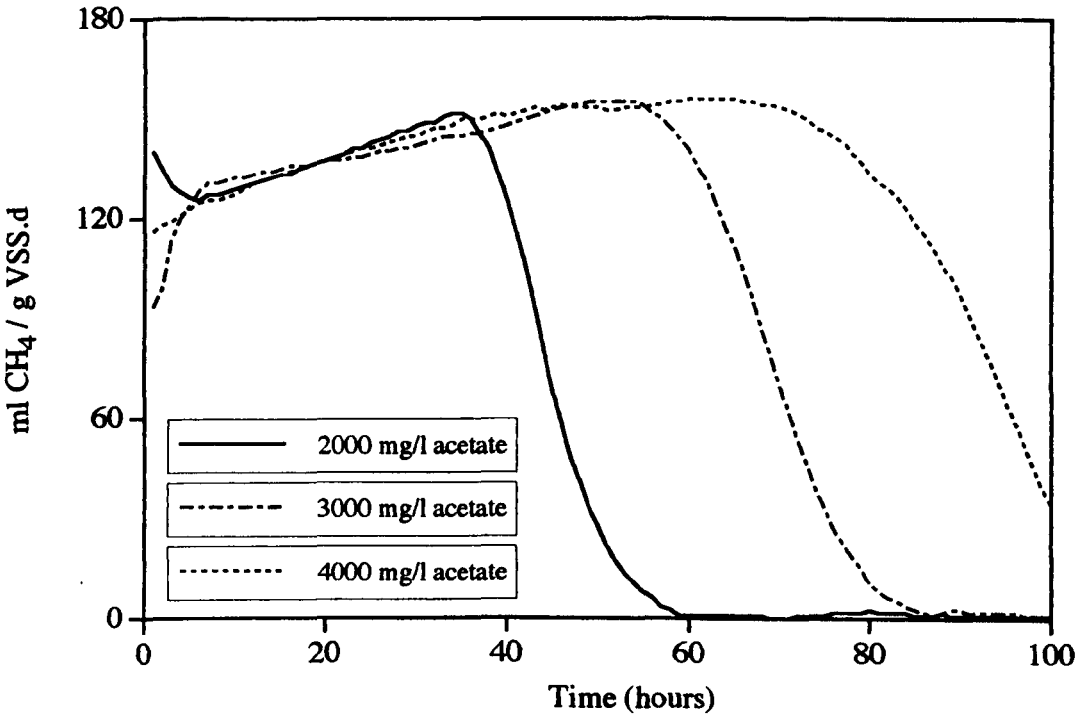


Figure 7.19 Plot of specific methanogenic activity at an organic loading rate of 27 kg COD/m^3.d (on day 460)

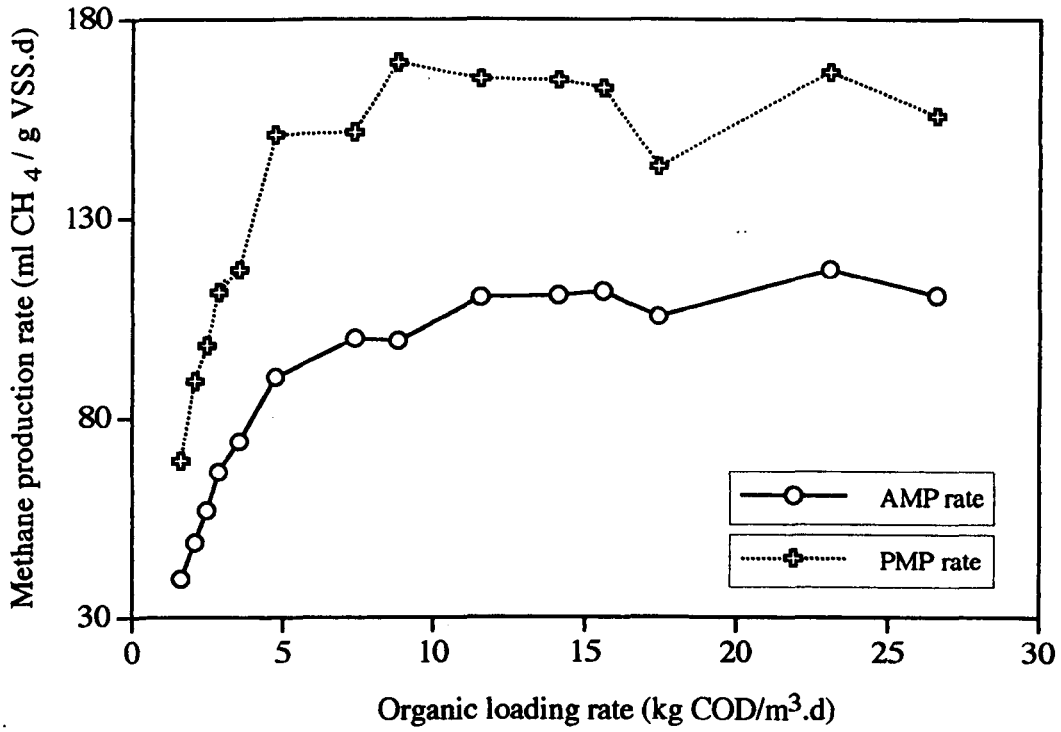


Figure 7.20 Actual methane production (AMP) and potential methane (PMP) production rates against organic loading rates

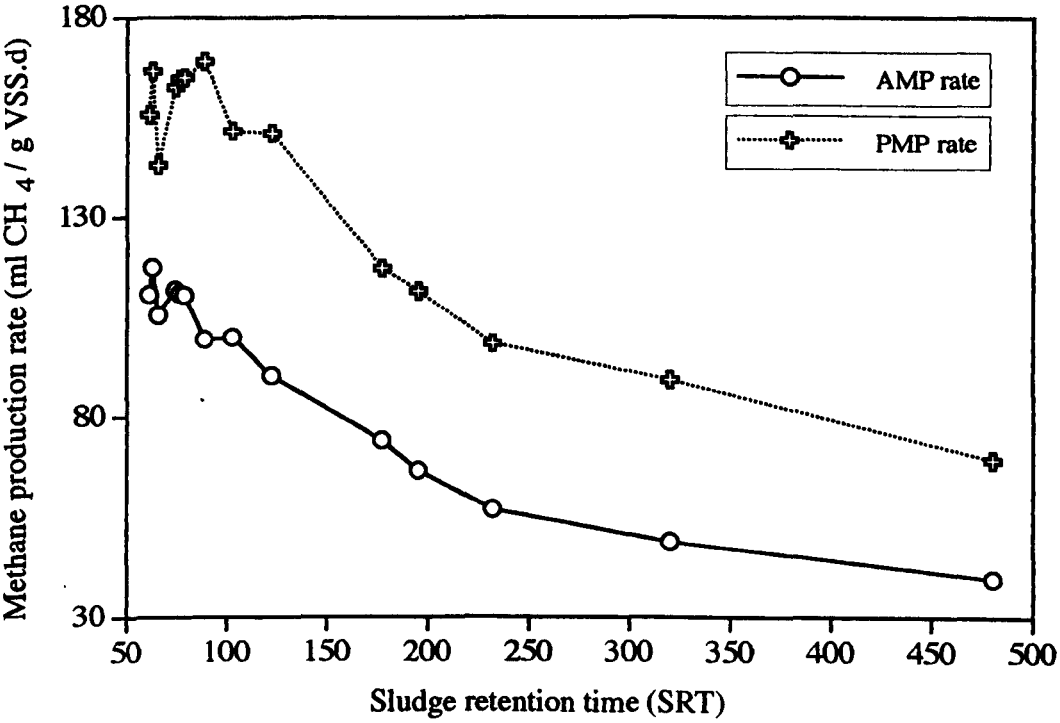


Figure 7.21 Actual methane production (AMP) and potential methane (PMP) production rates against sludge retention times

Table 7.1 Estimated values for AMP and PMP rates throughout the operation of CUMAR system

Cross Flow Ultrafiltration Membrane Anaerobic Contact Reactor						Specific Methanogenic Activity Test Results				
Time	OLR	CH_4 pro.	MLVSS	AMP	AMP x 0.70	MLVSS	Pulses	CH_4	PMP	AMP/PMP
d	kg COD/m^3.d	l/d	g/l	ml CH_4/g VSS	ml CH_4/g VSS	g/l		%	ml CH_4/ g VSS	
3	1.0	1.2	8.85	1.2	0.9	3.54	1	12	1	0.90
12	0.7	8.6	9.00	8	6	3.60	2	50	7	0.80
21	0.7	16.4	9.20	15	10	3.24	3	72	18	0.60
30	1.5	38.4	9.50	34	24	3.65	6	73	32	0.80
42	1.0	38.4	10.17	32	23	2.90	7	78	50	0.45
55	1.7	68.6	10.35	55	40	2.84	9	82	70	0.60
75	2.0	93.2	11.44	68	50	2.73	11	83	90	0.55
98	2.5	111	11.65	80	57	2.67	12	82	98	0.60
119	3.0	133	12.00	93	67	2.40	12	84	112	0.60
143	3.5	162	13.10	103	74	2.58	14	81	117	0.63
184	4.8	225	14.90	126	90	2.50	17	83	150	0.60
213	7.4	330	19.84	140	100	2.66	18	84	152	0.66

Table 7.1 Estimated values for AMP and PMP rates throughout the operation of CUMAR system (continued)

Cross Flow Ultrafiltration Membrane Anaerobic Contact Reactor						Specific Methanogenic Activity Test Results				
Time	OLR	CH_4 pro.	MLVSS	AMP	AMP x 0.72	MLVSS	Pulses	CH_4	PMP	AMP / PMP
d	kg COD/m^3.d	l/d	g/l	ml CH_4/g VSS	ml CH_4/g VSS	g/l		%	ml CH_4/ g VSS	
257	8.8	352	21.15	138	100	2.68	20	85	170	0.60
324	11.5	460	25.00	153	110	2.75	21	81	165	0.67
352	14.0	557	30.15	154	110	2.60	20	80	165	0.67
372	15.5	618	33.15	155	112	2.42	18	82	163	0.70
405	17.4	643	36.50	147	106	2.47	17	78	143	0.74
447	23.0	854	43.62	163	117	2.30	19	76	167	0.70
460	27.0	895	48.50	154	111	2.50	20	73	156	0.71

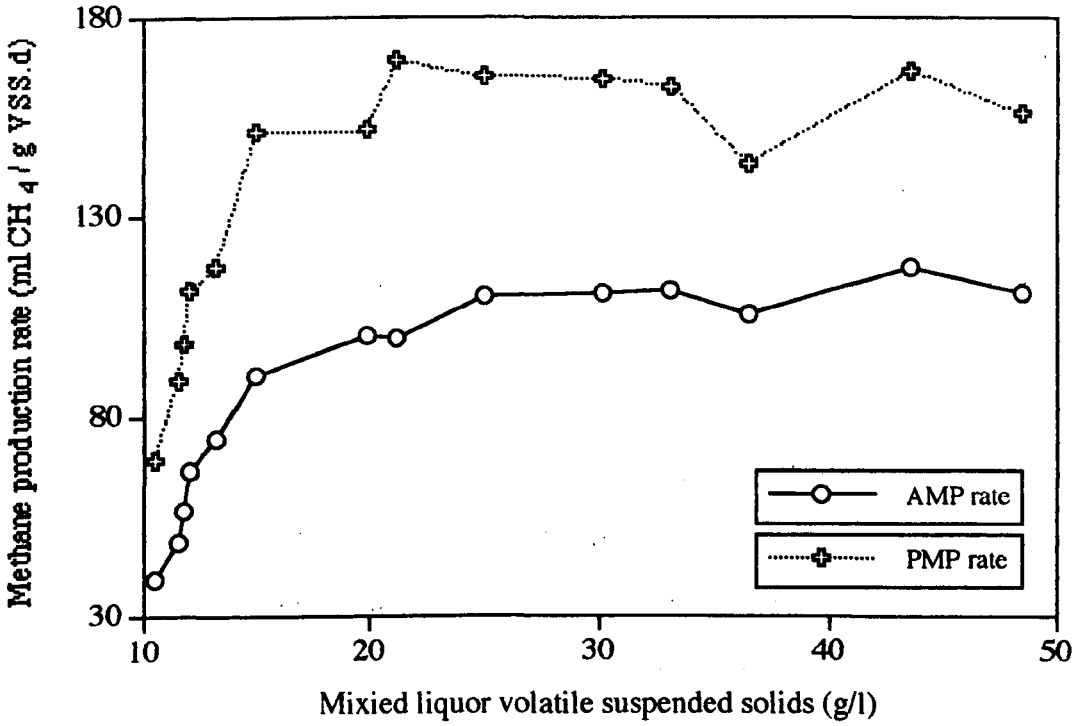


Figure 7.22 Actual methane production (AMP) and potential methane production (PMP) rates against mixed liquor volatile suspended solids

7.3. Discussion

An acceptable removal of organic matter in an anaerobic reactor depends upon the presence of an adequate level of methanogenic activity in which the methanogens are involved in the conversion of volatile fatty acids to end products such as carbon dioxide and methane. It is therefore, necessary to monitor any changes in the numbers or activities of the methanogenic bacteria in the digester using such available techniques as microscopic count, most probable number (MPN), ATP, coenzyme F_{420}, dehydrogenic activity and specific methanogenic activity (SMA) under controlled conditions.

Use of the MPN technique for enumeration of the various sub-populations present in anaerobic sludge is not a practical proposition due to the long doubling times, the strict anaerobic conditions required and the difficulty experienced in cultivating some of the species involved. Consequently, interest has focussed on alternative methods based either

on the quantification of specific molecular components exclusively associated with certain trophic groups or on a determination of the specific biological activity of key reference groups.

Since Coenzyme F_{420} is known to be exclusively present in methanogenic species (Cheeseman *et al.* ,1972; Keltjens and Vogels, 1981), its quantification as an index of the specific methanogenic activity of sludges was suggested by Delafontaine *et al.* (1979) and by de Zeeuw and Letinga (1983, 1980). However, due to the variability in F_{420} content of different methanogens and the effect of environmental conditions on the F_{420} level in individual species, it has not proved possible to use F_{420} determination as a reliable index of potential methanogenic activity (Dolfing and Mulder, 1985; Reynolds and Colleran, 1987). More meaningful data for the prediction of methanogenic activity may be obtained by extraction and separate quantification of individual F_{420} types or other unique cofactors and coenzymes (Gorris and van der Drift, 1986a,b) but the techniques employed are too complex for routine analysis.

The SMA technique is more rapid and reliable than the other tests. In this study,therefore, it was decided to use the SMA technique in order to determine the methane production capacity of the CUMAR system, thus allowing suitable OLRs to be applied and to assess the effect of MLVSS on the activity of acetoclastic methanogenic bacteria in the digester.

The PMP rates obtained from the SMA tests were used as a guideline to compare the AMP rates obtained in the CUMAR system at various organic loading rates. It should be noted, however, that the SMA test only measures the methane production from acetic acid, generally referred to as the acetoclastic methanogenic activity and does not include methane produced by hydrogen utilizing methanogenic bacteria. The AMP rates in the CUMAR system were corrected by a multiplying factor of 0.70.

A set of substrate concentrations was used in order to enable the maximum activity to be determined at which food would not be rate limiting during SMA tests. The most

favourable substrate concentrations were found to be in the range 2000-4000 mg/l (as acetic acid) as can be seen in Figures 7.1-20. Similar results were also reported by James *et al.* (1990) and Monteggia (1991).

De Zeeuv (1984) stated that at the beginning of the start-up of a new reactor, the specific activity of the seed sludge together with the amount of sludge present determines the permissible initial organic loading rate. Subsequent measurements of SMA and total volatile suspended solids would provide a safe guideline for further increases in organic loading rate during the start-up period.

At the beginning of the start-up an SMA test was therefore carried out in order to determine the most acceptable initial organic loading rate. According to the previous SMA test results further increases in organic loading rate were applied during the start-up and the steady-state operations of the CUMAR system. The SMA result obtained at the end of the start-up period is given in Table 7.2 along with the other values reported by Valcke and Verstraete (1983). In their study different anaerobic sludges were used in an upflow anaerobic sludge blanket (UASB) reactor treating wastewater from a flax retting industry during the start-up period. As can be seen from Table 7.2 the PMP rate of 50 ml CH_4/g VSS.d obtained from this study was lower than those reported values. The maximum potential methane production rate of enriched cultures cultivated on acetate has been reported to be approximately 1000 ml CH_4/g VSS.d (Valcke and Verstraete,1983) if all the biomass (measured as VSS) consists of acetoclastic methanogens. In this study the acetoclastic methanogens constituted 5% of the VSS of the digester sludge from the CUMAR whilst that was found to be 9.9 to 10.3% of the VSS of the sludge obtained from their study. This could be due mainly to the high recirculations of the digester contents of the CUMAR system, use of a different type of feed and seed and the reactor configurations. However, this system performed very well, achieving over 98% COD removal efficiency. The ratio of the actual methane production rate of the CUMAR system to potential methane production rate determined by the SMA test was found to be

0.45 which ensured that the CUMAR system could be loaded at higher organic loading rates for the remainder of the operating period.

The SMA results obtained from steady-state operations of the CUMAR system are compared in Table 7.3 with the results reported by the above authors. They did the SMA measurements when the reactor was fully operational. As can be seen from the Table 7.3 both the AMP and the PMP rates of the digester sludge of the CUMAR system at an organic loading rate of 28.5 kg COD/m^3.d were found to be higher than those obtained from the full-scale completely mixed reactors treating different types of wastewater but lower than those obtained from the full-scale upflow sludge blanket reactors treating the wastewaters. This could be explained by the level of methanogens or acetoclastic methanogens per kg VSS of each reactor being different resulting in different quantities of methane production per kg VSS per day as could be seen in Table 7.3. The acetoclastic methanogens in the CUMAR system at the end of the operation were found to be increased approximately 3.5 times to 17% of the VSS of the sludge when compared to its previous value obtained at the end of the start-up period. The ratio of the actual methane production rate of the CUMAR system to the potential methane production rate was found to be 0.7 showing the system had not reached its maximum loading capacity.

During the operation of the CUMAR system the numbers of methanogens gradually increased in the digester as seen in Figure 8.13 resulting in an increase in the numbers of methanogens expressed as a percentage of the non-methanogens from 6.5% to 9.5% (Figure 8.3 and Figure 8.11). In addition to this, viable methanogens determined by the MPN technique sharply increased up to an OLR of approximately 10 kg COD/m^3.d then levelled-off, resulting in a decrease in the ratio of total methanogens to viable methanogens, i.e. 400 to 15. The AMP and PMP rates expressed as ml methane/g VSS.d showed a similar trend during this period. It can be seen from the above results that the numbers of methanogens per gram VSS increased significantly, resulting in proportional increases in the AMP and the PMP rates of the CUMAR system.

The time required to reach steady-state after an increase in OLR was decreased maintaining the AMP/PMP ratios less than 0.7. For example, the COD removal efficiency was approximately 80% in the third week operation of the system while the AMP/PMP rate was 0.6. It was then decided to be double the OLR (1.5 kg COD/m^3.d) in the following week. The COD removal efficiency decreased to 70% and the TVFAs increased to 900 mg/l in the reactor on the same day when the OLR had been increased yielding an AMP/PMP ratio of 0.76. This indicated that the system was operating at its maximum load. Therefore, the OLR was decreased to 1 kg COD/m^3.d (after 12 hours) and maintained at the same level for the following two weeks operation after which the system performed very well, i.e. a COD removal efficiency of 96% and TVFAs of less than 15 mg/l in the permeate. At this point the AMP/PMP ratio was found to be 0.45. If the third week's AMP/PMP of 0.6 had been considered the OLR should not have been doubled and the length of time required to reach steady-state would have been shorter. For example, a further OLR of 1.7 kg COD/m^3.d was decided according to the AMP/PMP ratio. However, this increase in OLR neither increased the effluent COD nor increased the effluent TVFAs and the system reached a steady-state after 6 days at which point the AMP/PMP ratio was found to be 0.6. Therefore, further increases in OLRs were carried out according to the AMP/PMP ratio during the remainder of the operation.

Table 7.2 Comparison of methane production rates of two different reactor types

Reactor type	Sludge concentration (g VSS/l)	Initial concentration of HAc (g/l)	Initial sludge load (g HAc/g VSS)	Gas production (ml CH_4/g VSS.d)
UASB*	4.4	1.24	0.3	76
UASB*	4.4	2.48	0.6	80
UASB*	4.4	3.72	0.9	99
UASB*	6.1	1.24	0.2	71
UASB*	6.1	2.48	0.4	98
UASB*	6.1	3.72	0.6	103
CUMAR**	3.0	1.50	0.5	50

* Valcke and Versatraete (1983); **Ince (this study)

Table 7.3 Comparison of operational characteristics of the anaerobic sludges from full-scale digesters and CUMAR system

Substrate	Reactor type	Temp. °C	kg VSS/m^3	Reactor loading rate		CH_4 production of reactor		Acetoclastic biomasss		Potential CH_4 production rate $m^3 \cdot CH_4$/kg VSS.d (B)	P+
				kg COD/ m^3.d	kg COD/ kg VSS.d	m^3/m^2.d	m^3/kg VSS.d (A)	% VSS	kg/m^3		
Rettery wastewater*	upflow 12 m^3	33	13	8.0	0.60	3.2	0.25	33	4.2	0.329	0.76
Enzyme wastewater*	upflow 12 m^3	35-40	12	6.0	0.50	2.0	0.18	39	4.6	0.395	0.46
Distillery wastewater*	upflow 6 m^3	33	18	22.0	1.20	3.3	0.18	31	5.5	0.310	0.60
Sugarbeat wastewater*	upflow 1500 m^3	35	11	5.0	0.46	1.8	0.17	63	6.9	0.635	0.27
Secondary domestic sludge*	completely mixed 80 m^3	20-30[a]	39	0.6	0.02	0.07	0.002	8	3.1	0.082	0.02
Secondary domestic sludge*	completely mixed 6 500 m^3	33	11	1.6	0.14	0.23	0.02	6	0.6	0.064	0.34
Screened piggery manure*	completely mixed 10 l	30	8	1.5	0.18	0.60	0.08	13	1.0	0.132	0.60
Piggery manure*	completely mixed 70 m^3	30	43	1.8	0.04	0.40	0.01	6	2.7	0.066	0.14
Brewery wastewater**	CUMAR 120 l	36±1	51	28.5	0.55	7.2	0.11	17	8.7	0.170	0.65

a : 20 °C in winter, 30 °C in summer ; P^+: ratio of actual CH_4 production rate (A) over the potential production rate (B)

Source: * Valcke and Versatraete (1983) ; **Ince (this study)

CHAPTER 8

CHANGES IN NUMBERS OF METHANOGENS

The physical parameters which affect the operation of an anaerobic digester, e.g., temperature, pH, substrate feed rates, as well as gas production rates and its composition, can be readily controlled. However, there is little published information which enables numbers of species of viable microorganisms to be controlled. Although one may anticipate that a digester will continue to function well if feed rates and feed composition remain unchanged, these requirements are normally unattainable in practice. It is in the area of control under variable conditions that advances should be made as a result of the very considerable efforts being conducted into microbiological studies of methanogens.

The main objective of biological process control must be to achieve a microbial population that is able to respond more rapidly to increased loading than is presently the case. While early recognition of problems using improved instrumentation will undoubtedly be of value, it would be clearly more suitable to utilize a population able to respond quickly so that problems do not occur.

Previous studies have not considered the possible effects that membrane systems may have on the microbial population in the reactor. In this study, therefore, microscopic examinations have frequently been carried out in order to determine the effects of the new configuration on any variation in the morphology or on the proporties of methanogens as well as any changes in the number of non-methanogens present throughout the operation of the CUMAR system.

8.1 Changes in Species Composition During Start-up Period

The results of microbiological analyses obtained from the CUMAR system during the start-up period are given in Appendix 8.1. Figure 8.1 shows the changes in the number of methanogens and non-methanogens in the digester during an 7 week operation of the system. The variations in morphology of the methanogens are shown in Figure 8.2. All bacterial counts were expressed per mg VSS instead of counts per ml in order to avoid the effect of changes in the concentration of biomass in the digester. During the start-up period the total number of autofluorescent methanogens remained at between 6.8 and 7.5% of the total population and can be seen in Figure 8.3. Figure 8.4 shows that the total number of methanogens in the reactor almost doubled during the start-up period, after which very high COD removal efficiencies of the system were achieved.

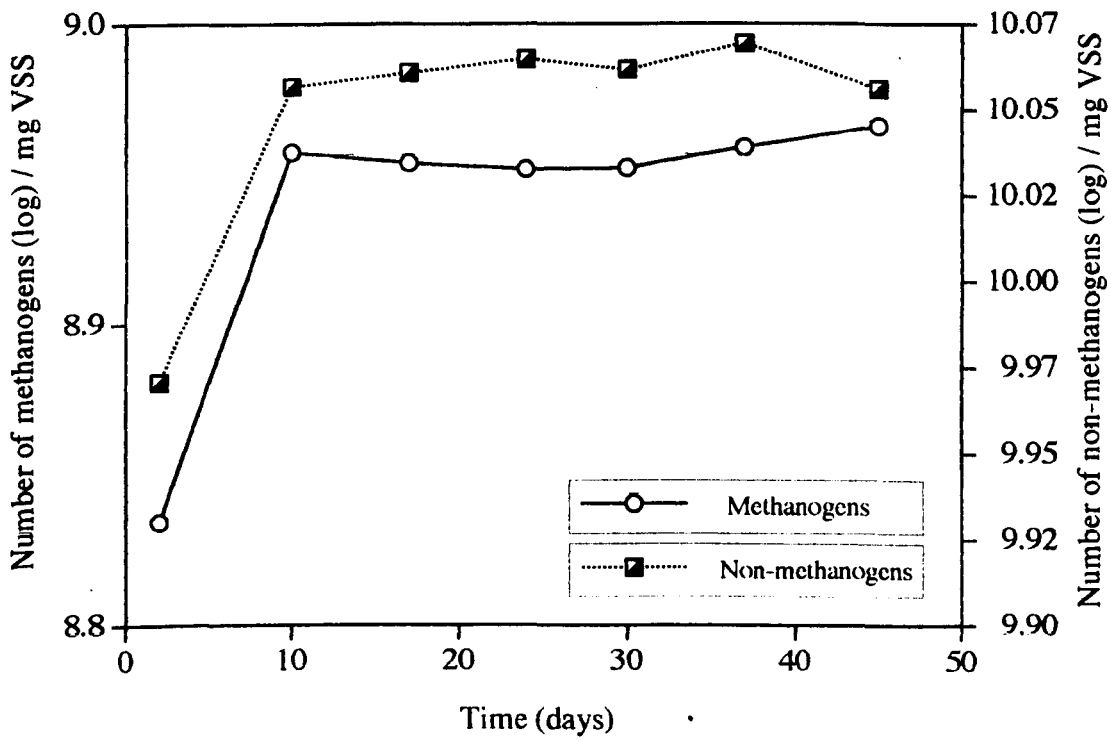


Figure 8.1 Changes in number of methanogens and non-methanogens in digester during start-up

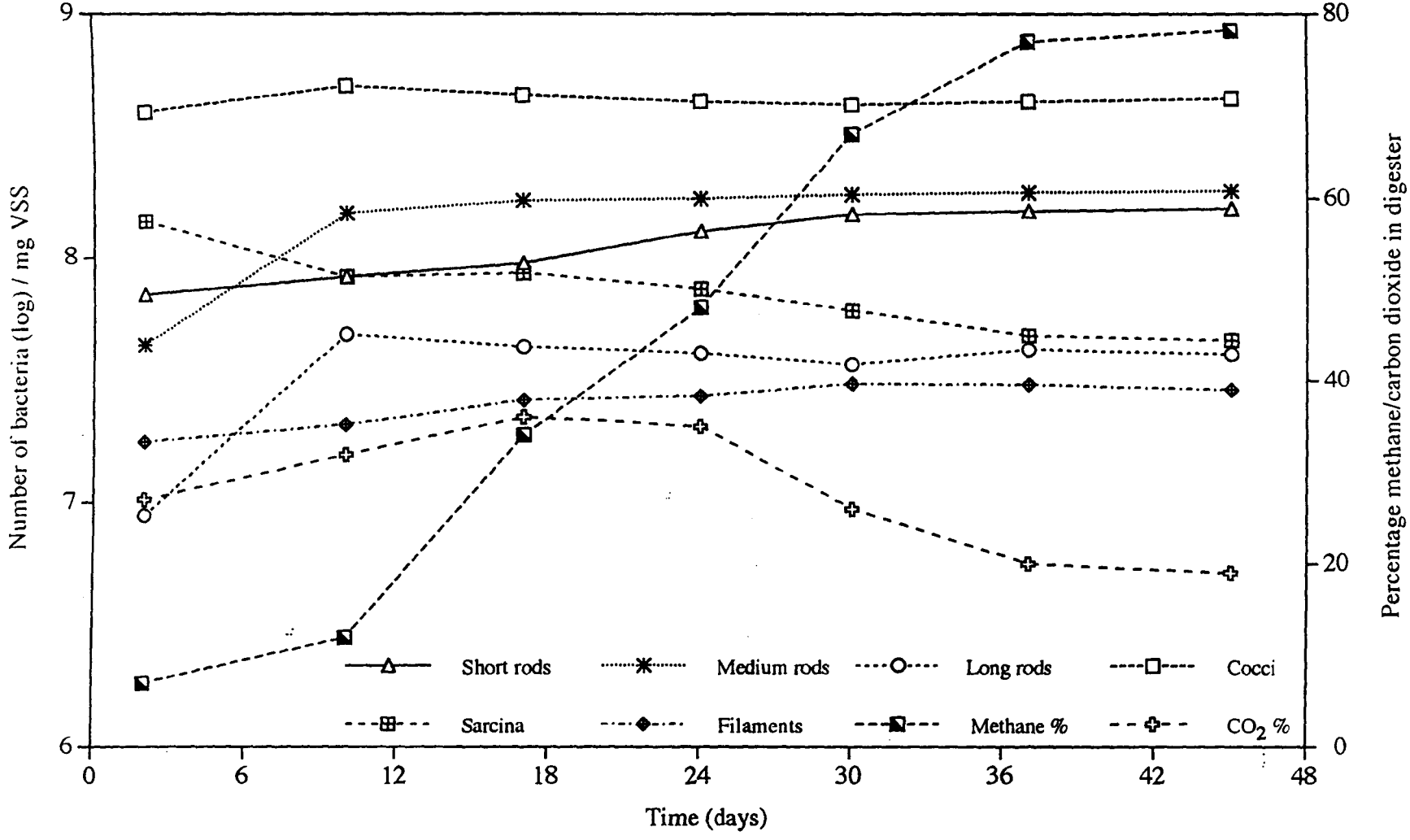


Figure 8.2 Changes in morphology of methanogens and biogas composition in digester during start-up period

N.B. During initial period the gas contained residual N_2 from flushing

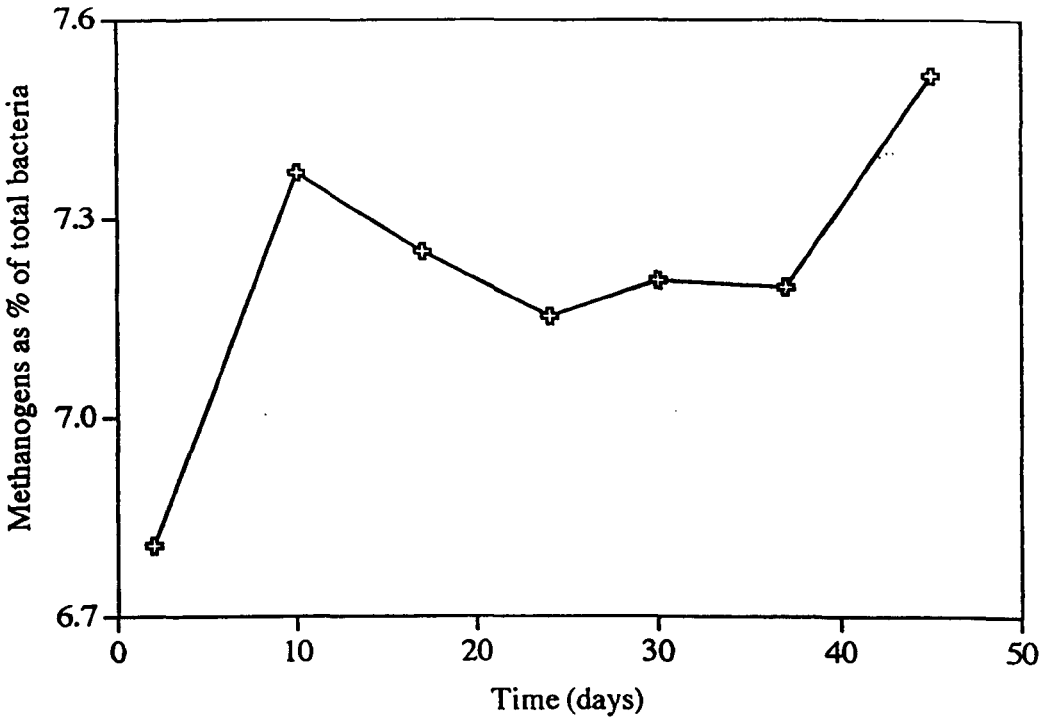


Figure 8.3 Changes in ratio of methanogens to total bacteria in digester during start-up

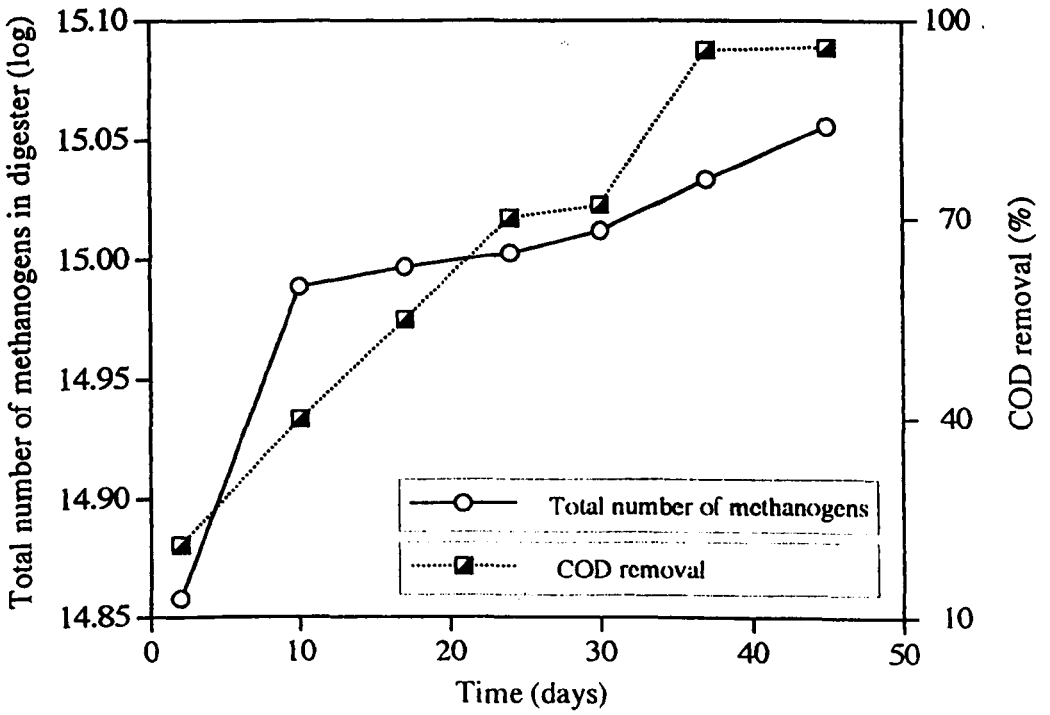


Figure 8.4 Changes in total number of methanogens in digester and COD removal efficiency of system during start-up

The Most Probable Number (MPN) technique was used to count the number of viable methanogens in the digester and the results are given in Table 8.1 along with the 95 % confidence limits, while the trend can also be seen in Figure 8.5. Throughout the study, an increase in the number of viable methanogens resulted in a proportional increase in the efficiency of the membrane system, i.e. to over 95 % which can be seen in Figure 8.4. Figure 8.6 shows the variations in the ratio of the number of viable methanogens to the number obtained by microscopic count during the start-up period. As can be seen from Figure 8.6 the ratio was 1:400 at the beginning, increasing to 1:50 at the end of start-up period.

Table 8.1 Most Probable Numbers of methanogens showing 95% confidence limits during start-up period

Operation Time (d)	Methanogens (No.per.mg VSS)	95% Confidence Limit	
		Lower	Higher
2	$1.6x10^6$	$4.2x10^5$	$3.9x10^6$
10	$2.4x10^6$	$6.3x10^5$	$7.8x10^6$
17	$3.8x10^6$	$1.3x10^6$	$1.1x10^7$
24	$4.6x10^6$	$1.6x10^6$	$1.2x10^7$
30	$7.3x10^6$	$2.4x10^6$	$1.8x10^7$
37	$1.3x10^7$	$3.5x10^6$	$3.0x10^7$
45	$1.8x10^7$	$4.2x10^6$	$4.9x10^7$

The seed sludge examined under UV light showed that methanogenic species such as *Methanococcus* , *Methanosarcina* as well as a range of short and medium rod shaped methanogens were present in high numbers whilst long rod and filamentous methanogens were only present in low numbers. Figure 8.2 shows a comparison of the

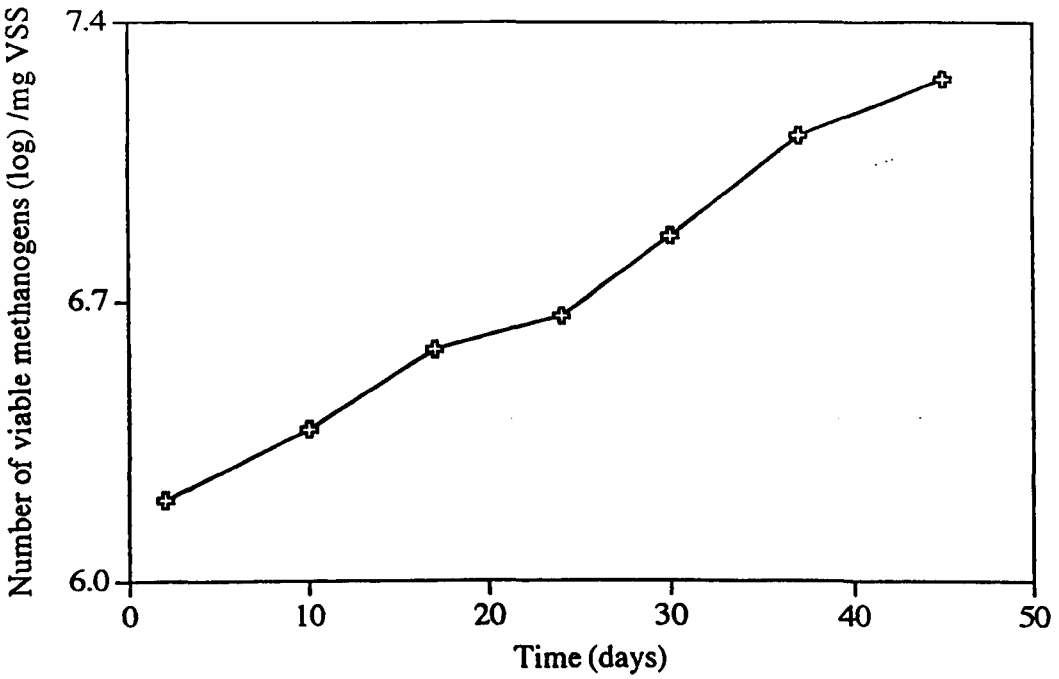


Figure 8.5 Changes in viable methanogens in digester during start-up

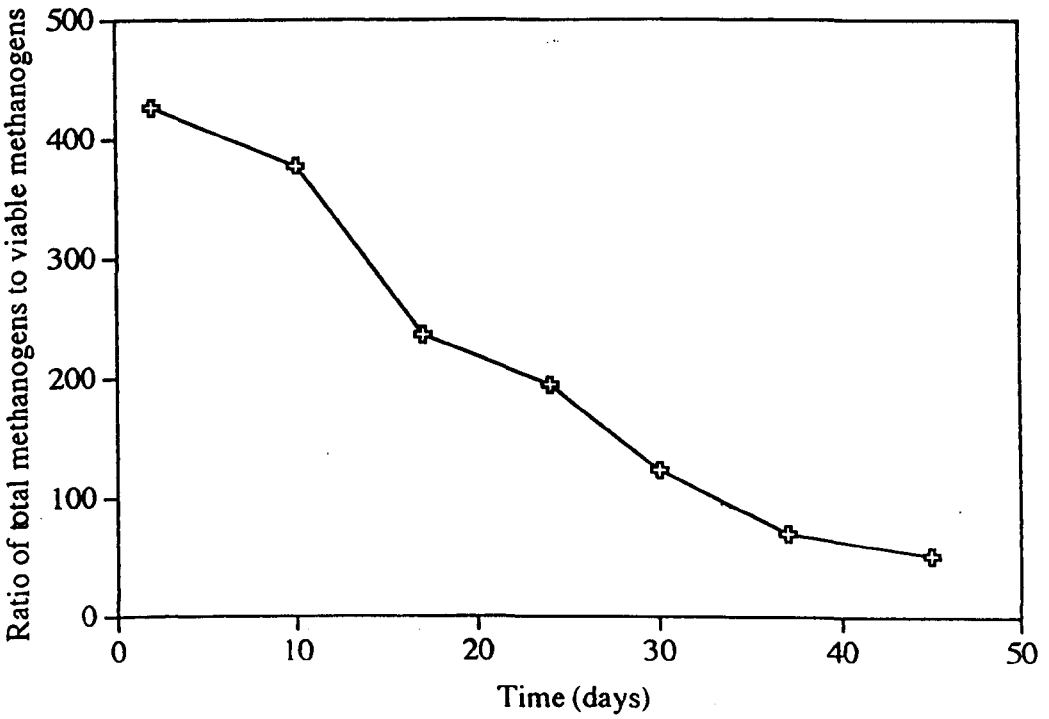


Figure 8.6 Changes in ratio of total methanogens to viable methanogens in digester during start-up

species present in the total methanogenic population together with the changes in methane and carbon dioxide content of the biogas produced in the digester. As can be seen in Figure 8.2, *Methanococcus* species were the most dominant group throughout the start-up period, while the medium rod shaped species became the next most dominant group after 2 weeks followed by the short rod shaped species. On the other hand only slight increases in the numbers of long rod shaped species and flamentous bacteria were observed during the start-up period. As the numbers of rod shaped species increased, a slight decrease in the number of *Methanosarcina* was noted which may be explained by the decrease in concentration of volatile fatty acids which can be seen in Figure 8.7.

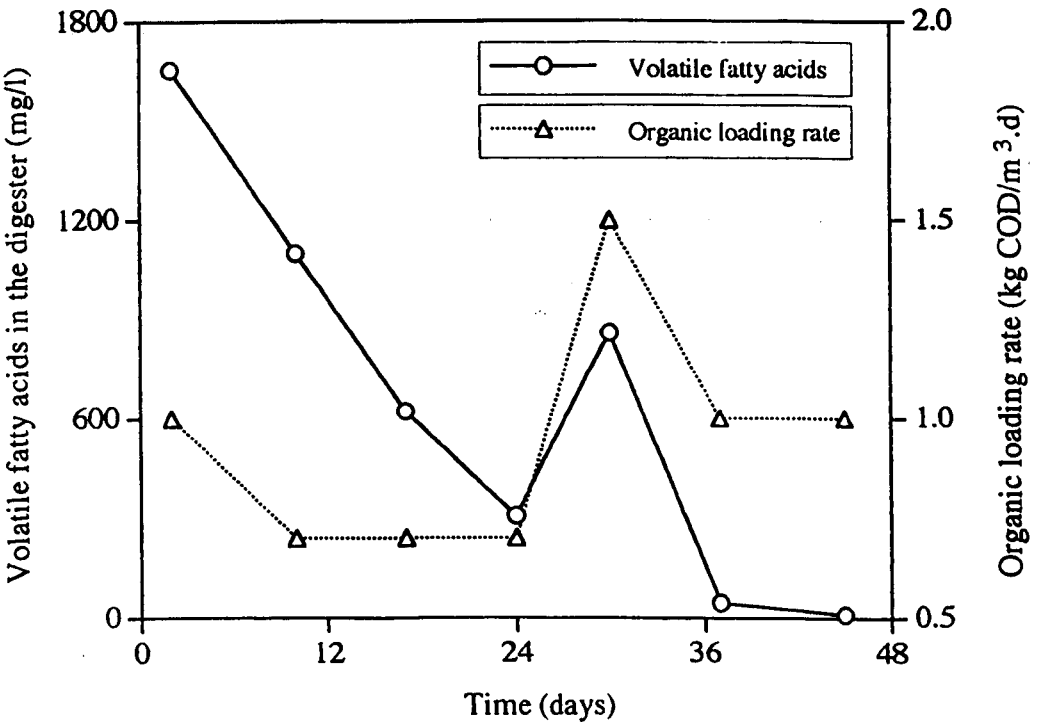


Figure 8.7 Changes in concentration of volatile fatty acids in digester during start-up

The results of the metabolic activity of the methanogenic population in the digester are given in Table 8.2 while the trend can also be seen in Figure 8.8. At the end of the start-up period the metabolic activity of the methanogens was found to be increased over 20 times compared to its initial value. This sharp increase in metabolic activity may have

Table 8.2 Changes in metabolic activity of digester sludge during start-up period

Time (d)	Methanogens (No.per mgVSS)	MLVSS (g)	Methanogenic Population	Methane Production Rate (l/d)	Metabolic Activity (ml CH_4/cell/d)
2	$6.83x10^8$	1060	$7.20x10^{14}$	1	$1.66x10^{-12}$
10	$9.06x10^8$	1080	$9.75x10^{14}$	7	$7.18x10^{-12}$
17	$9.00x10^8$	1100	$9.93x10^{14}$	13	$1.30x10^{-11}$
24	$8.94x10^8$	1120	$1.00x10^{15}$	22	$2.20x10^{-11}$
30	$8.95x10^8$	1150	$1.03x10^{15}$	38	$3.70x10^{-11}$
37	$9.10x10^8$	1190	$1.08x10^{15}$	38	$3.50x10^{-11}$
45	$9.24x10^8$	1230	$1.14x10^{15}$	42	$3.70x10^{-11}$

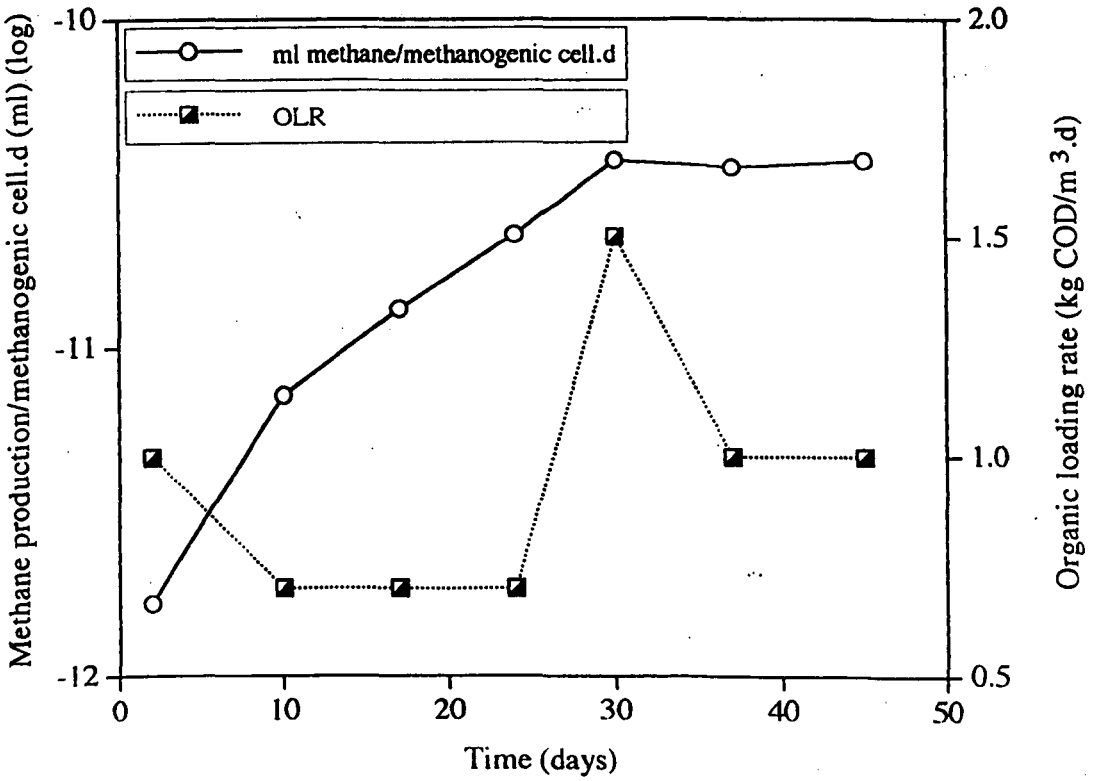


Figure 8.8 Changes in metabolic activity of methanogenic population in digester during start-up

been affected by either an increase in the number of viable methanogens or an increase in the acetoclastic methanogenic activity (SMA) of the sludge in the digester which was discussed in Chapter 7.

The methane content of the biogas produced in the digester, which can be seen in Figure 8.2, reached approximately 80% at the end of start-up period showing that a sufficient quantity of methane producing bacteria had been established in the digester.

8.2 Changes in Species Composition During Steady-state Operation

The results of microbiological analyses obtained from the CUMAR system throughout the steady-state operation are given in Appendix 8.1. The changes in number and the composition of the microbial population in the CUMAR system were also studied during steady-state operations. Increases in both the number of methanogens and the non-methanogens were found in the digester with variations in the morphology of the methanogens occuring throughout the study.

Figure 8.9 shows the changes in the numbers of methanogens and non-methanogens in the digester throughout the steady-state operation while the variations in the morphology of methanogens with the changes in the methane and carbon dioxide contents of the biogas produced in the digester can be seen in Figure 8.10. It can be seen from Figure 8.9 that the number of methanogens per mg VSS in the digester doubled towards the end of the operation compared to its initial value whilst that of the non-methanogens increased 1.5 times. The changes in the ratio of the numbers of methanogens/total bacteria throughout the study were also plotted in Figure 8.11 showing that the number of autofluorescent methanogens increased from 7.5% to 9.5% of the total population towards the end of the operation.

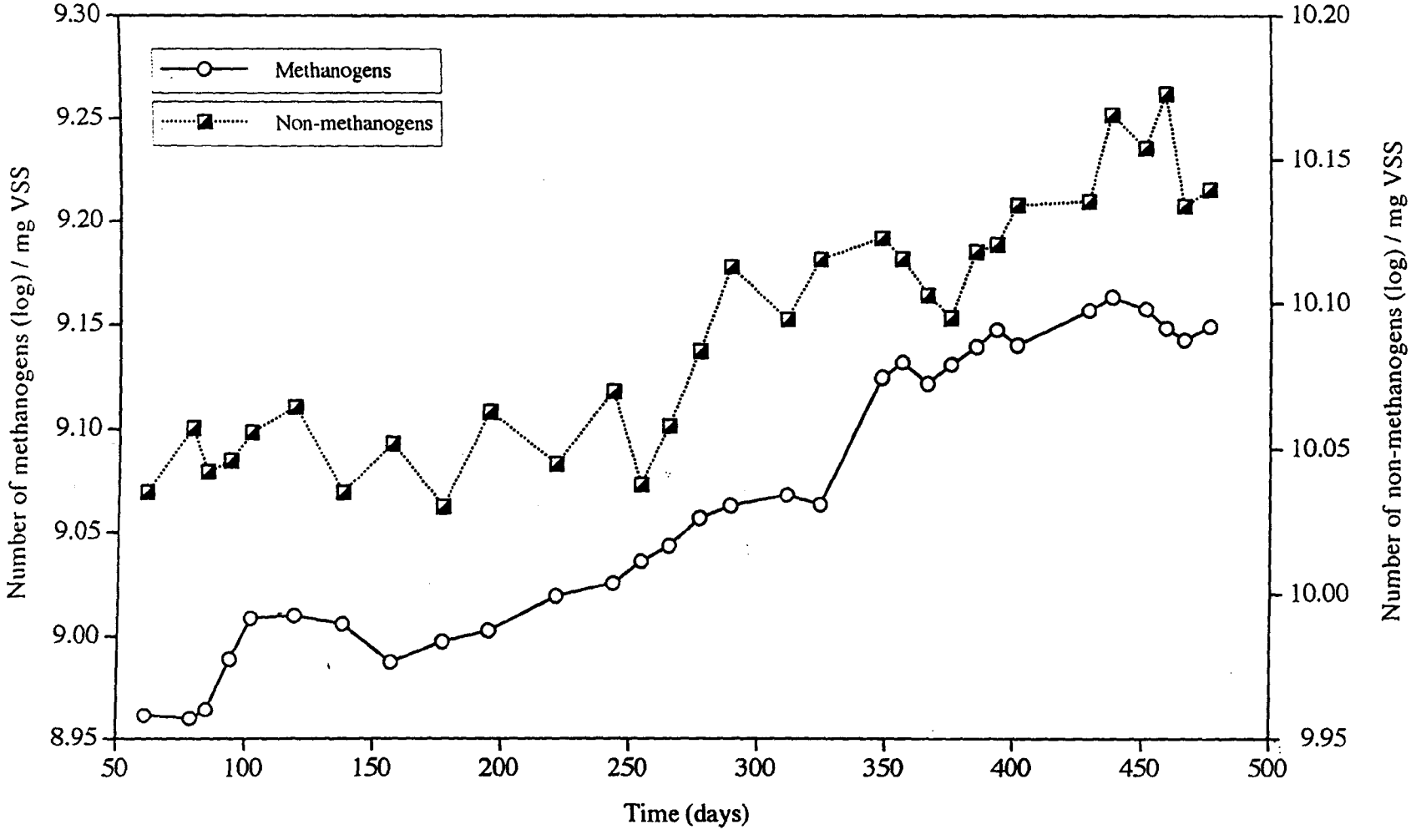


Figure 8.9 Changes in number of methanogens and non-methanogens in digester during steady-state operation

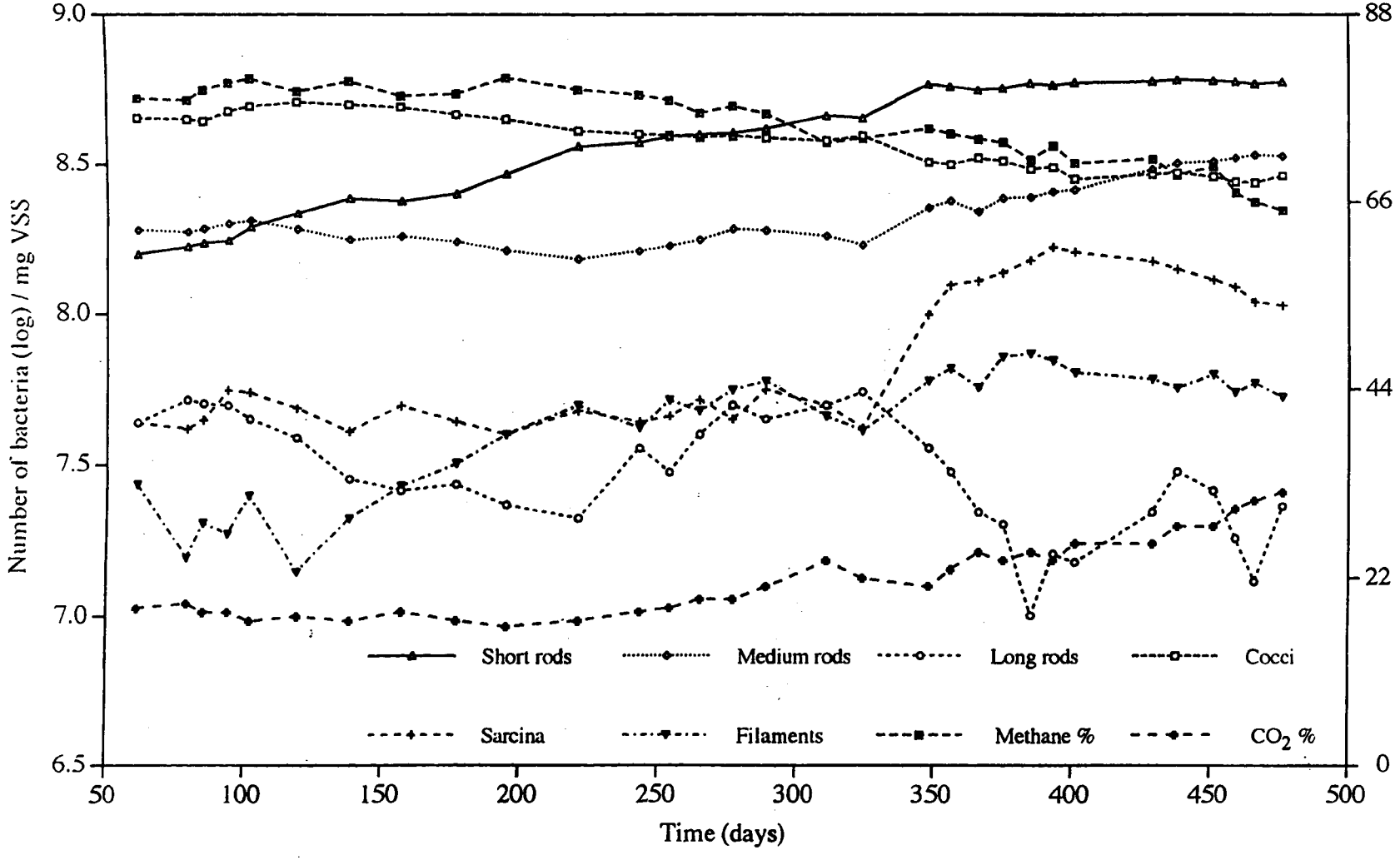


Figure 8.10 Changes in morphology of methanogens and biogas composition in digester during steady-state operation

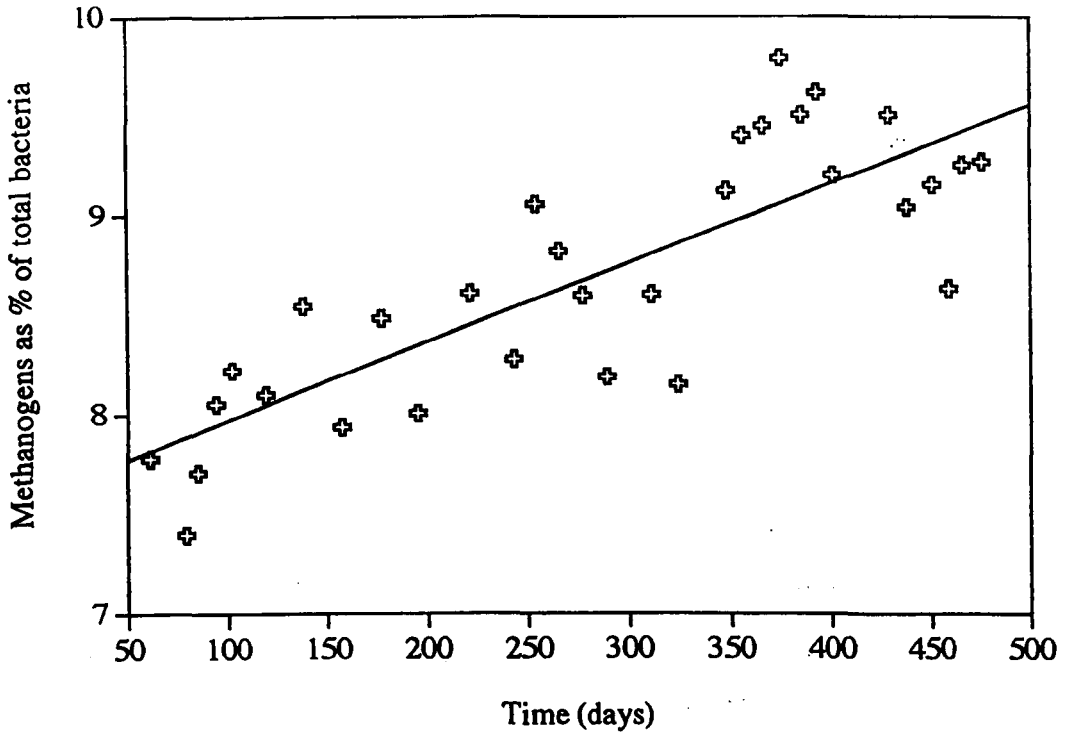


Figure 8.11 Changes in ratio of methanogens to total bacteria in digester during steady-state operation

After the start-up period microscopic examination of the digester sludge showed (Figure 8.10) that the most dominant group was found to be *Methanococcus* species followed by medium rods, short rods, *Methanosarcina* , long rods and filamentous species. Slight decreases in the numbers of *Methanococcus* species occured throughout the study becoming the third dominant group towards the end of the operation whilst sharp increases in the numbers of short rod species were observed up to an OLR of 14 kg COD/m^3.d (on day 350) after which the number of short rods remained fairly constant. Short rod species became the most dominant group after an OLR of 9 kg COD/m^3.d (on day 265). On the other hand the number of medium rod species showed slight decreases up to an OLR of 7.5 kg COD/m^3.d (on day 221) then increased sharply for the remainder of the operation and became the second dominant species towards the end of the study. Slight variations in the numbers of *Methanosarcina* species were observed up

to an OLR of 12 kg COD/m^3.d (on day 324) after which significant increases in the number of *Methanosarcina* species were found up to an OLR of 17 kg COD/m^3.d (on day 393) then slight decreases were observed towards the end of study becoming the fourth dominant group. Long rod species showed variations throughout the operation while the numbers of filamentous species increased and became the fifth dominant group. Long rod species were found to be the least dominant group towards the end of the operation.

The methane content of the biogas produced in the digester was found to be approximately 79% up to an OLR of 8 kg COD/m^3.d (on day 250) after which slight decreases in the methane content were observed for the remainder of the operation becoming 65% at the end of the operation while carbon dioxide content decreased to a minimum value of 16% at an OLR of 5 kg COD/m^3.d then gradually increased with the increases in OLRs reaching 32% at the end of the study. This might have been due to the changes in the dominant species and their numbers throughout the operation of the CUMAR system.

The changes in the metabolic activity of the digester sludge during steady-state operation are given in Table 8.3. The methane production rates per methanogenic cell were also plotted against OLRs in Figure 8.12 from which it can be seen that the methane production rate significantly increased up to an OLR of 8 kg COD/m^3.d after which a slight decrease was observed. The maximum methane production rate per cell was found to be 1.35 x 10^{-10} ml methane/d (on day 220) while this decreased to 1.0 x 10^{-10} ml methane/d at the end of the study. Although decreases in the metabolic activity after an OLR of 8 kg COD/m^3.d were observed the COD removal efficiencies of the system at higher OLRs were not adversely affected. This could be explained by the increases in the levels of methanogenic bacteria in the digester throughout the operation which can be seen in Figure 8.13.

Table 8.3 Changes in metabolic activity of digester sludge during steady-state operation

Time (d)	Methanogens (No.per mgVSS)	MLVSS (g)	Methanogenic Population	Methane Production Rate (l/d)	Metabolic Activity (ml CH_4/cell/d)
61	$9.15x10^8$	1290	$1.18x10^{15}$	72	$6.08x10^{-11}$
79	$9.12x10^8$	1380	$1.26x10^{15}$	95	$7.56x10^{-11}$
85	$9.20x10^8$	1380	$1.27x10^{15}$	110	$8.65x10^{-11}$
94	$9.75x10^8$	1400	$1.36x10^{15}$	110	$8.07x10^{-11}$
102	$1.02x10^9$	1400	$1.43x10^{15}$	110	$7.70x10^{-11}$
119	$1.02x10^9$	1440	$1.47x10^{15}$	130	$8.85x10^{-11}$
138	$1.00x10^9$	1500	$1.52x10^{15}$	145	$9.52x10^{-11}$
157	$9.72x10^8$	1580	$1.54x10^{15}$	157	$1.02x10^{-10}$
177	$9.94x10^8$	1690	$1.68x10^{15}$	194	$1.16x10^{-10}$
195	$1.00x10^9$	1800	$1.80x10^{15}$	225	$1.24x10^{-10}$
221	$1.05x10^9$	2390	$2.50x10^{15}$	335	$1.34x10^{-10}$
243	$1.06x10^9$	2420	$2.56x10^{15}$	345	$1.35x10^{-10}$
254	$1.10x10^9$	2500	$2.72x10^{15}$	350	$1.30x10^{-10}$
265	$1.11x10^9$	2540	$2.80x10^{15}$	355	$1.26x10^{-10}$
277	$1.14x10^9$	2610	$3.00x10^{15}$	380	$1.27x10^{-10}$
289	$1.16x10^9$	2740	$3.17x10^{15}$	385	$1.20x10^{-10}$
311	$1.17x10^9$	2950	$3.45x10^{15}$	405	$1.17x10^{-10}$
324	$1.16x10^9$	3000	$3.47x10^{15}$	460	$1.33x10^{-10}$
348	$1.33x10^9$	3610	$4.80x10^{15}$	520	$1.08x10^{-10}$
356	$1.35x10^9$	3700	$5.00x10^{15}$	555	$1.10x10^{-10}$
366	$1.32x10^9$	3860	$5.10x10^{15}$	570	$1.12x10^{-10}$
375	$1.35x10^9$	3980	$5.40x10^{15}$	600	$1.12x10^{-10}$

Table 8.3 Changes in metabolic activity of digester sludge during steady-state operation (continued)

Time (d)	Methanogens (No.per mgVSS)	MLVSS (g)	Methanogenic Population	Methane Production Rate (l/d)	Metabolic Activity (ml CH_4/cell/d)
385	$1.38x10^9$	4090	$5.64x10^{15}$	620	$1.10x10^{-10}$
393	$1.40x10^9$	4190	$5.88x10^{15}$	660	$1.12x10^{-10}$
401	$1.38x10^9$	4230	$5.83x10^{15}$	635	$1.10x10^{-10}$
429	$1.43x10^9$	4710	$6.75x10^{15}$	660	$9.77x10^{-11}$
438	$1.46x10^9$	5080	$7.40x10^{15}$	770	$1.04x10^{-10}$
451	$1.44x10^9$	5280	$7.58x10^{15}$	800	$1.05x10^{-10}$
459	$1.41x10^9$	5350	$7.52x10^{15}$	780	$1.04x10^{-10}$
466	$1.40x10^9$	5810	$8.07x10^{15}$	880	$1.10x10^{-10}$
476	$1.41x10^9$	6130	$8.63x10^{15}$	880	$1.02x10^{-10}$

The changes in the number of viable methanogens in the digester determined by the MPN technique are given in Table 8.4 along with the 95% confidence limits while the trend can also be seen in Figure 8.14. Significant increases in the numbers of viable methanogens were observed up to an OLR of 12 kg COD/m^3.d (on day 348) at which point the number of viable methanogens was found to be 5 times higher than the first value after the start-up period. After that, slight decreases were noticed but this did not adversely affect the system performance in terms of COD removal efficiency which can be seen in Figure 8.14. Figure 8.15 shows the changes in the ratio of the numbers of viable methanogens to total methanogens in the digester throughout the operation. the ratio was found to be 1:50 after start-up period (on day 61) increasing to 1:15 at the end of the study (on day 476).

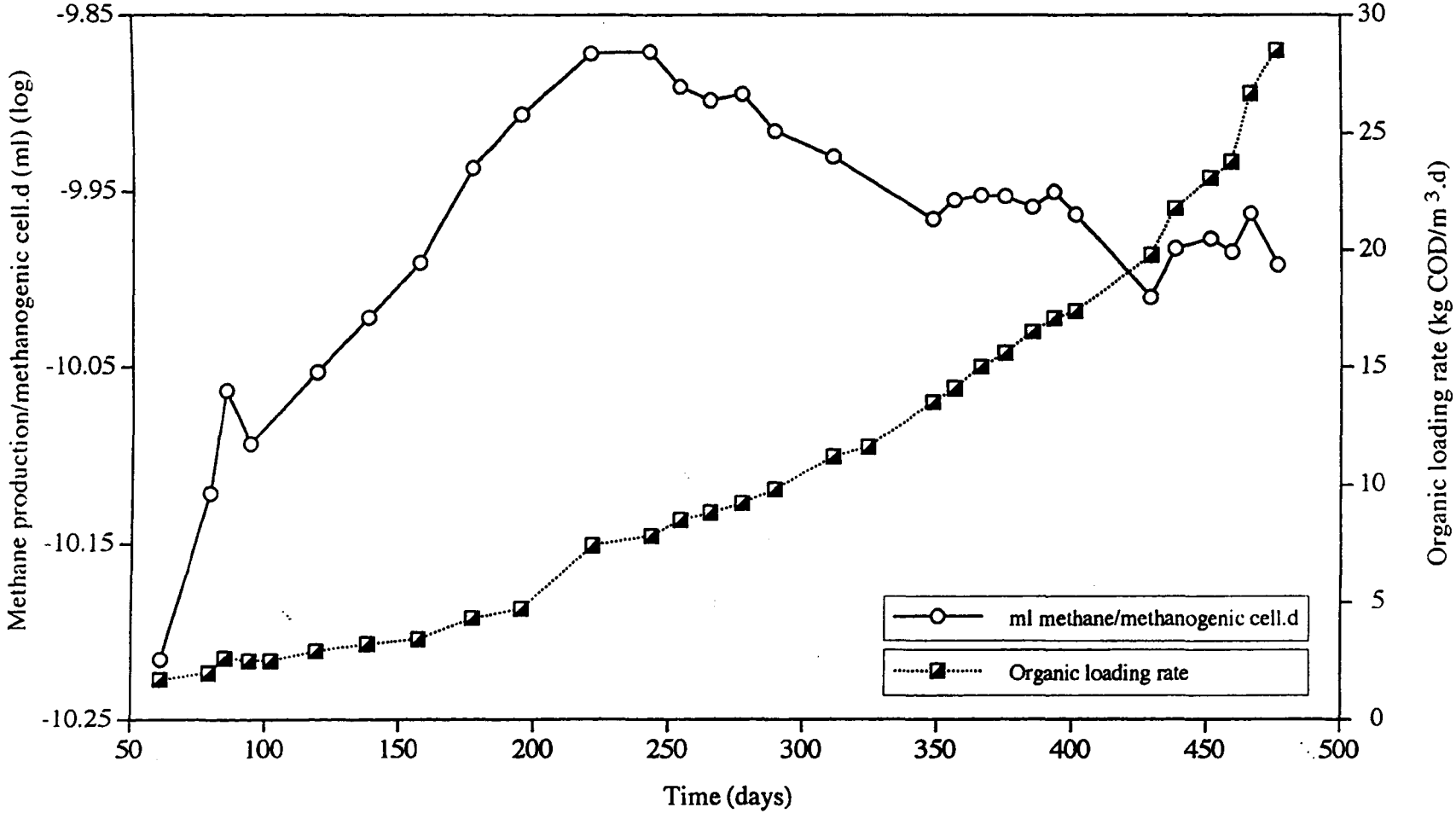


Figure 8.12 Changes in metabolic activity of methanogenic population in digester during steady-state operation

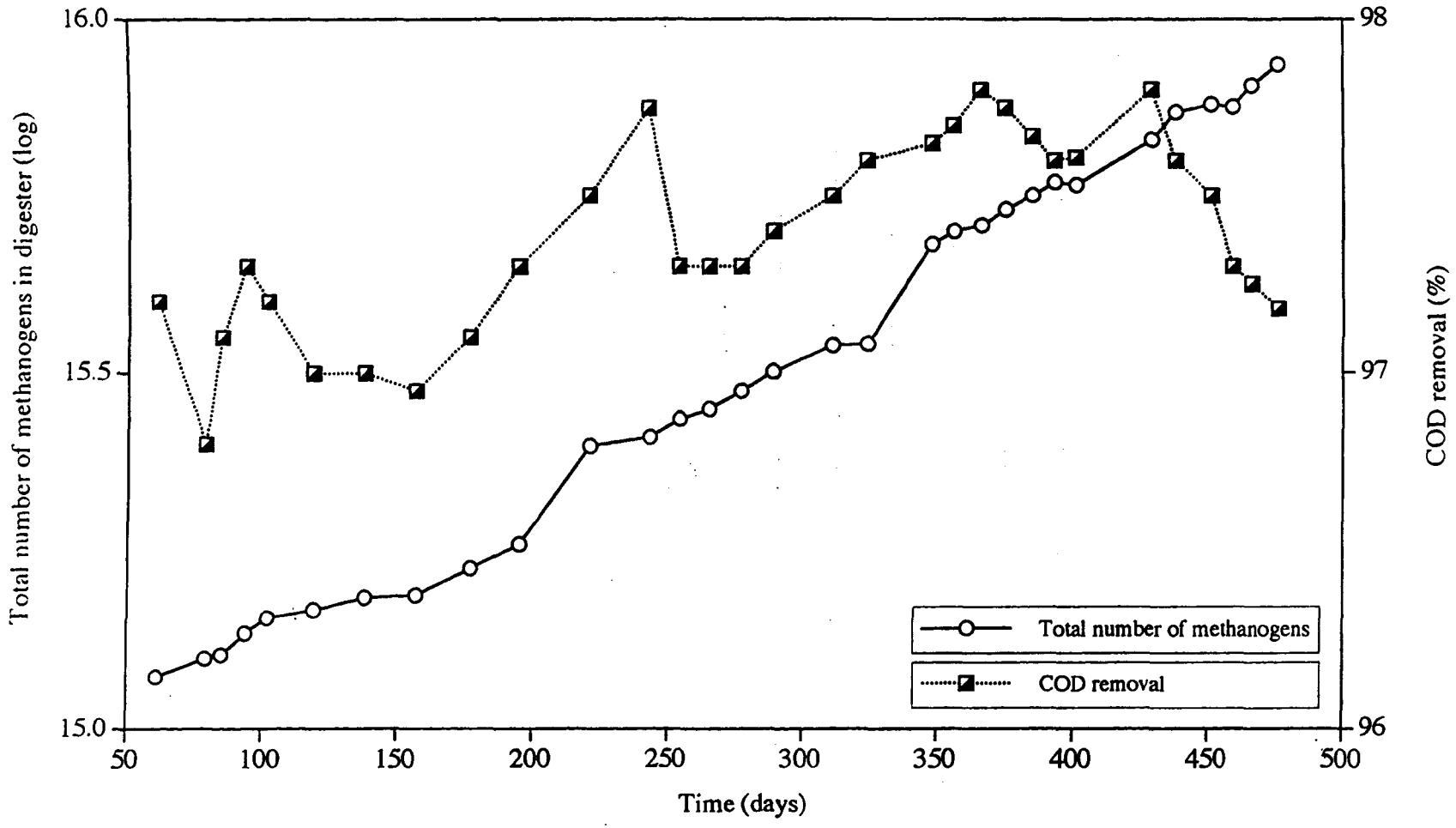


Figure 8.13 Changes in total number of methanogens in digester and COD removal efficiency of system during steady-state operation

Table 8.4 Most Probable Numbers of methanogens showing 95% confidence limits during steady-state operation

Operation Time (d)	Methanogens (No.per.mg VSS)	95% Confidence Limit	
		Lower	Higher
61	$2.0x10^7$	$5.2x10^6$	$6.4x10^7$
79	$2.4x10^7$	$7.7x10^6$	$7.3x10^7$
85	$2.7x10^7$	$9.6x10^6$	$7.8x10^7$
94	$2.8x10^7$	$9.3x10^6$	$7.8x10^7$
102	$3.0x10^7$	$1.0x10^7$	$8.6x10^7$
119	$3.8x10^7$	$1.3x10^7$	$9.9x10^7$
138	$4.3x10^7$	$1.4x10^7$	$1.1x10^8$
157	$5.3x10^7$	$1.7x10^7$	$1.3x10^8$
177	$5.6x10^7$	$1.8x10^7$	$1.4x10^8$
195	$6.3x10^7$	$1.9x10^7$	$1.5x10^8$
221	$5.5x10^7$	$1.6x10^7$	$1.3x10^8$
243	$6.5x10^7$	$1.8x10^7$	$1.5x10^8$
254	$6.7x10^7$	$1.8x10^7$	$1.6x10^8$
265	$8.0x10^7$	$2.0x10^7$	$2.3x10^8$
277	$7.8x10^7$	$2.0x10^7$	$2.3x10^8$
289	$7.9x10^7$	$1.9x10^7$	$2.2x10^8$
311	$9.4x10^7$	$2.9x10^7$	$2.9x10^8$
324	$9.6x10^7$	$2.7x10^7$	$3.0x10^8$
348	$9.3x10^7$	$3.0x10^7$	$2.8x10^8$
356	$8.4x10^7$	2.9E+7	$2.5x10^8$
366	$8.7x10^7$	$2.8x10^7$	$2.6x10^8$
375	$9.3x10^7$	$3.3x10^7$	$2.7x10^8$

Table 8.4 Most Probable Numbers of methanogens showing 95% confidence limits during steady-state operation (continued)

Operation Time (d)	Methanogens (No.per.mg VSS)	95% Confidence Limit	
		Lower	Higher
385	$9.1x10^7$	$3.2x10^7$	$2.6x10^8$
398	$9.4x10^7$	$3.1x10^7$	$2.7x10^8$
401	$8.8x10^7$	$3.1x10^7$	$2.5x10^8$
429	$8.4x10^7$	$2.8x10^7$	$2.4x10^8$
438	$8.3x10^7$	$2.8x10^7$	$2.4x10^8$
451	$8.0x10^7$	$2.7x10^7$	$2.3x10^8$
459	$7.9x10^7$	$2.7x10^7$	$2.3x10^8$
466	$8.9x10^7$	$3.1x10^7$	$2.3x10^8$
476	$8.4x10^7$	$2.9x10^7$	$2.2x10^8$

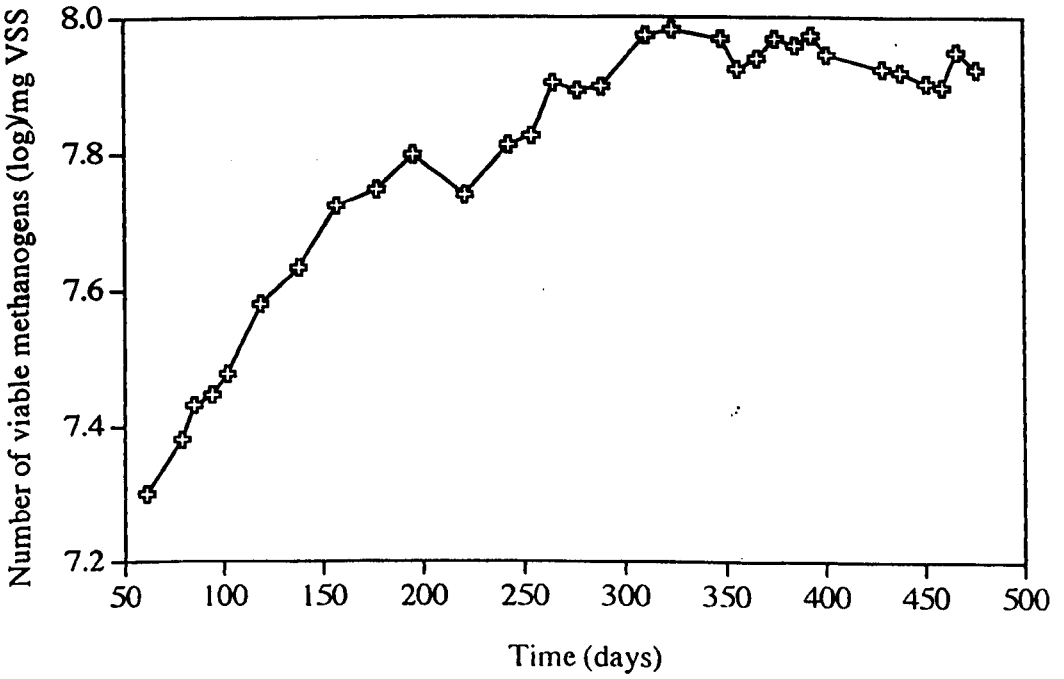


Figure 8.14 Changes in viable methanogens in digester during steady-state operation

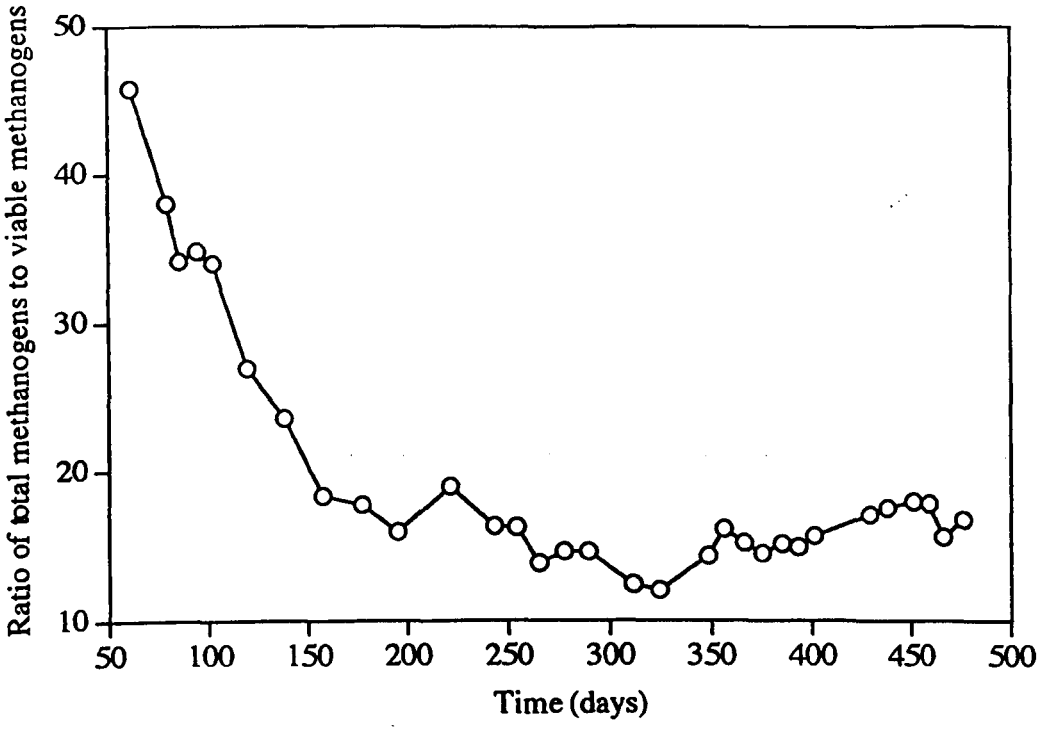


Figure 8.15 Changes in ratio of total methanogens to viable methanogens in digester during steady-state operation

Figure 8.16 shows the changes in the concentration of volatile fatty acids (VFAs) in the reactor throughout the operation. It can be seen from Figure 8.16 that VFAs were found to be less than 40 mg/l up to an OLR of 11 kg COD/m^3.d (on day 311) after which significant increases in the concentration of VFAs were observed reaching 200 mg/l towards the end of the study. The increases in the concentration of VFAs in the reactor might have been a reason for the significant increases in the number of *Methanosarcina* species after an OLR of 12 kg COD/m^3.d and which can be seen in Figure 8.10.

8.3 Comparison of Microbial Population in Two Different Anaerobic Reactors

The treatment capacity of any anaerobic system is primarily determined by sufficient

numbers of active methanogens being retained within the system, which in turn is influenced by the configuration and operation of the reactor. A comparison between the numbers of bacteria and their composition in a pilot-scale, crossflow ultrafiltration membrane anaerobic contact reactor system (CUMAR) and a laboratory-scale, two-stage anaerobic digestion system (TSAD) was therefore made during a 12 week operation period.

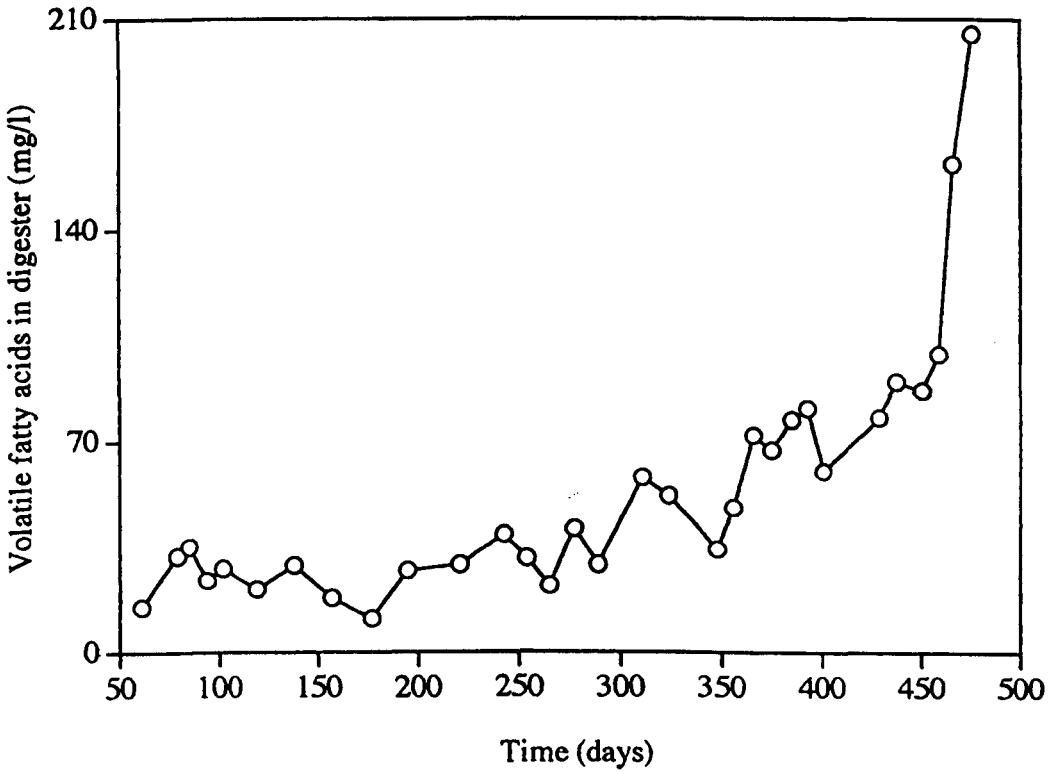


Figure 8.16 Changes in concentration of volatile fatty acids in digester during steady-state operation

8.3.1.Two-Stage Anaerobic Digestion System (TSAD)

The laboratory-scale, two-phase anaerobic treatment system consisted of a continuous stirred tank reactor (CSTR) as the pre-acidification reactor and an upflow filter as the methanogenic reactor (UFAF) and is shown in Figure 8.17.

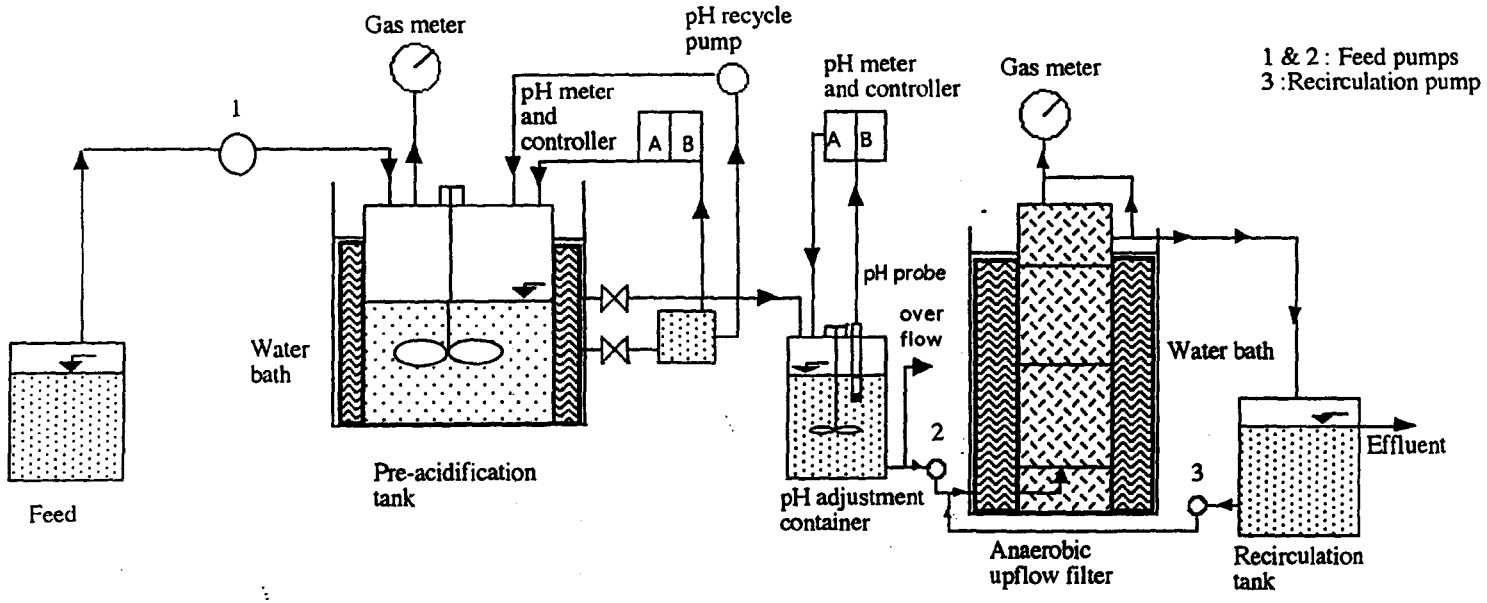


Figure 8.17 Schematic diagram of a two-stage anaerobic digestion system

The liquid volume of the CSTR was 10 litres but could be increased to a maximum of 15 litres. Hydraulic retention times (HRT) were maintained constant at approximately 0.5 day in the pre-acidification reactor and 1.5 days in the upflow filter throughout the start-up period. The two reactors were located in a water bath maintained at 35±2 °C using a thermostatically controlled water recirculation system. The pre-acidification reactor was equipped with a pH probe and a stirrer to provide good mixing while pH was controlled within the range of 5.0 - 5.5 by the automatic addition of 3N HCl, whereas that of the UFAF was controlled within the range 7.2-7.5 by addition of 0.5 N NaOH.

The drain line at the bottom of the upflow filter was connected to the recirculation line in order to increase the methanogenic activity by recirculating the sludge present below the influent port (which was rich in methanogenic bacteria) back into the filter.

8.3.2. Feed and Seed

Wastewater from a milk bottling plant was used as feed throughout the study of the TSAD. The chemical characteristics of the wastewater from a milk bottling factory were presented in Table 8.5. In order to establish an active anaerobic bacterial population both systems were seeded with digesting sludge taken from the same source, a primary sludge digester at a local domestic wastewater treatment plant.

8.3.3. Operation and Performance of CUMAR

The operation and performance of the CUMAR system have been widely discussed in Chapter 5.

Table 8.5. Chemical characteristics of dairy wastewater used

Parameter	Concentration (mg/l)
COD	2000 - 6000
BOD_{ATU}	1200 - 4000
Suspended Solids	350 - 1000
Volatile Suspended Solids	330 - 940
PO_4-P	20 - 50
TKN	50 - 60
Alkalinity (as $CaCO_3$)	150 - 300
Total Fattty Matter	300 - 500
Sodium	170 - 200
Potassium	35 - 40
Calcium	35 - 40
Magnesium	5 - 8
Ferrous	2 - 5
Cobalt	0.05 - 0.15
Nickel	0.50 - 1.00
Manganese	0.02 - 0.10
pH (units)	8 - 11

8.3.4. Operation and Performance of TSAD

During the start-up period the two-stage anaerobic digestion system was continuously fed with diluted wastewater at a strength of 500 mg/l COD to give an OLR of approximately 1kg COD / m^3.d in the pre-acidification stage and approximately 0.3 kg

COD / m^3.d in the upflow filter. During the start-up period, the OLR was increased to 5 kg COD / m^3.d in the pre-acidification reactor and 1.5 kg COD /m^3.d in the upflow filter. After establishing satisfactory operation the OLR was increased to 9.6 kg COD/m^3.d in the pre-acidification reactor and 3 kg COD / m^3.d in the upflow filter. This was achieved after 12 weeks. COD and BOD_5 removal efficiencies of 85 % and 92 % were obtained at this OLR with the overall performance of the system during this period being given in Figure 8.18. The upflow velocity (UFV) was initially maintained at 5 m/day in order to provide sufficent attachment to the media. It was then increased to 12 m/day and finally 17 m/day at which point the OLR had been increased to 2 kg COD.m^3.d. Figure 8.19 shows the changes in biomass concentration in the pre-acidification reactor and upflow filter at different levels during over 12 weeks of operation.

8.3.5. Evaluation of the Results

Figures 8.1 , 8.9 and 8.20 show the changes in the number of methanogens and non-methanogens in the two systems over the 12 weeks operation with the variations in morphology of methanogens being shown in Figures 8.2 , 8.10 , 8.21a and 8.21b. During the operation the percentage of total numbers of autofluorescent methanogens in the total population remained in between 6.5-8.5% in the crossflow ultrafiltration reactor, 1-9% in the upflow filter (at different depths) and 0.01-1.0 % in the pre-acidification reactor.

The MPN technique was also used to determine the numbers of viable methanogens in two-stage anaerobic system. The results are given in Tables 8.6a-d along with the 95% confidence limits while the trend can also be seen in Figure 8.22. Throughout the study, the number of viable methanogens remained fairly constant at about 10^5-10^6 /mg VSS

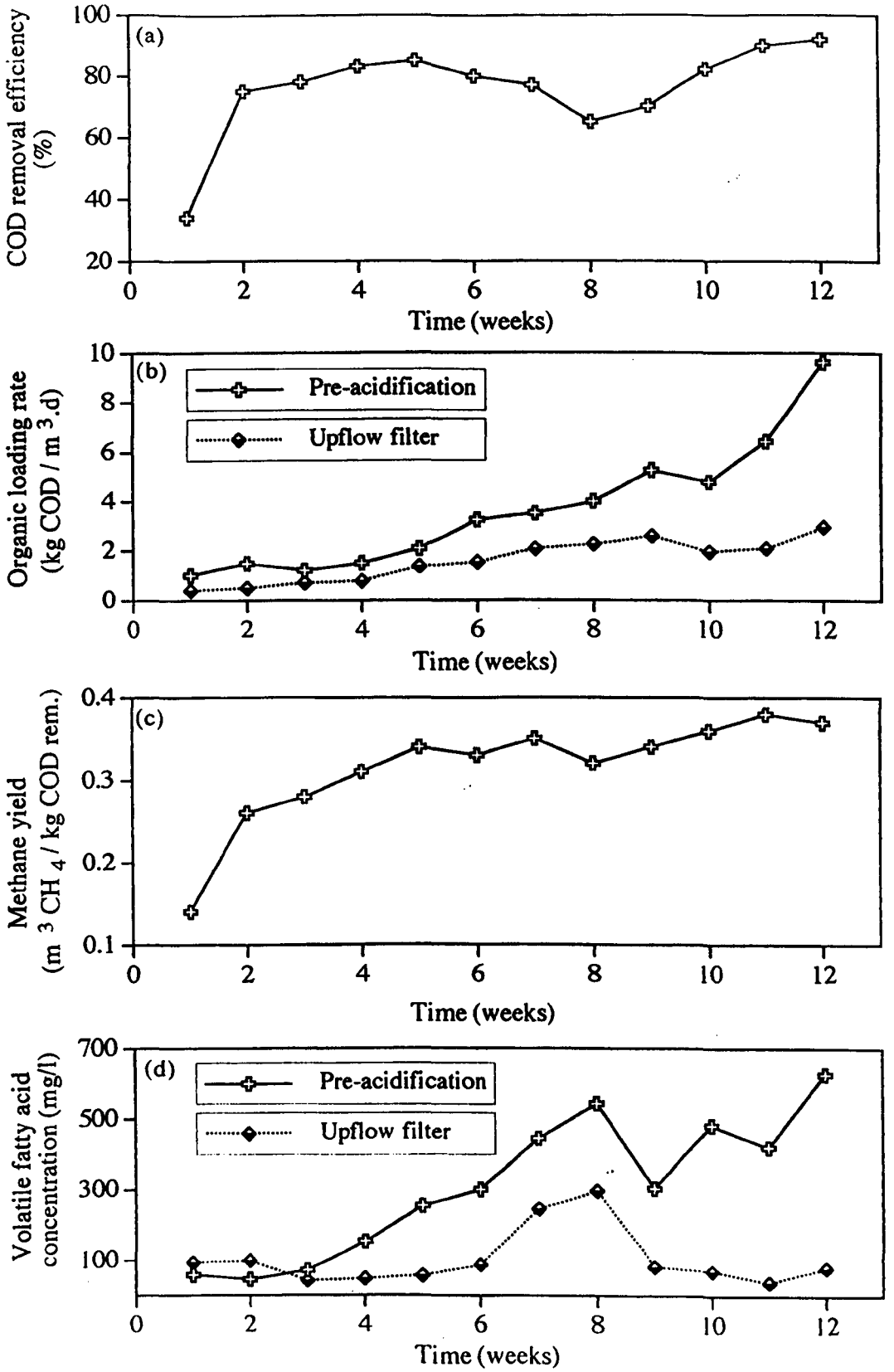


Figure 8.18. Performance of TSAD (a) Overall COD removal (%). (b) Organic Loading Rate (kg COD/m^3.d). (c)Methane Yield (m^3 CH_4/kg COD removed). (d) VFA concentration (mg/l)

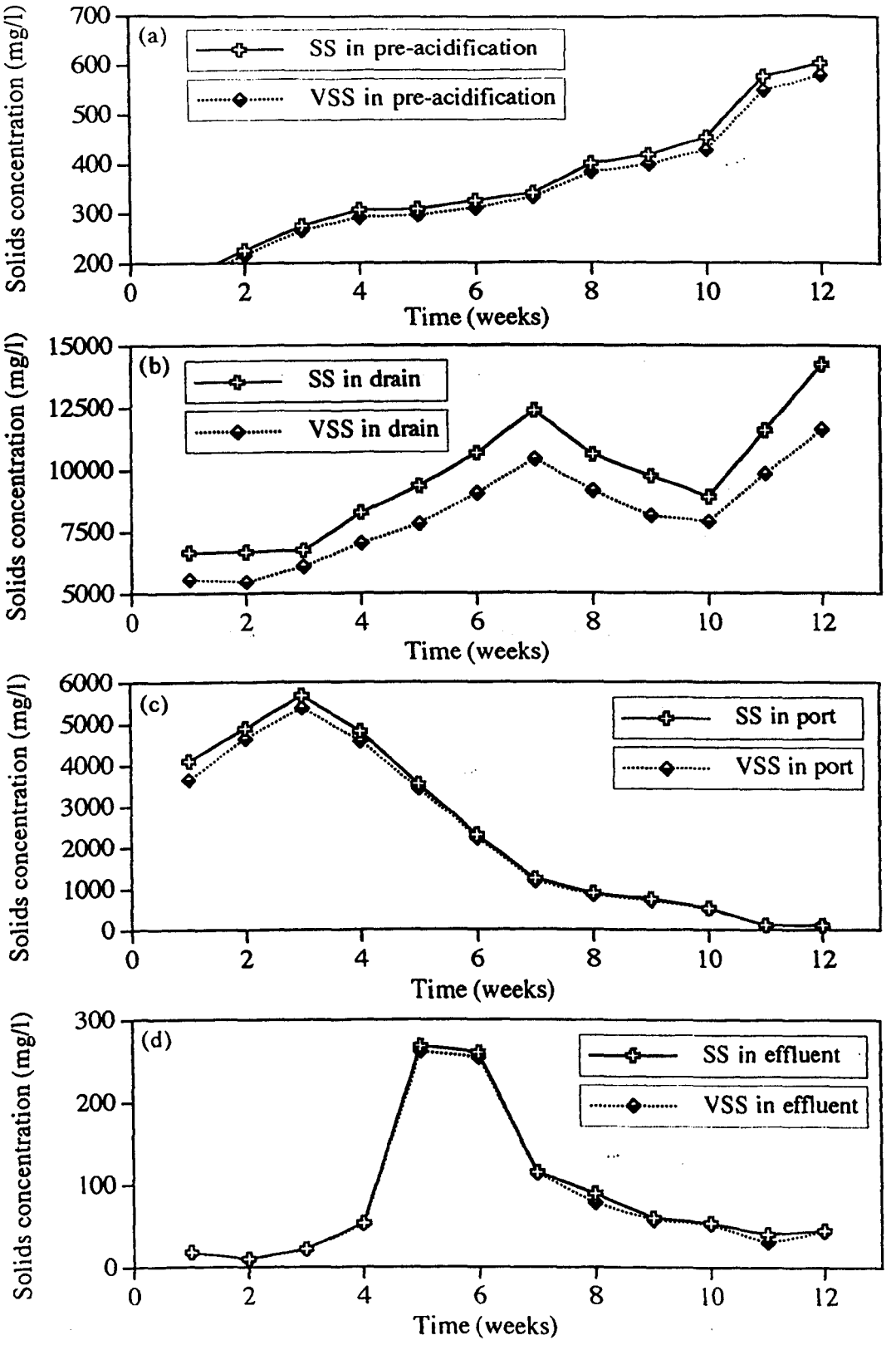


Figure 8.19. Suspended solid (SS) and volatile suspended solid (VSS) concentration (mg/l) in the pre-acidification reactor and upflow filter

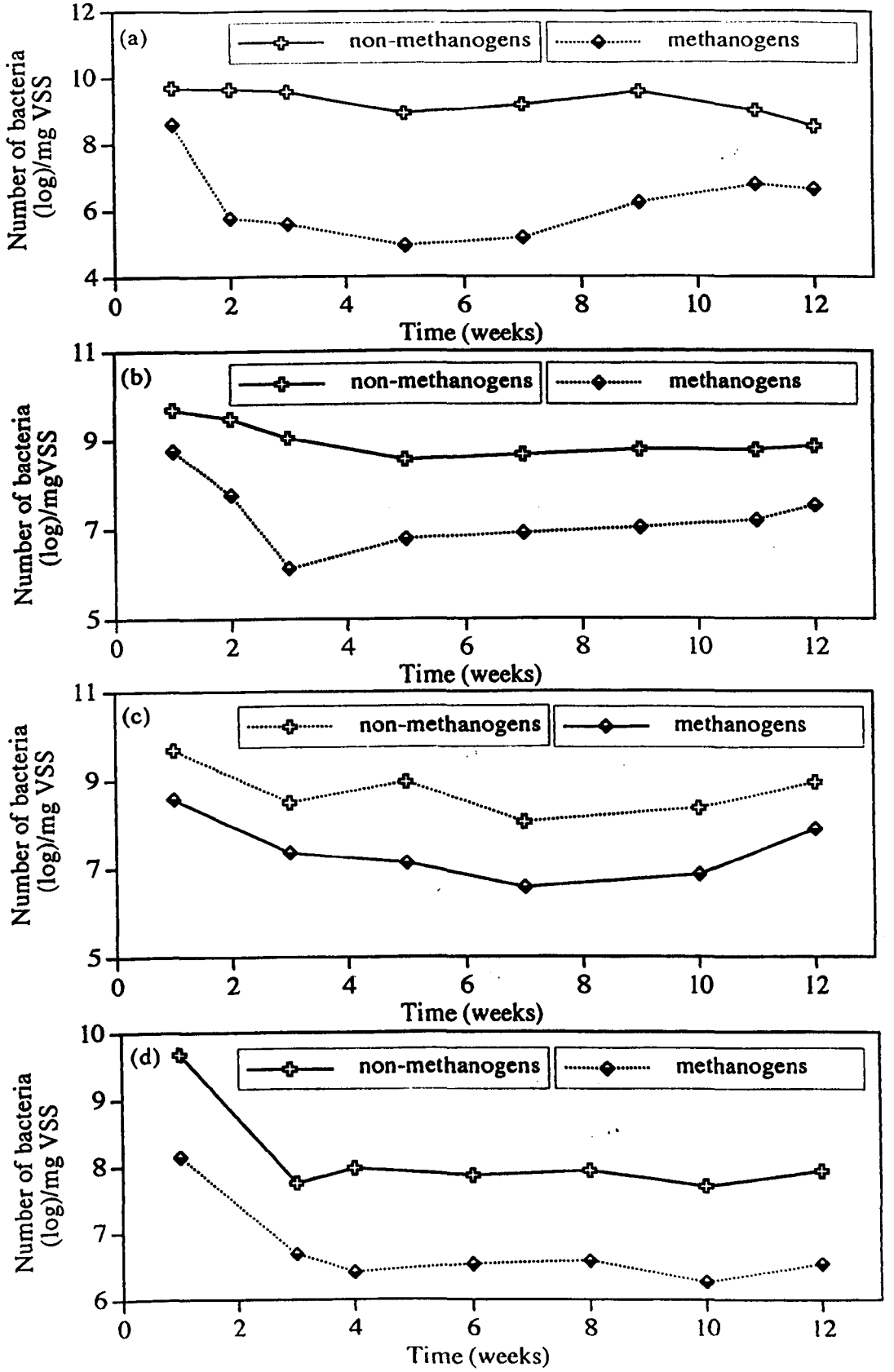


Figure 8.20. Changes in number of methanogens and non-methanogens in TSAD (a) Pre-acidification (b) Drain (c) Port (d) Effluent

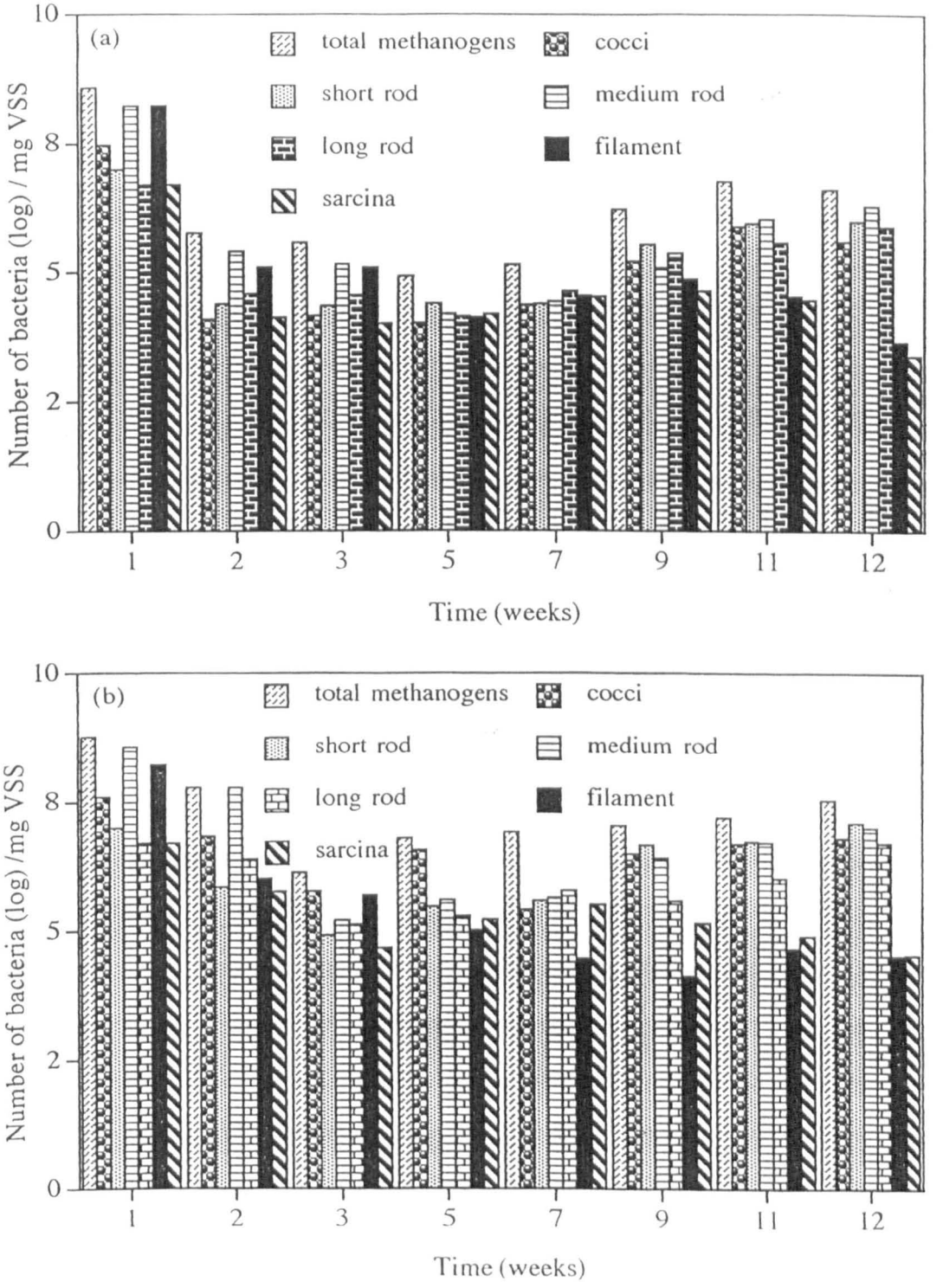


Figure 8.21a Changes in morphology of methanogens in TSAD (a) Pre-acidification (b) Drain

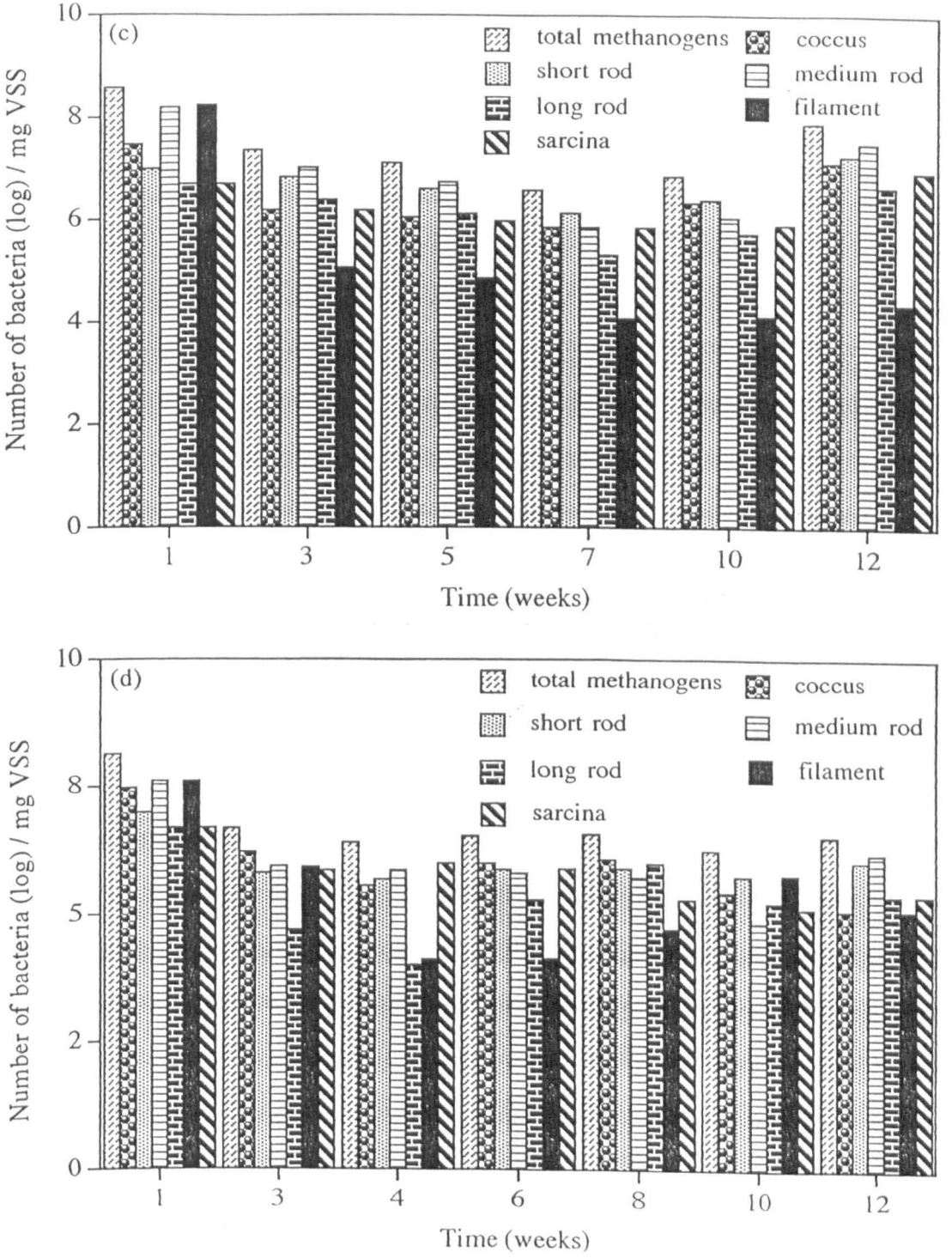


Figure 8.21b Changes in morphology of methanogens in TSAD (c) Port (d) Effluent

Table 8.6a. Most Probable Numbers of methanogens in pre-acidification reactor showing 95% confidence limits.

Week No.	Methanogens (No.per.mg VSS)	95% Confidence Limit Lower	Higher
1	1.7×10^7	5.5×10^6	5.2×10^7
2	6.5×10^3	1.7×10^3	1.6×10^4
3	2.5×10^4	7.4×10^3	5.8×10^4
5	6.1×10^3	1.5×10^3	1.7×10^4
7	1.0×10^4	3.6×10^3	3.0×10^4
9	1.4×10^5	4.5×10^4	3.5×10^5
11	5.1×10^5	1.6×10^5	1.5×10^6
12	6.0×10^5	2.1×10^5	1.7×10^6

Table 8.6b. Most Probable Numbers of methanogens in drain of upflow filter showing 95% confidence limits.

Week No.	Methanogens (No.per.mg VSS)	95% Confidence Limit Lower	Higher
1	1.4×10^7	4.5×10^6	3.4×10^7
2	5.1×10^6	1.6×10^6	1.5×10^7
3	1.0×10^5	3.3×10^4	2.4×10^5
5	6.3×10^5	2.1×10^5	1.6×10^6
7	6.7×10^5	2.2×10^5	1.6×10^6
9	4.3×10^5	1.5×10^5	1.2×10^6
11	1.4×10^6	3.7×10^5	3.4×10^6
12	2.4×10^6	7.7×10^5	7.3×10^6

Table 8.6c Most Probable Numbers of methanogens in effluent port of upflow filter showing 95% confidence limits.

Week No.	Methanogens (No. per. mg VSS)	95% Confidence Limit Lower	Higher
1	1.4×10^6	4.0×10^5	4.4×10^6
3	1.6×10^5	5.2×10^4	4.4×10^5
4	1.5×10^5	4.8×10^4	3.6×10^5
6	1.7×10^5	5.8×10^4	4.8×10^5
8	2.2×10^5	5.5×10^4	6.3×10^5
10	5.4×10^4	1.7×10^4	1.6×10^5
12	5.1×10^4	1.3×10^4	1.6×10^5

Table 8.6d. Most Probable Numbers of methanogens in mid-height port of upflow filter showing 95% confidence limits.

Week No.	Methanogens No.per.mg VSS)	95% Confidence Limit Lower	Higher
1	1.5×10^6	4.9×10^5	3.8×10^6
3	1.5×10^6	4.6×10^5	3.5×10^6
5	7.0×10^5	2.0×10^5	2.2×10^6
7	2.4×10^5	7.7×10^4	7.2×10^5
10	4.3×10^5	1.1×10^5	1.4×10^6
12	8.5×10^5	2.5×10^5	2.0×10^6

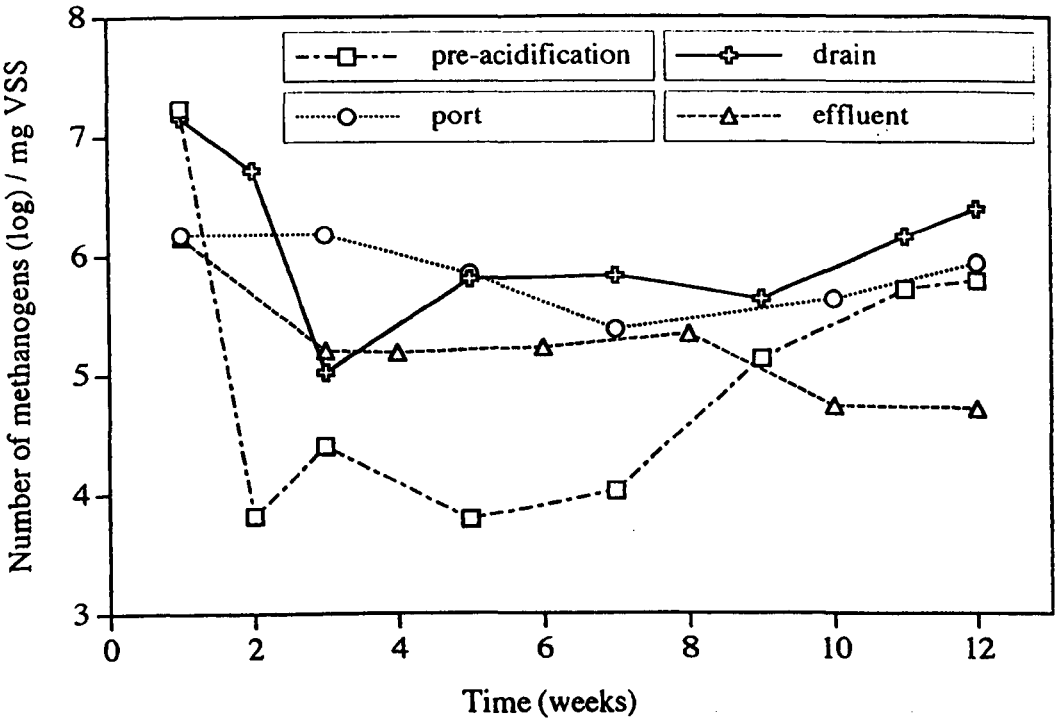


Figure 8.22 Changes in number of viable methanogens in pre-acidification and upflow filter

in the upflow filter samples taken from the drain and from the port (Table 8.7) but in the effluent samples this figure varied at around 10^5 /mg VSS depending on the biomass washout. In the pre-acidification reactor the number of viable methanogens was fairly constant at about 10^4 /mg VSS whereas in the CUMAR system the MPN was found to be in the range of 10^6 -10^7 /mg VSS.

8.4 Discussion

The significance and advantages of membrane systems have received considerable recognition in recent years. This has encouraged intensive research activity concerned with various aspects of the process, however changes in the microbial population of the

system have not yet been investigated. This study therefore, was carried out to determine the effects of a newly developed membrane system configuration on the species composition in a digester during both the start-up period and its steady-state operation.

Table 8.7. Design specifications of two-stage anaerobic digestion system

Reactor	Working Volume (l)	Dimensions diameter x length (mm)	Sample Port	Height from base (mm)
Pre-acidification	10	280 (inner ø) conical bottom 30 cm (side wall)	effluent	150
Upflow filter	30	20 x 100	drain port effluent	20 500 900

A microbial population which is able to respond more rapidly to increased organic loading rates will result in a more efficient digester performance in terms of gas yield and effluent quality. Microscopic examination of the seed sludge has shown that a wide range of methanogens was present, namely, rods, filaments, cocci and sarcina shaped species and throughout the study COD removal efficiencies, methane concentration and methane production gradually increased up to the anticipated values. When the organic loading rate was almost doubled to an OLR of 1.5 kg COD/m^3.d the efficiency of the CUMAR system was not adversely affected and soon after increased to over 95 %. This may be explained not only because of the existence of satisfactory microbial population but also the stability of the membrane system under load changes.

High treatment efficiencies for a wide range of wastes may be achieved as long as a sufficient quantity of active methanogens exists in the digester. As a result, the identification of methanogenic species as a control parameter of a digester is becoming increasingly attractive. For instance, any deterioration in the performance of a digester may have been due to the change in the dominant species or a decrease in the quantity of active methanogens.

As discussed in Chapter 7 there are a number of different methods for determining the numbers and activities of a methanogenic population, including microscopic count, most probable number (MPN), ATP, coenzyme F_{420}, dehydrogenic activity and specific methanogenic activity (SMA) under controlled conditions. The methods which have been given above, have a number of limitations and difficulties.

The microscopic technique, which is rapid and more reliable than the other methods, was used to determine the morphological changes in the methanogenic species and to count the number of methanogens and total bacteria. Some types of methanogens however, do not fluoresce under UV light. Dolfing *et al.* (1985) also pointed out that 20-30 % of methanogenic bacteria, such as *Methanotrix*, do not exhibit fluoresence.

Studies by other research workers including Morgan (1991) have shown that the proportion of methanogens in a total anaerobic bacterial population may vary from 1 to 10%. In this study the maximum ratio of methanogens to total bacteria was found to be 9.5%. The numbers of methanogens counted using direct microscopic techniques however, do not completely reflect the methanogenic activity in the digester. Another disadvantage of this technique is that the viable and non-viable methanogens cannot be distinguished from each other, and as a result, the MPN technique was used to count viable methanogens, although it has been reported (Dubourguier et al., 1988) that the numbers of viable methanogens determined using MPN is 10-1000 times less than the

numbers resulting from a microscopic count. One of the reasons for this lies in the MPN technique itself because only three different methanogenic groups are identified by this technique, namely *Methanosarcina* , *Methanobacterium formicicum* and *Methanococcus* species. In this study the ratio of the number of viable methanogens to the number obtained by microscopic count was initially 1:400 but becoming 1:12 towards the end of the study.

An earlier study by Morgan *et al.* (1991) claimed that the design of the reactor influences microbial selection less than factors which directly influence the physiology of the cells such as reactor environment which is subjected to the simultaneous influence of many variable parameters and the slight variations in the bacterial numbers and composition between reactors were probably associated with sampling techniques and enumeration methods. It was concluded that bacterial composition of the anaerobic biomass was influenced by composition of the wastewater rather than the reactor design. The results obtained from this laboratory study however showed that microbial ecology is very much influenced by both composition of the wastewater and configuration of the system .

The treatment capacity of any anaerobic digester is primarily determined by the active biomass retained in the system, either in suspension or by attachment. Another study was also carried out in order to compare the changes in the morphology of methanogenic bacteria in the two different anaerobic systems. Variations in the numbers and composition of the methanogenic and non-methanogenic bacteria were found in both systems .

For over 12 weeks of operation, both systems described in Sections 8.3.2 and 8.3.3 were very stable up to OLRs of 2.5 kg COD /m^3.d in the crossflow ultrafiltration reactor , 9.6 kg COD/ m^3.d in the pre-acidification reactor and 3 kg COD / m^3.d in the

upflow filter. These excellent performances may be explained by the fact that bacterial retention in the two systems was adequate. The development of a biofilm on the support media in the upflow filter should also be considered, however difficulties in obtaining samples of media prevented such a study. Since there is no biomass washout in the crossflow ultrafiltration system an excellent quality effluent was produced, eventually reaching over 98% and 99% COD and BOD removal efficiencies at an OLR of 2.5 kg COD / m^3.d at which that was at a hydraulic retention time of approximately 2.7 days. The TSAD system performed well at OLRs of up to 9.6 kg COD / m^3.d with no signs of instability in either of the reactors with 85% and 92% COD and BOD removal efficiencies being achieved at this OLR. The ratio of the number of viable methanogens to the number obtained by microscopic count was initially 1:400 , becoming 1:10 after 12 weeks operation of the CUMAR system while in the TSAD system at start -up this ratio was 1:100, 1:100, 1:200 and 1:1000 in the pre-acidification reactor, the drain, the port and the effluent respectively, becoming 1:5 in the pre-acidification reactor , 1:10 in the drain, 1:100 in the port and 1:50 in the effluent after 12 weeks operation.

CHAPTER 9

SUMMARY

In the first stage of this study the development of a new method of operating a completely mixed anaerobic digester using a crossflow membrane ultrafiltration unit for biomass retention and for the production of a clear final effluent was investigated and the following summarizes the individual discussion of the various aspects of the study:

(i) the results obtained from this study showed that the CUMAR system had not reached its maximum capacity. Overall COD and BOD removal efficiencies of 99% and almost 99% were achieved respectively at an OLR of 28.5 kg COD/m^3.d at a hydraulic retention time of 4.2 days.

(ii) based upon the SMA tests, MPN , microscopic count and plate count results almost 100% of the bacterial population was recovered by means of the UF membrane which in turn allowed higher digester loading rates compared to conventional anaerobic digesters,

(iii) the system demonstrated its capacity to positively control both the solid retention time and hydraulic retention time,

(iv) during the operation period, no significant adverse effects on the treatment capacity were encountered at high MLSS concentrations,

(v) the UF membrane exhibited a consistent, stable performance over the whole period of the study indicating that membrane fouling by MLSS will reach a limiting level, and

(vi) this newly developed membrane anaerobic digestion process could help to solve the problem of retaining an adequate amount of active biomass in an anaerobic digester, produce a clear, final effluent and achieve a high-rate treatment of high strength wastewaters.

In the second stage of the study the kinetics of the CUMAR system were investigated at different MLSS levels and the following was found:

(vii) the results of the kinetic studies showed that the kinetic coefficients estimated from the four steady-state runs had slight variations from each other which could mainly be due to the changes which took place in the bacterial numbers and the dominant species during the operation of the system,

(viii) the CUMAR system demonstrated the potential for having high biomass concentrations with no adverse effects on its treatment capacity provided the digester sludge could be recirculated and be maintained under good physical conditions (heating, mixing, anaerobic conditions, etc.), and

(ix) the differences in the values of the kinetic coefficients compared to reported values might have been due to the different reactor configurations employed, different seed sludges and different substrates used.

It is important to recognize that the active biomass in a reactor is the critical factor in achieving efficient wastewater treatment. A long sludge retention time, as reflected by the traditional VSS measurements, may not be the best indicator of potential system performance. From the standpoint of the design and operation of anaerobic processes, biomass "activity" is of great importance. In the third stage of this study the biomass "activity" in the CUMAR system was therefore measured and controlled by the use of the SMA test. The following comments summarize the results obtained from both the SMA tests and the system during the start-up and steady-state operations:

(x) both the actual methane production and the potential methane production rates of the system did not proportionally increase with the increases in biomass concentration in the digester after an organic loading rate of approximately 9 kg COD/m^3.d although a linear relationship was observed between organic loading rate and biomass concentration in the digester. However, they (the AMP and the PMP rates) increased almost proportionally with the increase in sludge wastage rates of the system (decrease in sludge retention times). Maintaining high biomass levels in the digester may not be conducive to high acetoclastic methanogenic levels,

(xi) any deterioration in acetoclastic methanogenic capacity of the system can be improved by increasing the sludge wastage rate,

(xii) the maximum actual methane production and potential methane production rates were found to be 110 ml CH_4/g VSS.d and 170 ml CH_4/ g VSS.d in this study while the maximum potential methane production rate of enriched culture cultivated on acetate has been reported to be approximately 1000 ml CH_4/ g VSS.d (Valcke and Verstraete, 1983). This high value was assumed to be the maximum acetoclastic

activity when all the biomass (measured as VSS) consists of acetoclastic methanogens, and

(xiii) the SMA test results showed that the most appropriate use of this technique compared to conventional parameters is its ability to predict the potential organic loading rates which may be applied to anaerobic digestion reactors.

In the final stage of the study the effects of reactor configuration on the dominant bacterial species were investigated and summarized as follows:

(xiv) the ratio of total methanogens to non-methanogens slightly increased throughout the study although the number of viable methanogens sharply increased up to an OLR of 10 kg COD/m^3.d, as did the specific methanogenic activity,

(xv) a slight increase in total methanogens occurred except for *Methanosarcina* species during the start-up period. The decrease in the number of *Methanosarcina* species may have been influenced by the presence of a low concentration of acetic acid in the digester,

(xvi) short rod species were found to be the most dominant group towards the end of study followed by medium rods, *Methanococcus*, *Methanosarcina*, filaments and long rods,

(xvii) within the fluorescent methanogenic population , *Methanosarcina*, *Methanococcus* , *Methanobacterium* and *Methanobrevibacteria* species have been tentatively identified,

(xviii) comparison studies have also showed that system configuration did have an apparent effect on the dominant species during the 12 weeks of operation,

(xix) the comparative study results obtained from microscopic examination of the early stage of start-up showed that *Methanococcus* species were the dominant group followed by *Methanosarcina* species in the CUMAR system whereas in the upflow filter, medium rods and cocci shaped species in the drain, medium and short rod species in the port and medium rod and cocci shaped species in the effluent were found to be the dominant groups. The numbers of filaments and sarcina species noticeably decreased at the three levels in the upflow filter which indicated that they possibly became attached to the biofilm, and

(xx) at a later stage of the study, in the CUMAR system, short and medium rod shaped species became the second dominant group after *Methanococcus* species . On the other hand, in the upflow filter, short rod species in the drain and in the effluent and medium rod species in the port were dominant. The MPN in the effluent of the upflow filter drastically decreased which implies considerably lower biomass washout. As a result, studying the changes in the number of viable methanogens and the dominant species may help to determine a reason for the deterioration in performance of a digester.

CHAPTER 10

CONCLUSIONS AND RECOMMENDATIONS

10.1 Conclusions

The major objective of this project has been to the control of biomass in anaerobic reactors using ultrafiltration membranes. The following summarize the conclusions drawn from the investigation:

(i) the increase in MLSS concentrations did not significantly affect the kinetics of the system,

(ii) the acetoclastic methanogenic bacteria were found to be affected at high biomass levels in the digester especially at a MLVSS concentration in excess of 25000 mg/l.

(iii) ratios of actual methane production rate to potential methane production rate of less than 0.7 were found to be satisfactory in order to run the system efficiently in terms of COD removal and methane yield,

(iv) the membrane system configuration had an apparent effect on the dominant methanogenic species throughout the operation of the CUMAR system,

(v) the treatment of brewery wastewater by the CUMAR system, at pilot-scale, showed that 99% COD and almost 100% BOD removal efficiencies were possible at an OLR of 28.5 kg COD/ m^3.d at a hydraulic retention time of 4.2 days,

(vi) the UF membrane almost completely prevented any biomass loss in the permeate, resulting in excellent biomass separation and increased digester loading rates compared to conventional anaerobic digesters,

(vii) the system demonstrated its capacity to positively control both the SRT and HRT,

(viii) the UF membrane exhibited a consistent, stable performance over the whole period of the study indicating that membrane fouling by MLSS will reach a limiting level, and

(ix) the CUMAR system demonstrated the capability of having high biomass concentrations with no adverse effects on its treatment capacity.

10.2 Recommendations For Further Works

(i) the crossflow ultrafiltration membrane anaerobic reactor system is a new technology and does not have a long track record for full-scale application. For this reason, there are many questions which cannot be answered at this stage of its development. To date, succesful pilot-scale and prototype studies have been carried out on brewery, wine distillery, malting, egg processing, chemical-processing, fruit-processing, and maize-processing effluents,

(ii) no long-term predictions can yet be made on the stability of the membrane flux and the membrane system life. An advantage of the membrane reactor, as compared to other industrial membrane applications, is that digestion occurs at neutral pH, at lower pressures (450 kPa) and temperatures (35 °C) and with a minimal chemical cleaning regime and hence with less detriment to the membranes or the support structures. High membrane flux and long membrane life naturally will have a direct bearing on process costs,

(iii) the membrane reactor cannot be advocated for the treatment of every type of industrial effluent. The organic industrial effluents investigated to date originated mainly from the food and beverage industries and were typically soluble or collodial in nature with fairly high biodegradable COD concentrations. Effluents containing recalcitrant components that do not respond well to anaerobic digestion will cause fouling of the membranes. Each effluent has unique chemical and physical characteristics and pilot-scale studies are advocated to establish the feasibility of the treatment process and to determine the rheological properties of the MLSS for membrane design purposes,

(iv) the effect of higher loading rates than those applied in this study should be investigated and the treatment efficiency of the process should also be analyzed, and

(v) a cost comparison study between the new membrane anaerobic process and other current anaerobic processes is required.

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Appendix 4.1

DESIGN SPECIFICATIONS FOR 120 LITRE ANAEROBIC CONTACT REACTOR

Internal diameter :	500 mm
External diameter :	520 mm
Height :	700 mm
Flange diameter :	640 mm
Diameter of raised section on reactor cover :	300 mm
Height of raised section of reactor cover :	10 mm
Height of skid :	50 mm
Material of construction :	PVC

CONNECTIONS / ATTACHMENTS

V1 : Gas outlet (+GF+Type 342 16 mm)

V2 : Feed (+GF+Type 342 20 mm)

V3 : Connection for pH probe (+GF+Type 342 16 mm)

V4 : Hot water outlet (+GF+Type 342 16 mm)

V5 : Hot water inlet (+GF+Type 342 16 mm)

V6 : Overflow (+GF+Type 342 20 mm)

V7 : Connection for filtration unit (+GF+Type 342 20 mm)

V8 : Connection for filtration unit (+GF+Type 342 20 mm)

V9 : Drain (+GF+Type 342 32 mm)

T1 : Connection for temperature probe

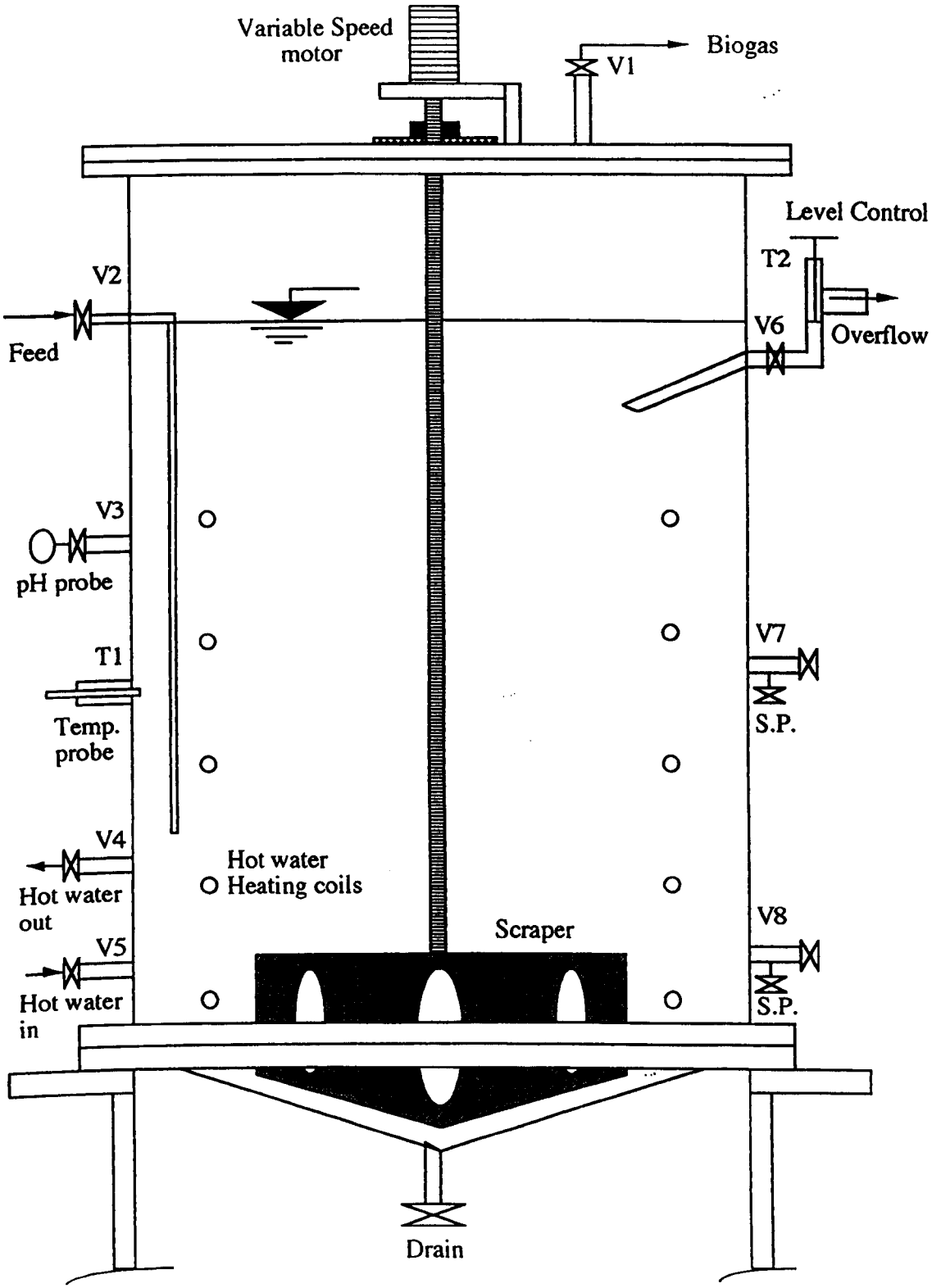
T2 : Connection for level controller (50 mm diameter)

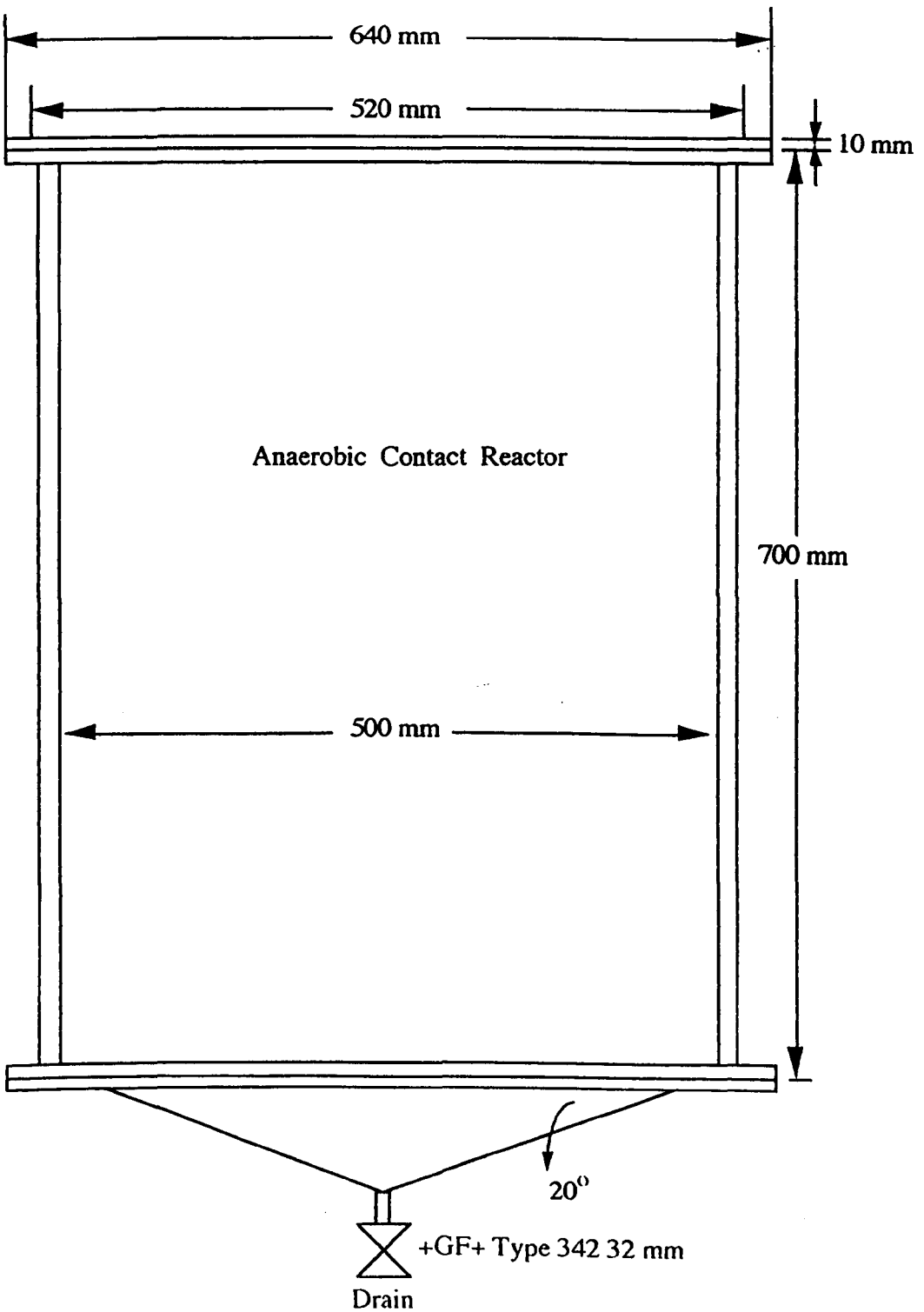
ANCILLARY EQUIPMENT

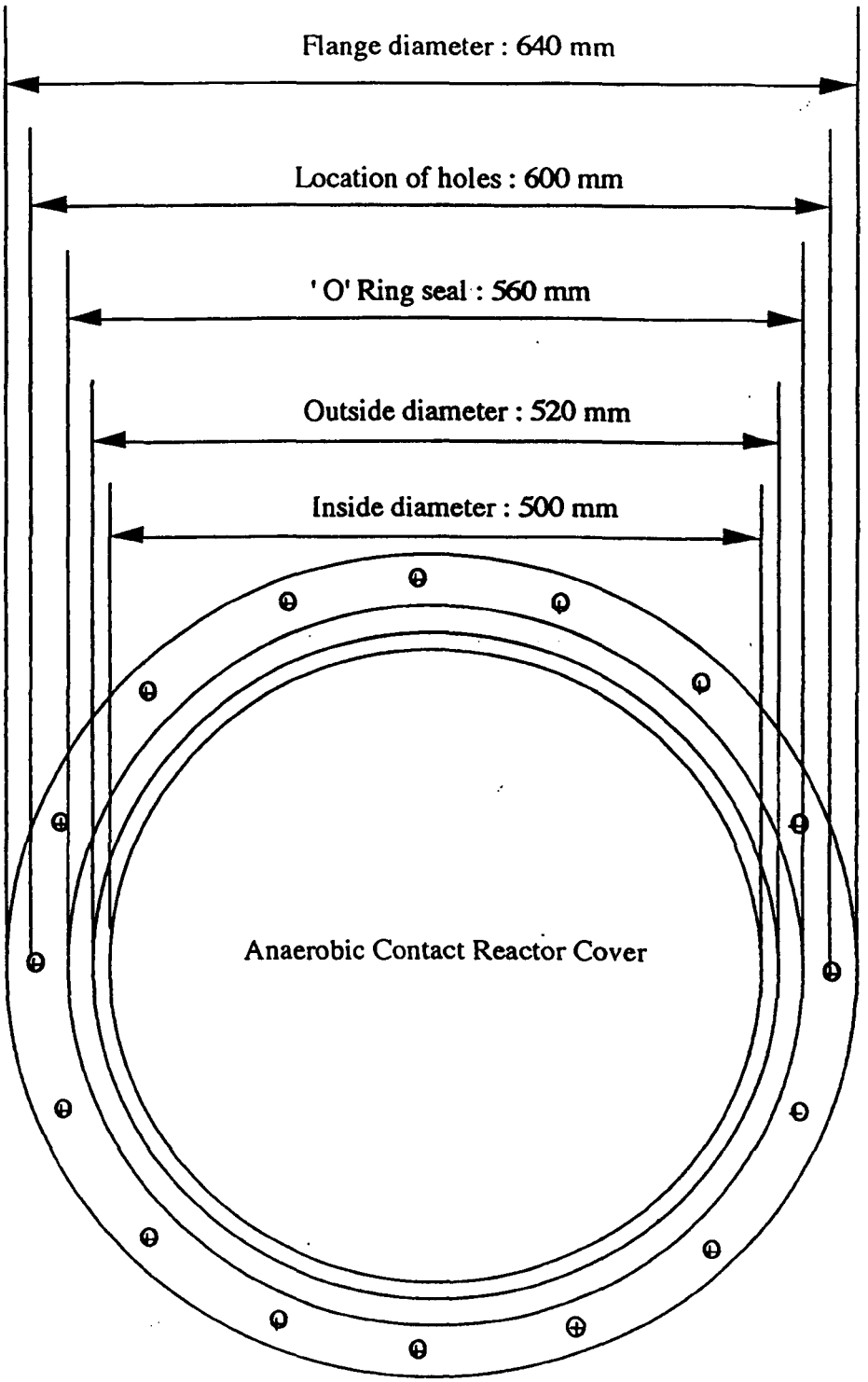
1 x 1/8 hp variable speed motor for scraper

1 x Anchor scraper (lightweight)

1 x Oil gland seal for scraper


Variable Speed motor
Biogas
V1
Level Control
T2
V2
Feed
V6
Overflow
V3
pH probe
T1
Temp. probe
V7
S.P.
V4
Hot water out
Hot water
Heating coils
Scraper
V8
V5
Hot water in
S.P.
Drain


640 mm
520 mm
10 mm
Anaerobic Contact Reactor
700 mm
500 mm
20°
+GF+ Type 342 32 mm
Drain


Flange diameter : 640 mm
Location of holes : 600 mm
' O' Ring seal : 560 mm
Outside diameter : 520 mm
Inside diameter : 500 mm
Anaerobic Contact Reactor Cover

Appendix 4.2

MEMBRANE CLEANING

A chemical solution recommended by the UF manufacturer, Peterson Candy International was used to clean the membranes. After the membranes were taken off they were flushed with tap water then put into the chemical solution which contained the following chemicals;

Cleaning solution = 2 g NaOH pellets + 5 ml sodium hypochloride to a litre

Residual chlorine levels were maintained no higher than 300 mg/l in order to prevent any deterioration on the membrane structure.

Membrane spares were stored horizontally in a storage area at a temperature of +4 ^{o}C. The sealed plastic tube were kept closed until immediately prior to use.

Cleaning with back-washing was not carried out since the earlier studies in this department showed that this technique causes membrane tube collapse.

Appendix 4.3

GENERAL MEDIA FOR METHANOGENS

Yeast extract	3000 mg
Sodium formate	1200 mg
Sodium acetate	1200 mg
Mineral solution I	15 ml
Mineral solution II	15 ml
Trace mineral solution	6 ml
Vitamins (Balch)	6 ml
Ferrous sulphate (1 % w/v)	0.6 ml
Resazurin (0.01 % w/v)	0.6 ml
Reducing solution	9 ml
Sodium carbonate (8 % w/v)	15 ml

Make up to 600 ml with distilled water (1 l boiled down to 600 ml should have negligible dissolved oxygen). Adjust the pH to 6.8 using concentrated HCl or NaOH. Replace headspace gas with 20 % CO_2 and 80 % N_2 then autoclave (121°C , 103.5 kN / m^2, for 20 min.)

MINERAL SOLUTION I

Dipotassium hydrogen orthophosphate	0.6 (w/v)
Distilled water	1 litre

MINERAL SOLUTION II

Potassium dihydrogen orthophosphate	6000 mg
Ammonium Sulphate	12000 mg
Sodium chloride	12000 mg
Calcium chloride	2400 mg
Magnesium sulphate	2500 mg
Distilled water	1000 mililitre

Dissolve ingredients separately before making up to 1 litre flushing oxygen out by 80% nitrogen and 20% carbon dioxide carrier gas throughout the procedure. Store at 4 °C.

TRACE MINERAL SOLUTION

Nitrilotricetic acid	1500 mg
Magnesium sulphate	3000 mg
Manganese sulphate	500 mg
Sodium chloride	1000 mg
Ferrous sulphate	100 mg
Cobalt sulphate	100 mg
Calcium chloride	100 mg
Zinc sulphate	100 mg
Copper sulphate	10 mg
Aluminium potassium sulphate	10 mg
Boric acid	10 mg
Sodium molybdate	10 mg
Nickel chloride	10 mg
Sodium selenate	10 mg
Distilled water to	1000 (mililitre)

Dissolve nitrilotriacetic acid separately with potassium hydoxide to pH 6.5, then add the minerals whilst flushing oxygen out by 80% nitrogen and 20% carbondioxide carrier gas. Store at 4 °C.

VITAMINS (BALCH)

D-Biotin	2.0 mg
Folic acid	2.0 mg
Pyridoxine hydrochlorie	10.0 mg
Thiamine hyrochloride	5.0 mg
Riboflavin	5.0 mg
Nicotinic acid	5.0 mg
D-Pantothenic	5.0 mg
Vitamin B-12	0.1 mg
p-Aminobenzoic acid	5.0 mg
DL-6 8 Thiotic acid	5.0 mg
Distilled water to	1000 mililitre

Dissolve in distilled water and replace head-space gas with oxygen free nitrogen.

REDUCING SOLUTION

Cysteine hydrochloride/sodium sulphide	2.5%

Dissolve 2.5 g cysteine hydrochloride in 40 ml of distilled water. Adjust the pH of the solution to 10 using NaOH pellets. Dissolve 2.5 g sodium sulphide in 40 ml of distilled water. Mix the solutions and make up to 100 ml. Heat and cool under oxygen-free nitrogen gas. Dispense into hungate tubes. Store at 4 °C until required.

Appendix 4.4

1/4 strength Ringer Solution Tablets

Code : BR52

Compounds	mg/l
Sodium chloride	2250
Potassium chloride	105
Calcium chloride $6H_2O$	120
Sodium bicarbonate	50
pH	7.0

To prepare quarter-strength Ringer solution, dissolve 1 tablet on 500 ml of distilled water. Sterilize by autoclaving at 121 C for 15 minutes.

Appendix 4.5

Reinforced Clostridial Agar

Compounds	Concentration (g/l)
Yeast extract	3
'Lab-Lemco' powder	10
Peptone	10
Glucose	5
Soluble strach	1
Sodium chloride	5
Sodium acetate	3
Cysteine hydrochloride	15
Agar	15

Adjust the pH to 6.8 using concentrated HCl or NaOH.

Appendix 5

Time (days)	Influent COD (mg/l)	Effluent COD (mg/l)	Reactor COD (mg/l)	Hydraulic retention time (days)	Organic loading rate (kg COD/m^3.d)
1	2500			2.5	1
3		2000	2150	2.5	
5	2350	1725	1860	2.7	0.9
8	1780	1060	1160	2.8	0.6
12	2050	725	1030	2.8	0.7
17	2060	650	930	3	0.7
21	2060	475	730	3	0.7
24		365	525	3.1	
26	2200	290	500	3.2	0.7
28	2220	270	400	3	0.7
30	4800	200	1350	3.2	1.5
33	3250	600	550	3.1	1
37	2800	300	125	2.8	1
40	2600	57	100	2.7	1
42	2500	35	110	2.5	1
45	2550	35	110	2.5	1
46	4180	35		2.5	1.7
47		60	140	2.5	
48		55	150	2.5	
49	4120		140	2.5	1.7
50	4180	50	135	2.5	1.7
55	4080	60	150	2.5	1.6
60	4170	50	145	2.5	1.7
61	5350	55	150	2.5	2.2
63		80	185	2.5	
65	5280		170	2.5	2.1
70	5310	67		2.6	2.1
71	5350	70	165	2.6	2.1
75	5300		165	2.6	2.1
79	5310	60		2.6	2.1
83	5350	65	170	2.6	2.1
84	6690	60		2.6	2.6
85		85	200	2.6	
86		90	185	2.6	
87	6610	80	190	2.6	2.5
88		70	180	2.7	
89	6650		172	2.7	2.5
90	6650	80	180	2.7	2.5
94	6570	70		2.7	2.4
98	6620	80	190	2.7	2.5
102	6630	80	190	2.7	2.5
103	8650		180	2.8	3.1
104		95	285	2.9	
106	8650	100	265	2.9	2.9
109	8670		250	3	2.9

110	8640	80	260	3	2.9
113	8680	90	265	3.1	2.8
116	8640	80		3	2.9
119	8600	85	260	3	2.9
120	10100		270	3	3.3
121		115	355	3.1	
122	10120	100	310	3.1	3.3
124	10160	90		3.1	3.2
126	10180	100	310	3.2	3.2
129	10160	90		3.2	3.2
132	10120	100	300	3.2	3.2
135	10160	90	315	3.2	3.2
138	10140	100	305	3.2	3.2
139	11800		300	3.2	3.6
140		145	365	3.2	
143	11750	125	290	3.3	3.5
145	11600	120	370	3.4	3.5
148	11650	125	360	3.4	3.4
151	11705	130	340	3.4	3.4
154	11660		265	3.4	3.4
157	11680	120	350	3.4	3.4
158	12870		360	2.8	4.6
159		120		2.8	
162	12720	165	390	2.9	4.4
165	12680	150	375	2.9	4.3
168	12700	165		2.9	4.3
171	12650	150	370	3	4.3
174	12680	160	375	2.9	4.3
177	12670	155	370	3	4.3
178	15050		380	3	5
179	14850	180		3	4.9
181	14620	200	420	3.1	4.8
184	14580		385	3.1	4.7
186	14660		390	3.1	4.7
189	14580	190		3.1	4.7
192	14540	170	380	3.1	4.7
195	14560	180	390	3.1	4.7
196	25100		385	3.2	7.9
197		200		3.3	
199	24560	265	1200	3.3	7.4
201	24660	315	960	3.4	7.3
203			810	3.5	
205	24620		760	3.5	7.1
207	26600	330		3.5	7.6
209	26510	260	665	3.5	7.5
211		220	645	3.6	
213	26500	220	660	3.6	7.4
215	26450			3.6	7.4
217	26550	230	660	3.6	7.5
219	26430	210		3.6	7.4
221	26500	220	655	3.6	7.4
223		215	645	3.6	
225	26430	210	660	3.6	7.4
227		200	640	3.6	
229	26410	210	635	3.6	7.3

230	26520	220	625	3.6	7.3
231		215	620	3.6	
232	26480	210	630	3.6	7.3
233	28700	210	640	3.7	7.8
234		220		3.7	
235	28740	260	700	3.6	8
236	28760	280	710	3.5	8.1
237	28710	255	700	3.7	7.8
239	28640	265	700	3.6	8
241	28600	250		3.6	7.9
243	28600	260	700	3.7	7.8
244	31850		715	3.7	8.7
245		265		3.7	
247	31800	300	850	3.7	8.5
248	31600	260	860	3.7	8.4
250		265		3.7	
252	31650	260	850	3.8	8.4
254	31650	270	860	3.7	8.5
255	33520		850	3.7	9
256		310		3.8	
258	33470	300	925	3.8	8.9
259	33400	315	910	3.8	8.8
261		300		3.8	
263	33440	300	920	3.8	8.8
265	33460	310	900	3.8	8.8
266	35460		910	3.8	9.4
267		345		3.8	
269	35320	365	940	3.8	9.3
270	35250	340	950	3.8	9.3
271	35200	360	955	3.8	9.2
273	35180	350	935	3.8	9.2
275	35230	360		3.8	9.2
277	35200	350	945	3.8	9.2
278	38050		950	3.8	9.9
279		360		3.8	
280	38120	380	1030	3.9	9.9
281	38160	390	1025	3.9	9.8
283		390		3.9	
285	38160	380	1020	3.9	9.8
287	38120	400		3.9	9.8
289	38100	390	1010	3.9	9.8
290	41600			3.9	10.6
291		450		3.9	
292	41620	450	1055	3.9	10.6
293	41550	460	1065	4	10.5
295	41480	440	1060	3.9	10.5
297	41520	430	1045	4	10.5
299	41500	430	1070	4	10.5
300	43450		1060	3.7	11.7
301		440		3.7	
303	43200	450	1090	3.8	11.4
304	43100	440	1120	3.8	11.3
305	43000	450		3.8	11.2
307	43070	440	1115	3.9	11.2
309		440		3.8	

311	43050	450	1100	3.8	11.2
312	46560			3.9	12
313		460	1150	3.9	
314	46000	450	1110	3.9	11.8
316		460		4	
317	45900	470	1110	4	11.5
318	46000	470		4	11.6
320	45900	460		4	11.5
322	45900	460	1130	4	11.5
324	45960	460	1125	4	11.5
326		470	1120	4	
328	45920	480	1110	4	11.5
330	45920	450	1045	4	11.5
333		450	1065	4	
336		430	1080	4	
338	45930	450	1070	4.1	11.3
340	45900	460	1060	4	11.6
341	48400	450	1085	3.9	12.4
342		460		3.4	
343	48340	460	1155	3.4	14.1
344		490		3.5	
345	48270	480	1140	3.6	13.6
346	48350	480	1130	3.6	13.6
347	48320	490		3.6	13.5
348	48340	490	1140	3.6	13.5
349	51960		1150	3.6	14.5
350		490		3.6	
351	51500	500	1175	3.6	14.2
352	51480	510	1190	3.7	14.1
353		490	1170	3.7	
354	51500			3.6	14.1
355	51480	510	1160	3.7	14.1
356	51500	500	1185	3.7	14.1
357	58860			3.7	16
358		520		3.7	
359		540	1270	3.7	
360	56800	530	1245	3.8	15.1
361	56420		1260	3.8	15
362		530		3.8	
363	56500	520	1235	3.8	15
364	56440	530		3.8	15
365	56500			3.8	15
366	56480	520	1250	3.8	15
367	61130		1265	3.8	16.1
368		570		3.8	
369	60180	620	1365	3.8	15.6
370	60150			3.9	15.5
371	60100	610	1340	3.9	15.6
372	60120	590	1360	3.9	15.6
373	60150		1340	3.9	15.5
374		600		3.9	
375	60130	590	1350	3.9	15.6
376	64120			3.9	16.6
377		580	1425	3.9	
378	64200	600	1510	3.9	16.6

379	64180	630	1500	3.9	16.5
380	64230	630	1485	3.9	16.5
381	64220		1500	3.9	16.4
382	64220	640	1480	3.9	16.4
383		620	1500	3.9	
384	64240			3.9	16.5
385	64260	630	1500	3.9	16.5
386	66550		1500	3.8	17.5
387		660		3.8	
388	66340		1570	3.8	17.3
389		640		3.9	
390	66320	650	1565	3.9	17.1
391	66380	660	1580	3.9	17.2
392	66320	660	1590	3.9	17.1
393	66350	650	1575	3.9	17.1
394	68720		1570	3.9	17.7
395		650		3.9	
396	68200	680	1670	3.9	17.4
397	68240	670	1645	3.9	17.4
398	68250	670	1650	3.9	17.3
399	68200	680	1635	3.9	17.4
400	68240	670	1645	3.9	17.4
401	68200	670	1630	3.9	17.4
405	68230	660	1610	3.9	17.4
407		670	1585	3.9	
409	68270	680	1620	4	17.3
411	68240	650	1630	3.9	17.3
413	68200	650	1620	3.9	17.3
415	68230	640	1610	4	17.3
417	68180	650	1610	4	17.2
418	82160	650		4	20.6
419		660		4	
420	81780	730	1780	4	20.3
421	81630	810	1910	4.1	20.1
422		830	1935	4.1	
423		820	1880	4.1	
424	81570		1825	4.1	19.9
425	81520	770	1840	4.1	19.8
426	81560			4.1	19.8
427	81500	770	1820	4.1	19.7
428	81580	790		4.1	19.8
429	81540	780	1820	4.1	19.8
430	88350	780	1920	3.9	22.7
431		830		3.9	
432	88360		2120	4	22.3
433	88040	820	2135	4	21.9
434	88000	830	2115	4	21.9
435		810		4	
436	88040	810	2110	4	21.8
437	88050	820	2125	4	21.8
438	88000	820	2110	4	21.8
439	95360		2230	4.1	23.3
440		870	2285	4.1	
441	95300	870	2315	4.1	23.5
442	95120	880	2380	4.1	23.3

443	95050	850		4.1	23.1
444	95180			4.1	23
445		870	2370	4.1	
446		870	2370	4.1	
447	95120			4.1	23.1
448		870	2380	4.1	
449	95100	850	2370	4.1	23.1
450		860		4.1	
451	95160	850	2400	4.1	23.1
452	100460	910		4.1	24.8
453		920	2700	4.1	
454	101200		2730	4.2	24.1
455		930		4.2	
456		930	2720	4.2	
457	101200	940	2720	4.3	23.8
458	101200	920	2690	4.2	23.8
459	101200	930	2700	4.2	23.8
460	108600			4.1	26.6
461	108600		2960	4.1	26.7
462		990	2980	4.1	
463	108680	980	2950	4.1	26.8
464		970		4.1	
465	108620	990	2970	4.1	26.6
466	108650	980	2950	4.1	26.7
467	119330			4.1	28.9
468		1030	3300	4.2	
469	119120	1180	3360	4.2	28.6
470		1200	3370	4.2	
471	118950			4.2	28.4
472		1140	3350	4.2	
473	119000	1160	3330	4.2	28.5
474	119000	1200	3340	4.2	28.4
475		1170	3300	4.2	
476	119050	1150	3350	4.2	28.5

Appendix 5

Time (days)	MLVSS (mg/l)	MLSS (mg/l)	Sludge wastage (ml)	Solid retention time (days)	Biogas production (l/d)
1					
3	8850				
5					
8					
12	9000				
17	9170	13000			
21	9200				
24					
26					
28					
30	9500				53
33					
37	9900	14100			50
40	9960				
42	10170				48
45	10250	14050			53
46			50		96
47					
48					
49					91
50	10400				
55	10360	14450	1250		88
60	10380	14530	1250	480	91
61					121
63					
65					113
70	11380	15800			117
71	11470				121
75	11440	15950	1500		119
79	11460		1500		122
83	11450	15900	1500	320	120
84					153
85					
86					
87			300		139
88			250		
89	11620				139
90	11650				
94	11680		2080		
98	11660	16000	2080		137
102	11700	16100	2080	232	136
103			1800		174
104			1600		
106			1000		165
109	11900				166

110	11950	16400			171
113	11970		1845		165
116	11940		1845		
119	11960	16450	1845	195	170
120					192
121					
122			100		190
124					190
126	12460				187
129	12500		2040		
132	12540	17000	2040		183
135	12500	16900	2040		180
138	12530	16900	2040	177	184
139					204
140					
143	13100				213
145	13060				200
148	13140	17800	2250		192
151	13180	17900	2250		198
154	13150		2250		206
157	13170	17840	2250	160	199
158			1000		269
159			1500		
162			1200		251
165	13900	18900	2670		246
168	14060		2670		
171	14030		2670		245
174	14080	18750	2670		247
177	14050	18820	2670	135	247
178					294
179			100		295
181					294
184	14900	19970			282
186	15000	20100			289
189	15030		2940		291
192	15050		2940		283
195	15000	20000	2940	122	278
196					483
197					
199					
201					438
203					
205					421
207					463
209					421
211					
213	19850		2340		420
215	19900		2340		418
217	19880	26700	2340		417
219	19940	26600	2340		434
221	19900	26650	2340	102.5	423
223			1900		
225			1900		422
227			1600		
229			1500		414

230			1560		
231			1300		
232			1500		
233			1500		435
234			1000		
235			900		439
236			900		452
237	20170				431
239	21000		2440		453
241	20200	27050	2440		456
243	20150	26950	2440	98	432
244			1000		491
245			1000		
247	20780	27000	800		467
248	20840	27260			
250	20870		2640		
252	20900		2640		456
254	20860	27320	2640	91	459
255			1800		480
256			1600		
258			1600		459
259	21150				457
261	21180	27430	2700		
263	21130	27500	2700		461
265	21170	27460	2700	88.6	465
266			1500		498
267			1500		
269			1500		493
270	21700				491
271	21790	28300			502
273	21760	28400	2780		485
275	21800	28260	2780		517
277	21780	28300	2780	86.3	493
278			250		528
279			250		
280			300		501
281	22670				488
283	22840		2860		
285	22870	28850	2860		490
287	22900		2860		514
289	22850	28800	2860	84	500
290			700		552
291			500		
292	23460				559
293	23600				549
295	23600	29900	2980		
297	23630	30000	2980		540
299	23600	29870	2980	80.5	536
300			1000		609
301			1000		
303			1200		
304	24430				561
305	24570	30160			
307	24540	30240	3020		532
309	24580		3020		

311	24560	30200	3020	79.7	555
312			2000		
313			2000		
314			2000		614
316			1600		
317	24600		1000		602
318	24950				637
320	25000	31100	3060		658
322	24960	31140	3060		618
324	25000	31200	3060	78.4	625
326			1500		
328			1500		623
330			1800		
333			1800		
336			1800		
338			1500		611
340			1500		603
341			1300		
342			1300		
343					717
344	29800				
345	30060	37200	1555		692
346	30030	37300	1555		691
347	30000	37350	1555		712
348	30050	37300	1555	77.2	698
349			1500		738
350					
351			1800		767
352	30500				759
353	30800		1580		
354	30830	37900	1580		761
355	30850	37820	1580		
356	30800	37800	1580	76	749
357			1000		883
358			1000		
359			1500		
360			1400		765
361			1400		776
362	31860	38500			
363	32120	38900	1590		773
364	31160	38770	1590		807
365	32100		1590		783
366	32150	38860	1590	75.5	776
367			1500		
368			1500		
369			1400		813
370			1300		829
371	33100	39405			832
372	33150		1620		845
373	33200		1620		819
374	33200	39520	1620		
375	33170	39640	1620	74	826
376			1500		
377			1500		
378			1500		882

379			1600		
380			1600		
381	34045				840
382	34100		1670		844
383	34070	41080	1670		
384	34030	41000	1670		875
385	34100	40970	1670	72	870
386			1600		938
387			1600		
388			1600		884
389			1600		
390	34900		1710		904
391	34900	41300	1710		
392	34840	41200	1710		902
393	34900	41260	1710	70.1	913
394			1700		894
395			2000		
396			2500		886
397					857
398	35200	41050	1730		858
399	35180		1730		847
400	35250		1730		878
401	35220	41300	1730	69.5	898
405	36500		3000		918
407			3000		
409			3000		878
411			3000		
413			3000		870
415			2500		
417			2500		833
418					997
419			2000		
420			2000		
421			2000		979
422					
423			1500		
424					950
425	38900		1830		931
426	39180	45800	1830		948
427	39140	45900	1830		
428	39150		1830		
429	39220	45870	1830	65.6	927
430			1700		1063
431			1700		
432			1700		
433			1800		1044
434	42100		1890		1088
435	42300		1890		
436	42300	48600	1890		1130
437	42350		1890		1115
438	42340	48700	1890	63.5	1109
439			1800		1187
440					
441			3500		
442			1800		1161

443					1213
444			3500		1232
445			1500		
446					
447	43620		1920		1200
448	44040	49800	1920		
449	43960	49900	1920		1178
450	44000	49800	1920		
451	43980	49800	1920	62.6	1147
452					1220
453			1800		
454					
455	44200	50300	3700		
456	44600		1900		
457	44550	50600	1970		1176
458	44600	50560	1970		1204
459	44570	50620	1970	61	1161
460			1900		1374
461					1246
462			3000		
463	48500		2015		1314
464	48460	54200	2015		
465	48500		2015		1372
466	48440	54250	2015	59.6	1395
467					1463
468			900		
469			1000		
470			1200		
471					1498
472					
473	51100	57900	2055		1423
474	51180	58000	2055		1392
475	50100		2055		
476	51050	58100	2055	58.4	1414

Appendix 5

Time (days)	Methane Production (l/d)	Methane Percentage (%)	Methane yield (m^3 CH_4/kg COD removed)	Influent Alkalinity (mg/l)	Effluent Alkalinity (mg/l)	Influent VFA (mg/l)	Effluent VFA (mg/l)
1				2880		20	
3	1	7	0.08	2560	2250		1650
5	2			2670	2250		1600
8				2700	2160	50	1270
12	9	14	0.2	2450	2200		700
17				2560	2000	70	620
21	16	42	0.31	2000			420
24				2200			310
26				2060	2060		280
28		55		2030	2050		230
30	38	67	0.3	2050	2050	100	860
33		70		1900	1900		280
37	38	77	0.34	1600	1600		50
40				1800		30	60
42	38	79	0.34			60	15
45	42	78	0.35	1500	1500		10
46	76	79	0.37	2150		30	50
47		79	0.37			40	40
48		80	0.38			40	30
49	72	79	0.37	1000	1000		20
50	72		0.37	1150			20
55	69	78	0.37	1050			20
60	72	79	0.38	1120	1250	50	10
61	95	78	0.37	1500		60	15
63		79	0.37		1560		20
65	91	80	0.38	1320			70
70	94	80	0.38	1420		100	90
71	96	79	0.39	1600			70
75	93	78	0.39	1720			50
79	95	78	0.38	1500	1900		30
83	95	79	0.39	1620	1960		30
84	122	80	0.39	1120		140	30
85		79	0.38			80	35
86			0.38			80	30
87	111	80	0.38	1000			20
88		81	0.38			110	45
89	113	81	0.39	1000			65
90				1040		200	40
94		80			1550		25
98	111	81	0.38	1220		190	30
102	109	81	0.38	1220	1450		30
103	138	80	0.38	960			40
104		78	0.37			65	50

106	128	77	0.37		1080	80	40
109	129	78	0.38	880			30
110	133	78	0.4	1030	1300		20
113	128	78	0.39	900			25
116				960			15
119	133	79	0.4	960	1160	130	20
120	150	78	0.39	770			25
121		77	0.38			85	35
122	149	78	0.39	1240	1300		30
124	151	80	0.39			80	15
126	149	80	0.4	900			40
129				800		150	50
132	145	79	0.39	1000			30
135	144	80	0.39			165	20
138	148	80	0.4	1050	1360		30
139	162	79	0.38	960		70	30
140		77	0.38	880			35
143	162	76	0.39	1200	760		20
145	154	77	0.38	1500		165	25
148	151	79	0.38		1000		20
151	157	79	0.39	1220		310	30
154	161	78	0.4			310	20
157	157	78	0.4	1140	1000		20
158	210	78	0.39	850		185	25
159		77	0.38	760			65
162	191	76	0.37		1500	100	40
165	187	76	0.37	640		55	20
168				640			30
171	190	78	0.38			110	20
174	193	78	0.38			220	20
177	194	79	0.39	660	1400		10
178	230	78	0.39	780	1300		15
179	236	80	0.4	900		350	40
181	231	78	0.41			320	30
184	224	80	0.41		1260		35
186	232	80	0.42			110	20
189	236	81	0.42		1300	170	20
192	226	80	0.41	1000		150	20
195	224	81	0.41	800	1080		25
196	387	80	0.41	760		110	30
197		78	0.4	800			90
199				800	820		160
201	311	71	0.37	900	930	425	200
203		72	0.37		880	460	180
205	306	73	0.37			450	170
207	346	75	0.38			500	100
209	324	77	0.37	1000		400	100
211		77	0.38	1000	1180		80
213	330	79	0.38		1140		35
215	330	79	0.37			230	40
217	328	79	0.38	840		310	50
219	343	79	0.39			175	35
221	335	79	0.38	800	1000		30
223		80	0.39	970		325	20
225	329	78	0.38	1500		400	15

227		78	0.38	1500		510	10
229	326	79	0.38			600	15
230				1500	1660	250	60
231				1400			45
232				1100			40
233	340	78	0.37	1250			50
234		78	0.37	1400		410	40
235	350	80	0.37		1300		30
236	361	80	0.38	1050		570	40
237	347	80	0.38			490	35
239	358	79	0.38		1350	670	30
241	355	78	0.38			850	30
243	339	79	0.37	1120	1360		40
244	383	78	0.38	1000			30
245		77	0.36			725	50
247	361	77	0.36	760		720	55
248				480			50
250		78	0.37			840	30
252	354	78	0.36	600		900	30
254	358	78	0.36	600	1450	820	30
255	370	77	0.35	500		770	30
256		76	0.34				25
258	349	76	0.34			460	40
259	352	77	0.34	520		500	50
261		76	0.35			500	30
263	351	76	0.34	660			30
265	356	76	0.34	640	1860		20
266	384	77	0.35	420		440	25
267		77	0.35		1720	500	40
269	374	76	0.34			430	40
270	380	77	0.35	360		665	40
271	387	77	0.36	460			35
273	378	78	0.35			660	30
275	396	76	0.36			740	35
277	381	77	0.35	460	1600		40
278	407	77	0.35	300			50
279		75	0.35			670	60
280	380	76	0.33	160	1820	580	50
281	372	76	0.32		1960		55
283				130		500	40
285	377	77	0.33	150		720	30
287	390	76	0.33	150		780	35
289	382	76	0.34	100	2000		30
290	423	77	0.33			980	25
291					2080	1020	35
292	421	75	0.34		2250	870	55
293	415	76	0.34			910	40
295						930	40
297	405	75	0.33		2280		50
299	403	75	0.33		2160		40
300	455	75	0.33			665	45
301		74	0.31			670	50
303					2300	650	70
304	411	73	0.31			650	60
305					1800		55

307	391	74	0.3			740	60
309		73	0.31			820	50
311	405	73	0.31		1770	820	60
312							50
313					1600		45
314	448	73	0.32			600	40
316		72	0.33		1660	640	50
317	446	74	0.33			660	50
318	473	74	0.34		1900	600	40
320	474	72	0.34			600	30
322	452	73	0.34			630	45
324	460	74	0.34		1680		50
326		72	0.34			880	55
328	458	74	0.34			880	60
330					2000	945	50
333						990	45
336						940	40
338	459	75	0.34		2700		35
340	447	74	0.33			770	40
341						640	40
342		75	0.33			645	50
343	532	74	0.32			660	50
344		73	0.33		2460		45
345	508	74	0.32			1020	40
346	513	74	0.32				40
347	527	74	0.32			1020	30
348	520	75	0.33		2600	1160	35
349	547	74	0.32			1000	35
350		73	0.33				50
351	569	74	0.34		2660		60
352	557	73	0.34			880	70
353		74	0.34			940	60
354	568	75	0.34		2800		50
355						1000	60
356	554	74	0.34		2850	970	50
357	644	73	0.34			1060	50
358		71	0.32			1020	60
359		70	0.31		2550		70
360	541	71	0.31		2500		90
361	555	72	0.31			1240	90
362						1240	80
363	564	73	0.32			1300	75
364	582	72	0.32			1310	70
365	574	73	0.32			1310	80
366	570	73	0.32		2400		70
367						1560	55
368					2300	1500	90
369	577	71	0.31		2100		130
370	597	72	0.32			1440	75
371	606	73	0.33			1440	85
372	618	73	0.34			1400	80
373	602	74	0.33			1460	70
374		72	0.33			1460	65
375	603	73	0.33		2200	1500	65
376						1340	70

377		70	0.31		1900		85
378	622	70	0.32			1645	100
379						1630	75
380					2000		50
381	588	70	0.31		2100		55
382	601	71	0.31			1240	70
383		70	0.31			1130	85
384	614	70	0.31			1180	80
385	618	71	0.32		2000		75
386	664	71	0.32			1300	110
387		70	0.32		2000		110
388	627	71	0.31		2050		90
389		72	0.31			1320	70
390	651	72	0.32			1290	90
391					2450		80
392	653	72	0.33			1360	90
393	663	73	0.33		2330		80
394	644	72	0.31		2200	1460	60
395					2200		70
396	615	70	0.3			1500	65
397	601	70	0.29			1520	55
398	609	71	0.3		2450	1520	50
399	594	70	0.29			1535	60
400	622	71	0.31		2700		70
401	634	71	0.31		2770		60
405	642	70	0.31			1660	50
407		71	0.31		2560		60
409	628	72	0.31		2640	1710	40
411						1710	40
413	614	70	0.3		2280		50
415					2200		50
417	575	69	0.28		2300		50
418	694	70	0.28			1680	65
419		70	0.29			1770	110
420						1700	130
421	695	71	0.29			1750	150
422		70	0.29			1800	165
423					2100	1790	150
424	677	71	0.29		2060		100
425	654	70	0.28			1800	90
426	664	70	0.28			1760	85
427					2270	1800	90
428							80
429	659	71	0.28		2330		80
430	747	70	0.28			1180	90
431		69	0.28			1200	110
432						1200	80
433	718	69	0.28		2000	1250	70
434	750	69	0.29			1310	50
435					2060		60
436	782	69	0.31		2080		90
437	768	69	0.3			1390	100
438	767	69	0.3		2200		90
439	833	70	0.31			1540	110
440		69	0.31			1510	110

441					2840		120
442	808	70	0.3		3150	1600	90
443	849	70	0.31		3000		80
444	868	70	0.31			1660	95
445		70	0.31		3000	1680	100
446		71	0.31			1660	110
447	853	71	0.31		2880		90
448		71	0.31			1700	85
449	836	71	0.31				80
450			0.3				85
451	805	70	0.3		3050		85
452	833	68	0.28			1110	120
453		67	0.28		2700	1120	120
454					2650		100
455		66	0.27			1200	100
456		67	0.28		2870		90
457	794	68	0.29			1200	110
458	807	67	0.29				110
459	779	67	0.28		2930		100
460	894	65	0.28			1410	90
461	810	65	0.26				115
462		66	0.26		3250		140
463	844	67	0.27			1560	155
464		65	0.27		3330		150
465	870	66	0.28		3400		140
466	879	66	0.28				160
467	938	64	0.27				160
468		65	0.28			1650	190
469					3400		230
470					3560		360
471	914	66	0.27		3640	1780	290
472		64	0.26		3470		235
473	882	64	0.27			1650	210
474	865	64	0.27			1710	210
475		65	0.28		3720		220
476	877	65	0.28		3660		210

Appendix 5

Time (days)	Influent TKN (mg/l)	Effluent TKN (mg/l)	Effluent NH_3-N (mg/l)	Influent PO_4-P (mg/l)	Effluent PO_4-P (mg/l)	Influent BOD (mg/l)	Effluent BOD (mg/l)
45						2000	10
60	56	30	27	11	8	3200	20
83	70	38	35	14	10	4200	10
102	88	48	41	17	13	5000	20
119	110	60	50	22	20	7200	10
138	130	70	65	26	23	8800	30
157	150	82	77	31	26	9000	10
177	200	100	92	33	29	10500	15
195	240	115	105	38	33	13000	10
221	390	210	182	65	53	22000	20
243	450	250	220	74	60	22500	20
254	500	280	260	82	67	23600	20
265	530	280	250	86	75	24300	30
277	560	300	270	90	73	26000	40
289	620	310	280	97	79	27500	20
299	660	330	310	102	81	30000	20
311			355	105	88	31000	40
324			400	113	92	33000	50
348			350	125	107	35400	20
356			430		124	37000	30
366			380	148	126	42000	40
375			460	157	134	44000	20
385			570	135	108	47000	30
393			780	125	102	48000	20
401			680	160	132	50000	70
429			840	172	148	68000	40
438			930		112	74000	70
447			1000		107		
451						80000	100
457			1180		141		
459						84000	70
466						90000	100
476						102000	130

Appendix 5

Time (days)	Influent turbidity (NTU)	Effluent turbidity (NTU)	Influent colour (Hazen)	Effluent colour (Hazen)
8			125	
21			75	
26			75	25
30	10	0.37	125	50
45			150	50
46	12	0.39	125	
49			100	
60	15	0.35	150	
71	30	0.34	175	
84			200	
85	18	0.35	200	100
90	62	0.38	300	125
103			300	
110	66	0.36	400	150
124	105	0.4	500	150
132	72	0.41		
139	55	0.37	500	200
154	70	0.36	500	200
158	60	0.34		
159	62	0.33	500	
171	120	0.31		
177	90	0.31	600	250
189	130	0.3	700	300
192	140	0.28		
195	150	0.27		
203	85	0.28	900	400
211	95	0.3	900	400
219	110	0.33		
229	260	0.31	1000	400
234	180	0.36		
242			1250	450
245	160	0.41		
250	190	0.37		
254	240	0.37	700	400
259	160	0.35		
261		0.34		
263	210	0.35	800	300
273	310	0.34		
275	330	0.34	880	400
280	180	0.31	1000	400
281		0.29		
283	160	0.29		
285		0.28		
290	300	0.27	2500	800

295	360	0.26		
303	240	0.28	2800	850
305	320	0.27		
309			3100	900
312	460	0.3		
314			1500	700
317	480	0.29		
322	540	0.3		
326	320	0.33		
330			1800	750
333	300	0.36		
338	660	0.35		
343	540	0.4	1900	700
344	530	0.42		
346	420	0.41		
350			2200	800
352	320	0.44		
354	300	0.41		
357	720	0.4		
359	700	0.46		
360			2600	800
363	320			
366	640	0.41		
367	660	0.43		
368		0.43		
370	740		3200	1000
371	820	0.41		
373	580	0.4		
377	460	0.4		
378	400	0.39		
380			3400	1000
381	480	0.34		
383	400	0.33		
387			3600	1000
388	460	0.36		
390	570	0.37		
393	680		3500	1100
397	720			
399	550	0.39		
400		0.39		
407	700	0.35		
411	750	0.34		
413	750	0.39	3100	1150
420	630	0.4		
421	600	0.42		
422			3350	1200
425	900	0.45		
428	720	0.42		
431	700			
432	760	0.41	3500	1350
435	640	0.4		
436	600			
439	540			
440	530			
441	600	0.39		

442		0.41		
444	680	0.42		
446			3300	1400
447	760			
450	800	0.47		
453	800	0.45	3600	1350
456	700	0.51		
457	730	0.52		
458	720	0.48		
460	700	0.44		
461	770	0.45	3400	1400
463	800	0.47		
465	860	0.49		
467	860	0.44	3500	1500
469	820			
470	880	0.41		
471	960	0.4		
472	900	0.38	3600	1500
474	820	0.48		

Appendix 7

Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3
1	0	0	0	55	0	0	0	109	1	0	0
2	0	0	0	56	1	1	0	110	1	1	0
3	0	0	0	57	0	0	1	111	1	1	1
4	0	0	0	58	1	0	0	112	1	1	0
5	0	0	0	59	1	1	0	113	1	1	0
6	0	0	0	60	0	0	1	114	1	1	0
7	0	0	0	61	1	0	0	115	1	0	0
8	0	0	0	62	0	0	0	116	1	0	1
9	0	0	0	63	1	0	0	117	1	1	0
10	0	1	0	64	1	1	0	118	1	1	0
11	0	0	1	65	1	0	1	119	1	1	0
12	0	0	0	66	0	0	0	120	1	1	1
13	0	0	0	67	1	0	0	121	0	0	0
14	1	0	0	68	1	1	0	122	0	1	0
15	0	0	0	69	0	0	0	123	0	0	0
16	0	0	0	70	1	0	1	124	1	1	0
17	0	0	0	71	1	0	1	125	0	0	0
18	0	1	1	72	1	1	1	126	1	1	0
19	1	0	0	73	1	0	1	127	1	1	0
20	0	0	0	74	0	0	1	128	1	1	0
21	0	0	1	75	1	0	1	129	0	1	0
22	0	0	0	76	1	0	1	130	1	1	0
23	0	0	0	77	0	1	1	131	0	1	0
24	0	0	0	78	1	1	1	132	1	1	0
25	1	0	0	79	1	0	1	133	1	0	1
26	0	1	0	80	1	0	1	134	1	0	0
27	0	0	0	81	1	1	1	135	1	0	0
28	0	0	1	82	1	0	0	136	1	0	0
29	0	0	1	83	1	0	0	137	0	1	0
30	0	0	0	84	1	1	0	138	1	1	0
31	0	0	0	85	0	0	0	139	0	1	0
32	1	0	0	86	1	0	0	140	1	0	0
33	0	0	0	87	1	0	0	141	1	0	0
34	1	1	0	88	1	1	0	142	0	1	0
35	0	0	1	89	0	1	0	143	1	1	1
36	0	0	0	90	1	1	1	144	1	1	0
37	0	0	0	91	1	0	0	145	1	1	0
38	0	0	1	92	1	1	0	146	1	1	0
39	1	0	0	93	1	0	0	147	1	0	0
40	0	0	1	94	0	1	0	148	1	0	0
41	0	1	0	95	1	0	1	149	1	1	0
42	1	0	1	96	1	0	0	150	1	1	0
43	1	0	1	97	0	0	1	151	0	1	0
44	0	0	0	98	1	1	0	152	1	1	0
45	1	0	1	99	0	1	0	153	1	1	0
46	0	1	1	100	1	1	0	154	1	1	0
47	0	1	0	101	1	1	0	155	0	1	0
48	1	1	0	102	1	1	0	156	1	0	1
49	0	0	1	103	1	1	1	157	1	0	0
50	1	0	1	104	1	1	0	158	1	0	0
51	1	0	1	105	1	1	0	159	1	0	0
52	0	0	0	106	1	1	1	160	1	0	0
53	0	0	0	107	1	1	0	161	1	1	0
54	1	1	1	108	1	1	0	162	1	1	0

SMA test on day 3, CH 1=Channel 1 (1000 mg/l acetate), CH 2=Channel 2 (1500 mg/l acetate), and CH 3=Channel 3 (2000 mg/l acetate).

Appendix 7

Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3
1	0	0	0	55	1	1	0	109	1	1	0
2	0	0	0	56	1	0	0	110	2	1	0
3	0	0	0	57	1	1	0	111	2	1	0
4	0	0	0	58	1	1	0	112	2	1	0
5	0	0	0	59	1	1	0	113	1	1	0
6	0	0	0	60	0	1	0	114	2	1	0
7	0	0	0	61	1	1	0	115	2	1	0
8	0	1	0	62	1	1	0	116	2	1	0
9	0	0	0	63	1	1	0	117	2	0	0
10	1	0	0	64	1	1	0	118	1	1	0
11	0	0	1	65	1	1	0	119	2	1	0
12	0	0	0	66	1	1	0	120	2	1	1
13	0	0	0	67	1	1	0	121	2	1	0
14	0	0	0	68	1	1	0	122	2	1	0
15	0	0	0	69	1	1	0	123	1	1	0
16	0	0	0	70	1	1	0	124	2	1	0
17	0	0	0	71	1	1	0	125	2	0	0
18	1	0	1	72	1	1	0	126	2	1	0
19	0	0	0	73	1	1	0	127	2	1	0
20	0	1	0	74	1	1	1	128	1	1	0
21	0	0	0	75	1	1	0	129	2	1	0
22	0	0	0	76	1	1	0	130	1	0	0
23	0	0	1	77	2	1	0	131	2	1	0
24	0	0	0	78	1	1	0	132	2	1	0
25	1	0	0	79	1	1	0	133	1	0	1
26	0	0	0	80	1	1	0	134	2	0	0
27	0	0	0	81	1	1	0	135	2	0	0
28	0	1	0	82	1	2	0	136	2	1	0
29	1	0	1	83	1	1	0	137	2	1	0
30	0	1	0	84	1	1	0	138	2	0	0
31	0	0	0	85	2	1	0	139	1	0	0
32	1	1	1	86	1	1	0	140	1	0	0
33	0	1	0	87	1	2	0	141	1	0	0
34	1	0	1	88	1	1	0	142	2	0	0
35	0	0	1	89	2	1	0	143	2	1	1
36	1	0	0	90	1	1	0	144	2	0	0
37	0	1	1	91	1	1	0	145	2	0	0
38	1	1	1	92	1	1	0	146	2	0	0
39	0	0	1	93	1	1	0	147	2	0	0
40	1	0	1	94	2	1	0	148	2	0	0
41	1	0	0	95	1	1	0	149	2	0	0
42	0	0	1	96	1	1	0	150	2	0	0
43	1	0	1	97	2	1	1	151	2	1	0
44	1	1	1	98	1	1	0	152	2	0	0
45	1	0	1	99	2	2	0	153	2	0	0
46	1	1	1	100	1	1	0	154	2	0	0
47	0	0	0	101	1	1	0	155	2	0	0
48	1	0	1	102	2	1	0	156	2	0	1
49	1	0	1	103	1	1	0	157	2	0	0
50	1	1	1	104	2	1	0	158	2	0	0
51	1	1	1	105	1	1	0	159	2	0	0
52	0	0	0	106	1	2	1	160	2	0	0
53	1	1	0	107	2	1	0	161	2	0	0
54	1	1	0	108	2	1	0	162	1	0	0

SMA test on day 12, CH 1=Channel 1 (1000 mg/l acetate), CH 2=Channel 2 (1500 mg/l acetate), and CH 3=Channel 3 (2000 mg/l acetate).

Appendix 7

Time (h)	CH 1	CH 2	CH 3
163	2	0	0
164	1	0	0
165	1	0	0
166	2	0	1
167	1	0	0
168	1	0	0
169	1	0	0
170	1	0	0
171	1	1	0
172	2	0	0
173	1	0	0
174	1	0	1
175	1	0	0
176	1	0	0
177	1	0	0
178	1	0	0
179	2	0	0
180	1	0	0
181	1	0	0
182	1	1	0
183	1	0	0
184	1	0	0
185	1	0	0
186	1	0	0
187	1	0	0
188	1	0	0
189	1	0	1
190	1	0	0
191	1	0	0
192	1	0	0
193	0	0	0
194	1	0	0
195	1	0	0
196	1	0	0
197	1	1	0
198	1	0	1
199	1	0	0
200	0	0	0
201	1	0	0
202	1	0	0
203	1	0	0
204	1	0	0
205	1	0	0
206	0	0	0
207	1	0	0
208	1	0	0
209	1	0	0
210	0	0	0
211	1	0	0
212	1	0	1
213	0	0	0
214	1	0	0
215	1	0	0
216	0	1	0

SMA test on day 12. CH 1=Channel 1 (1000 mg/l acetate), CH 2=Channel 2 (1500 mg/l acetate), and CH 3=Channel 3 (2000 mg/l acetate).

Appendix 7

Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3
1	0	0	0	55	1	1	1	109	2	2	1
2	0	0	0	56	2	1	1	110	3	2	2
3	0	0	0	57	1	1	1	111	2	1	1
4	1	0	0	58	1	1	1	112	2	2	1
5	0	0	0	59	2	1	0	113	3	2	1
6	0	0	0	60	1	1	1	114	2	2	1
7	0	1	0	61	1	2	1	115	3	2	2
8	1	0	0	62	2	1	1	116	2	3	1
9	0	0	0	63	1	1	1	117	3	2	1
10	0	0	0	64	2	1	1	118	3	2	1
11	1	0	0	65	2	1	0	119	3	2	2
12	0	1	0	66	1	2	1	120	2	2	2
13	1	0	0	67	2	1	1	121	3	3	2
14	0	0	0	68	2	1	0	122	3	2	1
15	1	0	1	69	1	2	1	123	3	2	2
16	1	1	0	70	1	1	1	124	2	2	1
17	0	0	0	71	2	1	1	125	3	2	2
18	1	0	0	72	2	2	1	126	3	2	1
19	1	0	0	73	1	1	1	127	3	2	2
20	0	1	0	74	2	2	0	128	2	2	1
21	0	0	0	75	2	1	1	129	3	3	1
22	1	1	1	76	2	2	1	130	3	2	1
23	1	0	0	77	1	1	1	131	3	2	1
24	1	1	0	78	2	2	1	132	3	2	1
25	1	0	0	79	2	1	1	133	3	2	1
26	0	1	0	80	2	2	1	134	2	2	2
27	1	1	0	81	2	2	1	135	3	2	1
28	1	0	0	82	1	2	1	136	3	2	1
29	1	1	0	83	2	2	1	137	3	3	1
30	1	1	0	84	2	1	1	138	3	2	2
31	1	1	0	85	2	1	1	139	3	2	1
32	1	1	0	86	2	2	1	140	3	2	1
33	0	0	1	87	2	2	2	141	3	2	1
34	1	1	0	88	2	2	1	142	2	3	1
35	1	1	0	89	2	2	1	143	3	2	2
36	1	1	0	90	2	2	1	144	3	2	1
37	1	0	1	91	2	2	1	145	3	2	1
38	1	1	0	92	2	2	1	146	2	2	1
39	1	1	0	93	2	1	2	147	3	3	1
40	1	1	1	94	1	2	1	148	3	2	1
41	1	1	0	95	2	2	1	149	3	2	1
42	1	0	0	96	2	2	1	150	3	3	2
43	2	1	1	97	3	2	1	151	3	2	1
44	1	1	0	98	2	2	1	152	3	2	1
45	1	1	1	99	2	2	1	153	3	3	1
46	1	1	0	100	2	2	1	154	3	2	1
47	1	1	1	101	3	1	2	155	3	3	1
48	2	1	0	102	2	2	1	156	3	2	2
49	1	1	1	103	2	2	1	157	3	3	1
50	1	1	0	104	2	2	1	158	3	2	1
51	1	1	1	105	2	2	2	159	3	3	1
52	1	1	0	106	3	2	1	160	3	2	1
53	2	1	0	107	2	2	1	161	3	3	1
54	1	2	1	108	2	2	1	162	3	2	1

SMA test on day 21, CH 1=Channel 1 (1000 mg/l acetate), CH 2=Channel 2 (1500 mg/l acetate), and CH 3=Channel 3 (2000 mg/l acetate).

Appendix 7

Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3
163	3	3	1	217	1	2	0
164	3	3	1	218	1	3	0
165	3	3	1	219	1	2	0
166	3	3	1	220	0	2	0
167	3	3	1	221	0	2	0
168	3	3	1	222	1	2	1
169	3	3	1	223	1	2	0
170	3	3	1	224	0	3	0
171	2	2	1	225	1	2	0
172	3	3	1	226	0	2	0
173	2	3	0	227	0	2	1
174	3	3	0	228	0	2	0
175	2	3	1	229	0	1	0
176	2	3	0	230	0	2	0
177	3	2	0	231	1	2	0
178	3	3	0	232	0	2	0
179	2	3	1	233	0	2	0
180	2	3	0	234	0	1	0
181	2	3	0	235	0	2	0
182	2	3	0	236	0	2	1
183	2	3	1	237	0	2	0
184	2	2	0	238	0	2	0
185	3	3	0	239	0	1	0
186	2	3	0	240	0	2	0
187	2	3	0	241	0	1	0
188	2	3	0	242	1	1	0
189	2	3	0	243	0	1	1
190	2	3	0	244	0	1	0
191	2	3	0	245	0	1	0
192	2	3	0	246	0	1	0
193	1	3	0	247	1	2	0
194	2	3	1	248	0	1	0
195	1	3	0	249	0	1	1
196	2	2	0	250	0	1	0
197	2	3	0	251	0	1	0
198	1	3	0	252	0	1	0
199	1	2	0	253	1	1	0
200	1	3	0	254	0	1	0
201	2	2	0	255	0	0	0
202	1	3	0	256	0	0	0
203	1	2	0	257	0	1	0
204	1	3	0	258	0	0	0
205	1	2	1	259	0	0	0
206	1	2	0	260	0	0	0
207	1	2	0				
208	1	2	0				
209	1	2	0				
210	1	3	0				
211	2	2	0				
212	1	3	0				
213	1	2	0				
214	1	2	0				
215	1	2	0				
216	1	2	1				

SMA test on day 21, CH 1=Channel 1 (1000 mg/l acetate), CH 2=Channel 2 (1500 mg/l acetate), and CH 3=Channel 3 (2000 mg/l acetate).

Appendix 7

Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3
1	0	0	0	55	2	1	1	109	4	4	4
2	0	0	0	56	2	1	2	110	5	3	4
3	0	0	0	57	2	1	2	111	4	3	3
4	1	0	0	58	3	1	1	112	4	4	4
5	0	0	0	59	2	2	2	113	5	3	4
6	0	0	1	60	2	2	2	114	4	3	4
7	0	1	0	61	3	1	2	115	4	3	4
8	1	0	0	62	3	2	2	116	5	4	5
9	0	0	1	63	3	2	2	117	4	4	4
10	0	0	0	64	2	2	2	118	4	4	4
11	1	1	0	65	2	1	1	119	5	3	4
12	0	0	1	66	2	2	2	120	5	4	4
13	1	0	0	67	2	2	2	121	5	4	4
14	1	0	1	68	3	2	2	122	4	3	5
15	1	1	1	69	3	1	2	123	5	4	4
16	1	0	1	70	3	2	3	124	5	4	4
17	0	1	1	71	3	2	3	125	5	3	5
18	1	1	0	72	3	2	2	126	5	4	4
19	1	1	0	73	2	2	2	127	5	4	4
20	1	0	1	74	3	1	2	128	5	4	5
21	0	0	0	75	3	2	3	129	5	5	5
22	1	0	0	76	3	2	2	130	5	4	5
23	1	1	1	77	3	3	3	131	6	4	5
24	1	0	1	78	3	2	3	132	5	4	5
25	1	1	1	79	4	2	3	133	5	4	5
26	1	0	1	80	4	2	3	134	5	5	4
27	1	1	0	81	3	2	3	135	5	4	5
28	1	1	1	82	3	3	3	136	6	4	5
29	1	0	1	83	4	2	3	137	6	5	5
30	2	1	1	84	4	2	2	138	5	5	5
31	1	1	1	85	4	2	3	139	5	4	5
32	1	1	2	86	4	3	3	140	5	4	5
33	1	1	1	87	3	3	3	141	5	5	4
34	1	1	1	88	3	3	3	142	5	5	5
35	2	1	1	89	4	3	4	143	6	5	4
36	1	2	1	90	4	2	3	144	5	4	5
37	1	1	1	91	4	2	3	145	6	5	5
38	1	1	1	92	4	2	3	146	6	5	5
39	1	1	2	93	4	3	3	147	6	5	5
40	1	1	1	94	3	2	4	148	6	5	4
41	2	1	2	95	4	2	3	149	6	5	5
42	1	1	1	96	4	3	3	150	6	4	4
43	1	1	1	97	4	2	3	151	5	5	5
44	2	1	1	98	3	2	3	152	6	5	5
45	2	2	1	99	4	3	4	153	6	5	4
46	2	1	2	100	4	3	3	154	6	5	5
47	2	1	1	101	4	3	3	155	6	5	4
48	2	1	1	102	4	2	3	156	6	6	5
49	2	2	2	103	4	3	4	157	6	5	4
50	1	1	1	104	4	3	3	158	6	5	5
51	2	1	1	105	5	3	4	159	6	5	4
52	2	1	2	106	4	3	3	160	6	6	4
53	2	1	2	107	4	3	4	161	6	5	4
54	3	2	2	108	4	4	3	162	6	5	4

SMA test on day 30, CH 1=Channel 1 (1000 mg/l acetate), CH 2=Channel 2 (1500 mg/l acetate), and CH 3=Channel 3 (2000 mg/l acetate).

Appendix 7

Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3
163	6	5	5	217	1	3	1
164	6	6	4	218	0	4	2
165	6	5	4	219	0	4	1
166	6	5	5	220	1	4	1
167	5	6	4	221	0	3	1
168	6	5	4	222	0	4	2
169	6	6	4	223	0	3	1
170	5	5	4	224	0	4	1
171	5	6	4	225	0	3	1
172	5	5	4	226	0	4	1
173	6	6	3	227	1	4	1
174	5	6	4	228	0	4	0
175	5	5	4	229	0	3	1
176	5	6	4	230	0	3	0
177	4	6	3	231	0	3	1
178	5	5	4	232	0	3	1
179	5	6	4	233	0	2	1
180	4	6	4	234	1	3	0
181	4	5	4	235	0	3	0
182	4	5	4	236	0	2	1
183	4	6	3	237	0	2	0
184	4	5	3	238	0	2	1
185	3	5	3	239	0	2	0
186	4	6	4	240	0	3	1
187	4	6	4	241	1	2	0
188	4	6	3	242	0	2	0
189	3	6	3	243	0	2	0
190	4	6	3	244	0	1	0
191	4	5	3	245	0	2	0
192	3	6	3	246	1	2	0
193	3	6	2	247	0	2	1
194	3	5	3	248	1	1	0
195	3	6	3	249	0	2	0
196	3	5	3	250	0	2	0
197	4	5	2	251	0	2	0
198	3	6	3	252	0	2	0
199	3	5	3	253	0	1	0
200	3	5	2	254	0	2	0
201	2	5	3	255	1	1	0
202	3	4	3	256	0	2	1
203	2	4	3	257	0	2	0
204	2	4	2	258	0	1	0
205	2	5	2	259	0	1	0
206	3	5	3	250	1	1	0
207	2	4	2	261	0	1	1
208	2	5	2	262	0	0	0
209	1	4	3				
210	2	4	2				
211	2	4	1				
212	1	3	2				
213	1	3	2				
214	2	3	1				
215	1	4	1				
216	1	4	1				

SMA test on day 30. CH 1=Channel 1 (1000 mg/l acetate), CH 2=Channel 2 (1500 mg/l acetate), and CH 3=Channel 3 (2000 mg/l acetate).

Appendix 7

Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3
1	0	0	0	55	4	4	3	109	7	6	6
2	0	1	0	56	4	4	4	110	6	6	7
3	1	1	0	57	4	4	4	111	7	7	7
4	1	0	0	58	4	4	4	112	6	6	6
5	0	1	0	59	3	4	3	113	7	7	7
6	0	1	1	60	3	5	4	114	7	6	6
7	1	1	1	61	3	4	4	115	7	7	7
8	1	1	1	62	4	4	3	116	6	6	6
9	1	1	0	63	4	4	4	117	7	7	6
10	1	2	1	64	4	5	4	118	7	6	6
11	1	1	1	65	4	4	4	119	7	6	6
12	1	1	1	66	4	4	5	120	7	7	6
13	2	1	1	67	4	4	5	121	7	7	6
14	1	1	1	68	4	4	4	122	7	7	7
15	1	1	1	69	4	4	5	123	7	6	6
16	1	1	1	70	5	5	5	124	7	7	6
17	2	1	1	71	4	4	6	125	7	7	6
18	1	1	2	72	4	5	6	126	7	6	7
19	2	1	1	73	4	4	6	127	7	6	6
20	1	2	1	74	5	4	5	128	7	7	6
21	2	1	1	75	4	5	5	129	7	7	6
22	1	1	1	76	4	4	6	130	7	7	6
23	1	1	1	77	4	5	6	131	6	7	7
24	2	2	1	78	4	4	6	132	7	7	6
25	2	2	1	79	5	5	6	133	6	7	6
26	2	2	2	80	4	4	5	134	7	7	7
27	2	2	1	81	5	5	5	135	6	7	7
28	3	2	1	82	4	5	6	136	7	7	7
29	2	2	1	83	5	4	6	137	7	7	6
30	2	2	1	84	5	5	6	138	6	7	6
31	2	3	2	85	5	5	7	139	6	7	6
32	2	2	1	86	5	6	6	140	6	7	7
33	3	2	2	87	6	5	6	141	6	7	7
34	2	3	2	88	5	5	7	142	6	7	7
35	3	2	2	89	5	6	7	143	5	7	6
36	2	3	2	90	6	5	7	144	6	7	6
37	2	3	2	91	6	5	7	145	6	7	6
38	2	2	3	92	6	5	7	146	6	7	6
39	2	3	2	93	6	6	6	147	5	7	6
40	3	3	2	94	6	6	6	148	6	7	5
41	3	3	2	95	7	5	6	149	6	7	6
42	3	4	3	96	6	5	6	150	6	7	6
43	2	3	2	97	6	5	7	151	5	6	5
44	2	3	2	98	6	6	6	152	5	7	6
45	3	3	3	99	7	6	7	153	5	7	6
46	3	3	3	100	6	6	6	154	6	6	6
47	3	3	3	101	6	7	7	155	5	7	6
48	3	3	3	102	6	6	6	156	5	6	5
49	2	4	3	103	7	6	7	157	6	6	6
50	3	3	3	104	6	6	6	158	5	7	6
51	3	4	3	105	7	6	7	159	5	6	5
52	3	3	4	106	6	6	6	160	5	6	5
53	3	4	3	107	6	6	7	161	5	6	6
54	3	4	3	108	7	7	6	162	4	5	5

SMA test on day 42, CH 1=Channel 1 (1000 mg/l acetate), CH 2=Channel 2 (1500 mg/l acetate), and CH 3=Channel 3 (2000 mg/l acetate).

Appendix 7

Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3
163	5	6	5	217	0	0	3
164	5	6	5	218	0	1	3
165	4	6	5	219	0	0	2
166	4	5	5	220	0	0	3
167	5	6	6	221	1	0	3
168	4	6	5	222	0	0	3
169	4	5	5	223	0	0	2
170	4	5	5	224	0	0	2
171	4	5	5	225	0	1	1
172	3	5	5	226	0	0	1
173	4	4	6	227	1	0	1
174	4	5	5	228	0	0	2
175	3	5	5	229	0	0	1
176	3	4	5	230	0	0	1
177	3	4	4	231	0	0	0
178	4	4	5	232	0	1	1
179	3	4	5	233	1	0	0
180	3	3	4	234	0	0	0
181	3	4	5				
182	2	4	5				
183	2	3	4				
184	2	3	5				
185	2	3	4				
186	3	4	4				
187	2	3	4				
188	2	3	4				
189	2	2	4				
190	1	2	4				
191	1	3	3				
192	1	3	3				
193	1	2	4				
194	0	2	4				
195	0	2	4				
196	1	2	3				
197	0	2	4				
198	0	2	4				
199	0	1	4				
200	0	2	3				
201	0	1	3				
202	0	1	4				
203	0	1	3				
204	0	1	3				
205	0	0	3				
206	0	0	4				
207	0	1	3				
208	0	0	3				
209	0	0	3				
210	1	0	4				
211	0	0	3				
212	0	0	3				
213	1	0	3				
214	0	0	3				
215	0	0	2				
216	0	0	3				

SMA test on day 42, CH 1=Channel 1 (1000 mg/l acetate), CH 2=Channel 2 (1500 mg/l acetate), and CH 3=Channel 3 (2000 mg/l acetate).

Appendix 7

Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3
1	0	0	1	55	4	4	4	109	8	8	7
2	1	1	1	56	4	3	4	110	9	8	8
3	1	0	1	57	4	3	5	111	8	8	7
4	1	1	0	58	4	4	4	112	9	8	8
5	0	1	0	59	4	5	4	113	9	7	8
6	1	1	1	60	3	5	5	114	8	8	7
7	1	1	1	61	3	4	5	115	9	8	7
8	1	2	1	62	5	5	5	116	9	8	8
9	2	1	2	63	4	4	6	117	9	8	8
10	1	2	1	64	4	5	5	118	9	9	8
11	1	1	1	65	4	4	5	119	9	8	7
12	1	1	1	66	5	5	5	120	9	9	8
13	2	1	1	67	4	4	6	121	9	9	8
14	2	1	2	68	4	5	6	122	8	9	8
15	1	2	1	69	4	4	6	123	9	9	8
16	2	2	1	70	5	5	6	124	8	9	9
17	2	1	2	71	4	4	6	125	7	9	8
18	1	1	1	72	5	5	7	126	8	9	9
19	2	2	1	73	5	5	6	127	6	9	8
20	2	1	2	74	5	5	7	128	6	9	9
21	2	2	2	75	4	5	6	129	5	9	9
22	2	2	2	76	4	5	6	130	5	9	9
23	2	2	1	77	5	6	6	131	6	9	8
24	2	2	1	78	5	5	6	132	5	9	9
25	3	2	2	79	5	6	6	133	4	9	9
26	2	3	2	80	5	5	6	134	4	9	9
27	2	3	2	81	6	6	6	135	2	9	9
28	3	2	1	82	5	5	7	136	2	9	9
29	2	3	2	83	5	6	6	137	2	9	9
30	2	3	1	84	6	5	6	138	1	9	9
31	2	3	2	85	5	6	7	139	1	9	9
32	2	3	2	86	6	6	7	140	0	9	9
33	3	3	2	87	6	6	7	141	0	9	9
34	2	4	3	88	6	5	7	142	0	8	9
35	3	4	2	89	7	6	6	143	0	9	9
36	3	3	2	90	6	6	7	144	0	8	9
37	3	3	3	91	6	6	7	145	0	8	9
38	2	3	3	92	7	7	7	146	0	7	9
39	2	3	2	93	7	7	7	147	0	8	9
40	3	4	3	94	7	6	6	148	0	7	9
41	3	4	3	95	7	7	6	149	0	8	9
42	3	4	3	96	8	7	6	150	0	7	9
43	3	3	3	97	7	7	7	151	0	7	9
44	2	4	3	98	7	8	7	152	0	7	8
45	3	4	4	99	7	7	7	153	0	7	9
46	3	4	4	100	8	8	7	154	0	6	8
47	3	4	4	101	8	8	6	155	0	7	8
48	3	4	3	102	7	8	6	156	0	7	9
49	3	4	3	103	8	7	6	157	0	7	8
50	4	3	4	104	8	7	6	158	0	6	9
51	4	4	4	105	8	8	6	159	0	6	8
52	4	3	4	106	7	8	7	160	0	5	9
53	3	4	5	107	8	8	7	161	0	6	8
54	3	4	5	108	8	7	7	162	1	6	8

SMA test on day 56, CH 1=Channel 1 (2000 mg/l acetate), CH 2=Channel 2 (3000 mg/l acetate), and CH 3=Channel 3 (4000 mg/l acetate).

Appendix 7

Time (h)	CH 1	CH 2	CH 3
163	0	6	7
164	0	5	8
165	0	5	8
166	0	5	7
167	0	6	8
168	0	4	7
169	1	4	7
170	0	4	7
171	0	4	6
172	0	3	6
173	0	3	7
174	0	2	7
175	0	2	6
176	1	2	7
177	0	2	6
178	0	2	6
179	0	2	6
180	0	1	6
181	0	2	5
182	0	1	6
183	0	1	5
184	1	1	6
185	0	1	5
186	0	1	5
187	0	0	5
188	0	0	6
189	0	0	5
190	0	0	4
191	1	0	4
192	0	0	4
193	0	1	4
194	1	0	3
195	0	0	3
196	0	0	2
197	0	0	2
198	0	0	2
199	0	1	2
200	0	0	1
201	0	0	2
202	0	0	2
203	1	0	2
204	0	0	1
205	0	0	1
206	0	0	0
207	0	0	1
208	0	0	0

SMA test on day 56. CH 1=Channel 1 (2000 mg/l acetate), CH 2=Channel 2 (3000 mg/l acetate), and CH 3=Channel 3 (4000 mg/l acetate).

Appendix 7

Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3
1	2	2	1	55	6	7	7	109	11	11	9
2	1	2	1	56	7	7	7	110	11	11	9
3	2	1	2	57	6	8	7	111	11	11	9
4	2	2	2	58	6	7	7	112	11	11	9
5	2	2	1	59	7	7	7	113	11	11	10
6	1	2	2	60	6	7	6	114	11	11	9
7	2	2	2	61	6	7	7	115	11	11	9
8	2	3	3	62	7	7	7	116	11	11	10
9	2	2	3	63	6	8	7	117	11	11	10
10	2	2	2	64	7	7	7	118	11	11	9
11	3	3	3	65	7	7	8	119	11	11	10
12	3	3	3	66	7	7	8	120	11	11	10
13	2	3	4	67	7	8	8	121	11	11	10
14	3	3	4	68	6	7	8	122	11	10	10
15	3	4	3	69	7	8	8	123	11	11	10
16	3	4	3	70	7	7	7	124	11	10	9
17	2	4	4	71	8	8	8	125	11	11	10
18	3	3	4	72	7	8	8	126	11	11	10
19	3	3	3	73	8	8	8	127	11	10	10
20	4	4	4	74	8	8	7	128	11	10	10
21	3	4	4	75	8	9	8	129	10	11	9
22	4	4	5	76	8	9	8	130	10	10	10
23	4	5	4	77	8	8	8	131	11	10	10
24	4	5	4	78	7	9	8	132	10	10	10
25	3	5	5	79	8	9	7	133	10	11	9
26	4	5	5	80	8	9	8	134	9	10	9
27	4	4	4	81	9	10	8	135	10	10	9
28	4	4	5	82	9	9	8	136	9	10	10
29	5	5	5	83	9	10	9	137	10	10	10
30	4	5	5	84	8	10	8	138	9	11	9
31	5	5	4	85	9	9	8	139	9	10	9
32	4	5	5	86	8	10	8	140	8	10	9
33	4	5	5	87	9	10	8	141	9	10	9
34	5	5	5	88	9	10	9	142	8	10	10
35	4	5	6	89	10	11	8	143	8	10	9
36	5	6	6	90	9	10	8	144	9	9	9
37	4	5	5	91	10	10	8	145	8	10	9
38	5	5	5	92	9	10	8	146	7	10	9
39	5	5	6	93	9	11	9	147	8	10	9
40	4	6	6	94	10	11	8	148	7	10	9
41	5	5	6	95	10	11	8	149	7	9	8
42	5	5	7	96	10	11	8	150	7	10	9
43	5	6	6	97	9	11	8	151	6	10	9
44	6	6	6	98	10	11	9	152	7	9	9
45	5	6	6	99	10	11	9	153	7	9	8
46	5	5	7	100	10	11	8	154	6	10	9
47	6	6	7	101	11	11	8	155	7	9	9
48	6	7	7	102	10	11	9	156	6	9	8
49	5	6	6	103	10	11	9	157	6	10	8
50	6	7	6	104	10	11	9	158	5	9	8
51	6	6	6	105	11	11	9	159	5	9	9
52	5	6	6	106	10	11	9	160	4	9	8
53	6	7	6	107	11	11	9	161	4	8	8
54	6	7	6	108	11	11	9	162	4	9	8

SMA test on day 77. CH 1=Channel 1 (2000 mg/l acetate), CH 2=Channel 2 (3000 mg/l acetate), and CH 3=Channel 3 (4000 mg/l acetate).

Appendix 7

Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3
163	3	9	8	217	0	0	5
164	4	8	9	218	0	0	5
165	3	8	8	219	0	0	5
166	3	8	8	220	0	0	4
167	2	8	8	221	0	1	4
168	3	8	7	222	1	0	4
169	1	9	8	223	0	0	3
170	2	9	8	224	0	0	4
171	1	8	8	225	0	0	3
172	2	8	7	226	0	0	3
173	1	7	8	227	0	0	2
174	1	7	8	228	0	0	2
175	0	7	8	229	0	0	3
176	1	7	8	230	0	0	2
177	0	6	7	231	0	0	2
178	0	6	8	232	0	0	1
179	0	7	8	233	0	0	2
180	0	6	7	234	0	0	1
181	0	6	7	235	0	1	1
182	0	5	7				
183	0	5	7				
184	0	5	8				
185	0	6	7				
186	0	5	7				
187	0	5	7				
188	0	4	7				
189	0	5	7				
190	1	4	7				
191	0	4	8				
192	0	3	7				
193	0	3	7				
194	0	4	7				
195	0	3	6				
196	0	4	7				
197	0	3	7				
198	1	2	7				
199	0	2	7				
200	0	2	6				
201	0	2	7				
202	1	3	6				
203	1	2	7				
204	0	2	6				
205	1	2	7				
206	0	2	6				
207	0	2	6				
208	0	1	6				
209	0	1	6				
210	0	1	6				
211	0	0	7				
212	0	0	6				
213	0	1	6				
214	1	0	5				
215	0	0	5				
216	0	0	6				

SMA test on day 77, CH 1=Channel 1 (2000 mg/l acetate), CH 2=Channel 2 (3000 mg/l acetate), and CH 3=Channel 3 (4000 mg/l acetate).

Appendix 7

Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3
1	3	5	3	55	11	9	8	109	8	12	11
2	2	5	3	56	11	9	7	110	7	12	12
3	3	4	4	57	10	9	7	111	6	12	11
4	3	3	4	58	11	10	8	112	5	12	11
5	2	4	4	59	11	9	8	113	5	12	11
6	3	4	3	60	11	9	8	114	4	12	11
7	3	4	3	61	11	10	9	115	4	12	12
8	4	5	3	62	10	9	8	116	4	12	11
9	4	5	4	63	11	10	8	117	3	12	11
10	4	5	3	64	11	9	8	118	2	12	11
11	3	6	3	65	11	10	9	119	1	12	12
12	4	6	4	66	12	10	8	120	1	12	11
13	4	5	4	67	11	10	8	121	0	12	11
14	4	6	4	68	11	9	8	122	0	12	12
15	5	6	3	69	11	10	9	123	0	12	11
16	5	6	4	70	12	10	9	124	0	12	11
17	5	7	4	71	11	10	9	125	1	11	11
18	4	6	5	72	11	10	9	126	0	12	11
19	5	6	4	73	11	11	8	127	0	11	11
20	5	6	5	74	12	10	9	128	0	12	11
21	5	7	5	75	11	10	9	129	0	11	11
22	6	6	5	76	11	10	8	130	0	11	10
23	5	6	6	77	12	10	9	131	0	11	11
24	5	7	5	78	11	11	9	132	0	10	11
25	6	7	6	79	12	10	9	133	0	11	10
26	6	7	6	80	11	11	10	134	0	10	11
27	6	7	6	81	12	10	9	135	1	10	10
28	7	8	7	82	12	11	9	136	0	9	11
29	6	7	6	83	12	10	10	137	0	10	10
30	7	7	6	84	12	11	9	138	0	9	11
31	7	8	7	85	12	11	9	139	0	10	10
32	7	8	6	86	12	11	10	140	0	9	10
33	8	7	6	87	12	11	10	141	0	9	11
34	7	8	7	88	12	11	10	142	0	9	10
35	7	8	6	89	12	12	11	143	0	8	10
36	8	7	6	90	12	11	10	144	0	9	11
37	8	8	7	91	12	11	10	145	1	8	10
38	9	8	7	92	12	12	10	146	0	8	10
39	8	8	7	93	11	11	10	147	0	9	10
40	8	9	7	94	12	11	11	148	0	8	10
41	9	8	6	95	11	11	11	149	0	8	11
42	9	8	7	96	11	12	11	150	0	7	10
43	9	8	7	97	10	11	11	151	0	7	10
44	10	9	7	98	11	12	11	152	0	6	10
45	9	8	6	99	10	11	10	153	0	5	10
46	9	8	7	100	10	12	11	154	0	5	10
47	10	9	7	101	10	11	11	155	0	4	9
48	9	8	8	102	9	12	11	156	0	3	10
49	10	9	7	103	10	11	10	157	0	3	10
50	10	8	7	104	9	12	11	158	1	2	9
51	10	9	7	105	9	12	11	159	0	2	9
52	11	9	8	106	9	12	11	160	0	2	9
53	10	9	7	107	8	12	11	161	0	1	9
54	10	10	7	108	9	12	11	162	0	1	10

SMA test on day 96. CH 1=Channel 1 (2000 mg/l acetate), CH 2=Channel 2 (3000 mg/l acetate), and CH 3=Channel 3 (4000 mg/l acetate).

Appendix 7

Time (h)	CH 1	CH 2	CH 3
163	0	0	9
164	0	0	9
165	0	0	9
166	0	0	9
167	0	0	9
168	1	1	8
169	0	0	8
170	0	0	9
171	0	0	8
172	0	0	8
173	0	0	7
174	0	0	8
175	0	0	7
176	0	0	8
177	0	0	7
178	1	1	7
179	0	0	6
180	0	0	5
181	0	0	6
182	0	0	5
183	0	0	6
184	0	0	5
185	0	0	4
186	0	0	5
187	0	0	4
188	1	1	5
189	0	0	4
190	0	0	3
191	0	0	3
192	0	0	3
193	0	0	3
194	0	0	3
195	1	0	2
196	0	0	2
197	0	0	2
198	0	0	1
199	0	0	0

SMA test on day 96, CH 1=Channel 1 (2000 mg/l acetate), CH 2=Channel 2 (3000 mg/l acetate), and CH 3=Channel 3 (4000 mg/l acetate).

Appendix 7

Time (d)	CH 1	CH 2	CH 3	Time (d)	CH 1	CH 2	CH 3	Time (d)	CH 1	CH 2	CH 3
1	2	4	5	55	10	9	8	109	5	12	11
2	3	3	4	56	11	9	9	110	4	12	12
3	2	3	4	57	11	10	8	111	3	12	11
4	2	3	5	58	11	9	8	112	2	12	11
5	2	3	5	59	11	10	9	113	3	12	12
6	3	4	5	60	10	9	8	114	2	12	11
7	3	4	4	61	11	10	9	115	1	12	12
8	3	4	5	62	11	9	8	116	0	11	11
9	3	3	4	63	11	10	9	117	0	12	11
10	3	4	5	64	11	10	8	118	0	11	12
11	4	4	4	65	12	10	9	119	0	10	12
12	4	4	5	66	11	10	9	120	1	11	11
13	4	5	5	67	11	10	9	121	0	10	11
14	4	5	5	68	11	11	10	122	0	11	12
15	4	5	5	69	12	10	9	123	0	10	11
16	5	4	6	70	11	10	9	124	0	10	11
17	4	5	5	71	11	10	9	125	1	9	12
18	4	5	6	72	12	10	10	126	0	10	11
19	5	6	5	73	11	11	9	127	0	9	11
20	5	6	6	74	12	10	9	128	0	9	12
21	5	5	5	75	11	10	10	129	0	9	11
22	5	6	6	76	12	10	9	130	0	8	12
23	5	6	6	77	11	10	10	131	0	9	11
24	6	6	6	78	12	11	9	132	0	9	11
25	5	7	5	79	11	10	10	133	0	8	11
26	6	7	6	80	12	10	10	134	0	8	12
27	6	7	6	81	12	10	10	135	1	7	11
28	6	7	6	82	12	11	9	136	0	8	12
29	6	8	6	83	12	10	10	137	0	7	11
30	7	7	5	84	12	10	10	138	0	8	12
31	7	7	6	85	12	11	10	139	0	7	11
32	7	7	6	86	12	10	10	140	0	6	12
33	7	8	6	87	12	11	11	141	0	6	11
34	7	7	7	88	12	11	10	142	0	5	11
35	8	8	6	89	12	11	10	143	0	6	10
36	7	7	6	90	12	11	10	144	0	5	10
37	7	8	7	91	12	12	11	145	1	5	11
38	8	7	7	92	12	11	10	146	0	4	10
39	8	8	8	93	12	11	10	147	0	5	11
40	8	8	7	94	12	11	11	148	0	4	11
41	8	8	7	95	12	12	10	149	0	3	10
42	9	8	7	96	11	11	10	150	0	3	10
43	9	9	8	97	11	12	11	151	0	3	9
44	9	8	7	98	10	12	10	152	0	2	10
45	10	8	7	99	9	12	10	153	0	2	10
46	10	8	7	100	9	12	11	154	0	2	9
47	9	9	7	101	8	12	10	155	0	1	10
48	10	8	7	102	7	12	10	156	0	1	9
49	10	8	8	103	7	12	11	157	0	1	9
50	10	9	7	104	6	12	11	158	1	2	9
51	11	8	8	105	5	12	11	159	0	1	9
52	10	9	8	106	6	12	12	160	0	1	10
53	11	8	8	107	5	12	11	161	0	0	9
54	11	9	8	108	4	12	11	162	0	1	9

SMA test on day 119, CH 1=Channel 1 (2000 mg/l acetate), CH 2=Channel 2 (3000 mg/l acetate), and CH 3=Channel 3 (4000 mg/l acetate)

Appendix 7

Time (d)	CH 1	CH 2	CH 3
163	0	0	9
164	0	0	8
165	0	0	9
166	0	0	8
167	0	0	8
168	1	1	8
169	0	0	7
170	0	0	8
171	0	0	8
172	0	0	8
173	0	0	7
174	0	0	8
175	0	0	8
176	0	0	7
177	0	0	7
178	1	1	6
179	0	0	6
180	0	0	5
181	0	0	5
182	0	0	4
183	0	0	4
184	0	0	3
185	0	0	2
186	0	1	2
187	0	0	1
188	1	0	0

SMA test on day 119, CH 1=Channel 1 (2000 mg/l acetate), CH 2=Channel 2 (3000 mg/l acetate), and CH 3=Channel 3 (4000 mg/l acetate).

Appendix 7

Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3
1	8	6	9	55	12	11	10	109	0	14	13
2	7	6	8	56	12	11	10	110	0	13	14
3	6	6	6	57	13	11	10	111	0	13	14
4	6	5	6	58	12	12	11	112	0	12	14
5	7	6	6	59	12	11	10	113	0	12	14
6	7	6	6	60	13	12	11	114	0	11	13
7	7	7	6	61	12	12	10	115	1	10	14
8	7	7	6	62	13	12	11	116	0	10	14
9	8	7	6	63	12	12	11	117	1	9	14
10	7	6	6	64	13	12	11	118	0	8	14
11	8	7	6	65	13	13	12	119	0	9	14
12	8	7	6	66	13	12	11	120	0	7	14
13	8	7	7	67	13	12	11	121	0	7	14
14	8	7	6	68	13	13	12	122	1	6	14
15	8	8	6	69	14	13	11	123	0	5	14
16	9	7	6	70	13	13	11	124	0	5	14
17	9	7	6	71	14	12	11	125	0	4	14
18	9	7	6	72	13	13	12	126	0	4	14
19	9	8	7	73	14	13	11	127	0	4	14
20	9	8	6	74	14	13	11	128	0	3	14
21	8	8	6	75	14	12	12	129	0	2	14
22	9	7	7	76	14	13	12	130	0	2	14
23	9	8	6	77	14	13	12	131	0	1	14
24	9	8	6	78	14	13	11	132	1	1	14
25	9	8	7	79	14	13	12	133	0	1	14
26	10	8	6	80	14	14	12	134	0	1	13
27	10	8	7	81	13	13	12	135	0	1	14
28	10	9	7	82	13	13	12	136	0	1	13
29	10	8	7	83	12	13	11	137	1	0	14
30	10	9	6	84	11	14	12	138	0	0	13
31	11	9	7	85	10	13	12	139	0	0	13
32	11	8	6	86	8	13	12	140	0	0	13
33	11	9	7	87	7	14	13	141	0	0	12
34	10	9	7	88	7	13	12	142	0	1	13
35	11	9	7	89	6	14	13	143	0	1	12
36	11	10	8	90	5	14	13	144	0	0	12
37	11	9	8	91	4	14	13	145	1	0	12
38	11	10	7	92	5	13	12	146	0	1	11
39	11	10	8	93	3	14	13	147	0	0	11
40	12	10	8	94	2	14	13	148	0	1	11
41	11	9	8	95	2	14	13	149	0	0	11
42	11	10	8	96	1	14	13	150	0	0	10
43	12	10	8	97	0	14	13	151	0	1	10
44	11	10	9	98	0	14	14	152	0	0	9
45	12	11	8	99	0	14	13	153	0	0	10
46	11	10	9	100	1	14	13	154	0	0	10
47	11	11	9	101	0	14	14	155	0	0	9
48	12	10	9	102	0	14	13	156	0	0	9
49	12	11	9	103	0	14	13	157	1	0	8
50	12	11	10	104	0	14	14	158	0	0	8
51	12	11	9	105	1	14	13	159	0	0	7
52	11	11	10	106	0	14	14	160	0	1	8
53	12	11	9	107	0	14	13	161	0	0	6
54	12	12	10	108	0	13	14	162	1	0	7

SMA test on day 144, CH 1=Channel 1 (2000 mg/l acetate), CH 2=Channel 2 (3000 mg l acetate), and CH 3=Channel 3 (4000 mg/l acetate).

Appendix 7

Time (h)	CH 1	CH 2	CH 3
163	0	0	6
164	0	0	5
165	0	1	5
166	0	0	4
167	0	0	4
168	0	0	3
169	0	0	4
170	0	0	3
171	1	0	3
172	0	0	2
173	0	0	2
174	0	1	1
175	0	0	1
176	1	0	1
177	0	0	0

SMA test on day 144, CH 1=Channel 1 (2000 mg/l acetate), CH 2=Channel 2 (3000 mg/l acetate), and CH 3=Channel 3 (4000 mg/l acetate).

Appendix 7

Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3
1	12	14	11	55	16	17	16	109	0	0	16
2	11	13	12	56	14	17	16	110	0	1	15
3	12	13	12	57	13	17	16	111	0	0	14
4	12	14	12	58	11	17	17	112	0	0	15
5	13	15	12	59	9	17	16	113	0	0	14
6	13	15	12	60	7	17	16	114	0	0	14
7	13	15	13	61	5	17	16	115	1	0	13
8	14	16	13	62	2	17	17	116	0	0	13
9	14	15	12	63	2	17	16	117	1	0	13
10	14	16	13	64	1	17	16	118	0	0	12
11	15	15	13	65	1	17	16	119	0	0	12
12	14	16	13	66	0	16	17	120	0	1	11
13	15	16	13	67	0	17	16	121	0	0	10
14	15	15	13	68	0	16	16	122	1	0	9
15	15	16	14	69	0	17	17	123	0	1	9
16	16	16	13	70	1	16	16	124	0	0	7
17	16	16	13	71	0	16	17	125	1	1	6
18	16	16	13	72	0	15	16	126	0	0	5
19	15	16	14	73	0	16	17	127	1	0	6
20	16	16	14	74	0	15	16	128	0	0	4
21	16	17	14	75	1	14	17	129	0	0	4
22	16	16	14	76	0	14	16	130	0	1	3
23	16	16	15	77	0	13	17	131	0	0	3
24	16	16	14	78	0	12	17	132	1	0	3
25	17	17	14	79	0	13	17	133	0	0	2
26	16	16	15	80	0	12	17	134	0	1	1
27	16	16	15	81	0	11	17	135	0	0	1
28	16	16	15	82	0	10	17	136	0	0	0
29	17	17	14	83	0	10	17				
30	16	16	15	84	0	9	17				
31	16	16	15	85	1	8	17				
32	16	16	15	86	0	6	17				
33	16	17	15	87	1	4	17				
34	17	16	15	88	0	3	17				
35	16	16	15	89	0	3	17				
36	16	16	16	90	0	2	17				
37	16	17	14	91	0	2	17				
38	17	16	16	92	1	1	17				
39	16	16	16	93	0	1	17				
40	16	17	15	94	0	0	17				
41	17	16	16	95	0	0	17				
42	16	17	16	96	0	0	17				
43	17	16	15	97	0	0	16				
44	16	17	16	98	0	0	16				
45	17	16	16	99	0	0	17				
46	16	17	16	100	0	0	16				
47	17	16	16	101	0	0	16				
48	17	17	15	102	1	0	17				
49	17	16	16	103	0	1	16				
50	17	17	16	104	0	0	16				
51	17	16	16	105	1	1	16				
52	17	17	15	106	0	0	15				
53	17	17	16	107	0	0	16				
54	16	17	16	108	0	0	15				

SMA test on day 184. CH 1=Channel 1 (2000 mg/l acetate), CH 2=Channel 2 (3000 mg/l acetate), and CH 3=Channel 3 (4000 mg/l acetate).

Appendix 7

Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3
1	15	14	13	55	0	18	17	109	0	0	11
2	14	13	14	56	0	17	17	110	0	1	10
3	14	13	14	57	0	18	18	111	0	0	9
4	14	13	14	58	0	18	17	112	0	0	9
5	13	14	15	59	0	18	17	113	0	0	8
6	14	13	15	60	1	18	18	114	0	0	7
7	13	14	15	61	1	18	17	115	1	0	7
8	14	14	16	62	0	18	18	116	0	0	7
9	13	14	15	63	0	18	17	117	1	0	5
10	14	15	16	64	0	18	18	118	0	0	4
11	14	14	16	65	0	18	17	119	0	0	3
12	15	14	16	66	0	17	18	120	0	1	3
13	14	15	15	67	0	18	18	121	0	0	2
14	15	14	16	68	0	17	18	122	1	0	2
15	15	14	16	69	0	16	17	123	0	0	1
16	16	15	16	70	1	17	18	124	0	0	0
17	15	14	16	71	0	16	18				
18	16	15	15	72	0	15	18				
19	16	15	16	73	0	16	18				
20	17	15	16	74	0	14	18				
21	17	14	16	75	1	14	18				
22	16	15	16	76	0	13	18				
23	16	15	15	77	0	11	18				
24	16	15	16	78	0	10	18				
25	16	16	16	79	0	9	18				
26	17	15	16	80	0	9	18				
27	16	15	17	81	0	8	18				
28	17	15	16	82	0	6	18				
29	17	16	16	83	0	6	18				
30	17	16	16	84	0	6	18				
31	17	16	17	85	1	5	18				
32	16	16	16	86	0	3	18				
33	17	15	16	87	1	2	18				
34	16	16	16	88	0	2	17				
35	17	16	17	89	0	2	18				
36	16	17	16	90	0	1	17				
37	17	16	16	91	0	0	17				
38	16	16	17	92	1	0	17				
39	17	17	17	93	0	1	18				
40	16	17	17	94	0	0	17				
41	17	17	16	95	0	0	17				
42	17	18	17	96	0	0	17				
43	17	17	17	97	0	0	16				
44	17	17	17	98	0	0	17				
45	17	18	17	99	0	0	16				
46	16	17	16	100	0	0	17				
47	15	17	17	101	0	0	16				
48	11	18	17	102	1	0	15				
49	9	17	17	103	0	1	16				
50	6	18	18	104	0	0	15				
51	5	17	17	105	1	1	15				
52	3	18	17	106	0	0	14				
53	3	17	17	107	0	0	13				
54	1	18	18	108	0	0	13				

SMA test on day 213, CH 1=Channel 1 (2000 mg/l acetate), CH 2=Channel 2 (3000 mg/l acetate), and CH 3=Channel 3 (4000 mg/l acetate).

Appendix 7

Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3
1	16	18	17	55	4	20	20	109	0	0	6
2	16	17	15	56	3	20	19	110	0	1	5
3	16	16	15	57	3	20	20	111	0	0	3
4	17	17	15	58	2	20	19	112	0	0	3
5	16	17	16	59	2	20	20	113	0	0	3
6	17	17	15	60	2	20	20	114	0	0	2
7	16	17	16	61	1	20	19	115	1	0	1
8	16	18	16	62	0	20	20	116	0	0	2
9	17	17	16	63	0	20	20	117	1	0	1
10	17	17	17	64	0	20	20	118	0	0	1
11	17	17	16	65	0	19	20	119	0	0	0
12	18	18	17	66	0	20	20				
13	17	18	17	67	0	19	20				
14	17	18	17	68	0	20	20				
15	17	18	18	69	0	19	20				
16	18	18	18	70	0	19	20				
17	17	17	17	71	0	18	20				
18	17	18	18	72	0	19	20				
19	18	18	18	73	0	18	20				
20	17	18	18	74	0	17	20				
21	18	18	18	75	0	16	20				
22	18	18	17	76	0	16	20				
23	18	17	18	77	0	15	20				
24	18	18	18	78	0	14	20				
25	17	18	18	79	1	12	20				
26	18	18	19	80	0	11	20				
27	18	19	18	81	1	10	19				
28	19	18	18	82	0	8	20				
29	18	18	18	83	0	8	19				
30	18	18	18	84	0	8	20				
31	18	19	19	85	0	7	19				
32	19	18	18	86	0	6	19				
33	18	18	19	87	1	4	19				
34	18	19	18	88	0	4	18				
35	19	18	18	89	0	3	19				
36	19	19	18	90	0	2	18				
37	18	18	19	91	0	1	17				
38	19	19	18	92	1	1	18				
39	20	18	19	93	0	1	17				
40	19	19	19	94	0	0	17				
41	20	19	18	95	0	1	16				
42	19	19	18	96	0	0	16				
43	20	18	19	97	0	0	15				
44	19	19	19	98	0	0	14				
45	20	19	19	99	0	0	15				
46	19	19	19	100	0	0	14				
47	18	20	19	101	0	0	13				
48	17	19	18	102	1	0	12				
49	15	20	19	103	0	1	10				
50	11	20	19	104	0	0	10				
51	10	19	19	105	1	1	9				
52	9	20	19	106	0	0	9				
53	7	20	20	107	0	0	9				
54	6	20	19	108	0	0	7				

SMA test on day 259, CH 1=Channel 1 (2000 mg/l acetate), CH 2=Channel 2 (3000 mg/l acetate), and CH 3=Channel 3 (4000 mg/l acetate).

Appendix 7

Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3
1	18	19	17	55	0	21	21	109	0	0	2
2	18	19	16	56	0	21	20	110	0	1	4
3	18	19	14	57	0	20	21	111	0	0	2
4	17	18	15	58	0	21	21	112	0	0	2
5	18	17	15	59	0	20	21	113	0	0	1
6	18	18	16	60	0	19	21	114	0	0	1
7	17	18	16	61	0	19	21	115	1	0	0
8	18	18	17	62	0	18	21				
9	18	18	17	63	1	16	21				
10	19	19	16	64	0	16	21				
11	19	18	17	65	0	15	21				
12	19	18	17	66	0	16	21				
13	19	18	18	67	0	14	21				
14	18	19	17	68	1	13	21				
15	19	19	18	69	0	11	21				
16	19	19	18	70	1	10	21				
17	18	18	18	71	0	9	21				
18	19	19	17	72	0	7	21				
19	19	19	18	73	0	6	21				
20	19	19	18	74	1	5	21				
21	20	20	18	75	0	3	20				
22	19	19	19	76	1	3	21				
23	19	19	19	77	0	2	20				
24	20	19	18	78	0	2	21				
25	19	19	19	79	0	1	20				
26	20	20	19	80	1	0	21				
27	20	19	19	81	0	0	20				
28	20	19	18	82	0	0	19				
29	20	19	19	83	0	0	20				
30	21	20	19	84	1	0	19				
31	20	19	20	85	0	1	19				
32	21	19	19	86	0	0	18				
33	20	20	19	87	0	0	19				
34	20	19	20	88	0	1	18				
35	20	19	19	89	0	0	17				
36	20	20	20	90	0	0	18				
37	19	19	19	91	0	1	16				
38	20	20	20	92	1	0	15				
39	18	19	19	93	0	1	16				
40	17	20	20	94	0	0	15				
41	15	20	20	95	0	1	14				
42	12	20	20	96	0	0	13				
43	10	20	19	97	0	0	13				
44	9	21	20	98	0	0	12				
45	7	20	20	99	0	0	10				
46	6	21	20	100	0	0	10				
47	4	20	21	101	0	0	9				
48	2	21	20	102	1	0	9				
49	2	21	20	103	0	1	7				
50	1	21	20	104	0	0	5				
51	1	21	21	105	1	1	5				
52	1	21	20	106	0	0	4				
53	0	21	21	107	0	0	3				
54	0	21	20	108	0	0	3				

SMA test on day 324. CH 1=Channel 1 (2000 mg/l acetate), CH 2=Channel 2 (3000 mg/l acetate), and CH 3=Channel 3 (4000 mg/l acetate).

Appendix 7

Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3
1	14	16	17	55	1	20	19	109	0	0	9
2	14	15	16	56	1	20	19	110	0	1	8
3	13	15	14	57	0	19	20	111	0	0	7
4	14	16	14	58	1	20	19	112	0	0	5
5	14	16	15	59	0	20	20	113	0	0	2
6	15	15	14	60	1	20	19	114	0	0	2
7	14	16	15	61	0	19	20	115	1	0	1
8	15	16	16	62	0	20	19	116	0	0	1
9	15	16	16	63	0	20	20	117	1	0	0
10	15	16	15	64	1	20	19				
11	16	17	16	65	0	20	20				
12	15	16	16	66	0	20	19				
13	16	16	16	67	0	20	20				
14	16	17	17	68	0	20	20				
15	16	16	16	69	1	20	19				
16	15	17	17	70	0	20	19				
17	16	16	17	71	1	20	20				
18	16	17	17	72	0	20	19				
19	16	17	16	73	0	19	20				
20	17	18	17	74	0	17	19				
21	17	17	17	75	0	18	20				
22	18	17	18	76	0	17	19				
23	17	17	17	77	1	15	20				
24	17	18	17	78	0	15	20				
25	18	17	18	79	1	14	20				
26	18	17	18	80	0	13	20				
27	18	18	18	81	0	12	20				
28	17	17	18	82	0	11	20				
29	18	18	19	83	0	11	20				
30	17	18	18	84	1	10	20				
31	17	18	18	85	0	9	20				
32	18	17	18	86	0	9	20				
33	17	18	19	87	0	7	20				
34	18	18	18	88	1	6	20				
35	18	18	19	89	1	7	20				
36	18	19	18	90	0	4	20				
37	18	18	19	91	0	3	19				
38	19	19	19	92	1	3	20				
39	18	18	19	93	0	2	19				
40	19	19	19	94	0	1	19				
41	19	19	18	95	0	2	18				
42	19	19	19	96	0	1	17				
43	17	20	19	97	0	0	18				
44	14	19	18	98	0	1	17				
45	12	20	19	99	0	1	16				
46	10	20	19	100	0	0	17				
47	8	21	19	101	0	0	16				
48	7	19	19	102	1	0	15				
49	6	20	19	103	0	1	14				
50	4	19	20	104	0	0	15				
51	3	19	19	105	1	1	14				
52	3	20	19	106	0	0	12				
53	2	19	19	107	0	0	11				
54	2	19	20	108	0	0	9				

SMA test on day 352, CH 1=Channel 1 (2000 mg/l acetate), CH 2=Channel 2 (3000 mg/l acetate), and CH 3=Channel 3 (4000 mg/l acetate).

Appendix 7

Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3
1	16	15	13	55	16	18	16
2	15	14	12	56	15	18	17
3	15	13	12	57	12	18	17
4	14	13	12	58	11	18	17
5	14	13	12	59	9	18	17
6	15	13	12	60	8	18	18
7	15	14	13	61	6	18	17
8	14	13	13	62	5	18	17
9	15	14	12	63	5	18	18
10	15	14	13	64	4	18	18
11	16	14	13	65	2	17	17
12	15	14	14	66	2	18	18
13	16	15	12	67	1	17	18
14	16	14	13	68	0	18	18
15	15	14	14	69	1	17	18
16	16	14	13	70	0	16	18
17	16	15	14	71	0	15	18
18	17	14	14	72	0	16	18
19	16	15	14	73	0	14	18
20	16	15	14	74	1	13	18
21	16	15	13	75	0	13	18
22	16	16	14	76	1	11	18
23	17	16	14	77	0	9	18
24	16	15	15	78	0	9	18
25	16	16	14	79	0	8	18
26	17	16	14	80	0	6	18
27	17	16	15	81	0	5	17
28	17	15	14	82	1	6	18
29	16	16	15	83	0	3	17
30	17	16	14	84	1	2	16
31	16	17	15	85	0	2	17
32	17	16	14	86	0	1	16
33	17	17	15	87	0	2	17
34	18	16	15	88	0	1	15
35	17	16	15	89	1	0	15
36	17	17	14	90	0	0	14
37	18	17	15	91	0	0	14
38	17	17	15	92	1	1	13
39	17	16	15	93	0	0	14
40	18	17	16	94	0	0	12
41	18	16	15	95	0	1	10
42	18	17	15	96	0	0	10
43	17	17	16	97	0	0	8
44	18	17	16	98	0	0	7
45	18	18	16	99	0	1	6
46	18	17	15	100	0	0	6
47	18	17	16	101	0	0	5
48	18	18	16	102	1	0	3
49	18	17	16	103	0	1	3
50	18	18	16	104	0	0	2
51	17	18	17	105	1	0	2
52	17	18	16	106	0	0	2
53	16	17	16	107	0	1	1
54	17	18	17	108	0	0	2

SMA test on day 372, CH 1=Channel 1 (2000 mg/l acetate), CH 2=Channel 2 (3000 mg/l acetate), and CH 3=Channel 3 (4000 mg/l acetate).

Appendix 7

Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3
1	16	12	13	55	16	17	14
2	15	11	11	56	15	16	15
3	14	12	10	57	16	16	15
4	12	12	10	58	14	17	15
5	11	13	11	59	15	17	15
6	11	12	10	60	14	17	16
7	12	13	11	61	13	16	15
8	13	13	11	62	13	17	15
9	12	12	12	63	12	16	16
10	13	13	11	64	10	17	15
11	12	13	11	65	10	17	16
12	13	14	12	66	8	17	15
13	13	14	11	67	6	17	16
14	14	13	12	68	6	17	16
15	14	14	11	69	5	17	16
16	14	14	12	70	6	17	16
17	15	14	13	71	4	17	17
18	14	13	12	72	4	17	16
19	14	14	13	73	3	17	16
20	15	14	13	74	2	17	17
21	14	14	12	75	3	17	16
22	15	14	13	76	1	16	16
23	15	15	13	77	2	17	17
24	15	14	13	78	1	16	16
25	16	14	13	79	0	16	17
26	15	15	14	80	0	17	16
27	16	14	13	81	0	15	17
28	15	15	13	82	0	16	17
29	15	15	14	83	0	14	17
30	16	15	14	84	0	14	17
31	16	15	13	85	1	13	17
32	16	15	14	86	0	13	17
33	15	14	14	87	1	11	17
34	16	15	14	88	0	10	17
35	16	15	13	89	0	9	17
36	16	15	14	90	0	9	17
37	16	16	14	91	0	8	17
38	16	15	14	92	1	6	17
39	17	15	15	93	0	6	16
40	17	16	14	94	0	5	16
41	16	15	14	95	0	2	15
42	17	15	14	96	0	2	16
43	16	16	15	97	0	3	14
44	17	16	14	98	0	2	14
45	17	16	14	99	0	1	12
46	17	16	15	100	0	1	12
47	17	15	14	101	0	0	10
48	17	16	15	102	0	0	10
49	17	16	14	103	0	0	9
50	16	16	15	104	1	1	7
51	17	17	15	105	0	0	5
52	17	16	14	106	0	0	5
53	17	16	15	107	0	0	4
54	16	16	15	108	0	0	3

SMA test on day 405, CH 1=Channel 1 (2000 mg/l acetate), CH 2=Channel 2 (3000 mg/l acetate), and CH 3=Channel 3 (4000 mg/l acetate).

Appendix 7

Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3
1	15	12	14	55	8	19	17
2	16	11	12	56	6	19	17
3	15	13	12	57	6	19	18
4	16	14	13	58	4	19	17
5	16	13	13	59	5	19	17
6	15	14	13	60	3	19	18
7	16	14	14	61	2	18	17
8	16	14	13	62	2	17	18
9	16	13	13	63	1	18	18
10	17	15	13	64	1	17	18
11	16	15	14	65	2	15	17
12	17	15	14	66	1	15	18
13	17	15	13	67	1	14	18
14	17	16	14	68	0	12	19
15	17	15	14	69	0	11	18
16	16	16	14	70	1	10	18
17	17	15	15	71	0	8	19
18	17	16	14	72	1	8	19
19	17	16	14	73	0	6	19
20	18	16	15	74	0	5	19
21	17	17	14	75	0	3	19
22	17	16	14	76	0	3	19
23	18	16	15	77	0	2	19
24	17	17	14	78	0	3	19
25	17	17	15	79	1	1	19
26	18	17	15	80	0	1	19
27	17	17	14	81	0	0	19
28	18	17	15	82	1	0	19
29	17	18	15	83	0	0	19
30	18	17	15	84	0	1	19
31	18	17	15	85	0	0	18
32	18	17	14	86	0	0	19
33	17	18	15	87	0	1	18
34	18	17	15	88	0	0	17
35	17	18	15	89	0	0	18
36	18	17	16	90	0	1	17
37	18	18	15	91	0	0	18
38	18	18	15	92	1	2	16
39	18	18	16	93	0	1	15
40	19	17	15	94	0	1	16
41	18	18	16	95	0	0	14
42	19	18	15	96	0	1	15
43	19	18	16	97	0	1	14
44	18	17	15	98	0	0	13
45	19	18	16	99	0	0	10
46	18	18	16	100	0	0	11
47	18	18	16	101	0	0	10
48	17	19	17	102	1	0	10
49	15	18	16	103	0	1	9
50	16	18	16	104	0	0	7
51	14	19	17	105	1	1	8
52	11	18	16	106	0	0	5
53	10	19	17	107	0	0	6
54	9	19	17	108	0	0	4

SMA test on day 447, CH 1=Channel 1 (2000 mg/l acetate), CH 2=Channel 2 (3000 mg/l acetate), and CH 3=Channel 3 (4000 mg/l acetate).

Appendix 7

Time (h)	CH 1	CH 2	CH 3	Time (h)	CH 1	CH 2	CH 3
1	18	12	15	55	1	20	19
2	15	14	16	56	0	20	20
3	16	17	16	57	0	19	20
4	16	17	16	58	0	20	20
5	16	16	16	59	0	19	20
6	17	17	15	60	0	17	20
7	16	17	16	61	0	18	20
8	16	16	16	62	0	17	20
9	16	17	16	63	0	16	20
10	17	17	17	64	1	16	20
11	17	17	17	65	0	14	20
12	16	17	16	66	0	14	20
13	17	18	17	67	0	13	20
14	17	17	17	68	0	11	20
15	18	17	17	69	0	10	20
16	17	18	18	70	0	9	19
17	17	17	17	71	0	8	20
18	17	17	17	72	0	6	19
19	18	18	18	73	0	6	20
20	17	18	18	74	0	4	19
21	18	17	17	75	0	5	19
22	18	18	18	76	1	3	19
23	18	18	18	77	0	3	18
24	19	18	18	78	0	1	17
25	18	18	19	79	0	2	18
26	18	17	18	80	1	1	18
27	19	18	18	81	0	1	17
28	18	18	19	82	0	1	16
29	19	19	18	83	0	0	16
30	19	18	18	84	1	0	15
31	19	18	19	85	0	0	16
32	20	19	19	86	0	1	16
33	19	18	19	87	0	0	14
34	20	19	19	88	0	0	14
35	19	18	20	89	0	0	13
36	19	19	19	90	0	0	13
37	20	19	19	91	0	0	11
38	20	19	19	92	1	1	10
39	19	18	20	93	0	1	11
40	17	19	19	94	0	0	10
41	15	19	19	95	0	0	9
42	14	19	20	96	0	0	7
43	11	20	20	97	0	0	7
44	10	19	19	98	0	0	6
45	8	20	20	99	0	0	4
46	8	20	20	100	0	0	4
47	6	20	20	101	0	0	3
48	4	19	19	102	1	0	3
49	3	20	20	103	0	1	2
50	3	20	20	104	0	0	3
51	2	20	19	105	1	1	1
52	3	20	20	106	0	0	1
53	1	20	19	107	0	0	2
54	2	20	20	108	1	0	1

SMA test on day 460, CH 1=Channel 1 (2000 mg/l acetate), CH 2=Channel 2 (3000 mg l acetate), and CH 3=Channel 3 (4000 mg/l acetate)

Appendix 8

Results of Microbiological Studies

Time (days)	Short rods No./g VSS	Medium rods No./g VSS	Long rods No./g VSS	Cocci No./g VSS
2	$7.10x10^7$	$4.45x10^7$	$8.85x10^6$	$4.00x10^8$
10	$8.45x10^7$	$1.55x10^8$	$4.90x10^7$	$5.15x10^8$
17	$9.60x10^7$	$1.75x10^8$	$4.35x10^7$	$4.70x10^8$
24	$1.30x10^8$	$1.80x10^8$	$4.10x10^7$	$4.45x10^8$
30	$1.55x10^8$	$1.85x10^8$	$3.70x10^7$	$4.30x10^8$
37	$1.60x10^8$	$1.90x10^8$	$4.25x10^7$	$4.40x10^8$
45	$1.65x10^8$	$1.90x10^8$	$4.05x10^7$	$4.55x10^8$
61	$1.60x10^8$	$1.90x10^8$	$4.35x10^7$	$4.50x10^8$
79	$1.70x10^8$	$1.90x10^8$	$5.20x10^7$	$4.50x10^8$
85	$1.70x10^8$	$1.90x10^8$	$5.05x10^7$	$4.40x10^8$
94	$1.75x10^8$	$2.00x10^8$	$5.00x10^7$	$4.75x10^8$
102	$1.95x10^8$	$2.05x10^8$	$4.50x10^7$	$4.95x10^8$
119	$2.15x10^8$	$1.90x10^8$	$3.90x10^7$	$5.10x10^8$
138	$2.45x10^8$	$1.75x10^8$	$2.85x10^7$	$5.00x10^8$
157	$2.40x10^8$	$1.80x10^8$	$2.60x10^7$	$4.93x10^8$
177	$2.55x10^8$	$1.75x10^8$	$2.70x10^7$	$4.65x10^8$
195	$2.95x10^8$	$1.60x10^8$	$2.30x10^7$	$4.45x10^8$
221	$3.65x10^8$	$1.50x10^8$	$2.10x10^7$	$4.10x10^8$
243	$3.75x10^8$	$1.60x10^8$	$3.60x10^7$	$4.00x10^8$
254	$3.95x10^8$	$1.70x10^8$	$3.00x10^7$	$3.95x10^8$
265	$4.00x10^8$	$1.75x10^8$	$4.00x10^7$	$3.90x10^8$
277	$4.05x10^8$	$1.90x10^8$	$5.00x10^7$	$3.95x10^8$
289	$4.20x10^8$	$1.90x10^8$	$4.50x10^7$	$3.90x10^8$
311	$4.60x10^8$	$1.80x10^8$	$5.00x10^7$	$3.80x10^8$
324	$4.55x10^8$	$1.70x10^8$	$5.50x10^7$	$3.95x10^8$
348	$5.90x10^8$	$2.25x10^8$	$3.60x10^7$	$3.20x10^8$
356	$5.75x10^8$	$2.40x10^8$	$3.00x10^7$	$3.20x10^8$
366	$5.60x10^8$	$2.20x10^8$	$2.20x10^7$	$3.35x10^8$

Results of Microbiological Studies (continued)

Time (days)	Short rods No./g VSS	Medium rods No./g VSS	Long rods No./g VSS	Cocci No./g VSS
375	$5.70x10^8$	$2.45x10^8$	$2.00x10^7$	$3.30x10^8$
385	$5.90x10^8$	$2.50x10^8$	$1.00x10^7$	$3.05x10^8$
393	$5.85x10^8$	$2.60x10^8$	$1.60x10^7$	$3.10x10^8$
401	$5.95x10^8$	$2.60x10^8$	$1.50x10^7$	$2.80x10^8$
429	$6.00x10^8$	$3.10x10^8$	$2.20x10^7$	$2.90x10^8$
438	$6.10x10^8$	$3.20x10^8$	$3.00x10^7$	$2.95x10^8$
451	$6.05x10^8$	$3.25x10^8$	$2.60x10^7$	$2.90x10^8$
459	$6.00x10^8$	$3.35x10^8$	$1.80x10^7$	$2.80x10^8$
466	$5.90x10^8$	$3.40x10^8$	$1.30x10^7$	$2.75x10^8$
476	$6.00x10^8$	$3.40x10^8$	$2.30x10^7$	$2.90x10^8$

Appendix 8

Results of Microbiological Studies

Sarcina No./g VSS	Filaments No./g VSS	Methanogens No./g VSS	Total Bacteria No./g VSS	Non-methanogens No./g VSS
$1.40x10^8$	$1.77x10^7$	$6.85x10^8$	$1.00x10^{10}$	$9.35x10^9$
$8.45x10^7$	$2.10x10^7$	$9.05x10^8$	$1.25x10^{10}$	$1.15x10^{10}$
$8.73x10^7$	$2.62x10^7$	$8.99x10^8$	$1.24x10^{10}$	$1.15x10^{10}$
$7.52x10^7$	$2.73x10^7$	$8.94x10^8$	$1.25x10^{10}$	$1.16x10^{10}$
$6.13x10^7$	$3.06x10^7$	$8.95x10^8$	$1.24x10^{10}$	$1.15x10^{10}$
$4.85x10^7$	$3.03x10^7$	$9.10x10^8$	$1.26x10^{10}$	$1.17x10^{10}$
$4.60x10^7$	$2.90x10^7$	$9.24x10^8$	$1.23x10^{10}$	$1.14x10^{10}$
$4.36x10^7$	$2.72x10^7$	$9.15x10^8$	$1.18x10^{10}$	$1.10x10^{10}$
$4.17x10^7$	$1.56x10^7$	$9.12x10^8$	$1.23x10^{10}$	$1.14x10^{10}$
$4.46x10^7$	$2.05x10^7$	$9.20x10^8$	$1.20x10^{10}$	$1.10x10^{10}$
$5.60x10^7$	$1.87x10^7$	$9.75x10^8$	$1.20x10^{10}$	$1.10x10^{10}$
$5.50x10^7$	$2.50x10^7$	$1.02x10^9$	$1.24x10^{10}$	$1.14x10^{10}$
$4.90x10^7$	$1.40x10^7$	$1.02x10^9$	$1.26x10^{10}$	$1.16x10^{10}$
$4.10x10^7$	$2.10x10^7$	$1.00x10^9$	$1.20x10^{10}$	$1.10x10^{10}$
$5.00x10^7$	$2.70x10^7$	$9.72x10^9$	$1.22x10^{10}$	$1.13x10^{10}$
$4.40x10^7$	$3.20x10^7$	$9.95x10^9$	$1.17x10^{10}$	$1.07x10^{10}$
$4.00x10^7$	$4.00x10^7$	$1.00x10^9$	$1.26x10^{10}$	$1.16x10^{10}$
$4.80x10^7$	$5.00x10^7$	$1.05x10^9$	$1.20x10^{10}$	$1.10x10^{10}$
$4.40x10^7$	$4.20x10^7$	$1.05x10^9$	$1.28x10^{10}$	$1.17x10^{10}$
$4.60x10^7$	$5.20x10^7$	$1.10x10^9$	$1.20x10^{10}$	$1.10x10^{10}$
$5.20x10^7$	$4.80x10^7$	$1.10x10^9$	$1.25x10^{10}$	$1.14x10^{10}$
$4.50x10^7$	$5.60x10^7$	$1.14x10^9$	$1.33x10^{10}$	$1.20x10^{10}$
$5.60x10^7$	$6.00x10^7$	$1.16x10^9$	$1.40x10^{10}$	$1.30x10^{10}$
$5.00x10^7$	$4.60x10^7$	$1.17x10^9$	$1.36x10^{10}$	$1.24x10^{10}$
$4.20x10^7$	$4.10x10^7$	$1.16x10^9$	$1.42x10^{10}$	$1.30x10^{10}$
$1.00x10^8$	$6.00x10^7$	$1.33x10^9$	$1.46x10^{10}$	$1.33x10^{10}$
$1.25x10^8$	$6.60x10^7$	$1.35x10^9$	$1.44x10^{10}$	$1.30x10^{10}$
$1.30x10^8$	$5.70x10^7$	$1.32x10^9$	$1.40x10^{10}$	$1.27x10^{10}$

Results of Microbiological Studies (continued)

Sarcina No./g VSS	Filaments No./g VSS	Methanogens No./g VSS	Total Bacteria No./g VSS	Non-methanogens No./g VSS
$1.35x10^8$	$7.20x10^7$	$1.35x10^9$	$1.38x10^{10}$	$1.25x10^{10}$
$1.50x10^8$	$7.40x10^7$	$1.38x10^9$	$1.45x10^{10}$	$1.30x10^{10}$
$1.65x10^8$	$7.00x10^7$	$1.40x10^9$	$1.46x10^{10}$	$1.30x10^{10}$
$1.60x10^8$	$6.40x10^7$	$1.38x10^9$	$1.50x10^{10}$	$1.35x10^{10}$
$1.50x10^8$	$6.10x10^7$	$1.43x10^9$	$1.50x10^{10}$	$1.37x10^{10}$
$1.40x10^8$	$5.70x10^7$	$1.46x10^9$	$1.60x10^{10}$	$1.45x10^{10}$
$1.30x10^8$	$6.30x10^7$	$1.44x10^9$	$1.55x10^{10}$	$1.43x10^{10}$
$1.25x10^8$	$5.50x10^7$	$1.40x10^9$	$1.65x10^{10}$	$1.50x10^{10}$
$1.10x10^8$	$5.90x10^7$	$1.40x10^9$	$1.50x10^{10}$	$1.35x10^{10}$
$1.05x10^8$	$5.30x10^7$	$1.40x10^9$	$1.50x10^{10}$	$1.40x10^{10}$

Appendix 8

Results of Microbiological Studies

MLVSS (g)	CH_4 %	CO_2 %	CH_4 Production (l/d)	OLR (kg COD/m^3.d)	COD Removal %	VFA (mg/l)
1050	7	27	1	1.0	20	1655
1080	12	32	7	0.7	40	1100
1100	34	36	13	0.7	55	620
1120	48	35	22	0.7	70	300
1150	67	26	38	1.5	72	860
1190	77	20	38	1.0	95	50
1230	78	19	42	1.0	96	10
1290	78	19	72	1.7	97	15
1380	78	19	95	2.0	97	30
1380	79	18	110	2.6	97	35
1400	80	18	110	2.5	97	25
1400	81	17	110	2.5	97	30
1440	79	18	130	3.0	97	20
1500	80	17	145	3.2	97	30
1580	79	18	157	3.4	97	20
1680	79	17	194	4.3	97	10
1800	81	16	225	4.7	97	30
2390	79	17	335	7.4	98	30
2420	79	18	345	7.8	98	40
2500	78	19	350	8.5	97	30
2540	77	20	355	8.8	97	20
2610	77	20	380	9.2	97	41
2740	76	21	385	9.8	97	30
2950	73	24	405	11.0	98	60
3000	74	22	460	11.6	98	50
3600	75	21	520	13.5	98	30
3700	74	23	555	14.0	98	50
3860	73	25	570	15.0	98	70

Results of Microbiological Studies (continued)

MLVSS (g)	CH_4 %	CO_2 %	CH_4 Production (l/d)	OLR (kg COD/m^3.d)	COD Removal %	VFA (mg/l)
3980	73	24	600	15.6	98	70
4090	71	25	620	16.5	98	80
4190	73	24	660	17.0	98	80
4230	71	26	635	17.4	98	60
4710	71	26	660	20.0	98	80
5080	69	28	770	22.0	98	90
5280	70	28	800	23.0	98	90
5350	67	30	780	24.0	97	100
5810	66	31	880	26.7	97	160
6130	65	32	880	28.5	97	200