Intensification of Single Stage
Continuously Stirred Tank
Anaerobic Digestion Process using
Carriers

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I hereby declare that this thesis is my own account of my research and contains as its main content work which has not been previously submitted for a degree at any tertiary education institution. Any contribution made to the research by others is explicitly acknowledged in the thesis.

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The following paper has been published from this research:

Abstract

The Continuously fed Stirred Tank Reactor (CSTR) is a popular design for anaerobic treatment of wastewater. This reactor type is simple in design and operation, independent of biomass type and low in capital costs. The CSTR has, however, to be operated at long Hydraulic Retention Times (HRT) of the order of 16 to 30 days since biomass is continuously lost with the effluent. Various alternate concepts of reactor design have, therefore, been developed to allow more rapid treatment. Treatment can be enhanced by retaining biomass within the digester so that the HRT is decoupled from solid biomass retention time (SRT). Unlike in continuous stirred tank digesters where the SRT is equal to HRT, the SRT in other designs are much greater than the HRT. This allows the wastewater to be treated at high throughputs while retaining the biocatalyst (or biomass) mediating the treatment within the digester.

In this study the operation of a CSTR was intensified by separating SRT from HRT while taking into account the economical aspects. The intensification of operation is defined as increasing wastewater throughput or organic loading rate while at the same time maintaining efficiency of treatment and robustness to reject disturbances (changes in wastewater concentration and flow rate). The operation of existing CSTR was intensified by addition of carriers. It is hypothesized that by providing surfaces (or carriers) for bacterial attachment within the continuous stirred tank digester, biomass will be better retained and the wastewater throughput can be increased. The carriers or surfaces employed in this study were light carrier elements (shredded granular rubber tire having a density of 0.96 g/cm³) that move gently with the water in the reactor. This carrier material is much cheaper compared with other commercial
carrier materials. This reactor type, called an Anaerobic Moving Bed Reactor (AMBR), was applied in this study to treat high strength synthetic wastewater, containing molasses as the main substrate.

The improvement of reactor performance was clearly shown by the capability of the system to be operated without any difficulties at HRT of 6 days at an OLR of 5.8 g COD/l/d or at HRT of 1 day at an OLR of 4 g COD/l/d. The carriers were shown to be effective in retaining biomass aggregates.

The AMBR was further intensified by changing the feeding strategy. It was shown that in stirred tank digester without carriers an intermittent feeding strategy resulted in better microbial capacity to degrade higher chain volatile fatty acids like propionic and butyric acids than the continuous feeding mode. An increase in degrading capacity of the intermittently fed digester was shown via degradation rates of pulse additions of propionic and butyric acids and by its capability of handling all changes in loading rates imposed. The continuously fed digester, receiving constant feed, on the other hand, suffered more when loading rates were changed, and the degradation rates of propionic and butyric acids were slower.

The intermittent feeding mode was then implemented on the AMBR, and it was operated as a sequencing batch reactor with a fill, react, settle and decant period in each cycle. The sequencing batch mode when applied to the AMBR (now called an Anaerobic Moving Bed Sequencing Batch Reactor or AMBSBR) could increase capability of the digester to handle higher shock loads. At 3.8 d HRT the AMBSBR could handle an OLR of 10.8 g COD/l/d as opposed to 7.4 g COD/l/d by the AMBR. At 2.5 d HRT the AMBSBR could handle an OLR of 6.4 g COD/l/d while the AMBR
could only be loaded at an OLR of 4.2 g COD/l/d. The ratio of SRT to HRT was at least 15 for this reactor. The reactor was able to handle concentrated feed flow rates at longer cycles or more dilute feed flow rates at frequent shorter cycles.

The proposed operational strategies were verified by using a structured mathematical model which was developed based on the IWA ADM1 model. Several modifications were implemented to the model to obtain better predictions. The modified model was capable in predicting all the trends of the operating variables from both continuously and intermittently fed reactors. None of the two model versions (ADM1 and modified models) was, however, able to predict the increased propionate degradation capacity in intermittently fed digesters. The reason for this was the assumption of fixed stoichiometry of fermentative reactions for glucose mineralisation. By modifying the fractions of glucose mineralisation a better fit between experimental results and the model could be obtained.
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List of Abbreviations

AMBR: Anaerobic Moving Bed Reactor
AMBSBR: Anaerobic Moving Bed Sequencing Batch Reactor
ADM1: Anaerobic Digestion Model No. 1
COD: Chemical Oxygen Demand
CSTR: Continuously fed Stirred Tank Reactor
HRT: Hydraulic Retention Time
L/D: Length to diameter ratio
MPR: Methane Production Rate
STR: Stirred Tank Reactor
OLR: Organic Loading Rate
SRT: Solid Retention Time
SS: Suspended Solids
VSS: Volatile Suspended Solids
VFAs: Volatile Fatty Acids
$\Delta G^o$': The standard Gibbs free energy
h: hours
d: days
l: litres
M: molar (or mole/l)
mM: millimolar
Pa: Pascal
Chapter 1
Introduction

1.1. General Introduction

Liquid waste especially high strength wastewater from industries (such as abattoir, dairies, breweries, distilleries, sugar cane molasses, chemical and petrochemical, and pulp and paper) is still one of the major sources of pollution worldwide. Anaerobic digestion is increasingly used as an effective method for treating such wastes (Malina and Pohland, 1991; Macarie, H., 2000). This treatment method gives several advantages over the conventional aerobic process. Among of them are: production of a useful energy source in methane gas, lower energy consumption as aeration is not required, production of low quantities of well stabilized sludge, and odor-free treatment as anaerobic digestion has to be carried out in sealed vessels (Craveiro et al. 1986; Harper and Pohland, 1986; Lettinga et al., 1993; Bjornsson, 2000).

Traditionally single stage continuous stirred tank reactor design has been used for anaerobic digestion as it is simple to construct and operate and capital costs are lower. However, this type of reactor has to be operated at hydraulic retention time (HRT) in the order of 16 to 30 days (Lin et al., 1986; Kim and Speece, 2002) since biomass is continuously lost with the effluent. Various alternate concepts of reactor design have, therefore, been developed to allow more rapid treatment. Treatment can be enhanced by retaining biomass within the digester so that the HRT is decoupled from solid retention time (SRT). Unlike in continuous stirred tank digesters where the SRT is
equal to HRT, the SRT in other designs are much greater than the HRT. This allows the wastewater to be treated at high throughputs while retaining the biocatalyst (or biomass) mediating the treatment within the digester.

Biomass retention can be effected in two ways: attached and non-attached systems (Callander and Barford, 1983; Henze and Harremoes, 1983). Attached biomass digesters contain surfaces or carriers which act as supports on which bacterial attachment occurs. These surfaces or carriers are denser than water and tend to settle well, which prevent them from being washed out. Digesters employing attached growth can be of two types depending on whether the supports are stationary (e.g. down flow and up-flow anaerobic filters) or mobile (e.g., anaerobic fluidized bed). Non-attached biomass digesters (e.g. up-flow anaerobic sludge blanket digester, and expanded granular sludge bed digester) rely on the tendency of some species of biomass to form aggregates or flocs, having better settling properties than free culture. The flocs or aggregates can either be entrapped within the digester or be allowed to settle out in a settling tank outside the digester and then returned to the digester.

Digesters based on these designs have several drawbacks. Anaerobic filter digesters containing stationary supports are subject to clogging and unpredictable biofilm sloughing (Hickey et al., 1991; Borja and Banks, 1994 b). Anaerobic fluidized bed reactors which contain high density particles, like sand are maintained by high fluid velocities and recycle ratios in reactors which have a high length to diameter (L/D) ratio (Heijnen et al., 1989, Iza, 1991, Setiadi and Ginting, R. 1993 and 1994). Consequently, there is significant energy consumption.
Among the non-attached biomass digesters, the Up-flow Anaerobic Sludge Blanket (UASB) reactor is the most popular. This particular design relies on natural granulation properties of anaerobic biomass to enable them to be retained in the digester. Granular biomass has excellent settling properties. However, granulation can be affected by the influent wastewater characteristics as it is difficult to grow granular biomass on certain wastewater (Johns, 1995; van Starkenburg, 1997). Additionally, in the absence of readily available granular biomass to seed a new digester, lengthy start-up periods are required for granular biomass to develop in the digester (Lettinga et al., 1983; Borja and Banks, 1994 b; Borja et al., 1996). High rate reactor designs are proprietary (or patented) and are expensive to implement.

Before deciding the most appropriate design for treatment of certain wastewater type, basic knowledge of the processes occurring during anaerobic digestion is critical. During anaerobic digestion, organic matter in wastewater is mineralized to methane and carbon dioxide through the concerted and syntrophic action of several groups of microorganisms. The process occurs through a series of steps where the metabolic products of one group of organisms serve as the substrates for another group of organisms. There are two principal steps in this bioconversion process: 1) fermentation of organic matter to volatile fatty acids (VFAs) mainly acetic, propionic and butyric acids and 2) methanogenesis from acetic acid as well as hydrogen and carbon dioxide formed in the VFA fermentation step. Given that methanogenesis occurs only from acetic acid and hydrogen in wastewater treatment and that residual concentrations of higher chain VFAs like propionic and butyric acids are very low in the treated effluent; these higher chain VFAs have to be converted to precursors like acetic acid and hydrogen for methanogenesis. For efficient anaerobic digestion, the rate of production of VFAs should match its consumption rate. In other words the
growth of microbial populations that mediate various metabolic processes should be in balance. An imbalance in these growth rates could lead to an accumulation of VFAs leading to failure of digestion process.

Single stage systems are prone to failure. One school of thought attributes such failures to the following reason. Under nominal influent feed conditions, the initial fermentation reactions produce acetic acid as the primary VFA with very little propionic or butyric acids being produced. Hence the system fails to maintain bacterial population to convert the higher chain VFAs. Excursions from nominal conditions in the feed would cause higher chain VFAs to build up, which can not be degraded due to lack of sufficient bacterial populations.

Two stage systems where the acid forming phase and methanogenic steps occur in separate tanks (or reactors) are generally more robust and less susceptible to failure (Pohland and Gosh, 1971; Sutton and Li, 1983; Kida et al., 1994; Houbron et al., 2003). Industrial wastewater treatment system design usually incorporates a buffer (equalization) tank prior to the anaerobic digester. Unwittingly, this buffer tank serves as the first stage of a two stage system since in this tank wastewater naturally acidifies producing the entire suite of VFAs; acetic, propionic and butyric acids. Therefore, the second stage fed with these VFAs maintains microbial populations capable of converting all these acids.

The work addressed in this thesis concerns the options for intensifying the operation of single stage continuous stirred tank digesters treating high strength industrial wastewater. Intensification of operation is defined here as increasing wastewater throughput or organic loading rate while at the same time maintaining efficiency of
treatment and robustness to reject disturbances. Disturbances to normal digester operation considered here are changes in wastewater concentration and flow rate. When attempting to intensify the operations an economical option was sought. Therefore, options such as retrofitting or changing the mechanical components or structural design of the digesters and the incorporation of a buffer (or equalization) tank that will convert the single stage to two stage system were not considered.

It is hypothesized that by providing surfaces (or carriers) for bacterial attachment within the continuous stirred tank digester, biomass will be better retained and the wastewater throughput can be increased, and the feeding strategy can be changed from a continuous to discontinuous one to enhance the digester effectiveness to reject disturbances. It is postulated that deliberate discontinuous feeding would cause higher chain VFAs to accumulate which in turn cause microbial that degrade these higher chain VFAs to build up within the single stage system.

The carriers or surfaces employed in this study were light carrier elements that move gently with the water in the reactor. The use of the light carrier may result in retaining the active biomass in the reactor with minimum energy requirement for carrier movement. Heavy carriers, on the contrary, require considerable energy to keep them mobile and may also entail changing the L/D ratio of reactor to increase up-flow velocity of fluid. Carrier movement may also allow for good mass transfer into the biofilm and in the long run this movement can be maintained by circulating the methane gas produced (Odegaard et al., 1994; Jahren et al., 1999; Jahren and Odegaard, 1999). This reactor type, henceforth called an anaerobic moving bed reactor (AMBR), was applied in this study to treat high strength synthetic wastewater, containing molasses as the main substrate. The carrier material used in this study was
shredded granular rubber tire, giving higher surface area and is much cheaper compared with the carrier materials employed by other researchers.

1.2. Objective of the Study

1. To develop a novel reactor design (AMBR) which could overcome some of the drawbacks of single stage continuous stirred tank anaerobic digesters, whilst maintaining its simplicity and economical operation.
2. To examine the effectiveness of using shredded granular rubber tire as carriers for biomass attachment in digesters.
3. To investigate the treatment ability of this design when subjected to both organic and hydraulic shock loads.
4. To investigate the effect of feeding strategy to enhance the robustness of digestion process.
5. To implement the robust operational strategy on AMBR.
6. To verify the effectiveness of proposed operational strategies by using an established mathematical model that describes the anaerobic mineralization of carbohydrates; namely Anaerobic Digestion Model No. 1 (ADM1) developed by IWA Task Group for Mathematical Modelling of Anaerobic Digestion Processes (Batstone et al., 2002).

1.3. Organization of the Thesis

Chapter 2 presents a relevant literature review on the theoretical background of anaerobic digestion, most popular designs of anaerobic reactors and general overview
of digester failure. This is intended to provide background knowledge and research motivation to conduct this study.

Chapter 3 describes general materials and methods employed during the study while details of specific experimental studies are presented in the relevant chapters.

The novel reactor design and its performance can be found in Chapter 4. Details of reactor start-up as well as its progress can also be found in this chapter. The effectiveness of adding granular rubber tire carriers (to an existing stirred tank reactor) to retain active microbial populations is also presented in this chapter.

The robustness of the novel reactor to reject disturbances is detailed in Chapter 5. The chapter presents reactor performance under shock load conditions; purely organic shock loads, purely hydraulic shock loads, and combination of organic and hydraulic shock loads.

The effect of feeding strategy on the performance of single stage continuous stirred tank digesters in relation to achieving robustness is presented in Chapter 6.

The performance and response of the novel reactor when it was switched from a continuous to discontinuous mode of operation are described in Chapter 7. The effectiveness of this digester operating on the discontinuous feeding mode in handling increased organic loads, shorter HRTs and shorter cycles are also presented in this chapter.
First part of Chapter 8 presents the development of a structured mathematical model for anaerobic mineralization of carbohydrates, which is based on the model called Anaerobic Digestion Model No. 1 (ADM1). An evaluation to what extent the ADM1 model could predict the differences in behavior observed in laboratory reactors fed continuously and discontinuously is presented in the second part of Chapter 8.

Finally, Chapter 9 provides general discussion and conclusions of the thesis. Recommendation and directions for further research are also included.
2.1. Introduction

This chapter provides a brief review on the general concept of anaerobic wastewater treatment processes. The review covers mechanism of anaerobic digestion, various types of anaerobic reactor configurations for enhanced biomass retention, assessment of reactor performance, factors affecting digester failure, and model development in anaerobic wastewater treatment processes. The mechanism of anaerobic digestion is a must to understand the theoretical background of anaerobic digestion. For anaerobic reactor configurations, emphasis will be given to the continuously fed stirred tank reactor (CSTR) and high rate anaerobic reactors as well as their advantages and drawbacks. This will provide reasons on why an anaerobic moving bed reactor (AMBR) was chosen in this study. Factors affecting digester failure will be focused on the effects of organic and hydraulic shock loads. This information will be useful to interpret the experimental results obtained. A brief review on model development in anaerobic wastewater treatment processes will provide basic knowledge for the development of mathematical modelling addressed in this study.

2.2. Anaerobic Digestion

Anaerobic digestion is an effective method of treating agricultural, industrial and domestic wastes. It is a typical anaerobic ecosystem where complex organic polymeric
substances are enzymatically broken down into the final end products of methane (CH₄) and carbon dioxide (CO₂) by the action of different microbial populations in the absence of free oxygen (Fig. 2.1). There are at least four major groups of microbial populations responsible for the anaerobic degradation, occurring via three steps which are discussed as follows: (Zeikus, 1980; Gujer and Zehnder, 1983; Harper and Pohland, 1986).

Fig. 2.1 Steps in anaerobic digestion involving four groups of bacterial activities
2.2.1. Hydrolysis and Fermentation

Hydrolysis is the first step in the anaerobic degradation of most insoluble organic wastes. It breaks down complex organic compounds (such as carbohydrates, fats and protein) into their monomers (simple sugars like glucose). The breakdown of organic polymers is performed by extracellular enzymes, which are produced by both facultative and strictly anaerobic bacteria. The monomers resulting from hydrolytic bacteria are then fermented to volatile fatty acids (VFAs) such as acetic, propionic and butyric acids and alcohols, CO₂, H₂ and some lactic acid.

The significance of the hydrolysis is that, this step is regarded as the rate limiting step for insoluble polymers (Eastman and Ferguson, 1981; Noike et al., 1985; Endo et al., 1986; Ferreiro and Soto, 2003). For carbohydrates, cellulose is considered to be the rate limiting step but not starch which can be much easier hydrolysed (Pavlostathis et al., 1988). In their study they used insoluble cellulose in which the concentration of soluble reducing sugars was only less than 1%. They obtained that the hydrolysis and fermentation of cellulose by continuous cultures of *Ruminococcus albus* followed first order kinetics and the rate constant was equal to 1.18 day⁻¹.

Hydrolysis rate of carbohydrates under anaerobic conditions is generally faster than the hydrolysis of protein. Yu et al. (2003) showed about 31 to 65% of carbohydrates, 20 to 45% of protein, and 14 to 24% of lipid were acidified in an up-flow reactor with an agitator and gas-liquid-solid separator. Gujer and Zehnder (1983) and Tong and McCarty (1991) found the rate of hydrolysis of lipids is between 0.08 to 1.7 day⁻¹, that of protein ranges from 0.02 to 0.03 day⁻¹, cellulose 0.04 to 0.13 day⁻¹, and hemicellulose 0.54 day⁻¹.
Temperature and pH are two environmental factors affecting hydrolysis. Hydrolysis of cellulosic materials by enriched cultures at pH 6.7 was faster than at pH 5.1 and 5.2 (Eastman and Ferguson, 1981). When experiments conducted at a neutral pH and temperatures between 20 and 45 °C, temperature optimum was found to be 40 °C (Tong and McCarty, 1991). Dinopoulou et al. (1988) made similar observations and further observed that temperature also affected the acidogenic phase following the Arrhenius equation. However, bacterial death rates could increase at high temperatures besides the increase of energy required for maintenance (Zoetemeyer et al., 1982 a).

Carbohydrates such as starch and sugars are most commonly fermented by Bacteriodes, Clostridia, Butyri vibrio, Selenomonas, Micrococcus and Lactobacillus (Marty, 1984). Sugars are common energy sources for fermentative microorganisms. Biochemical pathway occurring within the cell during this breakdown is generally via pyruvate. Pyruvate is metabolised primarily to acetate, formate, hydrogen and carbon dioxide. In this metabolism other products may also be found such as propionate, butyrate, succinate, ethanol and lactate (Thauer et al., 1977).

Pyruvic acid, the pivotal compound in sugars catabolism, is a result of breakdown of sugars through the Embden-Meyerhof-Parnas (EMP) or glycolysis pathway. The glycolytic reactions in glucose fermenting bacteria also produce electron equivalents in the form of NADH which are required to be re-oxidized in order to continue substrate degradation. One pathway for the bacteria to regenerate these reducing equivalents are via anaerobic respiration using inorganic electron acceptors. In the absence of external inorganic electron acceptors, NADH is commonly recycled through $\text{H}^+$ to produce $\text{H}_2$ or through pyruvate to produce lactate, propionate or
butyrate. The type of end product produced depends on the bacterial types and thermodynamic conditions (Thauer et al., 1977; Mosey, 1983; McInerney and Beaty, 1988; Hoh, 1996).

Lactic acid is the most common product in the fermentation of sugars. In natural mixed population fermentations, homofermentative bacteria such as *Lactobacillus curratus* and *Lactobacillus plantarum* initiate acidification of the medium following the reaction (Linden, 1988):

\[
C_6H_{12}O_6 \rightarrow 2\text{CH}_3\text{CHOHCOO}^- + 2\text{H}^+
\]

Other species, the heterofermentative bacteria such as *Lactobacillus buchneri* and *Lactobacillus brevis* convert glucose according to the following reaction:

\[
C_6H_{12}O_6 \rightarrow 2\text{CH}_3\text{CHOHCOO}^- + \text{CH}_3\text{CH}_2\text{OH} + \text{CO}_2 + \text{H}^+
\]

Romli et al. (1995) observed high amount of lactic acid during shock loads. In stable reactors, however, lactic acid is rarely detected in the effluent which may be due to fast consumption of this organic acid. *Lactobacillus* and other mesophilic fermentative bacteria such as *Bacteroides*, *Clostridium*, *Butyrivibrio*, *Eubacterium*, and *Bifidobacterium* have an average minimum doubling time of 30 minutes (McInerney and Bryant, 1981; Gujer and Zehnder, 1983). Early anaerobic degradation modelling, therefore, did not account for this acid as an intermediate product. Costello et al. (1991 a) proposed the inclusion of lactic acid in their model which was considered to be particularly important to describe the behaviour of anaerobic reactors during shock loads.
The volatile fatty acids (VFA) produced by anaerobic fermentative bacteria grown on glucose are mainly acetic, propionic and butyric acids. Acetic acid is the most abundant followed by propionic and butyric acids (Toerien and Hattingh, 1969). The conversion reactions followed ADM1 (2002):

\[
\begin{align*}
C_6H_{12}O_6 + 2H_2O & \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \\
3C_6H_{12}O_6 & \rightarrow 4CH_3CH_2COOH + 2CH_3COOH + 2CO_2 + 2H_2O \\
C_6H_{12}O_6 & \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2
\end{align*}
\]

Under stable conditions, the first reaction to produce acetic acid from glucose is the preferred reaction (Mosey, 1983). The second intermediate metabolic product, propionic acid, may also be formed by the following reactions:

\[
\begin{align*}
C_6H_{12}O_6 + 2H_2 & \rightarrow 2CH_3CH_2COOH + 2H_2O \\
3 \text{CH}_3\text{CHOHCOOH} + \text{H}_2 & \rightarrow 2\text{CH}_3\text{CH}_2\text{COOH} + \text{CH}_3\text{COOH} + \text{CO}_2 + 2\text{H}_2\text{O}
\end{align*}
\]

The second reaction is a result of the work done by *Propionibacterium* consuming lactate, produced by lactic acid bacteria.

Propionic acid may also result from metabolism of long chain fatty acids that contain odd numbers of carbon atoms as an end product. Firstly, odd numbered fatty acids are metabolised through β-oxidation. Then, the three carbons remaining as propionyl-CoA are converted to succinyl-CoA and oxidised to CO₂ through the Tricarboxylic acid (TCA) cycle. Because anaerobic bacteria can not use the TCA cycle as a complete pathway, propionic acid would be produced as an end product (Gaudy and Gaudy, 1981).
Another end product, butyric acid is a result of anaerobic metabolism of *Clostridium* species bacteria, known as the butyric clostridia, such as *Clostridium butyricum, Clostridium tyrobutyricum*. In this acid formation Mosey (1983) assumed only two moles ATP produced. Gaudy and Gaudy (1981) and Sheehan (1981), however, indicated the possibility for an addition of 1 mole of ATP for each mole of butyric acid formed. This was adopted by Costello et al. (1991 a) and ADM1 (2002). The latter is the basis of the model developed in this study (presented in Chapter 8).

2.2.2. Acetogenesis and Homoacetogenesis

The fermentation products such as propionic and butyric acids as well as ethanol need to be converted to a simpler product, i.e. acetic acid before being utilised by methanogenic bacteria. The bacteria responsible for this conversion are known as acetogenic bacteria (or called H₂ producing bacteria). Two common types in anaerobic digestion are the alcohols and the fatty acids degrading acetogens such as *Acetobacterium, Acetobacter, Syntrophobacter, Syntrophomonas* and some *Desulfovibrio* species (Bryant, 1979; McInerney and Bryant, 1981). These bacteria grow very slowly due to the low free energy available from their metabolic substrate degradation (Table 2.1) with doubling time ranging from 1.5 to 4 days (Lawrence and McCarty, 1969).

Under standard conditions the Gibbs free energy change for most reactions during acetogenesis is positive (Table 2.1). This bacterial type is therefore hypothesised to suffer from product inhibition by hydrogen. The bacterial activities are dependent on the simultaneous removal of end products H₂ and acetate (Iannotti et al., 1973; Wolin, 1974). It can, moreover, be a potentially rate limiting step in the digesters under
stressed condition such as organic or hydraulic overloading. Propionate having the Gibbs free energy change more positive than butyrate and ethanol is more affected by product inhibition. Oxidation of propionate resulting in 3 moles H₂ compared to 2 moles H₂ produced from butyrate and ethanol also shows thermodynamically less favourable, which means the reaction will not proceed unless the end products are kept at low levels.

Table 2.1 Equations and standard Gibbs free energy changes during acetogenesis

(Thiele and Zeikus, 1988)

<table>
<thead>
<tr>
<th>Reaction</th>
<th>ΔG°° (kJ/reaction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol + H₂O → acetate⁻ + 2 H₂ + H⁺</td>
<td>+9.6</td>
</tr>
<tr>
<td>Lactate⁻ + 2 H₂O → acetate⁻ + 2 H₂ + HCO₃⁻ + H⁺</td>
<td>-3.96</td>
</tr>
<tr>
<td>Butyrate⁻ + 2 H₂O → acetate⁻ + 2 H₂ + H⁺</td>
<td>+48.1</td>
</tr>
<tr>
<td>Propionate⁻ + 3 H₂O → acetate⁻ + HCO₃⁻ + 3 H₂ + H⁺</td>
<td>+76.1</td>
</tr>
</tbody>
</table>

Acetogenesis could perform efficiently when the acetogens grow synthropically with methanogens since methanogenic bacteria maintain the acetogenic end products at a low level leading the reaction in a thermodynamically favourable direction (Marty, 1984; Boone, 1987; Fukuzaki et al., 1990; Warikoo et al., 1996). For example *Syntrophomonas*, *Syntrophobacter* and *Desulfovibrio* have been co-cultured with species of *Metanobacterium* or *Metanospirillum* (Mah et al, 1977; McInerney et al., 1979; McInerney and Bryant, 1981).

Another group of acetogens known as H₂–acetogenic and homoacetogenic bacteria convert H₂ and CO₂ to acetate, according to the reaction:
\[ 2 \text{CO}_2 + 4 \text{H}_2 \rightarrow \text{CH}_3\text{COOH} + 2 \text{H}_2\text{O} \]

*Acetobacterium woodee* and *Clostridium aceticum* are bacterial species capable to perform the above reaction (Braun et al., 1981). These bacteria are also capable to consume other substrates for growth such as fructose, pyruvate, and lactate.

### 2.2.3. Methanogenesis

Methanogenesis is the final step in anaerobic digestion to produce methane and carbon dioxide from acetate and \( \text{H}_2 \) produced in acetogenesis step. In all anaerobic ecosystems, methanogenesis is carried out by methanogenic bacteria. These bacteria are the most sensitive bacterial group in the anaerobic digester ecosystems to oxygen and pH (Zehnder and Wuhrmann, 1977; Goodwin et al., 1988; Barredo and Evison, 1991).

Methanogens are able to metabolise a very narrow range of substrates. Almost all methanogens (except 4 species which include *Methanotrix soehngenii*) can grow on \( \text{H}_2 \) and \( \text{CO}_2 \) while some genera can use formate. Formate utilising genera include *Methanobacterium*, *Methanobrevibacter*, *Methanococcus*, *Methanomicrobium*, *Methanogenium* and *Methanospirillum* (Balch et al., 1979; Sahm, 1984). *Methanosarcina* and *Methanotrich* are two bacterial groups, which can utilise acetic acid and present in high numbers in anaerobic digesters (Zehnder, 1978; Smith and Mah, 1980) but they can not use formate. The former is present abundantly in unstable digesters, containing high acetate concentrations whereas the latter is dominant in stable systems, containing less acetate (Fox et al., 1990; McMahon et al., 2001).
There are two types of methanogenic bacteria, i.e. aceticlastic methanogenic and H₂-ulising methanogenic bacteria (Zehnder, 1978; Zeikus, 1980; McCarty and Smith, 1987). The aceticlastic methanogenic bacteria perform the important function of carbon removal. These methanogens play an important role to control the pH during fermentation process by the removal of acetate to form CO₂ and CH₄ (Mosey, 1983). They are responsible for 60 to 70% of the methane produced in anaerobic digesters (Smith and Mah, 1980; McInerney and Bryant, 1981) with the reaction that proceeds as follows:

\[
\text{CH}_3\text{COO}^- + \text{H}_2\text{O} \rightarrow \text{CH}_4 + \text{HCO}_3^-
\]

Since the free energy derived from the above reaction (Table 2.2) almost equates to the energy needed to generate 1 mole of ATP (\(\Delta G^{\circ} = -30.6 \text{ kJ}\)), it explains the slow growth rate of these aceticlastic methanogens (Mosey, 1983). Minimum doubling times of this bacterial type are between 2 to 3 days (Lawrence and McCarty, 1969; Zehnder, 1978). Therefore, methanogenesis from acetate is limited by the amount of biomass present in the system even though the acetate degradation is thermodynamically favourable under most digester conditions. Imbalance between fermentative bacteria and methanogens, resulting in acetate and hydrogen accumulation is thought to be the major cause of digester failure during organic shock loading (Denac et al., 1988; Hickey and Switzenbaum, 1991; Strong and Cord-Ruwisch, 1994).

The H₂-ulising methanogenic bacteria, is responsible for 30% of the total methane produced in anaerobic digesters (McInerney and Bryant, 1981). The process involves
the reduction of CO₂ by H₂ according to the reaction as the third reaction in Table 2.2 (McInerney et al., 1979; Thiele and Zeikus, 1988).

**Table 2.2** Equations and standard Gibbs free energy changes during methanogenesis

(Thiele and Zeikus, 1988)

<table>
<thead>
<tr>
<th>Reaction</th>
<th>ΔG°' (kJ/reaction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH₃COO⁻ + H₂O → CH₄ + HCO₃⁻</td>
<td>-31.0</td>
</tr>
<tr>
<td>4 H₂ + HCO₃⁻ + H⁺ → CH₄ + 3 H₂O</td>
<td>-135.6</td>
</tr>
<tr>
<td>4 HCO₂⁻ + H⁺ + H₂O → CH₄ + 3 HCO₃⁻</td>
<td>-130.4</td>
</tr>
</tbody>
</table>

In the above reaction hydrogen transfer and utilisation regulate the rate of H₂-producing reactions by controlling the partial pressure of hydrogen (Mosey, 1983). The H₂ concentration strongly affects the metabolic pathways used by the fermentative bacteria and is responsible for the types of end-products formed (Thauer et al., 1977; McInerney and Bryant, 1981; Mosey, 1983; Gujer and Zehnder, 1983). The inhibition of some H₂-producing reactions by H₂ concentration has been known as one of the major causes of VFA accumulation leading to failure in digester operations (McInerney and Bryant, 1980; Harper and Pohland, 1986; Strong and Cord-Ruwisch, 1994). Only at very low levels of H₂ concentration through interspecies H₂ transfer degradation of VFAs such as propionate can occur (McInerney and Bryant, 1981; Conrad et al., 1985). Minimum doubling times for the H₂-ulising methanogens are in the range of 6 to 12 hours (Zehnder and Wuhrmann, 1977; Smith and Mah, 1978; Gujer and Zehnder, 1983) as shown in the above reaction which is far in favour of H₂ use.
2.3. Anaerobic Reactor Configurations for Enhanced Biomass Retention

The conventional (medium-rate) anaerobic system, such as the continuously fed stirred tank reactor (CSTR) is still more widely used for anaerobic digestion. The reasons are its simplicity of operation and design and independence of biomass type. This reactor, however, has to be operated at hydraulic retention times (HRT) of the order of 16 to 30 days (Lin et al., 1986; Kim et al., 2002) since biomass is continuously lost with the effluent. In this type of reactor, the value of HRT is, therefore, similar or equal to solid retention time (SRT). To intensify this simple technology and maintain a viable population of the slow-growing methanogens, the CSTRs are usually combined with an internal or external biomass separation and recycle system.

High-rate anaerobic systems rely on the principle of high biomass concentrations within the reactor by de-linking of cell retention time from hydraulic retention time. The cells are retained within the reactor by immobilizing them through one of three means, i.e. the formation of highly settleable sludge aggregates, bacterial attachment to high density carrier particles and entrapment of sludge aggregates between packing materials within the reactor (Lettinga, 1984; Bal and Dhaagat, 2001).

There are two reactor classifications based on the state of biomass, namely non-attached and attached biomass digesters (Callader and Barford, 1983; Henze and Harremoes, 1983). The non-attached biomass digesters, which are called floc-based digesters by Callader and Barford (1983), rely on the tendency of some species of
biomass to form aggregates or flocs, having higher settleability than free culture. The form of flocs entrapment can be either bacterial entrapment within the digester or the flocs/aggregates which are allowed to settle out in a settling tank and then returned to the digester. The attached biomass digesters contain surfaces which act as supports on which bacterial attachment occurs to prevent them from being washed out.

Microbial attachment on carrier particles is effected by several factors. Among them, surface roughness, porosity, and surface area of the carriers are widely cited in literature as the critical factors effecting biomass adherence.

A comparative study on surface roughness and detachment of biomass due to shear in expanded bed reactors was conducted by Fox et al. (1990). They employed three different types of carriers having almost the same diameter, i.e. granular activated carbon (GAC), anthracite, and sand. The roughest surface support (GAC) showed 3 to 10 times more biomass attached on the support surface than those attached on the support surface of anthracite or sand. It was also shown in their study that biomass losses due to shear in the reactors containing sand and anthracite were 6 to 20 times than biomass losses from the reactor containing GAC.

Similar findings were also obtained by Huysman et al. (1983) who observed the surface features of 29 packing media, and Harendranath et al. (1996) who compared 5 types of non porous supports and 4 types of porous carriers. They came to the conclusion that the quantity of attached bacteria is higher as the surface roughness increases.
Porosity plays an important role in influencing biomass attachment on the surface of the carriers. The presence of pores and crevices in the surface of the support material provides adequate conditions for microbial attachment where biomass was protected from shear forces (Fox et al., 1990 and Picanco et al., 2001).

A study performed by Anderson et al. (1994) reported strong correlation between porosity of the packing materials and the performance of anaerobic filters. They observed that the reactor filled with the porous packing was still stable at organic loading rates up to 21 g COD/l/d whereas the reactor containing the non-porous packing showed instability above an organic loading rates of 4 g COD/l/d. Moreover, they observed a heavy biomass attachment retained in the porous media while unattached biomass entrapped among the non-porous carriers. Another study conducted by Yee et al. (1992) observed Methanothrix type bacterial consortia were predominant in porous carriers whereas non-porous carriers attracted a mixture of Methanothrix and Methanosarcina.

Huysman et al. (1983) and Picanco et al. (2001) also found the same observation. Reactors filled with porous supports present better colonization matrix than those containing non-porous supports.

A surface area of the carriers has a crucial importance in the retention of the anaerobic bacteria. The large surface area is fundamental to provide a large amount of attached bacteria. Reactors containing supports with high surface area present better efficiencies than the reactors filled with support having low surface area (Breitenbucher et al., 1990 and Picanco et al., 2001).
The non-attached biomass digesters include CSTR, contact reactors and up-flow anaerobic sludge blanket reactors (UASB). Included in the attached biomass digesters are fixed-film, fluidized bed and moving bed reactors. However, there is no a clear cut difference between the two categories (attached and non-attached biomass digesters). The attached growth in fluidized bed reactor contains suspended bacteria whereas granules in the UASB also exhibit some characteristics of attached growth.

2.3.1. Non-Attached Biomass Digesters

The first attempt to increase the value of SRT in anaerobic digestion is by recycling the biomass washed out of the digester. The biomass separation can be done through one of several means such as gravity, centrifugation, floatation, degasification, or thermal shock. The process is named anaerobic contact process. In this reactor type HRT can be reduced as low as one day. However, the operation of this reactor has been in limited since early 1950s due to floc retention and stability problems (Callader and Barford, 1983).

The UASB reactor developed by Lettinga and co-workers (Lettinga et al., 1980 and 1983; Bal and Dhagat, 2001) is the most sophisticated version of the non-attached digesters. This reactor type was developed based on the concept of the reversed flow Dorr Oliver Clarigester. It consists of two parts; the gas solid separator at the top and digester compartment at the bottom of the reactor. The gas solid separator is a baffle system to separate the gas from the liquid containing granular biomass. The digester compartment consists of three regions: the dense sludge bed at the bottom, sludge blanket in the middle and upper settling zone above the sludge blanket. The dense sludge bed and upper settling zone are characterized by plug flow. The sludge blanket
contains fluidized particles which are kept well mixed by the production of gas in the sludge bed (Heertjes and van der Meer, 1978). The fluidized particles which are commonly called granules may contain 80 to 90% of active microbial biomass and has very good settling characteristics.

The concentration of biomass within the reactor is between 30 to 50 g VSS/l (Weiland and Rozzi, 1991). Torkian et al. (2003) observed an average bioparticle mass at the bottom of the reactor of 89 g VSS/l. The high concentrations of biomass are promoted by very low organic loading during long initial start-up periods, with slightly acidified feed and high calcium concentrations. The wash out of non flocculated biomass allows the system to select for the granulating biomass. Therefore, when subjected to high volumetric loading rates UASB reactors exhibit superior performance compared to others (Paula and Foresti, 1992). Organic loading rates (OLR) up to 15 kg COD m$^{-3}$ d$^{-1}$ could be degraded with removal rates between 70 and 90% at HRTs of 4 hours (Lettinga et al., 1980) and OLRs between 13 to 39 kg COD m$^{-3}$ d$^{-1}$ could be degraded with removal rates between 75 and 90% at HRTs in the range of 2 to 7 hours (Torkian et al., 2003).

With UASB, problems may occur with wastewater containing high concentrations of suspended solids and fat due to their accumulation within the reactor with subsequent loss of active sludge (Johns, 1995; van Starkenburg, 1997). Other main drawbacks are slow start-up and sensitivity to organic loads (Lettinga, 1984; Lettinga et al., 1984; Souza, 1986).

The anaerobic baffled reactor (ABR), initially named as a modified sludge blanket reactor, is described as a series of UASBs but requires no special granule formation
for its operation (Bachmann et al., 1985). The first application was to promote
generation of methane as an energy source (Chynoweth et al., 1985). This type of
reactor uses a series of vertical hanging and standing baffles to force water to flow
under and over them as it passes from inlet to outlet and divide the vessel into several
compartments. Slanting of the lower edges of the hanging baffles allows the flow of
liquid through the middle of the sludge bed, resulting in channelling effect reduction.
The down flow chambers are narrower than the up-flow chambers to avoid more
biomass collection in the up-flow chambers (Bachmann et al., 1985).

A comprehensive review done by Barber and Stuckey (1999) describes applicability
and the possible future application of the ABR. The following advantages of ABRs
over other anaerobic systems were listed. These include better resilience to organic
and hydraulic shock loads, lower sludge yields, ability to separate acidogenesis and
methanogenesis longitudinally down the reactor, allowing the reactor to behave as a
two-phase system without the associated costs and control problems, inexpensive and
simple construction as there are no moving parts.

2.3.2. Attached Biomass Digesters

Up-flow anaerobic filters (UAF) were introduced by Young and McCarty (1969). The
first application for the treatment of wheat starch wastewater was performed in 1972
(McCarty, 1982). The success of applying the UAF relies on the retention of active
biomass by entrapment of bacteria in the space between and within as well as
adhesion to the external surface of the packing material (Lettinga et al., 1984; Hickey
et al., 1991; Young, 1991). Since its inception, the AF and fixed film processes have
been applied to a variety of industrial wastewaters with COD ranging from 2, 000 to
20,000 mg/l (Harrison et al. 1990). Results showed its good adaptation to different type of wastewater, applicable to dilute and high strength wastewater, insensitive against load fluctuations and fast re-start after shut down. The possibility of plugging of the support media, difficulty in start-up, restriction to wastewater with low suspended solids, sensitivity to high calcium concentrations and high costs of support media have limited the use of the UAF (Weiland and Rozzi, 1991).

The problems of plugging of the support media in UAF have led to the development of down-flow fixed film reactors (DFF). DFF reactors, in which their development began in 1976, have oriented packing that forms vertical channels that run the length of the packing as compared to random packing used in UAF reactors (Kennedy and Droste, 1985 and 1991). Several types of support media in DFF was studied by van den Berg and Kennedy (1982 a) and Kennedy and Droste (1983) focusing on start-up behaviour and reactor responses towards intermittent and continuous loading. Lengthy periods of start-up, biofilm development dependency on the source of inoculum, support material and other operational conditions which are similar to the requirements of a UASB reactor are the drawbacks of this reactor type (Weiland and Rozzi, 1991). The biomass should be at levels greater than 20 kg VSS/m³ to ensure the presence of enough bacteria within the reactor region (Salkinoja-Salonen et al., 1983).

Anaerobic fluidized bed reactors (AFBR) combine the attached film and fluidization technology. This reactor type was developed by Switzenbaum and Jewell which was firstly called as an anaerobic fixed film expanded bed (McCarty, 1982). It is derived conceptually from a CSTR which is improved by the formation of biomass clumps/biofilm on the surface of small particles. Sand is the most commonly used
medium because the material is inexpensive and easily available (Bull et al., 1983; Iza et al., 1988; Mathiot et al., 1992; Yee et al., 1992). Other carriers which have been used with considerable success are activated carbon, synthetic carbonaceous adsorbent and synthetic resin (Pirbazari et al., 1990).

The bed of particles is fluidized by pumping up the liquid from the bottom of the reactor to replace agitation in the case of CSTR (Andrews, 1988). Therefore, biomass concentration can be maximized in the reactor without clogging and biofilm thickness for good mass transfer can be achieved (Jewel, 1983; Iza, 1991). Initial dilution of the influent with effluent, which provide alkalinity, reduces substrate concentration, and contributes to reduce the shock effects of toxicants (Iza, 1991). The application of this reactor type was to handle various wastewaters including beet sugar waste (Iza et al., 1988), meat extract (Dinopoulou and Lester, 1989), ice cream waste (Cayless et al., 1989; Morgan et al., 1991), molasses (Denac et al., 1990) and wine distillery wastes (Mathiot et al., 1992). However, slow reactor start-up and energy intensive nature of AFBR are the limitation of this reactor type (Olthof and Oleszkiewicz, 1982; Iza, 1991; Weiland and Rozzi, 1991).

Another reactor design included in this category is an anaerobic moving bed reactor (AMBR) employing support materials on which biomass attached. Since this reactor type was chosen in this study detailed information of this reactor will be provided in a separate section (2.3.4).
2.3.3. Other Classification of Anaerobic Reactors

Hybrid anaerobic reactors combine the attributes of a UASB (in the lower portion) and an anaerobic filter (in the upper portion). Hybrid reactors were introduced by Guiot and van den Berg (1985) as a means of retaining biological solids in UASB reactors where sludge did not granulate and as a means of further increasing the solid carrying capacity of up-flow reactors (Newland et al., 1991). Therefore, performance stability could be achieved because even if the granular sludge was lost, sufficient flocculent sludge was retained in the filter section to maintain a high rate of degradation (Johns, 1993).

The high biomass concentration in the hybrid reactor allows the treatment of dilute and high strength wastewater at high organic loading rates and low HRTs. Full scale operations have been implemented to treat sludge thermal conditioning liquor, landfill leachate and domestic sewage (Crawford and Teletztke, 1987; Young, 1991). The main drawback of this reactor type is lengthy periods of start-up. In the absence of sludge adapted to target wastewater a long acclimatisation period of more than 3 months was needed (Chang, 1989).

The two-phase anaerobic systems were introduced to minimize the problems of reactor stability occurring in anaerobic contact processes (Pohland and Gosh, 1971; Roy and Jones, 1983; McDougall et al., 1994). In the two-phase anaerobic system, wastewater flowing into the first stage, which serves as an equalisation or buffer tank, is partially acidified to VFAs primarily acetic, propionic and butyric acids. Since acidogenesis is allowed to occur in the first reactor this stage is referred to as the acidogenic reactor. The second reactor is referred to as the methanogenic reactor, in
which the partially acidified wastewater from the first reactor is pumped up and during this process the organic carbon is mineralised to methane and CO₂.

Roy and Jones (1983) employed an up-flow digester as the acid stage reactor running at low HRTs. Growth rates of acid degrading bacteria are much faster than those of methanogenic bacteria. The second stage digester employed was either a CSTR with a high hold-up time or smaller attached-film reactor. They observed that stage separation improved the reactor stability. The overall retention achieved was 5 days. Ghosh et al. (1983 and 1985) applied the selective entrapment of solid used to develop a sludge bed to treat particulate wastes using this two stage system. The first and second stage digesters in the above study were both unmixed up-flow reactors. This two stage system has also been studied extensively in stirred tank and up-flow reactors by Cohen et al. (1980 and 1982).

The two-phase anaerobic fluidized bed systems were introduced to obtain higher treatment efficiencies (Bull et al., 1984) so that a better final effluent quality such as lower suspended solid and total COD concentrations can be achieved (Sutton and Li, 1983; Li et al., 1985) and to improve reactor stability to handling shock loads (Cayless et al., 1989). It was also observed that the biomass in the methanogenic fluidized bed of the two systems was more adapted to volatile acid degradation than the biomass in the single stage beds (Bull et al., 1984). Commonly the two-phase anaerobic fluidized bed systems consist of a CSTR type for the first reactor and fluidized bed reactor for second reactor (Dinopoulou and Lester, 1989; Kida et al., 1992; Romli et al, 1995; Haris, 2001).
Anaerobic sequencing bath reactors (ASBR) are operated on an intermittent, fill and draw cycles. One cycle consists of 4 phases, i.e. fill, react, settle, and decant. This is a variation of the UASB and provides for staging of kinetics. During high substrates/feast conditions right after feeding, high rates of substrate conversion to biogas occur. During low substrates/famine conditions near the end of the react phase, better separation of biomass is achieved so that the suspended solids in the effluent can be reduced (Dague et al., 1992; Sung and Dague, 1995). The technique also results in reducing the tendency for biomass solids to float due to CO₂ release (Dague et al., 1992).

In this reactor type, the de-linking of SRT from HRT occurs by separating biomass from the liquid within the reactor rather than in an external clarifier (Dague et al., 1992; Sung and Dague, 1992; Chang et al., 1994; Ndon and Dague, 1997 a; Lee et al., 2001; Ruiz et al., 2001). To obtain better separation of SRT from HRT, this reactor type relies on biomass with good settling properties. Well settling biomass is more effectively retained in the reactor which may also result in reduction of duration of settle phase. Biomass with good settling characteristic are produced when they self immobilize and form granules. As mentioned before, granulation requires lengthy start-up periods and appropriate feed characteristics (Lettinga et al., 1983; Lettinga et al., 1984; Borja and Banks, 1994 b; Liu et al., 2002). Sung and Dague (1992) observed granulation in an ASBR fed with a soluble, synthetic substrate (non-fat dry milk) after nearly 300 days of operation. Moreover, it has been shown that granular biomass tend to break up, float and wash out at high organic loading rates or short HRTs (Ndon and Dague, 1997 b).
Retention of biomass on support material is an option to obtain well settling biomass. Ratusznei et al. (2003) and Rodrigues et al. (2003) employed inert supports of polyurethane foam (having particle sizes of 5 mm and density of 23 kg/m³) for biomass adhesion and biofilm formation. The ASBR, having 2.5 l volume, could be operated at 2d HRT and 8 hours cycles treating low strength (0.5 g COD/l) synthetic wastewater, mainly containing meat extract and soluble starch, at an OLR of 0.24 g COD/l/d. The COD removal efficiency obtained was 86%. Moreover, the use of inert supports resulted in elimination of the settling step and thus reducing the overall cycle time (Ratusznei et al., 2000).

Reactor configuration is another factor affecting development of well settling sludge. Tall, slender reactors were found to select for granular sludge better than the short, stout reactors. However, the tall, slender reactors accumulated fewer concentration of biomass than the short, stout reactors (Sung and Dague, 1995).

Modification of operational strategy also influences the performance of ASBR. Higher ratios of fill time to cycle time resulted in improved performance of this reactor type (Shizas and Bagley, 2002). On the contrary, operation stability and efficiency were impaired when fed-batch feeding (having longer feeding times) was performed than the batch feeding mode (Ratusznei et al. 2003). A study conducted by Rodrigues et al. (2003) did not observe differences in reactor performance resulting from different feeding strategies (batch and fed-batch modes).

Mixing is recognized as another important factor affecting ASBR performance. Intermittent mixing was found to be preferable to produce more methane and higher
COD removal than the continuously mixing (Sung and Dague, 1995). Ratusznei et al. (2001) found reduction in the total cycle time when agitation was used.

In this study the sequencing batch mode was applied to the anaerobic moving bed operation. The main objective was to achieve higher organic loading rates since with this type of operation wash out of bacteria along with the effluent withdrawal could be minimized; besides tendency for biomass solids to float due to CO₂ release could also be reduced. More detailed explanation can be found in the relevant chapter.

### 2.3.4. Anaerobic Moving Bed Reactor (AMBR)

Interests in biofilm processes both for municipal and industrial wastewater treatment is based on several reasons (Odegaard et al., 1994). Less space is required since the treatment plant itself may be much more compact, the treatment result is far less dependent upon the final sludge separation and the attached biomass may be utilized in a more specialized way because of the lack of sludge return. The anaerobic moving bed reactor design fulfills such conditions. This reactor type employs light carrier elements that move gently with the liquid in the reactor. The use of the light carrier results in retaining the active biomass in the reactor while maintaining a minimum energy required for carrier movement. The carrier movement allows good mass transfer into the biofilm and in the long run this movement can be maintained by circulating the methane gas produced (Odegaard et al., 1994; Jahren and Odegaard, 1999; Jahren et al., 1999).

The moving bed reactor design was also developed to avoid the draw-backs of other submerged biofilter reactors. Submerged biofilter reactors pose build-up of head loss
in the carrier material, resulting in the need for filter backwashing. The submerged biofilter is also sensitive towards slugs of sludge coming into the reactor due to the loss of sludge from clarifiers’ upstream (Odegaard et al., 1994). Therefore, the basic idea behind the development of moving bed reactor system is to have a non-cloggable biofilm reactor with low head-loss and high specific biofilm surface.

The anaerobic moving bed reactor, employing Kaldnes’ polyethylene carriers, was developed by a Norwegian company, Kaldnes Miljoteknologi A/S. Kaldnes’ polyethylene carriers are shaped like small cylinders with a cross inside and longitudinal fins on the outside with diameter of 10 mm and height of 7 mm, have a density of 0.95 g/cm³ and maximum specific growth area of 350 m²/m³ (Jahren et al., 1999). Initially, moving bed reactors were upgraded from the existing activated sludge systems for nitrogen removal with a minimum of construction and without expanding the existing reactor volumes (Rusten et al., 1994). They named it the KMT moving bed biofilm reactor (MBBR). The carrier movement in aerobic MBBR is performed by aeration whereas, that of anaerobic MBBR is performed by a mechanical stirrer (Odegaard et al., 1994; Jahren and Odegaard, 1999 and 2000).

Effectiveness of Kaldnes’ polyethylene carriers to retain biomass attached on the surface of the carriers was seen (Jahren and Odegaard, 1999; Jahren et al. 1999; Jahren and Odegaard, 2000). Pilot scale anaerobic MBBRs were used to treat whitewater. After 7 months of operation, biomass concentrations increased from 3.3 to 5.5 g VSS/l resulting in overall soluble COD removals of about 60% at organic loading rates up to 7 kg COD/ m³d (Jahren and Odegaard, 1999). During 33 months of period, suspended and attached biomass concentrations of about 3 g VSS/l were obtained, resulting in substrate utilisation rates up to 4.2 soluble COD/kg VSS d at
organic loading rates of 16.4 soluble COD/kg VSS d. When the same reactor was fed with molasses waste (Jahren and Odegaard, 2000), they observed substrate utilisation rates of 6.8 soluble COD/kg VSS d at organic loading rates of 27 soluble COD/kg VSS d. Biomass varied between 1.1 to 2.5 g VSS/l. Jahren et al. (1999) employed three types of laboratory scale anaerobic reactor fed with whitewater, namely hybrid, multi-stage and moving bed reactors. All reactors could achieve soluble COD removals up to 70%. The hybrid anaerobic reactor composing of a UASB and filter containing Kaldnes’ carriers could achieve degradation rates up to 10 kg COD/m³d at organic loading rates of 15 kg COD/m³d and HRT of 3.1 hours. The anaerobic multi-stage reactor comprising three compartments each packed with granular sludge and carrier elements gave degradation rates up to 9 kg COD/m³d at organic loading rates of 15 to 16 kg COD/m³d and HRT of 2.6 hours. The anaerobic moving bed reactor showed similar performance at organic loading rates of 1.4 kg COD/m³d.

In this study, the anaerobic moving bed reactor employed shredded rubber tire carriers. The main consideration to choose this support material is that the rubber tire is a waste material which can be recycled for beneficial use. More detailed explanation on this anaerobic moving bed reactor can be found in Chapter 4.

The definitions listed in Table 2.3 would be helpful to understand the performance of reactors mentioned in this thesis.
Table 2.3 Definitions of common terms used in this thesis

<table>
<thead>
<tr>
<th>No.</th>
<th>Terms</th>
<th>Definitions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Organic loading rate (OLR)</td>
<td>The rate at which organic matter is supplied to the reactor. It is expressed as the concentration of organic matter in the feed over the digester hydraulic retention time.</td>
</tr>
<tr>
<td>2</td>
<td>Hydraulic retention time (HRT)</td>
<td>Is the average time a fluid element resides in the digester. This is defined as digester operating volume over feed flow rate (assuming that the digester is operating at a constant volume).</td>
</tr>
<tr>
<td>3</td>
<td>Solid retention time (SRT)</td>
<td>SRT represents the amount of active biomass retained in the reactor. It is presented as VSS concentration in the reactor over VSS concentration in the effluent.</td>
</tr>
<tr>
<td>4</td>
<td>Organic overloading</td>
<td>An input of organic matter exceeding the degradation capacity of the microbial ecosystem.</td>
</tr>
<tr>
<td>5</td>
<td>Hydraulic overloading</td>
<td>Hydraulic overloading occurs whenever the effective retention time (HRT) is reduced to a point at which the microorganisms can not reproduce before being washed out.</td>
</tr>
<tr>
<td>6</td>
<td>Removal efficiency</td>
<td>The percentage of degraded organic matter to the organic matter added to the reactor. The value varies depending on wastewater types or the percentage of biodegradable matter contained in the wastewater.</td>
</tr>
<tr>
<td>7</td>
<td>Methane production rate (MPR)</td>
<td>The rate of methane produced per litre of reactor volume per day.</td>
</tr>
<tr>
<td>No.</td>
<td>Terms</td>
<td>Definitions</td>
</tr>
<tr>
<td>-----</td>
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<td>-------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>8</td>
<td>Specific methane yield</td>
<td>The amount of methane produced compared to the theoretical methane yield expected from degradable organic matter added to the reactor. At standard conditions the theoretical specific methane yield equals to 0.35 l CH₄/g COD&lt;sub&gt;removed&lt;/sub&gt;.</td>
</tr>
<tr>
<td>9</td>
<td>Continuously fed stirred tank reactor (CSTR)</td>
<td>A type of reactor which is continuously fed and stirred. This design is simple to construct and operate, and low in capital costs.</td>
</tr>
<tr>
<td>10</td>
<td>Stirred tank reactor (STR)</td>
<td>Same design as CSTR, the digester contents are stirred continuously but feeding may be intermittent or continuous.</td>
</tr>
</tbody>
</table>
2.4. Assessment of Reactor Performance

Reactor performance is usually assessed based on a condition of so called “steady state” (Graef and Adrews, 1974; Bachmann et al., 1983). Usually a reactor is considered to have reached a steady state by achieving constant effluent parameter such as COD, volatile fatty acids (VFA) concentrations and suspended solids over 3 HRTs. However, the intra-cellular enzyme activities monitored over 12 HRTs of the steady state (showing by constant effluent parameters) of a CSTR operated at 30 days of HRT were varied continuously (Kotze et al., 1968). The term steady state used in literature is actually a quasi-steady state in which changes of the microbial population may still occur.

In this study, reactor performance was verified against the value of total VFAs in the effluent. With total VFAs in the effluent in the range of 0.3 to 0.5 g COD/l, the digester operation was considered to be stable or normal (Grady et al., 1984; Kennedy et al., 1985; Chynoweth et al., 1994).

Stability of a reactor is usually measured by the recovery periods required by a system after the system being shock loaded. A recovery period is defined as the time required by digesters to regain normal levels of VFA concentrations. With higher and longer periods of shock loads prolonged recovery periods will be obtained and in severe cases, it may result in failure of digester operation. In this study, reactor stability was measured against recovery periods occurring within 24 hours.
2.5. General Overview of Digester Failure

In anaerobic processes, the substrate is degraded to volatile fatty acids, mainly acetic, propionic and butyric acids during normal operation. Acetic acid is usually the predominant volatile fatty acid in the system, followed by propionic and butyric acids (Toerien and Hattingh, 1969). Anaerobic treatment systems are, however, subjected to environmental changes. Under disturbances such as organic or hydraulic overloading, higher carbon-chained VFAs accumulate in the digester. This happens due to slow growing H₂-consuming methanogenic bacteria which can not consume the accumulation H₂ as fast at it is produced by the fast growing fermenting glucose bacteria. In severe cases, this situation can lead to failure in digester operations.

Schmidt and Ahring (1993), Moletta et al. (1994) and Strong and Cord-Ruwisch (1994) asserted that high H₂ concentrations stimulate the accumulation of acetate, propionate and butyrate whereas H₂ concentrations of less than 10 Pa favour the production of CO₂ and CH₄. It is known that propionic acid can not be directly converted to methane by aceticlastic methanogenic bacteria. It has to be broken down into acetic acid by acetogenic bacteria. During this degradation the concentration of hydrogen in the system has to be kept at extremely low levels. Kaspar and Wuhrmann (1978) observed that propionic acid degradation did not occur at concentrations of hydrogen in the gas phase in the range of 500 to 50 000 ppm. A 50% decrease in the rate of propionic acid was due to an elevated concentration of hydrogen to 670 ppm (Mosey, 1983).
During shock loads, the aceticlastic methanogens control the reactor pH by removal of acetic acid and production of CO₂ that dissolves to form a bicarbonate buffer solution. This bacterial type is not much affected by H₂ concentrations in the gas phase a part from their low doubling times. The H₂-utilising methanogens remove almost all of the H₂ produced in the system and thus control the redox potential of the digester. Under severe shock loads, however, they can not function properly (Attal et al., 1988).

At the stoppage of overloading, the recovery of the accumulated propionic acid is slower than that of acetic and butyric acids. Ozturk (1991) observed a considerable time was needed to recover the accumulated propionic acid but acetic acid was quickly metabolized as soon as the overloading was terminated. The rate of butyric acid removal was faster than that of acetic and propionic acids (Zoetemeyer et al., 1982 b; Pavlostathis and Giraldo-Gomez, 1991). However, degradation of butyrate is inhibited both by high H₂ partial pressure or concentration of acetate, the other end product of butyrate degradation. If acetate builds up in the system to a significant level, the degradation of butyrate is impared. Ahring and Westermann (1988) showed that acetate was degraded immediately when this acid was added together with butyrate to anaerobic digester sludge. Butyrate did not start to degrade whenever concentrations of acetate still high in the system.

Volatile fatty acids (VFAs) usually monitored during anaerobic digestion are acetic, propionic and butyric acids. By monitoring the most important intermediate products the conditions of the digester can be followed and occurrence of digester failure operation can be avoided.
2.5.1. Organic Overloading

Organic loading rate (OLR) is defined as the rate at which the organic waste is supplied to the reactor volume. It is expressed as the concentration of organic matter in the feed over the reactor retention time. There are two ways to increase the organic loading rate, i.e. by feeding more concentrated feed or by shortening the retention time at a given feed concentration. Increasing reactor organic loading rates will increase the methane production rate but also decrease the percentage of organic waste that is converted to methane (McInerney and Bryant, 1981). If input of organic waste exceeds the mineralisation capacity of microbial ecosystem, organic overloading occurs (Moletta et al., 1994).

Anaerobic digesters subjected to organic overloads demonstrate the accumulation of reducing equivalents generated from glycolysis and channelling the equivalents into the production of higher carbon-chained VFAs other than formate and acetate (McInerney and Bryant, 1981). Schink (1988) explained this phenomenon by using a rain barrel model of carbon and electron flow in methanogenic degradation (Fig. 2.2). The reducing equivalents generated from glucose degradation are first channelled into the production of acetate, \( \text{H}_2 \) and \( \text{CO}_2 \). When level of reducing equivalents builds up as \( \text{H}_2 \) the accumulation of propionate and butyrate then occurs. This is due to the \( \text{H}_2 \)-utilizing methanogen which is unable to consume \( \text{H}_2 \) as fast as it is being produced.

During normal loads or at consistently low hydrogen levels most of the electron and carbon flow of the fermentative bacteria proceeds via acetate and hydrogen, both of which are suitable substrates for methanogenic bacteria. At increased hydrogen levels as they occur under organic overloading, the fermentative bacteria shift their pathways
towards the production of more reduced organics such as propionic and butyric acids and less hydrogen (McInerny and Bryant, 1980). Since methanogenic bacteria can not consume the substrates as fast as they accumulate, propionate and butyrate accumulate in the system.

![Rain barrel model of carbon and electron flow in methanogenic degradation (Schink, 1988).](image)

The production of propionate (except from odd numbered skeletons), butyrate, and other VFAs could also occur as a result of back reactions (Boone and Mah, 1987). These are the reactions which use H₂ to condense CO₂ onto existing VFAs or to condense VFA molecules such as the following reactions (Dolfing, 1988):

\[
2\text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_3\text{COOH} + 2\text{H}_2\text{O}
\]

\[
\text{CH}_3\text{COOH} + \text{CO}_2 + 3\text{H}_2 \rightarrow \text{CH}_3\text{CH}_2\text{COOH} + 2\text{H}_2\text{O}
\]
The production of these more reduced organics is carried out by the obligate proton-reducing acetogens. Under the same conditions both reactions cannot be exergonic. However, $H_2$ concentrations may differ at the micro-environmental level as a result of its rapid turn over. Therefore, the back reactions as well as the hydrogen-producing acetogenic reactions could be exergonic in neighbouring microenvironments (Boone and Mah, 1987).

Organic overloading usually occurs in reactors treating concentrated wastes, containing easily degradable substrate (lactose, starch and sucrose). Sudden variation in waste composition can create imbalance between microbial activities in the digester, i.e. acetogenesis running faster than methanogenesis (Eng et al., 1986). This leads to an increase in $H_2$ partial pressure and hence a build up of VFAs with subsequence increase in proton concentration (Switzenbaum et al., 1990). The drop in pH caused by increased proton concentration likely results in the inhibition of methanogens. This leads lower biogas production and subsequently digester failure. Cord-Ruwisch et al. (1996) showed an elevated $H_2$ concentration resulting from organic shock loading leading to an increase in acetate production by homoacetogenic bacteria which eventually dropped the reactor pH and caused failure of the digester.

2.5.2. Hydraulic Overloading

One of the most important operational factors affecting the efficiency of an anaerobic digester is the hydraulic retention time (HRT), which is defined as reactor volume over feed flow rate. In a system (stirred tank reactor) that is fed a substrate of constant concentration, an increase of HRT means that a higher percentage of the organic matter is destroyed but rate of flow of organic matter is less. As a result, the rate of
methane production decreases. On the other hand, when the HRT is shortened by increasing the feed flow rate the methane production may increase. Hydraulic overloading in continuously fed mixed digesters may occur whenever the liquid throughput rate exceeds the growth rate of the bacteria and thus resulting in washout. Hydraulic overloading normally occurs in digesters treating dilute wastes (such as brewery and food processing wastes), which require a high flow rate to function efficiently. The high flow rate means that retention time is short and wash out of the slow growing methanogenic bacteria may occur. The doubling time of the acid forming bacteria is about 1 to 5 hours. However, the doubling time for methanogens and hydrogen-producing bacteria (HPB) is approximately 6 hours and 1.5 to 2.5 days, respectively (Mosey and Fernandez, 1989). If the dilution rate exceeds the growth rate of the methanogens or HPB present in the system, VFAs accumulate and causing the digester to sour and fail.

High levels of acetic and propionic acids during hydraulic overloading have been reported. Kennedy and van den Berg (1982 b) observed acetic and propionic acids which increased 8 and 10 fold from the normal level, respectively when an anaerobic fixed film reactor treating chemical industry waste was hydraulically overloaded to 0.78 day (from about 1.3 days HRT). This decrease in HRT caused overloading to the system about 60 to 70% higher than the normal load of 11 g COD/l/d. Conivas-Diaz and Howell (1988) found that propionic acid dominated in two types of cheese-whey-wastewater- fed anaerobic fixed film reactor (the packing being fully and half submerged) when a hydraulic shock load was imposed by decreasing the HRT from 10 to 7 days.
2.6. Mathematical Modelling of Anaerobic Processes

This section provides a short review on the development of mathematical modelling of anaerobic treatment processes. Emphasis is given on the treatment of carbohydrate-based wastewater, a liquid waste type chosen in this study. Modelling in anaerobic digestion processes is based on fundamental principles that are known to govern the behaviour of the biological processes. The model does not only empirically describe the processes but also allow the generation of a better understanding of the digestion processes and help to verify to what extent the system reflects the fundamental scientific principles used as a basis for the model. Therefore the model can be very useful for the optimization and control of anaerobic digester operation.

Most of the anaerobic digestion process models employ the Michaelis-Menten equation (equation 1.1). The equation was developed with the assumption of irreversible reaction as a reverse reaction is not considered (see Appendix 1 for derivation of the irreversible the Michaelis-Menten equation).

\[
\mu = \frac{\mu_{\text{max}} S}{K_s + S}
\]  

(1.1)

where \( \mu \): rate of reaction

\( \mu_{\text{max}} \): rate of reaction at substrate saturation

\( K_s \): half saturation constant

\( S \): substrate concentration
The equation is useful for prediction of the reaction rate characterized by high substrate concentration and constant end product concentration. In biological processes, however, the end products can accumulate to significant levels which can cause inhibition of the reaction rate. Lee and Zinder (1988), Fukuzaki et al. (1990), Schmidt and Ahring (1993) and Wu et al. (1993) show hydrogen inhibition on acetogenic degradation. Therefore, the inhibition factors have to be considered to obtain realistic modelling of the overall processes. These factors have been used and incorporated into the Michaelis-Menten equation by many researchers (Mosey, 1983; Costello et al., 1991 a; Siegrist et al., 1993).

There are 3 different mechanisms describing the occurrence of the inhibitory effect of end products on enzyme-catalysed reactions (Stryer, 1988; Lehninger et al., 1993; Zubay, 1993).

1. Irreversible inhibition (equation 1.2) typically resulting from damage of parts of the enzymatic catalysis system. For instance the damage of biological material results from extremely high concentrations of end products; i.e. acids or alcohols.

\[
\mu = \frac{(\mu_{max} - \frac{I}{K_i})S}{K_s + S} \quad (1.2)
\]

where \(K_i\): inhibition factor

\(I\) : concentration of inhibiting compound (product)
2. Reversible non-competitive inhibition (equation 1.3), resulting from interaction between end products and allosteric control site of the enzyme catalysing the reaction. This inhibition causes the organism to slow down certain reactions for the optimization of the overall metabolism and to avoid the accumulation of undesirable intermediary products.

\[
\mu = \frac{\mu_{\text{max}} S}{(K_s + S)(1 + \frac{I}{K_i})}
\]  

(1.3)

3. Reversible, competitive inhibition (equation 1.4) resulting from the competition between the inhibitory compound (in this case end-product) and the substrate for the same catalytic side of the enzyme. Usually the microbes can not control this type of inhibition (especially at low substrate concentrations) and as a result an undesired decrease in the net reaction rate occurs.

\[
\mu = \frac{\mu_{\text{max}} S}{S + K_s(1 + \frac{I}{K_i})}
\]  

(1.4)

In the model developed in this study, the reversible non competitive inhibition was chosen to model carbohydrate degradation in continuously and intermittently fed anaerobic stirred tank reactors with the emphasis on the prediction of differences in behaviour observed during experimental runs. The irreversible inhibition or reversible competitive inhibition was not considered in the model since the reactors were only shock loaded in a low range so it was assumed that no damage of parts of the
enzymatic catalysis system and it was also assumed no competition between the inhibitory compound and the substrate for the same catalytic side of the enzyme.

Mathematical modelling applied for the anaerobic treatment of carbohydrate-based wastewater was firstly developed with an inclusion of two bacterial types, i.e. acid producing and methanogenic bacteria. The aceticlastic methanogenesis was considered as the rate–limiting step. Models developed by Andrews (1969), Andrews and Graef (1971), Graef and Andrews (1974) and Hill and Barth (1977) were among the developed models, which were also frequently employed to study the effect of reactor shock loads.

A new feature of biomass decay was incorporated into the developed model by Carr and O’Donell (1977). Heyes and Hall (1981) included molecular hydrogen affecting the bacterial population in the system. Four bacterial groups were then introduced to describe the complex bacterial interactions (Mosey, 1983; Rozzi et al., 1985). Mosey’s model presented the generation and utilisation of acetate, propionate and butyrate which are regulated by the ratio of reduced and oxidized forms of Nicotinamide Adenine Nucleotide (NAD). The ratio is related to the partial pressure of hydrogen in the gas phase which regulates the formation of the acids. To simulate the accumulation of propionic and butyric acids during shock loads, these acids produced in a fixed ratio from glucose were proposed by Smith et al. (1988). However, methane from hydrogen was not modelled in their proposed model. Costello et al. (1991 b), therefore, improved Mosey’s model to obtain better model predictions by incorporating lactic acid; an intermediate which accumulates momentarily during shock loading. They found their model could predict well the lactic acid accumulation.
Recent models have been extended over various applications. Ramsay (1997) incorporated protein degradation pathway in his model, while Batstone (1999) refined it to predict the degradation of lipid and solid, in addition to carbohydrate and protein degradation. Nopharatana (2000) and Lai (2001) applied the model in the degradation of municipal solid waste. Haris (2001) incorporated sulphate reduction into the carbohydrate degradation in two-stage anaerobic reactors since sulphate is also present in many wastewater streams either due to the use of sulphuric acid during chemical processes or its presence in the influent water supply. As a result, numerous inhibition factors have been introduced into the anaerobic digester models to produce a more realistic simulation in different applications.

With the emergence of complexity of the models, it reduces the practicability of the models. Task Group for Mathematical Modelling of Anaerobic Digestion Processes (Batstone et al., 2002), therefore, simplified and limited the models to the main relevant processes to make the model as widely applicable as possible. Their effort was presented in a report titled Anaerobic Digestion Model No.1 (ADM1). The ADM1 was used as the basis for the model developed in this study.
Chapter 3
Materials and Methods

3.1. Introduction

This chapter covers the general materials and methods used in this study. These include experimental set up and apparatus, inoculum and feed, and analytical methods used during the study. Details of specific experimental studies can be found together with their results in the relevant chapters.

3.2. Experimental Set Up and Apparatus

For studies carried out in this thesis two types of reactor set ups were established, i.e. the AMBR and two stirred tank reactors (STR). The AMBR was employed to evaluate the performance of the reactor under normal loads, step changes and shock loads. The STR was used for evaluating the effect of feeding strategy towards reactor performance. The suitable feeding mode chosen could, therefore, be applied to the AMBR to conduct further studies. Details of these reactor types are given below. The anaerobic moving bed sequencing batch reactor (AMBSBR) was the same reactor as the AMBR but employed a sequencing feeding mode.
3.2.1. Anaerobic Moving Bed Reactor (AMBR)

This reactor was made of a plexiglass tank of 17 cm in internal diameter and 22 cm in height, which gave a maximum volume of 5 liters. Four liquid sampling ports were located at different heights of the reactor, i.e. 0, 6, 11, and 16 mm from the bottom of the reactor (see Fig. 3.1). Granular rubber tire having cube sizes about 2x2x2 mm obtained from Entyre Rubber, Bibra Lake, Western Australia were used as carriers (Fig 3.3). About 1.5 kg granular rubber tire carriers were placed in the reactor containing 2.2 l acclimatized sludge (see section 4.2.1 for start-up of the reactor). This active reactor volume of 2.2 l was kept constant by overflow. The calculations of organic loading rates (OLR) and hydraulic retention times (HRT) were, therefore, based on this active reactor volume. Mixing was performed by a mechanical stirrer with stirrer speed at 180 rpm. To maintain anaerobic condition a water seal was placed under the motor of the stirrer. The reactor was kept in a temperature-controlled water bath at 37 °C (Paton water bath model RW 1812). The reactor was equipped with 3 baffles to prevent vortex formation and enhance mixing. Feeding to the reactor was served by a Chemap AG peristaltic pump. All tubing was Masterplex tygon tube number 18 (Cole Parmer) except tubing attached to the feeding pump which was Marprene tubing. Total gas production and pH were monitored daily throughout the study, except when hourly or data at certain intervals were required. Experimental setup can be seen in Fig. 3.2.
Fig. 3.1 Schematic of the anaerobic moving bed reactor (AMBR)

Fig. 3.2 Experimental set up of the anaerobic moving bed reactor (AMBR)
3.2.2. Stirred Tank Reactor (STR)

Two stirred tank reactors (STR) were constructed from modified Schott bottles, each with an active reactor volume of 2 l. One reactor was used to be fed intermittently (once a day) and the other reactor was used to be fed continuously. A constant liquid volume of the reactor fed continuously was maintained by placing a stainless steel tube level sensor in the reactor lid (Fig. 3.4), connected to Biolab peristaltic pump type AF/1. When the liquid reached levels above 2 l reactor volume a signal was sent for the pump to withdraw the effluent. Conversely, the effluent withdrawal was automatically stopped if the reactor volume reached 2 l. Effluent withdrawals for the intermittently fed digester were done manually by using a 60 ml syringe. Two Dreschel bottles, one empty and the other one containing soda lime particles and silica gel were placed between the gas outlet and the water displacement gas meter. Masterplex tygon tubes (Cole Parmer) numbers 15 and 18 were used to make connections. The empty Dreschel bottle was used to collect small amount of water which got sucked (from water displacement gas meter) during manual effluent
withdrawals (see Fig. 3.5 and Section 3.2.3 for gas measurement). However, only a small quantity of water was usually sucked which just filled the connection tubing.

Feeding with the rate of 4.2 ml/h to the continuously fed digester was served by an Eyela Microtube peristaltic pump model MP-3. To ensure such low feeding rate the R-3603 Masterplex tygon tube having internal diameter of 3/32 inch was attached to the feeding pump rotor.

Fig. 3.4 Schematic diagram of the stirred tank reactors (STR)

Stirring was performed by using rod shaped magnetic stirrer bars and Stirrers model Thermolyne Cimarec 2. Both reactors were kept in a temperature-controlled aquarium tank at 37 °C using Thermomix MM heater (B Braun, Germany). Methane production and pH from the STRs were monitored daily throughout the study, except when
hourly or data at certain intervals were required. Experimental set up of these STRs is shown in Fig. 3.5.

![Experimental set up of STRs](image)

**Fig. 3.5** Experimental set up of STRs

### 3.2.3. Gas Measurement

Two types of displacement gas meter were used during this study, i.e. a displacement gas meter made from inverted cylinders and a U-tube displacement gas meter. There were 2 types of U-tube displacement gas meter: U-tube made from an inverted buret and the other U-tube made of plexiglass, connected to an electric circuit. The former was used in experiments performed in serum bottles whereas the latter was used in experiments performed in a 5 l AMBR. The inverted cylinder displacement gas meter was employed in experiments performed in two 2 l stirred tank reactors.
When methane gas was measured by the inverted cylinders displacement gas meter, the gas was passed through soda lime particles in order to remove CO$_2$ and silica gel to remove moisture. It was assumed that only traces of hydrogen presenting in total gas so that the gas measured by the meter was methane only.

Total gas was measured from the 5 l AMBR. Its methane production was estimated by multiplying the total gas with the methane gas composition measured by GA 2000 Gas Analyser (Geotechnical Instruments, UK). The gas compositions measured by the Gas Analyser were CH$_4$, CO$_2$ and O$_2$. Gas composition measurements were conducted for at least twice a day for daily sampling or every four hours during 2 hourly sampling.

Methane gas obtained from the serum bottles was estimated from the methane gas composition measured from 2 l digesters (from which sludge in the serum bottles was poured) by using GA 2000 Gas Analyser. Gas composition measurements were performed 4 times during 24 hours and values were averaged.

The U-tube made from an inverted buret consisted of a 50 ml buret on the one side and a plastic tube having the same internal diameter of buret on the other side. On the tip of the buret was connected small tubing attached on a syringe needle. The plastic tube was connected to a 3 way plastic connector to allow water over flow resulting from an increase of the water level on the other side during measurements of gas (Fig. 3.6).
The plexiglass U-tube (Fig. 3.7) consisted of a U-tube made of plexiglass, a relay, a float switch, a counter, a timer and a three-way solenoid valve. The U-tube unit contained silicone oil (Dow Corning Pty. Ltd.).
The mechanism of gas measurement using the plexiglass U-tube is as follows:

Gas produced from the reactor will accumulate on one side of the U-tube and displace the silicone oil on this side, resulting in an increase of the liquid level on the other side. When the oil reaches a certain point, it activates the float switch which then triggers three events simultaneously: a signal is sent to the counter to record the number of clicks and display it; the accumulated gas is then exhausted to atmosphere through solenoid valve which reset the liquid level, and the timer is activated to allow gas to escape and to allow liquid to reach a steady level. The timer is set manually at 3 seconds. At the completion of this time, the solenoid valve switches to its original position. During the vent cycle, the three-way solenoid valve isolates the reactor from
the meter (Haris, 2001). Gas production rate can be estimated by multiplying the 
number of clicks per day times the volume of one click divided by 24 hours. 
Calibration was carried out before and after an experimental run.

3.3. Inoculum and Feed

3.3.1. Inoculum

The initial seed sludge used in this study was obtained from anaerobic digesters at 
Woodman Point sewage treatment plant, Perth, Western Australia. The sludge was 
acclimatized for about 5 weeks, fed with molasses based synthetic wastewater at low 
organic loading of 0.5 g COD/l/d before experiments began.

3.3.2. Feed

Molasses based synthetic wastewater was used as the main substrate throughout the 
study. Molasses was obtained from the Pacific Terminal, North Fremantle, Western 
Australia. Molasses was diluted with de-ionized water to attain the required COD 
concentration to be used in each experiment. Raw molasses had a COD value of 797 g 
COD/kg molasses. Nitrogen and Phosphorus were supplied in the forms of NH₄Cl and 
KH₂PO₄, respectively. Sodium hydrogen carbonate was added to the feed to maintain 
buffer condition of the system. Table 3.1 shows the feed composition at the 
concentration of 16 g COD/l which was normal concentration used throughout the 
study. Other trace nutrient requirements were obtained from Trace Metal Solutions 
(TMS) added to the feed (Table 3.2).
Table 3.1 Chemical composition of the feed (16 g COD/l)

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Composition (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molasses</td>
<td>20.1</td>
</tr>
<tr>
<td>KH2PO4</td>
<td>0.5</td>
</tr>
<tr>
<td>NH4Cl</td>
<td>0.8</td>
</tr>
<tr>
<td>NaHCO3</td>
<td>6</td>
</tr>
<tr>
<td>TMS (trace metal solution)</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 3.2 Chemical composition of trace metal solution (TMS)

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Composition (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeCl3.6H2O</td>
<td>5.0</td>
</tr>
<tr>
<td>MgCl2.6H2O</td>
<td>1.0</td>
</tr>
<tr>
<td>MnCl2.4H2O</td>
<td>1.0</td>
</tr>
<tr>
<td>CaCl2.2H2O</td>
<td>1.0</td>
</tr>
<tr>
<td>CoCl2.6H2O</td>
<td>0.3</td>
</tr>
<tr>
<td>NiCl2.6H2O</td>
<td>0.2</td>
</tr>
<tr>
<td>CuSO4.5H2O</td>
<td>0.1</td>
</tr>
<tr>
<td>ZnSO4.7H2O</td>
<td>0.1</td>
</tr>
<tr>
<td>H3BO3</td>
<td>0.1</td>
</tr>
<tr>
<td>Na2MoO4.2H2O</td>
<td>0.1</td>
</tr>
<tr>
<td>AlCl3.6H2O</td>
<td>0.05</td>
</tr>
</tbody>
</table>

The TMS was prepared by dissolving the listed chemicals in 1 l de-ionized water. Two ml of this solution was added per liter of feed. The feed was kept in the fridge
and made up every two to three days to minimize the change in the feed composition due to growth of bacteria. Feed concentrations other than 16 g COD/l were prepared by multiplying all the chemicals listed in Table 3.1 as well as the added TMS. This was done to meet the ratio of TOC:N:P of the feed of 100:10:2 by weight (Haris, 2001).

3.4. Analyses

3.4.1. Solid Determination

The determination of total solids (TS), volatile solids (VS), suspended solids (SS) and volatile suspended solids (VSS) was performed according to Standard Methods (APHA, 1995) as tabulated below.

Table 3.3 Parameters determined using standard methods (APHA, 1995)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total solids (TS)</td>
<td>2540 B</td>
</tr>
<tr>
<td>Volatile solids (VS)</td>
<td>2540 E</td>
</tr>
<tr>
<td>Suspended solids (SS)</td>
<td>2540 E</td>
</tr>
<tr>
<td>Volatile suspended solids (VSS)</td>
<td>2540 D</td>
</tr>
</tbody>
</table>

3.4.2. Chemical Oxygen Demand (COD)

COD of the samples was determined by using the methods of Hach (1996). The samples were diluted to a concentration within a range of 0 and 500 mg COD/l. In a 10 ml Hach tube, 2.5 ml diluted sample, 1.5 ml digestion solution and 3.5 ml sulphuric
acid reagent were mixed and digested in a Hach COD Reactor for 2 hours at 150 °C. The absorbance was read by a Hach Spectrophotometer DR/2010 at a wave length of 620 nm using program number 435. In this kit the sample absorbance was converted into concentration.

3.4.3. Volatile Fatty Acids (VFAs)

Three main volatile acids analyzed during experiments were acetic, propionic and butyric acids. Prior to measurement, 1 ml samples were centrifuged for 10 minutes at 13 000 rpm using a Biofuce pico centrifuge to remove suspended solids. The supernatant was acidified by addition of 1% formic acid to convert the acids to free acid form. The three main acids were analyzed using a Varian Star 3400 Model Gas Chromatography. When the analysis was not carried out at the time of sampling, the samples were stored at – 20 °C. During operation the temperature of the column was ramped from 80 °C to 180 °C at 6 °C /minute and then further ramped to 250 °C at 30 °C /minute for column flushing. The operating parameters for the Gas Chromatography analysis were as tabulated in Table 3.4. Gas chromatograms were recorded and processed by using the Varian Star System Software, version 4.02. Peak area integration method was used for the chromatogram analysis and standard curves of each acid (Fig. 3.8, 3.9 and 3.10) were plotted and used to calculate the sample concentrations. The new standard curves were made several times during the study to minimize any deviation during measurements. Three mM external standard solutions were regularly measured to recheck the validity of the standard curves.
Table 3.4 Analysis conditions for VFAs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Capillary column, 15 m x 0.53 mm</td>
</tr>
<tr>
<td></td>
<td>EC-1000</td>
</tr>
<tr>
<td>Auxiliary temperature</td>
<td>250 °C</td>
</tr>
<tr>
<td>Carrier gas</td>
<td>High purity nitrogen</td>
</tr>
<tr>
<td>Oven temperature</td>
<td>250 °C</td>
</tr>
<tr>
<td>Detector</td>
<td>Flame Ionization Detector (FID)</td>
</tr>
<tr>
<td>Detector temperature</td>
<td>250 °C</td>
</tr>
<tr>
<td>Injection type</td>
<td>Automatic injection</td>
</tr>
<tr>
<td>Injection temperature</td>
<td>250 °C</td>
</tr>
<tr>
<td>Sample size</td>
<td>0.6 ml</td>
</tr>
<tr>
<td>Injection volume</td>
<td>1 µL</td>
</tr>
<tr>
<td>Total analysis time</td>
<td>23 minutes</td>
</tr>
</tbody>
</table>

![Graph showing the relationship between area (counts) and concentration (mM)](image)

\[ y = 1E-05x - 0.0861 \]
\[ R^2 = 0.9873 \]

Fig. 3.8 Acetic acid standard curve
3.4.4. pH

The sample pH was measured by glass electrode Jenco pH meter Model 6230.

3.4.5. Density of Carriers

Density of the carriers was estimated from the weight of the carriers over volume occupied by the carriers in a 5 ml syringe (Equation 3.1). The weight of 5 ml syringe was determined (a). Granular rubber tire supports were placed in this syringe and their
weight was measured (b). Water was then injected to the void volume in the syringe while holding the syringe plunger and they were weighed (c). Five in equation 3.1 denotes the volume of the syringe in ml and the units for other symbols are in gram.

\[
\text{Carrier’s density (g/cm}^3) = \frac{b-a}{\frac{5}{c-b}} \quad \text{……………… (3.1)}
\]

3.4.6. Specific Surface Area

The specific surface area of the granular rubber carriers was determined by using a Micromeritics Gemini instrument following ASTM C1069 – ’86 (CSIRO, Waterford, Western Australia). A representative sample was placed in a clean glass Gemini sample tube and outgassed in vacuum at 20 °C until vacuum level at 110 mTorr. The glass tube together with the sample was then connected to the Gemini analysis port and surface area of the sample was estimated by the Brunauer Emmett Teller (BET) theory using adsorption data obtained from the instrument.

3.4.7. Scanning Electron Microscopy

Attached bacteria on the surface of granular rubber tire were examined by Scanning Electron Microscope XL 20 (Philips). Samples for microscopy was prepared by dehydration in a series of ethanol (30, 50, 70, 80, 90 and 95%) and then drying at 40 °C in Balzers Union critical point drying chamber (Liechtenstein). Samples were coated with gold/palladium before microscopic examination.
4.1. Introduction

Common designs for anaerobic treatment systems are the continuously fed stirred tank reactor (CSTR), up-flow anaerobic sludge blanket (UASB), and anaerobic fluidized bed reactor (AFBR). Among these three reactor types a CSTR is more widely used due to its simplicity of operation and design and independent of biomass type. However, this type of reactor has to be operated at hydraulic retention times (HRT) of the order of 16 to 30 days (Lin et al., 1986; Kim et al., 2002) since biomass is continuously lost with the effluent. One of the special features of a UASB reactor is its ability to retain a high biomass concentration within the reactor of about 30 to 50 g VSS/l (Weiland and Rozzi, 1991). This, however, very much depends on the granulation properties of the sludge, which is very sensitive to organic loads and promoted by very low loading during a long start-up period (Lettinga et al., 1983; Lettinga et al.; 1984 a; Borja and Banks, 1994 b; Liu et al., 2002). An AFBR combines attached film and fluidization technology. This reactor contains small particles such as sand on which the biofilm forms. The bed is fluidized with the aims to maximize biomass concentration in the reactor without clogging and to achieve the optimum biofilm thickness for good mass transfer (Heijnen et al., 1989; Iza, 1991; Setiadi and Ginting, R. 1993 and 1994). However, a high recycle ratio must be maintained and energy consumption is high to maintain the bed fluidized (Olthof and Oleszkiewicz, 1982; Iza, 1991; Weiland and Rozzi, 1991).
To overcome some of the drawbacks mentioned above and to take advantage of the simplicity of a CSTR technology, this study intensified the operation of an existing CSTR by addition of granular rubber tire as carriers to promote the attached biofilm growth. The use of relatively light carriers may result in retaining the active biomass in the reactor with minimum energy required for carrier movement. This reactor type is henceforth called an anaerobic moving bed reactor (AMBR).

4.2. Experimental Methods

4.2.1. Acclimatization of the Sludge

Acclimatization of the sludge was performed as follows:
Two liter sludge was poured into a 5 l plexiglass vessel served as the AMBR. The reactor was fed once a day with 50 ml of 16 g COD/l molasses based feed for 2 weeks. Effluent was not withdrawn during this period. Over the next 3 weeks OLR was gradually increased by increasing feed flow rates to the digester. Effluent was still not withdrawn during this period. At the end of the period the volume of the liquid in the digester was 4 l. Three liters of this acclimatized sludge was used for a toxicity test and rest of the sludge in the AMBR was used for start-up experiment.

4.2.2. Characteristics of the Carriers and Toxicity Tests

Characteristics of the carriers measured were density and surface area. This was conducted to compare these parameters with the same parameters of the carriers employed by similar reactor design called the moving bed biofilm reactor developed
by a Norwegian company, Kaldnes Miljoteknologi A/S. Methods to measure both parameters can be found in sections 3.4.5 and 3.4.6, respectively.

The toxicity test was performed in two 2 l modified Schott bottles. Into these 2 bottles, one of which contained 300 g of granular rubber tire, 1.5 l of acclimatized sludge (mentioned in Section 4.2.1) was poured. Both Schott bottles were placed in an aquarium tank water bath maintained at 37 °C. Mixing in a bottle containing sludge and granular rubber carriers was performed by using 3 cm oval shaped magnetic stirrer bar and for the other bottle containing sludge only was performed by using 1 cm rod shaped magnetic stirrer bar to obtain the same rotation of 180 rpm. The OLR was 1.6 g COD/l/d and experiment was performed for 7 days and methane accumulation was followed during this period.

4.2.3. Start-up and Operations of Anaerobic Moving Bed Reactor (AMBR)

The plexiglass AMBR containing about a liter of acclimatized sludge (mentioned above) was added with 0.5 l fresh sludge and acclimatized with the same procedure until the volume of the liquid in the digester reached 2.2 l. No effluent withdrawal was done during this period. Granular rubber tire carriers (1.5 kg) were then added to the reactor and effluent was started to withdraw to maintain a constant active reactor volume of 2.2 l. The calculations of OLR and HRT were now based on the active reactor volume of 2.2 l. This starting point was defined as day zero for start-up operation.

Start-up operation was conducted over a 100 day period. During this period, the HRT was stepwise decreased from 32 to 16 days by increasing the OLR from 1.2 to 2.2 g
COD/l/d. The stepwise decrease in HRT in the range of 10 to 30% was implemented only when stable reactor performance (indicated by constant gas productions) had been attained.

After the reactor start-up was accomplished, the value of HRT, which was 16 days, was decreased to 6 days by increasing OLR from 2.2 to 5.8 g COD/l/d. The stepwise decrease in HRT in the range of 10 to 30% was again implemented and reactor performance during this decrease in HRT was then observed.

During reactor start-up and reactor progress mentioned above total gas production rates, COD removal efficiencies and pH were used as parameters. However, since it was found that at a particular time gas composition may have changed with the increase of methane content (even though the amount of total gas produced was the same) for the next experiment methane production rates were measured instead of total gas production rates.

**4.2.4. Reactor Performance at Low HRTs**

During this experiment, the HRT was reduced gradually from 6 days to 12 hours. The digester was operated for duration of 4 HRTs at each HRT to ensure that the system approached conditions close to steady state. The organic loading rate (OLR) was kept the same at 4 g COD/l/d. It was done to avoid severe organic shock loads brought about by decreases of HRT especially at lower values of HRT. Parameters measured were methane production rate (MPR) in l/l/d and total VFA concentrations in the effluent in g COD/d.
Methane gas production was measured by using a displacement gas meter after passing the gas through soda lime particles to remove CO₂. Therefore, the gas measured by the meter was methane only as it was assumed that only traces of hydrogen was present in the gas (Section 3.2.3).

4.2.5. Biomass Retention in AMBR

The purpose of this experiment was to highlight biomass retention within the digester. The retention of biomass was observed from solid concentrations retained in the digester compared with the solid concentrations washed out in the effluent. To achieve this objective the following approach was taken:

**Solids Concentration in the Digester and Effluent**

The amount of biomass retained among the carriers and that of biomass washed out along with effluent were determined by measuring volatile suspended solids (VSS) of sludge aggregates in the digester and effluent, respectively. The aggregate samples were collected from 4 different heights of the reactor ports without stopping the mixing. Four ports in the reactor are the port at the bottom, at 6 cm from bottom, at 11 cm from bottom, and at 16 cm from bottom or effluent port. VSS from each port was determined and their values from each port were averaged to obtain VSS value of the aggregates. Experiments were performed on Day 180, 272 and 363. Solid distribution along the height of the reactor during these periods was also followed.

Unlike VSS of aggregates which was determined during certain periods mentioned above, the VSS of the effluent was determined every 3 weeks and variations in
effluent solid concentrations were followed. Exception was with the second measurement which was determined after 1 month of operation.

**Significance of Carriers**

This experiment was performed after the digester had been operated for more than 12 months. The reactor was run at 3.8 d HRT and OLR of 4.2 g COD/l/d until the steady state was reached. The rubber carriers were then removed from the digester after separating the sludge aggregates that were loosely adhered to these carriers. The sludge aggregates remained in the digester. This digester was now called a stirred tank reactor (STR). The STR was run at the same HRT and organic loading rate of 3.8 d HRT and OLR of 4.2 g COD/l/d, respectively during 3 days or 72 hours.

A simple test was also conducted to observe the settling nature of biomass aggregates. One of the two 100 ml measuring cylinders was filled with 100 ml sludge aggregates; the other one was filled with 40 ml carriers and 60 ml sludge aggregates to make the same volume of 100 ml. Both cylinders were stirred gently before settling velocity of biomass aggregates in both cylinders was measured.

**4.2.6. Contribution of Attached Bacteria to Whole Population**

In this study the term “attached bacteria” is defined as the bacteria that adhere firmly to the surface of carriers which can only be separated mechanically e.g. by sonication. The objective of the study was to determine how significant the contribution of the attached bacteria to the whole population in the digester. To achieve the objective several approaches were made.
Growth of the attached bacteria

The first approach was to check for any growth of attached biomass by determining the volatile suspended solids (VSS\(_{\text{att}}\)) of the bacteria attached on the carriers. Extent of the attached bacteria (on the surface of the carriers) was observed by determining VSS\(_{\text{att}}\) on the 190\(^{\text{th}}\), 283\(^{\text{rd}}\) and 374\(^{\text{th}}\) days of reactor operation. The procedure to dislodge attached bacteria followed similar methodology used by Meraz et al. (1995). The carriers were separated from aggregates by using a 2 mm mesh size plastic screen. The carriers were washed gently with de-ionized (DI) water for 2 to 3 times to separate any suspended bacteria. The attached bacteria were dislodged from the carriers by using Branson sonifier at 50% duty cycle, which could allow the bacterial detachment but did not cause any breakage of the carriers. The sample water (containing attached bacteria) was dried over night at 103 °C and then ignited at 550 °C for 6 hours. The clean carriers were dried at 55 °C for 2 days and their weight was measured. The concentration of the attached bacteria is expressed in g/kg carriers.

Methanogenic activity from bacterial aggregates in the presence and in the absence of carriers

The second approach was to determine the difference in methanogenic activity from reactor containing only biomass aggregates and that containing biomass aggregates plus attached biomass (i.e. whole population). The methanogenic activity from bacterial aggregates was performed on Day 200, 290 and 380. This experiment was conducted in 100 ml serum bottles and samples were duplicated. During each period, 50 ml of aggregates from the AMBR were poured into 4 serum bottles and a scoop of carriers free from suspended aggregates (after washing) was added into 2 of these 4
serum bottles. Acetate at saturating concentrations was added to the bottles and to ensure anaerobic condition the bottles were purged with nitrogen before incubation. They were incubated at 37 °C for 2 hours and gas produced was measured every hour. VFA concentrations were also checked hourly and VSS concentrations were measured at the end of experiment. Methane gas composition obtained from the AMBR measured by GA 2000 Gas Analyzer was used here to convert methane from total gas produced from the serum bottles. Methanogenic activity was expressed in ml CH₄/g VS/h.

Scanning Electron Micrographs

The third approach was to observe the occurrence of attached bacteria using Scanning Electron Microscopy. Photos were taken on 0, 90 and 180 days after the carrier addition following the method described in section 3.4.7.

4.3. Results

4.3.1. Characteristics of the Carriers and Toxicity Tests

Density of the rubber tire carriers was 0.96 ± 0.02 g/cm³ while their surface area was 30 ± 1.5 m²/m³.

Both reactors, one with carriers and the other one without carrier addition, produced the same methane accumulations (Fig. 4.1).
4.3.2. Start-up and Operations of AMBR

It should be noted that total gas was measured during start-up of AMBR. Therefore, total gas production efficiency was presented in this section.

During the first week biogas production was about 0.2 l/l/d and it became almost constant at about 0.35 l/l/d until Day 23 (Fig. 4.2). The specific methane yield was about 0.16 l CH₄/g COD degraded. During Day 24 to Day 62 a gradual increase of gas production rates (0.35 to 0.90 l/l/d) and that of organic removal efficiency (32 to 80%) occurred. The calculated specific methane yield changed from 0.16 to 0.31 l CH₄/g COD degraded. It shows that the process was becoming stable and the biomass was adapting to the feed conditions used. From Day 63 onwards a total gas production of about 1.2 l/l/d and constant COD removal efficiency of about 80% were evident. The steady state specific methane yield obtained during this period ranged from about 0.31 to 0.38 l CH₄/g COD removed. This showed the achievement of a stable operation.
After the reactor start-up was accomplished, the value of HRT of 16 days was stepwise (10 to 30%) decreased to 6 days by increasing OLR from 2.2 to 5.8 g COD/l/d. During this period gas production rates increased from 1.2 to 2.7 l/l/d while maintaining a COD removal of about 80% (Fig. 4.3). The calculated specific methane yield ranged between 0.31 to 0.38 l CH₄/g COD removed and residual total VFA in the effluent remained low about 0.2 g COD/l. From this period of operation it can be concluded that the AMBR could perform satisfactorily at a 6 days HRT and an OLR of 5.8 g COD/l/d.
It is shown in Fig. 4.3 that total gas produced was not proportional to loading increases. This might be because at a particular time gas composition may have changed with the methane content increasing even though the amount of total gas produced was the same. It is the methane production rate that increases more or less proportionally with organic loading rate whereas, gas production rate is in addition influenced by CO₂ composition as well which in turn can be influenced by the physico-chemical equilibrium condition in the liquid phase.
The methane gas composition, which was measured two to four times during each loading showed an increase from 55 to 72% of total gas over the duration of operation represented in Fig. 4.3. When the methane production rate (MPR) was used to check the performance of the digester during step increase in OLR (Section 5.4.2; page 111), it showed that a 20% increase in OLR resulted in a proportional increase in MPR.

4.3.3. Reactor Performance at Low HRTs

MPR and total VFAs in liquid during this study were plotted against HRT (Fig. 4.4). Three data points at 7 to 9d HRT obtained from Fig. 4.3 were included in this figure to indicate values at higher HRT. The figure shows that the MPR was almost constant at about 1.2 l/l/d when the digester was operated under HRTs of 1.5d and above. The rate dropped drastically when the digester was run at HRTs shorter than 1.5d. This indicated that the AMBR was capable of handling HRTs of 1.5d and higher at an OLR of 4 g COD/l/d.

Total VFA concentrations in the effluent were remained approximately the same at 0.17 g COD/d for experiments conducted at and higher than 3.8 d HRT. The total VFAs doubled at 2.5d HRT and rose drastically at HRTs lower than 2.5d HRTs and eventually reached 2.7 g COD/l when the system was pushed to run at 0.5d HRT.
Fig. 4.4 Performance of AMBR at lower HRTs

4.3.4. Biomass Retention in AMBR

*Solid Concentrations in the Digester and Effluent*

The average values of VSS aggregates and those of effluent are tabulated in Table 4.1. The increase of the aggregates concentrations in digester and decrease of effluent solid concentrations by time (represented by their VSS values) were observed. It indicates that as the digester was continued to be operated more biomass was retained within the digester and less wash out occurred with time.

<table>
<thead>
<tr>
<th>VSS concentration (g/l)</th>
<th>Period (day)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>180</td>
<td>272</td>
<td>363</td>
<td></td>
</tr>
<tr>
<td>Aggregates inside the AMBR</td>
<td>9.4 ± 3%</td>
<td>10.6 ± 0.2%</td>
<td>11.4 ± 3%</td>
<td></td>
</tr>
<tr>
<td>Treated effluent</td>
<td>4.3 ± 4%</td>
<td>3.0 ± 2.8%</td>
<td>2.2 ± 10%</td>
<td></td>
</tr>
</tbody>
</table>

The measurement of VSS effluent was conducted every 3 weeks (except the second measurement which was done a month after the first measurement) to observe
variations in effluent solid concentrations. As shown in Fig. 4.5 the amount of aggregates washed out decreased with time even though HRTs were decreased or organic loading rates applied were increased.

**Fig. 4.5** Change in effluent VSS concentrations and corresponding OLR and HRT

Solid distribution along the height of the reactor was followed on Day 180, 272 and 363 (Fig. 4.6). It is clearly shown that more bacteria were present in the middle of the reactor shown by higher VSS values. The least biomass was present at the height around the effluent port (16 cm from bottom). This demonstrated again that more bacteria were retained inside the reactor than the bacteria washed out along with effluent.
Decreases of VSS at the effluent port may be due to increasing of settling nature of biomass aggregates by time. Fluctuation of the amount of bacterial aggregates at the bottom of the digester may be caused by non uniform mixing on the bottom part. This resulted in non uniform solid sampling. In addition, the bottom part might have contained the largest amount of inorganic solids which were deposited and never got removed.

**Significance of Carriers**

Time zero in Fig. 4.7 represents results from the digesters with the presence of carriers and from 24 h onwards are the results from the digesters with the absence of carriers. With the presence of carriers, gas production efficiencies of about 96% were obtained. With the absence of carriers gas production efficiencies dropped to 42%, 29% and 26% over 24, 48, and 72 h of digester operation, respectively. Decreases in gas production which were also accompanied by accumulations of VFAs indicated that in the absence of carriers, wash out of the aggregates occurred. pH, which only dropped from 7.2 to 6.8 during observation might be due to excellent buffer capacity of feed.
**Fig. 4.7** Performance of digester after removal of carriers at 3.8d HRT and 4.2 g COD/l/d

The settling nature of biomass was observed by measuring settling velocities of the biomass aggregates in the presence and absence of carriers. With the presence of carriers, the settling velocity of the aggregates was very rapid (about 30 ml/min) while in the settling velocity of the aggregates in the absence of carriers was only 3 ml/min. Taking into account of the sludge aggregates of only 60 ml in a cylinder containing carriers, the settling velocity of the aggregates in the absence of carriers was equal to 5 ml/min.

### 4.3.5. Contribution of Attached Bacteria to Whole Population

As mentioned in Section 4.2.6 the term “attached bacteria” in this study is defined as the bacteria that adhere firmly to the surface of carriers which can only be separated mechanically, e.g. by sonication.
**Growth of the attached bacteria**

Extent of the attached bacteria (on the surface of the carriers), observed by determining VSS\(_{\text{att}}\) on the 190\(^{\text{th}}\), 283\(^{\text{nd}}\) and 374\(^{\text{th}}\) days of reactor operation, was presented in Table 4.2.

<table>
<thead>
<tr>
<th>Concentration (g/kg carriers)</th>
<th>190 days</th>
<th>283 days</th>
<th>374 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>VSS(_{\text{att}})</td>
<td>1.22 ± 2%</td>
<td>1.49 ± 16%</td>
<td>1.23 ± 5%</td>
</tr>
</tbody>
</table>

Values are average of 2 measurements

**Methanogenic activity from bacterial aggregates in the presence and in the absence of carriers**

Increases of methanogenic activity by time were seen from both aggregates in the presence and in the absence of carriers. The difference in methanogenic activity between these two aggregates ranged between -2 to 6% (Table 4.3).

**Scanning Electron Micrographs**

Figures 4.8 to 4.10 present scanning electron micrographs taken to check bacterial attachment on 0, 90 and 180 days after the carrier addition, respectively. No evidence of significant attached growth was observed during these periods. It confirmed that only a few of biomass formed as biofilm attachment on the surface of the carriers.
Table 4.3 Activity differences due to the addition of the carriers after 200, 290 and 380 days of operational periods

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Specific methanogenic activity from biomass aggregates and carriers (ml CH4/g VS/h)</th>
<th>Specific methanogenic activity from biomass aggregates only (ml CH4/g VS/h)</th>
<th>Activity difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>7.9 ± 2%</td>
<td>8.1 ± 0%</td>
<td>-2</td>
</tr>
<tr>
<td>290</td>
<td>10.8 ± 0%</td>
<td>10.8 ± 2%</td>
<td>0</td>
</tr>
<tr>
<td>380</td>
<td>13.1 ± 2%</td>
<td>12.3 ± 1%</td>
<td>6</td>
</tr>
</tbody>
</table>

Values are average of 2 measurements

Fig. 4.8 Scanning Electron Micrographs of the plain carriers (0 day)
Fig. 4.9 The Scanning Electron Micrograph of the carriers at 90 days of operation.

Fig. 4.10 The Scanning Electron Micrograph of the carriers at 180 days of operation.
4.4. Discussion

4.4.1. Characteristics of the Carriers and Toxicity Tests

As mentioned in the introduction, reactor simplicity and low energy cost were the main aims in developing a novel reactor design called the AMBR. Researchers from Norway have employed a similar reactor type called moving bed biofilm reactors (MBBR) which was developed by a Norwegian company, Kaldnes Miljøteknologi A/S for biological wastewater treatment (Jahren et al., 1999; Odegaard et al., 1994; Jahren and Odegaard, 2000). The MBBR uses polyethylene carriers to which the granular rubber tire carriers employed in this study were compared (Table 4.4).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Granular rubber tire carriers</th>
<th>Kaldnes carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density (g/cm³)</td>
<td>0.98</td>
<td>0.92 – 0.96</td>
</tr>
<tr>
<td>Surface area (m²/m³)</td>
<td>30</td>
<td>350</td>
</tr>
<tr>
<td>Shape</td>
<td>Irregular mostly cube, retained in a screen with a mesh size of 2.8 mm</td>
<td>cylinders, 10 mm diameter, 7 mm height, a cross inside the cylinder and longitudinal fins on the outside</td>
</tr>
<tr>
<td>Cost (AUDS/kg)</td>
<td>0.10</td>
<td>2.00</td>
</tr>
</tbody>
</table>

Granular rubber tire carriers were chosen because it is an inexpensive or no cost waste material and readily available. With a specific surface area of one tenth of the Kaldnes carriers the amount of rubber carriers needed to get the same area of bacterial attachment would be more than 10 times. However, considering that granular rubber
tire costs only a twentieth of the Kaldnes carriers the larger reactor volume that will be required for the granular rubber tire may be justified. Moreover, with the rubber tire a waste material is being recycled for beneficial use.

Bigger sizes of granular rubber tire carriers were not chosen since they might provide either less protection for biomass entrapment or less surface areas for biomass attachment. Whereas smaller sizes of the carriers would float on the surface of the liquid in the reactor, lifted by the gas produced, and may result in ineffective biomass entrapment or attachment.

A toxicity test was performed to investigate any toxic effects of the rubber on anaerobic bacteria. There was no difference in gas produced from the 2 reactors; one reactor with addition of carriers and the other one without addition of carriers (Fig. 4.1) indicated that no toxicity effects of the rubber on the biomass occurred during observation.

4.4.2. Start-Up and Operations of AMBR

During this study step increases in OLR in the range of 10 to 30% were applied by increasing feed flow rates. It was performed to avoid system instability or ‘sour’ condition (Bull et al., 1983 a; Perez et al., 1997; Michaud et al., 2002) created by sudden variation of organic loading rates.

The percentage of compounds in molasses that are anaerobically non biodegradable (in % g COD) was calculated from a COD balance measured in a continuously fed stirrer tank anaerobic digester (CSTR) fed with molasses and operated at steady state
conditions. It has been reproducibly found that molasses has non biodegradable compounds of about 15% (observed simultaneously in Experiments 6.2.1, see Appendix 2 for detail calculations). Therefore, calculations of gas production efficiencies were based on 85% of biodegradable compounds in molasses feed. Degradability of molasses was reported to be 90% by Denac and Dunn (1988) and 76% by Yeoh (1997). The composition of molasses may vary, the COD removal efficiency of 80% obtained from the start-up period in this study was, therefore, considered to be adequate.

Total gas production of about 1.2 l/l/d, constant COD removal efficiency of about 80% and residual total VFAs of 0.4 g COD/l were evident from Day 63 onwards (Fig. 4.2) signified an achievement of stable operation. Therefore, the start-up of the digester to treat molasses based feed at an OLR of 2.2 g COD/l/d was accomplished over a 100 day period.

The start-up period of about 3 months obtained in this study is in the range of start-up periods reported in literature. Camilleri et al. (1988) observed start-up periods for anaerobic fixed film reactors fed with wastes from beet sugar refining or sugar molasses processing ranged between 3 to 4 months. The start-up periods for a UASB ranged between 4 weeks to 6 months of operation (Lettinga, 1984; Lettinga et al., 1984; Souza, 1986) and that of an AFBR was about 2 months (Balaguer et al., 1992).

The steady state specific methane yield obtained during this period (from Day 63 onwards) ranging from about 0.31 to 0.38 l CH₄/g COD removed was in agreement with values reported in literature, around 0.35 l CH₄/g COD degraded (Bull et al., 1983; Hsu and Shieh, 1993; Borja and Banks, 1994 c).
After reactor start-up was accomplished, attempt to decrease HRT from 16 to 6 days was successful (Fig. 4.3). It signified that biomass retention by rubber tire carriers was obtained. Usually digesters with no biomass retention fail to operate at HRT less than 16 days (Lin et al., 1986; Kim et al., 2002) because of the slow growth of aceticlastic methanogenic bacteria (Section 2.2.3). From this experiment it can be concluded that the AMBR could perform satisfactorily at a 6 days HRT and an OLR of 5.8 g COD/l/d.

It is shown in Fig. 4.3 that total gas produced was not proportional to loading increases. This might be because at a particular time gas composition may have changed with methane content increasing even though the amount of total gas produced was the same. It is the methane production rate that increases more or less proportionally with organic loading rate while gas production rate is in addition influenced by CO₂ composition as well which in turn can be influenced by the physico-chemical equilibrium condition in the liquid phase.

The methane gas composition, which was measured two to four times during each loading showed an increase from 55 to 72% of total gas over the duration of operation represented in Fig. 4.3. When the MPR was used to check the performance of the digester during step increases in OLR it showed that a 20% increase in OLR resulted in a proportional increase in MPR. For the next experiment MPR (instead of total gas) was used as parameter.
4.4.3. Reactor Performance at Lower HRTs

As expected, the lower the HRT imposed the higher the VFAs accumulated in the system. However, as seen in Fig. 4.4 total VFAs rose drastically starting from 2.5 d HRT but MPR only started to drop after operation at 1.5 d. This may be because a drastic increase from low total VFAs (of 0.35 g COD/d, occurring during 2.5 d HRT) would not cause very high total VFAs and thus not result in a severe drop in MPR.

Since doubling times of aceticlastic methanogenic bacteria are about 3 days (Lawrence and McCarty, 1969; Zehnder and Wuhrmann, 1977) and this type of bacteria contributes to about 70% of methane gas generation in anaerobic processes (Kaspar and Wuhrmann 1978 a; Gujer and Zehnder 1983) the conventional CSTR can not be operated at low HRTs. It has been demonstrated (Fig. 4.3) that the AMBR was able to be operated at 6 d HRT and OLR of 5.8 g COD/l/d and at 1 d HRT and OLR of 4 g COD/l/d. This would have been possible by the effective retention of biomass by carriers.

In terms of methane production efficiency and total VFA concentration at a 1 d HRT operation which were 87% and 12% of degradable COD in input, respectively (Fig. 4.4), it can be concluded that the system could still perform satisfactorily up to 1 d HRT.

The performance of AMBR in comparison with other reactor designs can be seen in Table 4.5. It is clearly shown in this Table that the AMBR can be classified as high-rate anaerobic reactor.
4.4.4. Biomass Retention in AMBR

A high viable retention of biomass is the main key to gain success in operating high-rate anaerobic reactors. In conventional processes, the retention of the required biomass was achieved by combining the mixed reactor with an internal or external biomass separation and a recycle system. In high-rate reactors, the retention of biomass is achieved by immobilization of biomass. The immobilization of biomass can be classified into 3 forms, namely settleable sludge aggregates, bacterial attachment on carrier particles and entrapment of sludge aggregates between packing materials within the reactor (Lettinga, 1984). The term “bacterial aggregates” in this study are defined as settleable aggregates which retained among carriers present in the digester. Retention of biomass in the digester was followed by measuring the above defined aggregates.

Solid Concentrations in the Digester and Effluent

In a CSTR type digester one would expect the biomass concentration (or VSS) within the digester and that in the effluent to be about the same. The VSS washed out along with the effluent (2.21 ± 9%) were much less compared with those washed out from CSTRs of about 8.99 ± 8% g/l (Table 6.2).

In this study, with much more bacterial aggregates retained within the digester compared to the less settled biomass washed out with effluent signified that more biomass would be retained within the digester when the digester was operated longer.
### Table 4.5 Comparison of the performance of AMBR with that of other reactor designs

<table>
<thead>
<tr>
<th>No.</th>
<th>Reactor design</th>
<th>Carriers</th>
<th>Wastewater type</th>
<th>OLR (g COD/l/d)</th>
<th>HRT (d)</th>
<th>Density (kg/l)</th>
<th>Removal Rate (%)</th>
<th>Total VFA (g/l)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>AMBR</td>
<td>Granular rubber tyre</td>
<td>Molasses</td>
<td>4</td>
<td>1</td>
<td>0.96</td>
<td>87</td>
<td>1.1</td>
<td>This study</td>
</tr>
<tr>
<td>2.</td>
<td>Anaerobic Moving Bed Biofilm Reactor</td>
<td>Kaldnes polyethene carriers</td>
<td>TMP whitewater</td>
<td>1.4</td>
<td>2</td>
<td>0.96</td>
<td>70</td>
<td>NM*</td>
<td>Jahren et al. (1999)</td>
</tr>
<tr>
<td>3.</td>
<td>Anaerobic Fixed Film Reactor</td>
<td>Endosphere™</td>
<td>Glucose</td>
<td>6</td>
<td>1.5</td>
<td>0.7</td>
<td>80</td>
<td>NM</td>
<td>Michaud et al. (2002)</td>
</tr>
<tr>
<td>4.</td>
<td>Anaerobic Packed Bed Reactor</td>
<td>Shredded rubber tires (3x3x1 cm³)</td>
<td>Cane molasses distillery slops</td>
<td>24</td>
<td>2</td>
<td>NM</td>
<td>85</td>
<td>2</td>
<td>Borja et al. (1996)</td>
</tr>
<tr>
<td>5.</td>
<td>Inverse Turbulent Bed</td>
<td>Endosphere™</td>
<td>Wine distillery</td>
<td>10</td>
<td>NM</td>
<td>NM</td>
<td>80</td>
<td>2</td>
<td>Buffiere et al. (1994)</td>
</tr>
<tr>
<td>6.</td>
<td>Up-flow Anaerobic Filter</td>
<td>SIRAN™</td>
<td>Dairy wastewater</td>
<td>21</td>
<td>0.5</td>
<td>NM</td>
<td>70</td>
<td>1.2</td>
<td>Anderson et al. (1994)</td>
</tr>
<tr>
<td>No.</td>
<td>Reactor design</td>
<td>Carriers</td>
<td>Wastewater type</td>
<td>OLR (g COD/l/d)</td>
<td>HRT (d)</td>
<td>Density (kg/l)</td>
<td>Removal Rate (%)</td>
<td>Total VFA (g/l)</td>
<td>Reference</td>
</tr>
<tr>
<td>-----</td>
<td>----------------</td>
<td>----------------</td>
<td>-----------------------------------------------------</td>
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<td>-----------------------------</td>
</tr>
<tr>
<td>7.</td>
<td>ASBR</td>
<td>Polyurethane foam</td>
<td>Synthetic wastewater (meat extract and soluble starch)</td>
<td>0.24</td>
<td>2</td>
<td>0.23</td>
<td>86</td>
<td>NM</td>
<td>Rodrigues et al. (2003)</td>
</tr>
<tr>
<td>8.</td>
<td>UASB</td>
<td>None</td>
<td>Slaughterhouse effluent</td>
<td>13</td>
<td>NM</td>
<td>90</td>
<td>NM</td>
<td>NM</td>
<td>Torkian et al. (2003)</td>
</tr>
</tbody>
</table>

*NM: not mentioned*
Significance of Carriers

The presence of carriers played an important role to retain the biomass aggregates within the digester. Two experimental results supported this claim. The first one was the evidence of fast decline of methane production rates with the absence of the carriers in the system (Fig. 4.7). The second one was the settling nature of biomass which was much faster with the presence of carriers than the settling nature of biomass without the presence of carriers (about 30 ml/min as opposed to only 5 ml/min). One can argue for the latter that VSS concentrations of aggregates in both cylinders were not taken into account. However, it should be noted that settling of biomass in the presence of carriers was as many as six times faster as compared to the case with no carriers.

4.4.5. Contribution of Attached Bacteria to Whole Population

Growth of the attached bacteria

During 283 days of operational period, an increase of about 15% of the attached bacteria occurred but at 374 days a decrease to almost the same values of VSS\textsubscript{att} as those at the Day 190 was obtained (Table 4.2). Optimum attachment may have occurred during the 190 day period, resulting in no significant increase in attachment after this period. From this experiment a conclusion to how much the proportion of attached bacteria to the whole population could not, therefore, be drawn.

Literature reported that the amount of bacteria attached on different support materials ranges between 6 to 104 g VSS\textsubscript{att}/kg carriers (Fox et al., 1990; Meraz et al., 1995; Perez et al., 1997; Pinanco et al., 2001). A proper unit to compare would actually be mass
per surface area but since there was limited data available, this comparison could not be performed. From these published results, however, it appears that only a few of biomass attached on the surface of the carriers used in this study. The carbohydrate degradation was mostly contributed from the activity of biomass aggregates retained among the carriers.

* Methanogenic activity from bacterial aggregates in the presence and absence of carriers *

The highest methanogenic activity difference which was only 6% (Table 4.3) showed that only a few of attached aceticlastic methanogens on the surface of the carriers were present. These results confirmed the above results obtaining few attached bacterial growth compared with whole bacterial population. Therefore, the highest contribution in bacterial activity may have come from the sludge aggregates retained among the carriers not from biofilm growing on carriers.

* Scanning Electron Micrographs *

Even after 180 days of operational period, only few attached growth was seen by scanning electron microscopy. This confirmed that only a few biomass formed as biofilm attachment on the surface of the carriers, which was also observed in the two experiments above. The sludge aggregates retained among carriers within the reactor may have mostly contributed to the degradation of the high strength carbohydrate compounds. Unique type of biomass attachment of the sludge aggregates found in this study may have outweighed concern about the low specific surface area of the rubber carriers mentioned in Section 4.4.1.
The surface characteristic of the granular rubber tire carriers, which is smooth surface, and their porosity might have been the reason why little biomass was observed on the surface of the carriers. Such factors have been discussed in Section 2.3. The result of this study is in agreement with the result of a previous study conducted by Anderson et al. (1994) observing unattached biomass retained among the non-porous carriers contained in anaerobic filters whereas a heavy biomass attachment was retained in the porous carriers.

4.5. Conclusions

- Within only about 2 months of start-up, the digester achieved in excess of 80% soluble COD reduction and total gas was about 1.2 l/l/d at an OLR of 2.2 g COD/l/d.
- The AMBR could be operated without any difficulties at 6 days HRT and OLR of 5.8 g COD/l/d. Methane content during observation was between 55 to 72% of the total gas composition.
- The minimum HRT that can be handled is 1 day at an OLR of 4 g COD/l/d.
- The microbial activity responsible for the degradation resulted from settleable sludge aggregates entrapped among the carriers rather than biofilm growing on carriers.
- The carriers played a significant role in preventing severe wash out of the aggregates.
- The unique type of biomass attachment outweighed concern about low specific surface area of the carriers.
Chapter 5

Performance of Anaerobic Moving Bed Reactor (AMBR) under Disturbances

5.1. Introduction

Organic and hydraulic shock loads are difficult to be avoided in wastewater treatment plants. The stability of the process under adverse operating conditions such as organic and hydraulic overloads or combination of both is one factor determining the success of a biological wastewater treatment. Organic overloading occurs whenever an input of organic matter exceeds the degradation capacity of the microbial ecosystem (Mathiot et al., 1992; Moletta et al., 1994). In anaerobic digestion, it also can be defined as a situation where the reducing equivalents generated from glycolysis accumulate and get channelled into the production of higher carbon-chained volatile fatty acids (VFAs) other than formate and acetate (McInerney and Bryant, 1981; Schink, 1988). Whereas, hydraulic overloading occurs whenever the effective retention time, which is defined as reactor volume over its feed flow rate, is reduced to a point where the microorganisms can not reproduce before being washed out (Graef and Andrews, 1974).

Two types of disturbances that were imposed to the anaerobic moving bed reactor (AMBR) were organic and hydraulic alterations. The aim of this study was to observe the performance and response of the AMBR in dealing with these disturbances.
5.2. Experimental Methods

5.2.1. Reactor Performance during Normal Loads, when Organic Loading and Hydraulic Loading were altered and during Combination of Organic and Hydraulic Shock Loads

The digester was operated at an OLR of 4.2 g COD/l/d and HRT of 3.8 d for a month before reactor performance during normal loads was observed over a day (24 hours). The performance of the digester at this condition, which was defined as a normal base condition (OLR of 4.2 g COD/l/d and HRT of 3.8 d) was then used as the basis for comparison.

Two types of organic load alterations were imposed to the digester. These were a step increase from 4.2 to 5.1 g COD/l/d and three 24 hour organic shock loads from the normal load of 4.2 to 6.4, 7.4 and 10.8 g COD/l/d, respectively. The HRT was maintained the same at 3.8 d. Following each 24 hour organic shock load, the organic loading rate to the digester was returned to normal and operated at normal condition for 2 to 3 HRTs (i.e. 8 – 12 days) before the next shock load was applied.

Before reactor performance was observed during hydraulic alterations, the AMBR was operated at normal base condition for a month. Experiments were then conducted at the same OLR of 4.2 g COD/l/d but at different HRTs. Initially the HRT was dropped to 2.5 d from the normal HRT of 3.8 d for only 8 h and the retention time was returned to normal value thereafter. After ascertaining that the digester can handle a change in HRT for short duration, hydraulic step changes (from 2.5 to 1.5 d, 1 to 0.75 d, and 1 to 0.5 d) were applied for a period of 24 hours or longer. After each HRT
alteration, the digester was operated at normal load for 2 to 3 HRTs (i.e. 8 – 12 days) before the next HRT alteration.

The AMBR, which was operated under an OLR of 4.2 g COD/l/d at 3.8 d of HRT, was now operated at an OLR of 6.4 g COD/l/d at 2.5 d HRT. This value even though conservative was chosen based on the results obtained above which showed that the AMBR could handle well the organic shock load of 6.4 g COD/l/d and a hydraulic shock load of 2.5 d.

### 5.2.2. Sampling and Analysis

Volatile fatty acids (acetic, propionic and butyric acids) concentrations, methane production rate (MPR), methane production efficiency and pH during operational periods were used as parameters to observe the digester performance. In experiments here if the total VFAs in the effluent were equal or less than 0.5 g COD/l the digester operation was considered to be stable or normal. Grady et al. (1980), Kennedy et al. (1985) and Chynoweth et al. (1994) have classified total VFA values in the range of 0.3 to 0.5 g COD/l as indication of normal digester operation. Methane production rates (MPR) were averaged from 4 measurements taken every hour for 4 hours since the digester was fed every 4 hours. The methane production efficiency was calculated as the percentage of the MPR over the theoretical or expected MPR. Calculations for theoretical or expected MPR can be seen in Appendix 3.
5.3. Results

5.3.1. Reactor Performance during Normal Loads

As mentioned previously, the digester was fed once every 4 hours. Such a feeding pattern is bound to change VFA concentrations over a day (Fig. 5.1). The MPR was averaged over a four hour period from hourly measurements (Section 5.2.2). As the readings were noted manually it was not possible to record readings at night. Therefore, two values at 4 hours and 8 hours are shown, the next reading is the value after 24 hours.

During normal operation acetic acid concentration was between 1.8 to 2.6 mM, propionic acid was in the range of 1.7 to 2.1 mM and butyric acid was around 0.1 mM. The highest concentrations of both acetic and propionic acids were found every 4 hours (reflecting the feeding strategy). The total VFAs in the effluent were about 0.2 g COD/l. The pH during observation was about 7.2. Daily MPR was about 1.2 l/l/d or equivalent to 98% methane production efficiency.

![Fig. 5.1 Response of the digester at an OLR of 4.2 g COD/l/d and 3.8d HRT](image-url)
5.3.2. Reactor Performance when Organic Loading was Increased

*Step increase from 4.2 to 5.1 g COD/l/d.* During the first 24 hours after the step load increase (i.e. 24 to 48 h in Fig. 5.2), acetic and propionic acids increased from 1.9 to 4.2 mM and from 1.7 to 2.9 mM, respectively. From 48 to 72 hours acetic and propionic acids decreased from 4.2 to 1.8 mM and from 2.9 to 1.6 mM, respectively. From 72 to 96 h lower concentrations of both acetic and propionic acids were maintained, and eventually the concentrations of acetic and propionic acids dropped to 1.5 and 1.3 mM, respectively at 96 hours. Almost no changes in the concentration of butyric acid (0.1 mM) were found during observation. The pH remained around 7.

Table 5.1 summarizes the total VFAs after step increases. An accumulation of total VFAs of 0.61 g COD/l only occurred at the end of the first day after the step loading was applied (at 48 hours). At the end of the second day the total VFA value decreased to the normal value of 0.3 g COD/l or equal to the VFA value before the step loading was applied. It signified that the process had become stable and the biomass adapted to the increased feed load used and the organic loading rate applied was below the maximum loading capacity of the digester.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>OLR (g COD/l/d)</th>
<th>Total VFAs (g COD/l/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>4.2</td>
<td>0.31</td>
</tr>
<tr>
<td>48</td>
<td>5.1</td>
<td>0.61</td>
</tr>
<tr>
<td>72</td>
<td>5.1</td>
<td>0.31</td>
</tr>
<tr>
<td>96</td>
<td>5.1</td>
<td>0.26</td>
</tr>
</tbody>
</table>
MPR increased sharply from 1.2 to 1.5 l/l/d, 4h after the step change was applied but it then decreased to constant values of about 1.44 l/l/d thereafter. Methane production efficiency was in the range of 94 to 97%.

![Graph](image1)

**Fig. 5.2** Response of the system to a step change in organic load from 4.2 to 5.1 g COD/l/d at the same HRT of 3.8d

**Organic Shock Load from 4.2 to 6.4 g COD/l/d.** Upon application of a 50% shock load, the VFA concentrations in the effluent changed from low levels (Fig. 5.3, 1 to 24 hours) to higher concentrations (Fig. 5.3, 24 to 48 hours). A sharp increase of both acetic and propionic acids occurred during the first 8 hours, i.e. from 2.5 to 5.2 mM and from 1.7 to 2.7 mM, respectively. From 32 to 48 hours acetic acid further
increased to 6.4 mM whereas, propionic and butyric acids leveled off at 2.7 and 0.2 mM, respectively. VFA concentrations returned to normal levels of 1.5, 1.3 and 0.1 mM 24 hours after shock load was terminated. During shock load, the total VFAs in the effluent increased more than double than that at normal load to 0.77 g COD/l. However, the total VFAs returned to normal level of 0.30 g COD/l within 24 hours of switching the digester operation to normal organic loading rate.

The MPR, stabilizing at about 1.2 l/l/d during normal load, now increased to 1.8 l/l/d first 4 hours during shock load. It then decreased and maintained a steady rate of 1.65 l/l/d from 32 to 48 hours. When the organic loading rate to the digester was switched to normal load at 48 hours, the MPR returned to 1.2 l/l/d.
to normal, the MPR returned to normal level of 1.2 l/l/d. Methane production efficiency was in the range of 90 to 94%.

**Organic Shock Load from 4.2 to 7.4 g COD/l/d.** Acetic acid doubled from 2.5 to 5.0 mM, propionic acid increased from 2 to 2.7 mM and butyric acid from 0.1 to 0.2 mM eight hours after application of shock load (32 h in Fig. 5.4). Sixteen hours later (i.e. at 48 h in Fig. 5.4) further increase occurred for acetic acid to 6.7 mM, propionic acid to 4.3 mM but butyric acid stayed at 0.2 mM.

Total VFAs in the effluent increased from 0.41 to 0.67 g COD/l during the first 8 hours and increased further to 0.96 g COD/l 16 hours later. The pH decreased to 6.9. However, when the loading was returned to value of 4.2 g COD/l/d, the VFA level dropped to 0.41 g COD/l within 16 hours and the reactor pH returned to 7.

The MPR increased from 1.2 to 2.1 l/l/d four hours after the organic load was increased. It stayed at 2.0 l/l/d for the rest of shock load period. It then dropped to the range of 1.2 to 1.4 l/l/d after the shock load was terminated. Methane production efficiency during this shock load was in the range of 89 to 94%.
Fig. 5.4 Response of the system to an organic shock load of 7.4 g COD/l/d

**Organic Shock Load from 4.2 to 10.8 g COD/l/d.** Twenty four hours after the digester being shock loaded (48 h in Fig. 5.5), acetic acid increased from 2.1 to 8.5 mM, and propionic acid from 1.9 to 5.7 mM but no increase of butyric acid, which was still at 0.1 mM, was observed. Total VFAs in effluent reached 1.2 g COD/l at this point and pH decreased to 6.9. However, 24 hours after the loading was returned to normal base load of 4.2 g COD/l/d (72h in Fig. 5.5) acetic acid dropped to 3.2 mM, propionic acid decreased to 2.7 mM and no butyric acid was detected. The total VFAs dropped to 0.52 g COD/l and the pH was back to 7.
MPR increased from 1.2 to 2.4 l/l/d four hours after the organic load was increased (28 hours in Fig. 5.5). The MPR fluctuated between 2.2 to 2.3 l/l/d within 16 hours (32 to 48 hours in Fig. 5.5). Methane rates higher than normal level were always found until it reached 1.4 l/l/d 24 hours after the shock load was terminated. Methane production efficiency during shock load was in the range of 68 to 75%.

![Graph showing VFA (mM) and MPR over time](image)

![Graph showing OLR (g COD/l/d) over time](image)

**Fig. 5.5** Response of the system to an organic shock load of 10.8 g COD/l/d

During increases in organic loading, accumulation of metabolites other than the measured VFAs (acetic, propionic and butyric acids) were checked based on a theoretical COD balance. There was no accumulation of these metabolites except during the first 2 hours after the organic loading rates were increased. During this 2
hour period the highest accumulation was observed about 0.15 g COD/l for an increase load from 4.2 to 10.8 g COD/l/d (Fig. 5.6). Other lower shock loads (6.4 and 7.4 g COD/l/d) resulted in the same profile of accumulations.

![Graph showing accumulation of intermediate metabolites](image)

**Fig. 5.6** Accumulation other intermediate metabolites during organic shock load of 10.8 g COD/l/d

### 5.3.3. Reactor Performance when Hydraulic Loading was Altered

**Change in HRT from 3.8 to 2.5 days for short duration.** As a response to the change in HRT from 3.8 to 2.5 days for 8 hours, acetic and propionic acids concentrations which were in the range of 2.1 to 2.3 mM, and 1.6 to 2.0 mM respectively (0 to 24 hours in Fig. 5.7), rose to the range of 2.1 to 5.3 mM and 1.5 to 3.4 mM, respectively (24 to 32 hours). Butyric acid concentration did not change and was always less than 0.1 mM. VFA concentrations returned to their normal levels within 16 hours after HRT was returned to normal. Total VFAs in the effluent increased from 0.41 to about 0.70 g COD/l during change and dropped to 0.32 g COD/l at termination of HRT change. pH remained 7 during observations. MPR decreased to 1.12 l/l/d from normal
level of 1.2 l/l/d during the change and regained this normal level when change was terminated. Methane production efficiency was between 90 to 96%.

![Graph showing VFA and MPR over time](image1)

![Graph showing HRT over time](image2)

**Fig. 5.7** Response of the digester to a decrease in HRT from 3.8 to 2.5 d for 8 hours

**Step change in HRT from 2.5 to 1.5 days.** After ascertaining that the digester could handle a change in HRT for short duration, the HRT was lowered to 1.5 d from 2.5 d for 48 hours. Before the step change the VFA concentrations were in the range of 1.8 to 2.2 mM and 1.5 to 2.0 mM for acetic and propionic acids, respectively and less than 0.1 mM for butyric acid. Twenty four hours after HRT was decreased, acetic, propionic and butyric acids increased to the range of 1.8 to 3.8 mM, 1.7 to 3.2 mM
and 0.1 to 0.5 mM, respectively (i.e. 24 to 48 hours in Fig. 5.8) and they returned to normal level of about 2 mM for both acetic and propionic acids and 0.2 mM for butyric acid on the second day (i.e. 48 to 72 hours). Total VFA which was about 0.3 g COD/l during 2.5 d HRT increased to the range of 0.3 to 0.6 g COD/l during the first 24 hours and returned to normal range of 0.3 g COD/l 24 hours later. Methane rates were not affected much during observation. It fluctuated in the range of 1.13 and 1.22 l/l/d. pH did not change, remaining around 7. Methane production efficiency was between 90 to 96%.

**Fig. 5.8** Response of the digester to a decrease in HRT from 2.5 to 1.5d
**Step change in HRT from 1 to 0.75 day.** During 1 d HRT (i.e. 0 to 24 hours in Fig. 5.9) the concentrations of acetic, propionic and butyric acids were in the range of 1 to 1.3, 0.6 to 0.8 and 0 to 0.1 mM, respectively. Upon imposing the change, higher concentrations of both acetic and propionic acids were observed during the first 24 hours but both acids concentrations then decreased to normal levels on the second day. That is, these concentrations increased to the range of 1.1 to 1.8 and 0.8 to 1.9 mM for acetic and propionic acids, respectively but no changes (in the range of 0 to 0.1 mM) were observed in butyric acid concentration. On the second day after alteration (i.e. 48 to 72 hours), the acetic and propionic acids dropped to the range of 1.7 to 0.9 mM and 1.9 to 1.0 mM, respectively and low level of about 0.1 mM of butyric acid was maintained.

Total VFA concentration which was 0.17 g COD/l/d before the change increased to 0.34 g COD/l/d after 24 hours and then dropped to 0.22 g COD/l/d after 48 hours. MPR dropped a little bit from 1.22 to 1.12. pH was between 6.9 to 7. Methane production efficiency was in the range of 88 to 96%.
Fig. 5.9 Response of the digester to a decrease in HRT from 1 to 0.75d

*Step change in HRT from 1 to 0.5 days.* During operation at 1 d HRT the acetic and propionic acid concentrations were in the range of 1.3 to 1.7 mM and 0.6 to 0.9 mM, respectively and butyric acid concentration was always less than 0.1 mM. Different VFA accumulation profile was observed during this 100% HRT alteration. Higher concentrations of propionic acid than those of the acetic and butyric acids were measured (Fig. 5.10). Acetic, propionic and butyric acid concentrations increased to the level of 1.8 to 3.9 mM, 2.4 to 3.8 mM and 0.3 to 0.4 mM, respectively (i.e. 24 to 48 hours in Fig. 5.10).
The pH in the digester dropped to 6.7. Total VFA in the effluent increased from 0.18 to 0.45 g COD/l which equates to 25.2% of degradable COD of influent. Consequently, methane production efficiency was only about 75%. MPR dropped to 0.9 from 1.22 l/l/d.

5.3.4 Reactor Performance under Combination of Organic and Hydraulic Shock Loads

In the previous experiments, only one parameter was changed i.e. either the organic loading rate or hydraulic loading rate, while the other parameter was kept unchanged.
In this experiment, the feed concentration was kept constant while decreasing the HRT therefore both parameters were altered simultaneously.

The AMBR, which was operated under an OLR of 4.2 g COD/l/d at 3.8 d of HRT, was now operated at an OLR of 6.4 g COD/l/d at 2.5 d HRT. This value even though conservative was chosen based on the results obtained above which showed that the AMBR could handle well the organic shock load of 6.4 g COD/l/d and a hydraulic shock load of 2.5 d.

Acetic acid concentration of about 4.4 mM during normal operation (3.8d HRT & 4.2 g COD/l/d) increased to 5.9 mM 8 hours after change (i.e. 32 hours in Fig. 5.11) and it further increased to 8.6 mM within 24 hours (i.e. 48 hours in Fig. 5.11). During these periods propionic acid concentration increased from 2.3 to 2.7 mM and further increased to 3.8 mM while butyric acid was always less than 0.2 mM. Twenty four hours after termination of the shock load (i.e. 72 hours in Fig. 5.11) the concentration of acetic, propionic and butyric acids dropped to 3.1, 2.7 and 0.1 mM, respectively.

Total VFAs which were 0.55 g COD/l during normal operation increased to 1.02 g COD/l in 24 h (i.e. 48 hours in Fig. 5.11) and it returned to a safe level of 0.52 g COD/l 48 h after the digester was brought back to normal (i.e. 72 hours in Fig. 5.11).

Four hours after the change (i.e. 32 hours in Fig. 5.11), methane production rate increased from 1.2 to 1.7 l/l/d but it decreased and was maintained in the range of 1.4 to 1.6 l/l/d thereafter. It stayed at 1.4 l/l/d after the shock load was terminated. Efficiency of methane production during alteration was between 88 to 95%.
5.4. Discussion

5.4.1. Reactor Performance during Normal Loads

The normal pH, low concentrations of VFA and high methane production efficiency during normal loads indicated the synergistic and well-balanced cooperation between acid producers and consumers (Kim and Speece, 2002).
5.4.2. Reactor Performance when Organic Loading was increased

Upon applying a step increase in OLR, there was a sharp increase in MPR to a value which was proportional to the increased load applied. For the next 44 hours, slight fluctuation of MPR occurred with the lowest value occurring during the highest accumulation of VFAs. That is when VFAs accumulated to 0.6 from 0.3 g COD/l the MPR slightly decreased corresponding to methane production efficiency of 94%. The MPR then stabilized at a value yielding 97% methane production efficiency. This trend shows that at the beginning of step increase the balance between the acid production and methane formation reactions had been slightly disrupted. Therefore, substrate entering the system could not all be converted to methane gas and thus resulted in an accumulation of VFAs. Once biomass adapted to the new load condition, the accumulated VFAs were degraded and consequently the MPR started to increase and eventually regained a level proportional to the increased load applied. The digester reached a new steady state as seen by constant MPR and low level of VFAs within 3 days. This shows that a certain period is required for the digester to adapt to a step increase in OLR.

Carnaje (1995) made similar observations in a CSTR fed with glucose. A 10-30% increase in the normal organic loading rate of 1 g COD/l/d resulted in a proportional increase in the gas production rate. On the other hand, a 40-50% increase did not result in a proportional increase in gas production rate, which indicated that a fraction of the organic compounds in the feed was not converted to methane. However, the author did not observe the dynamic profiles during the step changes so that the time needed to achieve new steady state conditions could not be compared with this study.
A 20% increase in OLR was a proper strategy to develop the right number of the bacterial population to avoid system instability or ‘sour’ condition. It is in agreement with results shown in Section 4.3.2. The reactor was successfully started up by increasing the organic loading rate by 10 to 30%.

In all cases when the digester was shock loaded, the MPR increased and attained steady values within 8 hours. For the case where the organic shock load was increased by 50 and 75% (i.e. from 4.2 to 6.4 and 7.4 g COD/l/d, respectively) there was a sharp increase in MPR to values proportional to the load applied within 4 hours. However, after 4 hours the MPR decreased and stabilized at values yielding 90 and 89% methane production efficiency for 50 and 75% organic shock loads, respectively.

When a 75% shock load was applied the MPR steadied out to 2.0 l/l/d, however, when a 160% shock load was imposed, the MPR increased to only 2.4 l/l/d. In the next 4 hours the MPR maintained a value around 2.2 l/l/d until the 160% shock load was terminated. It appears that the maximum methane producing capacity of the digester was around 2.2 l/l/d, which corresponds to methane production potential from 8.4 g COD/l/d.

Since an accumulation of butyric acid was hardly seen in all cases only accumulations of acetic and propionic acids are discussed below. Romli (1993) also found that only accumulations of these two acids occurred in a molasses fed fluidized bed reactor which organic loading rate was doubled from a normal organic loading rate of 13 g COD/l/d. Butyric acid accumulation was only observed at more than 5 times normal load of 7 g COD/l/d of sucrose wastewater fed to a down flow stationery fixed-film reactor (Kennedy et al. 1985).
In general, the increase of net accumulation rate of acetic acid due to the increase of shock load was slower than net accumulation rate of propionic acid (see Table 5.2). With 50 and 75% organic shock loads, the net accumulation rates of acetic acid were the same, i.e. around 0.2 mM/h. However, net accumulation of propionic acid was double at 75% organic shock load compared with that at 50% organic shock load (i.e., 0.1 compared to 0.05 mM/h).

<table>
<thead>
<tr>
<th>Shock load applied (%)</th>
<th>Net rate of accumulation (mM/h)</th>
<th>Net rate of disappearance (mM/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acetate</td>
<td>Propionate</td>
</tr>
<tr>
<td>50</td>
<td>0.18</td>
<td>0.05</td>
</tr>
<tr>
<td>75</td>
<td>0.20</td>
<td>0.10</td>
</tr>
<tr>
<td>160</td>
<td>0.27</td>
<td>0.16</td>
</tr>
</tbody>
</table>

It is a typical reactor response to a stress situation where the accumulation of VFA occurs during shock loadings (Kennedy and van den Berg, 1982; Kennedy et al., 1985). The accumulation of VFAs can be related to accumulation of hydrogen during shock load in two ways. Increase hydrogen levels control which end products formed by the fermentative or acidogenic organisms. At consistently low hydrogen levels most of the electron and carbon flow of the fermentative bacteria proceeds via acetate and hydrogen, both of which are suitable substrates for methanogenic bacteria. At increased hydrogen levels as they occur under shock loads, the fermentative bacteria shift their pathways towards the production of more reduced organics such as propionic and butyric acids and less hydrogen (McInerny and Bryant, 1980).
Degradation of propionate is dependent on the concentration of hydrogen present in the system. At low H₂ partial pressure little or no inhibition of propionate degradation occurs. At high H₂ partial pressure, inhibition of degradation of this acid occurs, which results from blockage of the enzyme activity sites by hydrogen. Hoh (1996) observed very little inhibition of propionate degradation when H₂ partial pressure in the system was lower than 4 Pa but the inhibition increased to 90% at H₂ partial pressure of 15 Pa.

Degradation of butyrate is inhibited both by high H₂ partial pressure or concentration of acetate, the other end product of butyrate degradation. If acetate concentration builds up in the system to a significant level, the degradation of butyrate will not occur. Ahring and Westermann (1988) showed that acetate was degraded immediately when this acid was added together with butyrate to anaerobic digester sludge. Butyrate did not start to degrade whenever concentrations of acetate still high in the system. However, butyrate did not accumulate during shock load experiments performed here.

As shown in Table 5.2 the net disappearance rates of the accumulated acids were higher than the net accumulation rates of these acids. It indicated that the digester could handle well all shock loads imposed during this study. With disappearance rates of accumulated acids lower than the acid accumulation rates prolonged recovery periods will be needed and in severe cases, it may result in failure of digester operation. It should be noted that the net disappearance rates of the accumulated propionic acid were more than double than the net accumulation rates of this acid indicating that significant numbers of propionate utilizing acetogens were present in the digester.
Generally, in continuously fed stirred tank reactors (CSTR) propionic acid that accumulates as a result of organic shock loads is not readily degraded for the following reason. With CSTR running at steady state, the digester produces mainly acetate and very little propionate. It means that the number of propionate utilizing bacteria is limited. Therefore, when CSTRs are overloaded, propionate accumulates and does not degrade very rapidly. Pullammanappallil (1993) observed the degradation rates of accumulated acetic and propionic acid of 0.03 and 0.01 mM/h, respectively when a CSTR fed with glucose at an OLR of 0.025 g COD/l/d was doubled. In this study, the increase in propionate degrading capacity may be caused by the presence of carriers, which were able to retain a higher concentration of bacterial biomass including propionate utilizing acetogens. It was shown in section 4.3.3 that the AMBR could be operated stably up to 1.5 d HRT with an OLR of 4.2 g COD/l/d through effective retention of biomass. One may also argue that the feeding strategy applied in this study (once every 4 hours) which deliberately shock loads the digester every 4 hours would cause propionic acid concentration to build up when the digester is fed resulting in the development of significant numbers of propionate utilizing bacteria. However, the result shown in Fig. 5.1 does not support this argument. The propionic acid only fluctuated in the range of 1.7 to 2.1 mM with the higher values being measured when the digester was fed. Therefore, by providing carriers for biomass attachment a greater number of propionic acid utilizing acetogens was retained in the digester enabling the digester to cope well with shock loads.

The ability of biomass to degrade the accumulated propionic acid found in this study is in agreement with study conducted by Conivas-Diaz and Howell (1988). They found that the disappearance rate of propionic acid accumulated in two types of downflow anaerobic fixed film reactor (the packing being fully and half submerged) was
0.23 mM/h (converted from available data). Propionic acid was the dominant acid in all reactors, which was in the range of 1.2 to 1.8 g/l. The digesters which were fed by propionic acid resulted in the development of high concentration of propionic acid utilizers.

After the application of shock loads, the recovery period, which was defined as the time required by the digester to regain the normal level of VFA concentrations equal or less than 0.5 g COD/l/d, occurred within 24 hours. It could be expected that prolonged recovery periods would be needed if the intensity of shock loads were higher. Kennedy et al. (1985) showed that recovery periods in the range of 24 to 48 hours were needed in a down flow stationery fixed-film reactor fed with sucrose wastewater when a 24 hour shock load of 3 to 6 times of the normal load of 7 g COD/l/d was administered. With 7 times shock load an even longer recovery period of 144 hours was observed (the concentrations of acetic, propionic and butyric acids on the second day after the termination of shock load were high at about 4, 2.4 and 3.2 g COD/l, respectively). Mathiot et al. (1992) and Moletta et al. (1994) found that a 20 hour recovery period was required for an anaerobic fluidized bed reactor treating wine distillery wastewater which was shock loaded 20 times of the normal load of 12.8 g COD/l/d for 15 minutes.

Fig. 5.6 shows the accumulation of metabolites or substrates other than acetic, propionic and butyric acids based on a theoretical calculation. As seen in the figure there was a transient increase in the metabolites. Accumulation of these metabolites was also observed by Romli et al. (1995) who employed a molasses fed two-stage high rate anaerobic reactor. The reactor was 100% shocked loaded either by increasing the feed concentration or the feed flow rate. Both disturbances resulted in
an accumulation of lactic acids in the acidification reactor. Costello et al. (1991 a&b) suggested that lactic acid is an important intermediate in an anaerobic digester which may accumulate only temporarily for a short time under shock loading conditions.

Glucose is degraded by acid-forming bacteria into acetic, butyric and lactic acids. Lactic acid is degraded further to acetic and propionic acids by lactic acid-consuming bacteria. During normal digester operation lactic acid is not detected but this acid accumulates during shock loads and appears only for a short time before further degradation takes place (Costello et al., 1991 a).

5.4.3. Reactor Performance when Hydraulic Loading was Altered

One of the most important operational factors affecting the efficiency of an anaerobic digester is the hydraulic retention time (HRT). In a system that is fed a substrate of constant concentration, an increase of HRT means that a higher percentage of the organic matter is destroyed but rate of flow of organic matter is less. As a result the rate of methane production may decrease. On the other hand, when the HRT is shortened by increasing the feed flow rate the methane production may increase. Hydraulic overloading in continuously fed mixed digesters may occur whenever the liquid throughput rate exceeds the growth rate of the bacteria and thus resulting in washout.

Methanogens and acetogens are especially susceptible to wash out as they have low growth rates among the consortia. Doubling time of hydrogen utilizing methanogens is in the range of 6 to 12 hours (Zehnder and Wuhrmann, 1977; Gujer and Zehnder, 1983), that of aceticlastic methanogens is in the range of 2 to 3 days (Lawrence and
McCarty, 1969; Zehnder, 1978), and that of acetogens is between 1.5 to 4 days (Lawrence and McCarty, 1969). The doubling time of acidogens, on the other hand, is between 30 minutes to 5 hours (Lawrence and McCarty, 1969). Therefore, during hydraulic overloading the balance between microbial populations is disrupted which generally results in VFA accumulation, pH drop and the decrease of the methane production rate. In severe cases, the pH drop may result in failure of digester operation.

In general, in experiments performed here the response of the digester towards decreases in HRT was an increase in the range of values of VFAs concentrations. However, higher acetic acid concentration than that of propionic and butyric acids was observed at HRTs of 1 d and higher. At HRTs equal to or less than 0.75 d a different VFA accumulation profile with propionic acid as the dominant VFA was observed.

It should be noted that while most researchers observed the effect of HRT on the performance of digester without maintaining the same OLR value (substrate concentration was always kept the same regardless of HRT values imposed) this study adjusted the substrate concentration to keep the same OLR at different HRTs imposed to the system. With this strategy, pure effects due to HRT changes could be observed (without interference of loading increase brought about by decrease of HRT). Therefore, the increase range values of the VFA concentrations observed in this study might not be due to the response of microbial population to overloading caused by higher substrate concentrations but rather the wash out of the acetogenic and methanogenic bacteria. Washout might have been caused by a hydrodynamic effect resulting from higher flow rates as a consequence of decreases of HRT. At higher feed flow rates, the effluent contained more biomass shown by higher VSS concentrations.
(Table 5.3). The effluent did not contain carriers; the solids in the effluent being flocs which were either dislodged from carriers or removed from digester due to higher velocities. It appears that the spatial arrangement of bacterial population had been disrupted and caused VFA concentrations in the effluent to increase.

**Table 5.3** Effect of decreasing HRT to VS concentrations in the effluent

<table>
<thead>
<tr>
<th>HRT (d)</th>
<th>VSS\textsubscript{effluent} (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.8</td>
<td>2.2</td>
</tr>
<tr>
<td>2.5</td>
<td>2.3</td>
</tr>
<tr>
<td>1.5</td>
<td>2.6</td>
</tr>
<tr>
<td>1</td>
<td>2.8</td>
</tr>
<tr>
<td>0.75</td>
<td>3.0</td>
</tr>
<tr>
<td>0.5</td>
<td>3.2</td>
</tr>
</tbody>
</table>

The MPR did not drop much during the decrease of HRT until 1.5 d HRT. The drop in MPR might be caused by higher VFA concentrations accumulating in the digester. Gas production efficiencies were still above 90%. It indicated that the HRTs applied were above the minimum HRT that could be handled by the digester. When HRT was decreased from 1 to 0.75 d, propionic acid started dominating in the digester. At this HRT perhaps more propionic acid utilizing bacteria were washed out due to higher feed flow rate imposed. When HRT was further decreased to 0.5 d, the MPR dropped to 75%. This drop was due to the VFAs accumulation which accounted for 25.2% degradable COD of influent.

An observation by Conivas-Diaz and Howell (1988) showed that propionic acid dominated in two types of cheese-whey-wastewater- fed anaerobic fixed film reactor (the packing being fully and half submerged) when a hydraulic shock load was
imposed. This was the response to higher organic loads (in the range of 6.5 to 10.5 kg COD/l/d) brought about by decreases in HRT from 10 d to 7 d.

Kennedy and van den Berg (1982) observed propionic and acetic acids which increased 10 and 8 fold from the normal level, respectively when an anaerobic fixed film reactor treating chemical industry waste was hydraulically overloaded to 0.78 d (from about 1.3 d HRT). This decrease in HRT caused the system to be overloaded about 60 to 70% higher than the normal load of 11 g COD/l/d. All acids returned to normal levels within 16 to 18 hours, 24 hours after overload was removed.

Nandy and Kaul (2001) found decreases in removal efficiency with lower HRTs applied to a fixed-film anaerobic reactor fed with herbal-based pharmaceutical wastewater. Base condition was an operation of digester at 2.5 d HRT which resulted in a substrate removal efficiency of 94%. When HRTs were decreased to 1.3, 0.8 and 0.6 days the removal efficiencies dropped to 86, 74 and 72% of the normal condition, respectively. This was mainly due to the system being more organically overloaded.

The AMBR showed high tolerance towards hydraulic shock loads signifying that this type of reactor possesses high stability and it may be suitable for treating industrial wastewaters with highly varying flow rates.

5.4.4 Reactor Performance under Combination of Organic and Hydraulic Shock Loads

The MPR which was not affected much when the system was organically and hydraulically shock loaded showed that the balance in the microbial population had
only been slightly disrupted. The lowest methane production efficiency was found when the highest VFA accumulations occurred. On the stoppage of shock load the MPR which was maintained at the level corresponding to 17% higher than normal load showed the degradation of accumulated VFAs being converted to gas.

Acetic acid which dominated during the shock load and the capability of the digester to return to normal condition indicated that the shock load applied could be lower than the maximum shock load that could be handled by the digester. Results obtained from this experiment proved useful for observing both pure organic and hydraulic shock loads and that of combination of organic and hydraulic shock loads.

Net rates of accumulation of acetic and propionic acids were 0.17 and 0.05 mM/h, respectively while net rates of disappearance of both acids were 0.35 and 0.20 mM/h, respectively. It clearly shows that the degradation of accumulated acetic and propionic acids occurred rapidly.

Even though there were no further loads applied, results obtained from pure organic shock load experiments clearly showed that the degradation of propionic acid occurred or that its degradation was not limited by the number of propionic acid utilizing bacteria present in the system.

5.5. Conclusions

- At normal steady state operation i.e., organic loading rate of 4.2 g COD/l/d and HRT of 3.8 d, residual VFAs were primarily acetic acid and propionic acid at
concentrations of about 2 mM and 1.5 mM, respectively; the methane production rate was 1.2 l/l/d yielding a conversion efficiency of 98%. Butyric acid concentration was around 0.1 mM.

- Upon imposing organic or hydraulic shock loads an imbalance between the volatile fatty acid producing reactions and methanogenic reactions occur. Acetic acid was the dominant VFAs during most shock loads. Significant amounts of propionic acid accumulated at higher shock loads (10.8 g COD/l/d). Butyric acid did not accumulate during shock loads.
- The maximum methane production capacity of the digester was around 2.2 l/l/d.
- Propionic acid that accumulated during shock loads was consumed rapidly at rates comparable to that observed in high rate treatment systems.
- Carriers were effective in retaining biomass enabling the microbial population to handle shock loads and to recover to the normal condition upon the stoppage of disturbances.
- Wash out of the acetogenic and methanogenic bacteria during decreases in HRTs may be due to a hydrodynamic effect caused by higher flow rates. Severe wash out occurred during HRTs equal or less than 0.75 d as indicated by propionic acid being the dominant volatile fatty acid in the effluent.
- A transient accumulation of metabolites other than acetate, propionate and butyrate occurred during the first 2 hours after imposing the shock load
Chapter 6

Effect of Feeding Patterns on the Performance of Single
Stage Stirred Tank Anaerobic Digesters

6.1. Introduction

As stated in Chapter 1, intensification of single stage continuously stirred tank digesters may be accomplished by introduction of carriers or changing feeding patterns. It was demonstrated in earlier chapters that organic loading rate of single stage continuously stirred tank digesters may be increased by adding carriers. Feeding patterns can affect the nature of intermediate products produced. As these intermediates are degraded into methane and carbon dioxide, feeding patterns can influence the bacterial populations present in digesters. Consequently it may be possible to increase wastewater throughput or organic loading rate by changing feeding pattern.

This chapter compares the performance of anaerobic digesters fed continuously and intermittently to identify the better option. As generally accepted, propionic acid is the most difficult VFA to be removed (Kaspar and Wuhrmann, 1978 a; Inanc et al.; 1996; Angenent et al., 2002). Excessive build up of this acid and other VFAs may lead to failure of digester operation.
Thus after comparison, the best feeding pattern would be implemented on the AMBR. Further improvement of the AMBR especially to handle increases in organic loading rates could be obtained.

6.2. Experimental Methods

6.2.1. Reactor Start-up and Operation

Two identical, continuously stirred tank reactors, constructed from modified Schott bottles, each with a working volume of 2 l were operated in parallel. One reactor was fed intermittently (once a day) and the other reactor was fed continuously. Both were fed with the same volume and concentration of molasses based feed in each experimental run. Stirring was performed by using rod shaped magnetic stirrer bars. Both reactors were kept in a temperature-controlled aquarium tank at 37 °C. More details of reactor set up and operation are provided in Section 3.2.2.

Initially, the digesters were operated for a month at an organic loading rate (OLR) of 1 g COD/l/d with 20 g COD/l molasses based feed. Following a month of start-up period, the OLR was increased from 1.0 to 1.3 COD/l/d, and then from 1.3 to 1.9 and from 1.9 to 3.8 g COD/l/d after 2 months of operation at each organic loading rate (see Table 6.1 for chronological listing of OLR and experiments). The response of digesters to handle increases of organic loading rate was monitored for 10 hours after the increase. VSS contents were also measured during each loading.

Hydraulic retention time (HRT) was kept constant at 20 days for all runs. Increases in loading were performed by increasing feed concentration. It was assumed that
conditions close to steady state had been reached after 2 months of operation or 3 HRTs at constant organic loading rates.

6.2.2. Propionic Acid, Glycerol and Butyric Acid Pulses

The propionate degrading capacity of the digesters was measured by adding propionic acid and following its consumption in a batch mode. Pulses of propionic acid at concentrations of 3.8 and 5.5 mM were applied after each steady state (Table 6.1). A glycerol pulse (at a concentration of 20 mM) was performed to confirm results from pulses of propionic acid as substrate. Propionic acid is the main intermediate from glycerol degradation. Butyrate degrading capacity of the digesters was observed by pulsing the digesters with 6.5 mM butyric acid following the same procedure as described above for propionic acid pulses (see Table 6.1 for detailed time frames). The pulses of propionic and butyric acids as well as glycerol were added after the feed to the digesters was switched off. The digesters were operated in this batch mode for the duration of monitoring the consumption of pulsed substrate.

6.2.3. Ability of Biomass to Handle Shock Loads

In addition to the pulse experiments, the differences in ability of biomass to handle shock loads from intermittently and continuously fed digesters was assessed by batch tests, conducted in serum bottles. Twelve serum bottles were set up; with one half of the bottles, each containing 50 ml sludge from intermittently fed digester and the other half, each containing 50 ml sludge from continuously fed digester. Two and a half ml of feed was added into each bottle. The concentration of molasses in the feed was different. The concentrations chosen were such that it yielded organic loading of
2, 3 and 4 times normal loading of 3.8 g COD/l/d which equated to 0.38, 0.57 and 0.76 g COD being added to the serum bottles, respectively. For each organic loading and sludge from each digester 2 sets of serum bottles were set up. This yielded in a total of 12 assays. VFA concentration and total gas production were followed for 80 to 120 hours and samples were taken regularly. Prior to using the sludge for batch assays, the 2 l digesters had been operated for 128 days at 3.8 g COD/l/d (Table 6.1).

6.2.4. Revival of Activity after Shutdown

Biomass sludge from both digesters was poured into a container then the sludge was mixed. Afterwards the sludge was divided equally into the two digesters. Both digesters were unfed for 3.5 months and restarted afterwards. The OLR was increased step wise from 0.6 to 1.9 g COD/l/d during 3 months of operation. Pulses of propionic acid at concentration of 5 mM were added every month and performance of the digester fed intermittently and that fed continuously was observed (Table 6.1).

6.2.5. Sampling and Analysis

Liquid samples (5 ml) were collected for VFA analysis and pH measurement. When experiment was performed in 2 l reactors methane gas production was measured by using water displacement equipment made from inverted cylinders after the gas was passed through soda lime particles in order to remove CO₂. When experiment was performed in serum bottles total gas production was measured by using U tube water displacement equipment (Fig. 3.6). Methane content was estimated from measuring gas composition from 2 l digesters from which sludge was taken (section 3.2.3).
<table>
<thead>
<tr>
<th>Day</th>
<th>OLR (g COD/l/d)</th>
<th>Concentration of VFA or glycerol pulse (mM)**</th>
<th>Experiment/note</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 30</td>
<td>1</td>
<td></td>
<td>Start-up</td>
</tr>
<tr>
<td>31</td>
<td>1.3</td>
<td></td>
<td>Load increase from 1 to 1.3 g COD/l/d *</td>
</tr>
<tr>
<td>32 to 64</td>
<td>1.3</td>
<td></td>
<td>Steady state experiment</td>
</tr>
<tr>
<td>65</td>
<td>3.8</td>
<td></td>
<td>Propionic acid spike*</td>
</tr>
<tr>
<td>66</td>
<td></td>
<td></td>
<td>No feed was fed</td>
</tr>
<tr>
<td>67 to 103 (101)*</td>
<td>1.3</td>
<td></td>
<td>Steady state experiment</td>
</tr>
<tr>
<td>104</td>
<td>1.9</td>
<td></td>
<td>Load increase from 1.3 to 1.9 g COD/l/d *</td>
</tr>
<tr>
<td>105 to 136</td>
<td>1.9</td>
<td></td>
<td>Steady state experiment</td>
</tr>
<tr>
<td>137</td>
<td>5.5</td>
<td></td>
<td>Propionic acid spike *</td>
</tr>
<tr>
<td>138</td>
<td></td>
<td></td>
<td>No feed was fed</td>
</tr>
<tr>
<td>139 to 168 (166)*</td>
<td>1.9</td>
<td></td>
<td>Steady state experiment</td>
</tr>
<tr>
<td>169</td>
<td>3.8</td>
<td></td>
<td>Load increase from 1.9 to 3.8 g COD/l/d *</td>
</tr>
<tr>
<td>170 to 202</td>
<td>3.8</td>
<td></td>
<td>Steady state experiment</td>
</tr>
<tr>
<td>203</td>
<td>5.5</td>
<td></td>
<td>Propionic acid spike *</td>
</tr>
<tr>
<td>204</td>
<td></td>
<td></td>
<td>No feed was fed</td>
</tr>
<tr>
<td>205 to 230 (228)*</td>
<td>3.8</td>
<td></td>
<td>Steady state experiment</td>
</tr>
<tr>
<td>231</td>
<td>20</td>
<td></td>
<td>Glycerol addition *</td>
</tr>
<tr>
<td>232 to 233</td>
<td></td>
<td></td>
<td>No feed was fed</td>
</tr>
<tr>
<td>234 to 263</td>
<td>3.8</td>
<td></td>
<td>Steady state experiment</td>
</tr>
<tr>
<td>264</td>
<td>6.5</td>
<td></td>
<td>Butyric acid addition</td>
</tr>
<tr>
<td>265</td>
<td></td>
<td></td>
<td>No feed was fed</td>
</tr>
<tr>
<td>266 to 296</td>
<td></td>
<td></td>
<td>Steady state experiment</td>
</tr>
<tr>
<td>297 to 301</td>
<td></td>
<td></td>
<td>Batch tests on sludge (2, 3 and 4 times normal load of 3.8 g COD/l/d) *</td>
</tr>
<tr>
<td>302 to 407</td>
<td></td>
<td></td>
<td>No feed was fed</td>
</tr>
<tr>
<td>408 to 498</td>
<td></td>
<td></td>
<td>Step wise increased of OLR from 0.6 to 1.9 g COD/l/d for reactivation of the digesters</td>
</tr>
<tr>
<td>(439, 470 &amp; 499)*</td>
<td>5.0</td>
<td></td>
<td>Second set of propionic acid spike experiment</td>
</tr>
</tbody>
</table>

(*) two hourly data (VFAs and CH₄ production) were collected up to 10 hours, thereafter another data point at 24 hours. At other times data was collected daily.

(**) the concentration indicated is the resulting concentration in the digesters after addition of pulse.
For shock load experiments, propionic acid, glycerol and butyric acid pulses, and when reactor steady state was observed, 2 hourly liquid sampling for 10 hours and at 24 hours were taken together with gas measurement (shown by asterisk symbols in Table 6.1). Whereas, daily sampling and gas measurement were carried out during other times. Table 6.1 details the experimental time schedule. Propionic and butyric acid utilisation rates were calculated from two first data points after the introduction of pulse.

6.3. Results

6.3.1. Reactor Performance during Normal Loads and Increases in Organic Loading Rates

In general, VFA concentrations oscillated in the intermittently fed digester during normal operations. Acetic and propionic acids accumulated in the first 2 hours but were degraded in the next 8 hours and reached levels similar to the beginning of the cycle (Fig. 6.1). Methane gas production reflected the feeding mode in which substrate was available at the beginning of the day and none was fed during the rest of the day. For instance at an OLR of 1.3 g COD/l/d methane was produced during the first 10 hours at a rate of 32 ml/l/h and later at a rate of 6 ml/l/h (Fig. 6.1a). Total VFA concentrations and methane production efficiency 24 hours after feeding were about 0.2 g COD/l and 96%, respectively for all organic loading rates applied (Table 6.2). VSS measured during each loading was also included in the table to be able to calculate specific activities.
In the continuously fed digester, VFA concentrations were always at low levels at all times during observation (Fig. 6.2). Since the feed is supplied continuously, the substrate availability is the same throughout the day. This resulted in a steady state methane gas production rate of about 16 ml/l/h at an OLR of 1.3 g COD/l/d (Fig. 6.2a). Total VFA concentrations of 0.2 g COD/l and methane production efficiency of 91% were obtained for all organic loading rates applied (Table 6.2).

<table>
<thead>
<tr>
<th>OLR (g COD/l/d)</th>
<th>Total VFA (gCOD/l)</th>
<th>Efficiency CH₄ (%)</th>
<th>VSS (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R1*</td>
<td>R2</td>
<td>R1</td>
</tr>
<tr>
<td>1.3</td>
<td>0.20 ± 0.02</td>
<td>0.17 ± 0.02</td>
<td>96 ± 3.2</td>
</tr>
<tr>
<td>1.9</td>
<td>0.15 ± 0.02</td>
<td>0.15 ± 0.01</td>
<td>95 ± 2.4</td>
</tr>
<tr>
<td>3.8</td>
<td>0.23 ± 0.01</td>
<td>0.22 ± 0.03</td>
<td>96 ± 4.1</td>
</tr>
</tbody>
</table>

(*️⃣) Total VFA prior to addition of feed

During step changes in load (from 1.0 to 1.3 COD/l/d, from 1.3 to 1.9 and then from 1.9 to 3.8 g COD/l/d) two hourly data collected soon after the organic loading rate was increased were used to observe reactor performance (Fig. 6.3 and 6.4). Data points on Day 31 in Fig 6.3a and 6.4a show data collected soon after increasing load from 1 to 1.3 g COD/l/d for intermittent and continuously fed reactors, respectively. Data points on Day 104 in Fig 6.3b and 6.4b show data collected soon after increasing load from 1.3 to 1.9 g COD/l/d while data points on Day 169 in Fig 6.3c and 6.4c were data collected soon after increasing load from 1.9 to 3.8 g COD/l/d for intermittent and
Fig. 6.1 VFA profiles and methane accumulation in the intermittently fed digester at an OLR of 1.3 g COD/l/d (a), 1.9 g COD/l/d (b), and 3.8 g COD/l/d (c).
Fig. 6.2 VFA profiles and methane accumulation in the continuously fed digester at an OLR of 1.3 g COD/l/d (a), 1.9 g COD/l/d (b), and 3.8 g COD/l/d (c).
continuously fed reactors, respectively. The other data in the figures show data collected daily before and after the changes in organic loading rate.

In the intermittently fed reactor small peaks in acetate and propionate were found after each step increase. This showed that the digester was not overloaded and could handle well two times load increases or at an OLR of 3.8 g COD/l/d. The gas productions were proportional to the organic loads imposed.

The continuously fed reactor, on the other hand, showed less stability than its counterpart. It could handle the increased load from 1 to 1.3 and the load from 1.3 to 1.9 g COD/l/d. However, when the organic loading rate was doubled from 1.9 to 3.8 g COD/l/d, it showed failure symptoms when acetic and propionic acids accumulated to 15 and 4 mM, respectively (Fig. 6.4) and pH dropped from 7.1 to 6.8. The digester had to be unfed for 2 days to allow utilisation of accumulated VFAs to take place. After this, it appeared that the biomass was acclimatized to the increased load and the digester could handle the load of 3.8 g COD/l/d afterwards.
Fig. 6.3 Profiles of VFA in the effluent and methane gas production during continuous operation in the intermittently fed reactor at an increase of OLR from 1 to 1.3 (a), from 1.3 to 1.9 (b) and from 1.9 to 3.8 g COD/l/d (c). Two hourly data were collected during loading increases (Day 31, 104 and 169) and daily data were collected during constant organic loading rates.
Fig. 6.4 Profiles of VFA in the effluent and methane gas production during continuous operation in the continuously fed reactor at an increase of OLR from 1 to 1.3 (a), from 1.3 to 1.9 (b) and from 1.9 to 3.8 g COD/l/d (c). Two hourly data were collected during loading increases (Day 31, 104 and 169) and daily data were collected during constant organic loading rates.
6.3.2. Effect of Propionic Acid Pulses

After 64 days of operation, propionic acid degradation rates in the intermittently fed digester were two to four times higher than those in the continuously fed digester (Table 6.3). In the intermittently fed digester the propionate degradation rates increased with loading and initial propionic acid concentrations whereas in the continuously fed digester the rates remained constant regardless of loading and magnitude of propionic acid pulses imposed to the system. The results indicate that the intermittent feeding operation selected for higher numbers of propionate degrading bacteria (Table 6.3).

<table>
<thead>
<tr>
<th>Operation time (days)</th>
<th>OLR (gCOD/l/d)</th>
<th>Propionic acid added (mM)</th>
<th>Intermittently fed reactor</th>
<th>Continuously fed reactor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>r_PA (mM/gVSS/h)</td>
<td>Total VFA (gCOD/l)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>r_PA (mM/gVSS/h)</td>
</tr>
<tr>
<td>65</td>
<td>1.3</td>
<td>3.8</td>
<td>0.04</td>
<td>0.23</td>
</tr>
<tr>
<td>137</td>
<td>1.9</td>
<td>5.5</td>
<td>0.06</td>
<td>0.20</td>
</tr>
<tr>
<td>203</td>
<td>3.8</td>
<td>5.5</td>
<td>0.08</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Further observation showed that methane accumulation obtained from the intermittently fed digester was proportional to the added propionic acid (Fig. 6.5) while lower methane accumulation obtained from the continuously fed digester may have been caused by higher residual VFAs (Fig. 6.6). In the digester fed continuously, total VFA concentrations at the end of the day after the pulse was applied were about
1.6, 2 and 3 times total VFAs in the intermittently fed digester at loading of 1.3, 1.9 and 3.8 g COD/l/d, respectively (Table 6.3).

**Fig. 6.5** VFA profiles and methane accumulation in the intermittently fed digester after 5.5 mM propionic acid pulse. Similar profiles were observed after 3.8 mM and another 5.5 mM propionic acid pulses (Appendix 4).

**Fig. 6.6** VFA profiles and methane accumulation in the continuously fed digester after 5.5 mM propionic acid pulse. Similar profiles were observed after 3.8 mM and another 5.5 mM propionic acid pulses (Appendix 4).
6.3.3. Effect of Glycerol Pulse

To test whether the improved capacity of propionate degradation in the intermittently fed digester also enables improved degradation of typical waste compounds that readily give rise to propionate degradation, a spike of 20 mM glycerol was added.

In the intermittently fed reactor, propionic acid initially accumulated to 2.8 mM within 7 hours but was utilised gradually over 40 hours (Fig. 6.7).

![Fig. 6.7 VFA profiles and methane accumulation after 20 mM glycerol pulse in the intermittently fed reactor](image)

In the continuously fed digester, propionic acid accumulated to 3.9 mM over 40 hours of operation without signs of degradation (Fig. 6.8). Final total VFAs were about 4 times higher (0.50 g COD/l) than in the intermittently fed digester (of only 0.12 g COD/l). The higher total VFAs in the continuously fed digester may have caused lower methane accumulation (0.62 l/l as opposed to 0.77 l/l in the intermittently fed digester).
Results showed that glycerol, a typical product from fat hydrolysis was also degraded more readily by the intermittently fed digester than the continuously fed digester.

![Graph of VFA profiles and methane accumulation after 20 mM glycerol pulse in the continuously fed reactor](image)

**Fig. 6.8** VFA profiles and methane accumulation after 20 mM glycerol pulse in the continuously fed reactor

### 6.3.4. Effect of Butyric Acid Pulses

The intermittently fed reactor could degrade added butyric acid faster (Fig. 6.9) than its counterpart (Fig. 6.10). Again the intermittently fed reactor showed more than two fold higher capacity of VFA degradation.

Total amount of gas produced from both reactors were the same (0.27 l/l reactor) at the end of observation. The gas production profiles reflected residual substrate in the system over time. No gas was produced after 25 hours from the reactor fed intermittently while gas was still produced over 40 hours from the reactor fed continuously.
Fig. 6.9 VFA profiles and total gas accumulation after 6.5 mM butyric acid addition in the intermittently fed reactor

Fig. 6.10 VFA profiles total and gas accumulation after 6.5 mM butyric acid addition in the continuously fed reactor

6.3.5. Ability of Biomass to handle Shock Loads

The results presented are from the serum bottle assays. In the serum bottles containing sludge from intermittently fed digester, temporary VFA accumulation was found during shock loads (Fig. 6.11). Here, propionic acid was the dominant acid. The
higher the loading imposed the higher was the VFA peak. Up to 27 mM propionic acid was observed which was utilised during the period of observation (120 hours). Surprisingly, butyric acid was almost undetected during all shock loads applied.

In the serum bottles containing sludge from continuously fed digester, similar profiles of VFA accumulation and VFA consumption as those occurring in the serum bottles containing sludge from intermittently fed digester were observed however acetic acid was the dominant acid in the system. Propionic acid, which was usually at low levels during normal load, accumulated and returned to its levels at the beginning of experiment after 50 and 70 hours during 2 and 3 times normal load, respectively. At 4 times normal loading, propionic and butyric acids accumulated and did not return to their levels at the beginning of experiment after 120 hours (Fig. 6.12). Total VFAs in the effluent were about 20 times higher than the total VFAs at lower loads of 2 or 3 times loading (Table 6.4).

**Table 6.4** Total VFAs and methane production resulting after shock loading 2, 3 and 4 times the normal load of 3.8 g COD/l/d sludge from intermittently and continuously fed digesters

<table>
<thead>
<tr>
<th>Loading</th>
<th>COD added into serum bottles (g COD)</th>
<th>End of observation*</th>
<th>End of observation*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Intermittently fed reactor</td>
<td>Continuously fed reactor</td>
</tr>
<tr>
<td></td>
<td>Total VFAs (g COD/l)</td>
<td>CH₄ (l/l)</td>
<td>Total VFAs (g COD/l)</td>
</tr>
<tr>
<td>2 x normal</td>
<td>0.38</td>
<td>0.06</td>
<td>2.34</td>
</tr>
<tr>
<td>3 x normal</td>
<td>0.57</td>
<td>0.06</td>
<td>3.58</td>
</tr>
<tr>
<td>4 x normal</td>
<td>0.76</td>
<td>0.96</td>
<td>4.58</td>
</tr>
</tbody>
</table>

* 80, 90 and 120 hours for 2, 3 and 4 times normal load, respectively.
Fig. 6.11 Profiles of VFA and methane accumulation during batch tests in serum bottles containing sludge from intermittently fed digester at 2 times (a), 3 times (b) and 4 times (c) normal load of 3.8 g COD/l/d
Fig. 6.12 Profiles of VFA and methane accumulation during batch tests in serum bottles containing sludge from continuously fed digester at 2 times (a), 3 times (b) and 4 times (c) normal load of 3.8 g COD/l/d.
Methane accumulations were proportional to the loading increases (Table 6.4). Methane accumulations from intermittently fed digester were more than 10% higher than the accumulations in the continuously fed digester. Differences in VFA accumulation might result in differences in methane production.

6.3.6. Revival of Activity after Shutdown

The objective of this experiment was to observe duration required for microbial population to adapt to the feeding patterns imposed. As mentioned before, biomass sludge from both digesters were poured into a container then the sludge was mixed. Afterwards the sludge was divided equally into the two digesters. The digesters were unfed for 3.5 months before this experiment was started. During the first month of reactivation, the propionic acid degradation rates were the same for both reactors fed by different feeding modes (Table 6.5). After about 2 and 3 month of operation, the propionic acid degradation rates increased 2 to 3 times, respectively than the rates in the continuously fed reactor (Table 6.5).

Table 6.5 Propionic acid degradation rates ($r_{PA}$) and total VFA in the intermittently and continuously fed digesters during restart-up period

<table>
<thead>
<tr>
<th>Operation time (days)</th>
<th>OLR (gCOD/l/d)</th>
<th>Propionic acid added (mM)</th>
<th>Intermittently fed reactor</th>
<th>Continuously fed reactor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>$r_{PA}$ (mM/gVSS/h)</td>
<td>Total VFA (gCOD/l)</td>
</tr>
<tr>
<td>31</td>
<td>0.6</td>
<td>5</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>65</td>
<td>1.3</td>
<td>5</td>
<td>0.04</td>
<td>0.06</td>
</tr>
<tr>
<td>94</td>
<td>1.9</td>
<td>5</td>
<td>0.06</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$r_{PA}$ (mM/gVSS/h)</td>
<td>Total VFA (gCOD/l)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.02</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.02</td>
<td>0.15</td>
</tr>
</tbody>
</table>
6.4. Discussion

6.4.1. Reactor Performance during Normal Loads and Increases in Organic Loading Rates

Methane production efficiency was 5% lower in the continuously fed digester than that in the intermittently fed digester (Table 6.2). Given that no difference in residual VFAs was observed, differences in methane accumulation may be due to accumulation of intermediates other than the measured VFAs (acetic, propionic and butyric acids) or production of more biomass in the continuously fed digester or simply due to errors in gas measurement.

Considering the possibility of accumulations of metabolites other than measured VFAs is likely to occur during intermittent feeding, this reason will fail to account for higher gas productions during intermittent feeding. For example accumulation of lactic acid (an important intermediate in anaerobic digestion) was observed during shock loading conditions by Romli et al. (1995). In this study, intermittently fed digester is being shock loaded daily. Unfortunately, checking of these accumulations (from theoretical COD balance, such conducted in Section 5.3.2) could not be performed since data which were collected 2 hourly would give low accuracy in predictions. In the previous chapter (Fig. 5.6) the accumulation metabolites other than measured VFAs were indicated during the first hour of feed addition.

VSS levels in the intermittently fed digester were 10% higher than that in the continuously fed digester and no difference in residual VFAs were observed (Table 6.2). This would have resulted in lower gas production efficiencies in the
intermittently fed digester (to obtain a balance in COD or carbon). However, the experiments provided contradicting results showing the intermittently fed digester yielded higher gas production efficiencies during all loading applied. Theoretically, continuously fed digesters should produce more biomass. For instance, second row Table 8.7 shows glucose utilisation reactions. Glucose utilisation through acetate produces 4 ATP but the utilisation through propionate produces $4/3$ ATP. As will be described in Section 6.4.2, the intermittently fed digester produced more propionate degrading bacteria than the continuously fed digester. It means that the glucose utilisation through propionate dominated in the digester fed intermittently. Taking into account that glucose utilisation through propionate only produces 33% of the ATP produced from the glucose utilisation through acetate, the intermittently fed digester would have resulted in lower VSS than its counterpart. Consequently, to balance the COD, methane production would be lower in the continuously fed digester as seen in experiments here.

Visual observation showed that sludge biomass in the intermittently fed digester was darker and tended to settle quicker than the biomass in the continuously fed digester. Even though both digesters were stirred at the same rate, biomass may have not been distributed the same way in both digesters. As effluent was withdrawn from just below the liquid surface, less quickly settling biomass from the intermittently fed digester may have been withdrawn leading to more accumulation of biomass. More obvious difference in the colour of biomass from both digesters was seen during moisture or suspended solids (SS) measurement. Orange dried residue was obtained from the continuously fed digester where black dried residue was obtained from the intermittently fed digester.
The third reason for this could be errors in methane measurement. Due to the nature of feeding in the intermittently fed digester, it produces large amount of gas soon after feed is added, it is possible that during the release of gas some carbon dioxide which was supposed to be entrapped in the CO$_2$ absorber escaped and was measured and accounted as methane gas (see Section 3.2.3 for methane measurements).

During increases in organic loading rates the intermittently fed digester was capable of handling all changes in organic loading rates imposed (Fig. 6.3), whereas the continuously fed digester was more affected by organic loading rate increases (Fig. 6.4).

Results of this study were in agreement with a study conducted by van den Berg and Kennedy (1982 b). In that study, a once daily fed anaerobic fixed film reactor could handle pear peeling waste at an OLR of 9.3 g COD/l/d whereas, the continuously fed reactor could only be loaded at the rate of 6.4 g COD/l/d. When bean blanching waste was treated by the same reactor fed intermittently, an OLR of 13.7 g COD/l/d could be handled as opposed to 10 g COD/l/d for the fixed film reactor fed continuously.

6.4.2. Reactor Performance after Propionic Acid, Glycerol and Butyric Acid Pulses

Glycerol is mainly degraded to propionic acid (Barbirato et al.; 1997, Himmi et al.; 2000). The product profiles of glycerol degradation by *Propionibacterium acidipropionici* comprise propionic acid (84%) and succinic, acetic and formic acids and *n*-propanol as remainder. Glycerol produces higher yield of propionic acid compared with other conventional substrates. Glucose and lactic acid produce 17%
and 13% lower propionic acid conversion yields, respectively than glycerol (Barbirato et al., 1997). Therefore, glycerol was chosen in this study to test propionic acid degradation capacity of the digesters.

As mentioned above, butyric acid degradation is inhibited by high hydrogen partial pressure but it also inhibited by high concentration of acetate, the other end product of butyrate degradation. If acetate concentration builds up in a system to a significant level, the degradation of butyrate will not occur. Ahring and Westermann (1988) showed that acetate was degraded immediately when this acid was added together with butyrate to anaerobic digester sludge. Butyrate did not start to degrade whenever concentrations of acetate were still high in the system.

In this study acetic acid concentrations (during butyric acid pulses) in both intermittently and continuously fed digesters were similar at low levels of about 1 mM. The lower degradation rate of butyric acid in the continuously fed reactor was not caused by high acetic acid but may be a result of inhibition of high hydrogen partial pressure. The explanation below describes how higher hydrogen may be produced in the continuously fed reactor.

An explanation for the increase in propionic and butyric acid degrading capacities in the intermittently fed anaerobic digester can be based on inhibition of the majority of reactions by their end products. It is generally accepted (McInerney and Bryant, 1980) that overloading digesters results in the build up of hydrogen, a principal metabolite of the process. Accumulating hydrogen affects the accumulation of VFA and digester acidification by two separate mechanisms:
1) Even very low levels of molecular hydrogen inhibit the degradation of VFA by the obligate hydrogen producing acetogens, causing the accumulation of VFA.

2) Increased hydrogen levels also control which end products are formed by the fermentative organisms. At consistently low hydrogen levels (e.g. continuous feeding without overloading) most of the electron and carbon flow of the fermentative bacteria proceeds via acetate and hydrogen, both of which are suitable substrates for methanogenic bacteria. At increased hydrogen levels as they occur under temporary overloading such as intermittent feed regimes, the fermentative bacteria shift their pathways towards the production of more reduced organics such as propionic and butyric acids and less hydrogen.

The above mechanisms imply that intermittent feeding causes a larger proportion of the electron flow via propionic and butyric acids. The higher availability of propionic acid in the intermittently fed reactor is expected to favour the development of propionate degrading acetogens. With higher numbers of propionic acid degrading bacteria any accumulated propionate originating from step increases in organic loading rate could accordingly be degraded faster by biomass developed under intermittent feeding compared to biomass that was continuously fed and has never been exposed to much propionate.

6.4.3. Reactor Performance during Shock Loads

Since it was not certain whether the 2 l digesters could handle all the shock loads imposed this experiment was conducted in serum bottles. This was also done in this
manner to avoid the after effects of shock loading the 2 l digesters affecting subsequent experiments using these digesters.

Shock loads in the intermittently fed reactor resulted mainly in propionic acid accumulation (Fig. 6.11), while acetic acid was the principle VFA in the continuously fed reactor (Fig. 6.12). Using the above mentioned mechanisms of degradation pathways of intermittent shock loads and continuous feeding, one would expect faster rates of propionate degradation capability in the intermittently fed reactor. The theoretically expected and practically demonstrated (Fig. 6.5) increase number of propionate degrading acetogens in the intermittently fed reactor should have resulted in less propionate accumulation. The fact that the biomass with more propionate degrading capacity has accumulated more propionate can be explained as follows:

1. During the presence of excess fermentable material propionate degradation will be close to zero due to accumulation of inhibitory H2.
2. Once propionate had accumulated the intermittently fed reactor showed again that propionate degradation was indeed faster.
3. As a conclusion the increased build up of propionate in the intermittently fed reactor can be explained by a faster production rate of propionate. This could be due to increased proportions of propionic acid bacteria that ferment either sugar directly or convert lactic acid from lactic acid bacteria to propionate.
4. Alternatively it could be explained by increased populations of homo-acetogenic bacteria in the continuous fed reactor. Homo acetogens are not capable of producing propionic acid even under overloading conditions.

In conclusion, the differences in feeding regime can not only affect the populations of VFA degrading acetogens but also the populations of fermentative bacteria.
During 4 times normal load residual total VFAs in both digesters were above 0.5 g COD/l (0.96 g COD/l in the intermittently fed digester and 1.66 g COD/l in the continuously fed digester, Table 6.4). The levels were defined above normal in this study. The loads applied may have exceeded the degradation capacity of the biomass in the digesters. The residual total VFAs were 70% higher in the digester fed continuously indicated that propionic and butyric acid degraders in the continuously fed digester were less than these acid utilisers in the intermittently fed digester.

6.4.4. Revival of Activity after Shutdown

In the first month of reactivation the loss of propionic acid degrading capacity in the intermittently fed digester was seen (Table 6.5) shown by no difference in the rate of propionic acid degradation in both digesters fed by different feeding modes.

The rate at which the observed changes in population occurred was tested after reactor shut down. A change in microbial population, in particular the build-up of increased potential to degrade propionic acid was achieved after about 2 months of operation or 3 HRTs (Table 6.5). This showed the time required for the digesters to adapt or build up microbial populations that reflect feeding modes. In case of unavailability of new sludge after digester shut down, restart-up of unfed digesters to obtain certain populations seems possible after a period of adaptation.
6.5. Conclusions

- The intermittently fed reactor showed cycles of VFA accumulation followed by VFA consumption, while the continuously fed reactor showed consistently low levels of volatile fatty acids during steady state or normal loads.
- The intermittently fed reactor had higher capacity to degrade propionic acid added to the digester either from pure acid form or a main product of glycerol degradation.
- Intermittently feeding mode resulted in higher microbial butyrate degrading capacity.
- An accumulation of propionic and butyric acids as it occurs in typical step increases of organic loading rate could be more effectively dealt with by the digester fed intermittently.
- The reactor fed intermittently was shown to have higher resistance to shock loading than the digester fed continuously.
- A period of at least 3 HRTs was needed to obtain an adaptation in microbial population.
Chapter 7

Operation of Anaerobic Moving Bed Reactor as a Sequecing Batch Reactor (AMBSBR)

7.1. Introduction

Anaerobic batch reactors have been extensively studied as alternatives to continuous systems due to improved retention of biological solids and better process control (Zaiat et al. 2001). Batch reactors may be operated in sequencing or fed batch modes, according to the way feed is delivered. Sequencing batch reactor operation is characterized by four distinct phases per cycle, i.e. fill, react, settle, and decant phases (Dague et al., 1992). Section 2.3.3 reviewed in detail the anaerobic sequencing batch reactor (ASBR) also outlining its advantages and disadvantages.

For efficient operation, a sequencing batch reactor relies on biomass with good settling properties. Well settling biomass is more effectively retained in the reactor and the duration of settle phase can be reduced. Biomass with good settling characteristic are produced when they self immobilize and form granules. However, granulation requires lengthy start-up periods and appropriate feed characteristics (Lettinga et al., 1983; Lettinga et al., 1984; Borja and Banks, 1994 a; and Liu et al., 2002). Sung and Dague (1992) observed granulation after nearly 300 days of operation in an ASBR fed with a soluble, synthetic substrate (non-fat dry milk). Moreover, it has been shown that granular biomass tend to break up, float and wash out at high organic loading rates or short HRTs (Ndon and Dague, 1997 b).
Ratusznei et al. (2003) and Rodrigues et al. (2003) employed inert supports of polyurethane foam (having particle sizes of 5 mm and density of 23 kg/m³) for biomass adhesion and biofilm formation. The ASBR, having 2.5 l volume, could be operated at 2 d HRT and 8 hours cycles treating low strength (0.5 g COD/l) synthetic wastewater, mainly containing meat extract and soluble starch, at an OLR of 0.24 g COD/l/d with COD removal efficiency of 86%. The use of inert supports also resulted in elimination of the settling step and thus reducing the overall cycle time (Ratusznei et al., 2000).

The anaerobic moving bed reactor (AMBR) described in Chapters 4 and 5 employed granular rubber tires as inert supports and was shown to retain biomass very efficiently. Moreover, results obtained in Chapter 6 indicated that an intermittently fed digester built up a higher propionate utilization activity than a continuously fed digester, thereby preventing build up of propionic acid when overloaded. Perhaps the AMBR when operated as a sequencing batch reactor (henceforth referred to as anaerobic moving bed sequencing batch reactor or AMBSBR) might be able to handle higher organic loads.

The aim of this study was to observe the performance and response of the AMBR when it was switched from continuous to sequencing mode of operation and AMBSBR effectiveness in handling increased organic loads, shorter HRTs and shorter cycles.
7.2. Experimental Methods

7.2.1. Switch from AMBR to AMBSBR

The operation of digester described in section 4.2.3 was switched from AMBR, which was fed 6 times per day with effluent withdrawal by gravity, to a sequencing batch mode with feeding and decant phases once per cycle with decanting being carried out manually by gravity. A cycle time of 24 h was initially chosen to compare with results of the AMBR which was intensively monitored over a 24 h operational period. One 24 h cycle included 6 minutes of feeding, 22 hours and 50 minutes of reaction, 1 hour of settling time, and 4 minutes of decant phase. During each cycle, 580 ml of molasses feed with an organic concentration of 16 g COD/l was fed into the digester. As before, total VFAs in the effluent, methane production efficiency and pH were used as parameters. Total VFAs were determined from concentrations of the 3 main intermediate products: acetic, propionic and butyric acids. VFA concentrations and gas production were recorded during the first day and second day of the switch from AMBR operation to AMBSBR operation. The next set of data collected was taken after a month of operation of the digester which was continued to be fed at the same OLR of 4.2 g COD/l/d and 3.8 d HRT.

Hydraulic retention times (HRT) for the sequencing batch reactor was determined from the frequency of sequencing and the decanted volume processed with each sequence. Solid retention times (SRT) was estimated from VSS concentration in the reactor over VSS concentration in the effluent (Shizas and Bagley, 2002). Organic loading rates (OLR) were based on the frequency of sequencing and the concentration
of feed processed with each sequence (Dague et al., 1992). Mathematical expressions of HRT, SRT and OLR are shown in equations 7.1 to 7.3.

\[
HRT = \frac{\text{reactor volume}}{(\text{volume decanted per cycle})(\text{cycles per day})} \quad (7.1)
\]

\[
SRT = \frac{(\text{VSS conc. in reactor})(\text{reactor vol.})}{(\text{effluent VSS})(\text{vol. decanted per cycle})(\text{cycles per day})} \quad (7.2)
\]

\[
OLR = \frac{(\text{feed conc. per cycle})(\text{cycles per day})}{(\text{reactor vol.})} \quad (7.3)
\]

Therefore, feeding 0.58 l molasses based feed at a concentration of 16 g COD/l to the AMBR, having an active volume of 2.2 l and operated in sequence at 24 h per cycle resulted in an OLR of 4.2 g COD/l/d and HRT of 3.8 days. The condition at these OLR and HRT was referred as the base condition for comparison of various variables tested.

### 7.2.2. Effect of Settle Phase Duration on Quality of Decanted Liquid

The effect of settle phase duration on quality of decanted liquid was verified. This experiment was performed 5 weeks after switching AMBR to AMBSBR. Each experiment was repeated 7 times or conducted for 7 cycles. The settle duration was varied from 0.5 to 4 hours while total cycle time was 24 hours. All other phases in a cycle were the same except that the reaction phase became shorter with increase in settle phase duration. For example when settling phase was 0.5 hours the reaction
phase was 23 hours and 20 minutes and when the settling phase was 4 hours the reaction phase was 3.5 hours shorter i.e., 19 hours and 50 minutes. Suspended solids (SS) and unfiltered COD of the decanted liquid were measured during each cycle. During these experiments, OLR and HRT were maintained at the base condition of 4.2 g COD/l/d and 3.8 d, respectively.

In addition, the SS and unfiltered COD of the decanted liquid were measured over the duration of operation of the AMBSBR, i.e. for 9 months. During the first 3 months, the experiments were performed at a settling phase of 1 hour while during 6 and 9 months they were performed at a settling phase of 20 minutes. Each data was averaged over at least 7 measurements.

7.2.3. Settling Characteristics of Biomass

Settling times of biomass aggregates were observed after 3 months of operation. This was quantified by averaging time taken for aggregates to settle to a preset reactor level after which settling became much slower (in this case until aggregates reached the reactor volume of 3 l). The reactor was operated at the base OLR and HRT.

7.2.4. Effect of Increased Organic Loads, Shorter HRTs and Shorter Cycles

To verify the potential of AMBSBR to handle increased capacity it was subjected to increased organic loads, shorter HRTs and shorter cycles. The organic loading rates were increased from 4.2 to 6.4, 7.4 and 10.8 g COD/l/d, respectively by increasing feed concentration. Each increased organic loading was performed for 1 cycle of 24 hours after the AMBSBR was run at the base condition for duration of 4 HRTs or
about 2 weeks. During organic loading rate changes, the HRT was maintained at 3.8 d and the cycle time was kept at 24 h, i.e. 6 minutes of feeding, 22 hours and 50 minutes of reaction, 1 hour of settling phase, and 4 minutes of decant phase.

When the system was subjected to shorter HRTs, the decrease of HRT was done by increasing fill volume which also meant increasing the decant volume. The base HRT of 3.8 d was shortened to 2.5 and 1.8 d, respectively. The cycle time was maintained at 24 h as before. The feed concentration used in this experiment (of 16 g COD/l) was the same as that used during base condition. Therefore, organic loading rates increased accordingly with decreases of HRT.

During shorter cycles, the cycle time was shortened from 24 hours to 16 hours and 8 hours. This study was performed after 5 months of operations. Organic loading rates increased accordingly with decreases of cycle time since the same feed concentration of 16 g COD/l was maintained during this experiment. The settle phase which was maintained at 1 hour was now shortened to 20 minutes only. This was based on faster settling time observations after 3 months of operational period. Feeding and decant phases were kept at 6 and 4 minutes, respectively. The difference was at reaction phases which were at 15 hours and 30 minutes for 16 h cycle and at 7 hours and 30 minutes for the 8 h cycle. During 16 h cycle, the experiment was run for 2 cycles and afterwards it was returned to 24 h cycle for 4 HRTs before 8h cycle experiments were performed. During 8 h cycle, the experiment was run for 2 cycles and afterwards it was returned to 16 h cycle since it was not certain how this reactor will respond to this drastic change. Data was collected manually every 2 hours for 8 h cycles but data for 16 h cycle, performed soon after the second cycle of 8 h cycle (as mentioned above), was only collected at the end of the cycle (which was in the next morning).
7.3. Results

7.3.1. Switch from AMBR to AMBSBR

First day after the switch

As expected, the profiles of intermediates changed from consistently low levels of VFA during AMBR operation (Fig. 5.1) to cycles of VFA accumulation followed by VFA consumption (Fig. 7.1) following fill, react, settle, decant mode of operation. Over 4 hour period after feeding, acetic acid jumped from 2.3 to 7.8 mM, propionic acid rose from 0.8 to 3.2 mM and butyric acid increased from undetectable level to 0.6 mM. All the detected volatile acids, however, started to drop significantly and reached low levels of 4, 2 mM and undetectable level for acetic, propionic and butyric acids, respectively at 10h into reaction phase. At the end of 24 h cycle, both acetic and propionic acids further dropped to 1 mM resulting in total VFAs of less than 0.2 g COD/l.

![Graph showing VFA profiles and accumulation methane on the first day after the switch from AMBR. OLR: 4.2 g COD/l/d, HRT: 3.8 d.](image)

**Fig. 7.1** VFA profiles and accumulation methane on the first day after the switch from AMBR. OLR: 4.2 g COD/l/d, HRT: 3.8 d.
pH during observation was above 7 except during the first 4 hours when all VFA peaks were found, pH dropped to 6.9. At the end of 24 h cycle, pH returned to 7 and methane production efficiency for the cycle was about 95%.

**Second day after the switch**

Fig. 7.2 clearly shows that VFA peaks occurring after the first 4 hours were lower than the peaks observed during the same period on the first day of switching AMBR to AMBSBR. The peak of acetic acid decreased from about 8 to 6 mM, that of propionic acid from 3 to 2.6 mM and that of butyric acid almost the same. This showed good adaptation of the system within very short period of time. Total VFAs and methane production efficiency at the end of the cycle were 0.2 g COD/l and 97%, respectively.

![Fig. 7.2 VFA profiles and accumulation methane on the second day after the switch from AMBR. OLR: 4.2 g COD/l/d, HRT: 3.8 d.](image-url)
After 1 month of operation

The same profiles of intermediates obtained during second day after the switch of AMBR to AMBSBR were observed with acetic acid as the dominant VFA followed by propionic and butyric acids (Fig. 7.3). The highest levels of acetic, propionic and butyric acids were 6.0, 2.4 and 0.9, respectively. As usually observed, these peaks were observed after 4 hours of operation. Methane production efficiency was 97% and total VFAs at the end of 24 h cycle was 0.17 g COD/l.

After 20 hours of reaction phase total VFA concentration was about 0.2 g COD/l signifying that cycle operation times could now be shortened. Fig. 7.3 was now used as a base condition for comparison with other conditions such as higher loads, shorter HRTs or shorter cycles.

Fig. 7.3 VFA profiles and accumulation methane after 1 month of AMBSBR operation. OLR: 4.2 g COD/l/d, HRT: 3.8 d.
7.3.2. Effect of Settle Phase Duration on Quality of Decanted Liquid

Effect of settle phase duration on quality of decanted liquid is shown in Table 7.1. Time 0 represents an AMBR operation (data taken from Table 5.3). It was seen that settle phase in the operation of AMBSBR resulted in lower concentrations of suspended solid (SS) than that observed without a settle phase such as that occurring in AMBR operations. As shown in this table similar quality of decanted liquid with SS content less than 2 g/l and unfiltered COD less than 2.5 g COD/l were obtained when experiments were performed with settling times in the range of 0.5 to 1 hour. For 1 hour of settling time SS in the AMBSBR was about 60% of SS in the AMBR.

Longer settle phase duration (2 and 4 hours) resulted in poorer quality of effluent shown by higher SS and unfiltered COD. It was observed that residual gas entrapped between shredded rubber carriers was released which lifted up some aggregates and few carriers to overflow when longer settling times were imposed. Therefore, a settling time of 1 hour was considered to be optimal.

Table 7.1 Effect of settle phase duration on quality of decanted liquid

<table>
<thead>
<tr>
<th>Settle phase duration (h)</th>
<th>SS (g/l)</th>
<th>Unfiltered COD of decanted liquid (g COD/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.21 ± 0.09</td>
<td>2.75 ± 0.15</td>
</tr>
<tr>
<td>0.5</td>
<td>1.74 ± 0.04</td>
<td>2.45 ± 0.15</td>
</tr>
<tr>
<td>1</td>
<td>1.36 ± 0.08</td>
<td>2.45 ± 0.15</td>
</tr>
<tr>
<td>2</td>
<td>2.13 ± 0.03</td>
<td>2.60 ± 0.20</td>
</tr>
<tr>
<td>4</td>
<td>2.29 ± 0.02</td>
<td>2.75 ± 0.25</td>
</tr>
</tbody>
</table>
Suspended solid (SS) content and unfiltered COD values in the decanted liquid over 9 months of operation are tabulated in Table 7.2. The quality of decanted liquid over this period was similar shown by SS and unfiltered COD values in the range of 1.15 to 1.55 g/l and 2.10 to 2.55 g COD/l, respectively. It signified that the lowest SS concentration that could be obtained in this reactor was about 1.15 g/l. Compared with SS contents observed in this study of 8.99 and 2.21 g/l for CSTR and AMBR, respectively, a sequencing mode in AMBSBR operations resulted in about one eighth and half of SS concentrations obtained from CSTR and AMBR operations.

<table>
<thead>
<tr>
<th>Duration of reactor operation as AMBSBR (months)</th>
<th>SS (g/l)</th>
<th>Unfiltered COD of decanted liquid (g COD/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.36 ± 0.08</td>
<td>2.45 ± 0.15</td>
</tr>
<tr>
<td>2</td>
<td>1.61 ± 0.12</td>
<td>2.55 ± 0.25</td>
</tr>
<tr>
<td>3</td>
<td>1.30 ± 0.06</td>
<td>2.45 ± 0.15</td>
</tr>
<tr>
<td>6</td>
<td>1.55 ± 0.03</td>
<td>2.25 ± 0.15</td>
</tr>
<tr>
<td>9</td>
<td>1.15 ± 0.03</td>
<td>2.10 ± 0.20</td>
</tr>
</tbody>
</table>

7.3.3. Settling Characteristics of Biomass

As shown in Table 7.3, the settleability of biomass improved over the duration of operation. Fluctuation of SS concentrations in the range of 1.15 to 1.55 g/l during the same period of 3 to 9 months (see Table 7.2) indicated that rates of settling did not affect the SS value much but they affected time needed by aggregates to settle. Settle phase could even be shortened from 1 hour to 5 minutes.
Table 7.3 Increase of settling time of biomass aggregates due to sequencing batch of operation

<table>
<thead>
<tr>
<th>Duration of reactor operation as AMBSBR (months)</th>
<th>Settling time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>20.6</td>
</tr>
<tr>
<td>6</td>
<td>7.3</td>
</tr>
<tr>
<td>9</td>
<td>4.7</td>
</tr>
</tbody>
</table>

7.3.4. Performance of AMBSBR at Increased Organic Loads

*Performance at an OLR of 6.4 g COD/l/d*

Acetic, propionic and butyric acids increased from 1.3, 1.2 mM and undetectable level, respectively to 9.7, 3.9 and 1.7 mM, respectively within 6 hours (Fig 7.4). At the same time pH of the system dropped to 6.9. After 20 hours of feeding or reaction, however, VFA concentrations dropped to 3.7, 2.1 mM and undetectable level for acetic propionic and butyric acids, respectively. At the end of 24 h cycle further drop of these acids occurred resulting in total VFAs of 0.36 g COD/l, pH returned to normal and methane production efficiency of 97% was observed for the cycle. This shows the capability of the system to handle 50% higher organic loading without any severe effect of intermediate accumulations at the end of the cycle.
Fig. 7.4 VFA profiles and accumulation methane at an OLR of 6.4 g COD/l/d and HRT of 3.8 d

Performance at an OLR of 7.4 g COD/l/d

An increase of 75% organic loading rate resulted in propionic acid as a dominant VFA in the system followed by acetic and butyric acids (Fig. 7.5). Within 5 hours propionic acid reached a peak of 11 mM while acetic acid accumulated to 7 mM and butyric acid reached a peak of 1.1 mM. At this condition the pH of the system dropped to 6.8. However, over the next 5 hours all intermediates dropped significantly and at the end of 24 h cycle they dropped to 0.8 and 2.6 mM for acetic and propionic acids, respectively with undetectable level of butyric acid. At the end of the cycle, the total VFAs were 0.35 g COD/l and pH remained 7. Methane production efficiency was 96% for the cycle.
Fig. 7.5 VFA profiles and accumulation methane at OLR of 7.4 g COD/l/d and HRT of 3.8d

**Performance at an organic load of 10.8 g COD/l/d**

Similar profiles as those occurring during organic loading of 7.4 g COD/l/d with propionic acid as the dominant VFA were observed during an increase of 160% organic loading rate (Fig. 7.6). Over 8 hours, VFAs reached a peak of 13.8, 11.2 and 1.2 mM for propionic, acetic and butyric acids, respectively. At this point pH of the system dropped to 6.5 but at the end of the cycle the pH became normal again. At the end of the 24 h cycle propionic acid concentration dropped to 4.3 mM whereas acetic and butyric acids dropped to 1.1 mM and 0.6 mM, respectively resulting in total VFAs of 0.65 g COD/l. Methane production efficiency for the cycle was 81%.
When the loading was returned to normal load of 4.2 g COD/l/d the response was prompt (Fig. 7.7). At the end of the cycle the total VFA was very low at 0.05 g/l and methane production efficiency was higher than 100%. This signified that the residual substrate and undegraded metabolites were consumed during this period.

**Fig. 7.6** VFA profiles and accumulation methane at OLR of 10.8 g COD/l/d and HRT of 3.8 d

**Fig. 7.7** VFA profiles and methane accumulation over two cycles, the first one at an OLR of 10.8 g COD/l/d and the second cycle at 4.2 g COD/l/d
7.3.5. Performance of AMBSBR at Shorter HRTs

A conservative settle phase duration of 20 minutes was chosen based on settling time measured after 3 months of operation (Table 7.3). As mentioned in section 7.2.4, the decrease of HRT was done by increasing fill volume of the base feed concentration of 16 g COD/l.

**Performance at an OLR of 6.4 g COD/l/d and HRT of 2.5 d**

Similar VFA profiles as those occurring during the same OLR of 6.4 g COD/l/d at HRT of 3.8 d were observed here (Fig. 7.8). Within 6 hours VFAs jumped from 2.7, 3.3 mM and undetectable level to 9.5, 4.0 and 1.8 mM for acetic, propionic and butyric acids, respectively. All these intermediates, however, returned to low levels after 20 hours. At the end of 24 h cycle, total VFAs were 0.47 g COD/l, methane production efficiency for the cycle was 95% and pH of the system was in the normal range of 7.

![Fig. 7.8 VFA profiles and methane accumulation at OLR of 6.4 g COD/l/d and HRT of 2.5 d](image-url)
Performance at an OLR of 8.9 g COD/l/d and HRT of 1.8 d

An attempt was performed to conduct an experiment at 1.8 d HRT. This brought the system to an OLR of 8.9 g COD/l/d. Since the decrease of HRT was performed by increasing fill volume, it resulted in less gas space available. Therefore, the large amount of gas produced caused foaming and lifted the liquid and some carriers to the gas measuring device and blocked the effluent gas line.

7.3.6. Performance of AMBSBR at Shorter Cycles

Performance at 16 h cycle

As mentioned in section 7.2.4, organic loading rates increased accordingly with the decreases of cycle times. This brought the OLR and HRT to 6.4 g COD/l/d and 2.5 d, respectively at 16 h cycle. Data presented was the first cycle in the 2 cycles (section 7.2.4). During the first 4 h, acetic acid increased from 3.1 to 6.9 mM, propionic acid increased from 3.1 to 4.1 and butyric acid increased from undetectable level to 1.2 mM (Fig. 7.9). Within 12 hours acetic and propionic acids reached the same concentrations at the beginning of experiment of about 3mM, respectively. At the end of 16h cycle further drop of all VFAs was obtained resulting in total VFA of 0.48 g COD/l and methane production efficiency was 95%.

During this study, an OLR and HRT equated to 9.5 g COD/l/d and 1.7 d, respectively. Since VFA levels after 10 hours were higher than those at 8 hours of operation (Fig. 7.10) the digester was run at a 16 h cycle after 2 cycles of 8 h. As shown in Fig. 7.10, the system could not handle this loading capacity shown by higher accumulations of VFAs at the end of second cycle. Total VFAs were 0.76 g COD/l at the end of the first 8 h cycle and 0.97 g COD/l at the end of the second 8 h cycle. Gas production efficiencies at the end of the first and second 8 h cycles were 95 and 82%, respectively. At the end of 16 h cycle total VFAs were 0.56 g COD/l and methane production efficiency reached 102% indicating that degradation of residual VFA left in the system had been occurred.
7.4. Discussion

7.4.1. Performance of AMBSBR

When the sequencing mode was applied to the AMBR, the rates of VFA production initially exceeded their consumption rates causing VFA accumulation as expected (McInerney and Bryant, 1980). The intermediates profiles (Fig. 7.1) are similar to those obtained in Chapter 6 when the CSTR was fed intermittently (once a day). However, higher VFA concentrations were observed during this study, corresponding to more than 2 times higher organic loading applied than that applied during study in the previous chapter (Fig. 6.1).

Compared with VFA peaks occurring after the same period of 4 hours during the first and second day of the switch (of AMBR to AMBSBR operation), significant drop in maximum levels of VFA during a cycle showed good adaptation of the system within very short period of time. Activity of synthrophic bacteria entrapped among the carriers may be the reason for increased capability to degrade large amount of
organics entering the system. As shown in section 5.3.2, the AMBR was capable of handling 75% shock load imposed. During sequencing mode of operation, almost all bacteria could be kept entrapped among the carriers and only the poorly settled biomass was washed out along with the decanted liquid.

After 1 month of reactor operation as AMBSBR, total VFA concentration of about 0.2 g COD/l was obtained within 20 hours of reaction phase (Fig. 7.3). This signified that cycle operation times could now be shortened. Observation of settling phase times on the quality of decanted liquid was performed in the range of 0.5 to 4 hours. Results showed that settling phase times above 2 hours resulted in poorer quality shown by higher SS in the decanted liquid. Therefore, a settling phase time of 1 hour was considered to be optimal and this value was chosen for the rest of experiments with AMBSBR, unless stated otherwise. A comparison with available literature could not be made since literature on the effect of settling phase duration on the quality of decanted liquid in an ASBR could not be found.

Over 9 months of operation (Table 7.2), the SS contents were about 1.15 ± 0.03 g/l, about half of SS concentrations obtained from AMBR (2.21 ± 0.21 g/l; Table 5.3) or about one eighth of SS concentrations obtained from CSTR operations (8.99 ± 0.69 g/l; Table 6.2). A study performed by Ratusznei et al. (2003) and Rodrigues et al. (2003), employing biomass immobilized on polyurethane foam, obtained SS and COD concentrations in the effluent of about 0.1 g/l and 0.1 g COD/l, respectively. The values indicated are much lower than the values of SS and COD in the effluent obtained in this study. However, the SS contents of the influent were also low about 0.1 g/l and the synthetic substrates (contained mainly meat extract and soluble starch) they employed also contained only 0.5 g COD/l. The SS effluent concentrations in
their study were almost the same as the SS contents of the influent. With very dilute feed and at low organic loading rate of about 0.2 g COD/l/d less biomass growth and so less SS in the effluent would be expected. Much higher SS and feed concentrations were employed in this study, i.e. 1.1 ± 0.03 g/l and 16 g COD/l, respectively. This certainly resulted in higher SS (about 1 g/l) and COD contents (about 2 g COD/l) in the decanted liquid.

7.4.2. Performance of AMBSBR at Increased Organic Loads

In general, the response of the digester towards increases of organic loading rates was an increase of VFA peaks over the first 4 to 8 hours and VFAs being consumed by the end of the cycle (Fig. 7.4 to 7.7). At an OLR of 6.4 g COD/l/d peak conditions were dominated by acetic acid whereas, at organic loading rates of 7.4 and 10.8 g COD/l/d peak conditions were dominated by propionic acid. However, all the accumulated acids dropped significantly after 10 hours. Results obtained in Chapter 6 showed intermittent feeding resulted in much higher propionic acid utilizing activity compared with this activity in a continuous feeding system. Similarly, the AMBSBR must have build up a higher propionic acid utilization capacity. This might have been the reason for the increased capability of the AMBSBR to degrade accumulated propionic acid in the system.

Comparison of the performance of AMBSBR with that of AMBR is tabulated in Table 7.4. It is obviously seen that the AMBSBR could be operated at an OLR of 7.4 g COD/l/d, 3.8 d HRT with total VFAs in the decanted liquid of less than 0.5 g COD/l. On the other hand, the operation of AMBR at a lower OLR of 6.4 g COD/l/d at the same HRT had resulted in total VFAs in the effluent of higher than 0.5 g COD/l. More
obvious difference is the operation at an OLR of 10.8 g COD/l/d. Even though propionic acid dominated in the system the AMBSBR could achieve methane production efficiency of 81% compared with the methane production efficiency of 68% during AMBR operations.

Based on theoretical COD balance, there was still a 15% unmeasured total COD out missing in the calculation when the AMBSBR was shock loaded at an OLR of 10.8 g COD/l/d. Drop in methane production efficiency of about 16% (compared with the base load) therefore did not result from increases of measured VFAs (acetic, propionic and butyric acids). The accumulation of metabolites or substrates other than measured VFAs may have occurred in the system. This situation was also found during the AMBR operation when it was shock loaded at an OLR of 10.8 g COD/l/d (see Fig. 5.6 and discussion in Section 5.4.2).

Romli et al. (1995) and Costello et al. (1991 a and b) suggested that lactic acid is an important intermediate in an anaerobic digester which may accumulate only temporarily for a short period of time under shock loading conditions. During normal digester operation this metabolite is not detected.

The cyclic feeding operation such that occurring during sequencing modes had resulted in the build-up of increased concentrations of propionate degrading acetogens, an observation that is in line with anaerobic digestion processes (McInerney and Bryant, 1980; Hoh and Cord-Ruwisch, 1997 a). Temporary shock loads cause a shift in product formation by the fermentative bacteria away from acetate and hydrogen, towards more reduced products such as propionate and
Table 7.4 Comparison of the performance of AMBSBR and AMBR at the same HRT of 3.8d

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OLR = 4.2*</th>
<th>OLR = 6.4*</th>
<th>OLR = 7.4*</th>
<th>OLR = 10.8*</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMBR</td>
<td>AMBSBR</td>
<td>AMBR</td>
<td>AMBSBR</td>
<td>AMBR</td>
</tr>
<tr>
<td>Total VFA (g COD/l)</td>
<td>0.32</td>
<td>0.21</td>
<td>0.77</td>
<td>0.37</td>
</tr>
<tr>
<td>CH₄ prod. efficiency (%)</td>
<td>98</td>
<td>97</td>
<td>86</td>
<td>97</td>
</tr>
<tr>
<td>Dominant VFA</td>
<td>AA</td>
<td>AA</td>
<td>AA</td>
<td>AA</td>
</tr>
</tbody>
</table>

* Unit of OLR is in g COD/l/d, AA: acetic acid, PA: propionic acid.

butyrate. The higher availability of propionate and butyrate in the system is expected to favor the development of propionate and butyrate degrading acetogens. As a result, a sequencing batch mode could increase capability of the digester to handle higher shock loads.

On the other hand, the AMBR, which received consistent lower shock loading (feeding 6 times a day), accumulated more acetic acid in the system, caused by lower levels of hydrogen partial pressure, favoring the production of acetate and hydrogen as the main fermentation products. Both acetate and hydrogen are suitable substrates for methanogenic bacteria (McInerney and Bryant, 1980 and 1981). Unlike accumulation of metabolites (or substrates other than measured VFAs) which occurred during 2.5 times normal loads during AMBR operation, the 20% drop in methane production efficiency during shock loading of 7.4 g COD/l/d mostly resulted from the accumulation of measured VFAs. This again showed that the sequencing batch mode
resulted in a better degrading capacity to utilize accumulated VFAs especially propionic acid (Section 6.3.2) and butyric acid (Section 6.3.4).

Most sequencing batch reactors described in literature relied on granulation or self-immobilized biomass instead of on flocs or aggregates attached or entrapped among the support carriers. Among these studies, an observation found by Shizas and Bagley (2002) showed propionic acid dominating in the system when their reactor was shock loaded 50% higher than the normal load of 2.1 g COD/l/d at the same conditions of 2 d HRT and 24 h cycle at various feeding times with constant settle and decant phases of 90 and 30 minutes, respectively. At feeding phase performed during 6 hours (compared with only 6 minutes performed in this study), total VFAs at the end of 24h cycle were above 1 g COD/l. The presence of the carriers employed in this study may have contributed to the increased retention of propionic acid utilizing acetogens (as observed in Section 5.4.2) besides as a result from sequencing batch modes preventing wash out of active biomass and promoting increased concentrations as well.

7.4.3. Performance of AMBSBR at Shorter HRTs and Shorter Cycles

Performance at shorter HRTs

The system could perform well at 2.5 d HRT and OLR of 6.4 g COD/l/d shown by high methane production efficiency of 95% and 0.5 g COD/l of total VFAs at the end of 24 h cycle. Compared with the results of AMBR performed at the same OLR of 6.4 g COD/l/d and 2.5 d HRT, a better performance resulted from a sequencing mode. The AMBSBR produced higher methane production efficiency and lower total VFAs (Table 7.5). This means that a sequencing batch mode could increase capability of the digester to handle shock loads.
Table 7.5 Performance of AMBSBR and AMBR at the same OLR of 6.4 g COD/l/d and 2.5 d HRT

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AMBSBR</th>
<th>AMBR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total VFA (g COD/l)</td>
<td>0.47</td>
<td>1.0</td>
</tr>
<tr>
<td>Methane production efficiency (%)</td>
<td>95</td>
<td>76</td>
</tr>
</tbody>
</table>

The same methane production efficiency of 95% and total VFAs in the decanted liquid of 0.5 g COD/l were also observed when the digester was run at 16 h cycle at the same organic loading rate of 6.4 g COD/l/d and 2.5 d HRT (Table 7.6). Therefore, sequencing batch mode could be applied either for treating lower strength feed flow rates at longer cycles or more dilute feed flow rates at frequent shorter cycles. This also proved the flexibility of the sequencing batch mode mentioned in the introduction.

Table 7.6 Performance of AMBSBR at the same OLR of 6.4 g COD/l/d and 2.5 d HRT at different cycle periods

<table>
<thead>
<tr>
<th>Parameter</th>
<th>16 h cycle</th>
<th>24 h cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total VFA (gCOD/l)</td>
<td>0.48</td>
<td>0.47</td>
</tr>
<tr>
<td>Methane production efficiency (%)</td>
<td>95</td>
<td>95</td>
</tr>
</tbody>
</table>

Performance at shorter cycles

Problems of gas measurement due to blockage of effluent gas line by the lifted carriers were observed when HRT was shortened by increasing fill volume to the digester (Section 7.3.5). Therefore, to obtain lower HRTs cycles of operation were shortened. The cycle of the system was shortened to 16 hours and 8 hours based on results
indicating that normal total VFA level of about 0.2 g/l could be achieved within 20 hours (Section 7.3.1.), that a settle phase could be shortened to less than 20 minutes (Section 7.3.4), and that the digester could handle well an OLR of 6.4 g COD/l/d at 2.5 d of HRT (Section 7.3.5). In this experiment, the digester could handle well the change from 24 h cycle to 16 h cycle, showing no difference at all either in methane production efficiency or the total VFAs in the decanted liquid (Table 7.6).

Compared with the previous experiment conducting at the same feed concentration of 16 g COD/l but at 24 h cycle (Fig. 7.2), the VFA profiles during 16 h cycle (Fig. 7.9) mirrored their VFA profiles. Acetic acid dropped much lower at the end of cycle. Higher VFA peaks observed at the 16 h cycle than the peaks at 24 h cycle (shown in the brackets) were probably due to their higher initial values (Table 7.7). These were 6.9 mM (6.2 mM) for acetic acid, 3.9 mM (2.4 mM) for propionic acid, and 1.2 mM (0.9 mM) for butyric acid. The current experiment could achieve levels of VFAs lower than their levels at the beginning of the cycle (Table 7.7). Therefore, the current experiment performed slightly better in achieving levels of VFA at the end of cycle. This may be because the capability of syntrophic bacteria to degrade accumulated VFAs might have improved after 5 months of operation.

**Table 7.7** Profiles of VFA concentrations at 16 h and 24 h cycles at the same feed concentration of 16 g COD/l

<table>
<thead>
<tr>
<th>VFA</th>
<th>Concentration (16 h cycle)</th>
<th>Concentration (24 h cycle)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>End of cycle</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>3.1</td>
<td>1.8</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>3.1</td>
<td>2.8</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>
However, at 8 h cycles which brought the system to be operated at an OLR of 9.5 g COD/l/d and HRT of 1.7 d, the digester could not perform well. It showed that a bacterial imbalance has retarded the methanogenic population. The loading applied may have exceeded the maximum digester capacity. Cycle periods between 10 to 12 hours which equates to organic loading rates of about 7.4 g COD/l/d (the maximum loading could be handled well by the digester) could therefore be said as the lowest cycle for the AMBSBR during observation.

### 7.4.4. SRT in the AMBSBR

The solid retention time (SRT) was calculated by using equation 7.2. The concentration of biomass aggregates within the digester was assumed to be the same as that obtained during AMBR operations of about 11.35 g VSS/l. With an AMBSBR which had been operated for about 9 months this VSS value chosen was actually much lower than the actual value, which unfortunately was not measured. Therefore the SRT of 37 days was the minimum SRT obtained during observation.

Reactors capable to produce SRT higher than 20 days at lower HRTs are classified as high-rate systems (Bal and Dhagat, 2001). The UASB, the most sophisticated version of the non-attached biomass digesters could achieve biomass concentration within the reactor between 30 to 50 g VSS/l (Weiland and Rozzi, 1991) which also meant providing higher SRT. Table 7.8 shows the ratio between SRT and HRT which categorizes high rate from conventional or medium rate systems. Unfortunately, only very few publications were available from which SRT could be estimated.
**Table 7.8** Comparison between SRT and HRT ratio of CSTR and high-rate anaerobic reactors

<table>
<thead>
<tr>
<th>Reactor</th>
<th>SRT/HRT</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSTR</td>
<td>1</td>
<td>Binot et al. (1983); Kroiss and Svardal (1999)</td>
</tr>
<tr>
<td>UASB</td>
<td>10 to 60</td>
<td>Azbar et al. (2001); Martinez et al. (2001); Torkian et al. (2003)</td>
</tr>
<tr>
<td>AFBR</td>
<td>10 to 12</td>
<td>Costello (1989); Stronach et al. (1986); Prakash and Kennedy (1996)</td>
</tr>
<tr>
<td>ABR*</td>
<td>8</td>
<td>Chen and Shyu (1996)</td>
</tr>
<tr>
<td>Anaerobic Filter</td>
<td>3</td>
<td>Chen and Shyu (1996)</td>
</tr>
<tr>
<td>AMBSBR</td>
<td>15**</td>
<td>This study</td>
</tr>
</tbody>
</table>

* Anaerobic baffled reactor, ** minimum ratio

It clearly showed from this Table that minimum ratio between SRT and HRT of AMBSBR obtained was 15. De-linking of cell retention time from hydraulic retention time of this reactor type was successful. Therefore, the objective mentioned in the introduction to intensify the existing CSTR (having an SRT and HRT ratio of 1) by addition of granular rubber tire carriers could be achieved. The AMBSBR could be classified as a high-rate reactor.

### 7.5. Conclusions

- When the operation of AMBR was switched to AMBSBR the intermediate profiles changed from consistently low levels of VFA to cycles of VFA accumulation followed by VFA consumption.
- At 3.8 d HRT the AMBSBR could handle OLR up to 10.8 g COD/l/d at 24 h cycle whereas the AMBR could perform well for organic loading rates up to 7.4 g COD/l/d. Whereas AMBSBR could handle OLR of 6.4 g COD/l/d at 2.5 d HRT
the AMBR could only be loaded at an OLR of 4.2 g COD/l/d at the same HRT of 2.5 d.

- The sequencing batch mode could increase capability of the digester to handle higher shock loads either by employing longer cycles (24 hours) at concentrated feed (24 g COD/l) flow rates or frequent shorter cycles (16 hours) at more dilute feed (16 g COD/l) flow rates.

- The minimum SRT that could be achieved during observation was 37 days or minimum ratio of SRT and HRT of 15 and thus the AMBSBR could be classified as a high-rate anaerobic reactor.
Chapter 8

Modelling Anaerobic Degradation of Carbohydrates
under Different Feeding Strategies

8.1. Introduction

The first part of this chapter describes the development of a structured mathematical model for carbohydrate degradation in continuously and intermittently fed anaerobic stirred tank reactors. The second part presents parameter estimations and validation of the model as well as simulations of responses of the systems during shock loads. The basis for the model is the model developed by IWA Task Group for Mathematical Modelling of Anaerobic Digestion Processes that was reported in a scientific and technical report titled Anaerobic Digestion Model No.1 (ADM1, Batstone et al. 2002). The developed model contains a structured set of algebraic and differential equations describing mass and mole balances and physico-chemical reactions involved in the anaerobic degradation of soluble carbohydrates, represented by glucose in the molasses based feed. Experimental results from both Chapters 6 and 7 have indicated that intermittently fed reactors developed a change in microbial population, in particular the build-up of increased potential to degrade propionate. We attribute the increased propionic acid degradation capacity to a shift in population that has occurred in the intermittently fed reactor. The objective of this study was to evaluate to what extent the ADM1 model predicts the differences in behavior observed in laboratory reactors fed continuously and intermittently.
8.2. Model Description

8.2.1. Description of Original Kinetic Model

The kinetic model developed in ADM1 assumed that the degradation of complex organic material occurred in four steps. The first step, known as disintegration and hydrolysis mediates the breakdown and solubilisation of particulate and complex organic material to soluble substrates. In this study, this step was not accounted for since the molasses based feed contained soluble carbohydrate. The soluble carbohydrate substrate was represented as glucose in model. The second step, i.e. acidogenesis is the step where degradation of soluble organic matter into a number of simpler products like volatile fatty acids takes place. The next step, acetogenensis, is the degradation of higher carbon chain organic acids to acetate. Acetate and hydrogen are then converted to methane by two groups of anaerobic bacteria in the methanogenesis step. A schematic diagram of the relationships between each group of bacteria in the anaerobic ecosystem model is shown in Fig. 8.1. The features of the model developed in this study are listed as follows:

- Units of glucose, organic acids, and bacterial components were represented as g COD/l whereas units of all components in the gas phase were based on their partial pressures (bar). Dissolved CO$_2$ and H$_2$, were in the unit of mole/l.
- Bacterial growth within the reactor followed Monod growth kinetics.
- Bacterial decay rate is first order with respect to the bacterial concentration.
- All bacterial and product yield values were obtained from ADM1 except product yields from glucose utilization which were calculated from catabolic reactions.
• Products from glucose degradation are acetate, acetate and propionate, and butyrate (Table 8.1).

<table>
<thead>
<tr>
<th>Table 8.1 Glucose utilization stoichiometry used in the model</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$</td>
</tr>
<tr>
<td>$3C_6H_{12}O_6 \rightarrow 4CH_3CH_2COOH + 2H_2O + 2CO_2 + 2CH_3COOH$</td>
</tr>
<tr>
<td>$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2$</td>
</tr>
</tbody>
</table>

8.2.2. Assumptions used in the model

• the reactors were operated at a constant temperature of 37 °C;
• constant volume of 2 liters;
• constant pressure of 1 atm (or 1.013 bar);
• fully mixed;
• no biomass in feed;
• no physical losses;
• no oxygen contamination;
• growth associated product formation;
• inhibition of growth of propionic and butyric acid degrading bacteria by H₂;
• a fixed ratio of glucose utilisation reactions;
• non equilibrium transfer of CO₂ from liquid to gas;
• all gases behave as ideal gas;
• gas in head space is well mixed and at constant volume, temperature, and pressure;
• methane and hydrogen gas are insoluble in water;
• carbon dioxide is soluble in liquid phase and its production was equal to its transfer rate between the gas and liquid phase;
• only considered carbohydrate degradation;
• molasses contained 85% degradable materials on a COD basis;
• constant pH of 7.2, therefore pH inhibition was ignored.
Fig. 8.1. A schematic diagram of the relationships between each group of bacteria in the anaerobic ecosystem model.
8.3. Model Development

8.3.1. Constitutive Relations

The following set of equations show all the constitutive relations in developing the model. The list of nomenclature are provided in Tables 8.2 to 8.6 which show the list of constitutive variables, state variables, biomass and product yields, constant and fitting parameters, respectively.

**Individual reaction rates for all bacterial groups:**

\[
\begin{align*}
    r_1 & = \frac{\mu_{max} \cdot S_G \cdot X_G}{(Y_{XGSG})(K_S + S_G)} \\
    r_2 & = \frac{\mu_{max}^{AA} \cdot S_{AA} \cdot X_{AA}}{(Y_{XAASA})(k_S^{AA} + S_{AA})} \\
    r_3 & = \frac{\mu_{max}^{PA} \cdot S_{PA} \cdot X_{PA} \cdot \frac{1}{1 + I_{H2}}}{(Y_{XPASPA})(k_S^{PA} + S_{PA})} \\
    r_4 & = \frac{\mu_{max}^{BA} \cdot S_{BA} \cdot X_{BA} \cdot \frac{1}{1 + I_{H2}}}{(Y_{XBA5BA})(k_S^{BA} + S_{BA})} \\
    r_5 & = \frac{\mu_{max}^{H2} \cdot S_{H2} \cdot X_{H2}}{(Y_{XH2SH2})(k_S^{H2} + S_{H2})}
\end{align*}
\]
Rates of bacterial decay:

\[ r_d(1) = k_d(1) * X_g \]  \hspace{1cm} (8.6)

\[ r_d(2) = k_d(2) * X_{AA} \]  \hspace{1cm} (8.7)

\[ r_d(3) = k_d(3) * X_{PA} \]  \hspace{1cm} (8.8)

\[ r_d(4) = k_d(4) * X_{BA} \]  \hspace{1cm} (8.9)

\[ r_d(5) = k_d(5) * X_{H_2} \]  \hspace{1cm} (8.10)

Generation rates of biogas:

\[ r_{CO_2} = Y_{CO_2G} * r_1 + Y_{CO_2AA} * r_2 + Y_{CO_2PA} * r_3 - Y_{CO_2H_2} * r_5 \]  \hspace{1cm} (8.11)

\[ r_{H_2} = Y_{H_2G} * r_1 + Y_{H_2PA} * r_3 + Y_{H_2AA} * r_4 - r_5 \]  \hspace{1cm} (8.12)

\[ r_{CH_4} = Y_{CH_4AA} * r_2 + Y_{CH_4H_2} * r_5 \]  \hspace{1cm} (8.13)

Transfer rate of dissolved gasses to their respective gaseous phases:

\[ r_{H_2^T} = (k_1 a)_{H_2} * \left( S_{H_2} - K_{HH} * S_{gas,H_2} \right) \]  \hspace{1cm} (8.14)
\[ r_{\text{CO}_2, T} = (k_L a)^{\text{CO}_2} * \left( S_{\text{CO}_2} * S_H / (K_{\text{dissCO}_2} + S_H) \right) - K_{\text{HCO}_2} * S_{\text{gas}, \text{CO}_2} \]  

(8.15)

**Gas flow rate:**

\[
F_g = \frac{R * T * V_L}{1.013 - P_{\text{gas}, \text{H}_2\text{O}}} * (r_{\text{CH}_4} + r_{\text{CO}_2, T} + r_{\text{H}_2, T})
\]  

(8.16)

where:

\[
P_{\text{gas}, \text{H}_2\text{O}} = 0.013 \exp \left( 5290 \left( \frac{1}{298} - \frac{1}{T} \right) \right)
\]  

(8.17)

**Hydrogen inhibition:**

\[
I_{pH(i)} = \exp \left( -3 \left( \frac{pH - pH_{ul}(i)}{pH_{ul}(i) - pH_{ll}(i)} \right)^2 \right)
\]  

For pH<pH_{ul} (i)  

(8.18)

\[
I_{pH(i)} = 1
\]  

For pH>pH_{ul} (i)  

(8.19)

**Yields calculated from catabolic reactions:**

\[
Y_{\text{AAG}} = \frac{2\eta + 0.67\eta_z}{C_1 / C_4}
\]  

(8.20)

\[
Y_{\text{RAG}} = \frac{1.333\eta_z}{C_2 / C_4}
\]  

(8.21)
\[ Y_{BG} = \frac{1-\eta_1 - \eta_2}{C_3 / C_4} \]  

(8.22)

\[ Y_{CO_2} = \frac{2 - 1.333\eta_2}{C_4} \]  

(8.23)

\[ Y_{H_2,G} = \frac{2\eta_1 - 2\eta_2 + 2}{C_4} \]  

(8.24)

where: \( \eta_1 + \eta_2 + \eta_3 = 1 \)

(8.25)

**Table 8.2** List of Constitutive Variables used in the Model

<table>
<thead>
<tr>
<th>Constitutive Variables</th>
<th>Description</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>( r_1 )</td>
<td>acidogenesis rate</td>
<td>g CODS/l/d</td>
</tr>
<tr>
<td>( r_2 )</td>
<td>methanogenesis (acetate) rate</td>
<td>g CODS/l/d</td>
</tr>
<tr>
<td>( r_3 )</td>
<td>propionate utilization rate</td>
<td>g CODS/l/d</td>
</tr>
<tr>
<td>( r_4 )</td>
<td>butyrate utilization rate</td>
<td>g CODS/l/d</td>
</tr>
<tr>
<td>( r_5 )</td>
<td>methanogenesis (hydrogen) rate</td>
<td>mole H2/l/d</td>
</tr>
<tr>
<td>( r_d )</td>
<td>death rate</td>
<td>g CODX/l/d</td>
</tr>
<tr>
<td>( r_{CO_2} )</td>
<td>CO2 generation rate</td>
<td>mole CO2/l/d</td>
</tr>
<tr>
<td>( r_{CH_4} )</td>
<td>CH4 generation rate</td>
<td>mole CH4/l/d</td>
</tr>
<tr>
<td>( r_{H_2} )</td>
<td>H2 generation rate</td>
<td>mole H2/l/d</td>
</tr>
<tr>
<td>( F_g )</td>
<td>Total gas flow rate</td>
<td>l/d</td>
</tr>
<tr>
<td>( r_{CO_2,T} )</td>
<td>CO2 transfer rate</td>
<td>mole/l/d</td>
</tr>
<tr>
<td>( r_{H_2,T} )</td>
<td>H2 transfer rate</td>
<td>mole/l/d</td>
</tr>
<tr>
<td>( l_{H_2} )</td>
<td>Hydrogen Inhibition</td>
<td></td>
</tr>
</tbody>
</table>
Table 8.3 List of state variables used in the model

<table>
<thead>
<tr>
<th>Constitutive Variables</th>
<th>Description</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S_G$</td>
<td>Substrate</td>
<td>g CODS/l</td>
</tr>
<tr>
<td>$S_{AA}$</td>
<td>Total Acetic Acid</td>
<td>g CODS/l</td>
</tr>
<tr>
<td>$S_{PA}$</td>
<td>Total Propionic Acid</td>
<td>g CODS/l</td>
</tr>
<tr>
<td>$S_{BA}$</td>
<td>Total Butyric Acid</td>
<td>g CODS/l</td>
</tr>
<tr>
<td>$S_{CO2}$</td>
<td>CO$_2$(aq)</td>
<td>mole/l</td>
</tr>
<tr>
<td>$S_{H2}$</td>
<td>H$_2$(aq)</td>
<td>mole/l</td>
</tr>
<tr>
<td>$X_G$</td>
<td>Acidogens</td>
<td>g CODX/l</td>
</tr>
<tr>
<td>$X_{AA}$</td>
<td>Acetoclastic methanogenic bacteria</td>
<td>g CODX/l</td>
</tr>
<tr>
<td>$X_{PA}$</td>
<td>Propionate utilizing bacteria</td>
<td>g CODX/l</td>
</tr>
<tr>
<td>$X_{BA}$</td>
<td>Butyrate utilizing bacteria</td>
<td>g CODX/l</td>
</tr>
<tr>
<td>$X_{H2}$</td>
<td>Hydrogenotrophic methanogenic bacteria</td>
<td>g CODX/l</td>
</tr>
<tr>
<td>$S_{gas,H2}$</td>
<td>Hydrogen in gas phase</td>
<td>bar</td>
</tr>
<tr>
<td>$S_{gas,CH4}$</td>
<td>Methane in gas phase</td>
<td>bar</td>
</tr>
<tr>
<td>$S_{gas,CO2}$</td>
<td>CO$_2$ in gas phase</td>
<td>bar</td>
</tr>
</tbody>
</table>
Table 8.4 List of product and biomass yield coefficients used in the model

<table>
<thead>
<tr>
<th>Yield</th>
<th>Description</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yxgsg</td>
<td>Acidogens yield on glucose</td>
<td>gCODX/gCODS</td>
</tr>
<tr>
<td>Yxaasaa</td>
<td>Acetoclastic methanogenic bacteria yield on acetic acid</td>
<td>gCODX/gCODS</td>
</tr>
<tr>
<td>Yxpaspa</td>
<td>Propionic acid utilizing bacteria yield on propionic acid</td>
<td>gCODX/gCODS</td>
</tr>
<tr>
<td>Yxbasba</td>
<td>Butyric acid utilizing bacteria yield on butyric acid</td>
<td>gCODX/gCODS</td>
</tr>
<tr>
<td>Yxh2sh2</td>
<td>Hydrogenotrophic methanogenic bacteria yield on hydrogen</td>
<td>gCODX/gCODS</td>
</tr>
<tr>
<td>Yco2g</td>
<td>Carbon dioxide yield on glucose</td>
<td>moleCO2/gCODS</td>
</tr>
<tr>
<td>Yco2aa</td>
<td>Carbon dioxide yield on acetic acid</td>
<td>moleCO2/gCODS</td>
</tr>
<tr>
<td>Yco2pa</td>
<td>Carbon dioxide yield on propionic acid</td>
<td>moleCO2/gCODS</td>
</tr>
<tr>
<td>Ych4aa</td>
<td>Specific methane yield on acetic acid</td>
<td>moleCH4/gCODS</td>
</tr>
<tr>
<td>Ych4h2</td>
<td>Specific methane yield on hydrogen</td>
<td>moleCH4/gCODS</td>
</tr>
<tr>
<td>Yh2g</td>
<td>Hydrogen yield on glucose</td>
<td>moleH2/gCODS</td>
</tr>
<tr>
<td>Yh2pa</td>
<td>Hydrogen yield on propionic acid</td>
<td>moleH2/gCODS</td>
</tr>
<tr>
<td>Yh2ba</td>
<td>Hydrogen yield on butyric acid</td>
<td>moleH2/gCODS</td>
</tr>
<tr>
<td>Yaag</td>
<td>Acetic Acid yield on Glucose</td>
<td>gCODS/gCODS</td>
</tr>
<tr>
<td>Yaapa</td>
<td>Acetic Acid yield on Propionic Acid</td>
<td>gCODS/gCODS</td>
</tr>
<tr>
<td>Yaaba</td>
<td>Acetic Acid yield on butyric Acid</td>
<td>gCODS/gCODS</td>
</tr>
<tr>
<td>Ypag</td>
<td>Propionic Acid yield on glucose</td>
<td>gCODS/gCODS</td>
</tr>
<tr>
<td>Ybag</td>
<td>Butyric Acid yield on glucose</td>
<td>gCODS/gCODS</td>
</tr>
<tr>
<td>Yco2h2</td>
<td>Carbon dioxide yield on hydrogen</td>
<td>moleCO2/moleH2</td>
</tr>
</tbody>
</table>
**Table 8.5** List of Constants used in the model

<table>
<thead>
<tr>
<th>Constant</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>Universal Gas constant</td>
<td>0.08314 l<em>bar/(mole</em>K)</td>
</tr>
<tr>
<td>V_L</td>
<td>Liquid Volume</td>
<td>2 l</td>
</tr>
<tr>
<td>V_G</td>
<td>Gas Volume</td>
<td>0.976 l</td>
</tr>
<tr>
<td>T</td>
<td>Reactor Temperature</td>
<td>310 K</td>
</tr>
<tr>
<td>K_{AA}</td>
<td>Equilibrium Constant for dissociation of Acetic Acid</td>
<td>1.62.10^{-5} mole/l</td>
</tr>
<tr>
<td>K_{PA}</td>
<td>Equilibrium Constant for dissociation of Propionic</td>
<td>1.25.10^{-5} mole/l</td>
</tr>
<tr>
<td>K_{BA}</td>
<td>Equilibrium Constant for dissociation of Butyric Acid</td>
<td>1.31.10^{-5} mole/l</td>
</tr>
<tr>
<td>K_{CO2}</td>
<td>Equilibrium Constant for dissociation of Carbon Dioxide</td>
<td>5.03.10^{-7} mole/l</td>
</tr>
<tr>
<td>K_{w}</td>
<td>Equilibrium Constant for dissociation of Water</td>
<td>2.48.10^{-14} mole/l</td>
</tr>
<tr>
<td>K_{HH}</td>
<td>Henry’s Law Constant – Hydrogen</td>
<td>7.8.10^{-4} mole/l-bar</td>
</tr>
<tr>
<td>K_{HCO2}</td>
<td>Henry’s Law Constant – Carbon Dioxide</td>
<td>0.04 mole/l-bar</td>
</tr>
<tr>
<td>C_1</td>
<td>Conversion factor (mole acetic acid to g COD)</td>
<td>64</td>
</tr>
<tr>
<td>C_2</td>
<td>Conversion factor (mole propionic acid to g COD)</td>
<td>112</td>
</tr>
<tr>
<td>C_3</td>
<td>Conversion factor (mole butyric acid to g COD)</td>
<td>160</td>
</tr>
<tr>
<td>C_4</td>
<td>Conversion factor (mole glucose to g COD)</td>
<td>192</td>
</tr>
<tr>
<td>η_1</td>
<td>Reaction 1 fraction of glucose fermentation</td>
<td>0.45</td>
</tr>
<tr>
<td>η_2</td>
<td>Reaction 2 fraction of glucose fermentation</td>
<td>0.35</td>
</tr>
<tr>
<td>η_3</td>
<td>Reaction 3 fraction of glucose fermentation</td>
<td>0.20</td>
</tr>
</tbody>
</table>
Table 8.6 List of Fitting Parameters used in the model

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu_{\text{max}}$</td>
<td>Maximum growth rate of acidogens</td>
<td>8.780 /day</td>
</tr>
<tr>
<td>$\mu_{\text{max,AA}}^*$</td>
<td>Maximum growth rate of acetoclastic methanogenic bacteria</td>
<td>0.450 /day</td>
</tr>
<tr>
<td>$\mu_{\text{max,PA}}$</td>
<td>Maximum growth rate of propionic acid utilizing bacteria</td>
<td>0.800 /day</td>
</tr>
<tr>
<td>$\mu_{\text{max,BA}}$</td>
<td>Maximum growth rate of butyric acid utilizing bacteria</td>
<td>2.700 /day</td>
</tr>
<tr>
<td>$\mu_{\text{max,H2}}$</td>
<td>Maximum growth rate of Hydrogenotrophic methanogenic bacteria</td>
<td>2.600 /day</td>
</tr>
<tr>
<td>$k_s$</td>
<td>Maximum velocity constant for acidogens</td>
<td>1.28 g COD/l</td>
</tr>
<tr>
<td>$k_{s,AA}^*$</td>
<td>Half velocity constant for acetoclastic bacteria</td>
<td>0.384 g COD/l</td>
</tr>
<tr>
<td>$k_{s,PA}^*$</td>
<td>Half velocity constant for propionic acid utilizing bacteria</td>
<td>0.373 g COD/l</td>
</tr>
<tr>
<td>$k_{s,BA}$</td>
<td>Half velocity constant for butyric acid utilizing bacteria</td>
<td>0.280 g COD/l</td>
</tr>
<tr>
<td>$k_{s,H2}$</td>
<td>biomass death rate of hydrogen utilizing bacteria</td>
<td>0.02 /day</td>
</tr>
<tr>
<td>$k_d(1)$</td>
<td>Biomass death rate of acidogens</td>
<td>0.02 /day</td>
</tr>
<tr>
<td>$k_d(2)$</td>
<td>Biomass death rate of acetoclastic bacteria</td>
<td>0.02 /day</td>
</tr>
<tr>
<td>$k_d(3)$</td>
<td>Biomass death rate of propionic acid utilizing bacteria</td>
<td>0.01 /day</td>
</tr>
<tr>
<td>$k_d(4)$</td>
<td>Biomass death rate of butyric acid utilizing bacteria</td>
<td>0.03 /day</td>
</tr>
<tr>
<td>$K_{\text{CO2}}$</td>
<td>Biomass death rate of hydrogen utilizing bacteria</td>
<td>0.009 /day</td>
</tr>
<tr>
<td>$K_{\text{H2}}$</td>
<td>CO2 transfer coefficient</td>
<td>100 /day</td>
</tr>
<tr>
<td>$K_{\text{H1}}$</td>
<td>H2 transfer coefficient</td>
<td>100 /day</td>
</tr>
<tr>
<td>$K_{\text{IH}}$</td>
<td>Inhibition Factor</td>
<td>$8.10^6$ g COD/l</td>
</tr>
</tbody>
</table>

*parameters that required modification

8.3.2. Conservation Balances

Conservation balances used in developing the model were grouped into their phases, i.e. solid (bacterial components), liquid phase (substrate components) and gas phase (gas components).
Bacterial components:

\[
\frac{dX_G}{dt} = (Y_{XGSG})*(r_1) - r_d(1) - D*X_G
\]  \hspace{1cm} (8.26)

\[
\frac{dX_{AA}}{dt} = (Y_{XAASAA})*(r_2) - r_d(2) - D*X_{AA}
\]  \hspace{1cm} (8.27)

\[
\frac{dX_{PA}}{dt} = (Y_{XPASPA})*(r_3) - r_d(3) - D*X_{PA}
\]  \hspace{1cm} (8.28)

\[
\frac{dX_{BA}}{dt} = (Y_{XBASBA})*(r_4) - r_d(4) - D*X_{BA}
\]  \hspace{1cm} (8.29)

\[
\frac{dX_{H2}}{dt} = (Y_{XH2SH2})*(r_5) - r_d(5) - D*X_{H2}
\]  \hspace{1cm} (8.30)

Liquid components:

\[
\frac{dS_G}{dt} = D*S_G^{in} - S_G - r_1
\]  \hspace{1cm} (8.31)

\[
\frac{dS_{AA}}{dt} = Y_{AAG} * r_1 + Y_{AAPA} * r_3 + Y_{ABA} * r_4 - r_2 - D*S_{AA}
\]  \hspace{1cm} (8.32)

\[
\frac{dS_{PA}}{dt} = Y_{PAG} * r_1 - r_3 - D*S_{PA}
\]  \hspace{1cm} (8.33)
\[
\begin{align*}
\frac{dS_{BA}}{dt} &= Y_{BAG} \cdot r_1 - r_4 - D \cdot S_{BA} \quad (8.34) \\
\frac{dS_{CO_2}}{dt} &= r_{CO_2} - r_{CO_2,x} - D \cdot S_{CO_2} \quad (8.35) \\
\frac{dS_{H_2}}{dt} &= r_{H_2} - r_{H_2,x} - D \cdot S_{H_2} \quad (8.36)
\end{align*}
\]

Gas components:

\[
\begin{align*}
\frac{dS_{H_2}}{dt} &= -y_{H_2} \cdot \frac{F_{gas}}{V_{gas}} + r_{H_2,x} \cdot \frac{V_{liq}}{V_{gas}} \cdot R \cdot T \quad (8.37) \\
\frac{dS_{CH_4}}{dt} &= -y_{CH_4} \cdot \frac{F_{gas}}{V_{gas}} + r_{CH_4} \cdot \frac{V_{liq}}{V_{gas}} \cdot R \cdot T \quad (8.38) \\
\frac{dS_{CO_2}}{dt} &= -y_{CO_2} \cdot \frac{F_{gas}}{V_{gas}} + r_{r,CO_2} \cdot \frac{V_{liq}}{V_{gas}} \cdot R \cdot T \quad (8.39)
\end{align*}
\]

8.4. Model Implementation

The conservation balances were incorporated into a function block in the software MATLAB 6.1. The function block was then called by a driver function (Appendix 1) in which all the integration procedure was stated. The integration was performed by an ODE15s solver, which is a built-in function of MATLAB 6.1. In the driver function, minimum relative error tolerance and maximum step were chosen to maximize the
The difference between function block in the continuously feeding model and intermittently feeding model was only on the formulation of dilution rate. In the continuously feeding model, feed was fed continuously and effluent was withdrawn simultaneously. In the intermittently feeding model, the feeding was injected manually once a day within 2 minutes. The effluent was withdrawn the next day followed by two minutes feeding and degradation reactions (Section 6.2.1). When propionic acid spike experiments were performed, the influent of molasses substrate was stopped and replaced by propionic acid addition (Section 6.2.2). Therefore, the initial substrate concentration or $S_{in}$ in the function block was set to 0 and initial propionic acid concentration in the initial value matrix in the function driver was set according to the chosen experimental concentration.

### 8.5. Model Modification

It was noticed that the original ADM1 model when operated under continuous feeding regime showed a slight inconsistency in the COD balance. The model predicted the COD output to be about 11% higher than the input. This was attributed to two reasons:

1) The mass balance incorporates only catabolic reactions. The product yields are determined from stoichiometry, which does not incorporate biomass formation. Therefore, for a particular reaction only the masses (or COD) of the substrates and metabolic products will be conserved and any biomass produced would be in excess.

2) The model considers biomass decay, and the effect is incorporated as a first order rate with respect to bacterial concentrations. The decay rate is then subtracted from the growth rate, $\mu X_i$, to give the net growth rate. The biomass COD that is
released from microbial decay is not accounted. If biomass death rates, \( k_d(i) \), were set equal to 0, the inconsistency could be rectified.

To overcome the above inconsistencies the model was modified as follows:

1) The anabolic reactions for formation of biomass were included into the stoichiometry. An empirical formula of \( \text{C}_5\text{H}_9\text{O}_3\text{N} \) was assumed for biomass. It was also assumed that part of the substrate that is metabolised is used for building the cell components. From knowledge of the ATP yield of the catabolic reactions and assuming that 10 g of biomass can be produced from 1 mole of ATP, the catabolic and anabolic reactions (Table 8.7) are combined. For example, for aceticlastic methanogenesis assuming an ATP yield of 0.25 mole/mole acetate, the modified stoichiometry for this reaction is shown in Table 8.8. Similar stoichiometry can be written for other reactions as well. The yield coefficients for biomass and products (Equation 8.40 to 8.46) are now based on such stoichiometry.

2) A COD production from decayed biomass was incorporated as follows:

\[
\frac{dB_{\text{COD}}}{dt} = -DB_{\text{COD}} + \sum_{i=1}^{s} k_d(i)X_i
\]

where \( B_{\text{COD}} \): concentration of cellular components of decaying bacterial biomass (g COD/l), \( D \): dilution rate (1/d), \( k_d \): biomass death rate (1/d) and \( X \): biomass concentration (g COD/l).

The COD that is produced is assumed to be non degradable and decay of 1 g COD of microbial biomass produces 1 g COD of non degradable compounds.
Table 8.7 Stoichiometry of catabolic and anabolic reactions used in the model

<table>
<thead>
<tr>
<th>Acidogens</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 + 4ATP$</td>
</tr>
<tr>
<td>$3C_6H_{12}O_6 \rightarrow 4CH_3CH_2COOH + 2H_2O + 2CO_2 + 2CH_3COOH + 4/3ATP$</td>
</tr>
<tr>
<td>$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 + 3ATP$</td>
</tr>
<tr>
<td>$5C_6H_{12}O_6 + 6NH_3 \rightarrow 6C_5H_9O_3N + 12H_2O$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Propionic acid utilizing bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + 3H_2 + CO_2 + 1ATP$</td>
</tr>
<tr>
<td>$3CH_3CH_2COOH + CO_2 + 2NH_3 \rightarrow 2C_5H_9O_3N + 2H_2O + H_2$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Butyric acid utilizing bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>$CH_3CH_2CH_2COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2 + 2ATP$</td>
</tr>
<tr>
<td>$CH_3CH_2CH_2COOH + CO_2 + NH_3 \rightarrow C_5H_9O_3N + H_2O$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Aceticlastic methanogenic bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>$CH_3COOH \rightarrow CH_4 + CO_2 + 0.25ATP$</td>
</tr>
<tr>
<td>$5CH_3COOH + 2NH_3 \rightarrow 2C_5H_9O_3N + 4H_2O$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hydrogenotrophic methanogenic bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O + 1ATP$</td>
</tr>
<tr>
<td>$5CO_2 + 10H_2 + NH_3 \rightarrow C_5H_9O_3N + 7H_2O$</td>
</tr>
</tbody>
</table>
**Table 8.8** Combining catabolic and anabolic reactions for aceticlastic methanogenesis

\[
\begin{align*}
CH_3COOH & \rightarrow CH_4 + CO_2 + 0.25 \text{ATP} \\
0.0475CH_3COOH + 0.019NH_3 & \rightarrow 0.019C_5H_9O_3N + 2H_2 + 0.038H_2O \\
1.0475CH_3COOH + 0.019NH_3 & \rightarrow 0.019C_5H_9O_3N + CH_4 + CO_2 + 0.038H_2O
\end{align*}
\]

**Yields calculated from anabolic and catabolic reactions in the acidogenesis step:**

\[
Y_{X_{GSG}} = \frac{(0.229 + 0.076\eta_1 - 0.1951\eta_2)C_3}{(1.191 + 0.063\eta_1 - 0.1625\eta_2)C_4} \quad (8.40)
\]

\[
Y_{A_{AG}} = \frac{(2\eta_1 + 0.667\eta_2)C_1}{(1.191 + 0.063\eta_1 - 0.1625\eta_2)C_4} \quad (8.41)
\]

\[
Y_{P_{AG}} = \frac{(1.333\eta_2)C_2}{(1.191 + 0.063\eta_1 - 0.1625\eta_2)C_4} \quad (8.42)
\]

\[
Y_{B_{AG}} = \frac{(1 - \eta_1 - \eta_2)C_3}{(1.191 + 0.063\eta_1 - 0.1625\eta_2)C_4} \quad (8.43)
\]

\[
Y_{CO_{2, G}} = \frac{(2 - 1.333\eta_2)}{(1.191 + 0.063\eta_1 - 0.1625\eta_2)C_4} \quad (8.44)
\]
\[ Y_{H,G} = \frac{(2\eta_1 - 2\eta_2 + 2)}{(1.191 + 0.063\eta_1 - 0.1625\eta_2)C_4} \]  

(8.45)

Where:  \( \eta_1 + \eta_2 + \eta_3 = 1 \)  

(8.46)

The other yields were calculated similarly according to their reactions involved.

### 8.6. Parameter Estimation and Model Validation

Parameter estimation and model validation were carried out under two operational conditions: steady state and dynamic conditions. Steady state data were used to verify parameters and was only conducted for the continuously feeding runs. The steady state data are shown in Table 8.9 Columns 4 & 5. The experimental results were collected on Day 16 and 75 after steady state conditions were reached from the 2 l digesters, operated at 37\(^{\circ}\) C at an organic loading rate of 1.25 g COD/l/d (Section 6.2.1).

**Steady State Validation**

Kinetic parameters provided by ADM1 were initially used to observe the fit between simulated and experimental results. The variables tested include acetic, propionic and butyric acid concentrations in the effluent and methane gas production. The original kinetic model parameters were unfortunately unable to satisfactorily predict all the variables tested. The simulation over predicted methane gas production, and also
concentrations of both propionic and butyric acids and under predicted acetic acid concentration (Table 8.9, Column 2).

Modification to the model (as mentioned in Section 8.5) was therefore performed. This included yield coefficients for biomass and products calculated from a combination of catabolic and anabolic reactions, and an incorporation of COD production from decayed biomass which was all assumed to be non degradable.

Table 8.9 Comparison between predicted and observed intermediate, methane production rate, bacterial concentrations and gas composition.

<table>
<thead>
<tr>
<th>State variable</th>
<th>Original model</th>
<th>Modified model</th>
<th>Experiment Day 16</th>
<th>Experiment Day 75</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic acid</td>
<td>0.07</td>
<td>0.21</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>0.04</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>0.01</td>
<td>0.01</td>
<td>0.001</td>
<td>0.00</td>
</tr>
<tr>
<td>Methane production rate</td>
<td>0.40</td>
<td>0.30</td>
<td>0.30</td>
<td>0.29</td>
</tr>
<tr>
<td>Acidogenic bacteria</td>
<td>1.06</td>
<td>2.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aceticlastic methanogenic bacteria</td>
<td>0.48</td>
<td>0.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propionate utilizing bacteria</td>
<td>0.23</td>
<td>0.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butyrate utilizing bacteria</td>
<td>0.15</td>
<td>0.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrogen utilizing bacteria</td>
<td>0.36</td>
<td>0.78</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methane</td>
<td>61.6</td>
<td>65.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>38.4</td>
<td>349</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrogen</td>
<td>0.02</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: all units are in g COD/l except methane production rate is in l/l/d; and methane, carbon dioxide and hydrogen are in percentage.

In addition to modifying the model as described above, several fitting parameters required modification to obtain a better fit between simulated and experimental results. These were maximum growth rate of aceticlastic bacteria ($\mu_{maxAA}$), half velocity constant for aceticlastic bacteria ($K_{sAA}$) and half velocity constant for
propionic acid utilizing bacteria (K_{sPA}) as shown in Table 8.6 (asterisk symbols). The $\mu_{\text{maxAA}}$ was lowered from 0.45 to 0.35 per day; $K_{sAA}$ was increased from 0.384 to 0.8 g COD/L and $K_{sPA}$ was lowered from 0.373 to 0.1 g COD/l.

With this approach, the modified model could predict steady state values obtained from the continuously fed digester and produced data that were consistent with the experimental results (Table 8.9, Column 3).

**Dynamic Validation**

Dynamic validation was undertaken on intermittent feeding runs during normal loading and both continuous and intermittent feeding runs during propionic acid pulses (Chapter 6). As mentioned before, propionic acid was added to both reactors in a batch operation after the feed to the digesters was switched off.

When model validation was done for each different type of feeding strategy, all fitting parameters were kept unchanged but different dilution rates were applied according to its feeding type. Since butyric acid concentrations in the effluent from both reactors were always very low, a comparison between simulation and experimental results of this intermediate was not performed.

Due to cyclic shock-loading received by the intermittently fed reactor, the rates of VFA production initially exceed their consumption rates causing VFA accumulation. This trend could be predicted well by the modified model. However, levels of acetic acids returning (within 10 hours) to the levels at the beginning of cycle (about 0.2 g COD/l) could not predicted by the model (Fig. 8.2). With propionic acid levels which
always returned to low levels at the beginning of each cycle could be followed by the model.

Fig. 8.2. Comparison between model predictions and experimental data under intermittent feeding at an OLR of 1.25 g COD/l/d. AA: acetic acid, PA: propionic acid.

Propionate Degradation by Continuously Fed Digester Biomass

Satisfactory predictions of intermediate degradation by the modified model were observed even though acetic acid degradation occurred much slower in model simulations (Fig. 8.3). The somewhat slower acetate degradation might be due to pH inhibition of the aceticlastic bacteria, which was not simulated in the model (Mosey, 1983; Costello et al., 1991 a and b; Siegrist et al., 1993; Hoh and Cord-Ruwisch, 1997 a) or faster recovery of this type of bacteria, which might have occurred in reality (Costello et al., 1991 b). The modified model precisely predicted propionic acid degradation of about 0.2 g COD/l/d for the biomass originating from the continuously fed digester (Fig. 8.3).
**Fig. 8.3.** Comparison between model predictions and experimental data during propionic acid addition under continuous feeding. AA: acetic acid, PA: propionic acid.

*Propionate Degradation by Intermittently Fed Digester Biomass*

The digester biomass accustomed to intermittent feeding degraded added propionate significantly faster than the biomass fed continuously. This indicates that the cyclic feeding operation had resulted in the build-up of increased concentrations of propionate degrading acetogens. According to this established fundamental understanding, temporary shock loads cause a shift in product formation by the fermentative bacteria away from acetate and hydrogen towards more reduced products such as propionate and butyrate. This shift is known to occur under overloading conditions that trigger elevated hydrogen partial pressures in the digester (McInerney and Bryant, 1980; Hoh and Cord-Ruwisch, 1997 b). In contrast, under consistent digester operation the low levels of hydrogen partial pressure are known to favor the production of acetate and hydrogen as the main fermentation products.
The modified model could only predict the trends of degradation of both acetic and propionic acids. Much faster propionic acid degradation as well as higher steady levels of acetic acid could not be predicted (Fig. 8.4).

**Fig. 8.4.** Comparison between model predictions and experimental data during propionic acid addition under intermittent feeding. AA: acetic acid, PA: propionic acid.

The main difference in behavior between the continuously and intermittently fed biomass could not be predicted by any of the two (original and modified) model versions tested. Experimental results showed that intermittently fed biomass accumulated less propionate, presumably because the biomass was exposed to significant propionate levels during every feeding cycle, enabling the development of more propionic acid degrading acetogens. The incapability of both models to predict this phenomenon of increased propionate degradation capacity by intermittently fed digester biomass (Fig. 8.4) is probably because the ADM1 model assumes a fixed stoichiometry of fermentative reactions. The model cannot predict changes in the proportions of products formed from glucose fermentation. Hence the shift towards producing more reduced fermentation products (VFA) under shock loading explained above is not part of the model. As a consequence the expected shifts in biomass
development caused by shifts in fermentation stoichiometry were not predicted by the model.

The above problem may be overcome by adjusting fractions of glucose fermented via each reaction (Table 8.1, glucose fermentation reactions carried out by acidogens). Reaction 1 fraction was decreased from 0.45 to 0.005, reaction 2 fraction was increased from 0.35 to 0.95, and reaction 3 fraction was decreased from 0.2 to 0.045. By modifying the fractions, propionate utilization occurred over one day (Fig. 8.5) compared to 2 days prior to modification (Fig. 8.4). It should be noted that propionate production from glucose occurs via reaction 2 and by increasing the fraction of this reaction for simulating the intermittent feeding runs propionate production was increased. It resulted in a build up of propionate utilizing organisms which in turn increased the utilization of propionate when it was spiked into the digester.

![Graphs showing model predictions and experimental data](image)

**Fig. 8.5** Comparison between model predictions (with modification of fractions of glucose fermentation reactions) and experimental data during propionic acid addition under intermittent feeding. PA addition of 0.4 g COD/l on day 10. AA: acetic acid, PA: propionic acid and exp: experiment.
With adjusting fractions of glucose mineralization the modified model could precisely predict propionic acid degradation on Day 157 (Fig. 8.6).

Fig. 8.6 Comparison between model predictions (with modification of fractions of glucose fermentation reactions) and experimental data during propionic acid addition under intermittent feeding. PA addition of 0.5 g COD/l on Day 157. AA: acetic acid, PA: propionic acid and exp: experiment.

8.7. Model Simulation

Simulation during Over Load

Simulation during over load was performed by loading both reactors with ten times normal loads. As expected, continuously fed reactor suffered less from possessing high VFAs with acetic acid dominating in the system (Fig. 8.7). Intermittently fed reactor showed cycles of VFA accumulation followed by VFA consumption as which occurred during normal loads. But with such high loading, much higher propionic acid dominated in the system. This was in agreement with experimental results (Section 6.4.2) showing propionic acid dominating in the system and higher the loading imposed the higher VFA contents and peaks. Even though propionic acid dominated
in the system since sufficient amount of propionate degraders existed in the system this acid was degraded.

The experimental results show that 4 times normal load imposed to both reactors resulted in accumulations of total VFAs above the defined safe VFA range (Table 6.4). The loads applied may have exceeded the degradation capacity of the biomass in the digesters. On the contrary, results of the model show the capability of both digesters to return to normal conditions even after being overloaded ten times normal load. This was attributed from no pH inhibition included in the model. Inclusion of pH inhibition to further modify the model would provide more accurate and better fit model results to experimental results.

![Comparison between model simulations of intermediate degradation occurred in continuously feeding reactor and in intermittently feeding reactors.](image)

**Fig. 8.7** Comparison between model simulations of intermediate degradation occurred in continuously feeding reactor and in intermittently feeding reactors.

**Simulation during Propionic Acid Pulse**

Ten times of 0.4 mM (the concentration firstly added to the digesters) propionic acid additions were simulated in both systems. The intermittently fed reactor could degrade
the propionic acid added at a rate of 2.7 g COD/l/d while continuously fed reactor degraded propionic acid with the rate of 1.5 g COD/l/d (Fig. 8.8). This was probably a result of high concentration of propionate utilising bacteria in the intermittently fed reactor, which was almost 3 times as that in the continuously feeding reactor (Table 8.10). Different feeding strategies resulted in different compositions of bacterial community in the system. This is in agreement with the experimental results suggesting that the intermittent feeding resulted in the development of more propionic acid degrading acetogens, gained by training the bacteria to receive shock loads such as what occurs during this type of feeding.

**Fig. 8.8** Comparison between model simulations of propionic acid degradation occurred in continuously feeding reactor and in intermittently feeding reactors.

From the simulation it shows that acidogenic bacteria concentration in the continuously fed digester is 3 times higher than the acidogen concentration in the intermittently fed digester (Table 8.10). This was because glucose fermentation in the continuously fed digester mainly occurred through the acetic acid with its fraction of 0.45 (second row of Table 8.7). The glucose utilization through acetic acid produces 4 mole ATP or equates to 40 g of biomass. Glucose fermentation in the intermittently fed digester, on the other hand, mainly occurred through propionic acid with its
fraction of 0.95. This second reaction only produces 4/3 mole ATP or equates to 13.33 g biomass. The glucose utilization through acetic acid in this digester was set with reaction fraction of only 0.005. This certainly resulted in fewer acidogens in the intermittently fed digester. As mentioned before, the adjustment of fractions in glucose utilization reactions was performed to simulate propionic acid utilization in the intermittently fed digester to match with the propionic acid utilization obtained from experiments.

**Table 8.10** Comparison between predicted bacterial concentrations in the continuously and intermittently fed reactors

<table>
<thead>
<tr>
<th>State variable</th>
<th>Continuously fed reactor</th>
<th>Intermittently fed reactor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidogenic bacteria</td>
<td>113</td>
<td>3.6</td>
</tr>
<tr>
<td>Aceticlastic methanogenic bacteria</td>
<td>2.1</td>
<td>2.3</td>
</tr>
<tr>
<td>Propionate utilizing bacteria</td>
<td>2.3</td>
<td>6.1</td>
</tr>
<tr>
<td>Butyrate utilizing bacteria</td>
<td>1.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Hydrogen utilizing bacteria</td>
<td>4.1</td>
<td>4.4</td>
</tr>
</tbody>
</table>

Note: all units are in g COD/l

Similarly, three and half times higher in concentration of butyrate utilizing bacteria in the continuously fed digester than that in the intermittently fed digester was due to higher fraction in the glucose mineralization to butyric acid in the continuously fed digester (second row of Table 8.7). The fraction of this third reaction in the glucose mineralization in the continuously fed digester was 0.30 as opposed to 0.045 in its counterpart.

Experimental results observing butyric acid degrading capacity in the intermittently fed digester higher than the butyric acid degrading capacity in the continuously fed
digester is contradiction with the model prediction (Table 8.10). It indicates that further modification to the model is required to enable all matched predictions including butyric acid degrading capacity.

### 8.8. Conclusions

- The original (ADM1) model could not satisfactorily predict VFA concentrations and methane production rates during steady state. This model resulted in 10% higher of COD output stream than the COD input stream. The reasons for these are in the assumption of the model.

- The modified model was capable of predicting the digester behaviour during both steady state and dynamic conditions using the continuous feeding strategy. However the model could not predict the apparent changes in biomass composition caused by long term intermittent feeding of laboratory reactors. The reasons for this are in the assumption of the model.

- The modified model required further modification in fractions of the stoichiometry of glucose fermentation during acidogenesis to enable it to predict carbohydrate degradation under intermittent feeding operations.

- Model simulation showed higher amount of propionic acid degrading bacteria in the intermittently fed digester than the propionic acid degraders in the continuously fed digester.
Chapter 9
General Discussion, Conclusions and Recommendations

9.1. General Discussion and Conclusions

A common design of anaerobic reactor for the treatment of liquid wastes is the continuous stirred tank reactors (CSTR). The main reasons for its popularity are its simplicity of operation and design and independent of sludge type used. An obvious drawback of the CSTR is the operation at long Hydraulic Retention Times (HRT) of the order of 16 to 30 days. This study was, therefore, performed to develop methods to overcome the CSTR’s drawback and at the same time employing its advantages.

The anaerobic moving bed reactor (AMBR) is an intensification of an existing CSTR by addition of granular rubber tyre which act as supports for biofilm attachment. With a density of 0.96 g/cm$^3$, the active movement of the supports could be maintained with a less energy input. Within only about 2 months of start-up, the digester achieved in excess of 80% soluble chemical oxygen demand (COD) reduction and total gas of about 1.2 l/l/d at an OLR of 2.2 g COD/l/d. The improvement of reactor performance was clearly shown by the capability of the system to be operated without any difficulties at HRT of 6 days at an OLR of 5.8 g COD/l/d or at HRT of 1 day at an OLR of 4 g COD/l/d. This was achieved by the formation of settleable sludge aggregates entrapped among the carriers rather than biofilm growing on the surface of the carriers. The amount of biomass attached on the surface of the carriers was less than 15% of the whole population. This unique type of biomass attachment played a
significant role in preventing severe wash out of the biomass aggregates. This also outweighed concern about low specific surface area of the carriers of 30 m$^2$/m$^3$ compared to commercial carriers.

During shock loads, the carriers were effective in retaining biomass aggregates. The presence of carriers enabled the microbial population to handle shock loads and to recover (to the normal conditions) upon the stoppage of disturbances. Upon imposing organic or hydraulic shock loads an imbalance between the volatile fatty acid producing reactions and methanogenic reactions occurred as expected. Acetic acid was the dominant VFA during most shock loads. Significant amounts of propionic acid accumulated at higher shock loads (10.8 g COD/l/d). Butyric acid did not accumulate during the shock load range imposed. During decreases in HRT below 1 day wash out of the acetogenic and methanogenic bacteria occurred. This was due to a hydrodynamic effect caused by higher flow rates rather than the response of microbial population to overloading caused by higher substrate concentrations (feed concentration was kept constant). At HRTs equal or less than 0.5 day severe wash out occurred indicated by propionic acid being the dominant volatile fatty acid in the effluent. Combination of shock load and hydraulic load could also be handled well by the digester. This showed the robustness of the proposed operational strategies.

From pulses of propionic acid (either in the form of pure acid or a main product of glycerol degradation) and butyric acid, it was shown that an intermittently feeding mode resulted in better microbial capacity in degrading these acids than the continuously feeding mode.
When the sequencing batch mode was applied to the AMBR (named as an anaerobic moving bed sequencing batch reactor or AMBSBR) the intermediate profiles changed from consistently low levels of VFA to cycles of VFA accumulation followed by VFA consumption. The sequencing batch mode could increase capability of the digester to handle higher organic loads. At 3.8 d HRT the AMBSBR could handle an OLR of 10.8 g COD/l/d as opposed to 7.4 g COD/l/d capable of being handled by the AMBR. At 2.5 d HRT the AMBSBR could handle OLR of 6.4 g COD/l/d while the AMBR could only be loaded at an OLR of 4.2 g COD/l/d. It was shown that this reactor type could be employed to handle concentrated feed flow rates at longer cycles or more dilute feed flow rates at frequent shorter cycles. The sequencing batch mode could thus be a choice to handle high fluctuations of wastewater. The AMBSBR could be classified as a high-rate anaerobic reactor as it achieved an SRT to HRT ratio of 15.

Low cost of treatment using AMBR include low capital costs of the reactor design and almost no cost of rubber tire carriers employed. This is another reason to apply AMBR besides the technical reasons mentioned above, i.e. the robustness of the proposed operational strategies. This highlights the uniqueness of this research which provides a way for reuse of solid waste to treat liquid waste.

To verify the outcome of proposed operational strategies a structured mathematical model was developed. The model developed from ADM1 (2002) consisted of liquid and gas phase equations, bacterial growth and physico-chemical equations that were incorporated into a function block of MATLAB 6.1 software. Several modifications were implemented to the model to obtain better predictions. Model validations were carried out using experimental data obtained from continuously and intermittently fed reactors. These two reactors were run in parallel. The modified model was capable of predicting all the trends of the operating variables from both feeding strategies.
None of the two model versions (ADM1 and modified models) was able to predict the phenomenon of increased propionate degradation capacity by intermittently fed digester biomass. The reason for this was the assumption of a fixed stoichiometry of fermentative reactions of glucose mineralisation. As a result it was not able to predict changes in the proportions of products formed from glucose fermentation. By modifying the fractions of glucose mineralisation, especially by increasing more than double of the fraction of reaction for propionate production (from glucose), a better fit between experimental results and the model could be obtained.

9.2. Recommendations for Future Studies

9.2.1. Experimental Work

The largest amount of bacteria was found in the middle of the AMBR in areas around 2 port heights, located at 6 and 11 cm from bottom. Such bacterial distribution might be attributed from well mixing in the middle part where two impellers were located (at 4 cm and 8 cm from bottom). The location of the impellers, speed of agitation as well as continuous or intermittent mixing would be interesting subjects in future studies.

The mode of retention of sludge aggregates was biomass entrapment among the carriers. Concern of low surface area of the carriers, therefore, became insignificant. The effect of particle size of carriers and amount of carriers added as well as the effect of types of feed wastewater on the treatment performance of AMBR would be other areas of further study.
In the operation of AMBSBR, fill time duration was set constant and feeding mode was set once per cycle within the designated fill duration. Published studies show contradicting results from different feeding strategy imposed to the system (Ratusznei et al., 2003; Rodrigues et al., 2003). The effect of feeding strategy on the performance of the AMBSBR could be conducted to verify those results as well as to obtain the optimum fill time duration. Keeping in mind that a slow fill rate (as in a fed batch strategy) will not shock load the digester sufficiently well to culture a large population of propionate utilizing acetogens.

Non uniform samples obtained from the port at the bottom of the digester experienced in this study might be attributed to non uniform mixing at the bottom part. There was a suspected dead zone resulting from the reactor geometry. To avoid this dead zone simple modification of bottom part of the reactor to a conical bottom shape may be done. With such design dead zone can be minimized (Wibisono, 2002).

A simple technique such as embedding sludge sample on agar medium could be performed to observe the aggregate size to compare with the size of granules or other types of anaerobic aggregates observed by other researchers (Tenorio, 1995).

To be able to operate the AMBR successfully, the following limitations have to be considered:

a. Care must be taken to prevent washout of the carriers while start-up of digester when bio-carrier-aggregates are being formed. This was taken care of in the laboratory scale digester by placing a screen under the effluent port.

b. During start-up, mixing speed had to be reduced gradually to prevent carry over of carriers due to turbulence as more gas is produced.
c. A second impeller had to be placed under the screen to avoid clogging of the screen by bacterial clumps attached on the inside surface of the screen.
d. After the start-up period was achieved, mixing speed at 180 rpm was necessary to obtain proper mass transfer and to avoid breaking of the aggregates which were formed.

9.2.2. Modelling Work

The capability of the model to predict increased propionate degradation capacity by intermittently fed digester biomass relied on the fractions of glucose mineralisation reactions. By adjusting these fractions the model could satisfactorily predict results from experiments. Glucose mineralisation reactions proposed by other researchers could also be interesting variables for further study.

Results of the model show the capability of both digesters (continuously and intermittently fed digesters) to return to normal conditions even after being overloaded ten times normal load. The experimental results show that four times normal load were the maximum loads could be handled by the digesters. The capability of the model to handle very high loads was attributed from no pH inhibition included in the model. The inclusion of pH in the model would result in better and more realistic predictions.

The capability of the modified model to predict all the effects of the operating variables from both feeding strategies could result in better predictions if the feed for both reactors was glucose. Quality of model predictions is affected by feed characteristics. Ramsay and Pullammanappallil (2005) found that model predictions of
brewery wastewater degradation was improved by incorporating pathways for ethanol breakdown into the model as well as including the concentrations of VFA in feed. This synthetic wastewater and other types of raw wastewater need to be properly characterised in terms of constituents and degradation rates of carbohydrates to properly verify models.
References


Appendices

Appendix 1 Derivation of the Irreversible Michaelis-Menten Equation

(Huang, 1993)

The following reaction is adopted:

\[
E + S \iff ES \iff E + P
\]

where

\begin{align*}
E &: \text{enzyme concentration} \\
S &: \text{substrate concentration} \\
P &: \text{product concentration} \\
ES &: \text{enzyme-substrate complex concentration}
\end{align*}

\[k_1, k_2, k_{-1}: \text{rate constants}\]

With the assumption:

(1) Total enzyme: \(E_T = ES + E\)

(2) Maximum forward reaction: \(\mu_{\text{max}} = k_2 \times E_T\)

(3) Michaelis constant for substrate: \(K_S = (k_{-1} + k_2)/k_1\)

(4) \(\frac{\text{d}(ES)}{\text{d}t} = k_1.E.S - (k_2 + k_{-1}).ES\)

(5) At equilibrium: \(\frac{\text{d}(ES)}{\text{d}t} = 0\),

\[k_1.S.E - (k_{-1} - k_2).ES = 0\]

Substitute assumption (1): \(E = E_T - ES\)

\[k_1.S.(E_T - ES) - (k_{-1} - k_2).ES = 0\]

\[k_1.S.E_T - k_1.S.ES - (k_{-1} - k_2).ES = 0\]

\[k_1.S.E_T = (k_1.S + k_{-1} + k_2).ES\]

\[
ES = \frac{k_1.S.E_T}{k_1.S + k_{-1} + k_2}
\]

Rate of substrate degradation: \(\frac{\text{d}S}{\text{d}t} = k_2.ES\)
Substitute assumption (5): $ES = \frac{k_1 S \cdot E_T}{k_1 S + k_{-1} + k_2}$

$$\frac{dS}{dt} = \frac{k_2 k_1 S \cdot E_T}{k_1 S + k_{-1} + k_2}$$

divide $k_j$ throughout: $$\frac{dS}{dt} = \frac{k_2 k_1 S \cdot E_T}{k_1 S / k_1 + (k_{-1} + k_2) k_1}$$

Substitute assumption (3): $K_S = \frac{(k_{-1} + k_2)}{k_j}$

$$\frac{dS}{dt} = \frac{k_2 S \cdot E_T}{S + K_S}$$

Substitute assumption (2): $\mu_{\text{max}} = k_2 \times E_T$

Therefore: $$\frac{dS}{dt} = \frac{\mu_{\text{max}} \cdot S}{S + K_S}$$

**Appendix 2** Non Degradable Compound of Molasses

Feed flow rate = 0.6 l/d
Feed concentration = 16 g COD/l/d
Gas production = 6 l/d with methane composition of 60%
Methane production = 0.6 x 6 l/d = 3.6 l/d
= 3.6 l/d x (1 mole/24.45 l ($^{\circ}$)) x 64 g COD/mole
= 9.4 g COD/d
For feed of 0.6 l/d, methane production = (9.4 g COD/d)/0.6 l
= 15.7 g COD/l

With COD effluent obtained of 2.4 g/l, molasses degradability can be calculated:
\[
Degradability = \frac{15.7 - 2.4}{15.7} \times 100\% = 85\%
\]

(*) At standard condition (0°C & 1 atm) 1 mole gas occupies 22.4 l.
Assuming gas methane was measured at 25°C & 1 atm & it behaves as an ideal gas, 1 mole methane equal to 24.5 l is calculated as follows:

Ideal Gas Law: \( n = \frac{PV}{RT} \). Since R & P are the same for both gases & \( n_1 = n_2 \), so \((V_1/T_1) = (V_2/T_2) \rightarrow V_1/298 \text{ K} = 22.4 \text{ l}/273 \text{ K} \rightarrow V_1 = 24.5 \text{ l}\)

**Appendix 3 Calculation for Theoretical MPR**

\( \text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 3 \text{ CH}_4 + 3 \text{ CO}_2 \)

1 mole glucose = 3 mole CH\(_4\)

since molecular weight of glucose= 180 g/mole & 1 mole gas at 25°C & 1 atm = 24.5 l(*)

180 g glucose = 3mole*24.5 l/mole

1 g glucose = \((3*24.5)/180 \text{ l CH}_4 \) ………………… (1)

\( \text{C}_6\text{H}_{12}\text{O}_6 + 6 \text{ O}_2 \rightarrow 6 \text{ CO}_2 + 6 \text{ H}_2\text{O} \)

To degrade 1 mole glucose 6 mole O\(_2\) needed

180 g glucose = 6*32 g COD

1 g glucose = 1.07 g COD ………………… (2)

\((1) \& (2) 1.07 \text{ g COD glucose} = (3*24.5)/180 \text{ l CH}_4 \)

Therefore 1 g COD glucose = 0.38 l CH\(_4\)

Expected CH\(_4\) production rate (l/l/d) = OLR (gCOD/l/d) \* 0.38 l/g COD
Appendix 4 VFA Profiles and Methane Accumulation after Propionic Acid Pulses

Fig. Appendix 4.1 VFA profiles and methane accumulation in the intermittently fed digester after 3.8 mM propionic acid pulse.

Fig. Appendix 4.2 VFA profiles and methane accumulation in the continuously fed digester after 3.8 mM propionic acid pulse.
Fig. Appendix 4.3 VFA profiles and methane accumulation in the intermittently fed digester after 5.5 mM propionic acid pulse.

Fig. Appendix 4.4 VFA profiles and methane accumulation in the continuously fed digester after 5.5 mM propionic acid pulse.

Appendix 5 Matlab Code

Function Block for the Intermittently Fed Reactor Model

function f=interSA4_36(t,S)
global VL pH eta1 eta2
%SGin=21.25, ADM1 kds, no +rds in products, Vg=0.976, all cat.&ana.stch yields, %fitting para (ksAA=0.8, mumaxAA=0.35, ksPA=0.1, mumaxPA=0.8) %eta1=0.45, eta2=0.35 %F=100ml/2min or D=36 (original model) to suit conti model

format long
ks=1.280;% gCOD/L mumax=8.78;% /day
ksAA=0.8;% gCOD/L mumaxAA=0.35;%0.45 /day
pHul(1)=5.5;% from lower side only
pHll(1)=4;% from lower side only
ksPA=0.1;%0.15 0.373 gCOD/L mumaxPA=0.8;%0.80 2.725; /day
pHul(2)=7;% from lower side only
pHll(2)=6;% from lower side only
ksBA=0.28;%0.280;% gCOD/L mumaxBA=2.70;% /day
pHul(3)=5.5;% from lower side only
pHll(3)=4;% from lower side only
ksH=0.55e-5;% molH2/L mumaxH=2.6;%4 % /day
kaCO=5.036343438023773e-007;% 310K mole/L
kaAA=1.621810097358930e-005;% 310K mole/L
kaPA=1.258925411794166e-005;% 310K mole/L
kaBA=1.318256738556407e-005;% 310K mole/L
kw=2.489296150410740e-014;% 310K
KHH=0.00078;% 310K mol/L-bar
KHM=0.0014;%310K mol/L-bar
KHCO=0.04;%310K mol/L-bar
klaH=720;%72 /day
klaM=10;% /day
klaIC=100;%1000 /day
kd1=0.02;% 0.07 /day
kd2=0.02; % 0.101 /day
kd3=0.01; % 0.20 /day
kd4=0.03; % 0.07 /day
kd5=0.009; % 0.07 /day
pHul(4)=6;
pHll(4)=4;
pHul(5)=6;
pHll(5)=5;

% Bacterial yields from catabolic and anabolic reactions
Yxgsg=((0.229+0.076*eta1-0.1951*eta2)*160)/((1.191+0.063*eta1-0.1625*eta2)*192); %gCODX/gCODS
Yxaasaa=0.04535; % gCODX/gCODS
Yxpaspa=0.09746; % gCODX/gCODS
Yxbasba=0.13247; % gCODX/gCODS
Yxh2sh2=2.55462; % gCODX/moleH2

% Other yields from catabolic and anabolic reactions
Yaag=((2*eta1+0.667*eta2)*64)/((1.191+0.063*eta1-0.1625*eta2)*192); %gCODS/gCODS
Ypag=((1.333*eta2)*112)/((1.191+0.063*eta1-0.1625*eta2)*192); %gCODS/gCODS
Ybag=((1-eta2-eta1)*160)/((1.191+0.063*eta1-0.1625*eta2)*192); %gCODS/gCODS
Yco2g=(2-1.333*eta2)/((1.191+0.063*eta1-0.1625*eta2)*192); %moleCO2/gCODS
Yh2g=(2*eta1-2*eta2+2)/((1.191+0.063*eta1-0.1625*eta2)*192); %moleH2/gCODS
Ych4aa=0.01492; %moleCH4/gCODS
Yco2aa=0.01492; %moleCO2/gCODS
Yaapa=0.51295; %gCODS/gCODS
Yco2pa=0.00771; %moleCO2/gCODS
Yh2pa=0.02435; %moleH2/gCODS
Yaaba=0.69402; %gCODS/gCODS
Yh2ba=0.01084; %moleH2/gCODS
Yco2ba=8.2795e-4; %moleCO2/gCODS
Ych4h2=0.21008; %moleCH4/moleH2
Yco2h2=0.29412; %molCO2/molH2
KH=8e-6;%gCOD/L 8e-6
SH=10^-pH;
%inhibition terms due to pH are calculated in the loop below
for X=1:5,
    if (pH < pHul(X))
        IpH(X)=exp(-3*(((pH-pHul(X))/(pHul(X)-pHll(X)))^2));
    else
        IpH(X)=1;
    end
end
IH=1/(1+((S(6)*16)/KH));%inhibition caused by the hydrogen
%yields (ADM1)
%Yaag=(2*eta1+0.67*eta2)*(64/192);
%Ypag=(1.33*eta2)*(112/192);
%Ybag=(1-eta2-eta1)*(160/192);
%Yco2g=(2-1.33*eta2)/192;
%Yh2g=(2*eta1-2*eta2+2)/192;
%individual reaction rates for all bacterial groups
r1=mumax*S(1)*S(7)*IpH(1)/(Yxgsg*(ks+S(1))); 
 r2=mumaxAA*S(2)*S(8)*IpH(2)/(Yxaasaa*(ksAA+S(2))); 
 r3=mumaxPA*S(3)*S(9)*IpH(3)*IH/(Yxpaspa*(ksPA+S(3)))); 
 r4=mumaxBA*S(4)*S(10)*IpH(4)*IH/(Yxbasba*(ksBA+S(4)))); 
 r5=mumaxH*S(6)*S(11)*IpH(5)/(Yxh2sh2*(ksH+S(6)));

%first order decay rate of all five bacterial groups
rd1=kd1*S(7);
rd2=kd2*S(8);
rd3=kd3*S(9);
rd4=kd4*S(10);
rd5=kd5*S(11);
%rate of generation for carbondioxide
\[ r_{CO2} = Y_{co2g} * r_1 + Y_{co2aa} * r_2 + Y_{co2pa} * r_3 - Y_{co2ba} * r_4 - Y_{co2h2} * r_5; \]

%rate of generation for methane
\[ r_{CH4} = Y_{ch4aa} * r_2 + Y_{ch4h2} * r_5; \]

%rate of generation for hydrogen
\[ r_{H2} = Y_{h2g} * r_1 + Y_{h2pa} * r_3 + Y_{h2ba} * r_4 - r_5; \]

\[ R = 0.08314; \text{L*bar/(mol*K)} \] Universal Gas Constant
\[ T = 310; \text{K Temperature} \]

%D = 0.05; %/day Dilution Rate
\[ \text{if } ((1+t-\text{ceil}(t)) >= 0.0013888888889) \text{ } \%(100 \text{ ml/2min}) \]
\[ D = 0; \]
\[ VL = 2; \]
else
\[ D = 36; \%(100 \text{ ml/2min}) \]
\[ VL = 1.9; \]
end

%transfer rate of dissolved gases to their respective gaseous phases
\[ \rho_{H} = k_{laH} * ((S(6)) - (S(12) * K_{HH})); \]

%dissolved carbondioxide
\[ \rho_{IC} = k_{laIC} * (((S(5) * SH) / (kaCO + SH)) - (S(14) * K_{HCO})); \]

%Water Vapour Pressure (ADM1)
\[ pg_W = 0.0313 * \exp(5290 * (1/298-1/T)); \]

%HeadSpace total pressure
\[ pg = 1.013; \]

%Gas Flow Rate
\[ F_g = R \times T \times VL \times (\rho_{H} + r_{CH4} + \rho_{IC}) / (pg - pg_W); \]
\[ SG_{In} = 21.25; \text{85\% degradability of 25 gCOD/L} \]
\[ f(1) = -r_1 + D * (SG_{In} - S(1)); \]
\[ f(2) = Y_{aag} * r_1 + Y_{apa} * r_3 + Y_{aba} * r_4 - r_2 - D \times S(2); \]
\[ f(3) = Y_{pag} * r_1 - r_3 - D \times S(3); \]
\[ f(4) = Y_{bag} * r_1 - r_4 - D \times S(4); \]
\[ f(5) = r_{CO2} - \rho_{IC} - D \times S(5); \]
\[ f(6) = r_{H2} - \rho_{H} - D \times S(6); \]
\[ f(7) = Y_{xgsg} * r_1 - r_{dl1} - D \times S(7); \]
\[ f(8) = Y_{x\text{asaa}} r_2 - rd_2 - D * S(8); \]
\[ f(9) = Y_{x\text{pspa}} r_3 - rd_3 - D * S(9); \]
\[ f(10) = Y_{x\text{basba}} r_4 - rd_4 - D * S(10); \]
\[ f(11) = Y_{xh2s} r_5 - rd_5 - D * S(11); \]
\[ V_g = 0.976; \text{%(1/0.615*0.6 L) 0.990 Litre} \]
\[ f(12) = (-S(12) * F_g / V_g) + (\rho_H * R * T * (V_L / V_g)); \]
\[ f(13) = (-S(13) * F_g / V_g) + (r_{CH4} * R * T * (V_L / V_g)); \]
\[ f(14) = (-S(14) * F_g / V_g) + (\rho_{IC} * R * T * (V_L / V_g)); \]
\[ f = f'; \]

**Driver Function for the Intermittently Fed Reactor Model**

```
global VL pH eta1 eta2
SGin=21.25, ADM1 kds, no +rds in products, Vg=0.976, all cat.&ana. stch yields,
%fitting para (ksAA=0.8, mumaxAA=0.35, ksPA=0.1, mumaxPA=0.8)
%eta1=0.45, eta2=0.35
%F=100ml/2min or D=36 (original model) to suit conti model
```

```
S0=[-0.00000000000000 0.04179648507547 0.0000000000000040
  0.00000001988098 0.11456946298255 0.0000000000000003 2.10015119181016
  0.37253165825940 0.40255624445671 0.24946457821929 0.78348649529909
  0.00000000008873 0.61210314074732 0.33867042967847 0.33867042967847];
VL=2;%litre
eta1=0.45;% 0.99  0.5
eta2=0.35;% 0.009  0.3
Ych4aa=0.01492; %moleCH4/gCODS
mumaxAA=0.35;% /day
Yxaasaa=0.04535; % gCODX/gCODS
ksAA=0.8;% gCOD/L
Ych4h2=0.21008; %moleCH4/moleH2
mumaxH=2.6;%4 %/day
Yxh2sh2=2.55462; % gCODX/moleH2
ksH=0.55e-5;% molH2/L
```
disp('Getting there....be patient !')
tt=cputime;

options=odeset('RelTol',2.2204e-14,'MaxStep',0.001)
pH=7.17;

[t,S]=ode45('modelADM1',[0 2000],S0,options);
[t,S]=ode15s('interSA4_36',[0 10],S0,options,pH);
elap = cputime - tt;

fprintf('execution time = %5.3f seconds 
',elap)

steps=length(t);

fprintf('no. of steps = %4.0f
',steps)

box =[t S];

save C:\Mat-lab\inter\interSA4_36.dat box -ascii

MPR=0;

for I=1:steps,

MPR(I)=((Ych4aa*((mumaxAA*box(I,3)*box(I,9)/(Yxaasaa*(ksAA+box(I,3))))))+((Ych4h2*((mumaxH*box(I,7)*box(I,12))/(Yxh2sh2*(ksH+box(I,7))))))*24.45;

end

%CODout=0;

%for A=1:steps,

%CODout(A)=((MPR(I)/24.45)*2*64)+(box(I,2)+box(I,3)+box(I,4)+box(I,5)+box(I,8)+box(I,9)+box(I,10)+box(I,11)+box(I,12))*0.1;

%end

%plot(t,S(:,1),t,S(:,2),t,S(:,3),t,S(:,4),t,S(:,5),t,S(:,6),t,S(:,7),t,S(:,8),t,S(:,9),t,S(:,10),t,S(:,11),t,S(:,12),t,S(:,13),t,S(:,14));

subplot (3,1,1),plot(t,S(:,2),'r',t,S(:,3),'b',t,S(:,4),'g');

xlabel('Time (Day)')

ylabel('S (gCOD/l)')

legend('SAA','SPA','SBA')

subplot (3,1,2),plot(t,S(:,7),'r',t,S(:,8),'b',t,S(:,9),'g',t,S(:,10),'y',t,S(:,11),'k');

%plot(t,S(:,1),t,S(:,2),t,S(:,3),t,S(:,4),t,S(:,5),t,S(:,6),t,S(:,7),t,S(:,8),t,S(:,9),t,S(:,10),t,S(:,11),t,S(:,12),t,S(:,13),t,S(:,14));

subplot (3,1,1),plot(t,S(:,2),'r',t,S(:,3),'b',t,S(:,4),'g');

xlabel('Time (Day)')

ylabel('S (gCOD/l)')

legend('SAA','SPA','SBA')

subplot (3,1,2),plot(t,S(:,7),'r',t,S(:,8),'b',t,S(:,9),'g',t,S(:,10),'y',t,S(:,11),'k');
xlabel('Time (Day)')
ylabel('X (gCOD/l)')
legend('XAci','XAce','XPro','XBut','XH2')

subplot (3,1,3),plot(t,MPR,'r');
xlabel('Time (Day)')
ylabel('rCH4 (l/l/d)')
legend('rCH4')

%subplot (4,1,4),plot(t,CODout,'b');
%xlabel('Time (Day)')
%ylabel('COD out (gCOD/d)')
%legend('CODout')

Function Block for the Continuously Fed Reactor Model

function f=contiSA4(t,S)
global VL pH eta1 eta2

%SGin=21.25, ADM1 kds, no +rds in products, Vg=0.976, all cat.&ana.stch yields,
%fitting para (ksAA=0.8, mumaxAA=0.35, ksPA=0.1, mumaxPA=0.8)
%eta1=0.45, eta2=0.35

format long

ks=1.280;% gCOD/L
mumax=8.78;% /day
ksAA=0.8;% gCOD/L
mumaxAA=0.35;%0.45 /day
pHul(1)=5.5;% from lower side only
pHll(1)=4;% from lower side only
ksPA=0.1;%0.15 0.373 gCOD/L
mumaxPA=0.8;%0.80 2.725; /day
pHul(2)=7;% from lower side only
pHll(2)=6;% from lower side only
ksBA=0.28;%0.280;% gCOD/L
mumaxBA=2.70;% /day
pHul(3)=5.5;% from lower side only
pHll(3)=4;% from lower side only
ksH=0.55e-5;% molH2/L
mumaxH=2.6;%4 % /day
kaCO=5.036343438023773e-007;% 310K mole/L
kaAA=1.621810097358930e-005;% 310K mole/L
kaPA=1.258925411794166e-005;% 310K mole/L
kaBA=1.31825673856407e-005;% 310K mole/L
kw=2.489296150410740e-014;% 310K
KHH=0.00078;% 310K mol/L-bar
KHM=0.0014;%310K mol/L-bar
KHCO=0.04;%310K mol/L-bar
klaH=720;%72 /day
klaM=10;% /day
klaIC=100;%1000 /day
kd1=0.02;% 0.07 /day
kd2=0.02;% 0.101 /day
kd3=0.01;% 0.20 /day
kd4=0.03;% 0.07 /day
kd5=0.009;% 0.07 /day
pHul(4)=6;
pHll(4)=4;
pHul(5)=6;
pHll(5)=5;

% Bacterial yields from catabolic and anabolic reactions
Yxgsg=((0.229+0.076*eta1-0.1951*eta2)*160)/((1.191+0.063*eta1-0.1625*eta2)*160); %gCODX/gCODS
Yxaasaa=0.04535; % gCODX/gCODS
Yxpaspa=0.09746; % gCODX/gCODS
Yxbasba=0.13247; % gCODX/gCODS
Yxh2sh2=2.55462; % gCODX/moleH2
% Other yields from catabolic and anabolic reactions
Yaag=((2*eta1+0.667*eta2)*64)/((1.191+0.063*eta1-0.1625*eta2)*192); % gCODS/gCODS
Ypag=((1.333*eta2)*112)/((1.191+0.063*eta1-0.1625*eta2)*192); % gCODS/gCODS
Ybag=((1-eta2-eta1)*160)/((1.191+0.063*eta1-0.1625*eta2)*192); % gCODS/gCODS
Yco2g=(2-1.333*eta2)/((1.191+0.063*eta1-0.1625*eta2)*192); % mole CO2/gCODS
Yh2g=(2*eta1-2*eta2+2)/((1.191+0.063*eta1-0.1625*eta2)*192); % mole H2/gCODS
Ych4aa=0.01492; % mole CH4/gCODS
Yco2aa=0.01492; % mole CO2/gCODS
Yaapa=0.51295; % gCODS/gCODS
Yco2pa=0.00771; % mole CO2/gCODS
Yh2pa=0.02435; % mole H2/gCODS
Yaaba=0.69402; % gCODS/gCODS
Yh2ba=0.01084; % mole H2/gCODS
Yco2ba=8.2795e-4; % mole CO2/mole H2
Ych4h2=0.21008; % mole CH4/mole H2
Yco2h2=0.29412; % mol CO2/mol H2
KH=8e-6; % gCOD/L
SH=10^(-pH);
% inhibition terms due to pH are calculated in the loop below for X=1:5,
if (pH < pHul(X))
    IpH(X)=exp(-3*(((pH-pHul(X))/(pHul(X)-pHll(X)))^2));
else
    IpH(X)=1;
end
end
IH=1/(1+((S(6)*16)/KH)); % inhibition caused by the hydrogen

% yields (ADM1)
% Yaag=(2*eta1+0.67*eta2)*(64/192);
%Ypag=(1.33*eta2)*(112/192);
%Ybag=(1-eta2-eta1)*(160/192);
%Yco2g=(2-1.33*eta2)/192;
%Yh2g=(2*eta1-2*eta2+2)/192;

%individual reaction rates for all bacterial groups
r1=mumax*S(1)*S(7)*IpH(1)/(Yxgsg*(ks+S(1)));
r2=mumaxAA*S(2)*S(8)*IpH(2)/(Yxaasaa*(ksAA+S(2)));
r3=mumaxPA*S(3)*S(9)*IpH(3)*IH/(Yxpaspa*(ksPA+S(3)));
r4=mumaxBA*S(4)*S(10)*IpH(4)*IH/(Yxbasba*(ksBA+S(4)));
r5=mumaxH*S(6)*S(11)*IpH(5)/(Yxh2sh2*(ksH+S(6)));

%first order decay rate of all five bacterial groups
rd1=kd1*S(7);
rd2=kd2*S(8);
rd3=kd3*S(9);
rd4=kd4*S(10);
rd5=kd5*S(11);

%rate of generation for carbon dioxide
rCO2=Yco2g*r1+Yco2aa*r2+Yco2pa*r3-Yco2ba*r4-Yco2h2*r5;
%rate of generation for methane
rCH4=Ych4aa*r2+Ych4h2*r5;
%rate of generation for hydrogen
rH2=Yh2g*r1+Yh2pa*r3+Yh2ba*r4-r5;
R=0.08314; %L*bar/(mol*K) Universal Gas Constant
T=310; %K Temperature
D=0.05; % /day Dilution Rate

%transfer rate of dissolved gases to their respective gaseous phases
rhoH=klaH*((S(6))-(S(12)*KHH));
%dissolved carbon dioxide
rhoIC=klaIC*(((S(5)*SH)/(kaCO+SH))-(S(14)*KCO));
%Water Vapour Pressure (ADM1)
pgW=0.0313*exp(5290*(1/298-1/T));
%HeadSpace total pressure
pg=1.013;
%Gas Flow Rate
Fg=R*T*VL*(rhoH+rCH4+rhoIC)/(pg-pgW);
SGin=21.25;%85% degradability of 25 gCOD/L

f(1)=-r1+D*(SGin-S(1));
f(2)=Yaag*r1+Yaapa*r3+Yaaba*r4-r2-D*S(2);
f(3)=Ypag*r1-r3-D*S(3);
f(4)=Ybag*r1-r4-D*S(4);
f(5)=rCO2-rhoIC-D*S(5);
f(6)=rH2-rhoH-D*S(6);
f(7)=Yxsgs*r1-rd1-D*S(7);
f(8)=Yxaasaa*r2-rd2-D*S(8);
f(9)=Yxaspa*r3-rd3-D*S(9);
f(10)=Yxasba*r4-rd4-D*S(10);
f(11)=Yxh2sh*r5-rd5-D*S(11);
Vg=0.976;%1/0.615*0.6 L 0.990 Litre
f(12)=(-S(12)*Fg/Vg)+(rhoH*R*T*(VL/Vg));
f(13)=(-S(13)*Fg/Vg)+(rCH4*R*T*(VL/Vg));
f(14)=(-S(14)*Fg/Vg)+(rhoIC*R*T*(VL/Vg));
%f(15)=((f(1)+f(2)+f(3)+f(4)+f(7)+f(8)+f(9)+f(10)+f(11));
f=f;

Driver Function for the Continuously Fed Reactor Model

global VL pH eta1 eta2
%SGin=21.25, ADM1 kds, no +rds in products, Vg=0.976, all cat.&ana.stch yields,
%fitting para (ksAA=0.8, mumaxAA=0.35, ksPA=0.1, mumaxPA=0.8)
%eta1=0.45, eta2=0.35

S0=[0.01028702640642 0.20000000000003 0.01039426523407 0.01081765195667 0.11265601877990 0.00000012770563 2.11982984490843]
VL=2;%litre
etta1=0.45;% 0.99  0.5
eta2=0.35;% 0.009  0.3
Ych4aa=0.01492; %moleCH4/gCODS
mumaxAA=0.35;% /day
Yxaasaa=0.04535; % gCODX/gCODS
ksAA=0.8;% gCOD/L
Ych4h2=0.21008; %moleCH4/moleH2
mumaxH=2.6;%4 % /day
Yxh2sh2=2.55462; % gCODX/moleH2
ksH=0.55e-5;% molH2/L

disp('Getting there....be patient !')
tt=cputime;
options=odeset('RelTol',2.2204e-14,'MaxStep',0.05)
pH=7.17;
%[t,S]=ode45('modelADM1',[0 2000],S0,options);
[t,S]=ode15s('contiSA4',[0 10],S0,options,pH);
elap = cputime - tt;
fprintf('execution time = %5.3f seconds 
',elap)
steps=length(t);
fprintf('no. of steps = %4.0f
',steps)
box =[t S];
save C:\Mat-lab\conti\contiSA4.dat box -ascii
MPR=0;
for I=1:steps,

MPR(I)=((Ych4aa*((mumaxAA*box(I,3)*box(I,9)/(Yxaasaa*(ksAA+box(I,3))))))+
(Ych4h2*((mumaxH*box(I,7)*box(I,12))/(Yxh2sh2*(ksH+box(I,7))))))*24.45;


end

%CODout=0;
%for A=1:steps,
%
CODout(A)=((MPR(I)/24.45)*2*64)+(box(I,2)+box(I,3)+box(I,4)+box(I,5)+box(I,8)
+box(I,9)+box(I,10)+box(I,11)+box(I,12))*0.1;
%end

%plot(t,S(:,1),t,S(:,2),t,S(:,3),t,S(:,4),t,S(:,5),t,S(:,6),t,S(:,7),t,S(:,8),t,S(:,9),t,S(:,10),t,
S(:,11),t,S(:,12),t,S(:,13),t,S(:,14));
subplot (3,1,1),plot(t,S(:,2),'r',t,S(:,3),'b',t,S(:,3),'g');
xlabel('Time (Day)')
ylabel('S (gCOD/l)')
legend('SAA','SPA','SBA')
subplot (3,1,2),plot(t,S(:,7),'r',t,S(:,8),'b',t,S(:,9),'g',t,S(:,10),'y',t,S(:,11),'k');
xlabel('Time (Day)')
ylabel('X (gCOD/l)')
legend('XAci','XAce','XPro','XBut','XH2')
subplot (3,1,3),plot(t,MPR,'r');
xlabel('Time (Day)')
ylabel('rCH4 (l/l/d)')
legend('rCH4')
%subplot (4,1,4),plot(t,CODout,'b');
%xxlabel('Time (Day)')
%ylabel('COD 0ut (gCOD/d)')
%legend('CODout')