THE HONG KONG POLYTECHNIC UNIVERSITY

Department of Civil and Structural Engineering

Roles of Immobilized Biomass

In

An Anaerobic Hybrid Reactor

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A dissertation submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy

2003

DECLARATION

I hereby declare that this dissertation, entitle "Roles of Immobilized Biomass in an Anaerobic Hybrid Reactor", is my own work and that, to the best of my knowledge and belief, it reproduces no material previously published or written nor material which has been accepted for the award of any other degree or diploma, except where due acknowledgement has been made in the text.

(Cheung, Wing Leung Montgomery)

DEDICATION

In memory of

My Father and Mother

"Those who are wise will shine like the brightness of the heavens, and those who lead many to righteousness, like the stars for ever and ever. (Daniel 12:3)"

ABSTRACT

An anaerobic hybrid reactor (AHR), comprising an anaerobic filter (AF) upper section and an up-flow anaerobic sludge blanket (UASB) lower section, was used to treat synthetic wastewater under different loading conditions. The AF was then separately investigated for its role in contributing to the performance and process stability of the AHR. The immobilized biomass and population distribution of the different microbial species in the AF were also studied under steady operating and different shock loading conditions.

With the incorporation of the AF section in the upper portion of the system, the AHR presented a better capability against the environments of the shock loadings. The AF section of the AHR played a significant role in the removal of COD; particularly when the AHR system was operated under adverse conditions, such as shock loadings. The AF section of the AHR performed as a biofilter to maintain the temporarily inhibited microorganisms on the surfaces of the packing medium and thus prevented a wash out of the inhibited microorganisms under shock loadings. This rendered the AHR more tolerant to shock loadings. During shock loadings at different HRTs, the average COD removed by the AF section was about 36%, ranging from 49 to 21%, whereas the UASB section only accounted for about 10% of the COD removal, ranging from 10.4 to 8.2%. Even under critical shock loading at an HRT 0.5 day, the average COD removed by the AF section was maintained at about 21%; whereas the COD removed by the UASB section declined to about 9%. In response to the shock loadings, the AHR showed a temporary drop in the efficiency of COD removal, but resumed to steady state operations after the adverse situation ceased. As a general trend, the COD

removal rate decreased as the HRTs decreased from 5 to 0.5 days. The efficiency of removal of COD of the AF section was much higher than that of the UASB section during the transient state of shock loadings. The COD removal rate of the AHR was maintained at between 1.5 and 0.4 g COD/L-d at HRTs of 2.5 to 0.5 days before the failure of the AHR at an HRT of 0.25 day.

The AF section was then isolated to study its process stability and responses to hydraulic and organic shock loadings. For the hydraulic shock loading experiments, the AF was started-up with synthetic wastewater of 3000 mgCOD/L at 5.0 days of HRT, achieving 98.1% COD removal efficiency. Under 2, 4 and 5 times hydraulic shock loadings, the efficiency of COD removal was temporary reduced, ranging from 92.7 to 89.7%, the pH of the treated effluent and biogas production were also affected. The average pH value dropped from 7.3 to 6.0. The specific methane yield, with an average methane concentration around 69% (v/v), was generated at a rate of between 0.28 to 0.32 L/g COD. The AF recovered from a state of temporary inhibition resulting from the shock loadings, and resumed normal operation within 8 days. Under 10 times hydraulic shock loading, the treatment performance deteriorated drastically. Volatile fatty acids (VFAs) accumulated in the AF liquor, resulting in reactor souring and failure. When the HRT of the AHR was restored to 5 days, the AF recovered within a few days. The ability of the AF to recover from critical hydraulic shock loadings and system failure was attributed to the immobilized-biofilm design, which enabled the temporarily inhibited biomass to be retained in the AF and the temporarily inhibited biomass resumed its activity when favourable conditions were restored.

For the organic shock loading experiments, the operation of the AF was steady after 60 days of operation at an HRT of 1.25 days. The COD removal efficiency was 98.2%. The biogas production rate was maintained within the range of 1.9 to 2.0 L/day. Methane concentration was about 70% (v/v). Effluent pH ranged from 6.4 to 6.5. When the organic load of the AF was progressively increased to 115.2 gCOD/L-d, the total COD removal efficiency of the AF was still be maintained above 90%. The AF recovered from various shock loadings equivalent to a sixteen times increase in organic load, which showed that the AF possessed excellent anti-shock capacity. The specific methane yield, with an average methane concentration of 68% (v/v), was at a rate of between 0.22 to 0.12 L/g COD. The methane gas produced from each cubic meter of the trade effluent was equivalent to $2.2 * 10^5$ kJ of energy generation. This heat value was in excess of the amount required for the pre-heating of the effluent to 30 °C. Combining the mechanisms of both organic adsorption and biodegradation rendered the AF more stable under various shock loading conditions.

The anaerobic pathway of the organic degradation, converting acetate to carbon dioxide and methane by *Methanosaeta and Methanosarcina* spps., was prone to inhibition under the critical hydraulic shock environment. The results suggest that the hydraulic shocking loading distributed, to some extent, the physical contact between the syntrophs and methanogen, leading to an inhibition of the methanogenic bacteria. This inhibition leads to an imbalance of the microbial ecosystem, including an accumulation of VFAs and a decline in methane concentrations in the biogas. The anaerobic pathways of the conversion of the VFAs to acetate, hydrogen and carbon dioxide by (i) *Syntrophomonas* spp., the conversion of the acetate to methane and carbon dioxide by (ii) *Methanosaeta* spp., the conversion of the butyrate to methane

and water and the conversion of the carbon dioxide and reduction of the hydrogen mediated by (iii) *Methanococcus* spp., were prone to inhibition under the critical organic shock loading environment. The degree of inhibition of these three groups of bacteria was found to be different (e.g. iii > ii > i), as evidenced by the concentration of carbon dioxide that increased incrementally as the organic loading increased. As a result, the supply of organic acids, mainly acetate and butyrate, exceeded the assimilative capacity of the methane-forming bacteria, *Methanosaeta* spp., leading to an accumulation of VFAs. The content of the methane in the biogas decreased and the concentration of carbon dioxide increased. Generally, the failure of the AF under various shock loadings was attributed to process souring, as indicated by the low methane yield and an accumulation of VFAs.

The results suggest that the AF contributed a stable environment for the immobilized biomass in the AHR, and helped to improve the performance and process stability of the AHR. Under shock loading conditions, the AF also rendered the AHR more able to recover from unstable operating conditions or even from process failure.

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1. Introduction

1.1 Background

The anaerobic treatment process has long been recognized as one of the most effective methods for treating organic waste streams, including domestic and industrial effluents. The organic waste could be derived from various sources including agricultural (Ahring *et al.*, 1992; Souza *et al.*, 1992), industrial (Macarie *et al.*, 1992; Visser *et al.*, 1992; Borja *et al.*, 2001), and domestic effluents (Draaijer *et al.*, 1992; Vieira and Garacia, 1992; Schellinkhout and Collazos, 1992). The potential of anaerobic process for converting a wide range of industrial wastes into energy-rich biogas has also been appreciated (Stafford *et al.*, 1980a; Tentscher, 1988; Tekin and Dalgic, 2000; Hill and Bolte, 2000; Nandy & Kaul, 2001). Meanwhile, anaerobic treatment processes offer a number of advantages, such as (i) low operating costs, (ii) low bacterial growth yields, thus reducing the frequency and the associated costs for sludge disposal, and (iii) less energy consumption via the conversion of the biogas (e.g., methane) to produce a supply of electricity for the system (Lettinga, 1996).

Despite the advantages and widespread applications of the anaerobic process in waste treatment, difficulties were inevitably encountered in treating certain persistent industrial wastes (Schoberth, 1978; Speece, 1987; Hobson, 1988; Holliger *et al.*, 1988). The slow growth rate of methanogenic bacteria in an anaerobic system has been an inherent disadvantage of this process, resulting in a washout of the content of the reactor, particularly when the system was operated under shock load conditions. This led to an imbalance between production and consumption of the intermediates,

namely VFAs that would ultimately turn the reactors "sour" (Dague *et al.*, 1970; Kennedy *et al.,* 1985; Chartrain and Zeikus 1986; Kissalita *et al.* 1987).

Different types of anaerobic reactors have been developed and applied in the operation of the laboratory-scale, pilot plant-scale, and full-scale reactor studies in the past 20 years. These include, the upflow anaerobic sludge blanket (UASB) reactor (Lettinga, *et al.*, 1980), expanded granular sludge bed (EGSB) (van der Last, 1991; Zoutberg and Frankin, 1996), anaerobic rotating disc contractor (ARDC) (Stronach *et al.*, 1986), anaerobic filter (AF) (Young, 1968; Young *et al.*, 1969; Chua *et al.*, 1991), and anaerobic hybrid reactor (AHR) (Sun and Zhou, 1988; Lo *et al.*, 1994). The conventional anaerobic reactor usually required a relatively long HRT to stabilize the organic matter and to prevent biomass washout. The recent development of anaerobic treatment technology, particularly the anaerobic fixed-film technology, has rendered the process as one of the most feasible and promising options for on-site pre-treatment of trade effluents, such as food processing, or restaurant waste effluents. (Cho *et al.*, 1995; Chua *et al.*, 1992, 1995a,b; Perle *et al.*, 1995; Walsh *et al.*, 1995; Oliva *et al.*, 1995). The high-rate of anaerobic treatment seems to be a viable alternative for the treatment of both industrial and municipal waste effluents.

In 1968, Young developed the first AF reactor (Young, J.C., 1968; Young *et al.*, 1969) for the treatment of diluted organic wastewater. The upflow anaerobic filter is one of the earlier designs based on a relatively simple technology, and its characteristics are well defined (Henze and Harremoes, 1983). By means of immobilization, bacterial biomass was attached to the packed bed medium, which thus

prevents washout of biomass from the reactor under short hydraulic retention times (HRTs). It was reported that AF reactors were very tolerant to organic shock loadings, pH fluctuation, and toxicities (Hovious *et al.*, 1973; Jennet *et al.*, 1975; Young *et al.*, 1989). However, three typical problems were still commonly encountered by the AF reactors, including dead zone, short-circuits and low specific activity of the biomass presented on the bottom of the reactor. These latter two negative effects were probably due to the presence of the packing medium, which might be an obstacle to horizontal mixing and thus trap large amounts of suspended solids (SS) in the lower portion of the reactor where mixing, which resulted from gas evolution, was lowest (Tilche *et al.*, 1991).

The UASB reactor was first developed by Lettinga in the 1980s (Lettinga *et al.*, 1980; Lettinga, 1983) and it is by far the most widely used high-rate anaerobic reactor in the world, particularly for industrial wastewater and sewage treatment. Amongst these reactors, the influent of the waste stream flows upward through a sludge bed and an expanded sludge blanket. Despite the improved performance in wastewater treatment, there were limitations inherent in the design of the UASB, for example, a few months was required to start-up a UASB and to establish a stable sludge blanket and sludge bed (Christensen *et al.*, 1984). Additionally, it was reported that the sludge blanket might be disrupted if the up-flow velocity exceeded the settling velocity of the bacterial granules, or if biogas production was too vigorous. Consequently, the UASB reactors are usually susceptible to a loss of portions of the sludge bed during a hydraulic surge or toxic upset (Fannin *et al.*, 1987). Furthermore, it was reported that the UASB reactors were not suitable for treating particulate wastes, since particles

appear to interfere with flocculation and might also be accumulated in the bed and thus reduce the effective volume of the UASB.

In the late 1980s, an improved type of the conventional UASB, namely AHR, was developed by Sun and Zhou (Sun and Zhou, 1988; Lo *et al.*, 1994; Fang and Chui, 1994). It comprised a conventional UASB with an inserted AF section in a portion of the reactor. The AHR, at a lab-scale, has been applied for treating a variety of waste streams, including brewery wastewater (Sun and Zhou, 1988), fibrous wastewater (Fernandz *et al.*, 1995), diary effluent (Strydom *et al.*, 1995), starch particulates (Fang and Kwong, 1994), and pharmaceutical wastewater (Henry *et al.*, 1996), with treatment performances comparable to that of the UASB. In addition, the capability and stability of the reactors against adverse conditions, such as shock loadings, have also been improved.

Shock loading, due to a lack of balancing and equalizing facilities, is one of the common problems encountered in the context of congested urbanized areas, such as Hong Kong. It often results in a failure of the conventional treatment system. Anaerobic processes have been reported as being sensitive to shock loadings, such as hydraulic-, and organic-shock loadings. It has usually resulted in process souring and failure (Chua *et al.*, 1995c, Chua *et al.*, 1997). Massive sloughing of biofilm from the support medium and washout of the biomass from the reactor are the common phenomena under unfavorable conditions, causing organic inhibition to the anaerobic microbial or excessive hydraulic shear to the biofilm. Hydraulic shock loads, resulting in reduction of COD loads, are the common problems to be encountered in wastewater

treatment particularly when treating food-processing waste effluent, cleaning up equipment, and carrying out certain fermentation processes.

While the benefits of the AHR are obvious in a number of applications, the function and role of the inserted AF section still remains a controversy. Fang and Chui (1994) carried out a comparative study on AHR and UASB, and concluded that there was no obvious difference in the treatment performance between the AHR and USAB. However, Chua and his co-worker operated a pilot-scale AHR system for treating livestock wastewater (Chua *et al.*, 1999), and found that the AHR system was stable within 30 days after start-up of the system. The start up time required for AHR was much less than that of a conventional UASB reactor (e.g. 90 days).

This study was specifically devoted to delineate the function of the AF section in the AHR as well as the prominent role of an individual AF and its behavior against shock load conditions, namely hydraulic and organic shock loadings.

1.2 Objectives of the Study

The objectives of this study are as follows:

(a) To delineate the significant role of the AF section in the AHR when treating synthetic wastewater under shock loadings.

(b) To carry out an in-depth investigation on the stability of an AF when treating wastewater under critical hydraulic shock loadings. Process failure and the recovery of the system were also studied.

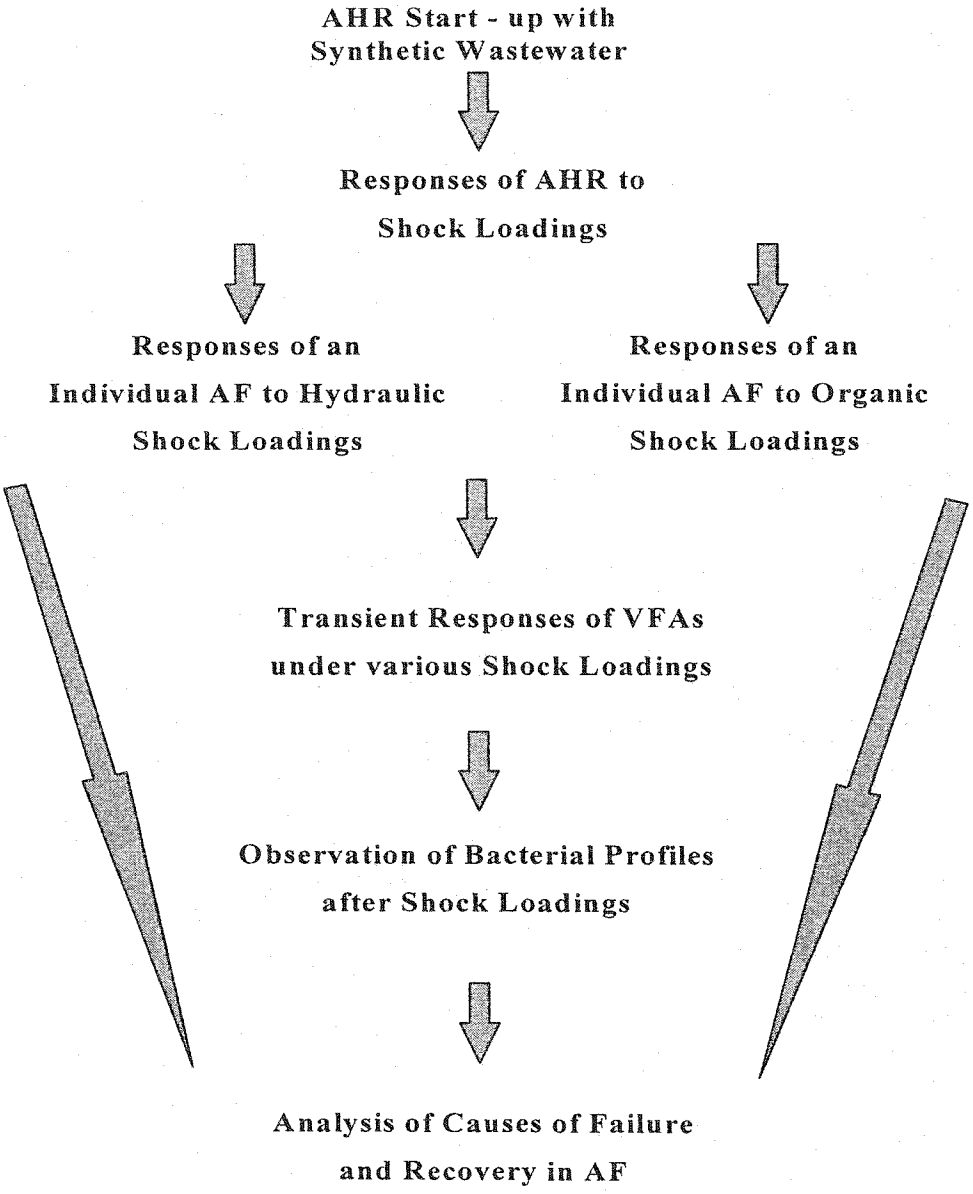
(c) To investigate the stability of the AF when treating synthetic wastewater under critical organic shock loadings. Process failure and the recovery of the system were also investigated.

(d) To study the transient responses of VFAs to hydraulic and organic shock loadings.

(e) To observe the changes in the composition and distribution of the bacterial population (if any) in the AF after the shock loadings.

1.3 Outline of the Study

The outline of this study is presented in the flow diagram as follows:



2. Literature Review

2.1 Development of Anaerobic Treatment Processes – An Overview

Anaerobic digestion is one of the naturally occurring processes involving decomposition and decay, in which complex organic matter is broken down into its simple chemical constituents. The mechanism of the anaerobic degradation of organic matter in ecological systems, e.g., rivers, lake sediments, and fresh water sediments, had been recognized for a long period of time (Balch *et al.*, 1979; Medrack *et al.*, 1987; Lay *et al.*, 1996; Tabassum and Rajoka, 2000). In nature, this type of degradation is typically associated with wet, warm and dark environments as characterized by the ooze of eutrophic lakes or the digestive tract of ruminant herbivores. Lay *et al.* (1996) estimated that the average experimental methane release rate from lake sediments was 19.9 mg $CH_4m^{-2}h^{-1}$ under batch culture conditions. Since the 19^{th} century, commercial batch and fed-batch anaerobic processes have been developed for and applied to the treatment of domestic and agricultural wastes (Buswell, 1958; Keenan *et al.*, 1977; Hobson *et al.*, 1977; Nyns *et al.*, 1979; McCarty 1981; Quano, 1981; Loehr, 1984; Wiycik *et al.*, 1980; Sun *et al.*, 1988; Zhao *et al.*, 1988; Broughton *et al.*, 1998; Pozo and Diez V., 2000).

Large-scale anaerobic digesters were extensively used not only for wastewater treatment, but also for stabilizing domestic sludge (McCarty, 1964; Young *et al.*, 1969; Hobson *et al.*, 1974). The conventional digesters were usually operated in a continuous mode in the form of a CSTR mode, because waste was produced continuously and there was a steady demand for biogas (Morris, 1980; Lehmann *et al.*, 1981; Tapp, 1981).

The conventional anaerobic reactors usually required long HRTs to stabilize the organic wastes being treated and to prevent washout of the biomass, resulting from the slow growth rate of methanogenic bacteria in the system (Henze and Harrenmoes, 1983; Chua *et al.*, 1994). Consequently, anaerobic treatments had been confined to treat the high-strength organic wastes produced from industrial or agricultural sources at low hydraulic loading rates. For instance, anaerobic processes were only used to treat the thickened sludge from domestic wastes while the liquid fraction of the waste, with lower COD content and larger volume, undergoes aerobic treatment. It would be economically unattractive if wastewater was treated anaerobically because a large reactor volume would be required (Young *et al.*, 1969).

Recently, the anaerobic treatment processes have been applied to the treatment of a variety of effluents such as municipal sewage (van Haandel and Lettinga, 1994; Lettinga, 1996; Kalogo *et al.*, 2001), food processing wastewater (Lettinga, 1994; Frankin *et al.*, 1992; Letttinga and van Haandel, 1993; Chua, & Cheng, 1996), aromatic-bearing wastewater (Borghans and van Driel, 1988; Macarie *et al.*, 1992), chlorinated hydrocarbons (Prakash and Gupta, 2000), dairy waste (Berg and Kennedy, 1983), slaughterhouse wastewater (Borja *et al.*, 1993; Pozo and Beltran, 2000; Pozo *et al.*, 2002), textile finishing wastewater (Yoo *et al.*, 2001), and effluent produced from the forestry industry (Lettinga *et al.*, 1991).

The success of the anaerobic treatment is mainly attributed to the high retention of active biomass that was entrapped on an appropriate packed-bed medium contained in high-rate reactors (e.g., UASB, and fixed-film anaerobic reactors). It allows anaerobic biomass to be more tolerant to toxic compounds present in the system or even adapt to

inhibitory substances during the treatment process (Blum, *et al.*, 1986; Dwyer *et al.*, 1986; Fedorak and Hrudey, 1986).

Anaerobic treatment processes have also been used for treating a wide range of waste streams, varying from very high to very low strength and relatively very hot (50 - 70 ^{o}C) to cold conditions (e.g. even < 10 ^{o}C). Recent developments in high rate anaerobic reactor technology, have demonstrated that an anaerobic process is feasible for treating cold or even very dilute wastewater (e.g., sewage) at a loading rate exceeding 10 kg/m^3 at a temperature even lower than 10 ^{o}C, when the hydraulic retention time was reduced to less than 1.5 hours (Lettinga 1996). Wang (1994) demonstrated that domestic sewage could be treated by a two-stage USAB system with an HRT of 3 hours for the first stage. The results of his study also showed that the first UASB could be considered as an improved primary settler. In addition to the high removal efficiency for suspended solids by filtration, some hydrolysis, acidification, and conversion to biogas also occurred in the first UASB reactor. Wastewater containing refractory compounds or even quite toxic substances could still be treated satisfactorily by an anaerobic treatment (Stafford *et al.*, 1980a; Henze *et al.*, 1983; Speece, 1983; Hao *et al.*, 1990).

Despite the drawback of a slow start-up, there are still a number of benefits of the anaerobic process as shown in Table 1 (Lettinga, 1996). During the early twentieth century, the potential of the anaerobic process for converting a variety of industrial wastes into energy-rich biogas was appreciated. Inevitably, difficulties were encountered in the treatment of certain persistent and recalcitrant, industrial wastes (Schoberth, 1978; Speece, 1987; Hobson, 1988; Holliger *et al.*, 1988). Persistent

wastes were only degradable under specific operational conditions, while recalcitrant wastes were inherently resistant to degradation (Brown *et al.*, 1987). For instance, food processing wastes, with high cellulose content, paper-mill wastes, distillery wastes (e.g. molasses) with high COD and high hydrocarbon content, and oily discharges from the petrochemical industries, are known to be persistent (Mudrack *et al.*, 1987; Hawkes *et al.*, 1987). Xenobiotic (or synthetic) compounds in chemical and pharmaceutical wastewaters might also be recalcitrant. These compounds were only present in the environment for a comparatively short period of time. Bacteria might not have evolved with the specific enzymatic mechanisms for their degradation. Certain xenobiotic compounds might inhibit bacterial activity, while degradation of others might produce inhibitors (Goodwin *et al.*, 1990). These persistent and recalcitrant wastes were often pre-treated by specially designed in-house treatment plants which were seeded with bacterial cultures acclimatized to the specific waste by enrichment techniques.

Research and development efforts have been devoted to retaining a high concentration of useful biomass in the reactor so as to achieve rapid and effective treatment. To this end, considerable technological developments in microbial floe formation and in microbial adhesion onto the pack-bed medium, which retains the biomass in the reactor, have been made. The innovations in the contact process, including the up-flow anaerobic sludge blanket reactor (UASB), anaerobic rotating disc contactor (ARDC), anaerobic fluidized-bed reactor (AFBR), anaerobic biofilter, anaerobic sludge bed reactor (ASB), fluidized bed (FB), egg-shaped anaerobic digester, expanded granular sludge bed reactor (EGSB), and horizontal-flow anaerobic immobilized sludge (HAIS) reactor have resulted in higher concentrations of biomass

being retained in the reactor. Some of the configurations of these reactors are illustrated in Figure 2-1 (Wheately *et al.*, 1997).

Despite anaerobic treatment processes, involving conversion of organic materials, various oxygen-demanding mineralized matter and reduced compounds (e.g. ammonium, phosphate and sulfide), still remained at concentrations usually higher than what are acceptable for discharges into receiving water. Post-treatments (e.g., aerobic or physicochemical processes) appear to be mandatory after the anaerobic treatment. They are usually used as an additional polishing stage to further reduce the organic content in the effluent of the anaerobic process in order to meet the effluent discharge standard for discharging into watercourses.

2.2 Comparisons of the Performances of Anaerobic Reactors

Figure 2-1 illustrates some of the innovative anaerobic reactors, based on different means of retaining the active biomass in the system, which are improvements on the conventional reactor. Other anaerobic reactors, such as the baffle-flow reactor (or plug-flow reactor), two-phase reactor, up-flow anaerobic sludge blanket reactor with biomass settler, expanded-bed reactor, and fluidized-bed reactor with culture recycling, are merely modifications of these reactors and have been described elsewhere (Hammer *et al.*, 1966; Borchardt, 1970; Pohland *et al.*, 1971; Massey and Pohland, 1978; Chandler *et al.*, 1983; Ng *et al.*, 1985; Schwitzguebel *et al.*, 1986; Ng and Chin, 1986; Tentscher,1987;1988; Gijzen *et al.*, 1988a;1988b; Schwitzguebel *et al.*, 1988; Kim and Speece, 2002a and 2002b).

Table 2.1 Beneficial Use of Anaerobic Treatment (after Lettinga 1996)

- Anaerobic treatment can be achieved at comparatively low cost. (e.g. installation and maintenance costs are relatively low)
- Less energy consumption, by recovering and using the biogas produced for the production of the electricity used in the system.
- Volume of excess sludge produced is generally significantly lower as compared to aerobic systems (e.g., both in kg sludge DS/kg $COD_{removed}$ and in m^3/kg sludge DS.)
- The excess sludge produced is usually well stabilized; it could be employed for soil conditioning.
- Anaerobic microorganisms can be preserved for a long period of time (e.g., exceeding one year) without any serious deterioration of their microbial activity.
- Very high space loading rates frequently can be applied in modern anaerobic wastewater systems.
- Anaerobic treatment can be (in principle) combined with post-treatment systems by which useful products like sulfur and ammonia can be recovered.

2.2.1 Contact Process

The contact process is the earliest improved type of conventional reactor (Obayashi *et al.*, 1985). The principle of the operation is similar to that of the activated sludge process. The contact process is a two-stage process. The first stage is similar to a conventional reactor, while the second stage is a liquid-biomass separator. A portion of the settled and thickened biomass from the second stage is recycled back to the first stage, thus improving bacterial retention without lengthening the HRT. Well-designed contact processes have been reported to have more than 10 days biomass retention time while operating at HRTs below 6 days, and the can maintain biomass concentrations of between 5 and 10 g VSS/L (Anderson *et al.*, 1977; Stronach *et al.*, 1986). A comparison of the performance of the anaerobic reactors is shown in Table 2.2, which shows that the loading rates and organic removal achievable by the contact process are better than the conventional reactors. The examples of contact stirred tank reactors (CSTR) treating food wastes are highlighted in Table 2.3. A comparison of loading rates used in anaerobic filters, UASB, and expanded beds reactors are shown in Table 2.4. Lesile and Grady (1998) compiled a table of comparisons, in terms of the benefits and drawbacks, for different options of organic stabilization using anaerobic treatment (Table 2.5).

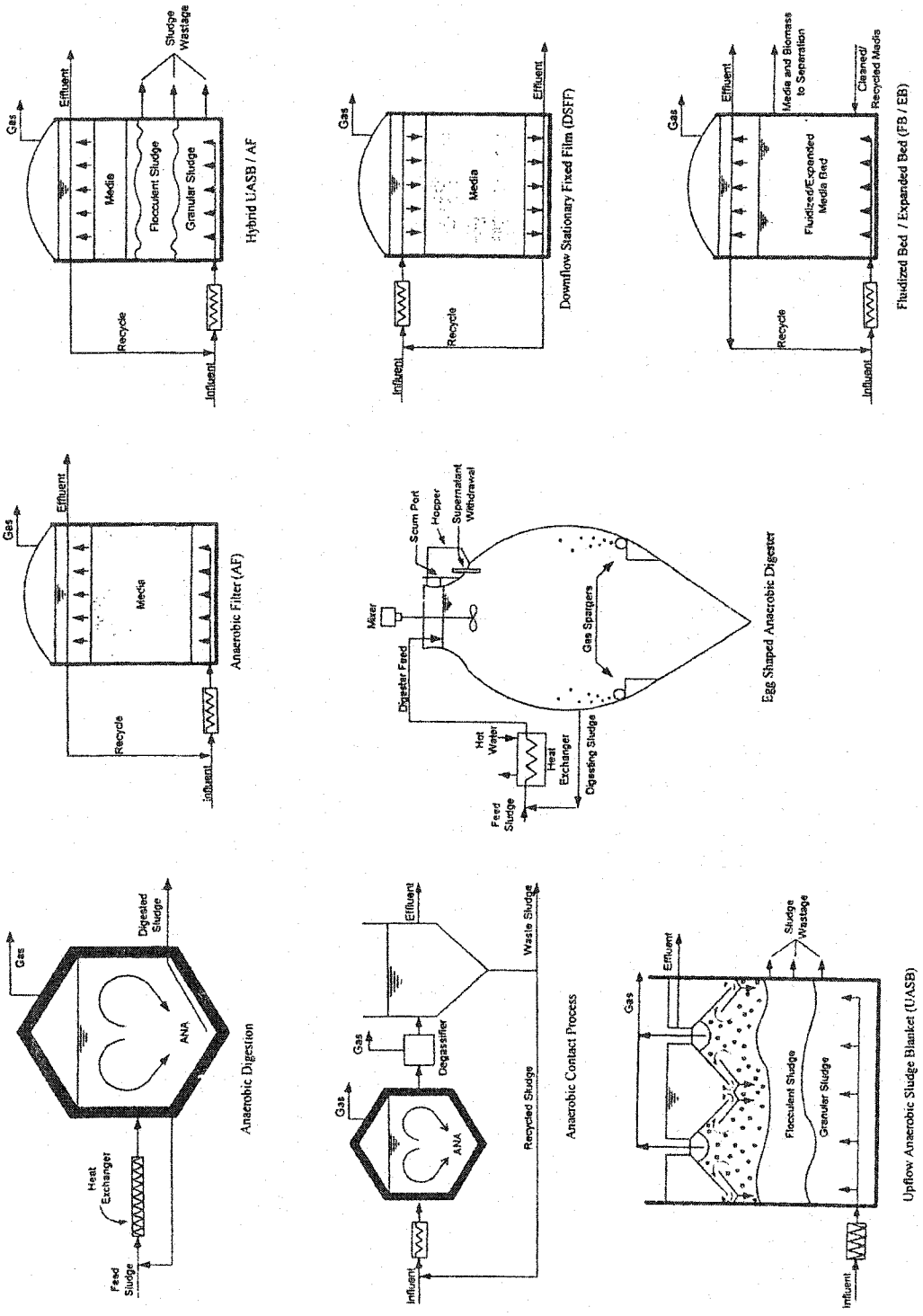


Figure 2.1 Configurations of Anaerobic Reactors (after Wheately *et al.*, 1997)

Table 2.2 Performances of Anaerobic Processes in Different Configurations of Reactors

Reactor Type	Waste Type	Organic Loading in COD (g/L-day) (day)	HRT (hr.)	Organic Removal (%)	Reference(s)
Conventional Reactor	Formaldehyde	10×10^{-2}	10	65	Lin *et al.* 1979
	Cane Sugar;	0.62	4	60	Bhaskaran *et al.*, 1966
	Slaughter- house Waste;	2.8	20	44	Kandler *et al.*, 1981
Contact Process	Domestic Sewage;	0.4-2.5	0.5-5	70-98	Stronach *et al.*,1986
	Rum Spillage	2	3-6	90	Heertjes *et al.*, 1979
UASB	Chemical Wastes;	5-25	0.2- 0.25	90	Lettinga *et al.*, 1981
	Dairy Wastes	5	0.2	90	Dohanyos *et al.*, 1985
	Sugar	22.5	0.25	91	Letting *et al.* 1980
	Vegetable & Fruit Processing Wastewater;	1.4	7.5	80	Campos *et al.*, 1987
	Chlorinated hydrocarbon;	2 - 6	24 - 8	93-96	Prakash and Gupta, 2000
	Municipal Wastewater;	1.12×10^{-5}	6.0	74-75	Draaijer *et al.*, 1992
ARDC	Synthetic Wastes;	4.1-32.6	0.09-0.73	46-96	Tait *et al.*, 1980
AFBR	Synthetic Waste	0.8 - 4.0	0.01- 0.25	80	Switzen- baum *et al.*, 1978
	Domestic Waste	8	0.075	80	Wang *et al.*, 1984
AF	Pharmaceutical Waste;	1.5	1.5	94	Sachs *et al.*, 1982
	Synthetic Waste;	1	1	90	Young *et al.,1961*
	Petrochemical Waste;	2.13	1.5	90-98	Havious *et al.*, 1973
	Landfill Leachate;	7	1	89	DeWalle *et al.*, 1976
	Municipal Waste;	0.6	0.08-0.44	50	Genung *et al.*, 1978
	Slaughterhouse Wastwater;	8000 - 35000	6-31	87 - 89	del Pozo *et al.*, 2000
	Meat Processing Wastewater;	1.4	24	80	Campos *et al.*, 1987
Fixed Film	Cheese Whey	5-22	3-13	92-97	Berg and Kennedy, 1983
	Dairy Waste	5-15	0.8-0.3	67-83	Berg and Kennedy, 1983
	Skim Milk Waste	5-10	0.8-0.4	-	Berg and Kennedy, 1983

Remarks:
UASB - up-flow anaerobic sludge blanket reactor
AF - Anaerobic Filter
ARDC - anaerobic rotating disc contactor
AFBR - anaerobic fluidized-bed reactor

Table 2.3 Anaerobic Contact Stirred Tank Reactor (CSTR) Treating Food Processing Wastes (after Nahle, 1991)

Type of Effluent	Organic Loading Rate (kg COD/m^3 d)	F: M (kg COD: kg TS)	COD Removal (%)
Citric Acid	1.3 - 4.0	0.2 - 0.3	75 - 80
Cellulose	1.3 - 1.8	0.1 - 0.2	95 - 98
Condensate			
Distillery	1.5 - 2.5	0.2 - 0.25	90 - 95
Meat Processing	0.8 - 4.8	0.5 - 1.1	90 - 94
Pectin Factory	1.7 - 5.3	0.03 - 0.2	88 - 93
Sauerkraut	1.5	0.4	96
Starch Factory	36	1.4	65
Sugar Processing	0.6 - 13.0	1.3 - 3.0	90 - 95
Vegetable Cannery	2.0 - 4.2	0.1 - 0.3	90 - 95
Yeast Production	3.0 - 4.0	0.2 - 0.4	77 - 80

Table 2.4 Comparison of Loading Rates used in Anaerobic Filters, USAB and Expanded Beds Reactors (after Hobson and Wheatley, 1993)

Type of Reactor	Organic Loading (kg COD/ m^3 d)	Retention Time (hr.)	COD Removal (%)	Critical Solid Concentration in Feed (mg/L)
Anaerobic Filter	2 – 10	10 - 15	70 - 80	450 - 1050
UASB	2 – 15	10 - 50	70 - 90	-
Expanded Bed	2 – 50	0.5 - 24	70 - 80	-

Table 2.5 Comparison of the Selected Anaerobic Treatment Process for Organic Stabilization – Benefits and Drawbacks (after Grady *et al.*, 1999)

Treatment options	Benefits	Drawbacks
Anaerobic Digestion	• Suitable for a wide range of wastewaters • Efficiently handles wastewaters with high level of suspended solids • Easy to mix and create uniform reaction environment. • Large bioreactor volume to dilute inhibitors. • Performance not dependent on ability of sludge to settle. • Capable of accepting waste aerobic biomass	• Large bioreactor volumes required. • Effluent quality can be poor if non-degradable organic matter is present or if a large concentration of anaerobic organisms is generated. • Process stability and performance poor at short SRTs. • Requires separate mechanical mixing.
Up-flow Anaerobic Sludge Blanket (UASB)	• High biomass concentrations and long SRTs achievable. • Small bioreactor volume due to high volumetric organic loading rates. • High-quality effluent achievable. • Mechanically simple • Compact system, relatively small land area. • Well-mixed conditions produced.	• Performance dependent on development of dense, settleable solids. • Much lower process loading required if wastewater contains suspended solids. • Special bioreactor configuration required which is based on experience. • Little process control possible. • Shorter bioreactor HRTs mean less equalization and dilution of inhibitors.
Anaerobic Filter (AF)	• High biomass concentrations and long SRTs achievable. • Small bioreactor volumes due to high volumetric organic loading rates. • High quality effluent achievable. • Mechanically simple. • Compact system, relatively small land area. • Performance not dependent on development of dense, settleable solids. • Well-mixed conditions produced in bioreactor.	• Suspended solids accumulation may negatively impact on performance. • Not suitable for wastewater high in suspended solids. • Little process control possible. • High cost for media and support. • Shorter bioreactor HRTs mean less equalization and dilution of inhibitors.

Cont.		
Hybrid UASB/AF	• High biomass concentrations *and long SRTs achievable. • Small bioreactors volumes due to high volumetric organic loading rates. • High quality effluent achievable. • Mechanically simple. • Compact system, relatively small land area. • Performance partially dependent on development of dense, settleable solids. • Well-mixed conditions generally produced in bioreactor. • Reduced media cost.	• Lower process loadings required if wastewater contains suspended solids. • Little process control possible. • Shorter bioreactor HRTs mean less equalization and dilution of inhibitors.
Down-flow Stationary Fixed Film (DSFF)	• High biomass concentration and long SRTs achievable. • High quality effluent achievable. • Mechanically simple. • Compact system, relatively small land area. • Performance not dependent on development of dense, settleable solids. • Well-mixed conditions generally produced in bioreactor.	• Suspended solids not generally degraded. • High cost for media and support. • Organic removal rate generally lower than other high-rate processes. • Little process control possible. • Shorter bioreactor HRTs mean less equalization and dilution of inhibitors.
Fluidized Bed/Expanded Bed (FB/ EB)	• High biomass concentrations and long SRTs achievable. • Small bioreactor volume due to high volumetric organic loading rates. • Excellent mass transfer characteristics. • High quality effluent achievable, often better than other high-rate processes • Most compact of all high-rate processes; requires smallest land area. • Performance not dependent on development of settleable solids. • Very well mixed conditions generally produced in bioreactor. • Increased process control capability relative to other high-rate processes.	• Lengthy start-up period required. • High power requirements for bed fluidization and expansion. • Not suitable for wastewaters high in suspended solids. • Mechanically more complex than other high-rate processes. • Increased process control required. • Cost of carrier media is high. • Shorter bioreactor HRT's mean less equalization and dilution of inhibitors.

However, problems still existed regarding the effectiveness and reliability of biomass-liquid separation in the second stage. The latter was essential because of the low specific gravity difference between the bacterial flocs and the reactor liquor caused by entrapment of the biogas in the bacterial flocs (van den Berg *et al.*, 1979). Lettinga *et al.* (1980) improved the sedimentation by adding flocculating agents and these added chemical agents ultimately improved the effluent's quality.

2.2.2 Up-flow Anaerobic Sludge Blanket Reactor (UASB)

The up-flow anaerobic sludge blanket (UASB) reactor was first developed by Lettinga in the 1970s (Lettinga, 1979; Lettinga *et al.*, 1980). It is by far the most widely used high-rate anaerobic reactor in the world, particularly for industrial wastewater and sewage treatment (Lin *et al.*, 2001; Kalogo *et al.*, 2001). Among these reactors, the influent waste stream flows upward through a sludge bed and an expanded sludge blanket (Figure 2-1). The latter comprises a spontaneously flocculating bacteria which are oval, or sometimes nearly spherical. A granular type of anaerobic sludge with a diameter of 1 - 5 mm is commonly formed in the system, and is maintained in suspension by the hydraulic flow. Such type of granular sludge is responsible for most of the organic removal (Christensen *et al.*, 1984). These granules have a high density and excellent mechanical strength together with a high settling velocity and a high specific methanogenic activity. The granulation process has been studied by a number of researchers, including. De Zeeuw,1984; Hulshoff Pol and Lettinga,1986; Hulshoff Pol *et al.*,1987. Razo-Flores *et al.* (1966) evaluated the effect of five different granular sludge sources for anaerobically biodegrading aromatic compounds, including phenol, 4-cresol, 2-aminobezoate (2-AB)s and 5-aminosalicylate (5-ASA)s and found that the

granular sludge, regardless of the source of the sludge used, had a universal capacity to degrade phenol and 4-cresol. In contrast, only some granular sludge sources could degrade N-substituted aromatics like 2-AB and 5-ASA.

Multiple feed nozzles at the base of the reactor ensure an even distribution of the wastewater entering through the sludge blanket. Mixing is affected by the waste stream flow and the biogas generation. Inclined louvers are included in the upper portion of the UASBs to improve the ability of the bacteria to settle and minimize biomass washout. Such a reactor configuration enhances retention of biomass without requiring sludge recycling from an external source. The biomass concentration in the sludge blanket is about 20 - 40 g VSS/L and that in the sludge bed is about 40 - 70 g VSS/L. These values are much higher than those of the contact process, showing performances that surpass both the conventional reactor and contact process as shown in Table 2-1.

Despite the improved performance of the UASB, there are limitations inherent in the UASB design. Christensen *et al.* (1984) reported that a few months is required to start-up a UASB and to establish a stable sludge blanket and sludge bed. These may still be disrupted if the up-flow velocity exceeds the settling velocity of the bacterial granules, or if biogas production is too vigorous. Consequently, UASBs are usually susceptible to a loss of portions of the sludge bed during a hydraulic surge or toxic upset (Fannin *et al.*, 1987). Unlike the conventional reactors and contact process, UASBs are not suitable for treating particulate wastes since particles appear to interfere with flocculation and may also accumulate in the bed and reduce the effective volume. Inefficient operation may also occur if the influent forms channels

through the sludge blanket. Consequently, a multiple inlet feed system is required which generally increases construction costs. As a general guideline, one feed inlet point per square meter for loadings exceeding 2 kg COD/m^3-day would be satisfactory. For a loading of below 1 kg COD/m^3-day, more feed points have to be incorporated into the system (Lettinga *et al.*, 1987). Significant parameters to be considered in USAB operation are floe diameter, microbial concentration, and the structure of the gas-solid separator, which effectively retains the microbial granules within the reactor. To achieve successful USAB operation, some of the criteria that need to be observed, include (a) selection of a suitable wastewater capable of granule formation; (b) start-up at a relatively low COD load; (c) use of waste containing calcium and barium ions; (d) operation of the reactor without mechanical agitation; and (e) avoidance of bulking caused by filamentous bacteria, because it is observed that granule formation in a UASB system is influenced by the growth of *Methanothrix* spp., which produces spherical granules.

Two variants of the UASB reactor (the expanded granular sludge bed (EGSB) reactor and the two-step (phase) UASB reactor) have been recently developed and these are discussed in the following sections.

2.2.2.1 Expanded Granular Sludge Bed (EGSB) Reactor

The expanded granular sludge bed (EGSB) reactor is usually operated under hydraulic up-flow velocities from 4 m/hr to 6 m/hr. As such, an almost complete contact between retained sludge and wastewater can be achieved, and, therefore, the internal

transport of substrate and reaction products within the aggregate is definitely enhanced. (Letttinga, 1996). This has led to higher rates of treatment and better resistance to hydraulic and organic shock loadings.

van der Last (1991) carried out an experiment using the so-called EGSB reactors. The granular sludge bed was operated in an expanded mode as the direct result of higher upward velocities (e.g. 6-12 m h^{-1}). The EGSB reactor has been shown to be efficient in the removal of soluble organic matter, even at low temperatures, which could be attributed to the intensive contact between the incoming organic matter and the sludge granules. The EGSB seems to be particularly useful at lower temperatures and relatively low strength wastewater, when the production rate of biogas and the mixing intensity induced are relatively low.

However, EGSB is inadequate for the removal of particulate organic matter due to the high up-flow liquid velocity. The influent suspended solids are usually brown in color throughout the granular bed and leave the reactor with the effluent. Consequently, the colloidal matter is partially removed due to the absorption of the sludge flocs.

Zoutberg and Frankin (1996) presented a new type of EGSB reactor, known as Biobed® EGSB, that is a new ultra-high-load, full-scale anaerobic treatment system. Its treats chemicals (e.g., concentration of formaldehyde to 5g/L and methanol to 10 g/L) and brewery wastewater (volumetric load =30 kg TCOD/m^3) and results in a decreased COD load to the aerobic post-treatment, lesser sludge production and lower energy consumption. It is possible to achieve a removal efficiency of more than 99% for both compounds %due to the specific configuration of the reactor.

A 120 L EGSB reactor was used by Last and Lettinga (1992) to treat domestic sewage. Under dry weather conditions, a removal efficiency of 90%% with respect to the maximum obtainable efficiency of the soluble COD fraction was achieved at an HRT of 3 hours. The removal efficiency, varying from 84% to 77%, at HRTs ranging from 2 to 1.5 hours, could be achieved at temperatures over 13 °C.

2.2.2.2 Two-Stage (Phase) UASB Reactor

Acidogensis occurs more frequently than methanogenesis, leading to the accumulation of inhibitory by-products, such as volatile fatty acids. A two-phase anaerobic treatment process has been developed to resolve this problem (Pohland, *et al.*, 1971; Fox and Pohland., 1994; Azbar and Speece, 2001; van Lier *et al.*, 2001). Partial stage (phase) separation was also observed in a variety of applications of anaerobic reactors, including the anaerobic filter, an expanded-bed anaerobic reactor, up-flow UASB, and anaerobic hybrid reactor (Bull *et al.*, 1984; Dold *et al.* 1987; Sun and Zhou, 1988).

When treating wastewater with a high organic particulate fraction (e.g. sewage), it is beneficial to employ a two-stage (phase) anaerobic process. In the first stage, the organic particulate matter is entrapped and partially converted into soluble compounds, which are then digested in the later reactor (Adrianus *et al.*, 1994). A two-stage (phase) reactor with spatial separation of acid formation and methane production can be used in a UASB reactor to degrade the suspended solids at the acidogenic phase as well as to prevent the methanogenic phase (Ghosh *et al.*, 1975). The acidogenic-phase reactor is a well-known for protecting the subsequent

methanogenic reactor from adverse conditions, such as inhibitory or toxic materials, shock loads, and variable conditions (Ghosh *et al.*, 1975).

The first stage hydrolytic reactor is typically preceeded with the formation of the flocculent sludge and is operated at a relatively low up-flow liquid velocity. Particulate influent organic matter can be adsorbed onto the sludge flocs, partially re-introduced into the liquid phase by hydrolysis, and discharged from the reactor. Methanogensis will be developed in the hydrolytic reactor when the environmental and operational conditions are not suitable for the process. Besides, the development of acid fermentation may lead to a depression of the pH of the reactor. Therefore, the entrapped matter will be partially hydrolyzed and the sludge age will remain relatively low and consequently the slow growing methanogens cannot develop well.

Shin *et al.* (1992) operated a two-phase UASB system treating concentrated distillery wastewater. The performance of the reactors showed that when the influent SS concentration was 4.1 g/L, the first phase (acidogenic) UASB reactor could effectively operate up to an organic loading rate of 16.5 kg COD/m^3 day and produce 3.9 g HAc/L day at an HRT of 1.8 days. In the second (methanogenic) UASB reactor, a loading rate up to 44 kg COD/m^3 day could be applied with 80% of the influent COD removed together with a specific gas production of 16.5 L/Lday. Wang (1994) treated domestic sewage by a two-stage UASB system with an HRT of 3 hours for the first stage; and demonstrated that the first UASB reactor could be considered as an improved primary settler. In addition to the high efficiency of removal of suspended solids by filtration, some hydrolysis, acidifications and conversion to biogas also occurred in the first USAB reactor.

2.2.3 Anaerobic Rotating Disc Contactor (ARDC)

Effective and reliable treatment of industrial wastes requires reactors that demonstrate wide-ranging tolerance to fluctuations in operational conditions. Attached-growth reactors, with the biomass immobilized as biofilms on support media, are generally more resistant to fluctuations, such as hydraulic and organic shocks, and toxicity. This is possible because bacterial immobilization promotes stable inter-bacterial associations (Chynoweth, 1987). As bacterial proliferation increases, the biofilm gradually thickens until food and nutrients are no longer effectively diffused into the inner layers that are in contact with the support medium. Consequently, the biofilm will slough off and remain in suspension until it is washed out with the effluent. New biofilm will then re-develop and the cycle recurs. This provides for very long cell retention times (> 20 days) and a resistance to washout even at short HRTs (Stronach *et al.*, 1986).

ARDCs are among the earliest designs that capitalize on the natural tendency of bacteria to adhere to surfaces and form biofilms. In these reactors, biofilms are formed on an array of small polyethylene discs that rotate on a horizontal axis at a typical velocity of 13 rpm and are submerged in an elongated vessel through which the waste stream flows (Winkler, 1981; Hao *et al.*, 1990). Loading rates higher than the non attached-growth reactors described previously have been reported and are shown in Table 2-1. Furthermore, the ground area required to accommodate an ARDC is only about 10% of the space required for accommodating an equivalent conventional reactor (Tait *et al.*, 1980; Winkler, 1981).

While the rotating discs and biogas generation may offer some degree of mixing, it is not sufficient to achieve a thorough distribution of substrate and prevent a localized accumulation of intermediate VFAs. Therefore, a plug-flow pattern generally predominates in these reactors. Consequently, several ARDC units, operating in series, are normally required for effective treatment (Winkler, 1981; Hao *et al.*, 1990).

2.2.4 Anaerobic Fluidized-bed Reactor (AFBR)

The AFBR is a column with a bed of small particles freely suspended in the upward flow of liquid. Sand, glass beads, PVC particles, and carbon granules of diameters between 0.2 and 3 mm are most commonly used as the support media because their low densities minimize the energy requirement for fluidization (Cooper *et al.*, 1980; Stronach *et al.*, 1987). The bacterial film attaches either to the external surface or within the porous structure of the support particles. These particles are in constant motion, thus preventing channeling or clogging. Thus, a very efficient substrate distribution is achievable.

The concept of AFBR was developed to treat particulate wastes with concentrations of up to 200 mg TSS/L, and the problem of clogging was alleviated (Cooper *et al.*, 1982). These reactors have been reported to be capable of achieving high organic loading rates and organic removal efficiencies at extremely short HRTs when treating low-strength wastewater of about 600 mg/L COD (Switzenbaum *et al.*, 1980; Wang *et al.*, 1984). Jeris *et al.* (1977) reported that an AFBR requires less than 5% of the space required by a conventional reactor.

However, the AFBR has not been widely applied in waste treatment. The main reason for this is that the design and control of the reactor are very complex as it is dependent on the size and density of the support medium (Cooper *et al.*, 1981; Price *et al.*, 1985b). These reactors often experience gradual bed expansion and washout of the support medium, even when the HRT is held constant, because of the change in overall particle density as the biofilm thickens (Miller, 1983). This may require the inclusion of a device to regularly withdraw some of the support particles for biofilm removal. An alternative method for preventing the washout of particles is to design the AFBR as a tapered column with an enlarged upper section (Cooper *et al.*, 1981).

The immense shear stress, produced after fluidization, may cause sloughing of the biofilm. Recent developments in immobilization techniques attempt to resolve this problem by entrapping the bacterial cells within the matrices of polymerizing or cross-linking gelling materials, such as alginate, or carrageenan gel beads (Cheetham *et al.*, 1984; Chibata *et al.*, 1986). However, this technique may seriously affect the ability of the substrates to diffuse to the entrapped cells and for their products (e.g., VFAs) to diffuse out into the liquid phase (Tanaka, *et al.*, 1984). Other disadvantages associated with the AFBR are the high-energy requirements for fluidization and the requirement of a long start-up period (Fannin *et al.*, 1987). These operational problems have rendered AFBRs less widely used than anaerobic biofilters.

2.2.5 Anaerobic Filter (AF)

The anaerobic filter was studied by Young in 1968 (Young, J.C., 1968; Young *et al.*, 1969) on the basis of the earlier work carried out by Coulter *et al.* (1957) for treating

soluble, dilute organic wastewater, and it has recently become popular for wastewater treatment. Immobilized bacteria on the packed bed of these column reactors (Figure 2-1) prevented biomass washout even under the situation of short HRTs. External separation and recycling of the biomass are often not necessary (Coulter *et al.*, 1977). Wastewater can either be passed in the up-flow or down-flow direction through the packed bed. Horizontal-flow biofilters are less common although they have also been used (Landine *et al.*, 1982). Up-flow biofilters are by far the most commonly used anaerobic reactor because the packed bed is completely submerged, thus providing better contact between the bacteria and the substrate (Sachs *et al.*, 1982; Yap *et al.*, 1991). In the down-flow biofliter, the reactor is fed from the top of the reactor. In contrast, in the up-flow AF, the wastewater is feed from the bottom of the reactor. The biological activity of the reactor is contributed by the biomass attached to the surface of the medium support as a thin biofilm and entrapped within the media matrix or suspended as a granulated or flocculated sludge mass beneath the media (Young and McCarty, 1969; van den Berg and Lentz, 1979). Soluble organic compounds pass through the close proximity of the biomass and diffuse into the surfaces of the attached or granulated solids where the biodegradation occurs with production of intermediates and final end-products; mainly methane and carbon dioxide. Due to the relatively large clearance between the channels in the vertically oriented media, the down-flow AF is able to treat waste effluent with a higher content of suspended solids, but the AF cannot be sustained (Mara-Alvarez and Llares 1988).

In some earlier designs, anaerobic filters were operated without mixing (Koepp *et al.*, 1985; Russo *et al.*, 1985; Mosey, 1978; Sachs *et al.*, 1982). Bubbles of biogas may provide a certain degree of mixing, but this is usually insufficient for achieving

mixed-flow condition. These AFs are generally considered to be in a plug-flow operation, often encountering local accumulations of intermediate VFAs and organic shock by toxic substances (Stafford, 1982; Casey, 1986). Additionally, the entrapped biogas and scum may cause clogging and reduce the effective volume of the reactor. Effluent or biogas recycled through the packed bed is a method commonly employed to counteract these problems (Ng *et al.*, 1987; Denac *et al.*, 1988). DeWalle *et al.* (1976) reported that an effluent recycle, while diluting the influent concentration, maintained the COD removal efficiency with respect to the diluted influent.

Chua *et al.* (1991) conducted a comparative study on the performance of a mixed- and a plug-flow biofilter for chemical waste treatment and such chemical waste was found to be inhibitory to the biofilters at high concentration. The superior performance of the mixed-flow biofilter with such chemical waste was attributed to the presence of the effluent recycle for diluting and distributing the feed. In a separate study, Thirumurthi (1988) used a 14.8 L up-flow anaerobic biofilter, packed with toroidal bio-rings, to study the effect of the effluent recycle rate on COD removal efficiency. The biofilter was found to perform best when the recycle velocity was maintained at between 66 and 660 cm/hr, which was equivalent to re-circulating the entire liquid content of the biofilter 0.4 - 4.2 times an hour. Sloughing of biofilm occurs when recycle velocities exceed 680 cm/hr.

Because of the increased biomass concentration, anaerobic filter shows high organic removal efficiency at high hydraulic loading rates (Tables 2.1 and 2.5). In a study that compared the performances of conventional reactors with an AF for treating screened manure, Lo *et al.* (1984) found that a significant reduction in volume was possible for

the latter. The anaerobic filter has also been applied in treating a very wide range of wastes including piggery wastes (Ng *et al.*, 1987), slaughterhouse wastewater (Pozo and Beltran, 2000), food processing wastewater (Plummer *et al.*, 1968), distillery wastewater (Russo *et al.*, 1985) and it has also been applied in biological denitrification processes (Seidel *et al.*, 1970; Tamblyn *et al.*, 1969). The anaerobic filter is very tolerant to organic shock loadings, pH fluctuations, and toxicities (Hovious *et al.*, 1973; Jennet *et al.*, 1975; Young et al., 1969). Anaerobic filters have the advantage over the UASB in that they are not susceptible to washout by high hydraulic shock loads (Dohanyos *et al.*, 1985). Two typical problems that are commonly encountered in AF reactors are (i) dead zones and short-circuits, probably due to the uneven horizontal distribution of the inflowing wastewater in the bottom of the reactor and (ii) low specific activity of the biomass present on the bottom of the reactor, where the greater part of the biomass is concentrated. Both of these negative effects are considered to be caused by the presence of the packing material, which is an obstacle to the horizontal mixing, and traps large amounts of SS, which is mainly confined in the lower portion of the reactor where the mixing due to gas evolution is lowest (Tilche *et al.*, 1991). However, the AFs are still simpler and cheaper in design, operation, and control than the AFBRs. Although problems of clogging and channeling occur when the filters are used to treat particulate wastes, they are generally an excellent choice for treating wastes with a wide range of organic strengths and low solid contents at high hydraulic loading rates.

2.2.6 Up-flow Anaerobic Sludge Bed Reactor (ASB)

It was observed that the operation of the up-flow anaerobic sludge bed (ASB) reactor is dependent on such factors as (i) the formation of immobilized - balanced microorganism in the reactor, (ii) the high settle-ability of the immobilized anaerobic aggregates and (iii) the prevalence of an excellent contact between sludge and wastewater. The ASB is usually operating under a high rate of mass transfer in and out of the aggregates. Hydraulic conditions, that favor the retention of the biomass, are imposed on the ASB reactors. This allows sludge to be formed with a very good sedimentation rate and promotes a very high methanogenic activity (Tilche *et al.*, 1991)

2.2.7 Horizontal-Flow Anaerobic Immobilized Sludge (HAIS) Reactor

A laboratory-scale horizontal-flow anaerobic immobilized sludge (HAIS) reactor was first developed in 1995 for treating the waste effluent produced by a kraft paper factory (Foresti *et al.*, 1995). After 15 days of operation, the results show that the performance of this reactor was slightly better than that of a full-scale UASB operated by the factory. The configuration of the HAIS reactor consists of a horizontal tube filled with anaerobic sludge immobilized in polyurethane foam cubic matrices. A perforated tube for gas collection is installed along the upper portion of the reactor (Zaiat *et al.*, 1994). This lab-scale HAIS reactor was also tested for its tolerance of cell washout from the reactor with the purpose of establishing the limits for the horizontal-flow velocities. It was reported that velocities as high as 0.5 cm/s reduce the loss of total solids and volatile suspended solids less than 9.0% and 6.0% (Zaiat *et al.*, 1994).

2.2.8 Anaerobic Hybrid reactor (AHR)

In the early eighties, a novel reactor type, known as an anaerobic hybrid reactor (AHR), was developed as an improvement over the conventional UASB (Maxham and Wakamiya, 1981; Sun and Zhou, 1988; Lo *et al.*, 1994; Fang and Chui, 1994; Fernandez *et al.*, 2001). The configuration of an AHR is basically an improved type of UASB reactor, comprising a conventional UASB with an inserted biofilter in the upper sludge separation zone. Since then active research work has been undertaken with both laboratory- and full-scale reactors to optimize the design and operating parameters (Chang, 1989; Harris *et al.*, 1992; Hawkes *et al.*, 1995; Tur and Huang, 1997; Sun and Zhou, 1988; Lo *et al.*, 1994; Fang and Chui, 1994). The AHR exhibits the capability of high treatment performance in the lower UASB portion. In addition, stability and tolerance to shock loadings is improved by inserting a BF in an upper portion.

The laboratory-scale AHR has been successfully applied to treat a variety of wastewaters including brewery wastewater (Sun and Zhou, 1988), fibrous wastewater (Fernandz *et al.*, 1995; 2001), diary effluent (Strydom *et al.*, 1995), palm oil mill effluent (Borja and Banks, 1994; Borja *et al.*, 1996), landfill leachate (Chang, 1989), starch particulates (Fang and Kwong, 1994), and pharmaceutical wastewater (Henry *et al*, 1996) with treatment performances that were comparable to that of the UASB, but with improved stability under adverse situations (e.g. shock loadings).

Although the benefits of the AHR are obvious in a number of applications, as mentioned above, the function as well as the role of the inserted AF still remains a

controversy. Fang and Chui (1994) conducted a comparative study and concluded that there was no obvious difference between the performances of the AHR and the UASB. Chua *et al.*, 1999 applied a pilot-scale AHR system for treating livestock wastewater. This AHR system started up with a very stable performance within 30 days, which was much faster than the normal start-up time (usually three months) required for a conventional UASB reactor.

Mulder *et al.*, (2001) highlighted the future perspective of the conversion capacities of both anaerobic and aerobic wastewater treatment systems in relation to the growth kinetic, hydrodynamic, and biomass concentration.

2.3 Biochemical Pathway and Microbial Population in Anaerobic Digestion - An Overview

Anaerobic digestion is a process by which a mixed microbiological culture attacks a complex organic matter in the absence of oxygen, resulting in generation of biogas (e.g., methane) together with solid and liquid residuals. The anaerobic process involves complex pathways and the synergistic actions of numerous groups of bacteria. Over the last 30 years, a broad outline of the degradation process has been established (McCarty, 1964; Lawrence *et al.*, 1969; Pfeffer, 1979; Pretorius, 1983; Gijer *et al.*, 1983; Zinder, 1984; Boone, 1985; Price, 1985; McCarty *et al.*, 1986; Harper *et al.*, 1986; Archer and Kirsop, 1990), and it is summarized in Figure 2.2 below.

Five main groups of bacteria are responsible for anaerobic reactions. They are, (i) fermentative bacteria, (ii) hydrogen-producing acetogenic bacteria, (iii) hygrogen-

consuming acetogenic bacteria, (iv) carbon dioxide-reducing methanogens, and (v) acetoclastic methanogens. The 4-phase process of anaerobic digestion involves hydrolysis of large, insoluble macromolecules, fermentation of the soluble organics to acetate, H_2 and other intermediates, and production of CH_4. Hydrolysis is the rate limiting step when treating insoluble organics (Eastman *et al.*, 1981; Boone, 1982; Sleat *et al.*, 1987), while in industrial wastewaters, containing mainly soluble organics, methane production becomes the rate limiting step of the reaction (Siebert *et al.*, 1968; Stadtman, 1967; Mosey, 1983).

It has been widely accepted that three main stages of reaction are involved in anaerobic digestion, namely hydrolysis, acidogensis and methanogensis. In the first stage, a group of microorganisms secrete enzymes, which hydrolyze polymeric materials to monomers, such as glucose, and amino acids, which are subsequently converted to higher VFAs, H_2 and acetate. In the second stage, hydrogen-producing acetogenic bacteria convert the higher VFAs (e.g., propionates and butyrates) produced to H_2, CO_2, and acetate. In the final stage, methanogenic bacteria convert H_2, CO_2, and acetate to CH_4 and CO_2.

2.3.1 Hydrolysis Phase

Hydrolysis of fats and oils is the most essential reaction in hydrolysis phase, because these compounds account for about 30% of the total organic matter in domestic sludge. The digestion of these compounds results in over 50% of the total organic removal and CH_4 production (O'Rourke, 1968; Chynoweth *et al.*, 1971; Stronach *et*

al., 1986). *Micrococcus* spp. is the lipolytic bacteria that produces lipases and esterases to hydrolyze fats and oils in a stepwise process. Rapid hydrolysis of the 2 ester bonds of triglycerides to form monoglycerides is followed by a slow hydrolysis of the monoglycerides to free glycerol and fatty acids (Toerien, 1967; Hobson, 1982; Price, 1985). Long straight-chain fatty acids, such as myristic (14-C), palmitic (16-C), stearic (18-C), oleic (unsaturated, 18-C), and linoleic (unsaturated, 17-C) acids are the major products (Novac *et al.*, 1970). However, some researchers indicate that hydrolysis is the action of enzymes secreted by the acid-forming bacteria (Crowther *et al.*, 1975; Hill *et al.*, 1977; Torre *et al.*, 1986a).

Hydrolytic bacteria are Gram-negative (G-ve) type bacteria, having a rod shape form, that are obligate or facultative anaerobes. Their population ranges from 10^8 to 10^9 cell/ml found in sewage sludge digesters (Zeikus, 1979). These bacteria secrete enzymes that hydrolyze and make soluble the organic matter, which is then taken up by other bacteria for further intracellular fermentation (Rogers, 1961). The enzymes, known as hydrolases, are secreted as a spontaneous response to the presence of complex degradable organics. Manual addition of pure enzymes to promote hydrolysis has been unsuccessful (Chamberlin, 1930; Heukelekian *et al.*, 1953). Certain *Clostridium* spp. and *Bacteroide* spp. produce carbohydrases to catalyze the hydrolysis of the glycosidic bonds of polysaccharides to form monosaccharides (Chan *et al.*, 1970; Hobson *et al.*, 1974). Other *Clostridium* spp., *Bacteroide* spp. and *Eubacterium* spp. secrete proteases for the hydrolytic cleavage of the polypeptide bonds of proteins to form amino acids (Frutton *et al.*, 1959; Blackburn, 1968a; Blackburn, 1968b; Hazelwood *et al.*, 1981).

2.3.2 Acid-producing Phase

The hydrolysed products are utilized and fermented by the highly diversified groups of acid-producing bacteria (acidogens), including *Clostridium* spp., *Eubacterium* spp., *Selenomonas* spp., *Micrococcus* spp. and *Staphylococcus* spp. (Bryant, 1956a; Bryant, 1956b; Bryant *et al.*, 1956; Hobson *et al.*, 1961; Peage *et al.*, 1956; Stieb *et al.*, 1985). However, as mentioned in the previous section, some researchers have referred to these organisms as hydrolytic bacteria.

Monosaccharides are fermented, through the Embden-Mayerhof-Parnas (EMP) pathway, to acetate, CO_2, and H_2 (Peage *et al.*, 1956; Dagley *et al.*, 1970). Different pathways to pyruvic and acetate and ammonia degrade amino acids. Pyruvic acid is further reduced, via lactic acid, to propinoate (Weng *et al.*, 1976; Price 1986). The most predominant products of fermentation are acetate and H_2, through which all degradation pathways must pass (Jeris, 1962). Other products include alcohols, other VFAs, and H_2S (Andrews *et al.*, 1964; Kaplovsky, 1951; Kaplovsky, 1952; Pohland *et al.*, 1963; Gottschalk, 1979).

The β-oxidation process, using the ^{14}C tracer studies, is the major mechanism adopted in the degradation of fatty acids under anaerobic condition (Jeris *et al.*, 1965). This reaction sequence results in sequential cleavage and removal of 2-carbon acetate groups from the carboxyl end of the carbon chain as summarized in Figure 2-3, by a repeating series of passes through a set of enzymes that removed one 2-carbon acetyl unit at a time in the form of acetyl-CoA. (Novak *et al.*, 1970; Weng *et al.*, 1976;

Mahler, 1964; McInerney *et al.*, 1979; Price, 1985; Boone, 1985). Fatty acids with an even number of carbon atoms are degraded to acetate and H_2; those having an odd number of carbon atoms are degraded to propionate, acetate and H_2. Unsaturated fatty acids, in contrast, are postulated to be hydrogenated before undergoing oxidation (Heukelekian *et al.*, 1958; Weng *et al.*, 1976).

It is noted that not much research has been devoted to studying the anaerobic degradation of branched-chain fatty acids, mainly because such fatty acids were not commonly found in domestic wastes. These organic compounds were discharged by certain industries where they are produced as intermediates during the degradation of other industrial wastes. Massey *et al.* (1976) reported that 3-methylbutanoic and 2-methylbutanoic acids were produced through the anaerobic degradation of amino acids such as leucine, isoleucine and valine. These branched-chain fatty acids were shown to be biodegradable in an anaerobic biofilter (Jimeno *et al.*, 1990; Chua *et al.* 1990).

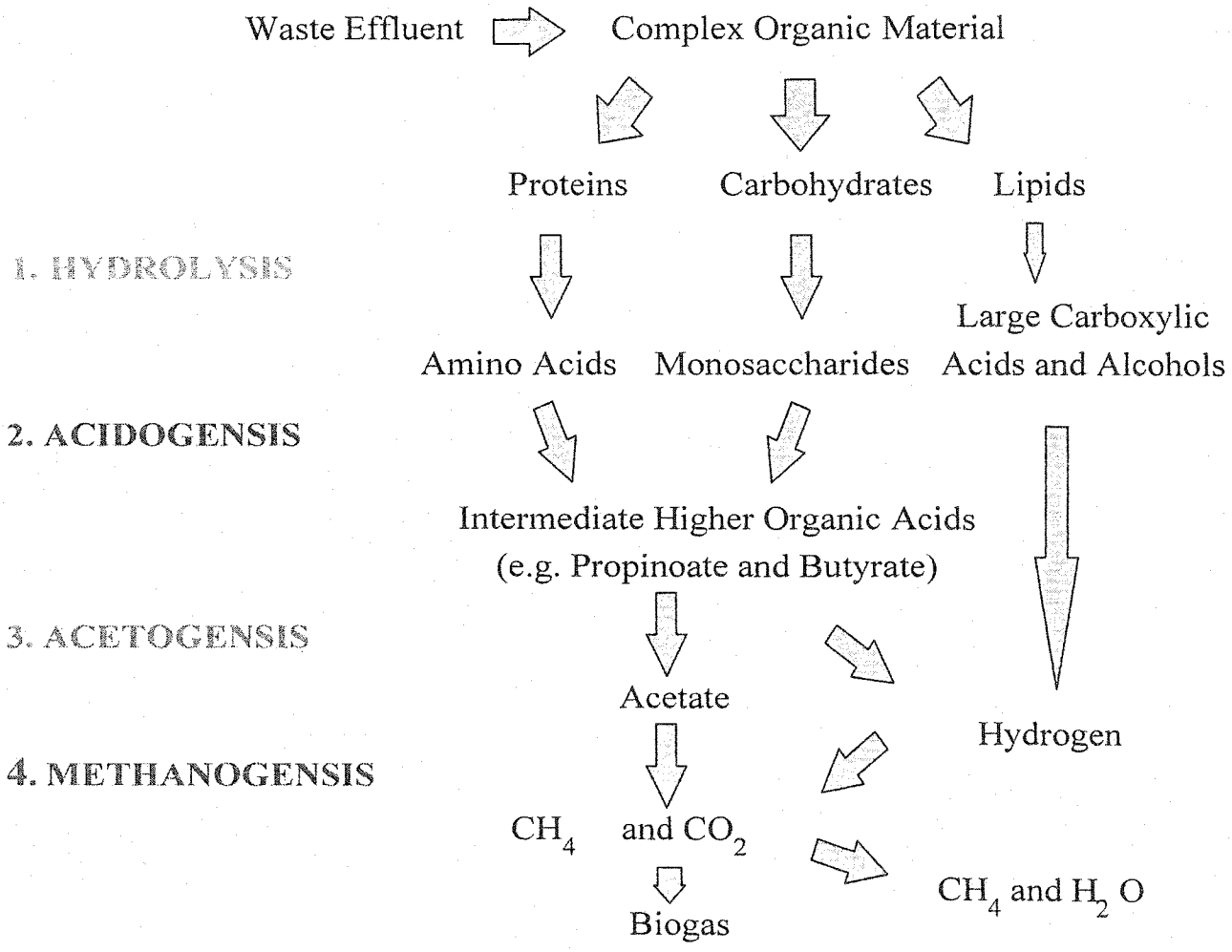


Figure 2.2 **Biochemical Steps in Anaerobic Digestion**

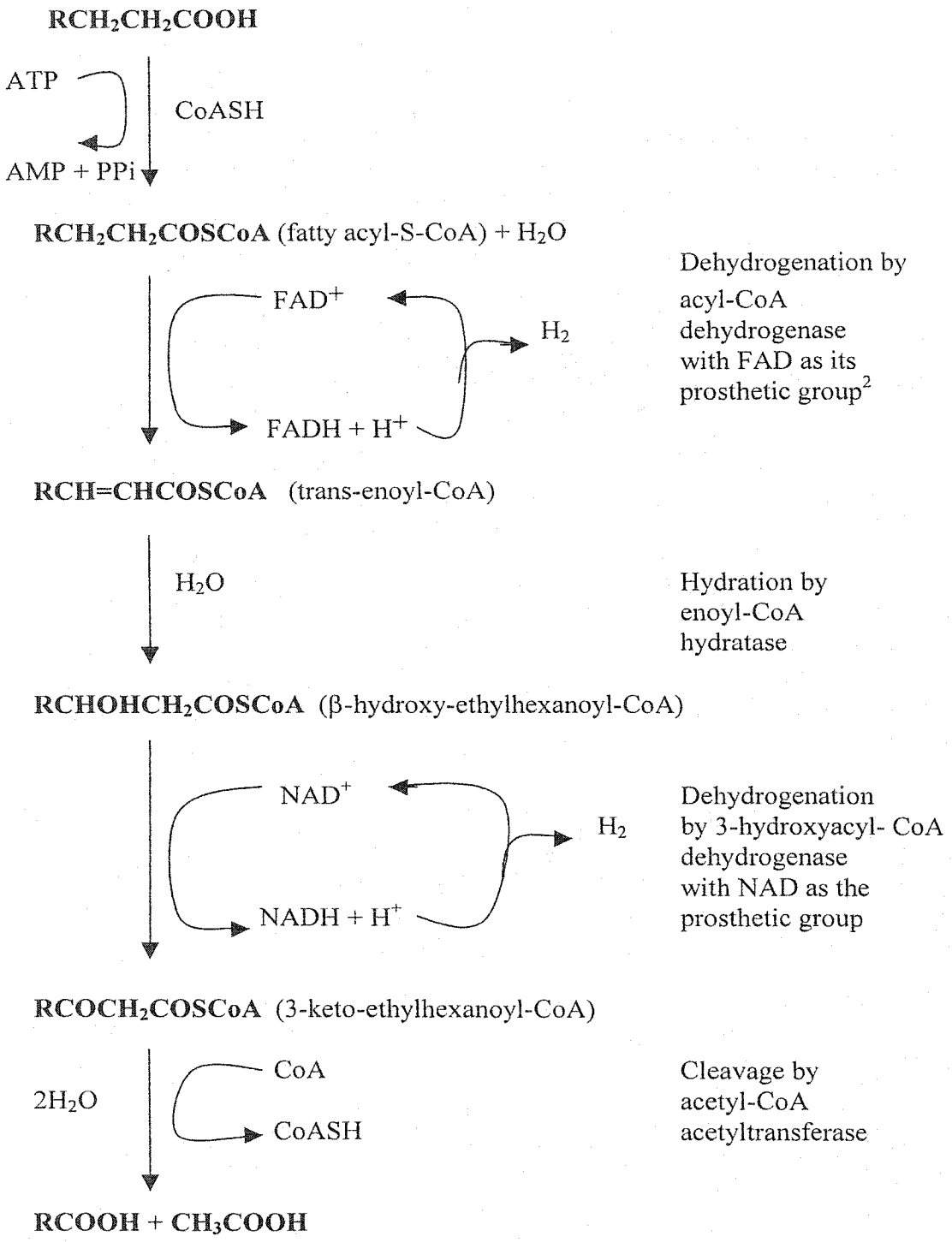


Figure 2.3 **β-Oxidation of Straight-Chain Fatty Acid**

[2] Prosthetic Group: An organic group (other than an amino acid) that is bound to a protein and serves as its active group.

Richardson *et al.* (1987) had isolated co-cultures from a cattle waste digester that were capable of degrading 2-methylbutanoic acids to acetate and propionate.

Several species of specialized acidogens, known as acetogens, are responsible for the degradation of long-chain fatty acids to acetate which is isolated from sewage sludge. *Syntrophomonas* spp. oxidizes straight-chain fatty acids to propionate, acetate and H_2, and it has been isolated from a co-culture with H_2-utilizing methanogens (McInerney *et al.*, 1979; 1981b). These are Gram-negative, curved rods with rounded or tapered ends. The cells ranged from 2.0 - 5.5 microns in length and 0.5 - 1.0 microns in width. Another species, *Syntrophobacter wolinii*, which degrades propionate to acetate and H_2, has been isolated in a co-culture with a sulfate-reducing species as the H_2-remover (Boone *et al.*, 1980; Boone *et al.*, 1987).

Literature shows that acidogens are also referred to as H_2-producing bacteria because their only means of regenerating the reduced coenzymes, nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH), is by oxidising these compounds through H_2 evolution (Bryant, 1967; Bryant *et al.*, 1977). A typical acidogenic reaction on butyrate, with H_2 evolution, is shown as follows:

$$CH_3CH_2CH_2COO^- + 2H_2O \longrightarrow 2CH_3COO^- + H^+ + 2H_2$$

$$\Delta G^o = +48.1 \text{ kJ/reaction} \quad (2\text{-}1)$$

This reaction is not energy yielding and is possible only if it is coupled with an energy yielding H_2-utilizing reaction which maintains a low partial pressure of H_2 (Bryant,

1967; Bryant *et al.*, 1977; Chung, 1976; Harper *et al.*, 1986; Thauer *et al.*, 1977). Acidogens are usually obligate syntrophs under such circumstances.

2.3.3 Methane-producing Phase

The methane-producing phase involves the action of two groups of bacteria, namely; H_2-utilizing and acetoclastic (ethanoate-utilizing) methanogens. A total of 36 species of methanogens under 13 genera have been discovered and isolated (Jones *et al.*; 1987) since the development of anaerobic techniques in the 1950s (Hungate, 1969). Biavati *et al.* (1988) isolated a novel species, *Methanosphaera cuniculi*, from the intestinal tracts of rabbits, which made a total of 37 species. Taxonomically, methanogens belong to a group of bacteria, the Archaebacteria, which has a number of unique features (Woese, 1982; Wolin *et al.*,1985; Ferugusan *et al.*, 1987; Konig *et al.*, 1989). Populations of 10^6 - 10^8 cell/ml were detected in digesters.

The majority of methanogens (e.g. at least 33 species) obtain their energy for growth by utilizing the H_2 for the reduction of CO_2 to form CH_4 as shown in equation (2-2) (Archer, 1984; Balch *et al.*, 1976; Thauer *et al.*, 1977; McInerney *et al.*, 1979; Stieb *et al.*, 1985).

$$4H_2 + HCO_3^- + H^+ \longrightarrow CH_4 + 3H_2O$$

$$\Delta G^o = -135.6 \text{ kJ/reaction} \quad (2\text{-}2)$$

The H_2-utilizing methanogens have a low saturation coefficient (K_S) for H_2 (e.g., 5 x 10^{-6} M), and they can maintain a H_2 partial pressure as low as 10^{-4} atm. in a healthy digester and induce the acid-producing bacteria to produce the "non-reduced" acetate, which is an essential substrate for acetoclastic methanogens.

The H_2-utilizing methanogens usually contain the coenzyme 420 (F^{420nm}), which is involved in electron transfer in the biochemical reduction of carbon dioxide (Mink *et al.*, 1977; Doddema *et al.*, 1978; Gorris, 1989). This coenzyme fluoresces when excited by an irradiation at 420 nm, which assists in the identification of the species.

Acetate is the precursor for 75% of the total methane production (Mah *et al.*, 1976; Mah *et al.*, 1977; Smith *et al.*, 1966; Winfrey *et al.*, 1977; Mountfort and Asher, 1978; McCarty *et al.*, 1986; Moletta *et al.*, 1986). Propionate and butyrate are also the important volatile fatty acids, which are essential for the further conversion into acetate and hydrogen. However, there are only a few species of acetoclastic methanogens, namely, *Methanosarcina* spp., and *Methanosaeta* (previously known as *Methanothrix*) spp., that derive their energy sources from propionate and butyrate (Smith *et al.*, 1978; Fathepure, 1983; Platen *et al.*, 1987). As indicated by some other researchers, *Methanosarcina* spp. could derive its energy from acetate in the absence of H_2. It grew faster on the preferred and more metabolizable substrate, H_2-CO_2 (Zeikus, 1977; Zeikus *et al.*, 1975). However, some researchers also report that *Methanosarcina* spp. are the most versatile methanogans and can use H_2/CO_2, acetate, methanol, and methylated amines as energy sources (Nishio *et al.*,1993; Boopathy, 1996; Chen and Hashimoto, 1996; Weijma and Stams, 2001) at fast rates. Their

affinity for acetate is only about one tenth that of *Methanosaeta* spp. (Huser *et al.*, 1978; Zehnder *et al.*, 1980). *Methanosaeta* spp. is commonly found in anaerobic ecosystems as acetate utilizers and it can only utilize acetate as the energy source (Platen *et al.*, 1987). Acetoclastic methanogens have very distinctive morphologies. *Methanosarcina backeri* are in packets of 8 cells while *Methanothrix soehgenii* are rods with truncated ends.

Acetate undergoes an energy yielding, decarboxylation reaction, in which the methyl group is reduced to CH_4 while the carboxyl group is oxidized to CO_2, as in equations 2-3 and 2-4 (Jeris *et al.*, 1965).

$$CH_3COO^- + H_2O \longrightarrow CH_4 + HCO_3^-$$

$$\Delta G^o = -31 \text{ kJ/reaction} \quad (2\text{-}3)$$

$$^*CH_3COOH \longrightarrow {}^*CH_4 + CO_2 \quad ^*C = \text{labeled carbon} \quad (2\text{-}4)$$

However, the standard change in free energy is barely sufficient to form one mole of ATP, which is equivalent to 30.6 kJ (Bailey *et al.*, 1986). This explains why the growth rate of acetoclastic methanogens was relatively slow.

2.3.4 Interactions of Microbials under Anaerobic Conditions

It was reported that sulfate-reducing bacteria (SRB) competed with both the methane-producing bacteria (MPB) and syntrophic accetogenic bacteria (SAB) for the intermediates as the sole carbon source for energy under anaerobic conditions (Cappenberg and Prins, 1974; Parkes *et al.*, 1989), including acetate (Parkin *et al.*, 1990; Gupta *et al.*, 1994; Isa *et al.*, 1986; Yoda *et al.*, 1987), propionate (Parkin *et al.*, 1990; Visser *et al.*, 1993), butyrate (Visser *et al.*, 1993; Mizuno *et al.*, 1994), and formate (Isa *et al.*, 1986; Gupta *et al.*, 1994).

Harada *et al.* (1994) studied the interaction between SRB and MPB, by using UASB reactors fed with a low strength synthetic wastewater, containing starch and sucrose at 500 mg COD/L, and at different sulfate levels. They found that methane production decreased with increasing sulfate levels. The SRB has played an important role in the degradation of propionate.

Some researchers also showed that the re-dox potential and feed organic to sulfate ratio were the important parameters to delineate the interactions between SRB and MPB in anaerobic processes. Cappenberg (1975) reported that the optimum re-dox potential values for lactate-fed SRB and MPB were - 140 and - 380 mV. Bhattacharya *et al.* (1996) also found that the percentage of acetate utilized by the SRB increased from 10 to 35% when the re-dox potential was changed from - 175 to - 75 mV.

The interactions between SRB and MPB in both natural environments and bioreactors have been studied extensively. SRB can easily out-compete the MPB under such

circumstances (Winfrey and Zeikus, 1977; Oremland and Plicin, 1982; Lovley and Klug, 1983) as SRB has a higher affinity for the substrate (e.g. acetate) than does MPB. Yoda *et al.* (1987) reported that the K_s (Monod half- velocity coefficient) values for SRB and MPB were 9.5 mg acetate/L and 32.8 mg acetate/L.

Table 2.6 shows a comparision of the e's, the K_s values of MPB and SRB for acetate as substrate, that were obtained from different studies. Comparatively, the K_s values of SRB are lower than those for MPB and, in that sense, the SRB has a higher affinity for substrate than does MPB. The reported k value for the acetate utilization rate obtained from different studies is shown in Table 2.7.

However, Isa *et al.* (1986) found that SRB do not completely out-compete MPB in high-rate anaerobic reactors. It is believed that with a high concentration of acetate in the reactors, both the SRB and MPB can utilize acetate at its own maximum rates and gives no significant advantage to either group. Mizuno *et al.* (1994) observed that the competition between sulfate reduction and methane production is dominated by the chemical oxygen demand (COD)/sulfate (S) ratio. Li *et al.* (1996) also observed that the interactions between SRB and MPB are strongly dependent on the ratio of COD/S in wastewater. It has been reported that the MPB consumed 99% of the available electron donors at a COD/S ratio of 60, but consumed only 69% at a ratio of 1.5, and 13% at 0.75.

Li *et al.* (1996) showed that at a high COD/S ratio (e.g. 3.0 or higher), the degradation pathway for an organic substrate (e.g., benzoate) is mainly to methane via the acetate and hydrogen/formate pathways. At a low COD/S ratio (e.g., 1.5 or lower), benzoate

is consumed mainly by SRB, converting sulfate into sulfide and thus suppressing methane production. The degradation of benzoate, under anaerobic conditions, is partially inhibited when the sulfide concentration in wastewater is high (e.g., total S^{-1} 330 mg/L and free H_2S 50 mg/L). In addition, the degradation of benzoate required the syntrophic acetogenic bacteria association between the hydrogen-producing acetogens, such as *Syntrophus buswellii,* and hydrogen-consuming MPB, plus *Methanothrix*-like MPB.

Table 2.6 : Comparison of the K_s value of SRB and MPB for Acetate

SRB (mg acetate/L)	MPB (mg acetate /L)	Reference (s)
9.5	32.8	Yoda *et al.* (1987)
12 (*Desulfobacter postgatei*)	180 (*Methanosarcina barkeri*)	Schonheit *et al.* (1982)
-	12	Bhattacharya and Parkin (1986)
102	116	Bhattacharya *et al.* (1996)

Table 2.7: Comparison of the k - acetate utilization rates of SRB and MPB for Acetate

SRB (d^{-1})	MPB (d^{-1})	Reference(s)
4.53 (*Desulfobacter postgatei*)	-	Ingvorsen *et al.* (1984)
-	2.07 - 2.29 (*Methanothrix soehngenii*)	Huser *et al.* (1982)
0.93	1.83	Yoda *et al.* (1987)
2.4	3.2	Bhattacharya *et al.* (1996)

The synergistic relationship between SRB and MPB has also been observed for wastewater containing substrates such as butyrate (Mizuno *et al.*, 1994), ethanol, (Kremer *et al.*, 1988), lactate (Bryant *et al.*, 1977), and propionate (Harada *et al.*, 1994). In all these cases, SRB played an important role in supporting the growth of MPB under sulfate-depleted conditions. However; with benzoate as substrate, the synergistic relationship between SRB and MPB was found insignificant and the competition for the available substrate was the critical interaction among SRB, MPB and SAB (Li *et al.*, 1996).

It has been reported that as the feed acetate/SO_4^{-2} ratio increased from 0.66 to 3.33, the percentage of acetate utilized by SRB decreased from 71 to only 4% in chemostates and from 40 to 12% in batch serum bottle conditions. In that sense, the ratio of feed organic to sulfate loading can be an important parameter in monitoring or controlling the relative growth of SRB and MPB (Bhattacharya *et al.*, 1996). The interaction between SRB and MPB has also been studied for acetate, methanol and formate in six chemostates containing mixed cultures, and the kinetic parameters for the degradation of these substrates are also evaluated (Gupta *et al.*, 1994a). During the treatment of sulfate-containing wastewater under thermophilic (e.g., 55 ^{o}C) conditions, sulfate reducers are found capable of using acetate as the substrate and even out compete acetaclastic methanogens. This competition is influenced by the pH. pH values $\geq$ 8 will strongly inhibit the methanogenesis, giving SRB sufficient advantage to out compete methanogens. However, an equilibrium can be established between these two microbials in a neutral pH environment (Visser *et al.*, 1992).

2.3.5 Distribution of Microbials in Anaerobic Treatment Processes

The changes in number and composition of the microbial populations have been examined during start-up of a two-stage anaerobic digestion system (Anderson *et al.* 1994). The number of methanogens and non-methanogens slightly decreased in an up-flow filter, while the number of acidogens was constant under the pre-acidification stage. Fukuzaki *et al.* (1991) characterized the granules from a lactate-fed UASB reactor, and found that *Methanosaeta* spp. appeared to be the dominant bacteria species in the 0.2 to 0.6 mm diameter granules. Fang *et al.* (1994, 1995) observed that granules from an UASB reactor treating brewery wastewater have a complex three-layered structure. In these three layers, acidogens dominated in the outer layer, the middle layer contained syntrophic micro-colonies that were composed mainly of hydrogen-consuming methanogens and hydrogen-producing acetogens, and the inner layer of the granule is dominated by *Methanosaeta* spp. Macleod *et al.*, (1990) suggested that the presence of *Methanosaeta*-like cells in the central core of the granules might function as nucleation centers that initiated granule developments. The microbial populations in four different laboratory-scale anaerobic reactors, including UASB, AF, anaerobic fluidized bed reactor (AFBR), and anaerobic contact process, are found to be similar during the start-up of these systems treating ice-cream wastewater. A *Methanobacterium formicicum*-like organism became the dominant fluorescent methanogen in all these reactors (Morgon *et al.*, 1991). *Methanococus* is found to dominate in the sludge applied in the reactors, including the pilot-scale, cross-flow and ultrafiltration membrane anaerobic reactor systems, treating brewery wastewater during startup and steady state operations (Ince *et al.*, 1995).

Regarding the distribution of biomass in the anaerobic reactors, Macarie *et al.* (1992) observed that the biomass is distributed along the column of the down-flow tubular fixed-film reactor, whereas, for the UASB reactors, the biomass is located at the bottom of the reactors. Moreover, it has been considered that resistance to aromatic toxicity (e.g. terephthalic acid) may be greater when the bacteria are fixed on a support medium (Marcarie *et al.*, 1992). Dwyer *et al.* (1986) determined that a consortium of bacteria, composed of a phenol oxidizing bacteria, a *Methanosaeta*-like bacteria and an H_2-utilizing methanogen, can tolerate higher concentration of phenol when it is immobilized in agar than in the form of suspended solids. With regard to the solid colonization of biofilm on supported medium, the following processes may be occurring, including (i) cell transportation to the surface by diffusion, convection and active movement, (ii) initial adhesion – a physical driven process, (iii) biofilm growth, when biopolymers bind strongly to the surface and superficial organic films are formed, (iv) colonization, while cells become firmly attached to the support, begins to reproduce and forming micro-colonies, (v) actual biofilm formation (Tessele *et al.*, 2002; van Loosdrech *et al.*, 1990; Charaklis, 1984; Meraz and Alvarez-Ramirez, 2000).

Tessele *et al.* (2002) observed that the initial adhesion of biofilm on polypropylene particles (< 4mm) in the down flow anaerobic fluidized bed reactors occurred within the first 6 hours and completion of the biofilm structure was achieved after the 44^{th} day. The presence of attached cells morphologically similar to *Methanotrix bacilli* and *Methanosarcina* spp. was observed under scanning electron microscopy.

Shin *et al.* (1992) examined the granular sludge obtained from a two-phase UASB reactor treating for a high concentration of distillery wastewater. The acidogenic granular sludge, formed after 90 days of operation, consisted of long chains of large rods, short plump rods and cocci of various sizes. The acidogenic granular sludge has different shapes and cytoplastic appearances that are commonly found in methanogenic bacteria. The long multi-cellular filaments of *Methanosaeta* spp., with diverse entrapped bacteria, are prevalent in the methanogenic granular sludge that was formed on 120th day of operation. Chua *et al.* (1996) examined the biofilm collected from an anaerobic filter treating a simulated pharmaceutical effluent, which contained a branched-chain fatty acid (BCFA). Chua *et al.* (1996) also observed that the biofilm was a consortium of (i) BCFA-degradating *Syntrophomonas* spp. that produced acetate and H_2, (ii) H_2-utilizing *Methanococcus* spp., and (iii) acetate-utilizing *Methanosaeta* spp.

Jawed and Tare (2000) conducted a post-mortem examination of down-flow (DAF) and up-flow anaerobic filters (UAF) after operating these two filters under similar sets of conditions, but varying organic loads, for more than 20 months. It has been revealed that a considerable volume of the filter (e.g. 42% in DAF and 49% of UAF) was entrapped by retained biomass, leading to significant reduction in operating HRT. Retained biomass, both suspended and entrapped in the packing media, appeared to be considerable black, brown-black and brown granular solid fractions. The brown granules were spongy in nature. The sludge solids in the packing media of the DAF were unevenly distributed when compared to the UAF. Solids retained in the DAF packing media were mostly composed of black granules (3-5 mm in size), whereas solids retained in the UAF packing media were brown-black granules (2-3 mm in

size). The retained granular solids in the DAF packing media appeared to be more compact as compared to those in the UAF. In addition, the estimated concentration of retained solids in the DAF packing media (e.g., 76.4 g TS/l) was significantly higher than that in the UAF (e.g., 69.9 g TS/l).

2.4 Factors Affecting the Anaerobic Process

There are a number of factors affecting the anaerobic process. These include temperature, pH, availability of nutrients, partial pressure and presence of toxic substances, and SS content in the system. Some of these factors are discussed in the following sections.

2.4.1 Temperature

An anaerobic treatment process, like all other biological processes, is strongly affected by temperature, which affects the microbial activity. Production of methane from an anaerobic process is also closely related to the temperature of the reactor. It has been observed that methane production is most favorable at temperatures ranging from 0 to 60 ^{o}C (Kotze *et al.*, 1969; Svensson, 1984). In general, at higher temperatures, faster reaction rates of the process can be attained, thus permitting the reactor to operate at higher loading rates without dampening its conversion or removal efficiency. For a mesophillic range of temperature (30 to 40 ^{o}C), the anaerobic process is best operated between 35 to 40 ^{o}C. The optimal temperature for the thermophilic bacteria in the range is around 55 ^{o}C.

A satisfactory level of performance for an anaerobic biofilter and other fixed-film reactors can usually be attained under the messophilic range of temperature, ranging from 25 to 38 °C. However, in the treatment for complex organic wastes, an initial hydrolysis is required , which needs to be carried out at temperatures above 25 °C. This hydrolysis process has been considered as the rate-limiting step affecting the overall reaction.

It is also feasible to operate an anaerobic treatment process under a thermophillic range of temperatures (e.g. 50 to 60 °C). However; it may not be justified for energy recovery. It is believed that energy consumption for raising the temperature of the reactor from 35 to 55 °C is much greater than the value of the additional methane recovered after the process (Young, 1991).

The influence of temperature on the rate and extent of the anaerobic process in the mesophillic range of temperature has been studied by a number of researchers (Van den Berg, 1977; Lettinga, 1978; Stander, 1976; van den Berg *et al.*, 1976; Kennedy *et al.*, 1981; and Dan Man, 1990). The effect is not only confined to the process rates, but also the extent of the anaerobic reaction (Rourke, O. 1968; Van der Last, 1991).

Rourke (1968) observed that there is a strong temperature-dependence relationship in primary sludge digestion. The removal rate of the organic material decreased from about 68% at 35 °C to about 38% at 15 °C. The decrease in the fraction of organic matter resulted in a low rate of hydrolysis. In that sense, suspended organic matter can be removed from the water phase at low temperature, even when it is not yet

metabolized. As a result, organic matter is entrapped in the sludge bed, becoming part of the sludge, and ultimately discharged from the system as excess sludge.

De Man *et al.*, (1988) demonstrated that specific sludge activity depended on temperature and re-circulation flow using expanded granular sludge bed reactors (ESGB). It has been reported that a UASB reactor fed with VFAs at 75 °C was less stable than a similar UASB reactor at 46 - 64 °C, and the process was limited by the wash-out of active biomass (Van Lier *et al.*, 1992a).

Lepostö and Rintala (1996) reported that removal of VFAs (mainly acetate and propionate) in hot wastewater by a USAB reactor operating at 76 - 80 °C is also feasible. However, the acetate and propionate removals are temperature dependent. The maximum removal efficiency of acetate at 76 °C is about 65% and at 80 °C is about 46%. Propionate removal cannot be achieved at 80 °C. However; about 10% of it can be removed at 76 °C. It is possibly true that 80 °C is above the upper temperature limit for thermophilic anaerobic propionate oxidation. The optimum temperature for thermophilic propionate oxidation is 55 - 60 °C, as reported by a number of researchers (Stams *et al.*, 1992; Van Lier *et al*, 1993a; Lepostö and Rintala, 1996). Propionate oxidation, under the thermophilic anaerobic conversion of VFA condition, has been reported to be susceptible to temperature changes (Van Lier *et al.*, 1992a; Van Lier *et al.*, 1993a).

Using experimental on-site USAB reactors for treating domestic wastewater anaerobically, Bogte *et al.* (1993) showed that the process efficiency is highly dependent on temperature. At temperatures below 12 °C, settling predominantly

contributes to the wastewater purification, and microbial degradation is significant when the temperature is above 12 °C. Ahn and Forestor (2000) made a comparison of mesophilic (35 °C) and thermophilic (55 °C) anaerobic up-flow filters in treating the starch based wastewater at a series of organic loading rates. At an organic loading up to 8.3 kg COD $m^{-3}d^{-1}$, there was no difference in the performance of these two types of reactors in terms of soluble COD (SCOD) and gas production. However, at the higher organic loading rates of 12.4 and 17 kg COD m^{-3} d^{-1}, the thermophilic filter was found to perform better than the mesophilic filter, with a SCOD removal of 93% compared to 78% at the former loading rate and of 88% compared to 55% at the latter one. The daily methane production from the mesophilic digester was also lower with 3.19 ld^{-1} compared to 4.98 ld^{-1} for the thermophilic digester at the loading rate of 12.4 kg COD m^{-3} d^{-1}, and 2.24ld^{-1} compared to 6.18 l d-1 at the loading rate of 17 kg COD m^{-2}.

2.4.2 pH

The influence of pH value on the stability of the anaerobic process is of paramount importance. This is because methogenesis proceeds at a high rate only when the pH is maintained in the neutral range, around 7. The optimum range of pH for the anaerobic process is from 6.6 to 7.6 (McCarty, 1964b). It is a good indication of the performance of the system when the pH deviates significantly from the desired optimum range, indicating an imbalance or failure of the system. When the pH value of the anaerobic system is higher than 7.8 or lower than 6.3, methogenesis decreases significantly and acidogensis is less sensitive to the fluctuation of pH value of the system. As a result, acid fermentation will prevail over the methanogenic fermentation, resulting in a souring of the system. Clark and Speece (1970) observed that low pH (e.g. < 6)

exerted the bacteriostatic effect on acetate- fermenting methanogens in an anaerobic filter, while a pH between 6 and 8 is not inhibitory.

Zoetemeyer *et al.* (1982) showed that propionate, as a major product of acidogensis of glucose, was not formed in an anaerobic digestor when the pH was above 6.0. The pH value of the system is also an important parameter in the post-treatment of anaerobically digested sewage. At high pH, the phosphate content present in the water phase tends to precipitate, while the nitrogen (e.g. ammonia) content can be removed by stripping or precipitation even by poorly mineral struvite (Mg (NH_4) PO_4) (Adrianus, *et al.*, 1994) .

Chen and Hashimoto (1996) determined the effects of inoculum to substrate ratio and initial medium pH on methane production from glucose as a substrate. Both substrate to inoculum solids ratio (SISR) and initial medium pH can affect the duration of inactivity of methane production. It decreased when SISR decreased or when a higher initial pH was used. With an SISR of 4.8 and a pH of 7.0 or 7.2, the inactivity period is eliminated, indicating satisfactory coupling of the acid - and methane - forming phases.

Inanc *et al.* (1996) found that the pH of the CSTR reactors was a useful parameter for selecting the dominant species of acidogenic bacterial populations. The propionate producing bacteria species are inhibited by low pH (e.g., pH around 5), while butyrate producers are favored under such circumstances.

Methanogensis is found to be inhibited at pH greater than 8 and sulfate-reducing predeominated when treating synthetic wastewater with VFAs and sulfate using a thermophilic (55 °C) USAB reactor (Visser *et al.*, 1993b).

2.4.3 Toxic Compounds

Anaerobic processes, like other biological processes, are extremely sensitive to a number of toxic substances in wastewater, including hydrogen ion concentration [H^+], heavy metals, chloro-organic compounds, sulfide, and oxygen.

2.4.3.1 Oxygen

Ambient free oxygen can be introduced into the anaerobic system via the influent of the distribution system. However, oxygen can normally be consumed under oxidative metabolism in the acidogenesis process, and thus no dissolved oxygen is maintained in the anaerobic reactor. Accidental leakage, due to a brakdown of the piping networks, or the loosening of the air-tight cover of the anaerobic reactor can also contribute a significant amount of toxic oxygen to the anaerobic system.

2.4.3.2 Ammonium

High concentrations of ammonium (> 1000 mg NH_4-N/L) present in the anaerobic system have been reported to inhibit the granulation process in the USAB reactors (Hulshoff Pol *et al.*, 1983). Other researcher also indicated that when the ammonium concentration is greater than 2000 to 3000 mg NH_4-N/L, significant inhibition of

methanogensis is induced by the digested sludge and granular sludge (Koster and Lettinga, 1984). In addition, a high concentration of sodium (e.g. 5 to 10 mg/L) has been observed to inhibit the utilization of acetate by methanogen growth in both granular sludge and digested sludge (Liu *et al.*, 1985; Rinzema *et al.*, 1988).

2.4.3.3 Sulfide and Sulfate

Sulfide is usually formed after the sulfate reduction process. Rinzema (1989) reported that up to 50 mg/L of sulfide was encountered in the anaerobic sewage system, but it was still far below the concentration required for a noticeable toxic effect on the system.

The common problems caused by the presence of sulfate that are commonly encountered in anaerobic treatment processes are (i) sulfate is a strong inhibitor of methanogenesis after reduction to hydrogen sulfide, (ii) H_2S release to the biogas stream causes corrosion downstream, (iii) sulfide is malodorous and exerts a high oxygen demand in the effluent and (iv) sulfate-reducing bacteria competed with other bacteria associated with methane production for the substrate. Li *et al.* (1996) reported the interactions between the methane-producing bacteria and sulfate-reducing bacteria were strongly dependent on the COD/S ratio in wastewater. Sulfate reduction is predominant when the COD/S ratio is significantly lower than 1.0. In an anaerobic reactor when treating wastewater containing a COD/S at a ratio of 6, methanogenesis is inhibited by the high concentration of total sulfide (e.g. 330 mg/L) and free hydrogen sulfide (e.g. 50 mg/L). Ram *et al.*, (1995) also observed that sulfate could inhibit methanogensis. However, there is no inhibitory effect on acetate degradation.

The toxicity of sulfide to methanogenic bacteria, due to the undissociated H_2S, has been studied extensively. A 50% methanogensis inhibition was reported at H_2S concentrations ranging from 50 to 250 mg/L (Kroiss *et al.*, 1983; Karhadkar *et al.*, 1987; Koster *et al.*, 1986; Oleskiewicz *et al.*, 1989). Complete inhibition on the growth of the sulfate-reducing bacteria (e.g. *Desulfovibrio spp.*) was observed at concentrations of 550 mg/L and 350 mg/L of H_2S, at pH levels of 6.2 to 6.7 and 7 (Reiss *et al.*, 1992; Okabe *et al.*, 1992). Parkin *et al.* (1990) reported that dissolved a sulfur content, at concentrations of 100 to 800 mg S/L, has a toxic effect on methane formation. It was also observed that free hydrogen sulfide concentrations, ranging from 50 to 200 mg S/L, inhibit degradation of substrates, including acetate, propionate, lactose, etc., as well as methane formation (Koster *et al.*, 1986; Hilton and Oleszkiewicz, 1988; Li *et al.*, 1996). Ascensiounoz *et al.* (1994) found that increasing the concentration of sulfate in the anaerobic treatment process exerted a negative effect on methane production from domestic sludge.

2.4.3.4 Heavy and Trace Metals

The effect of heavy metals, including copper, lead, cadmium, chromium, and zinc, at various concentrations, ranging from 20 to 200 mg/L, on the anaerobic metabolism of acetate, propionate, butyrate, and H_2, was evaluated in serum bottle microcosms (Kong *et al.*, 1994). The biodegradation of the organic acids was highly inhibited by chromium (IV) and copper and somewhat less by lead. For all the heavy metals tested, the degree of inhibition increased with increasing metal concentration, However, methanogensis was less inhibited by the metals tested at all concentrations.

Haghighi-Podeh *et al.*, (1996) studied the fate and toxic effect of nitrophenols (e.g. 2 - 4 nitrophenols and 2,4 nitrophenol) on anaerobic treatment systems. The results show that up to 10 ppm of 2 and 3 nitrophenols and up to 5 ppm of 4-nitrophenols and 2,4-nitrophenols did not caused any toxicity. However, higher concentrations of these toxicants either cause a reversible inhibition (with recovery after a few days) or an irreversible inhibition leading to system failure. It was also showed that 4-nitrophenol can be removed by methanogenic bacteria using the anaerobic filters, with a removal efficiency of about 81% and with no formation of amino-phenols as by-product (Bhattacharya and Nandipati, 1991).

Chacin and Forster (1994) evaluated the effect of copper and lead on the performance of the reactors by using a two-phase (e.g. methanogenic and acidogenic) anaerobic reactor fed with a starch-based substrate. The copper had a more significant effect on the performance of the acidogenic reactor than did the lead.

The effect of trace metals (e.g., Fe, Ni, Co, and Mo) on the performance of reactor, using a lab-scale UASB reactor that fed was with molasses spillage at OLR from 5 to 21.5 kg COD/m^3·d, has been studied (Espinosa *et al.*, 1995). Addition of trace metals to the influent reduced significantly the level of the accumulation of VFA by 94% (e.g. propionate (5291 mg/L to 251 mg/L), and acetate (1100 mg/L to 158 mg/L)). The COD removal efficiency increased from 44% to 58%, the biogas production increased from 10.7 to 14.4 L/d (NTP) and the specific sludge methanogenic activity increased from 0.085 to 0.32 g CH_4-COD/ g VSS · d with propionate as substrate (Espinosa *et al.*, 1995).

2.4.4 Alkalinity and Volatile Fatty Acids

The alkalinity and concentration of volatile fatty acids (VFAs), including acetate, propionate and butyrate, present in anaerobic system are inter-related. Alkalinity has the buffering capacity to maintain an ionic equilibrium of the system. Under stable conditions, bicarbonate alkalinity is approximately equal to total alkalinity. However, when the concentration of VFAs increases, the bicarbonate alkalinity is neutralized. The anaerobic process becomes unstable and toxic to methanogenic bacteria (methanogen) when the pH decreases, resulting from the accumulation of volatile fatty acids due to the depletion of bicarbonate alkalinity. McCarty (1964b) reported that the anaerobic reactor can still be operated if the bicarbonate alkalinity is in the range of about 2500 to 5000 mg/L, even there is a large increase in the concentration of VFAs fatty acids in the system.

Volatile fatty acids are formed after methane fermentation and these contribute about 70% of the total methane production from the anaerobic system (McCarty, 1964a). The formation and utilization of VFAs by methogenic bacteria are maintained in a balanced situation when anaerobic reactors are operated under healthy and stable conditions. Any factors that affect the imbalance of VFAs production or inhibition of the activity of methogenic bacteria could lead to accumulation of VFAs and thus decrease the pH and alkalinity of the system. To offset this undesirable effect, the buffering capacity of the system should be maintained with the presence of an adequate amount of alkalinity (Borja *et al.*, 1995). Duran and Speece (1998) demonstrated that the propionate concentration dropped significantly from 1050 mg/L to 300 mg/L after incorporation of the high F/M contact anaerobic reactor in the

preliminary stage. The results showed that the contact time and F/M ratio in the high F/M reactor were critical parameters in determining the efficacy of the two-stage configuration of the anaerobic reactor.

2.4.3.5 Others

Pavlostathis and Sridhar (1994) used batch reactors to study the inhibitory effect and the biodegradability of photo-processing wastewater under anaerobic conditions. A partial inhibition of methanogensis is observed in the initial phase of the study resulting from the competition between SRB and MPB. However, methanogensis can be recovered under prolonged exposures to this wastewater being treated. Semi-continuous flow anaerobic digesters fed with activated sludge and photo-processing wastewater have not shown any adverse effects.

Huang and Pinder (1995) reported that calcium could enhance the activity and stability of an anaerobic acidogenic biofilm reactor when it was operated under an optimum concentration of 100 to 120 mg/L. At a higher concentration of calcium, a decrease in specific activity is observed. Increased sloughing occurred when a biofilm acclimated to higher calcium concentrations was transferred to lower calcium concentration conditions.

Choo and Lee (1996) studied the mechanisms of membrane fouling, by using a membrane-coupled anaerobic bioreactor (MCAB) in the treatment of alcohol-distillery wastewater. It was observed that the external fouling is mainly attributable to the membrane fouling of the reactor. The major inorganic polutant responsible for

membrane fouling was identified as struvite (Mg (NH_4) PO_4 * 6 H_2O), limiting the membrane permeability by the deposition of inorganic polutants in the biomass attached to the membrane surface. It was also reported that the inhibition of propionate removal in the anaerobic treatment system is attributable to the presence of acetate (Fukuzaki *et al.*, 1990; van Lier *et al.*, 1993a). Nitrate and molybdate can also show the inhibition of both the sulfate reduction and methanogensis in the system (Ram *et al.*, 1995).

2.4.5 Long - Chain Fatty Acids

During anaerobic degradation, organic substrates, such as lipids, are readily hydrolyzed to long chain fatty acids (LCFA) and glycerol, whilst the degradation of LCFA via the β-oxidation process to acetate has been regarded as the rate-limiting step (Novak and Carlson, 1970; Rinzema *et al.*, 1994). The long chain fatty acids (LCFA) are well-known inhibitors to a number of anaerobic microbials even at milli-molar concentrations (Koster and Cramer, 1987; Hwu *et al.*, 1996). The presence of long chain fatty acids ultimately caused serious problems in the anaerobic treatment process (Rinzema, 1988). It is believed that both acetogens and methanogens suffered from LCFA inhibition since these two microbials are related to the β-oxidation process of straight chain fatty acids (Roy *et al.*, 1986). The effect of LCFA toxicity on an anaerobic treatment system is found to depend solely on the concentration, but not on the concentration to biomass ratio (Koster and Cramer, 1987; Angelidaki and Ahring, 1992; Rinzema *et al.*, 1994). Oleic acid and lauric acid are the most versatile inhibitors among the LCFAs (Galraith *et al.*, 1971; Koster and Cramer, 1987). Rinzema *et al.* (1994) studied the toxic effect of LCFA on the methanogenic and

acetogenic activities of granular methanogenic sludge. A concentration of capric acids, ranging from 6.7 to 9 mol/m^3, has a lethal effect on both methanogenic and acetogenic bacteria. The H_2-producing acetogenic and hydrogenotrophic bacteria recovered more quickly than did the acetotrophic methanogens. The mechanism of LCFA toxicity is probably due to the adsorption of the surface active LCFA onto the cell-membrane or cell wall thus affecting the function of ion-transportation across the cell membrane or the protective function provided by the cell-wall (Demeyer and Henderickx, 1967; Galbraith and Miller, 1973 a,b).

Hwu *et al.* (1996) determined that the LCFA toxicity varied with the type of anaerobic sludge being used. However, its toxicity is more correlated to their physical characteristics (e.g., settle-ability), specific surface area and size distribution than their biological ones (e.g., sludge origin or sludge adaptation). The selection of inoculums for full-scale anaerobic reactors, treating wastewater containing LCFA or lipids, should depend on sludge size rather than wastewater type. Granular sludge is found to be a more appropriate inoculum for start-up, because it showed less susceptibility to the toxicants. Shin *et al.*, (2003) reported that the inhibitory effect of major long-chain VFAs, with 16 or 18 carbons, not only on acetate degradation, but also on propionate degradation and β-oxidation, and the inhibitory effect of LCFA on β-oxidation was slower than that on methanogensis. Lalman and Bagley (2000) reported that linoleic acid, an 18 carbon acid with two double bonds (C 18:2), at concentrations of 30 mg/l or more, completely inhibited aceticlastic methanogensis, but only slightly inhibited hydrogenotrophic methanogensis.

2.4.6 Nutrients

The addition of nutrients to anaerobic reactors, in particular during start-up with wastewater that is deficient in certain trace minerals contents and with imbalanced C: N: P ratio, stimulates the initial biofilm development and the microbial growth, (van den Berg and Lentz, 1977; Mendz *et al.*, 1989). Methanol has been demonstrated to be one of the cheapest nutrients that can achieve this purpose (Fanin, 1984; Stephenson and Lester, 1986). Trace elements, like Co, Fe, Ni, and Mo, are essential for increasing the bacterial activity in order to maintain a high biomass concentration in the system (van den Berg *et al.*, 1980; Murry and van den Berg, 1981).

In the UASB process, a similar effect of trace elements on enhancing the performance of the reactors has also been observed. Trace elements (e.g., Ni, Mo, and Co) could enhance methanogenic activities in lab-scale anaerobic digesters (Takashima and Speece 1988), increased biomass in anaerobic filters (Murray and van den Berg, 1981), and stimulated granule formation in up-flow anaerobic sludge bed-filter reactors (Guiot *et al.*, 1988). Ram *et al.* (1995) observed that the presence of sulfate could enhance propionate, butyrate and valerate degradation during the anaerobic oxidation of VFAs in rabbit waste at 20 °C. Syutsuto *et al.* (2001) reported that changes in feed composition could affect the microbial community structure where thermophillic granular sludge was used as the inoculum source when operating a thermophilic UASB reactor at 55 °C.

2.4.7 Partial Pressure

The effects of partial pressure on the anaerobic process have been evaluated by some of the researchers. Fukuzaki *et al.*, (1990) reported that propionate removal could be inhibited under high H_2 partial pressure conditions. Ahring and Westermann (1988) found that thermophilic (60 ^{o}C) butyrate degradation is inhibited at a hydrogen partial pressure of 2 x 10 $^{-2}$ atm, and is partially inhibited at an acetate concentration of 1025 mg COD/L in the absence of acetate-utilizing methanogens in an anaerobic process carried out in a co-culture of butyrate-degrading bacterium in syntrophic association with *Methanobacterium thermoautotrophicum.*

An increase in hydrogen partial pressure in the anaerobic treatment system prevents the production of hydrogen by the acidogenic bacteria and affects the metabolic pattern of the fermentation, leading to more production of propionate (Sykes, 1970; Boone, 1982; Kaspar and Wujrmann, 1978; Barnes, *et al.*, 1983; Mosey, 1983; Fynn and Syafila, 1990; Harper and Poland, 1986; Mosey and Fernandes; 1989). A shift of the metabolic pattern towards the formation of more oxidized compounds was also observed when the hydrogen partial pressure was reduced (Poels *et al.*, 1985; Harper and Pohland, 1986; Fynn and Syafila, 1990; DeSantis and Friedman, 1989).

However, other studies showed that there was no significant effect on production and decomposition of propionate in an anaerobic biofilm by the external addition or physically removal of hydrogen in the gas phase and bulk liquid (Denac *et al.*, 1988; Harper and Pohland, 1990).

On the contrary, Inanc *et al.*, (1996) found that continuous removal of hydrogen by strong vacuum means or artificial elevation of the hydrogen partial pressure by the external addition of the gas did not shown any effect on the accumulation of propionate in glucose-fed CSTR reactors, and concluded that the production of propionate from glucose seems to be independent of the hydrogen partial pressure. The population dynamics in th acidogenic phase was the most likely factor to determine the end-product distribution as well as the propionate accumulation during organic overloading.

The effect of H_2 partial pressure on the sulfur dioxide reduction by *Desulfotomaculim orientis* had been studied (Lee and Sublette, 1994). At high H_2 partial pressures, SO_2 is completely reduced to H_2S, whereas at low H_2 partial pressures, SO_2 is oxidized to SO_4.

2.4.8 Suspended Solids

The USAB process has been reported as being susceptible to the presence of suspended solids (SS) in the wastewater. The SS may inhibit sludge granulation (Letting *et al.* 1980) and they can also affect the methanogenic activity of the sludge (Sayed, 1987). Under some extreme circumstances, a sudden acidification of the content of the reactor caused by SS has been reported (Lettinga, *et al.* 1980). To avoid the potential adverse effect of SS on the USAB process, Souza (1986) suggested that the SS:COD ratio in the wastewater and the SS level should be maintained at less than 0.5 and below 1000 mg/L. Lettinga and Hulshoff Pol (1991) also suggested that installing a settling pre-treatment device for SS removal or restricting the volumetric

loading ratio to 2 - 8 g COD/L-day. Kwong and Fang (1996) found that, despite the insoluble nature of cornstarch, cornstarch particulates wastewater treated by the UASB and the modified anaerobic filter reactors, has shown no effect on the inhibition of the sludge granulation nor impaired the COD removal efficiency of the reactors.

2.5 Impact of Shock Loadings on Anaerobic Process

2.5.1 Effect of Hydraulic Loading Rate (HLR) on Anaerobic Process

The anaerobic process was reported as being sensitive to shock loadings of both hydraulic and organic natures. Sachs *et al.* (1982) reported that anaerobic reactors, when treating pharmaceutical wastewater of 2000 mg COD/L, failed at hydraulic retention times (HRT) of about 2 days. This could be a serious disadvantage especially where moderating and balancing facilities are insufficient (Boardman *et al.*, 1995; Borja, *et al.*, 1995; Chua *et al.*, 1995a). When an anaerobic biofilter suffers hydraulic and organic shock loadings, it usually results in the souring of the biomass from the support media and process failure. (Chua *et al.*, 1995b)

Chua and Cheng (1995) operated an adsorption-anaerobiosis column treating wastewater with an inhibitory concentration of two branched-chain fatty acids (e.g., neophentanoic acid and 2-ethylhexanoic acid). When the system was maintained at 2 days HRT, the anaerobic treatment system eliminated 75% of the neophentanoic acid present in the contaminated water at a concentration of 50 mg/L and 98% of the 2-ethylhexanoic acid present in the contaminated water at a concentration of 100 mg/L. The column could be recovered from a shock loading condition, equivalent to a 150%

increase in hydraulic and organic loads, after a sudden decrease of HRT from 5 to 2 days. Additionally, Chua and Cheng (1996) operated a novel anaerobic biofilter, treating food-processing wastewater, with 4500 mg COD/L. The efficiencies of COD and grease removal were 85% and 53%. These were achieved when maintaining the anaerobic filter at an HRT of 1.5 days.

2.5.2 Effect of Organic Loading Rate (OLR) or Critical Shock Loading on Anaerobic Process

Coverti *et al.* (1993) observed that the efficiencies of COD removal and methane production rates are affected by the OLR of a fluidized-bed reactor fed with municipal wastewater supplemented with glucose. The maximum theoretical specific COD degradation rate is estimated to be 1.76 g/g·d of VSS.

The effect of HRT and OLR on the performance of an anaerobic membrane bioreactor has been studied by using a synthetic wastewater containing starch as the sole carbon substrate. The membrane flux ranged from 10 to 20 $L/h \cdot m^2$ is not limited when the volatile solids content is less than 40 g/L (Cadi *et al.*, 1994).

The floating of the granules in UASB reactors or the sloughing of the biomass from AF reactors are common problems encountered after the anaerobic reactors have suffered a shock load (Samson *et al.*, 1984; Kalyuzhni, *et al.*, 1996). It has been observed in laboratory-scale experiments that UASBs are more sensitive to shock loads than anaerobic filters (van den Berg *et al.*, 1991). Smith (1995) reported that anaerobic/aerobic reactors connected in sequence were resistant to hydraulic and organic shock loadings.

In 1995, Borja *et al.* studied the effect of organic loading rates (OLR) (2.9 to 54 g COD/L-day at HRT 0.5 to 8 hr, feed COD concentrations 250 to 4500 mg/L), on the anaerobic treatment of slaughterhouse wastewater in a fluidized-bed reactor, and found that (i) methane production was independent of the OLR applied in the reactor, (ii) the efficiency of COD removal decreased linearly with an increase of the OLR over the range tested, and (iii) the higher the OLR applied, the higher the volume of effluent COD obtained which is largely composed of the unused VFA produced in the reactor.

Kalyuzhnyi *et al.* (1996) studied the performance of a laboratory scale UASB reactor fed with synthetic wastewater, under various organic loading rates (OLRs), ranging from 3.4 to 44.9 g COD/L-day, for organic removal. The distribution of substrates, intermediate by-products, pH, VSS, and specific sludge activities (e.g., acidogenic (g glucose/g VSS-day), acetoclastic (mg CH_4 /g VSS-day), and lithotrophic (mg CH_4/g VSS-day) etc.) in the reactor were affected. For instance, under organic shock loading (e.g. immediately after changing the OLR), some disturbances, such as the appearance of large gas bubbles in the sludge bed, partial sludge flotation, and destruction of granules, are observed as the direct results of the temporary overloading of the reactor. Also, with an increase of the OLRs, a substantial increase of *Methanosarcina* in the granules has been observed. The undesirable disturbance caused by a shock loading may disappear after a period of time, and the anaerobic reactor can be recovered after temporary inhibition (Chua *et al.*, 1995b; Chua *et al*, 1996).

Kwong and Fang (1996) operated the UASB and the modified AF-reactors in parallel with a built-in gas liquid separator to treat wastewater containing a high concentration

of cornstarch. Both of these reactors removed 95.3% of the soluble COD containing starch as the sole organic substrate at organic loading rates from 3 g COD/L-day up to 90 g COD/L-day. There is no noticeable accumulation of starch particulates in the reactor. However, the reactors failed at an organic loading rate of 150 g COD/L-day due to a severe washout of sludge.

Ghangrekar *et al.* (1996) demonstrated that a higher loading rate, rather than the sludge loading rate (SLR) = 0.3 kg COD/kg VSS·d and OLR = 4.5 kg COD/m^3·d, might be detrimental to the performance of the UASB reactors. Such organic loadings were not acceptable for the start-up operation. They also observed that SLR influences the efficiency of COD removal at the steady state. An SLR of 0.6 kg COD/kg VSS·d can achieve about 50% of the COD removal under steady state conditions. The performance of the reactor cannot be improved even after three months of operation.

Tsuno *et al.* (1996) reported that an expanded-bed GAC anaerobic reactorm treating PCP-Na at concentrations of 100 and 400 mg/L, could successfully respond to a 4 times shock-load of PCP-Na without a serious increase of total effluent COD. Garcia-Morales *et al.* (2003) studied the influence of operational conditions on the biofilm specific activity of an anaerobic fluidized bed reactor. The results showed a dependence between the percentage of bed expansion and the specific activity of the methanogenic bacteria on the biofilm, and that there is a relationship between the percentage of bed expansion, the shear stress on the biofilm, and the hydrodynamic conditions in the system.

2.6 Physical Factors Affecting the Anaerobic Process

2.6.1 Reactor Configuration

Young (1989) reported that the most common configuration for reactors used in full-scale anaerobic filters are cylindrical and rectangular in shape, the diameters (or widths) of the tanks usually ranging from 6 to 26 m and the heights are in the range 3 to about 13 m. Media height ranges from the full-depth of the reactor to placements only in the upper 50 to 70% of the reactor height.

2.6.2 Media Types and its Placement

Muller and Mancini (1975) were the first to recognize the importance of the media-related factors on the performance of the up-flow anaerobic packed-bed reactors. A plastic medium of lightweight and high porosity allows for a high accumulation of biomass per unit of reactor volume. Hudson *et al.* (1978) observed the effects of packing media on APBR performance, and suggested that a medium with high porosity and specific area could achieve a much higher COD removal.

Many different types of support media have been tested for suitability of biomass retention in both up-flow (UAF) and downflow AF (DAF) reactors. The performance of these materials is most likely determined by the ease of entrapment or attachment of the biomass to the support media. Some critical factors need to be considered when choosing suitable support media. These factors include (i) surface roughness, (ii) porosity, and (iii) availability of leached trace nutrients (Young, J.C. 1991). Various packing media have been used in anaerobic biofilters (van den Berg *et al.*, 1981;

1982). Examples include gravel, commercially available PVC and ceramic supports, and glass beads (Plummer *et al.*, 1968; Sach *et al.*, 1982). Fire-expanded clay (otherwise known as potter's clay), needle-punched polyester beads, folded wire mesh, and polyester foams, of average diameter 0.6 mm - 6.0 cm, are most commonly used because the high porosity and rough surfaces promote bacterial attachment and entrapment (Atkinson *et al.*, 1979; Murray *et al.*, 1981; van den Berg *et al.*, 1982; Bryers, 1982; Fynn *et al.*, 1987; Chua *et al.*, 1990a). Calcium-rich media such as limestone chips and oyster shells are other favorable options because they contribute to the buffering capacity of the system (Price *et al.*, 1985). With a carefully selected medium, the specific surface area available for bacterial attachment can be as high as 200 m^2/m^3, accommodating a bacterial population exceeding 20 g VSS/L (Stronach *et al.*, 1986). The relationships between the types of packing medium and the types of waste being treated have not been explicitly documented. However, it is generally recognized that small-diameter media are not suitable for wastes with high solid content or of high-strength that allow rapid growth of biomass, which may result in the clogging of the packed bed (Young *et al.*, 1982).

Support media, including (i) pall rings, (ii) tubular (corrugated) type, and (iii) cross-flow type, are commercially available for use in anaerobic filters. The channels in the modular block may be either tubular or cross-flow type. The tubular type of support media usually has no lateral flow occurring throughout the height of a block, however; when it is placed counter-stacked, a cross-flow effect occurs at the contact points within the media matrix. When cross-flow media with various specific surface areas are available, the most common design is a corrugated shape, about 25 mm by 75 mm,

and the interstitial channels are placed on an incline of 60° relative to the horizontal (Young, J.C. 1991).

The average specific surface area of support media applied in the full-scale anaerobic filters was about 100 m^2/m^3 regardless of the type of media (Young, J.C. 1991). Young (1983), using corrugated modular blocks of different sizes and shapes, indicated that having media with a high capacity to prevent the washout of the biomass from the reactor is more important than the specific surface area (surface area ratio to volume ratio) of the media.

Oleszkiewicz and Thadai (1988) observed that when comparing the performance of 2.5 cm unglazed ceramic Rashig rings with vertically oriented PVC tubing (ID=7.5 cm) as the packed bed media for hybrid reactors at a loading rate of 1 kg COD/m^3-d, the Rashig rings, with a media volume to total volume ratio of 0.4, appeared to be more effective in retaining the biomass in the system.

Huysman (1983) tested and compared different type of non-porus media supports (e.g., glass beads, activated carbon, argex, zeolite, and sepholite) and porus media supports (e.g., non-reticulated polyurethane foam, reticulated polyurethane foam, polyurethane foam coated with a PVC layer, and natural sponge), and demonstrated that surface roughness, total porosity and pore size were the critical factors affecting the attachment of the bacteria to the media.

Bonastre and Paris (1988) compared different types of support media, including PVC rings, polyurethane foam, red brick, sepholite, and a nickel containing residue

material, and noticed that the red clay materials used have an inhibitory effect on methane production. This may be due to the leaching of some toxic compounds to the biomass of the system. Wilike and Colleran (1984) compared four different types of support media (e.g., plastic, clay, mussel shells, and coral), and observed that by using the clay media, the most rapid start-up of the process and the most effective steady-state performance were achieved. This media has the lowest specific surface area and porosity. Leaching of the inorganic nutrients from the support media that stimulated the growth and activity of methanogenic bacteria might have contributed to the high performance of the system.

Kennedy and Droste (1985) also observed, in the down-flow stationary fixed film reactor, that the needle punched polyster (NPP) and red drain tile clay showed more rapid biofilm development than the other support media used, like potters clay, PVC, or glass. These achievements are probably due to the surface roughness of the support media as well as the leaching of minerals from the clay, which can stimulate biomass activity and enhance its adhesion to the support media.

In fixed-bed reactors, biofilm production, with a thickness of about 1 to 3 mm on various plastic and wood supports, was reported, whereas no film was observed at the bottom or top portions of the reactors. This may be due to the effect of the high turbulence that exists in these areas (Robinson *et al.*, 1984; Albagnac, 1990). Verrier *et al.* (1988) tested various supports, including polytetrafluoroethyene (PEF), polypropylene (PP), polyethylene (PE), PVC, polyacetal (PAC), and polyamide (PAM), with various microorganisms, and concluded that adhesion was affected by both the critical surface tension of the support media and the bacteria itself.

Ascensiounoz *et al.* (1994) studied the effect of the support material siiolite on methane production from domestic sludge and showed that increasing concentration of sepiolite had a negative effect on methane production. Borja *et al.* (1995) studied the effectiveness of an anaerobic fluidized-bed reactor, treating slaughterhouse wastewater, with bentonite of 0.3-0.5 mm (clay particles) as the growth support media, and demonstrated that more than 94% of the feed COD can be removed up to an organic loading rate of approximately 27 g COD/L-day when the HRT is maintained at longer than 2 hours. Methane production is 0.32 L/g COD, that is equivalent to 94% of the theoretical maximum value when using glucose as the starting substrate.

The relationship between filter media and bacterial populations in an anaerobic fixed-bed reactor was studied (Miyahara *et al.,* 1995). It was found that the number of acidogenic bacteria is higher when the reactor is sparsely packed with the filter media than when it is closely packed. However, the opposite is observed with methanogenic bacteria. In addition, when there are more suspended acetogens attached to the media, it is predominately the methanogens which are attached (e.g., about ten times more than those in suspended conditions). Tay *et al.* (1996) examined the effects of media specific area, porosity, and pore size on the performance of up-flow anaerobic packed-bed reactors (APBRs), and reported that the highest COD removal efficiencies of 90% and 73% can be achieved at loading rates of 8 and 16 g/COC/L-day, when the APBR contained the media with the lowest surface area and with the largest pore size and porosity.

Balaguer *et al.* (1991) studied the feasibility of using pumice stone (0.53 mm dia.; sp. gr. = 1.53) as the support media in a laboratory-scale AFBR treating distillery wastewater. At an OLR of 24 kg COD/m^3 d, a COD removal efficiency of 84% is achieved. Allaoui and Forster (1994) compared different support media (e.g., sand, pumice, and sintered glass) used in anaerobic expanded-bed reactors. The porous media were found to be superior to the non-porous media, achieving higher colonization and lower inhibition of the system.

Camargo and Nour (2001) studied the feasibility of using bamboo as an anaerobic medium and its effect on filter column height, and concluded that the efficiency of the filters peaked at a height of approximately 40 cm, which suggested that relatively short columns in anaerobic filters were efficient. The bamboo ring as a medium for microfilm adherence proved to be a relatively efficient and financially feasible alternative for the packing medium. The bamboo ring materials only permitted the transport of solids to the upper layers of the anaerobic filter after biological and physical destruction of the interstitial particles (biological flakes). An equivalent performance was obtained no matter whether the whole or half rings of bamboo weree used. Picanco *et al.* (2001) reported that the porous supports (e.g., polyurethane foam and special ceramic) retained a higher quantity of the biomass than did the non-porous materials (e.g., PVC, and refractory brick). In addition, distinct methanogenic archeas were found to be colonized in different support materials. *Methanosarcia* prevailed in the polymeric supports, while the predominance of *Methanosaeta*-like microorganisms was established in the ceramic materials. The formation of bunches between the pores and cracks of the materials were observed.

2.6.3 The Media - Packing Ratio

The media-packing ratio is considered to be an important design parameter in an anaerobic process, particularly AHR. So far, the packing media ratio used in an AHR system is still controversial. Different researchers in their studies have adopted different packing media ratios. Some researcher used high media packing ratios (e.g., 60 – 70%) in an AHR treating landfill leachate, furfural by-products, and ice-cream wastewater (Chang, 1989; Harris *et al.*, 1992; Hawkes *et al.*, 1995). However, some other researchers used much smaller packing ratios, (e.g., 30%) in the design of the AHR (Fang and Kwong, 1994; Tilche *et al.*, 1994).

Oleszkiewicz *et al.* (1986) studied the effect of media-packing on the performance of an AHR by using four AHRs with total media volume/total reactor volume ratios of 0.5, 0.4, 0.25 and 0.05. They reported that a larger media/total volume ratio would improve the performance of an AHR by retaining more biomass inside the reactor.

Wu *et al.* (2000) also studied the influence of media-packing ratios (e.g. 75%, 60%, 40% and 20% of the total reactor height) on the performance of an AHR and concluded that the media-packing ratio had a significant effect on the performance of the AHRs at high loading rates (e.g. > 16 g COD/l/d). This probably resulted from the hydraulic overloading rather than the organic overloading. The packing ratio had little effect on performance at low loading rates (e.g., < 2 g COD/l/d). They also found that the media-packing ratio exerted an influence on the performance of AHRs at medium organic loading rates (e.g., 4 to 12 g COD/l/d). The distortion of the COD profiles revealed that it was strongly related to changes in the short-circuiting fractions.

In contrast, it has been reported that the depth of media-packing had little effect on the performance of the AHR when using an AHR with a 1/3 or 30% of media/total height ratio (Kennedy *et al.*, 1989; Chun and Choi, 1993).

3. Material and Methods

3.1 Anaerobic Hybrid Assembly

The anaerobic hybrid reactor (AHR) is a conventional up-flow anaerobic sludge bed (UASB) reactor, with an anaerobic filter (AF) inserted in the upper sludge separation zone (Figure 3.1). The column of the AHR is made of plexi-glass, with an internal diameter of 9 cm and a height of 2.1 m . Ten sampling ports that were extended into the axis of the AHR, were evenly distributed along the entire length of the column. The effective volume of the AHR was 6 L. The AF zone of the AHR was packed with to 1/3 of its volume with fire-expanded clay spheres (FECS) as the pack-bed medium and the remaining 2/3 of the volume was allocated as the sludge blanket section (UASB). Each FECS pellet had an average diameter of 1 cm (Plate 3.1).

The AHR was fed with a synthetic wastewater, containing reconstituted milk at a concentration of 1920 mg/L, equivalent to 3000 mg COD/L, to simulate food-processing wastewater. The synthetic wastewater was fed from the bottom of the reactor, through a distributor in an upward direction. Mixing was achieved by re-circulating the liquor at a flow rate of 6 L/h. The liquor was maintained within a temperature range of 20 to 25 ^{o}C throughout the entire operation.

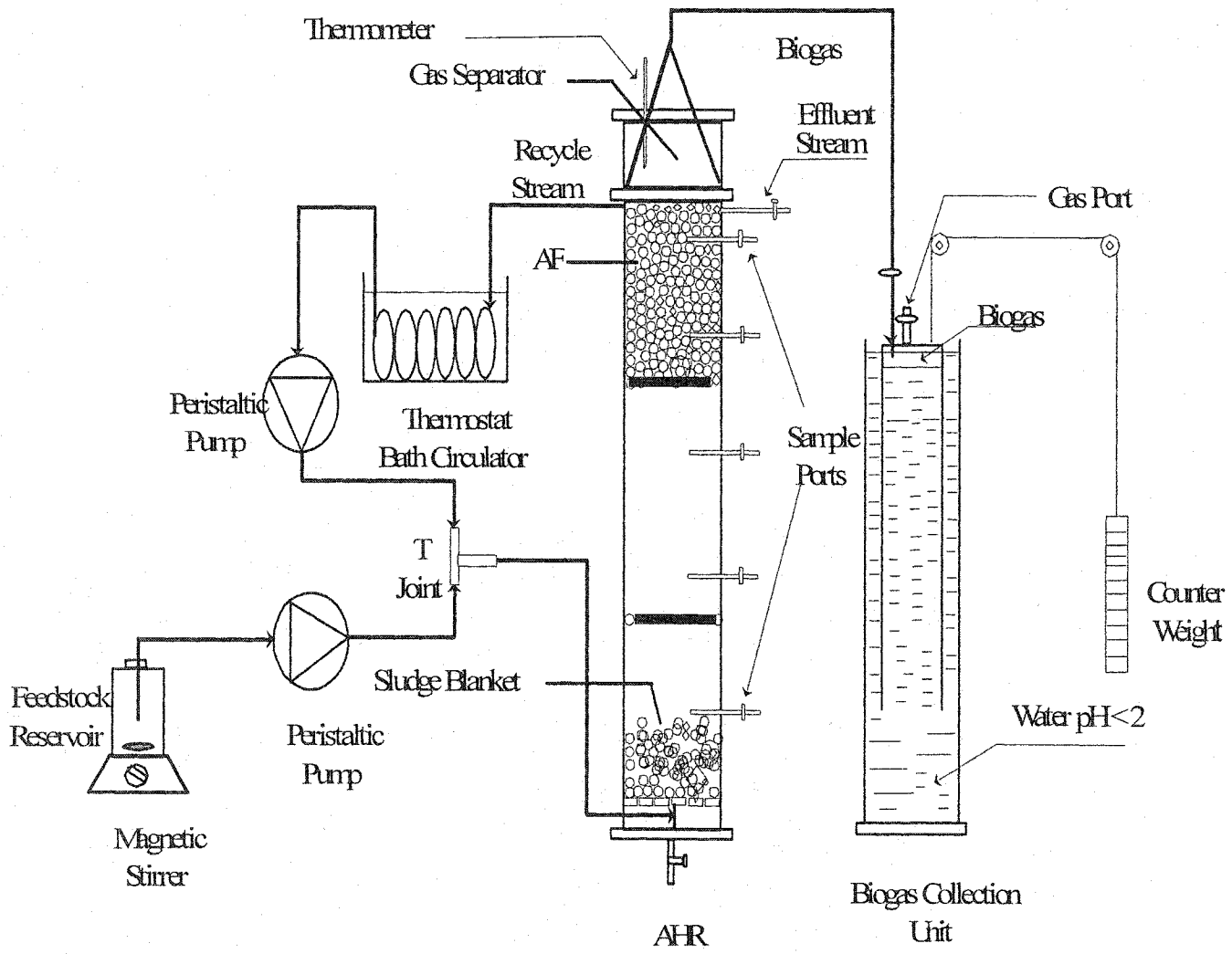


Figure 3.1 Anaerobic Hybrid Reactor

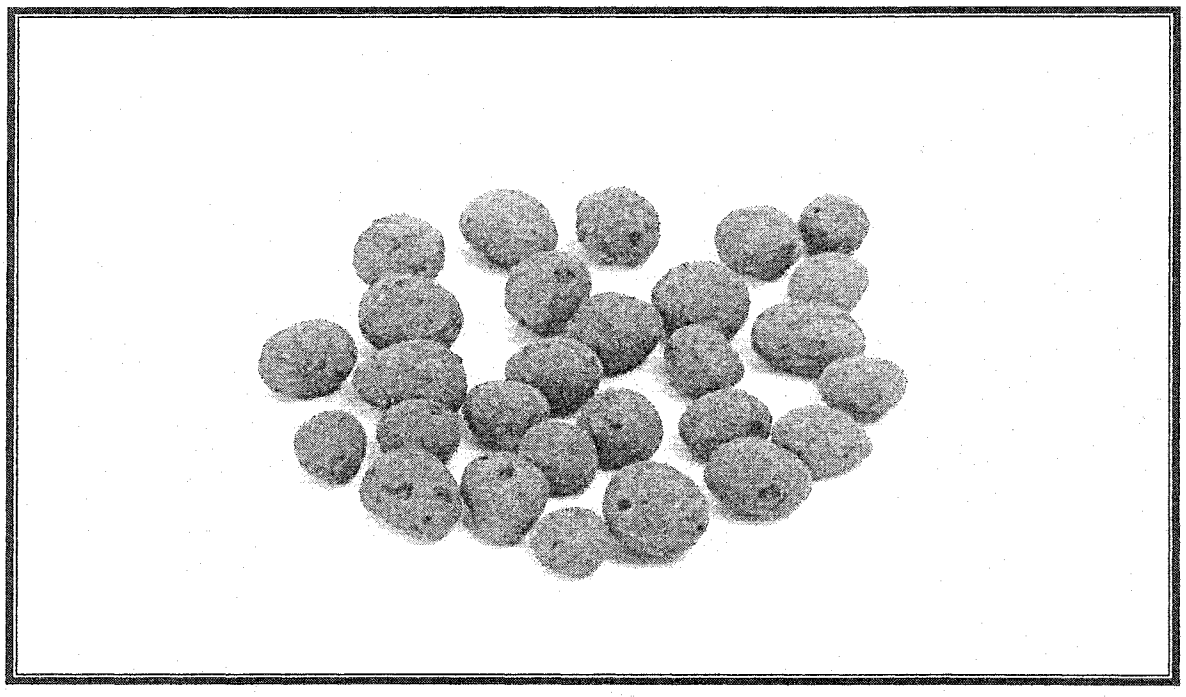


Plate 3.1 Fire-Expanded Clay Spheres

3.1.1 Start-up of AHR

The non-granular digested sludge collected from a sludge digestion reactor of a local sewage treatment works was used as the inoculum of the reactor. The properties of the digested sludge used, in terms of TSS, VSS/TSS and SVI, were 2850 mg/L, 79% and 34 ml/g. The seeding sludge was first screened with a 2 mm sieve then pumped with a multi-head peristaltic pump into the inlet at bottom of each reactor. About 2.4 L of digested sludge was seeded in each of the UASB sections of the AHR. After seeding, the digested sludge was kept under anaerobic conditions without any feeding for 48 hours to eliminate any residual dissolved oxygen prior to the operation.

3.1.2 Shock Loading Experiments

The stability of the AHR was studied using the shock loading method. The AHR became stable after 30 days of operation at an HRT of 5 days. The HRT of the AHR was sporadically adjusted from 5 to 2.5 to 1.25 to 1 to 0.5 and to 0.25 days, to create stepwise shock loadings. During the shock loadings, the organic loading rate (OLR) of the AHR was correspondingly increased stepwise from 1.15 to 46.08 g/Ld while the HRT was correspondingly reduced from 5 to 0.25 days.

3.2 Anaerobic Filter

The individual anaerobic filter is a plexi-glass cylindrical column with an internal diameter of 9 cm and a height of 1.2 m (Figure 3.2). The column was packed with FECS with an average diameter 1 cm. Ten sampling ports, that were extended into the

axis of the biofilter column, were evenly distributed along the entire length of the column. The effective volume of the biofilter was 3 L.

3.2.1 Start-up: Procedure and Operation

The anaerobic filter was seeded with an anaerobic digestion sludge collected from a local municipal sewage treatment plant. The AF was seeded two times over a week. Each time, 3 liters of the sludge sample were screened with a 2-mm sieve to remove coarse particles. The pH of the sludge was adjusted to 7.0 before it was pumped into the biofilter. The AF was stabilized after one month by re-circulating the waste stream. Biofilm, with dense layer of microbial biomass, formed on the surface of the packing medium and suspended flocs were observed in the interstices after 45 days of seeding. After that the biofilter was fed with reconstituted milk at an initial HRT of 10 days.

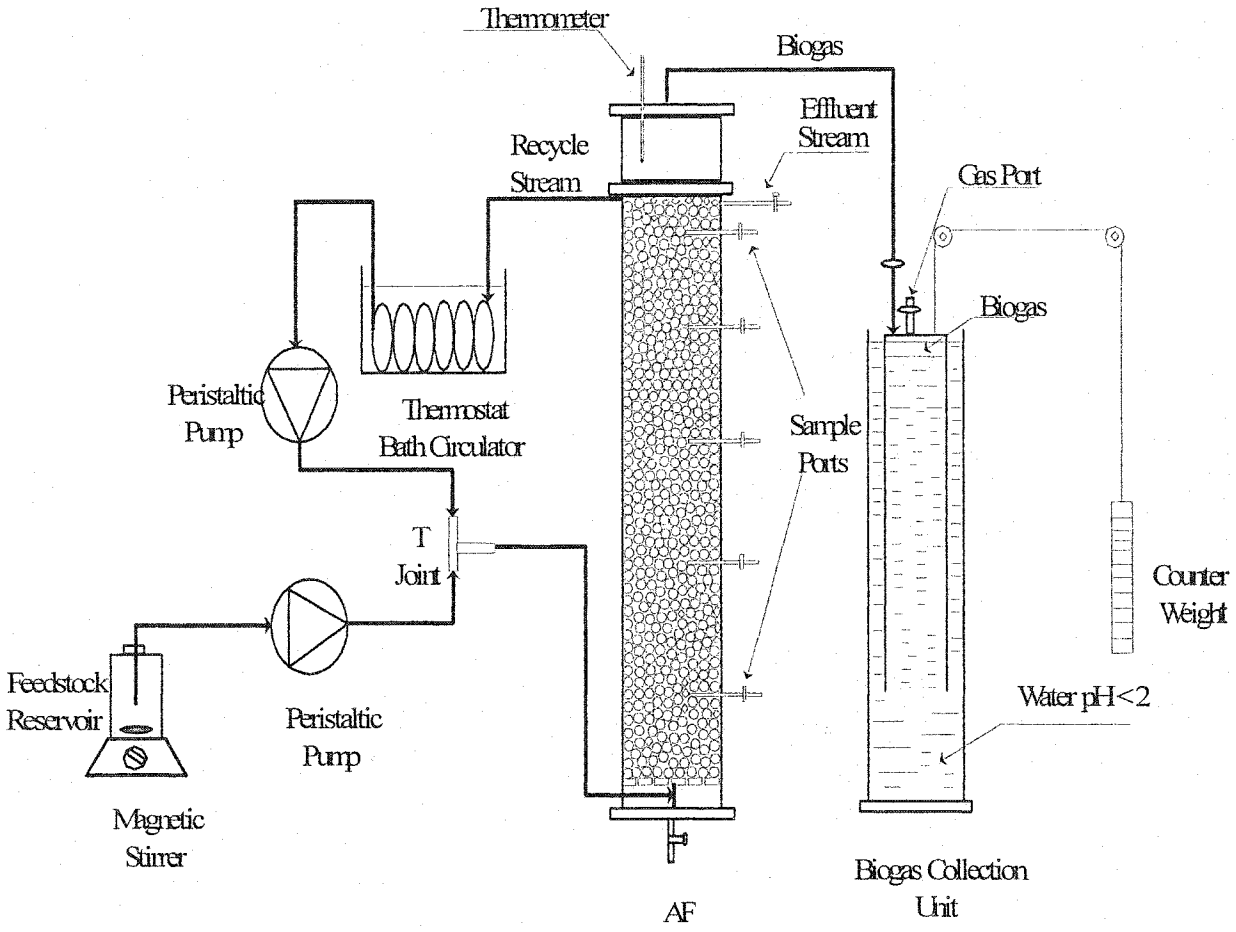


Figure 3.2 Schematic Diagram of Anaerobic Filter

Mixing of the reactor contents in the packed bed was achieved by re-circulating the AF liquor at 3 L/hr. in an up-flow direction. The re-circulation rate was equivalent to replacing the entire liquid content of the biofilter once an hour. The re-circulated stream passed through a coiled brass tube that was submerged in a 40 ^{o}C water bath. The AF was fed with a synthetic wastewater containing reconstituted milk, comprising protein (25.4%), sugars (38.9%), fats (26%), and salts (9.7%), at a concentration of 1920 mg/L, equivalent to 3000 mg COD/L. No other nutrients were provided. The BOD/COD ratio of the reconstituted milk was determined as 0.3 to 0.35, indicating that the components of the reconstituted milk were biodegradable. The AF liquor was maintained ata temperature of 28 - 30 ^{o}C throughout the entire operation. The collecting device for the biogas consisted of an inverted plexiglass tank of 30 L capacity, contained in a second, larger, plexiglass tank. To minimize the dissolution of carbon dioxides, water contained in the biogas collecting device was maintained at pH 2 by the addition of phosphoric acid (H_3PO_4). Measurement of the daily production of biogas was carried out after equilibrating the gas to atmospheric pressure. Sampling of the biogas for an analysis of its composition was made through a rubber septum gas port.

3.2.2 Hydraulic Shock Loadings Experiments

When the operation of the AF and the treated effluent quality were stable at 5 days HRT, the HRT of the reactor was reduced to 2.5 days for a period of seven days, and then returned to 5 days. In that sense, the organic strength of the synthetic wastewater had been reduced to half the initial concentration. This can result in a shock loading equivalent to a 2 times increment in the hydraulic loadings. Similarly, 4, 5 and 10

times hydraulic shock loadings were also generated by reducing the HRT from 5.0 day to 1.25, to 1.00 and to 0.5 days, with concomitant adjustments in the organic strength of the influent.

3.2.3 Organic Shock Loadings Experiments

Under the organic shock-loading experiments, the HRT of the system was adjusted to 1.25 days without any adjustment to the organic strength of 3,000 mgCOD/L. When the performance of the filter was stable, influent COD was increased to 6,000 mg/L for a period of 7 days, and then reduced to 3,000 mg COD/L. This represented a 2 times increment in the organic loading. Using a similar approach, 4, 8 and 16 times organic shock loadings wer introduced by increasing the influent COD from 3000 mg/L to 12,000, 24,000 and 48,000 mg/L, without any change in the HRT.

3.3 Transient Responses of Fatty Acids under Shock Loading Experiments

Analysis of the VFAs content in the treated effluent was carried out under various hydraulic (e.g., 2 to 10 times) and organic (e.g., 2 to 16 times) shock loadings as well as following the recovery period. The treated effluent was first filtered with a membrane filter with a 0.45 μm pore size. The samples were analyzed using the GC in accordance with the procedures as described in 3.4 below. All sample analyses were duplicated.

3.3.1 Exaction of Short-Chain Volatile Fatty Acids for GC Analysis

Two ml of the filtered sample was added to a centrifuge tube that contained 0.4 mL of 50% H_2SO_4. After mixing with a vortex mixer for about 30 seconds, 2 mL of ether and 2 mL of internal standard (ID) C_7, with a concentration of 50 mg/L[1], was added. The tube was sealed and centrifuged at 3000 rpm for 5 minutes in order to break the ether/water emulsion. The centrifuging allows separation of the aqueous and ether layers. 1 μL of the upper layer (ether) sample was injected into the GC for analysis of its VFAs content.

The sample was preserved by removing of the upper layer (ether) of the sample, and transferring it to a small tube containing a small amount of Na_2 SO_4. The tube was then sealed for later analysis.

3.4 Analytical Methods

Analyses for chemical oxygen demand (COD), volatile suspended solids (VSS), and the pH of the treated effluent were conducted in accordance with the Standard Methods of the American Public Health Association (APHA), 19 ed., (1995). All sample analyses were duplicated.

[1] 10 μl of C_7 stock solution (e.g., conc. 1000 mg/L) was diluted with de-ionized water to 2 mL, then the final concentration of ID was 50 mg/L.

The COD was selected as the principle parameter of analysis, because the time required for analyzing the organic content in the samples is only 3 to 4 hours, while the time required for the determination of other parameters, like biological oxygen demand (BOD_5), require 5 days for the completion of a sample analysis. The results of the efficiency of removal of COD are particularly useful for determining the performance of an AHR, under a shock load environment.

The biogas was determined as methane and carbon dioxide, using a Varian Model 3300 gas chromatograph with a 2 m column (3 mm ID) Prorpak Q (mesh size 80-100) support. Column temperature was maintained at between 30 °C and 230 °C during the experiments. A sample, of size 0.5 μL to 1 μL, was analyzed and helium, high purity grade at a flow rate of 30 ml $min^{-1,}$ was used as the carrier gas. Under such conditions, the retention times for the N_2, CH_4, and CO_2 in biogas were 6, 9 and 12 minutes.

The volatile fatty acids (VFAs) were analyzed using a Hewlett Packard Model 5890 gas chromatograph (GC) with the Chromosorb WAW 100/120 mesh column and the FFAF (15%) and H_3PO_4 (1%) column. Column temperature was maintained between 80 °C and 230 °C. System temperature was increased at a rate of 10 °C per minute. A sample of size of 0.5 to 1 μL was analyzed. The injection syringe was rinsed two times with pure grade ether that contained a small amount of Na_2SO_4 to remove any excess water that remained in the samples. High purity grade nitrogen gas, fed at a flow rate of about 47 (e.g., 46.4) ml/min,. was used as the carrier gas. The analyses of all samples were conducted in duplicate. The system was calibrated with a series of different dilutions of a standard mixture (125 mg/L) of VFAs (e.g., C_2 to C_6). The exception was the acetic acids which were at a strength of 250 mg/L. The retention

times of the volatile fatty acids with from two to six carbons were determined as 8.7, 9.8, 11.0, 12.4, 13.67, 14.9 minutes.

3.5 Scanning Electron Microscopy

The bacterial consortium was observed using scanning electron microscope (SEM) techniques. The anaerobic biofilm established on the FECS in the AF was examined at the end of each run of the operation using scanning electron microscopy. The FECS was crushed and a fragment with the immobilized biofilm was mounted on a support.

The samples were fixed with 2% v/v glutaraldehyde, buffered with 0.1 M sodium cacodylate, pH 7.2, for one hour and dehydrated with increasing concentrations of ethyl alcohol, remaining for 10 minutes in each of 25%, 50%, 75% and 100% solutions (Drier *et al.*, 1978).

After dehydration, the samples were transferred to numbered aluminum squares that contained circles of filter paper (2.5 cm in diameter.) soaked in 100% Et OH. The samples were arranged in a sandwich arrangement (e.g., aluminum foil, filter papers with 100% EtOH, and samples) to keep the samples sufficiently moist for when it was taken under the critical point dryer. The foil and filters with the samples were gently rolled and dried in liquid CO_2. The samples could not be permitted to air dry during any part of the fixing or dehydration process.

After fixing and drying, as described by Drier *et al.* (1978), the sample was coated with a mixture of gold-palladium to a thickness of 25 nm, using an ion sputter (Joel

Fine Coat Ion Sputter Type JFC-1100). The sample was examined with a Joel JSM-T330A scanning electron microscope (SEM) at 10 KV, 15 mm working distance and a zero degree tilt.

3.6 Fluorescence Microscopic Techniques

The fluorescence microscopic techniques used for bacterial identification were similar to that described by Birk (1984). A Leitz Ortholux 2 microscope, with 4-Lambda Ploem Opak for incident light fluorescence excitation, 250s mirror house, and 250 lamp house were used.

3.7 Key for Bacterial Identification

In additional to the cell morphology of the anaerobic bacteria, as illustrated in the SEM micrographs, references to the to the following sources were made for bacterial identification, including Patel and Sprott (1990) for *Methanosaeta concillii*; Huser *et al.* (1982) for *Methanothrix soeghngenii*; McInerney *et al.* (1981) for *Syntrophomonas wolfei*; Belay *et al.* (1984) for *Methanococcus thermolithotrophicus*; Smith and Mah (1978) for *Methanosarcina* strain 277; Balch *et al.* (1979) for a general classification of methanogens; Koster (1988); and Boone and White (1988).

In addition, any methanogen-like *methanoccocus* spp. which contained F_{420} co-factors, could be recognized by the emitted fluorescence (Edward and McBride, 1975) method under epi-fluorescent excitation at 420 nm.

4. Introduction

4.1 Background

In the early 1980s, an anaerobic hybrid reactor (AHR) was first introduced to a wastewater treatment environment (Maxham and Wakamiya, 1981; Sun and Zhou, 1988). The AHR is an improved type of a conventional Upflow Anaerobic Sludge Blanket (UASB) reactor, incorporating an anaerobic filter in the upper portion of the reactor. Since then, a number of studies have been conducted at both the laboratory-scale and full-scale reactors in order to optimize the performance of such an innovative type of reactor (Sun and Zhou, 1988; Chang, 1989; Harris *et al.*, 1992; Lo *et al.*, 1994; Fang and Chui, 1994; Hawkes *et al.*, 1995; Tur and Huang, 1997; Wu *et al.*, 2000).

Food-processing wastewater and dairy wastes are characterized by wide variations in hydraulic and organic loadings, resulting from the type of batch operations of cleaning and production. The laboratory-scale AHR has been applied for the treatment of various waste streams including brewery waste water (Sun and Zhou, 1988), fibrous waste water (Fernandz *et al.*, 1995; Fernandz *et al.*, 2001), diary effluent (Strydom *et al.*, 1995), ice-cream effluent (Hawkes *et al.*, 1995), starch particulates (Fang and Kwong, 1994), and pharmaceutical wastewater (Henry *et al.*, 1996), giving a treatment performance that was comparable to that of the UASB. In addition, the capability as well as the stability of the reactor in adverse conditions, namely hydraulic shock loadings, has also been significantly improved. Some researchers have indicated that the media-packing ratio is an important parameter in an AHR design. It was reported that high media/total volume ratio improves the performance of an AHR, resulting from the retention of greater quantities of biomass in the reactor (Oleszkiewicz *et al.*,

1986; Young and Yang, 1989). Other researchers, however, have reported that the media-packing ratio had no obvious effect on improving the performance of the AHR. These recommended that the media-packing ratio should be kept as low as 30% of the total volume of the reactor (Kennedy *et al.*, 1989; Chun and Choi, 1993; Wu *et al.*, 2000).

While the benefits of the AHR in wastewater treatment are obvious in a number of the applications highlighted above, the function as well as the role of an AF inserted in the AHR still remains controversial. Fang and Chui (1994) conducted a comparative study on the performance of the AHR and UASB, and concluded that there was no significant difference in treatment performance between these two types of reactor. However, when Chua and his co-workers applied a pilot-scale AHR for livestock wastewater treatment, the AHR achieved a very stable performance within 30 days after start-up, and it took much less than the normal time (e.g. three months) required for a conventional UASB reactor start-up (Chua *et al.*, 1999).

This study was conducted to examine and evaluate the stability of the AHR under different levels of shock loading. The treatment performance, in terms of COD removal, between the UASB section and the AF section was compared in order to elucidate the role played by the inserted AF section in COD removal towards attaining the overall treatment efficiency of the AHR during shock loadings.

Results and Discussion

4.2 COD Removal

The AHR was fed with synthetic wastewater containing reconstituted milk with the ingredients of protein (25.4%), sugars (38.9%), fats (26%) and salts (9.7%), at a concentration of 1920 mg/L, equivalent to 3000 mg COD/L. The BOD/COD ratio of reconstituted milk ranged from 0.3 to 0.35, indicating that the components of the reconstituted milk are biodegradable. The chemical oxygen demand (COD) is a parameter commonly used for the analysis of industrial wastewater. It indirectly measures the amount of electrons in the substrates available for oxidation. The COD was selected as the principle parameter for analysis in the present study, because the time required for the determination of the organic content in a sample is only three to four hours, whereas the time required for the determination of other parameters like the biological oxygen demand (BOD_5) is a full five days. As such, the results of COD removal are particularly useful in determining the performance of the AHR under shock loading environments so as to enable the operator to adjust the system appropriately in response to any adverse conditions encountered. In a strict anaerobic process, in general, no electron acceptor is added to the system. Under such circumstances, the influent COD content can be transformed into VFA, alcohol, hydrogen and biomass. However, the overall COD content should remain unchanged. As a result, in a strict anaerobic process, the amount of COD removed should be equal to the influent COD minus the COD in the biogas (e.g. methane and hydrogen), if any, and the COD content in the biomass.

As shown in Figure 4.1, after 200 days of operation, the average COD removal efficiency of the AHR at HRT 5 days was about 72% under steady state conditions. As a general trend, the COD removal efficiency, achieved by both UASB and AF sections, decreased as HRT was reduced from 5 days to 0.5 day. The capacity of COD removal achieved by AHR gradually declined from 65.5% to 34.7% when the HRT was reduced from 2.5 to 0.5 days. Table 4.1 shows the average COD removal efficiency of the individual UASB and AF sections during various HRTs. Under steady state operating conditions, the COD removal rate of the AHR was maintained between 1.5 to 0.4 gCOD/L-d at HRT of 5 days to 0.5 day respectively (Table 4.2).

When the HRTs decreased from 5 days to 0.5 day, the average COD removal efficiency, achieved by the UASB section, varied from 53.8% to 27.1%, which was much higher than that achieved by the AF section. Meanwhile, the AF section was responsible for only 42.2 to 10% of the COD removal when the HRTs were reduced from 5 to 0.5 days respectively.

Table 4.1 shows that 72.2% of the influent COD was removed by the AHR at HRT 5 days. Of this 72.2%, the UASB section removed 74.5% of the COD content, and the AF section removed about 25.48% of the remaining COD, with a ratio of 2.1 to 1 in COD removal. Figure 4.3 and Table 4.5 present the portion of total COD removed, in terms of percentage of COD, by the particular section of the AHR at different HRTs under steady state conditions. Table 4.5 shows that the average COD portion removed by the UASB section was about 75%, ranging from 74.5 to 78.0%, whereas the AF section contributed only about 44% of COD removal, ranging from 58.5% to 28.9%.

Under steady state conditions, the average ratio of COD removal achieved by UASB section and AF section respectively, was about 3 to 1.

In view of the above, the UASB section of the AHR is considered to be the major contributor in COD removal under steady state conditions, resulting from the retention of a dense and active biomass in the sludge blanket of the UASB section.

Table 4.1 Average COD Removal Efficiency of AHR, UASB Section and AF Section Under Steady State Conditions at Different HRTs

	COD Removal Efficiency (%)		
HRT (d)	UASB-Section	AF-Section	AHR
5	53.8	18.4	72.2
2.5	48.0	15.7	65.5
1.25	42.6	17.3	59.9
1	39.8	13.6	53.4
0.5	27.1	7.6	34.7

Table 4.2 Average COD Removal Rate of AHR, UASB Section and AF Section During Steady State Conditions

	COD Removal Rate (gCOD/L-d)		
HRT (d)	Re COD UASB	Re COD AF	Re COD AHR
5	1.1	0.4	1.5
2.5	1.0	0.3	1.3
1.25	0.8	0.3	1.1
1	0.5	0.1	0.6
0.5	0.3	0.1	0.4

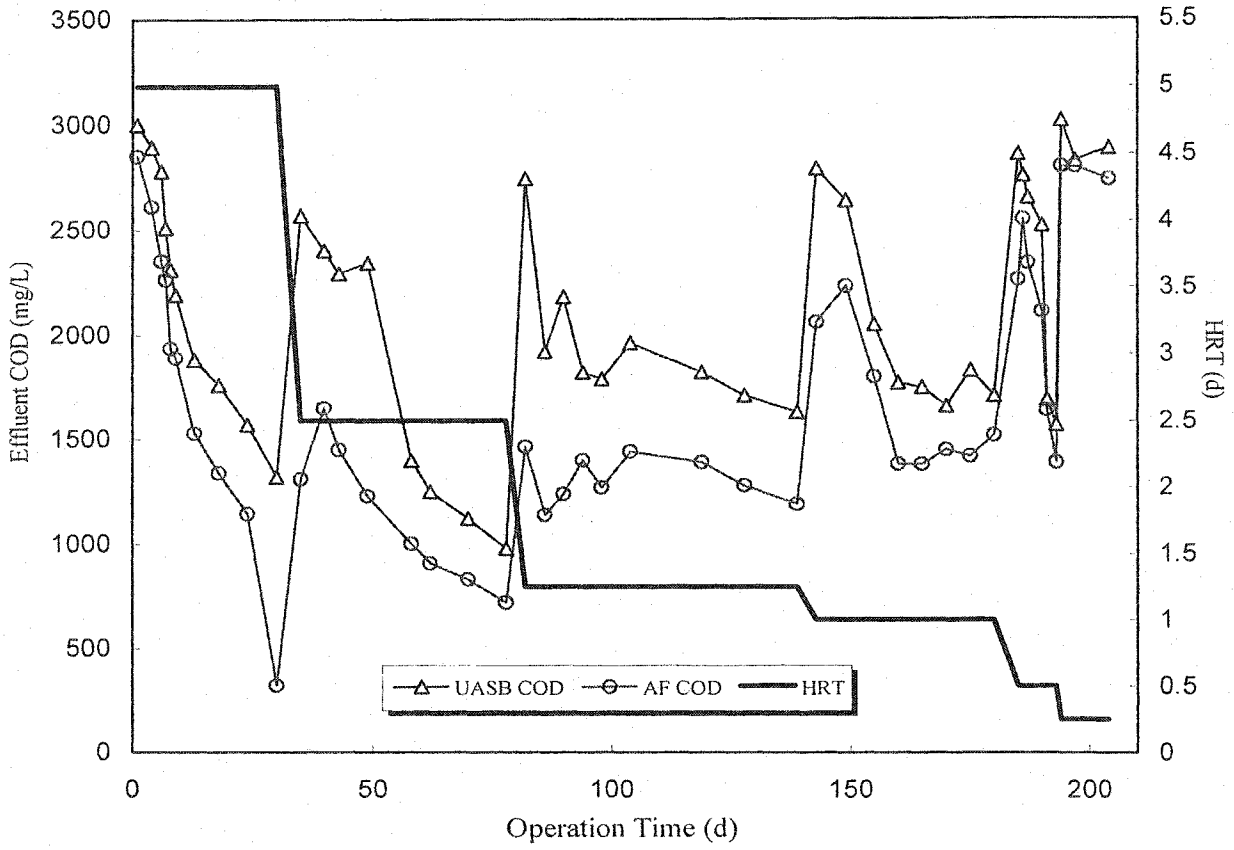


Figure 4.1 COD Removal During Shock Loadings

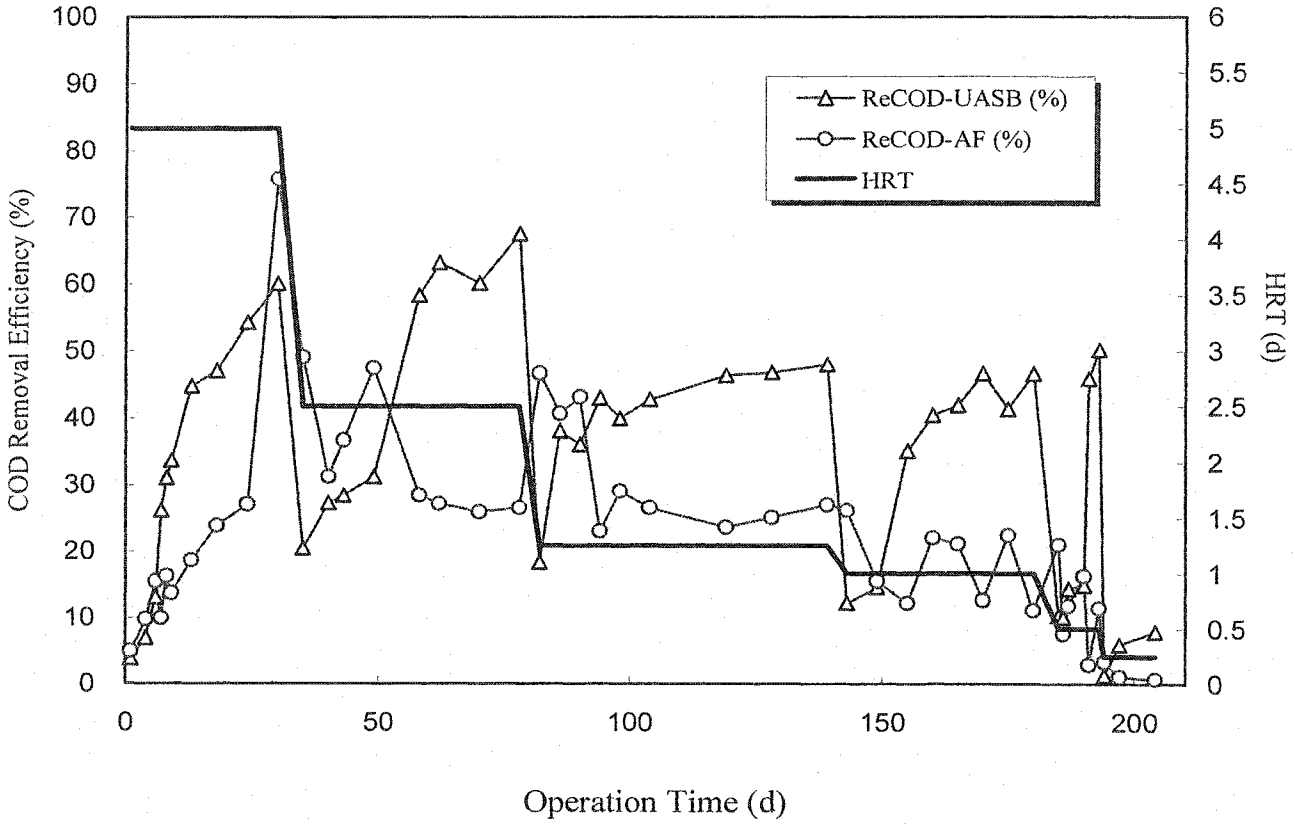


Figure 4.2 Stability of AHR During Shock Loadings

4.3 Stability and Failure of AHR Under Shock Loadings

Figure 4.2 shows the stability of the AHR in terms of COD removal under shock loadings. The responses of the AHR to the shock loadings included a temporarily drop in the COD removal efficiency, after which the AHR gradually resumed to stable operation state after the shock loading was terminated. The role of the AF section of the AHR became obvious and significant, particularly during the transient period of shock loading. Figure 4.4 and Table 4.6 present the portion of COD, in terms of percentage of total COD removal, removed by particular sections of the AHR at different HRTs during shock loadings. The average COD removed by the AF section was about 73%, ranging from 82.6 to 51.6%, whereas the UASB section only accounted for about 22% of the COD removal, ranging from 17.4 to 28.1%.

Even under a critical shock loading at HRT 0.5 day, the COD removed by the AF section still maintained a proportion of about 72% removal; whereas the COD removed by the UASB section declined to about 28% (Figure 4.4). As a general trend, the COD removal rate decreased as HRTs decreased from 5 to 0.5 days. The COD removal efficiency of the AF section was much higher than that of the UASB section during the transient state of shock loadings (Figure 4.2 and Table 4.3). The COD removal rate of AHR was maintained at between 14.3 and 6.2 g COD/L-d at HRTs 2.5 to 0.5 days respectively, prior to the failure of the AHR at HRT 0.25 day (Table 4.4).

It was observed that the sludge blanket of the AHR was slightly disrupted by the incoming flows under shock loading conditions. Under such circumstances, the function of the AF section, particularly in COD removal, became prominent and significant (Figure 4.4). In the AHR, the anaerobic microbial ecosystem, which consists of several groups of interactive micro-organisms, is responsible for converting the organic substrate to methane and carbon dioxide (Mah, 1981). It was believed that, when a shock loading was encountered, one or more groups of these anaerobes were inhibited, resulting in the reduction of the COD removal efficiency (Boardman *et al.*, 1995; Borja *et al.* 1995) (Table 4.3).

Due to the nature of the FES with many void spaces on the surface, the AF section performed as a biofilter, which retains the temporarily inhibited biomass on the surfaces of the packing medium, and thus prevents washout of the biomass under shock loadings (Guiot and Berg, 1984, Borja, *et al.*, 1998; Fernandez *et al.*, 2001). The presence of crevices and pores in the support materials (e.g. FECS) provides favorable conditions for biomass adherence (Verrier *et al.*, 1988; Picanco *et al.*, 2001). As a result, this renders the AHR more tolerant to adverse situations. Borja *et al.* (1988) observed that, in treating slaughterhouse wastewater, the packed bed (e.g. polyurethane foam) of the AHR reactor, significantly enhanced the retention of active biomass and allowed for a self-compacting of the biomass. The biomass concentration entrapped in the packed bed medium was found to increase from 0.5 gVSS/l at the beginning of the study to 5 gVSS/l at the end of the second run of the experiments. Dugan (1987) indicated that many living organisms have a tendency to form flocculent and to produce extra-cellular polymers (e.g. polysaccharides, polypeptides or peptidoglycans),

and this, as a result, enhanced the bio-sorption of organic particles. Sprouse and Rittman (1991) suggested that an anaerobic biofilm attached on the surface of the packing medium created favorable conditions for the capture of organic substances. Ince *et al.* (1999) observed that the support materials of anaerobic filters with high porosity and a high specific surface area achieved better removal efficiencies than that of the reactors filled with non-porous materials. Anderson *et al.* (1994) reported observing the extensive clogging of fatty residues on the support medium of an anaerobic filter treating dairy waste. This clogging of fatty residuals was not observed in the AF section of the AHR.

The COD removal efficiency of the AF section was reduced from 49% to 21% when the HRTs decreased from 2.5 to 0.5 days respectively. However, the COD removal efficiency of the AF section was drastically reduced to about 3% when the HRT was further reduced from 0.5 day to 0.25 day (Table 4.3). Under such circumstances, the total COD removal efficiency of the AHR dropped to about 5%, and the AHR was found to have failed.

As shown in Table 4.3, when the HRTs were decreased from 2.5 to 0.5 days, the capacity of the UASB section of the AHR to remove COD was significantly affected. The COD removal efficiency was maintained only in the range of 10.4% to 8.2% at an HRT of 2.5 to 0.5 days respectively. The COD removal efficiency of the UASB section further dropped to about 1% when the HRT was reduced to 0.25 day.

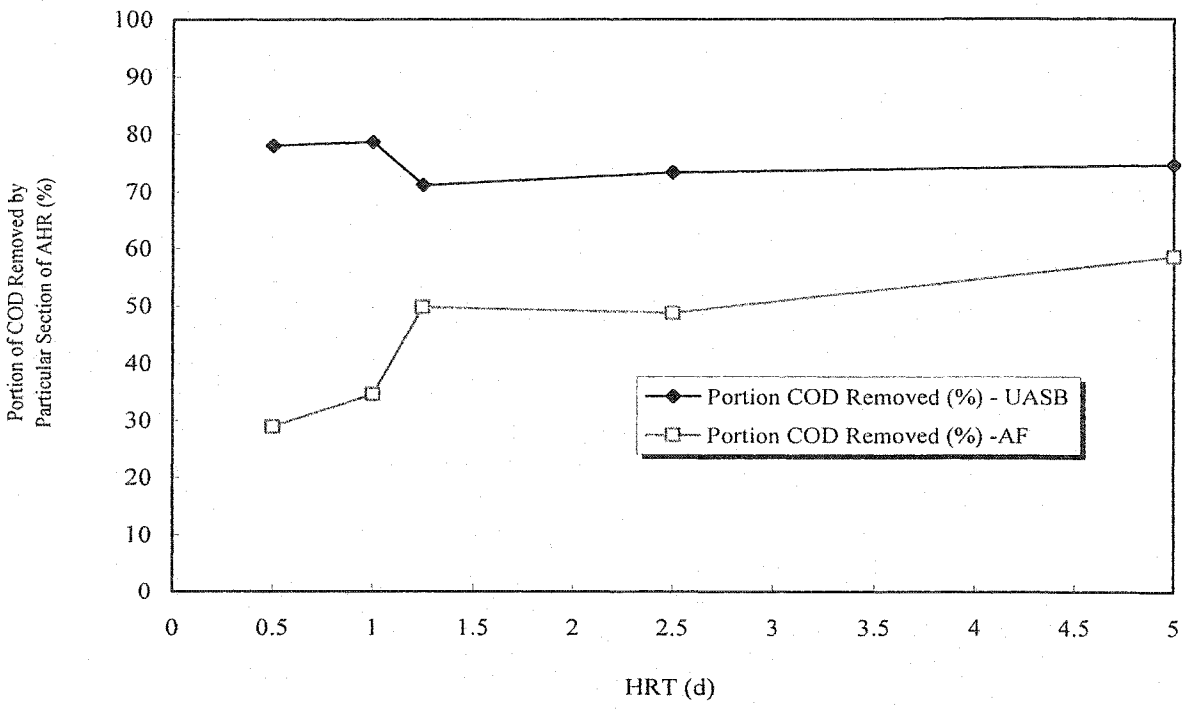


Figure 4.3 Portion of COD Removed by the UASB and AF Sections During Steady State Conditions

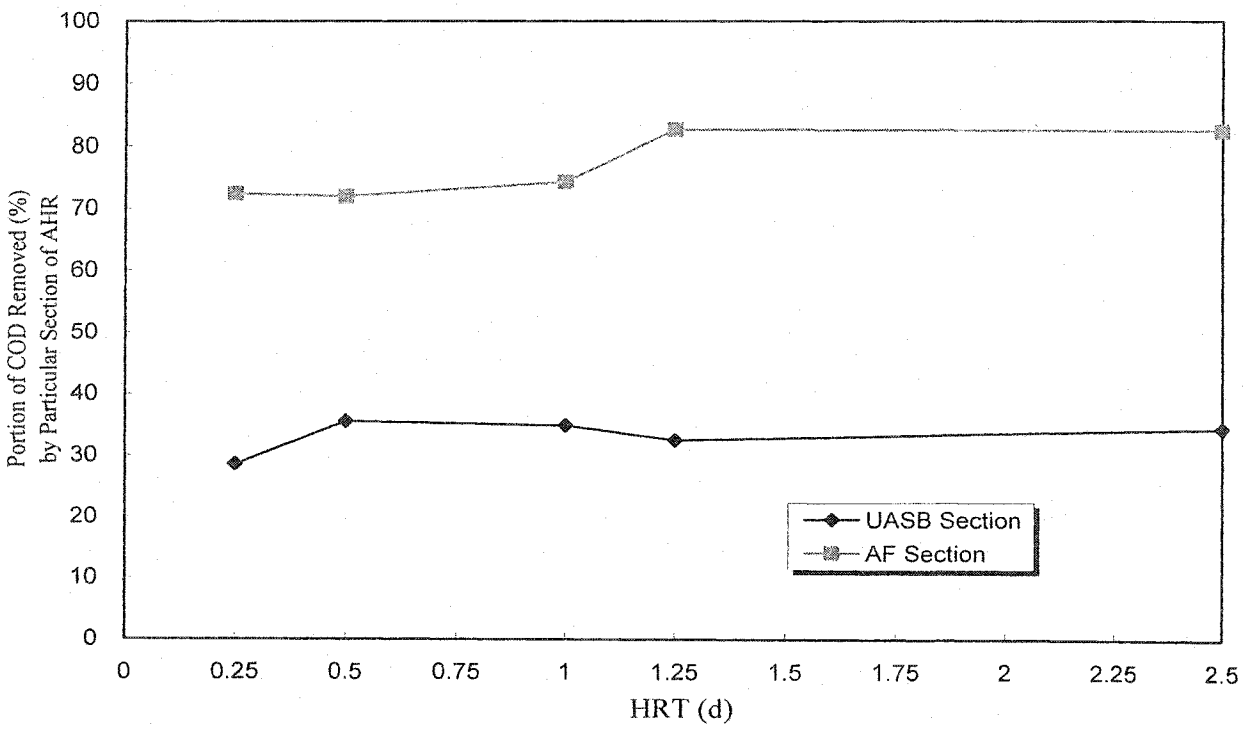


Figure 4.4 Portion of COD Removed by the UASB and AF Sections During Shock Loadings

Table 4.3 Average COD Removal Efficiency in AHR, UASB Section and AF Section During Shock Loadings

	COD Removal Efficiency (%)		
HRT (d)	UASB-Section	AF-Section	AHR
2.5	10.4	49.0	59.4
1.25	9.8	46.6	56.4
1	9.0	26.2	35.2
0.5	8.2	21.0	29.2
0.25	1.3	3.3	4.6

Table 4.4 Average COD Removal Rate in AHR, UASB Section and AF Section During Shock Loadings

	COD Removal Rate (gCOD/L-d)		
HRT (d)	Re COD UASB	Re COD AF	ReCOD AHR
2.5	0.4	0.8	1.2
1.25	0.3	0.8	1.1
1	0.1	0.3	0.4
0.5	0.1	0.2	0.3
0.25	0.0	0.1	0.1

Table 4.5 Ratio of COD, in Terms of the Percentage of Total COD Removal, Removed by UASB Section and AF Section Under Steady State conditions

	Ratio of COD Removed (%)	
HRT (d)	**UASB-Section**	**AF-Section**
5	2.9 (74.5)	1 (25.48)
2.5	3.1 (73.3)	1 (24.0)
1.25	2.46 (71.1)	1 (28.9)
1	3.1 (78.6)	1 (25.5)
0.5	3.56 (78.0)	1 (21.9)

Table 4.6 Ratio of COD, in Terms of the Percentage of Total COD Removal, Removed by UASB Section and AF Section Under Shock Loadings

	Ratio of COD Removed (%)	
HRT (d)	**UASB-Section**	**AF-Section**
2.5	1 (17.5)	4.7 (82.5)
1.25	1 (17.4)	4.7 (82.6)
1	1 (25.8)	2.9 (74.4)
0.5	1 (28.1)	2.6 (72.4)
0.25	1 (20.3)	2.5 (51.6)

Meanwhile, the COD removal efficiency achieved by the AF section was maintained within the range of 49% to 21% during shock loadings at HRTs of 2.5 to 0.5 days respectively. As shown in Figures 4.3 and 4.4, it is obvious that the UASB section of the AHR is the major contributor in COD removal under steady state conditions, whereas the role of the AF section of the AHR in COD removal became dominant, particularly when the AHR was operated under adverse conditions (e.g. shock loadings). In view of the above, with the insertion of the AF section in the upper portion of the AHR system, the AHR achieves a better capability against shock loadings. The AF section could also assist in the reduction of the COD, prevent the wash out of the biomass, and maintain the stability of the AHR under adverse situations. The results of this study demonstrate that, with incorporation of an upper AF section and a lower sludge bed section, the AHR system offers an interesting alternative for treating the waste effluent from food processing, because of its low sensitive to clogging or the loss of biomass due to floatation (Kennedy and Guiot, 1986; Tilch and Vieira, 1991; Fernandez *et al.*, 2001).

The prominent role of an individual AF and its behavior against hydraulic and organic shock loadings is discussed in detail in the following chapters.

4.4 Summary of Findings

With incorporation of the AF in the upper portion of the system, the AHR presented a better capacity against the environment of shock loadings. The AF section of the AHR played a significant role in COD removal; particularly when the AHR was operated under adverse conditions, namely shock loadings. Under such circumstances, the AF section performed as a biofilter for biomass adherence, and thus prevented the washout of the biomass under adverse situations. As such, this rendered the AHR more tolerant to shock loading environments. The UASB section of the AHR is considered to be the major contributor in the COD removal under steady state conditions, resulting in the retention of dense and active biomass in the sludge blanket of the UASB section. Under steady state conditions, the average ratio achieved by UASB section in COD removal, was about 3 to 1. However, the average ratio achieved by the AF section in COD removal was about 3.5 to 1 (3.48 to 1) under shock loading situations.

The average COD removed by the AF section during shock loading conditions was about 36%, ranging from 49% to 21%. The UASB section was responsible for only about 9.4% of the average COD removal, ranging from 10.4 to 8.2 %. Table 4.3 shows that the average COD removed by the AF section of the AHR was 21% when the HRT was 0.5 day under the critical situation. The average COD removed by the UASB section was 8.2%. In response to shock loadings, the AHR showed a temporary drop in COD removal efficiency, and resumed steady state operations after the adverse situation ceased. The results of this study also demonstrate that the AHR system, with the incorporation of an upper AF section and a lower sludge bed section, offers a

viable alternative for treating waste effluent from food processing and dairy wastes, because of its low sensitivity to clogging or loss of biomass due to floatation.

5. Introduction

5.1 Background

Recent developments in anaerobic fixed-film technology have rendered the anaerobic process a feasible option for on-site pre-treatment of trade effluents (Cho *et al.*, 1995; Chua *et al.*, 1992; 1995a, b, c; 1996a, b; Perle *et al.*, 1995; Walsh *et al.*, 1995). Borja *et al.* (1995) reported that a COD removal efficiency above 75% could be achieved in an anaerobic fluidised-bed reactor treating slaughterhouse wastewater at an HRT of 8 hours with an organic loading rate of 54000 mgCOD/L d. Chua *et al.* (1996a) demonstrated a COD removal efficiency of 85% and a grease removal efficiency of 53% from 4500 mgCOD/L food-processing wastewater with an anaerobic biofilter operating at a an HRT of 1.5 days. However, anaerobic processes have been reported as being sensitive to shock loadings. This is a serious disadvantage, especially when balancing and equalizing facilities are limited (Boardman *et al.*, 1995; Borja *et al.*, 1995; Chua *et al.*, 1995b). Hydraulic- and organic-shock loadings have often resulted in process souring and failure (Chua *et al.*, 1995c). Massive sloughing of biofilm from the support medium and washout of biomass from the reactor were common under unfavourable conditions that caused organic inhibition of the microorganisms or excessive hydraulic shear to the biofilm. Hydraulic shock loadings with reduced COD load are common in treatment plants treating food-processing wastewater, particularly during the washings of certain fermentation processes. However, there have been few investigations specifically devoted to the stability of anaerobic fixed-film processes under hydraulic shock loadings.

This chapter focus on the studies of the stability of an individual AF section of an AHR applied to treat a synthetic wastewater. The performance of the AF under various hydraulic shock loading conditions was also studied in detail. The causes of failure as well as the recovery of the AF were discussed.

Results and Discussion

5.2 Responses to Hydraulic Shock Loadings

The average COD removal efficiency was 98.1% throughout the steady-state condition during the start-up period, which was between days 39 and 87 (Figure 5.1). The organic loading rate was 1,800 mg COD/d and the average COD removal rate was 1764 mg/d. The biogas production rate remained at between 0.5 and 0.6 L/d throughout this period. The average methane concentration and methane yield were 73.5% and 0.25 L/g COD removed. The pH was maintained at between 7.0 and 7.4.

The responses to a 2 times hydraulic shock loading at a 2.50 days HRT, introduced on day 88, included a temporary drop in the efficiency of COD removal; the COD removal efficiency dropped to about 88.3% three days after the hydraulic shock loading was introduced. The average COD removal rate was also reduced, ranging from 1611 to 1668 mg COD/d (Figure 5.1). The effluent pH dropped from 7.2 to 6.4~6.0 (Figure 5.2). The averaged VSS in the treated effluent, biogas production rate, and methane yield, at different hydraulic shock loadings, are listed in Table 5.1. The VSS in the treated effluent during the initial period, from the point of seeding to day 100, was maintained at between 300 to 450 mg/L. The high VSS concentration due to

the initial seed was gradually reduced after day 100 following the establishment of a firm biofilm on the support medium and the washout of the suspended biomass.

During the entire period of stable operation, from day 120 onwards, the VSS was maintained at a relatively low level, between 40 and 70 mg/L (Figure 5.2). This coincided with the general characteristics of immobilized-cell systems, which produce treated effluents with low solid content. Even during the hydraulic shock loadings, when the treatment performance was affected, the VSS levels in the treated effluent remained low. The VSS yield was estimated to be 0.017~0.026 g $VSS_{produced}$/g $COD_{removed}$, which was lower than the reported range of 0.025~0.051 g $VSS_{produced}$/g $COD_{removed}$ (Pavlostathis and Giraldo-Gomez, 1991). The reason for the low VSS yield obtained from this study might be attributed to the effect of differences in the substrate. In previous studies, short chain VFAs (e.g., acetate, propionate, butyrate, and a mixture of these VFAs) were used as the sole carbon sources rather than reconstituted milk. When comparing the molecular structures of the VFAs and reconstituted milk, the VFAs had a simple molecule that was a ready-to-use energy source for the growth of anaerobic microbials, whereas the reconstituted milk contained large molecules of the substrate, including protein and carbohydrate as well as fat, that were not the ready-to-use energy sources that could be consumed by the anaerobes prior to the hydrolysis of the macro-molecules taking place.

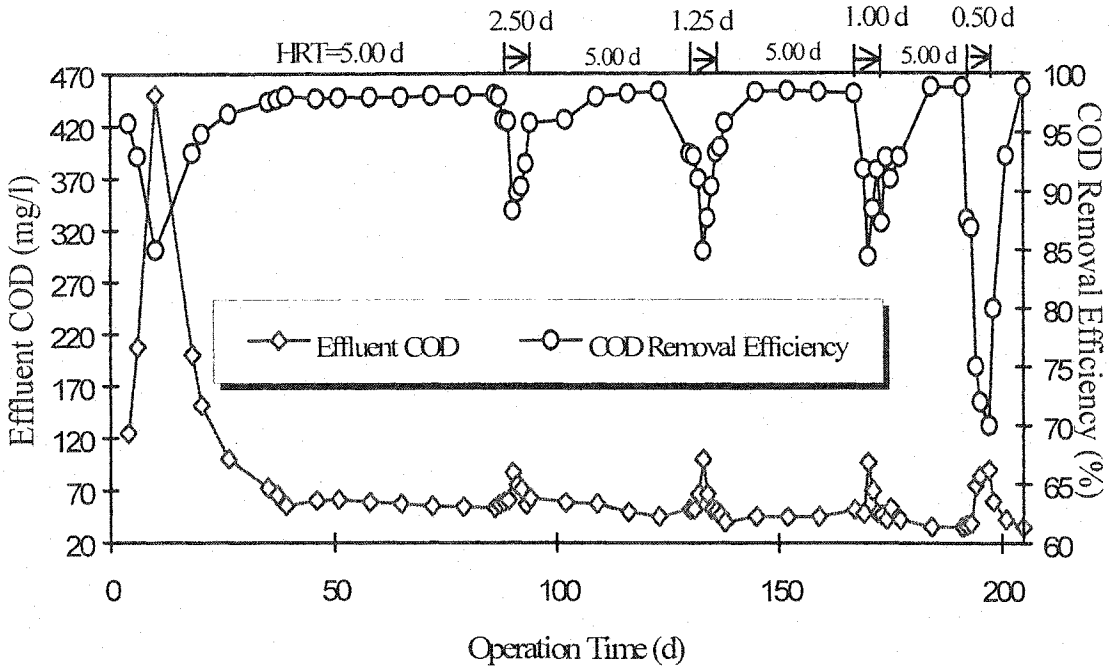


Figure 5.1 Effluent COD and COD Removal Efficiency During Hydraulic Shock Loadings

Table 5.1 Performance of the AF under various Hydraulic Shock Loadings

Hydraulic Shock Load (times)	0 (normal)	2	4	5	10**
Organic Load Rate (g COD/L-d)	1.8	1.8	1.8	1.8	1.8
Hydraulic Retention Time (day)	5	2.5	1.25	1	0.5
COD Removal Rate (g COD/L-d)	1.76	1.72	1.65	1.63	1.26
COD Removal Efficiency (%)	98.1	92.7	91.0	89.7	70.0
Specific Growth Yield ($g\ VSS_{produced}/g\ COD_{removed}$)	0.019	0.026	0.023	0.021	0.017
Biogas Production Rate (L/d)	0.60	0.54	0.38	0.51	0.25
Biogas Yield (L/L.d)	0.99	0.45	0.16	0.26	0.04
Methane Concentration (%)	74	69	64	70	50
Methane Production Rate (L/d)	0.44	0.37	0.24	0.36	0.13
Specific Methane Yield ($L_{produced}/g\ COD_{removed}$)	0.28	0.32	0.29	0.30	0.10
pH	7.2	6.8	6.0	6.3	5.5

** Critical shock load introduced on the day 197

Both the biogas production rate and methane concentration decreased during the 2 times hydraulic shock loading introduced on day 88 (Figure 5.3). The anaerobic microbial ecosystem is comprised of several groups of interactive microorganisms that convert organic matter into methane and carbon dioxide (Mah, 1981). In the reaction, organic matter is first converted to acetate and hydrogen by syntrophic acetogens. The acetate and hydrogen are then converted to methane and carbon dioxide by acetoclastic methanogens and hydrogenotrophic methanogens. Hydrogen may also be converted to acetate by homoacetogenic bacteria depending on the environmental conditions as well as the culture mix; however, the factors affecting these reactions have not yet defined (Wolf, 1983; Wilkie and Colleran, 1986). When hydraulic shock loading was introduced, one or more microbial groups were, presumably, inhibited, causing a drop in COD removal efficiency and biogas production. This implies that the close association between syntrophs and methanogens seems to be disturbed, to some extent, by the hydraulic shock loadings. An average 69% of methane was generated after the various shock loadings. This was equivalent to specific a methane production rate ranging from 0.28 to 0.32 L/g COD. These specific methane production rates were very close to the stoichiometric value of 0.35 L/g COD, giving rise to very low VSS concentrations in the treated effluent. The results suggest that a high proportion of the degraded organic constituents was converted to biogaswith much less being converted to biomass. The methane production rate was equivalent to 2.2×10^5 kJ of energy generation per cubic metre of the trade effluent. This heat value is in excess of the amount of energy required for pre-heating the effluent to 30 °C.

The response of the AF to a 4 times hydraulic shock loading, introduced on day 131, was similar to that for the 2 times hydraulic shock loading. The temporarily affected

treatment performance recovered within 6 days after the shock loading was introduced into the AF. Borja *et al.* (1994b) also observed a quick recovery of the treatment performance of an anaerobic filter after termination of the shock loading.

In contrast, the response of the AF to a 5 times hydraulic shock loading, introduced on day 169, took a slightly longer time, more than 8 days after the shock loading, to recover (Figure 5.2). As shown in Table 5.1, it is interesting to note that a high methane concentration and a high biogas production rate were obtained under a 5 times hydraulic shock loading. The reason for the high methane concentration and high biogas production rate resulting from a 5 times hydraulic shock loading was attributed to the reduced effect of pH, resulting from the lower levels of VFA produced u. As shown in Figure 5.2, the pH value ranged between 5.8 to 6 under a 4 times HSL, whereas the pH value ranged between 6 to 6.5 under a 5 times HSL. Iit was further observed that less VFAs were produced under a 5 times than under a 4 times HSL. Clark and Speece (1970) indicated that bacteriostatic to acetate fermenting methanogens were observed in an anaerobic filter system when the pH (e.g. < 6) was low. A pH of between 6 and 8 is not inhibitory to acetate fermenting methanogens. However, the recovery of the acetate fermentation was slow as the pH remained low for several days during the 4 times hydraulic shock loading. In contrast, under a 5 times hydraulic shock loading the pH value ranged between 6 to 6.5, and under such circumstances exerted no harmful effect on methanogensis with the result that a high methane concentration and high biogas production rate were obtained. The other possible reason for the high methane concentration and biogas production rate obtained under a 5 times HSL was that it might due to some sort of dynamic

adaptation of the anaerobic microbials in response to the 5 times hydraulic shock loading introduced. The exact reasons for such an outcome are unknown.

The ability of the AF to withstand the hydraulic shock loading at 1 day HRT surpassed that reported by Yap *et al.* (1992) who tested a similar AF system operating under similar conditions which tolerated a critical hydraulic loading at 1.5-day HRT, but beyond which the system failed and was unable to recover.

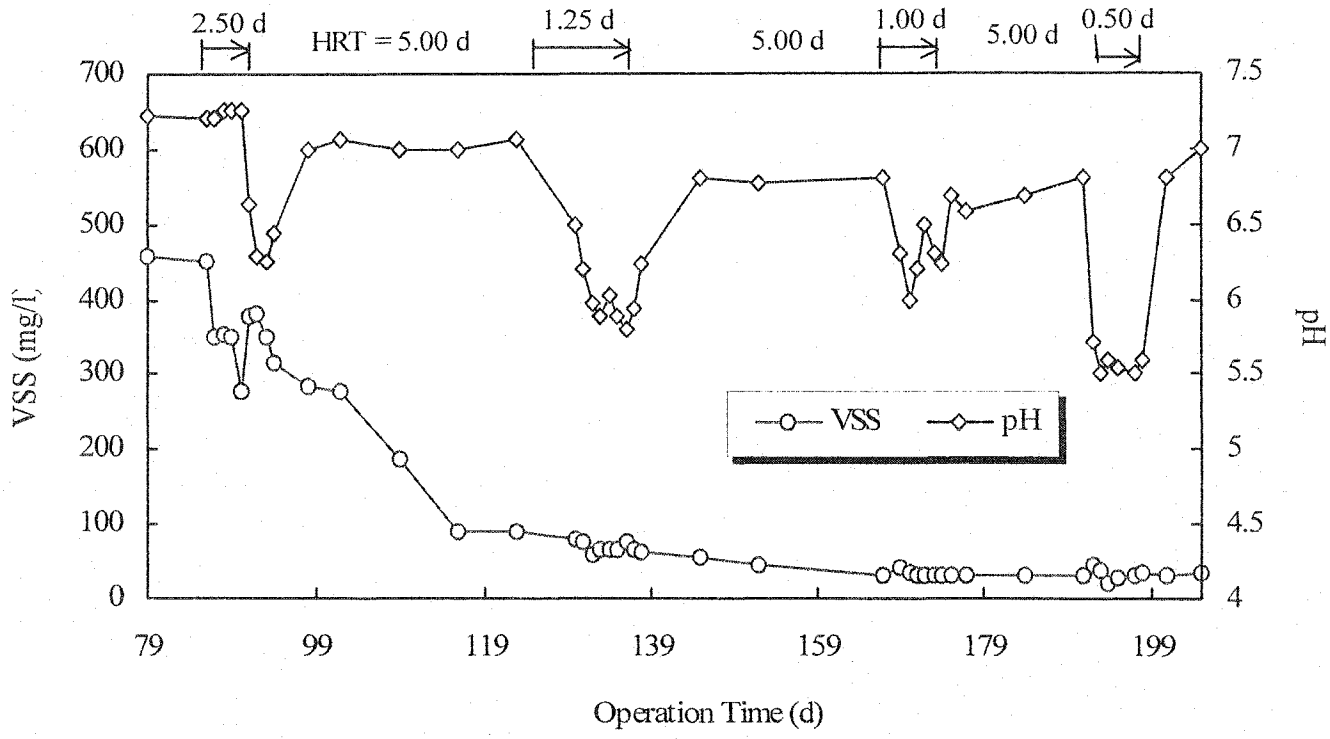


Figure 5.2 VSS and pH in AF Effluent during Hydraulic Shock Loadings

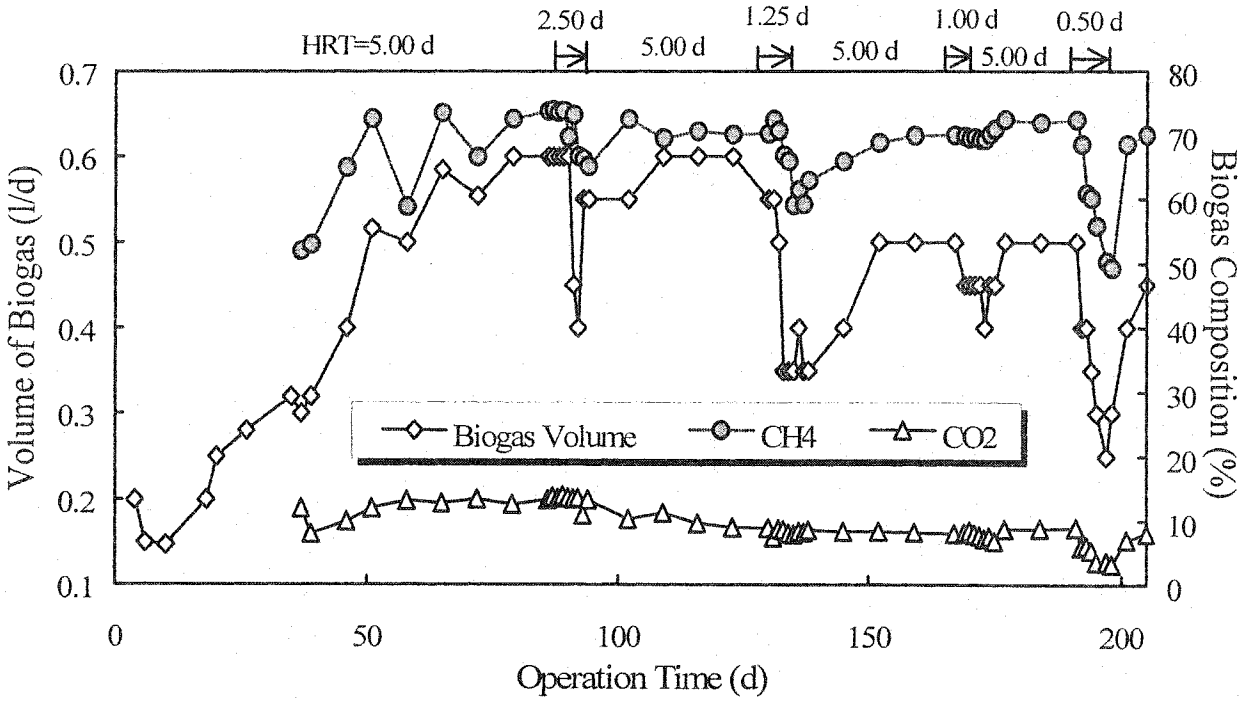


Figure 5.3 Biogas Production Rate and Composition During Hydraulic Shock Loadings

5.3 Transient Responses of Volatile Fatty Acids during Hydraulic Shock Loadings

Periodic analyses showed negligible concentrations of VFAs in the treated effluent at 5.0 and 2.50 days HRT. When the HRT was changed from 5.0 to 1.0 day, the predominate VFAs in the treated effluent were acetate and propionate. The average acetate and propionate concentrations in the treated effluent increased to 24.2 and 70.0 mg/L (Figure 5.4). The concentration of these VFAs rapidly dropped to negligible levels within 6 days of the shock loading. This behavior of a temporal accumulation of VFAs corresponded well with the observations of the temporal decline and rapid resumption in effluent pH, COD removal efficiency, biogas production rate, and methane concentration. This implies that the hydraulic shock loading distributed, to some extent, the physical contact between the syntrophs and methanogen. Thus, a hydraulic shock loading appears to inhibit the methanogenic bacteria, causing an accumulation of VFAs and a decline in methane production.

It was reported that when the process of methanogensis was inhibited, high levels of VFAs were accumulated in the treated effluent (Ashely and Hurst, 1981; Sorensen *et al.*, 1981). Unlike conventional suspended-growth reactors, the temporarily inhibited biofilm in the AF was not washed out from the reactor, and could rapidly regain its activity and re-establish a new balance of the microbial population in the ecosystem.

The immobilized biofilm that grew on the porous surface of the FECSs in the AF was examined using an SEM. A dense population of anaerobic bacteria, composed of mainly *Methanosaeta* (previously known as *Methanothrix*) spp. and *Methanococuss* spp, was observed on the surface and in the pores of the FECS (Plate 5.1).

5.4 Filter Failure and Recovery

When the HRT was further reduced from 5.0 to 0.5 days, on the 192th day, to create a 10 times hydraulic shock loading, the COD removal efficiency dropped immediately from 98.1 to 87.6% and continued to fall to 70% on the sixth day (Figure 5.1). There was no indication of recovery 7 days after the shock loading had been introduced. The effluent pH dropped to a low level of 5.5 (Figure 5.2) and the biogas production rate and methane composition declined drastically to 0.25 L/d and 50.3% (Figure 5.3).

Significant amounts of VFAs were accumulated during this critical shock load, equivalent to a 10 times hydraulic shock loading. The acetate and propionate concentrations drastically increased to maximum levels of 250 and 312 mg/L (Figure 5.4). These VFA concentrations exceeded the threshold concentrations that are inhibitory to methanogenic bacteria (Chynoweth *et al.*, 1970; Inoue *et al.*, 1988; Chua *et al.*, 1995b, 1996c). It has also been reported that the inhibition of propionate removal in the anaerobic treatment system is attributable to the presence of acetate (Fukuzaki *et al.*, 1990; van Lier *et al.*, 1993a).

The optimal pH for anaerobic reactors ranges from 6.6 to 7.6 (McCarty and Smith, 1986). When the pH value is less than 6.2, methanogensis is inhibited, but acetogenesis can still maintain its function under such circumstances. A 10 times hydraulic loading is considered to be the critical shock loading, causing the failure of the AF. While the AF failed, no massive sloughing of biofilm and washout of biomass was observed.

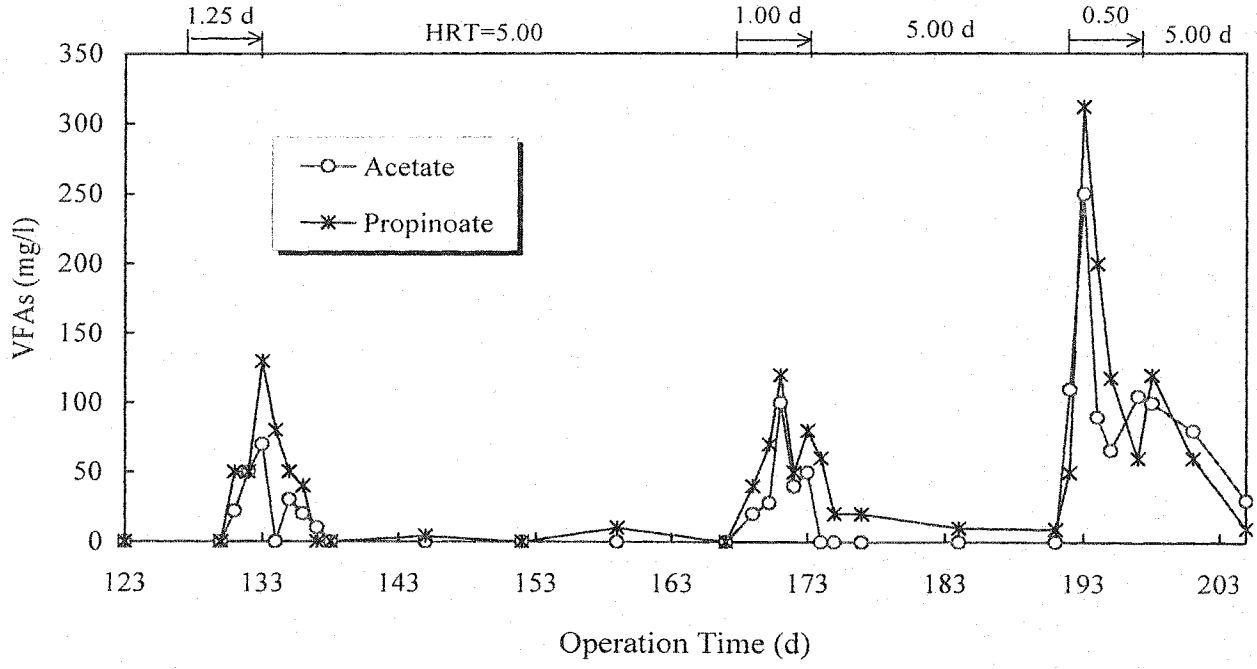


Figure 5.4 Transient Responses of VFAs in Effluent During Hydraulic Shock Loadings

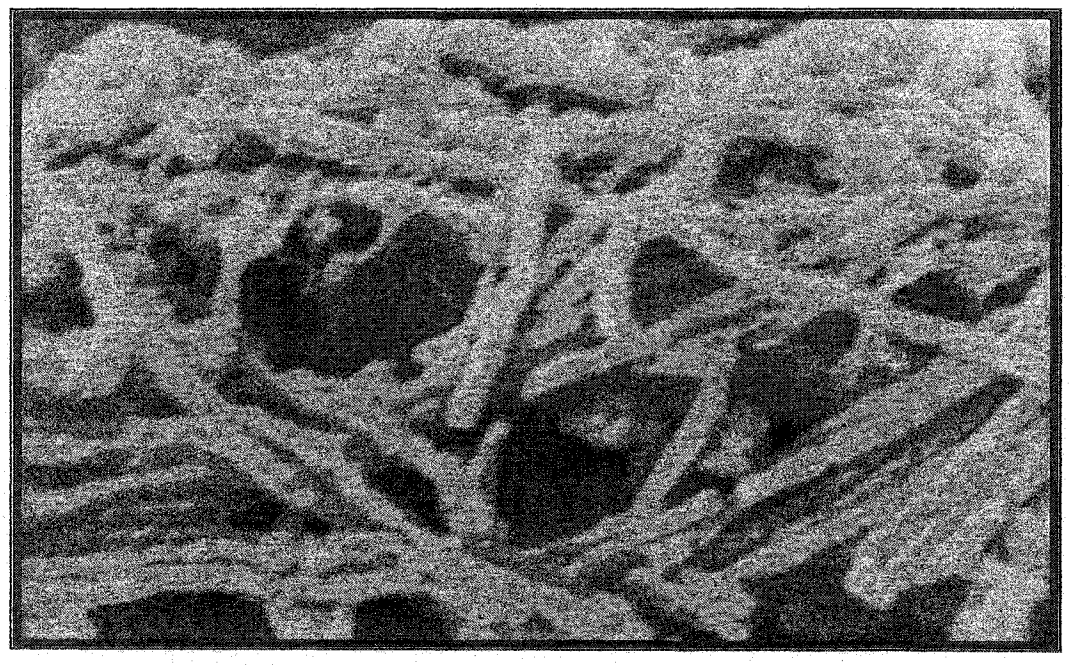


Plate 5.1 Electron Micrograph of Dense Biofilm on FECS Dominated with *Methanosaeta* spp. (10 KV, x 5000)

The VSS in the treated effluent during the 7 days following a 10 times hydraulic shock loading were maintained at a level below 70 mg/L. Seven days after the 10 times hydraulic shock loading was introduced, the AF failed. The HRT returned to 5.0 days and the process recovered. The COD removal efficiency increased from 70 to 98% within 3 days after the shock loading was alleviated. The pH returned to 6.8, biogas production and methane concentration returned to normal levels 3 days after the end of the 10 times hydraulic shock loading (Figures 5.1 and 5.3). VFAs in the treated effluent fell to negligible concentrations within 15 days (Figure 5.4), when the AF was fully recovered.

5.5 Summary of Findings

The immobilized biofilm in an individual AF was able to tolerate hydraulic shock loadings at constant COD loading. Under two, four and five times hydraulic shock loadings, the efficiency of COD removal was only slightly affected. The COD removal efficiency dropped from 98.1 to 84.4% when the hydraulic shock loadings were introduced, and returned to 98% after eight days. A 10 times hydraulic shock loading was the critical loading that caused reactor failure. However, treatment performance recovered in three days after the critical shock loading was alleviated. The stability of the AF and its ability to recover from inhibition and process failure were attributed to the immobilization of the biofilm. The temporarily inhibited biofilm was not washed out and was able to re-establish activity when favourable conditions were restored.

6. Introduction

6.1 Background

The mechanisms of anaerobic degradation of organic matters in natural ecological environments such as river and lake sediment were discovered about two centuries ago. Methane is one of the key components in biogas produced after anaerobic processes. The greenhouse effect induced by methane was reported to be 25 times than that of carbon dioxide in the atmosphere (Robert and Stephen, 1992). The urban atmospheric methane emissions are influenced by the anthropogenic sources, which originate from landfills, energy production and consumption (Veenhuysen *et al.*, 1998; Levin *et al.*, 1999; Padhy and Varshenym 2000; Ito *et al.*, 2000, 2001; Park and Shin, 2001). Lay *et al.* (1996) reported that the overall methane release rate from sediments was 19.9 mg CH_4 $m^{-2}h^{-1}$. Meanwhile, it has been observed that methane concentration increased from 1.52 parts per million by volume (ppmv) in 1978 to 1.68 ppmv in 1988, and it has also been rising at an average rate of 0.0165 ppmv per year (Steel *et al.*, 1987). As a result, methane is a promising potential source of energy, particularly in rural areas, for converting biogas into one of the usable forms of energy, and thus minimizing the greenhouse effect on the atmosphere.

Anaerobic processes have attracted renewed interest from scientists and engineers in recent years. The performance of the contemporary anaerobic bioreactor has been greatly improved due to the applications of new technologies and equipment. Another reason for the new development interest in the anaerobic process resides in its superior performance when treating waste streams with very high organic loading

rates, and its potential application to industrial waste-water streams containing persistent waste components.

Commercial batch and fed-batch anaerobic processes have been developed and applied to the treatment of domestic and agricultural waste (Cowan, 1992; Kalia *et al.*, 1992). In recent years, large-scale anaerobic digesters have been widely used, not only for treating waste water but also for stabilizing sludge from domestic waste-water treatment works. However, compared with the use of aerobic processes, the industrial application of the anaerobic process in waste water treatment areas is still very limited, mainly because of its slow treatment rate, which is attributed to the slow growth rate of methanogenic bacteria.

Waste-water streams containing persistent waste are the waste streams with a high cellulose content produced from food processing and the paper industries, oily waste water with a high carbon content from the petrochemical industry and the high COD waste water from the distilleries. Persistent waste can be degraded only under specific conditions and some waste may even inhibit bacterial activity in the degradation processes. In-house waste water treatment facilities are often needed for effluents containing specific persistent wastes.

Anaerobic processes have been reported as being sensitive to shock loading, especially where balancing and equalizing facilities are limited, and particularly in the context of a congested urban area like Hong Kong. Shock loading that result in a massive sloughing problem, often leads to the failure of the bioreactor. However, shock loading is a common and often unavoidable phenomenon, especially when

treating the waste streams produced in the food processing industry. This is a problem that needs to be resolved in order to optimize the design of an anaerobic reactor.

This chapter describes the investigation into the stability of an individual AF section of the AHR under various organic shock-loading conditions and the performance of the AF in response to organic shock loadings. The causes of failure resulting from critical organic shock loadings are also discussed.

6.2 Responses to Organic Shock Loadings

In a steady state operational condition, the residual COD value in effluent of the AF was around 130 mg/L, with a COD removal efficiency of 98.2%, and the biogas production rate ranged from 1.9 to 2.0 L/day. The methane concentration in the biogas was maintained at about 70% (v/v). The effluent pH ranged from 6.4 to 6.5.

The residual COD values in effluent temporarily jumped respectively from 130 to a maximum value of 335, 635 and 1300 mg/L, when organic loading rates in influent were increased as shock loading to 6000, 12000 and 24000 mg/L. However these unwanted high COD residual values decreased rapidly in a seven-day period and the performance of the AF was hacked into their original strengths, while a superior 90% COD removal efficiency was recorded. This means that the AF processed an excellent anti-shock loading capacity under this COD concentration range. These results are demonstrated in Figure 6.1.

However the AF looked reasonable and fragile if the shock loading brought as high as 16 times (COD=48000 mg/L) the level of organic substances in the influent. When the COD value in the influent further increased to 48000 mg/L, the COD value in the influent jumped and was almost a quarter (14000 mg/L) of the COD value in the influent stream. However, even in this circumstance, the AF was still able to restore its original performance, though this time it took a lengthy period to do so, as shown in Figure 6.1.

The production rate of the biogas is shown in Figure 6.2. It increased proportionally with the increment of the organic loading rate. The maximum biogas production rates at the COD values of 6000, 12000 and 24000 mg/L were respectively 4.5, 7.75 and 12.65 L/day. The biogas yield ranged from 0.81 to 4.59 L/Ld.

The percentage of methane in the biogas was reduced against the increase of the biogas production rate. The corresponding lowest methane concentrations in the biogas were respectively 70, 56.2 and 52.7%. In the meantime, the content of carbon dioxide in the biogas increased during each stage of the organic shock loading (Figure 6.2). The highest concentrations of carbon dioxide detected were 27.3, 40.1 and 45.3% for shock loading with COD values of 6000, 12000 and 24000 mg/L. It is believed that the increased concentration of carbon dioxide in the biogas resulted in the temporary inhibition of the activities of hydrogen-utilizing bacteria, such as hydrogentrophic methanogens, in the shock-loading environment.

It has been demonstrated that the biofilm in packing material tolerates organic shock loading well, except when the COD value in the influent jumped to 48,000 mg/L. Generally no massive sloughing problem occurred within the bioreactor.

6.3 Transient Response of Volatile Fatty Acids Under Shock Loadings

VFAs are intermediate products in anaerobic process. Their concentrations vary under different circumstances. Dindale (1997) found that the predominant VFA products in liquid phase of a thermophilic pre-acidification anaerobic bioreactor were n-butyrate, acetate and propionate. Using glucose as substrate at pH 5.8, Zoetemeyer *et al.* (1982b) observed that butyrate, acetate and ethanol were the most common VFA products in liquid phase of the bioreactor at 30°C, but ethanol, acetate, followed by propionate, became the most common VFA products at 55°C. Hawkes *et al* (1992) also observed that acetate and propionate were the most common VFA products produced under an eight-hour organic overload from 45000 to 20000 mg/L studies on two-stage and single-stage UASB bioreactors.

The predominant VFA products in the effluent of this anaerobic filter are acetate and propionate. Other VFAs also observed in the treated effluent were principally n-butyrate and n-valerate (Figure 6.3). The concentrations of n-butyrate, n-valerate and caproate were relatively low in the effluent, except during critical organic shock loadings. The results indicated that even-numbered carbon fatty acids degraded more easily than odd-numbered carbon fatty-acids. In addition, the degradation of the even-numbered fatty acids was not a rate-limiting step (Shin *et al.*, 2001).

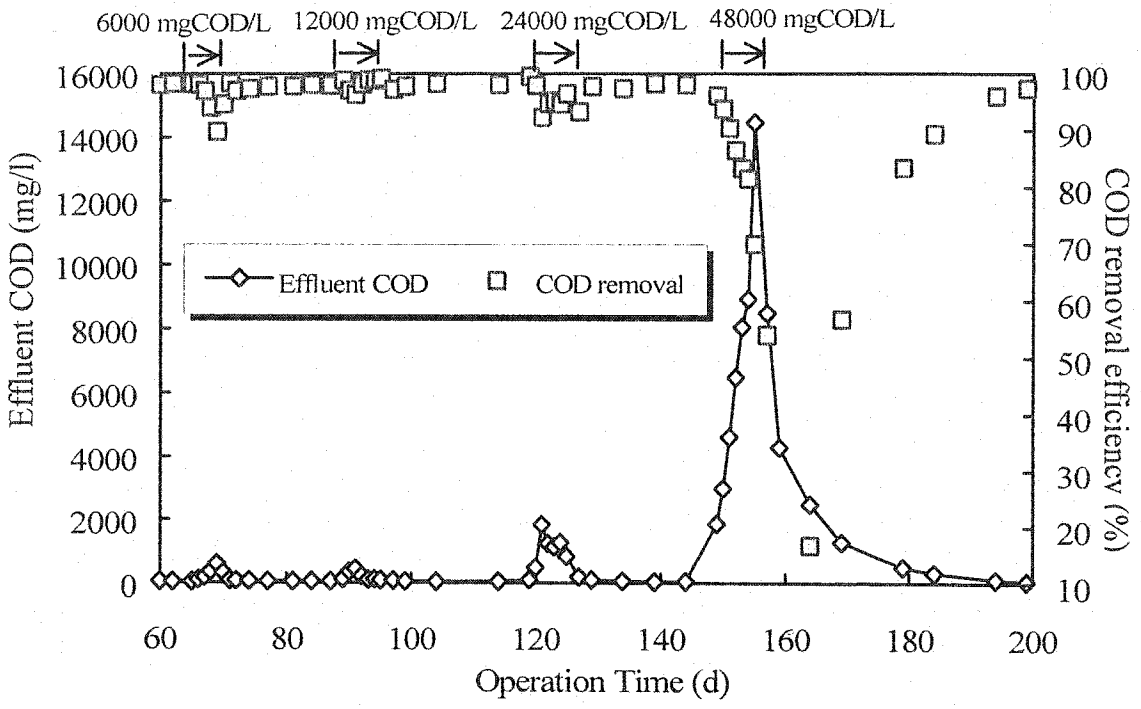


Figure 6.1 Effluent COD and Calculated COD Removal Efficiency During Various Organic Shock Loadings

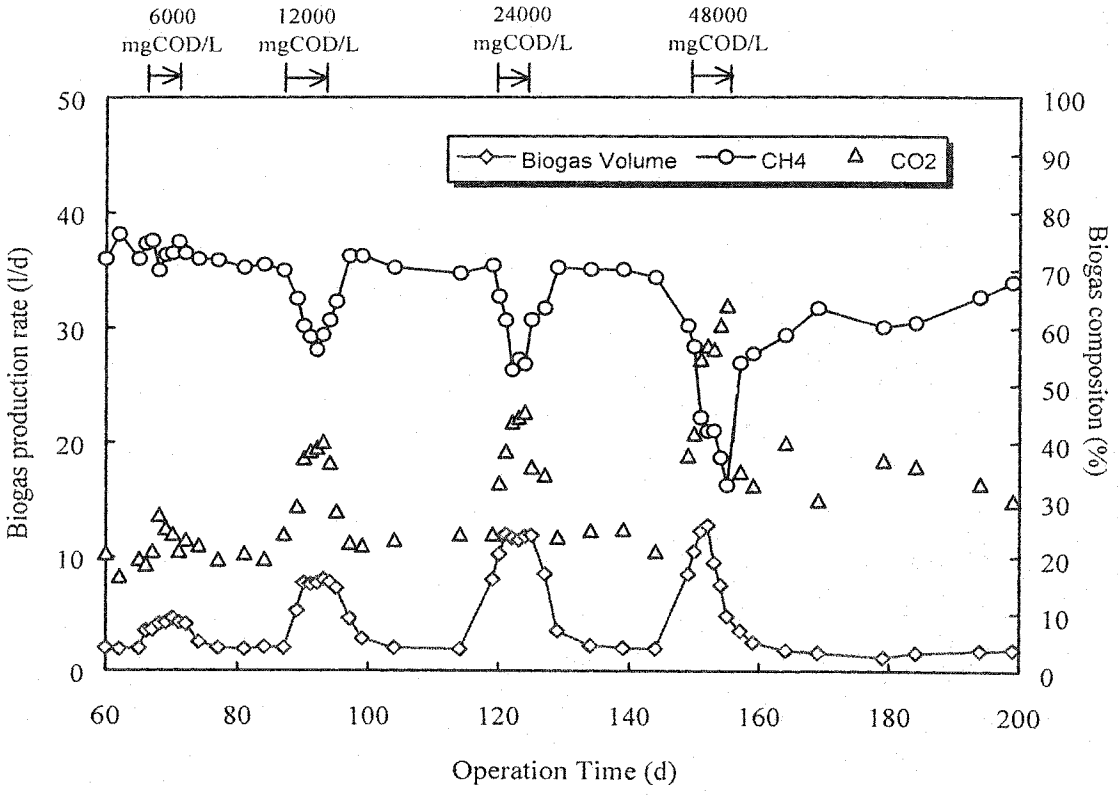


Figure 6.2 Biogas Production Rate and Composition During Various Organic Shock Loadings

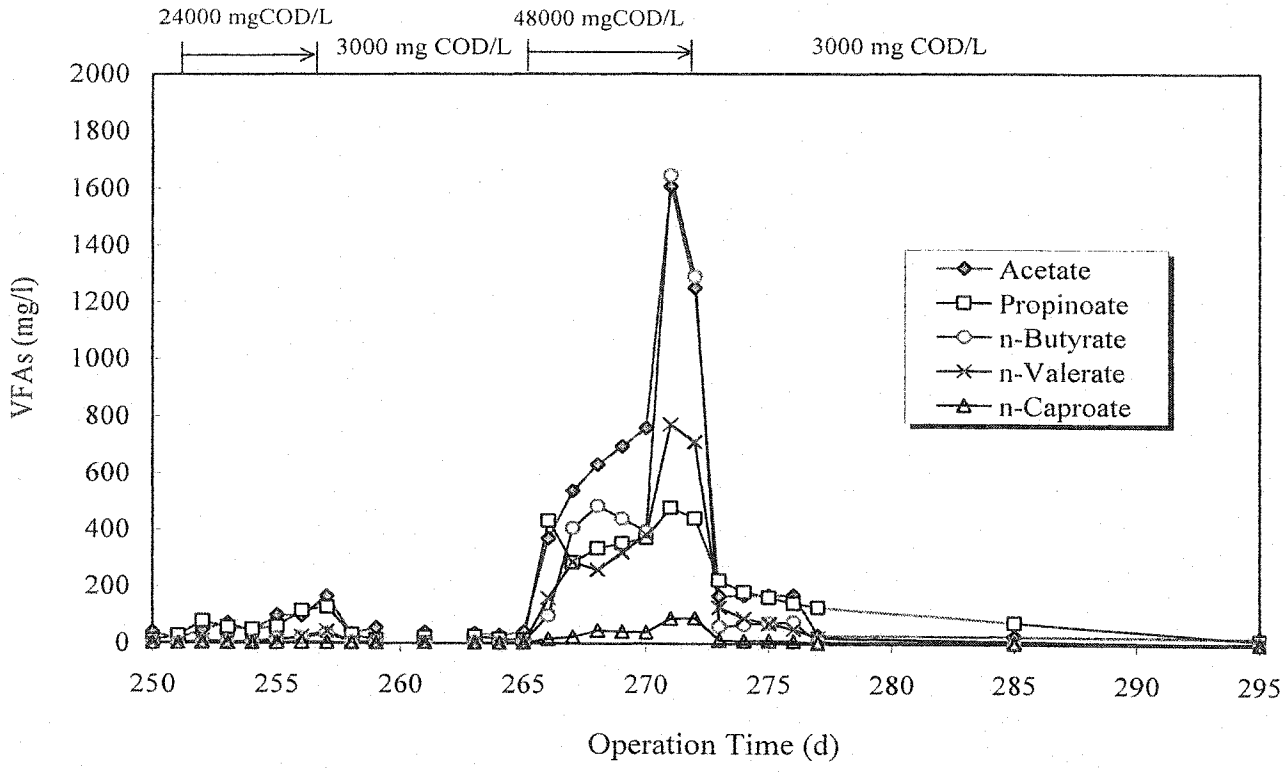


Figure 6.3 Transient Response of VFAs During Organic Shock Loadings

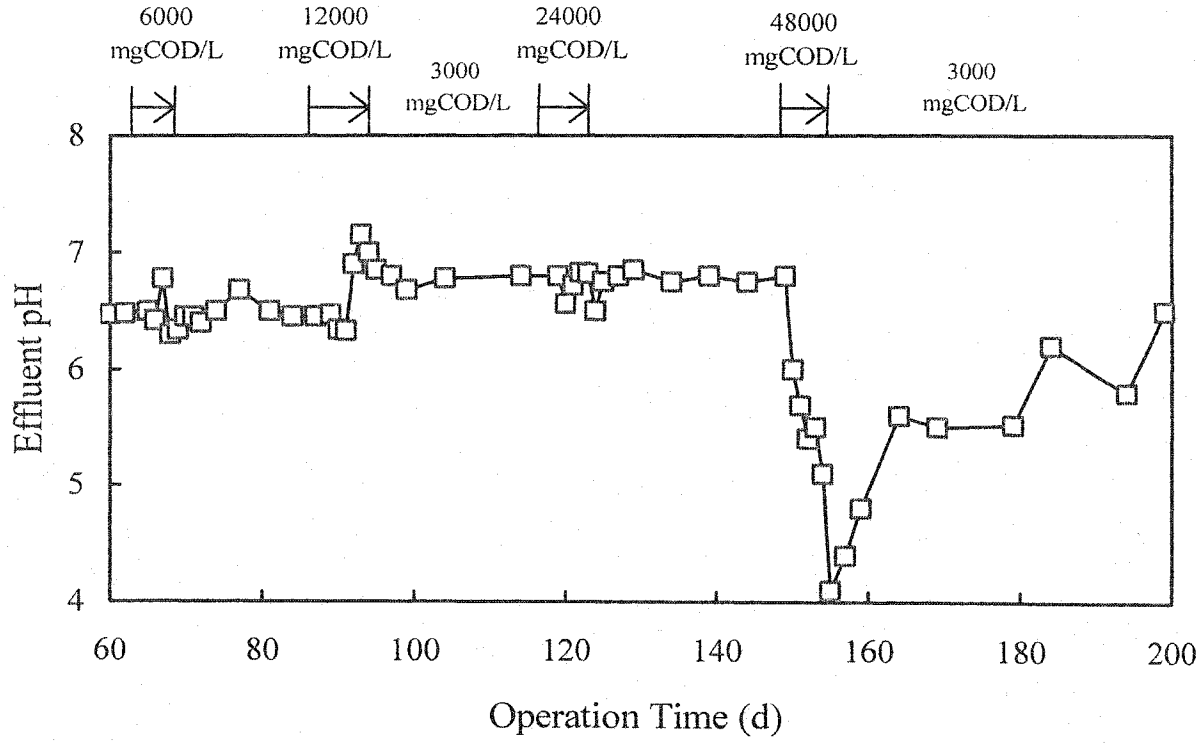


Figure 6.4 Effluent pH During Organic Shock Loadings

The results of the analysis indicated that the VFA contents in the effluent were at a very low level and were almost negligible under normal operating conditions. It has been reported that larger quantities of ethanol, butyrate and acetate than propionate would be produced during high organic loading conditions (Sixt and Sahm, 1986).

Propionate has been recognized as an intermediate, which is difficult to metabolize into methane. The degradation of propionate to acetate is thermodynamically unfeasible, (e.g. ΔG^{o}- +76 kJ), except that the hydrogen gas produced is consumed by hydrogen-consuming bacteria (Thauer *et al.*, 1977; Boone and Xun, 1987; Fang, 2000). The generation time for methane-forming bacteria like acetoclastic bacteria (e.g. a few days) is usually longer than that for acid-forming bacteria (e.g. a few hours). Acetoclastic bacteria comprise two main genera; *Methanosarcina* (Smith and Mah, 1978) and *Methanosaeta* (Fang, 2000), previously known as *Methanothrix*, (Huser *et al.*, 1982). It has therefore been suggested that an anaerobic digester should be designed with a three-day retention time in which the organic load should be sufficient to avoid the production of propionate (Dichtl, 1997). This indicates that converting propionate to acetate is a rate-limiting step during the acetogenesis process due to the thermodynamically unfavorable reasons that are indicated by the positive ΔG^{o} value (Fang *et al.*, 1995 b).

$C_3H_5COOH + 2\ H_2O \longrightarrow CH_3COOH + CO_2 + 3\ H_2$ ($\Delta G^{o} = +76.1$ kJ, pH 7, 1 atm., Thauer *et al.*, 1977)

Like propionate, conversions of butyrate to acetate are also thermodynamically unfavorable.

$C_3H_7COOH + 2\ H_2O \longrightarrow 2CH_3COOH + 2\ H_2$ (ΔG^o = + 48.1 kJ, pH 7, 1 atm., Thauer *et al.*, 1977)

Duran and Speece (1998) observed that the propionate concentration dropped significantly from 1050 mg/l to 300 mg/l, after incorporation of the high F/M contact reactor in the preliminary treatment step of the two-stage (e.g. CSTR and UASB) anaerobic processes.

In this study, the higher the VFA concentrations are in the effluent, the poorer the performance of the anaerobic biofilter is in COD removal. The concentrations of acetate, propionate, n-butyrate and n-valerate in the effluent were respectively increased to 76.5, 72.9, 13.6 and 22 mg/L when the AF was given a shock organic loading rate by increasing COD values from 3000 to 24000 mg/L. However, the VFA concentrations dropped to normal levels within seven days after the organic shock loading was introduced (Figure 6.3). This temporary accumulation of VFAs corresponds well with the observations of the temporal declination and rapid resumption of the value of pH, the COD removal efficiency, and the biogas production rate as well as the methane concentration in the biogas (Figures 6.1 to 6.3).

The reason for the appearance of the temporarily high VFA concentrations in the effluent was because the activities of the methanogensis bacteria were temporarily inhibited under the shock-loading environment. A similar phenomenon was also observed in other studies (Ashely *et al.*, 1981; Sorensen *et al.*, 1981).

6.4 System Failure and Recovery During Extremely High Organic Substance Shock Loading Rates

A very high organic shock-loading rate (when the COD was increased from 3000 to 48000 mg/L) was introduced at the 150th day of operation of the AF. As demonstrated in Figures 6.1 to 6.3, the performance of the bioreactor was severely affected. The COD value in the effluent increased sharply from 60 to 1875 mg/L on the second day after the introduction of the shock loading. After seven days, the COD level in the effluent was still very high, at 1446 mg/L, corresponding to a decrease in the COD removal efficiency from 98% to 69.8%. At the same time the pH value in the effluent decreased dramatically from 6.8 to 4.1 (Figure 6.4).

The theoretical biogas production rate at this time was 32 L/day. It was observed that the real biogas production rate was only 3.5 L/day on the seventh day of the shock loading. The methane concentration in the biogas before the very high shock loading was 60.5% dropping to 32.6% on the seventh day after the very high shock loading was introduced.

Under such high organic loading rates, significant amounts of VFAs accumulated in the treated effluent. After six days of shock loading, the VFA contents in the effluent, in terms of acetate, propionate, n-butyrate, n-valeroate and n-caproate, increased respectively to their maximum concentrations at 1606.9, 477.1, 1645.9 and 87.3 mg/L. At the same time the pH value dropped further to about 4. These VFA concentrations well exceeded the threshold limits that inhibit methanogenic bacteria (Chynoweth *et al.*, 1970; Inoue *et al.*, 1988).

The experimental results suggested that the activities of both methanogenic and acidogenic bacteria were inhibited due to the very high level of the organic shock loading rate (COD = 48000 mg/L). When shock loading was introduced, the activities of the hydrogen-utilizing methanogenic bacteria were the first to be affected. The excess VFAs produced and accumulated in liquor soon exceeded the assimilative capability of the methane-utilizing bacteria and this resulted in the decrease of the production rate of methane. The accumulation of the VFAs may also be the result of the drop of the pH value within the AF. The drop of the pH value further inhibited bacterial activity within the AF. As indicated by Zoetmeyer (1982b), the activity of the acidogenic bacteria is seriously damaged when the pH value is lower than 5.

Nevertheless, the AF showed a satisfactory anti-shock loading capacity. As shown in Figures 6.1 to 6.3, its original performance gradually recovered in a period of 20 days after the introduction of the very high organic shock loading. All operation parameters were restored, though the process was slow. On the 20th day after the introduction of the very high organic loading, the pH value in the effluent rose from 5.5 to 6.0. In the meantime, the biogas production rate was back to normal to 19 L/d. The methane concentration within the biogas also increased from 45% to 60% and the carbon dioxide concentration in biogas dropped from 40% to 30%.

6.5 Degradation of Organic Substances During Anaerobic Conditions

The anaerobic degradation of organic substances to methane and carbon dioxide takes four steps, namely hydrolysis, acidiogensis, acetogenesis and methanogenesis:

Complex organic substrates, such as neutral fats (triacylglycerols), which are esters of the alcohol glycerol with three fatty acids molecules, were initially degraded to glycerol and long-chain fatty acids. Further degradations of the long-chained fatty acids were mediated by β-oxidation through the syntrophic association of the hydrogen-producing and hydrogen-consuming bacteria. The degradation underwent a cleavage change to form a 2-carbon fragment, presumably acetate, and a fatty acid chain shorter by 2-carbon atoms (Speece, 1973; Weng *et al.*, 1976; Hanaki *et al.*, 1981).

The hydrolysis of the high molecular substrate is a slow process that can be accomplished by using extra-cellular enzymes (e.g., cellulase, protease and lipase). Hydrolytic or non-hydrolytic bacteria ferment the products, including amino acid, carbohydrates and fatty acids, to carbon dioxide, hydrogen, ethanol and some short-chain volatile acids such as propionate, butyrate, valeroate and caproate. This is often referred as the acidogensis or fermentation step.

The degradation of volatile fatty acids to methane was mediated by a group of anaerobes known as hydrogen-producing acetogens (Bryant, 1979). These bacteria convert volatile acids into hydrogen and acetate. Acetate is decarboxylated by the action of the acetotrophic methanogenic bacteria into methane, while hydrogen is utilized by hydrogenotrophic bacteria, leading to the production of methane (Thauer, *et al.*, 1977; Henzen and Harremoes, 1983).

$$CH_3COOH + H_2O \xrightarrow{\text{acetotrophic bacteria}} CH_4 + H_2CO_3 \quad (\Delta G^{o} = -104.81 \text{ kJ, Thauer } et\ al., 1977)$$

hydrogentrophic bacteria

$$4H_2 + H_2CO_3 \longrightarrow CH_4 + 3H_2O \quad (\Delta G^{o} = -135.6 \text{ kJ, Thauer } et\ al., 1977)$$

Research has indicated that about 70% of methane is produced from acetate by acetoclastic methanogen while the remaining 30% is produced by the reduction of carbon dioxide with hydrogen (Smith and Mah, 1966; Speece, 1973). The overall conversion of the organic substrate was mediated by methanogenic bacteria, in association with the production of methane and carbon dioxide. The overall anaerobic transformation of the higher intermediates (e.g. long chain VFAs and ethanol) in acetogenic and methanogenic processes is illustrated in Figure 6.5 (Young and Tabak, 1989; Smith and McCarty, 1989; Kim *et al.*, 1994; Lay *et al.*, 1996).

In contrast, homoacetogenic and hydrogenotrophic methanogenic bacteria competed with each other for consuming the H_2/CO_2 gases as a substrate (Cord-Ruwisch *et al.*, 1988; Vavilin *et al.*, 2000, Chen *et al.*, 2003). As reported elsewhere, homoacetogenic bacteria can take over the role of the H_2-utilizing methanogen if these are inhibited by high proton activity (Chen *et al.*, 2003). It has also been reported that more than 95% of all methane production was derived from acetate, indicating that the carbon and electron flows were extensively through homoacetogensis and acetate cleavage (Phelps and Zeikus, 1984). Cord-Ruwisch *et al.* (1988) conducted a trace study in a mildly acidic lake sediment (e.g. Knacack Lake, pH 6.1), and revealed that nearly the entire electron flux from biomass to methane goes through the acetate pool, due to the specific inhibition of the hydrogen-oxidation methanogens by the enhanced proton

activity. Cord-Ruwish *et al.* (1988) also reported that the threshold concentration for hydrogen by homoacetogens is substantially higher than that of the threshold concentration for hydrogen by H_2-utilizing methanogens (e.g. hydrogentrophic methanogens).

Chen *et al.* (2003) reported that the homoacetogenis was the significant process for hydrogen utilization in landfill, which was validated by the consumption of 4.01 to 5.43 moles of hydrogen per mole of acetate formed. Chen *et al.* (2003) also observed that significant amounts of acetate had accumulated in the landfill samples, and suggested that the homoacetogens out-competed the hydrogentrophic methanogens for hydrogen consumption under high H_2 partial pressure conditions.

$$4H_2 + 2H_2CO_3 \xrightarrow{\text{homoacetogenic bacteria}} CH_3COOH + 4H_2O$$

As reported, the homoacetogens are capable of utilizing a substrate other than hydrogen as a carbon source, including sugars (Fontaine *et al.*, 1942), fumaerate (Dorn *et al.*, 1978), mandelate (Dorner and Schink, 1991), methanol (Weijma and Stams, 2001) and vanillate (Harriott and Frazer, 1997). Their metabolic versatility is the reason why homoacetogens can out-compete other methanogens in an anaerobic system for hydrogen utilization (Chen *et al.*, 2003).

Lay *et al.* (1996) demonstrated that the production rates of acetate, ranging from 3.0 to 4.4 mg COD d^{-1}, were faster than those of methane, which ranges from 0.5 to 0.8 mg COD d^{-1}, indicating that most of the hydrogen was transferred to acetate, via

homoacetogenic bacteria, but not the methane in the lake sediment environment. Lay *et al.* (1996) also determined that the number of hydrogen-utilizing methanogens was substantially smaller than that of acetate-utilizing methanogens and homoacetogen in the lake sediments under psychrophilic conditions.

The anaerobes are proton reducers due to their inability to respire anaerobically or to ferment. As hydrogen inhibits the hydrogenase of the bacteria, the excess hydrogen gas must be eliminated. For example, the degradation of propionate and butyrate are thermodynamically unfavorable (for propionate, ΔG^{o}= + 76.1 kJ/mol, for butyrate, ΔG^{o}= + 48,1 kJ/mol) unless a partial hydrogen pressure as low as 10^{-3} atm is maintained (Thauer *et al.*, 1977; Gujer and Zehnder, 1983; Ahring and Westermann, 1988; Stams *et al.*, 1992; Duran and Speece, 1998).

Low hydrogen pressure results in the greater availability of energy for acidogens and acetogens, their increased substrate utilization, the displacement of unfavorable reaction equilibrates and the increased growth of bacteria consortium in treatment systems (Harper and Pohland, 1986). Low hydrogen pressure can be achieved in methanogenic conditions by means of interspecies hydrogen transfer with methanogen or other hydrogen-utilizing anaerobes. The syntrophic removal of the waste hydrogen by other bacteria is of paramount importance (McInerney *et al.*, 1980; Fang, 2000).

As indicated by some other researchers, a *Methanosaeta*-like colony (previously known as *Methanothrix* spp., Huser *et al.*, 1982) and a two-bacteria bacterium colony, namely, *Syntrophobacter*-like bacteria and *Methanobrevibacter*-like bacteria, were

observed in juxtaposition in a biogranule when treating brewery waste water (Alphenaar *et al.*, 1993; Grobicki and Stuckey, 1991; Fang, 2000). As a result, the hydrogen produced by the former, namely the *Syntrophobacter*-like bacteria, can readily be consumed by the latter, namely the *Methanobrevibacter*-like bacteria, without a build-up of hydrogen concentrations within the syntrophic association.

Due to the large hydrogen turnover rates and the small size of the hydrogen pool, most of the hydrogen produced during the reactions may not enter a common hydrogen pool at all (Corn *et al.*, 1985). The superiority of the biomass removal performance and its capability to resist shock loading both reside in its design.

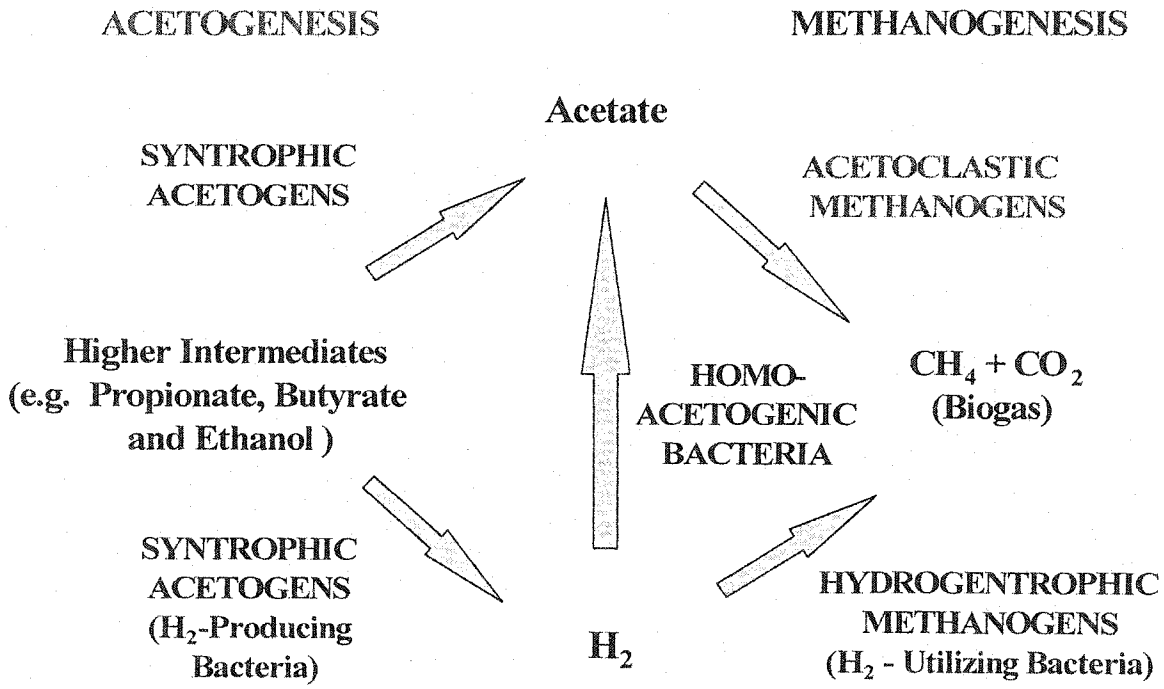


Figure 6.5 Transformation of Higher Intermediate in Acetogenic and Methanogenic Reactions

The success of interspecies-transfer is attributed to the formation of biofilm in the packing material within the anaerobic filter, because the proximity of the bacteria consortium enables the interspecies transfer to occur directly from one to another.

The packing materials also created a favorable environment for the growth and accumulation of anaerobic micro-organisms such as hydrogen-producing lipolytic bacteria, acetate-producing methanogenic bacteria and hydrogen-utilizing methanogenic bacteria in the void space of the packing material. Miyahara and Noike (1994) observed a similar phenomenon. The packing material environment also provides micro-organisms with an ideal environment for carrying out hydrolysis.

Grotenhuis (1991) indicated that the packing media environment also assists in creating a micro-environment favorable to interspecies-transfer, when the hydrogen-producing bacteria are surrounded by hydrogen-utilizing methanogenic bacteria in the biofilm.

6.6 Summary of Findings

The anaerobic filter, packed with fire-expanded clay pellets, was used to convert persistent waste, namely biological grease, into usable biofuel. The COD removal efficiency reached 90% during the entire operation period of 150 days. The AF section of AHR possessed an excellent anti-shock capacity, which could be 16 times that of normal organic loading loads, and it can readily recover from the shock loading. Biogas, with an average concentration of 68% (v/v) in methane, was generated at a rate of between 0.22 and 0.12 L/g COD. The biogas yield ranged from

0.81 to 4.59 L/Ld. This corresponded to an energy production rate at $2.2*10^5$ kJ/m^3 (e.g. 2.1 to 2.5* 10^5 kJ/m^3) of the trade effluent. Combining the mechanisms of both organic adsorption and biodegradation rendered the AF more stable under various shock loading conditions.

7. Introduction

7.1 Background

The potential of anaerobic processes for converting a variety of low and high strength industrial and municipal waste to energy-rich biogas has been appreciated in the literature (Chua *et al.*, 1995; Chua and Cheng, 1996; Staubmann *et al.*; 1997, Rajoka *et al.,* 1999; Nandy and Kaul, 2001). However, difficulties have also been encountered, particularly when treating certain types of persistent industrial waste.

Under normal circumstances, the bacterial biomass contained in an anaerobic treatment system has yet to evolve specific mechanisms for the degradation of certain persistent waste; such as wastewater from food processing and paper industries with a high cellulose content, oily and the high carbon content wastewater from the petrochemical industry and high COD wastewater from distilleries. It is therefore necessary to treat such persistent waste by specially designed in-house treatment facilities seeded with bacterial cultures. These bacterial cultures have been acclimatized by enrichment techniques to the specific waste being treated. Some of them may inhibit bacterial activity during degradation (Brown *et al.*, 1987), while the degradation of others may produce inhibitors (Goodwin *et al.*, 1990).

As compared with the aerobic process, the application of anaerobic process in wastewater treatment is still limited. The slow treatment rate is attributed to the slow growth rate of methanogenic bacteria. Conventional anaerobic reactors usually require a long HRT to prevent biomass washout, and to ensure organic stabilization.

Consequently, conventional anaerobic reactors have been applied only to the treatment of high-strength organic waste at low hydraulic loading rates (Young *et al.*, 1969).

Immobilized biofilm technology has given anaerobic reactors the capability necessary for retaining a high concentration of biomass within the system. As a result it has become popular, particularly when treating effluent with low suspended solids and a high organic content. This has led to a high rate of treatment as well as better resistance to hydraulic and organic shock loadings. Anaerobic processes have been applied to treating the entire stream of industrial waste effluent (Stafford *et al.*, 1980a; Henze *et al.*, 1983; Speece, 1983; Hao *et al.*, 1990; del Poze *et al.*, 2000; Borja *et al.*, 2001; Fang and Yu, 2001). Chua *et al.* (1996a) demonstrated a COD removal efficiency rate of 85% and a grease removal efficiency of 53% from 4.5 gCOD/L food processing wastewater with an anaerobic filter operating at a HRT of 1.5 days.

Anaerobic processes, however, have been reported as being sensitive to shock loading environments in terms of hydraulic and organic shock loadings (Chua *et al.*, 1995a). Shock loading due to the lack of balancing and equalizing facilities in the context of a congested urbanized area often results in the failure of a conventional treatment system (Boardman *et al.*, 1995; Borja *et al.*, 1995; Chua *et al.*, 1995b; Chua *et al.*, 1995c). In conventional single-stage anaerobic reactors, the excessive production of VFA resulting from shock loadings and sudden changes in processing conditions has been observed. As a result, reactors turn "sour" and methane production is terminated (Chartrain and Zeikus 1986; Kissalita *et al.*, 1987). The massive sloughing of the biofilm from the support medium and the washout of biomass from the reactor are the

phenomena commonly encountered under unfavorable conditions, causing organic inhibition to the micro-organisms or an excessive hydraulic shear force to the biofilm.

Hydraulic shock loading with low concentrations of the COD load is fairly common when treating the food-processing effluent produced during the washing of certain types of fermentation equipment or the generation of washing water from production lines. Organic shock loading commonly occurs when accidental overflows of high concentrations of COD load lead to the malfunction of the grease traps.

In this chapter, the performance and stability of the individual AF operated under critical hydraulic and organic shock loads are compared and evaluated and the causes of the failure and recovery of the AF under critical shock loading conditions are discussed. The inhibition of anaerobic degradation pathways for soluble organic matter resulting from critical shock loadings is also elucidated.

Results and Discussion

7.2 Performance of the AF Under Various Shock Loadings

7.2.1 COD Removal

The profiles of temporal variation in the effluent COD and the COD removal efficiency during various hydraulic and organic shock loadings are shown in Figures 7.1 and 7.2. The performance of the AF, which was operated under various hydraulic and organic shock loading conditions, is shown in Tables 7.1 and 7.2 respectively. Table 7.3 shows a summary of the AF's performance during normal and shock

loadings as well as under critical shock loadings. Table 7.4 shows a comparison of the performance of the AF in response to the critical hydraulic and organic shock loadings.

At steady state operation, the AF maintained a constant OLR of 1.8 gCOD/L-d. As the hydraulic shock loading increased (e.g. when HRT was reduced from 5 days to 1 day), the COD removal rate decreased from 1.76 to 1.63 g COD/L-d, and it dropped further to 1.44 gCOD/L-d under critical shock loading conditions, equivalent to ten times of hydraulic shock loading. On average, over 93% of the influent COD was removed during the hydraulic shock loading experiments. Process stability could be maintained during hydraulic shock loading at HRTs from 2.5 days to 1 day. However, the stability of the AF was reduced at an HRT of 0.5 day. The COD removal rate was maintained at only 1.26 gCOD/L-d under critical hydraulic shock loading conditions. Under these circumstances, the COD removal efficiency was reduced to around 70%. As suggested, when a high flow rate of influent is introduced during hydraulic shock loading, it may induce more and more channeling through the biomass bed, resulting in the poor contact of the substrate with the biomass, thus reducing the degradation of the incoming COD content (Grobicki and Stuckey; 1991).

In the present study of organic shock loadings, it was observed that the COD removal rate increased from 9.52 to 55.2 gCOD/L-d as an increment of the organic loading rate from 7.2 to 57.6 gCOD/L-d (Table 7.2). On average, about 97% of the COD removal efficiency was achieved under the organic shock loading environment. When the organic loading rate was further increased to 115.2 gCOD/L-d at HRT of 1.25 day, equivalent to a 16-fold organic shock loading, the AF became unstable. The COD

removal rate and the COD removal efficiency were respectively 80.5 gCOD/L-d and 69.9%.

In terms of the COD removal efficiency, the performance of AF showed no significant difference under the hydraulic and organic shock loading environment from normal. The AF achieved over 90% of the COD removal efficiency under all these situations except that it was under a critical shock loading environment (Table 7.3). In this environment the overall COD removal efficiency was reduced to around 70%. The reduction of this efficiency resulted in the accumulation of significant amounts of VFAs (e.g. propionate) under shock loading (Dermirel and Yenigun, 2002). The temporal variations in parameters like pH, effluent VSS and COD removal, in response to various shock loading conditions, are summarized in Table 7.4.

In summary, the general responses of the AF to the shock loadings introduced are characterized by an accumulation of VFAs, a decrease in removal efficiency, a decrease in methane concentration, an increase in biogas production rate (only applicable to organic shock loadings) and a decline of pH.

Table 7.1 Performance of the AF During Various Hydraulic Shock Loadings

Hydraulic Shock Load (times)	0 (normal)	2	4	5	10**
Organic Loading Rate (gCOD/L-d)	1.8	1.8	1.8	1.8	1.8
Hydraulic Retention Time (d)	5	2.5	1.25	1	0.5
COD Removal Rate (gCOD/d)	1.76	1.72	1.65	1.63	1.26
COD Removal Efficiency (%)	98.1	92.7	91.0	89.7	70.0
Specific Growth Yield ($gVSS_{produced}/gCOD_{removed}$)	0.019	0.026	0.023	0.021	0.017
Biogas Production Rate (L/d)	0.60	0.54	0.38	0.51	0.25
Biogas Yield (L/L-d)	0.99	0.45	0.16	0.26	0.04
Methane Concentration (%)	74	69	64	70	50
Methane Production Rate (L/d)	0.44	0.37	0.24	0.36	0.13
Specific Methane Yield (L/gCOD)	0.28	0.32	0.29	0.30	0.10
pH	7.2	6.8	6.0	6.3	5.5

** Critical hydraulic shock load introduced on the day 197 of the operation

Table 7.2 Performance of the AF During Various Organic Shock Loadings

Organic Shock Load (times)	0 (normal)	2	4	8	16**
Organic Loading Rate (gCOD/L-d)	7.2	14.4	28.8	57.6	115.2
Hydraulic Retention Time (d)	1.25	1.25	1.25	1.25	1.25
COD Removal Rate (gCOD/d)	9.52	13.79	28.29	55.2	80.5
COD Removal Efficiency (%)	98.1	95.5	98.2	95.8	69.9
Specific Growth Yield ($gVSS_{produced}/gCOD_{removed}$)	0.030	0.026	0.023	0.014	0.010
Biogas Production Rate (L/d)	2.0	4.0	7.3	11.0	9.4
Biogas Yield (L/L-d)	0.81	1.68	3.05	4.59	3.92
Methane Concentration (%)	73	73	65	60	45
Methane Production Rate (L/d)	1.46	2.92	4.45	6.6	4.23
Specific Methane Yield (L/gCOD)	0.22	0.19	0.16	0.12	0.05
pH	6.5	6.4	6.7	6.7	5.7

* Critical organic shock load introduced on the day 155 of the operation

Table 7.3 Performance of the AF During Various Hydraulic and Organic Shock Loadings : A Summary

	Normal (0 times HSL)	Normal (0 times OSL)	HSL (2–5 times)	OSL (2–8 times)	Critical HSL (10times)	Critical OSL (16 times)
COD Removal Efficiency (%)	98.1	98.1	92.7–89.7	98.2–95.5	70	69.9
Specific Growth Yield ($gVSS_{produced}/gCOD_{removed}$)	0.019	0.030	0.026–0.021	0.026–0.014	0.017	0.010
Biogas Yield (L/L-d)	0.99	0.81	0.54–0.38	1.68–4.59	0.26	3.92
Methane Concentration (%)	74	73	70–64	73–60	50	45
Specific Methane Yield (L/gCOD)	0.28	0.22	0.32–0.29	0.19–0.12	0.10	0.05
VFA Accumulation (mg/L)	NA	NA	24 (Acetate)	76.5 (Acetate)	250 (Acetate)	1607 (Acetate)
			70 (Propionate)	72.9 (Propionate)	312 (Propionate)	477 (Propionate)
			----	----	-----	1646 (Butyrate)
			----	13.6 (n-Valerate)	-----	771 (n-Valerate)
pH	7.2	6.5	6.8–6.0	6.7–6.4	5.5	5.7

7.2.2 Biogas and Methane Production

Both the quality and the yield of biogas are common parameters used for describing the status as well as monitoring the performance of an anaerobic system. Figures 7.3 and 7.4 show the daily biogas production rate and the composition of biogas under various hydraulic and organic shock loading environments respectively. In the hydraulic shock loading experiments, the biogas production rate, the biogas yield and the methane percentage in the biogas decreased progressively with the increment of hydraulic shock loadings (Figure 7.3 and Table 7.1). In contrast, the concentration of carbon dioxide in the biogas was maintained at a fairly low level during the various hydraulic shock loadings. The minimum biogas production rate at HRT 2.5, 1.25 and 1.0 days was 0.4, 0.35 and 0.4 L/d respectively. The biogas production rate further declined to 0.25 L/d under the critical hydraulic shock loading conditions at HRT 0.5 day. The corresponding lowest methane concentrations in the biogas were 66.7%, 59.1%, and 69.3% at HRT 2.5, 1.25 and 1.0 days respectively. The specific methane yield ranged between 0.28 and 0.32 L/gCOD. It was further reduced to 0.1 L/gCOD during the critical hydraulic shock loading condition.

In the organic shock loading experiments, the biogas production rate, biogas yield and carbon dioxide concentration increased in proportion to the increment of the organic loading rate (Figure 7.4). The maximum biogas production rates at COD values of 6000, 12000 and 24000 mg/L were 4.5, 7.75 and 12.65 L/d respectively. However, the percentage of methane in the biogas decreased against the increase of the biogas production rate. The lowest correspondent methane concentrations in the biogas were 70%, 56.2%, and 52.7% respectively. The specific methane yield ranged from 0.36 to

0.44 L/gCOD, and it dropped further to 0.05 L/gCOD during the critical organic shock loading.

As shown in Figures 7.3 and 7.4, the biogas yield exhibited a different trend of behavior in response to hydraulic and organic shock loadings. In the experiments of hydraulic shock loadings, the average biogas yield decreased with an increment of the hydraulic loading rate. In contrast, the biogas yield increased with an increment of the organic loading rate during organic shock loadings (Tables 7.1 and 7.2). It is suggested that the substrate removal is a direct function of biogas production. The results also indicated that a high biogas yield corresponds to the increase in the mass of substrate removed per unit volume per day of the reactor system (Nandy and Kaul, 2001). Grobicki and Stuckey (1991), in a study of the stability of the anaerobic baffled reactor (ABR, described as several UASB in series), observed that mass transfer limitations increased as the HRT decreased. It is suggested that the organic substrate may flow though the packed bed medium without being metabolized, resulting in a low contact time between the biomass and the substrate during the various hydraulic shock loadings. The biogas production rate has been found to be proportional to the COD removal rate during various organic shock loadings (McCarty and Smith, 1986). Both the biogas production rate and the COD removal rate were found to decrease as an increment of hydraulic shock loadings (Tables 7.1 and 7.2).

Theoretically, 0.35 L of methane is produced from each gram of organic substrate removed when the starting substrate is glucose (Lawrence and McCarty, 1969). The volume of biogas produced per day is assumed to be proportional to the amount of substrate consumed. Through the anaerobic degradation pathway, namely; β –

oxidation of fatty acids, a theoretical methane concentration of 68.75% in biogas is obtained (Yap *et al.*, 1992). The specific methane production rate of the AF was maintained within the range of 0.28 to 0.32 L/gCOD in various hydraulic shock loadings (Table 7.1). Such a value is much closer to the theoretical value of 0.35 L/g COD. The present results indicate that the activities of the methanogenic bacteria were not much affected by the hydraulic shock loadings introduced. On the other hand, under various organic shock loading situations, the specific methane production rate of AF was maintained at only between 0.22 to 0.12 L/g COD, which was much lower than that of the theoretical value, as reported elsewhere, indicating that the activities of the methanogenic bacteria were temporarily inhibited under critical organic shock loading conditions.

In view of the above, the decreased production of methane under organic shock loading may result in the inhibition of the methanogenic bacteria, causing an accumulation of VFA residuals in the effluent (Figure 7.6). If so, it appears that the organic shock loadings have a more significant impact, in terms of microbial inhibition, on methane production than the hydraulic shock loadings in the AF assembly.

Table 7.4 Performance of an Individual AF to Critical Hydraulic and Organic Shock Loadings

	Hydraulic Shock Loadings		Organic Shock Loadings	
Shock Load (times)	5*	10	8**	16
HRT (d)	1	0.5	1.25	1.25
Organic Loading Rate (gCOD/L-d)	1.8	1.8	57.6	115.2
Specific Methane Yield (L/gCOD)	0.30	0.28	0.12	0.05
Biogas Production (L/d)	0.51	0.25	11.0	9.4
Biogas Yield (L/L-d)	0.26	0.04	4.59	3.92
Methane Concentration (%)	70	50	60	45
Specific Methane Yield (L/gCOD)	0.30	0.10	0.12	0.05
COD Removal Rate (gCOD/d)	1.63	1.26	55.2	80.5
COD Removal Efficiency (%)	89.7	70.0	95.8	69.9
Specific Growth Yield ($g_{produced}VSS/gCOD_{removed}$)	0.021	0.017	0.014	0.010

Remarks: The maximum stable and critical shock load conditions are denoted by "*" and "**" respectively

Table 7.5 Temporal Variations in AF in Response to Hydraulic and Organic Shock Loadings - A Summary

Parameter	Hydraulic Shock Loadings (HSLs)	Organic Shock Loadings (OSLs)
pH	Slightly ↓ as ↑ of HSLs (e.g. from 7 to 5.5).	Slightly ↓ as ↑ of OSLs (e.g. from 7.15 to 6.4), except at critical OSL (e.g. 16 times), pH level dropped from 6.8 to 4.1
Effluent VSS	Maintained at a fairly low level as ↑ of HSLs.	Maintained at a low level as ↑ of OSLs.
COD removal (%)	↓ as ↑ of HSLs.	↓ as ↑ of OSLs
Biogas production (L/d)	↓ as ↑ of HSLs.	↑ as ↑ of OSLs.
CH_4 (%)	↓ as ↑ of HSLs.	↓ as ↑ of OSLs
CO_2 (%)	Maintained a fairly constant level as ↑ of HSLs, except at critical HSL (e.g. 10 times)	↑ as ↑ of OSLs.
Temporary inhibition on anaerobe	Acetoclastic methanogenic bacteria and H_2-utilizing bacteria	1. Acetoclastic methanogenic bacteria and H_2-utilizing bacteria. 2. The activity of acidogenic bacteria was also inhibited under critical organic shock loads.
Inhibition magnitude	Inhibition magnitude on both Acetoclastic methanogenic and H_2-utilizing bacteria was similar.	Inhibition magnitude on H_2-utilizing bacteria was higher than that of Acetoclastic methanogenic bacteria.
Critical shock loads	10 times HSLs.	16 times OSLs.
Phenomenon	1. VFAs accumulated, mainly acetate and propionate, and CH_4 production decreased. 2. System failure, no massive sloughing and no biomass washout 10 times HSLs.	1. VFAs accumulated, mainly acetate and butyrate, and CH_4 production decreased. 2. System failure, massive sloughing and biomass washout under 16 times OSLs .

Remarks:

The decrease or increase in magnitude are denoted by the symbol of " ↓ " or " ↑ " respectively

7.2.3 VFA Accumulation

Volatile fatty acids are intermediate products that are produced from anaerobic processes, whose concentration varies under different circumstances. As a general trend, the higher the VFA concentrations in the effluent, the poorer the performance of the AF is in removing COD.

Figures 7.5 and 7.6 show the profiles of VFA accumulation under various hydraulic and organic shock loading environment respectively. During hydraulic and organic shock loadings, the main VFAs accumulating in the effluent were acetate and propionate. Other VFAs accumulating in the effluent under critical organic shock loadings were mainly n-butyrate and n-valerate, but no other VFAs, except acetate and propionate, were observed under the critical hydraulic shock loading environment. Fang and Yu (2002) demonstrated that the VFAs products in effluent were highly influenced by the variation of HRT. They also suggested that short HRT favored the production of propionate. The results of the present studies coincide with the findings by other researchers (McCarty and Mosey, 1991; Fang and Yu, 2002), showing that propionate is one of the main VFAs that accumulate in the effluent under hydraulic shock loadings.

Yu and Pinder (1993) found that fatty acids (e.g. acetate, propionate and butyrate) have a different utilization rate in the methane-producing biofilm, because each acid is utilized by different types of bacteria. Of these, propionate is utilized at the lowest rate. Yu and Pinder (1993) also indicated that a high propionate concentration inhibits

butyrate utilization, which may be attributed to the competition between acetogenic bacterial types, including acetogenic bacteria, which convert high fatty acids like propionate and butyrate into acetate and hydrogen; acetophilic methane bacteria, which convert acetate to methane; and hydrogenophilic bacteria, which utilize H_2 and CO_2 to produce methane for hydrogen removal (Zeikus, 1977; Dolfing, 1988).

Other researchers indicated that the HRT have a significant effect on the distribution of effluent products (Dinopoulou *et al.*, 1988; Henry *et al.*, 1987, Elefsioniotis and Oldam, 1994; Fang and Yu, 2002). Dinopoulou *et al.* (1988) observed that the longer HRT resulted in an increased acetate concentration, whereas the concentration of propionate did not change under different HRTs. At high organic loading rates, Baloch and Akunna (2003) observed that the microbial ecology of the anaerobic system appeared to favor acetate and butyrate production. Such findings also coincide with the results of the present studies under critical organic shock loading. Contradictory results have been reported with regard to the effect of high organic loading rates. Bull *et al.* (1994) reported that more propionate was produced under higher organic loading rates. However, no effect of the HRT on the distribution of the VFAs was observed in the present study.

In accordance with McCarty and Mosey's (1991) hypothesis, the production of butyrate, in contrast to format, acetate or propionate is favored at low pH levels, which enhance the growth of butyrate-forming bacteria. The increased production of butyrate instead of acetate may be an adaptation by anaerobe consortia against the high acidity resulting in an accumulation of VFAs. The accumulation of acetate, propionate and

butyrate may be due to an increment of the hydrogen partial pressure that may limit the degradation of VFAs (Ferguson and Man, 1983; Huang *et al.*, 2000).

The average acetate and propionate concentrations increased to 24 and 70 mg/L respectively (Figure 7.5). However, it increased further to the highest concentrations of 250 and 312 mg/L respectively under critical hydraulic shock loading conditions. These concentrations exceeded the threshold limits that inhibited methanogenic bacteria (Chua *et al.*, 1995b, 1996c; Chynoweth *et al.*, 1970; Inoue *et al.*, 1988).

Figure 7.6 shows that the average concentrations of acetate; propionate, n-butyrate, n-valerate and n-caproate in effluent rise dramatically to 76.5, 72.9, 13.0, 13.6 and 22 mg/L respectively when the shock organic loading rate was increased from 3000 to 24000 mgCOD/L. Under high organic shock loading conditions such as the increase of COD from 3000 to 48000 mgCOD/L, significant quantities of VFAs accumulated in the effluent. The VFAs contents in the effluent, in terms of acetate, propionate, n-butyrate, n-valerate and n-caproate, increased to their maximum concentration of about 1607, 477, 1646, 771 and 87 mg/L respectively.

When comparing Figures 7.5 and 7.6, it was observed that the magnitude of the accumulation of the VFAs was different. A significantly greater amount of VFAs accumulated in the waste effluent during organic shock loadings than during the hydraulic shock loadings. Such a discrepancy may be attributed to the low availability of organics that results from the high volumetric flow-rate introduced under the various hydraulic shock loadings.

7.2.4 Growth Yield

A variation of effluent VSS was observed during the initial startup stage, resulting from the washout of the biomass after seeding. The VSS of the effluent became stable when a firm biofilm was established on the support medium after 100 days of operation. The VSS levels in the treated effluent remained at a fairly low concentration even during the hydraulic shock loading situations. The specific growth yield was estimated to be 0.017 to 0.026 $gVSS_{produced}/gCOD_{removed}$.

During the organic shock loadings experiments, no substantial variation in the effluent VSS was observed. On the other hand, the VSS content was maintained at a very low level during the entire period of the organic shock loading experiments. The specific growth yield was estimated to be 0.010 to 0.030 g $VSS_{produced}/gCOD_{removed}$. Both of these values were lower than that of the reported range of 0.025 to 0.051 gVSS/gCOD (Pavlostathis and Giraldo-Gopmez, 1991). The reason for this low specific growth yield obtained in the hydraulic shock loading studies was that it might be due to the effect of substrate difference. In the present study, reconstituted milk was used as the carbon substrate rather than fatty acids as the solo carbon sources (Pavlostathis and Giraldo-Gopmez, 1991). As compared with reconstituted milk, VFAs provide ready-to-use energy sources for the growth of anaerobic microbial, whereas reconstituted milk contains large molecules of substrate including protein, carbohydrate and fat that is not the form of a ready-to-use energy source without undertaking the further process of the hydrolysis of macro-molecules.

However, the low specific growth yield (e.g. 0.010 $gVSS_{produced}/gCOD_{removed}$) obtained in the organic shock experiments was partly due to the massive sloughing of insoluble fatty matter with the biomass from the support media, particularly during the very high organic shock loading (e.g. 16 times of organic shock loading).

The results confirmed that the immobilized biofilm established on the surface of the supporting medium could tolerate various hydraulic and organic shock loadings environment while, providing excellent anti-shock capability against various adverse situations.

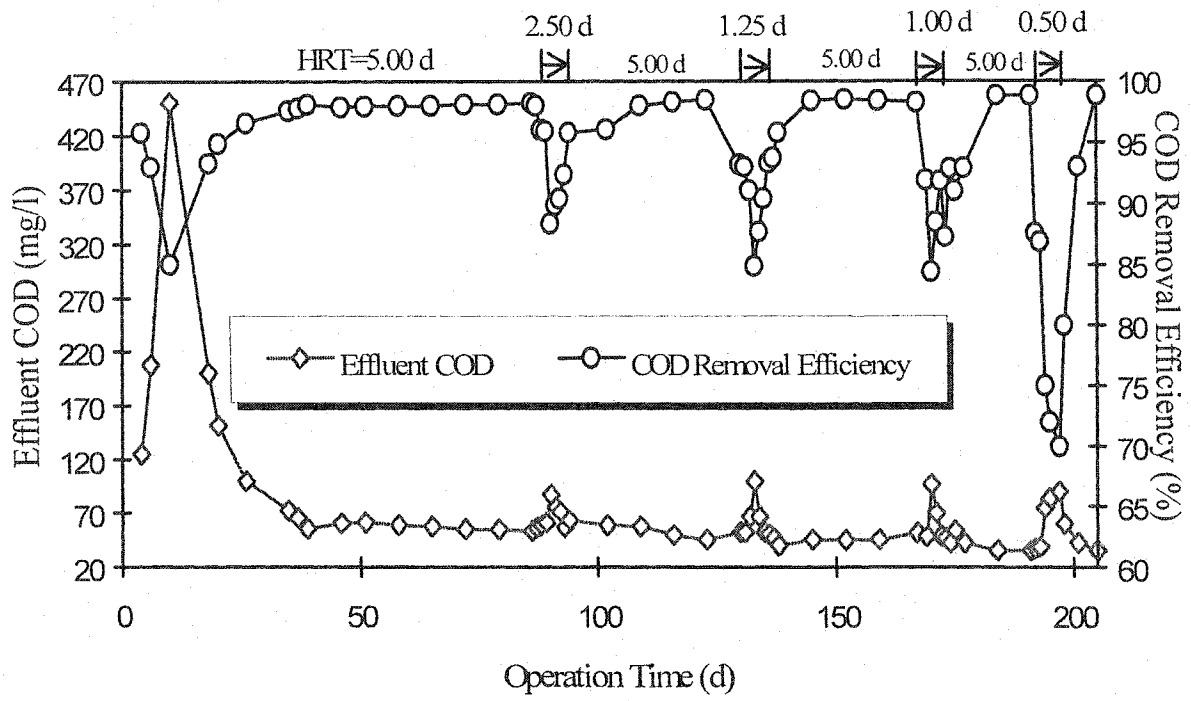


Figure 7.1 (5.1) Effluent COD and COD Removal Efficiency During Various Hydraulic Shock Loadings

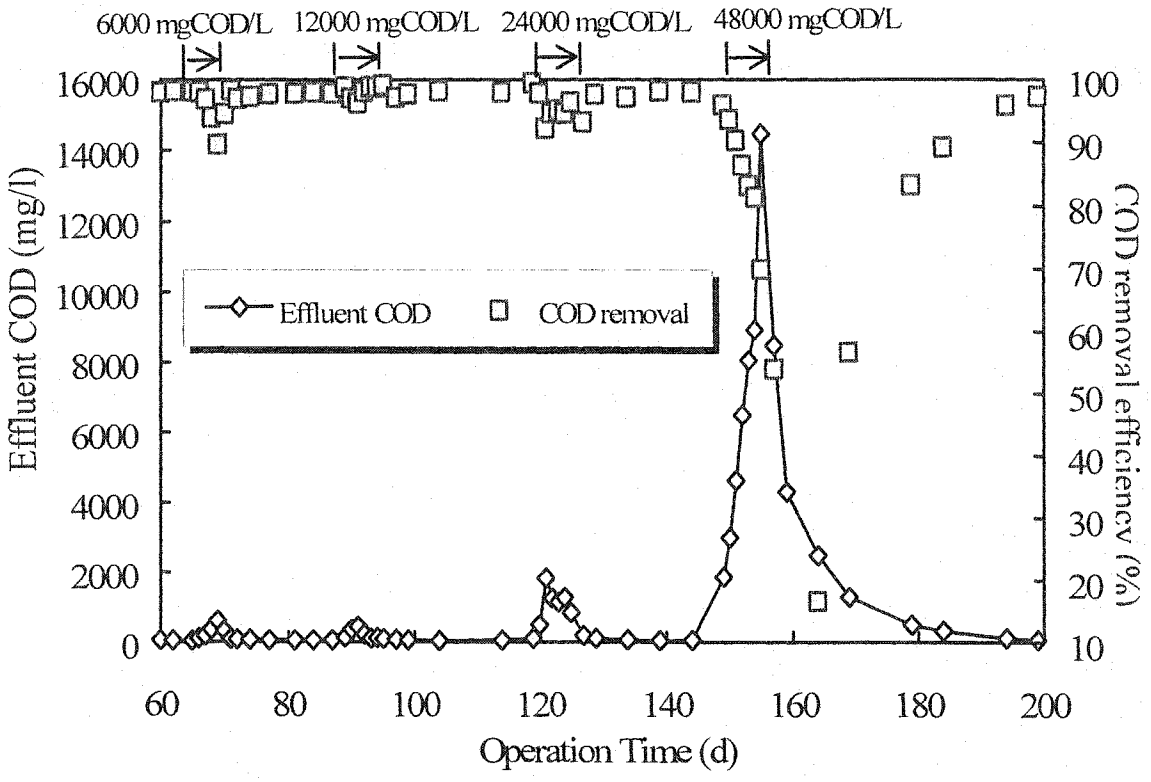


Figure 7.2 (6.1) Effluent COD and Calculated COD Removal Efficiency During Various Organic Shock Loadings

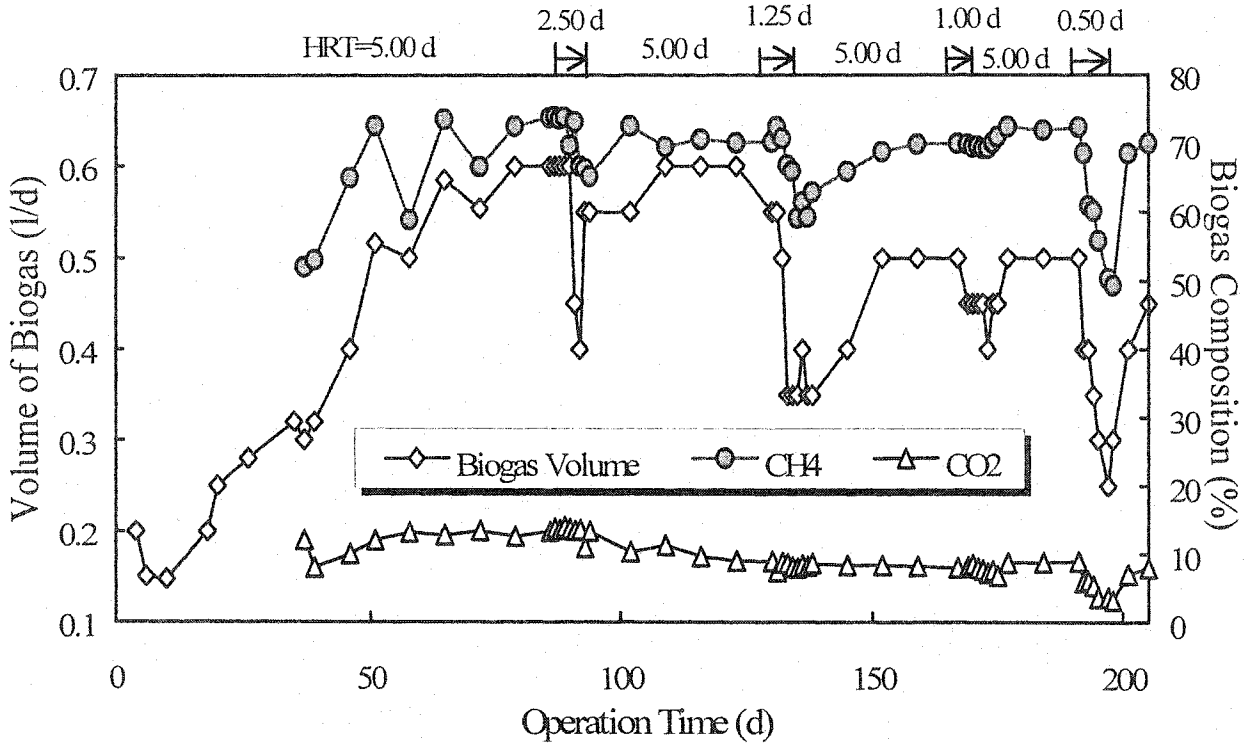


Figure 7.3 (5.3) Biogas Production Rate and Composition During Various Hydraulic Shock Loadings

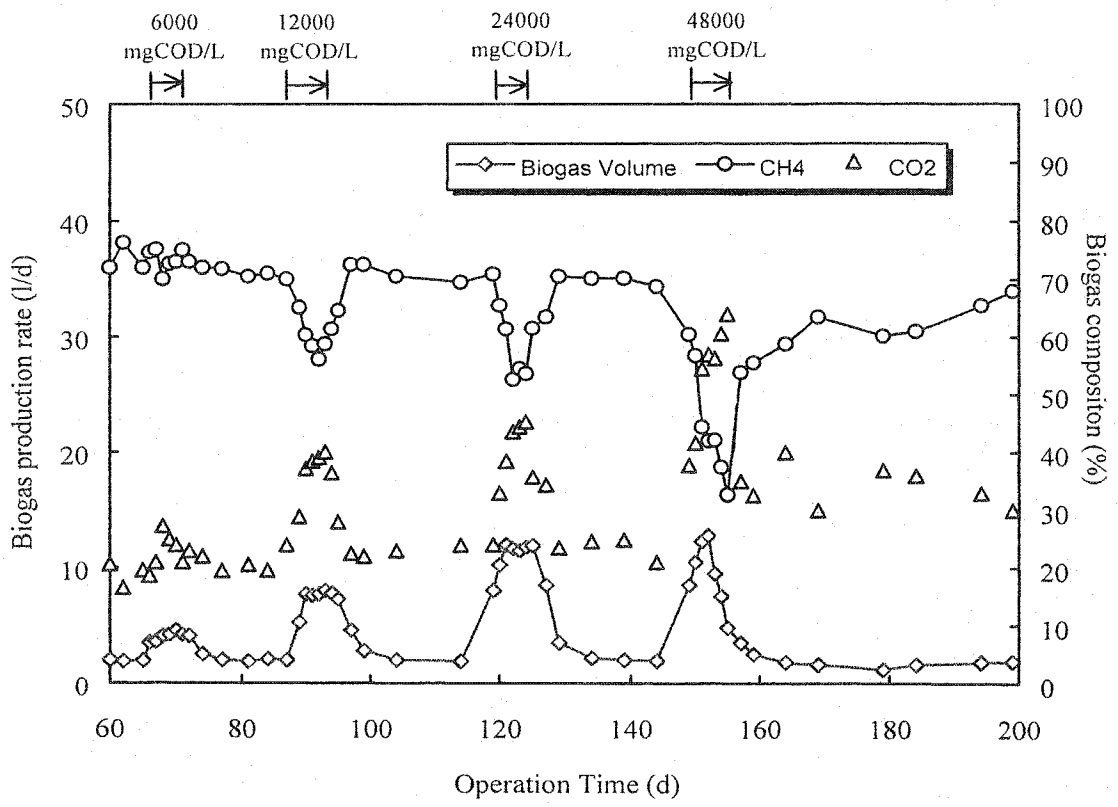


Figure 7.4 (6.2) Biogas Production Rate and Composition During Various Organic Shock Loadings

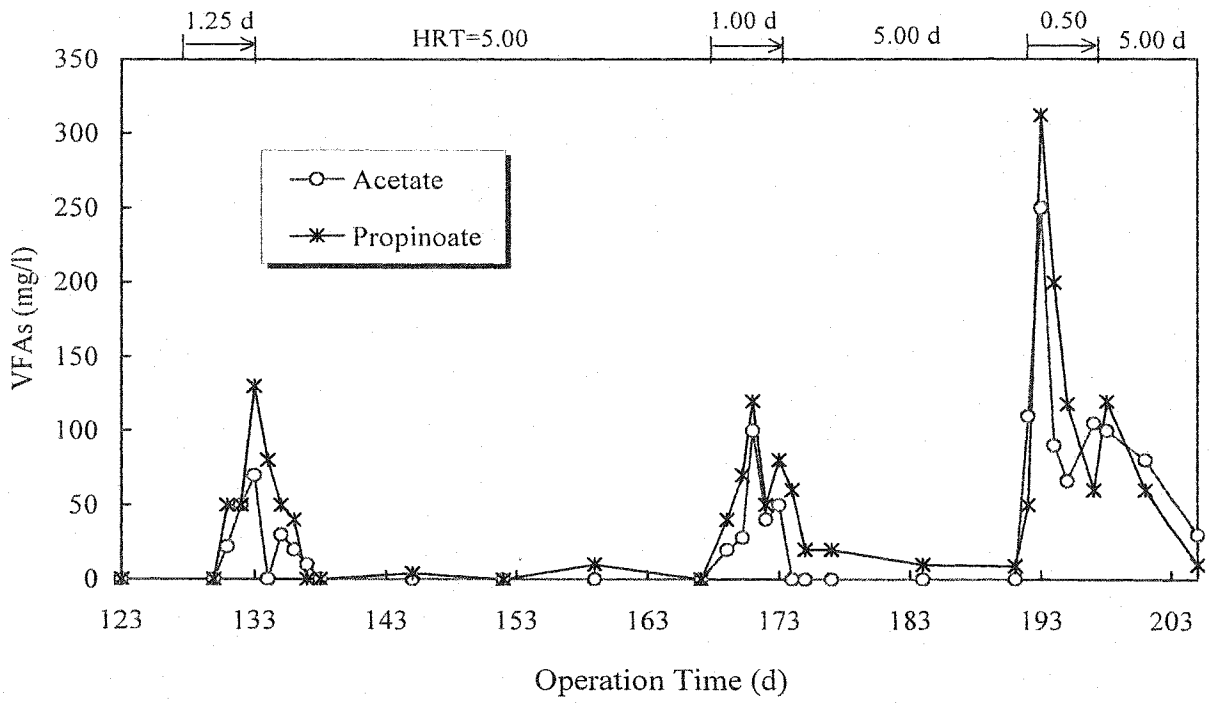


Figure 7.5 (5.4) Transient Responses of VFAs During Hydraulic Shock Loadings

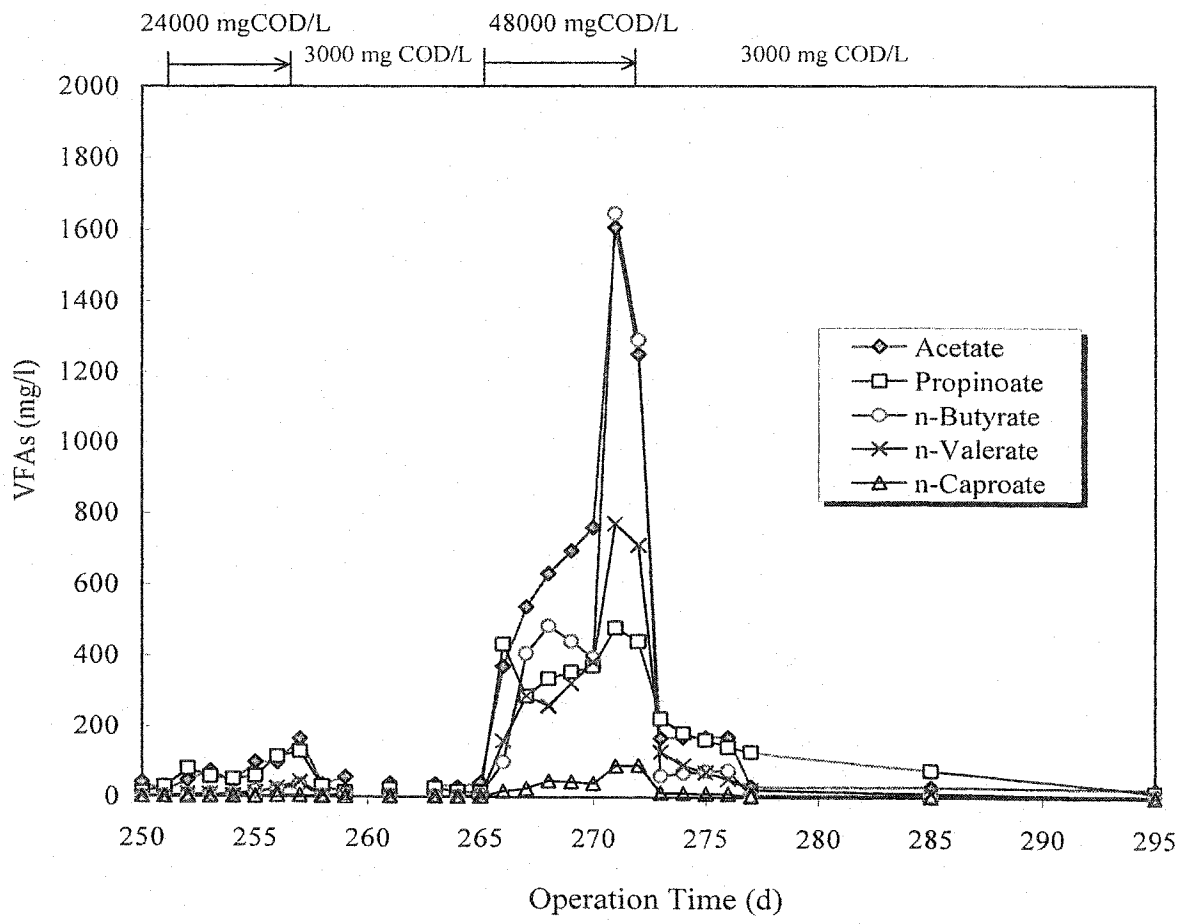


Figure 7.6 (6.3) Transient Responses of VFAs During Organic Shock Loadings

7.3 Bacterial Consortium on the Biofilm of the AF

The structure of the biofilm was examined under SEM. Samples were collected during the startup and after completion of the experiments of hydraulic and organic shock loadings. During the startup of AF at HRT of 5 days, a dense population of bacterial consortium was observed on both surface and voids/pores of FECS (Plate 7.1). The distribution of this bacteria consortium seems homogenous.

The SEM micrographs revealed that the bacterial consortium, contained in the AF, was composed of four morphologically distinctive species, identified as:

(i) *Syntrophmonas* spp. (McInerney *et al.*, 1981) – curved rod with round ends (0.3 to 2.0 μm in diameter and 1.5 to 6.0 μm in length),

(ii) *Methanosaeta* spp. (previously known as *Methanothrix* spp., Huser *et al.*, 1982; Patel and Sprott, 1990; Fang 2000) – rods and filaments with distinctive truncated ends (0.3 to 0.8 μm in diameter and 3–14 μm in length),

(iii) *Methanosarcina* spp. (Boone and Whitman, 1988) – readily form agglomerates of four cells in a spherical shape (1.2 to 1.6 μm in diameter) and,

(iv) *Methanococcus* spp. (Boone and Whiteman, 1988) – a cooci (0.5 to 1.2 μm), which is capable of being autofluoresced when excited at 420 nm for hydrogen utilization (Edwards and McBride, 1975; Jones *et al.*, 1987) (Plates 7.2 to 7.5).

Plate 7.1 shows the bacterial consortium that is composed of a microbial cluster of *Methanococcus* spp., *Methanosaeta* spp. and *Syntrophomonas* spp. The population profiles of the anaerobic consortium, mainly *Methanosaeta* spp. and *Methanococcus* spp., after hydraulic and organic shock loadings, are illustrated in Plates 7.6 and 7.7 respectively. In general, no significant difference between the appearance and arrangement of the anaerobic microbes was observed.

The observations made under SEM, demonstrate that the immobilized biofilm reactor possesses an excellent tolerance, in terms of hydraulic and organic shock loadings, to adverse conditions. They have also confirmed that the temporarily inhibited anaerobic bacteria were not washed out from the reactor even after shock loading, enabling the anaerobic bacteria to re-establish their activity when favorable conditions are restored.

As shown in Plate 7.7, the immobilized anaerobic bacterium attached to the support medium, substantially increased in cell retention time, with supportive evidence of the phenomenon of aging, particularly the *Methanosaeta* spp. As observed, some of these filaments even grew longer than 20 μm in length. Such observations corresponded to similar observations made by Endo *et al.* (1988) and Chua *et al.* (1996) in their studies of an anaerobic contact process and an anaerobic fixed-film reactor respectively.

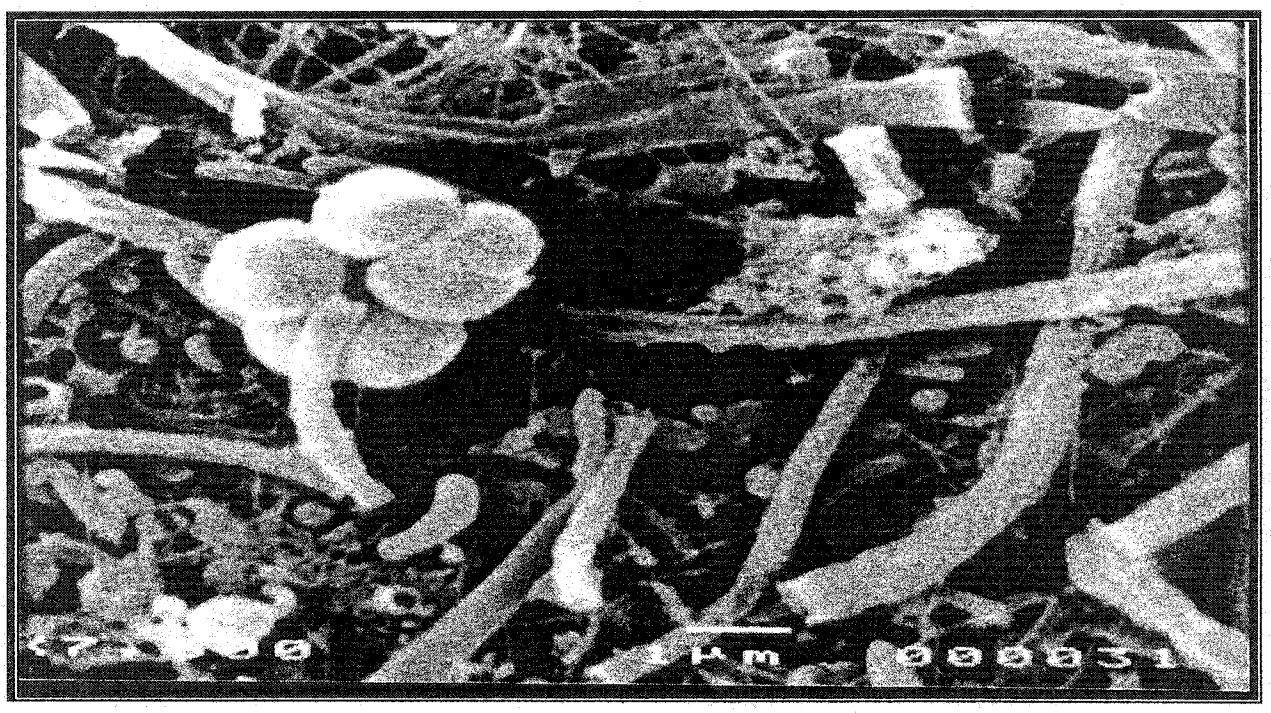


Plate 7.1 Electron micrograph of Bacterial consortiums on FECS (10KV, x7500)

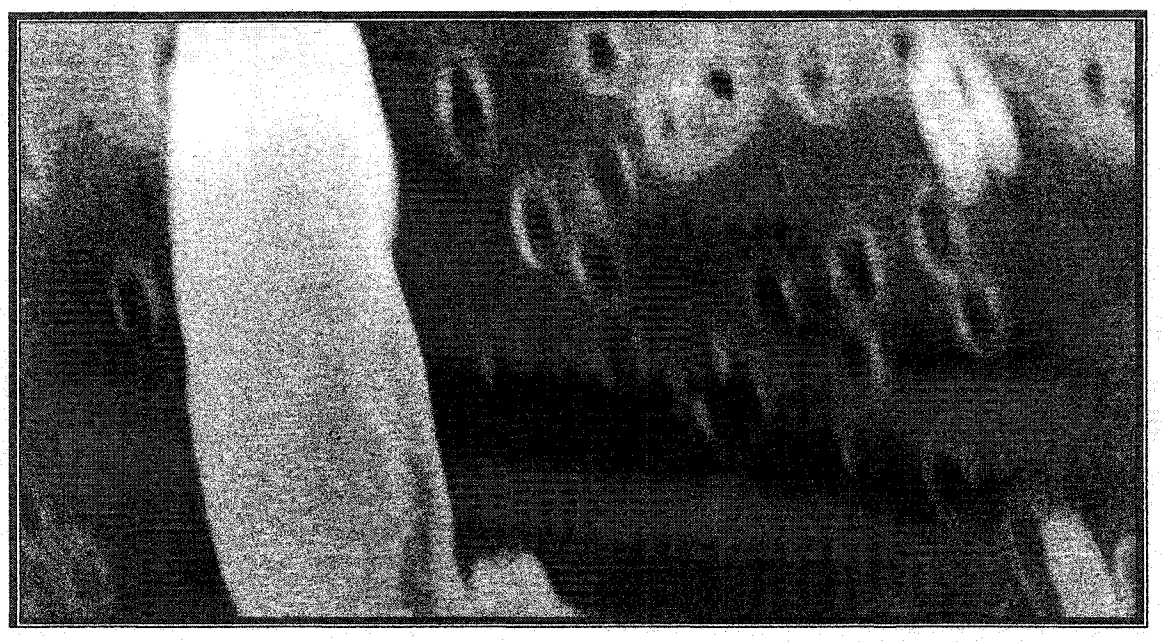


Plate 7.2 Electron micrograph of *Syntrophomonas* spp. (10KV, x10,000)

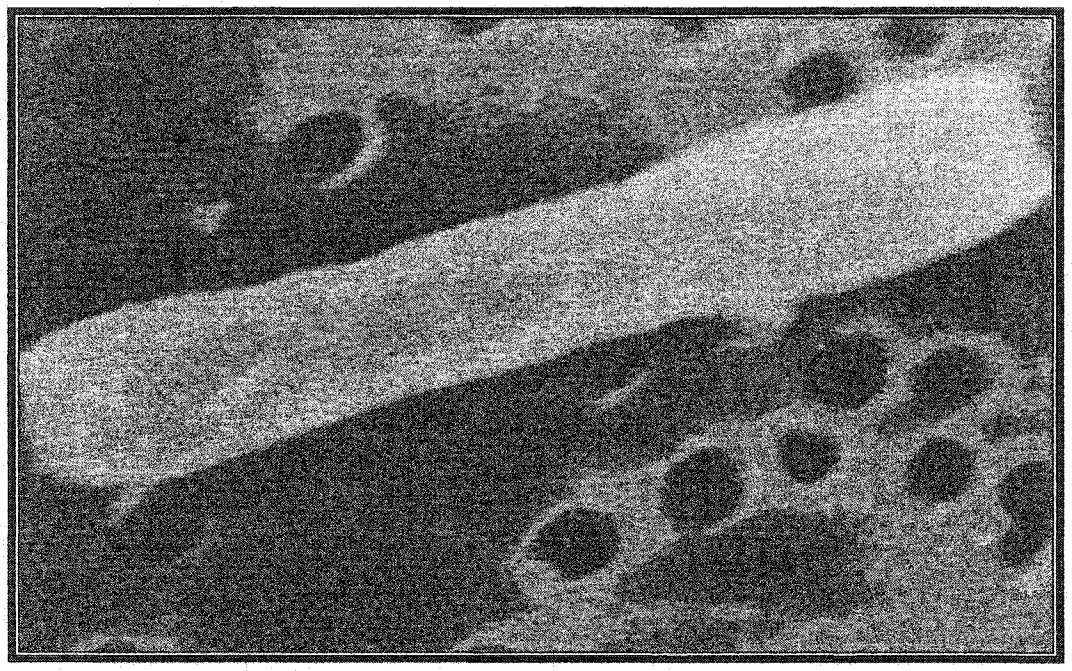


Plate 7.3 Electron micrograph of *Methanosaeta* spp.
(10 KV, x25,000)

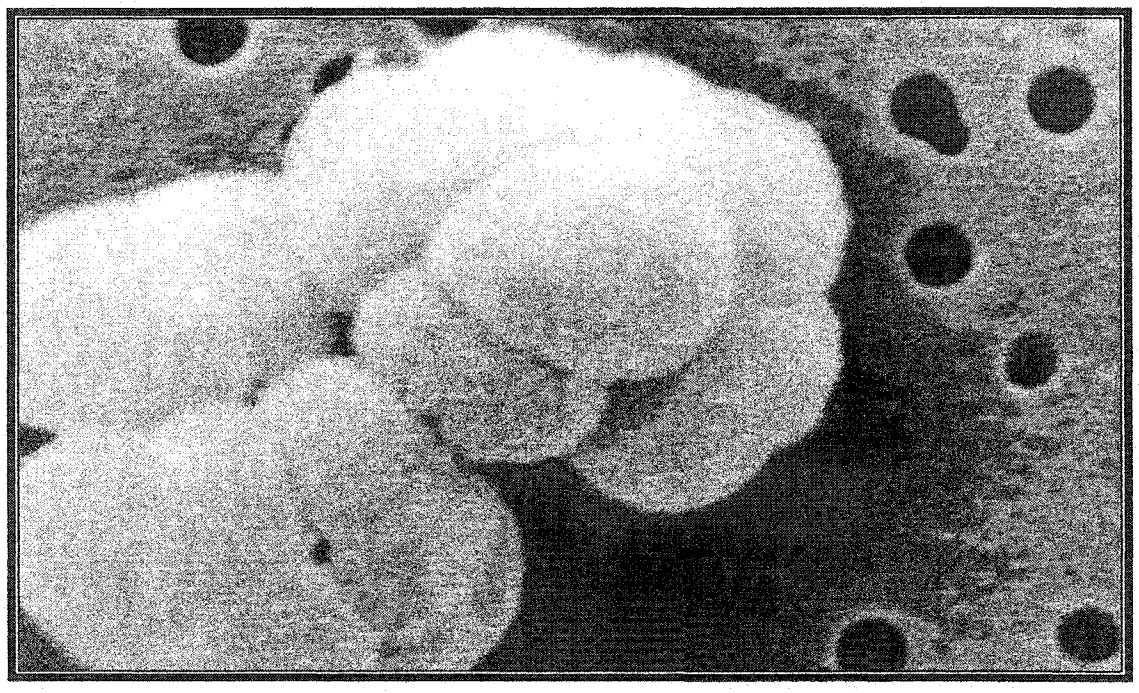


Plate 7.4 Electron micrograph of *Methanosarcina* spp.
(10 KV, x15000)

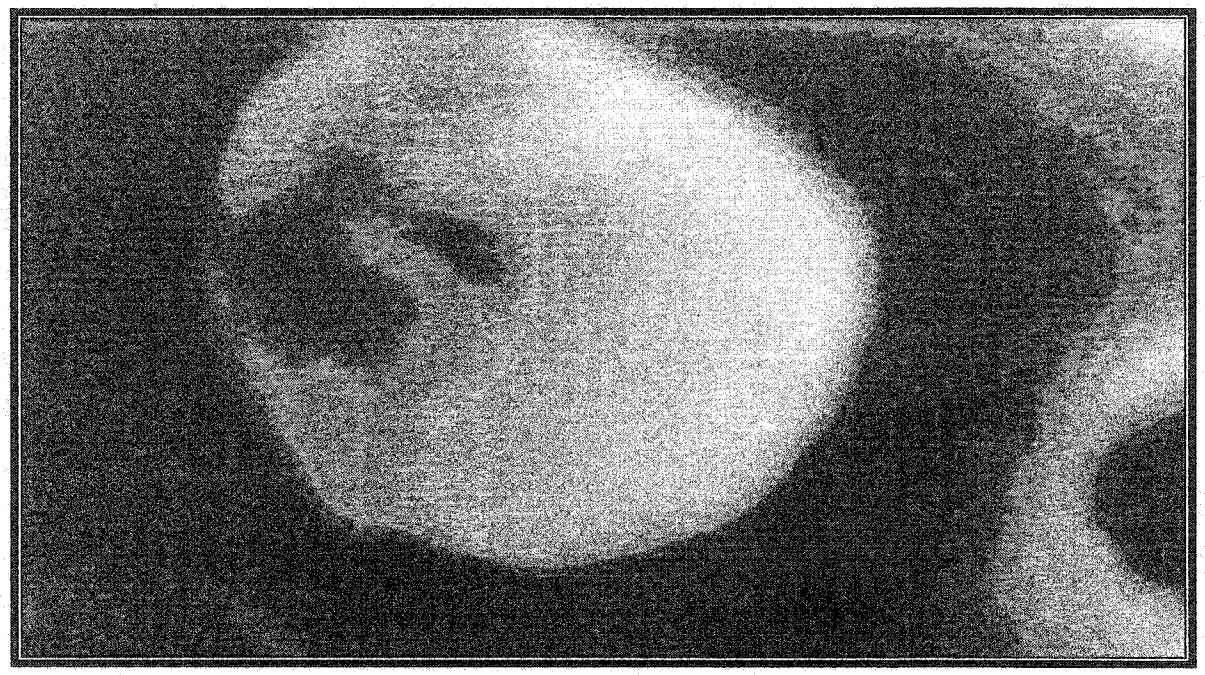


Plate 7.5 Electron micrograph of *Methanococcus* spp. (10 KV, x 60,000)

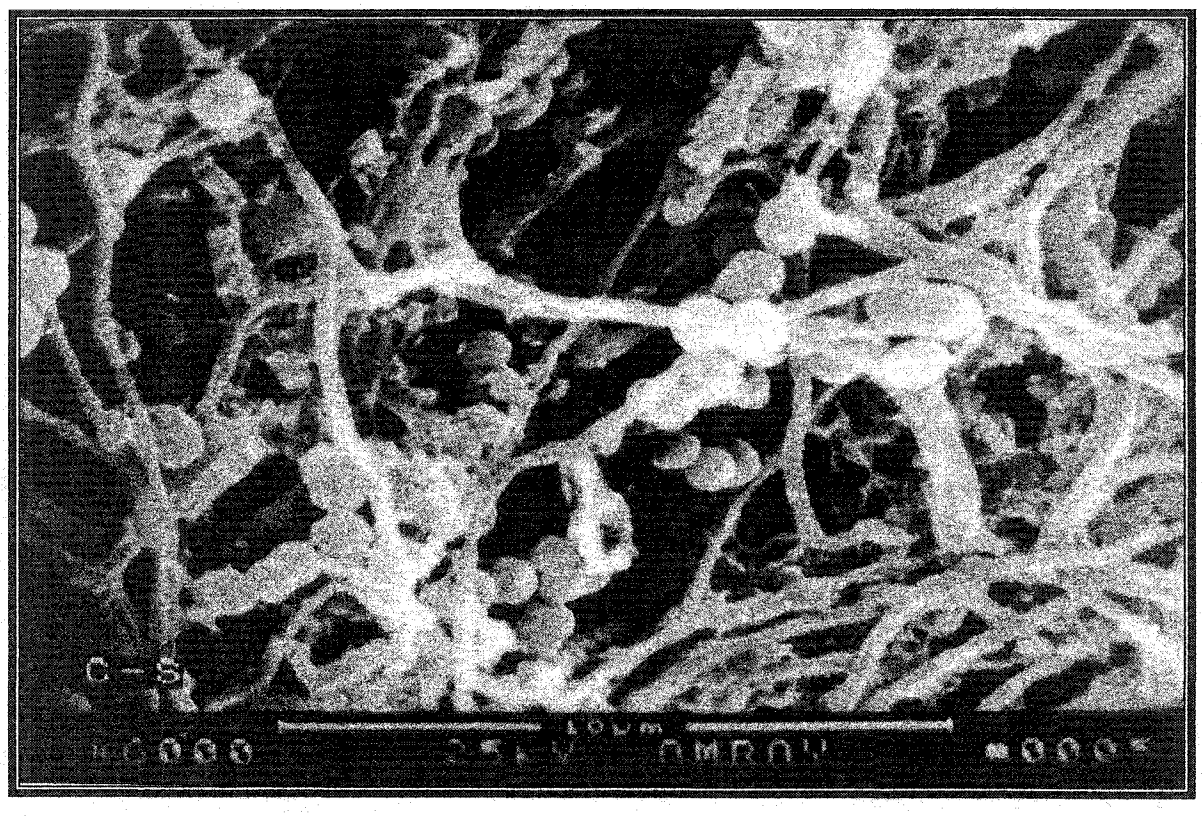


Plate 7.6 Electron micrograph of biofilm on FECS, dominated by *Methanosaeta* and *Methanococcus* spps., after hydraulic shock loadings (25 KV, x 6000)

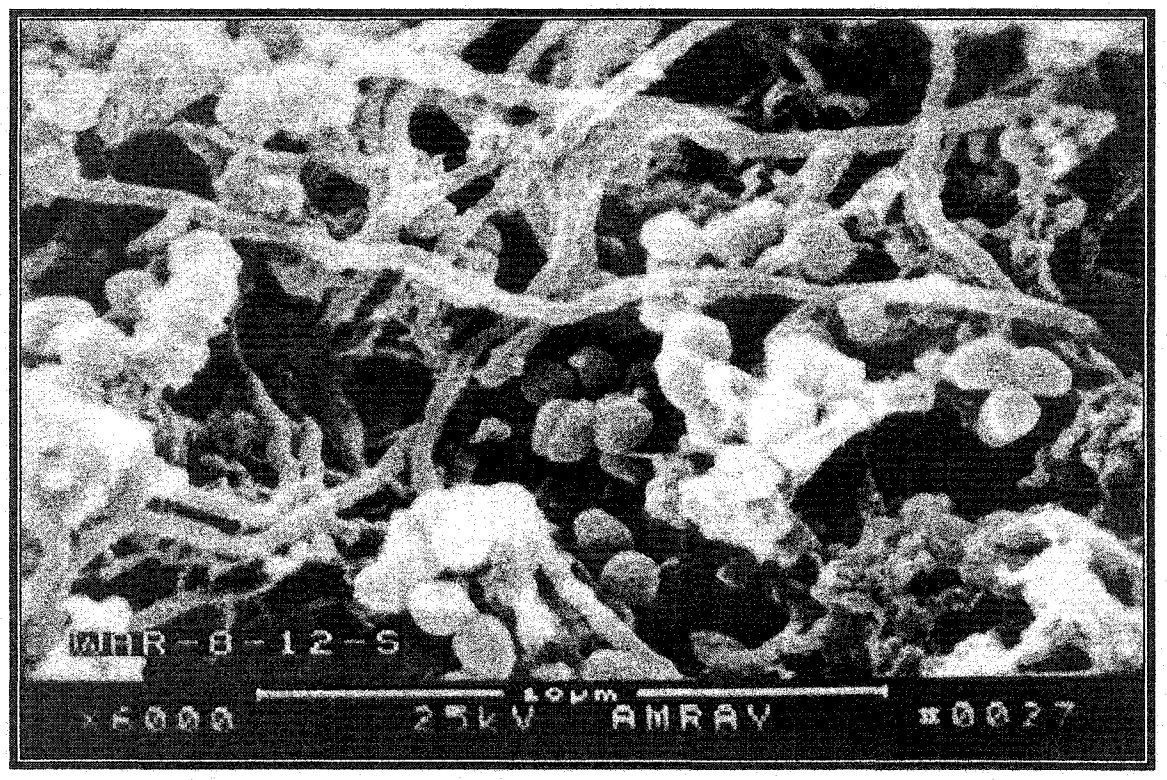


Plate 7.7 Electron micrograph of biofilm on FECS, dominated by *Methanosaeta* and *Methanococcus* spps., after organic shock loadings (25 KV, x 6000)

7.4 Failure Analysis in Critical Hydraulic and Organic Shock Loadings

7.4.1 The Failure of the AF Under Critical Hydraulic Shock Loadings

Figure 7.7 shows a schematic bi-phase pathway of anaerobic degradation for soluble organic substances under normal circumstances. Of these, VFAs were β-oxidized by *Syntrophomonas* spp., via intermediate VFAs, to acetate with concomitant H_2 production. Acetate was decarboxylated by *Methanosaeta* spp and *Methanosarcina* spp., to CH_4 and CO_2; H_2 was utilized by *Methanococcus* spp to reduce CO_2 to CH_4.

These anaerobes convert long-chain fatty acids to hydrogen and acetate, and in some cases, to odd-length chains of VFA (e.g. propionate). A group of anaerobes, known as hydrogen-producing acetogens, is responsible for the conversion of VFAs to methane and CO_2 (Bryant, 1979).

The failure of the AF under the situation of the critical hydraulic stock load at HRT of 0.5 days was attributed to process souring, which resulted in an accumulation of VFAs. The predominant VFAs accumulating in the effluent were acetate and propionate. It was reported that the accumulation of acetate and propionate in high concentrations is toxic to methanogens (Yu and Pinder, 1993; Kalia *et al.*, 1994; Dong *et al.*, 1994; Kato *et al.*, 1994; Vavilin and Lokshina, 1996b) because of the low number of *Syntrophomonas* spp. available in the system for the further conversion of VFAs to acetate. A temporary inhibition on methanogens, as indicated by a low specific methane yield and a low methane concentration (e.g. 50%), was observed in this study

under critical hydraulic shock loading. The average methane concentration dropped to about 50% (Figure 7.3) and the specific methane yield was only 0.1 L/gCOD. The optimum pH for an anaerobic treatment system ranged between 6.6 and 7.6 (McCarty and Smith, 1986). The average pH value was 5.5 under a critical hydraulic shock loading. When pH is less than 6.2, methanogensis is inhibited but acetogenesis can still maintain its function.

Besides this, the accumulation of acetate in high concentrations in anaerobic reactors can retard the degradation of propionate and butyrate in a synthrophic consortium (McInerney and Bryant, 1981; Ahring and Westermann, 1988; Goris *et al.*, 1989; Fukuzaki *et al.*, 1990; and Wu *et al.*, 1993). For thermodynamic reasons, the conversion of propionate (ΔG^{o} = +76 kJ, Thauer, *et al.*, 1977) to acetate, as indicated by a positive ΔG^{o} value, can not be directly utilized by methanogens (Fang, 2000) unless the hydrogen produced is effectively consumed by hydrogen-utilizing bacteria, which maintain a low level H_2 partial pressure (Bryant, 1967; Chung, 1976; Bryant *et al.*, 1977; Thauer *et al.*, 1977; Harper *et al.*, 1986; Boone and Xun, 1987). This indicates that the conversion of propionate to acetate is the rate-limiting step of the acetogenesis process (Fang *et al.*, 1995b; Shin *et al.*, 2001). Other researchers working with slaughterhouse effluent treatment have also confirmed that hydrolysis becomes a limiting step with an excess of propionate (Salminen *et al.*, 2000) and with a fat and high protein content (Bastone *et al.*, 2000) rather than with the habitual VFA accumulation. On the other hand, Chua *et al.* (1996b) confirmed that *Syntrophomonas* spp. could not utilize propionate as the sole carbon source for its survival under conditions of enrichment culture. Propionate belongs to a group of compounds, which can only be degraded by hydrogen-utilizing methanogen, unless the H_2 concentration

is kept at a low level under anaerobic conditions (Wiegant *et al.*, 1986). However, when pH drops to a certain level, the inhibition of methanogens occurs.

As reported elsewhere, *Methanosaeta* spp. has a higher affinity with a low concentration of acetate (e.g. μ_{max} = 0.1 day^{-1}, K_s=30 mg/L)(Gujer and Zehnder, 1983) than other acetoclastic methanogens, namely *Methanosarcina* spp. (e.g. μ_{max} = 0.3 day^{-1}, K_s=200 mg/L) (Zehnder *et al.*, 1980; Huser *et al.*, 1982; Gujer and Zehnder, 1983) which are known to grow only on acetate (Platen *et al.*, 1987; Schmidt and Ahring, 1996). *Methanosarcina* spp., the most versatile methanogen, consumes several methanogenic substrates including acetate, methanol, methylamines and sometimes H_2/CO_2 as its energy source (Nishio *et al.*, 1993; Boopathy, 1996; Chen and Hashimoto, 1996; Schmidt and Ahring, 1996), and its affinity for acetate is only about one tenth of that of *Methanosaeta* spp. (Huser *et al.*, 1978; Zehnder *et al.*, 1980). Bochem *et al.* (1982) reported that the specific methanogenic activity of the *Methanosaeta*-like bacteria was higher than that of the *Methanosarcina*-like bacteria in an environment with a low acetate concentration. Gujer and Zehnder (1983) also observed that *Methanosaeta* out-competed other methanogenic bacteria for acetate when the acetate concentration was low, as in the mixed liquor of an UASB reactor and in an interior granule with a very low half-rate constant (e.g. 30 mg COD/L). It has been postulated that the competition in favor of *Methanosaeta* was due to the lower acetate Ks value of this organism (Gujer and Zehnder, 1983; Koster, 1988).

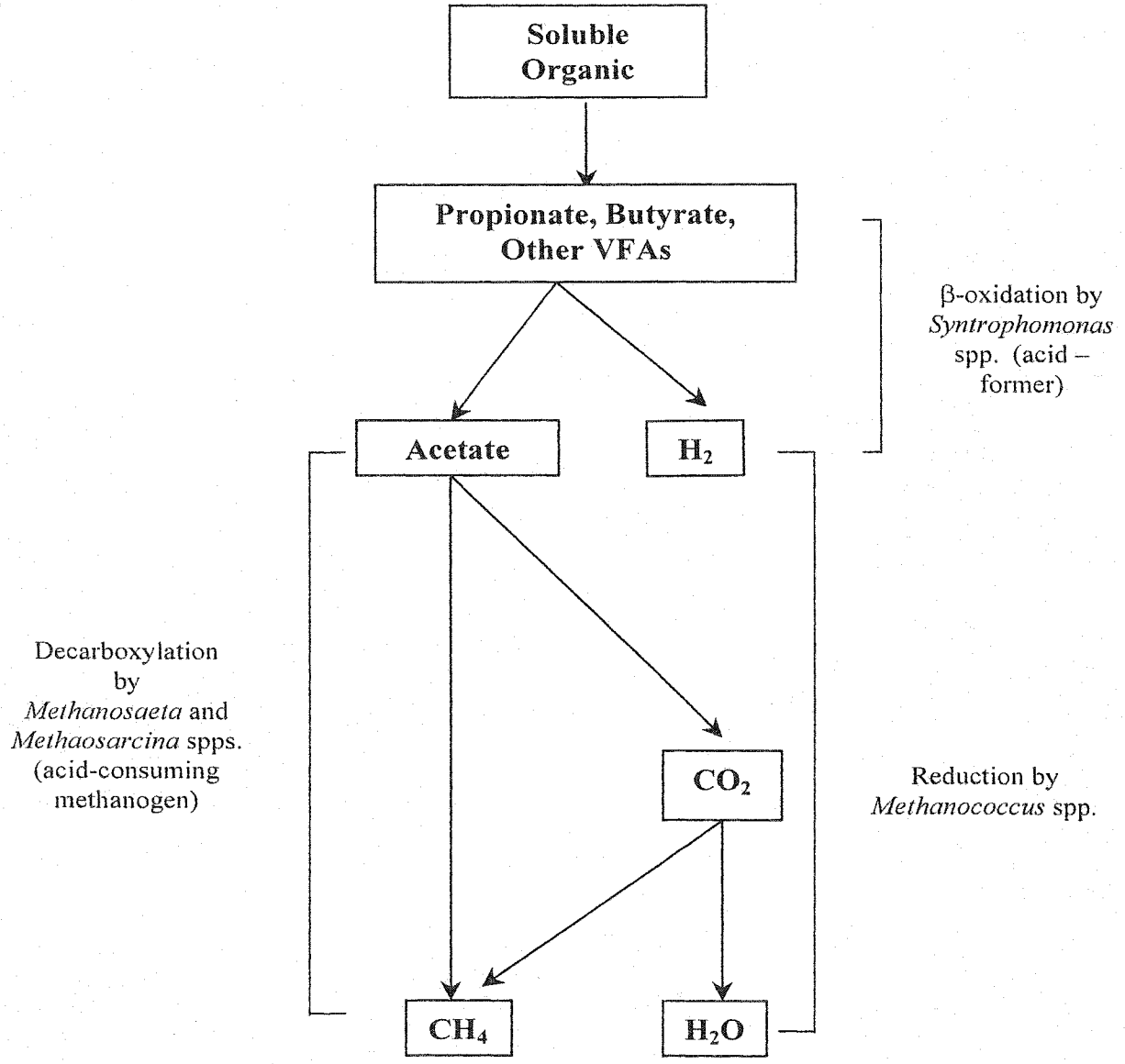


Figure 7.7 A Schematic Bi-Phase Pathway of Anaerobic Degradation of Soluble Organics

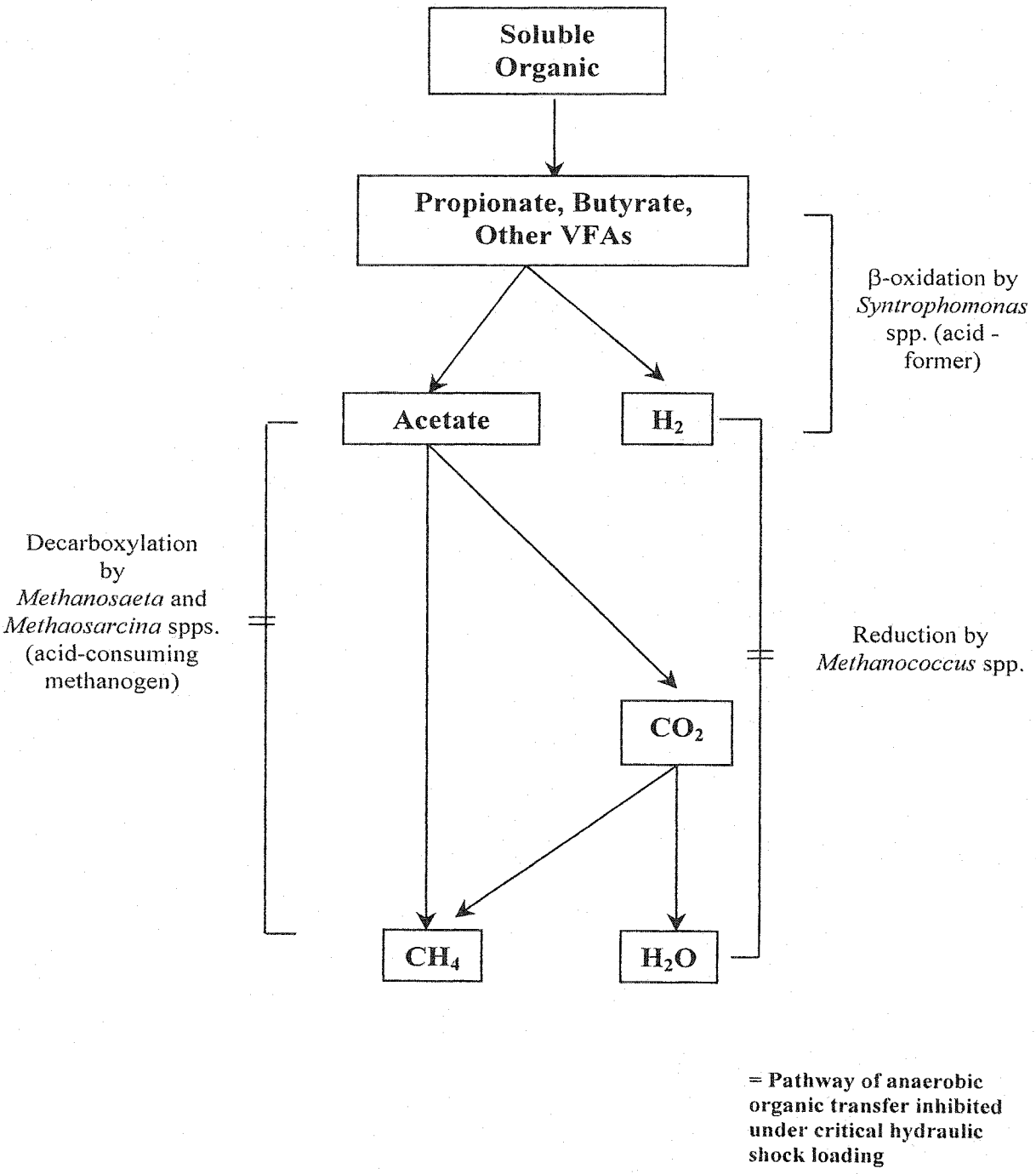


Figure 7.8 Inhibition of Anaerobic Organic Transfer Under Critical Hydraulic Shock Loading

The growth of *Methanosaeta* spp. relies upon the supply of acetate, and hence its growth indirectly depends upon the status of *Syntrophomonas* spp. In addition, the growth of *Syntrophomonas* spp. also relies on the status of *Methanosaeta* spp., hydrogen-utilizing bacteria, which provide a thermodynamically favorable environment for their survival. As a result, the symbiotic relationships of these two groups of microbes can then be maintained.

Meanwhile, *Methanococcus* spp. also plays an important role in an interspecies hydrogen transfer process. The growth of *Methanococcus* spp. depends on the existence and status of *Methanosaeta* spp., which provide the necessary carbon dioxide sources for their survival. Basically, the syntrophic association between acetogens and methanogens allow for the rapid removal of hydrogen. Due to the effect of the critical hydraulic shock loading, the degradation of propionate may be hindered. A critical hydraulic shock loading at HRT 0.5 day seems to disturb the syntrophic association between acetogens and methanogens that were maintained within the AF before the shock loading. In the event of critical hydraulic shock loading, a significant impact on the activities of both acetoclastic methanogenic bacteria (e.g. *Methanosaeta* and *Methanosarcina* spps.) and hydrogen-utilizing bacteria (e.g. *Methanococcus* spp.) was observed. However, the magnitude of the inhibition on these two groups of bacteria, in which no accumulation of carbon dioxide was observed (Figure 7.3), was presumed to be similar. The concentration of carbon dioxide in the biogas was maintained at a low level, resulting in a relatively dynamic equilibrium being maintained in the AF. The CO_2 level ranged between 10 and 15% (v/v) (Figure 7.3). Grobicki and Stuckey (1991) observed that mass transfer limitations increased as the HRT decreased. Pozo *et al.*

(2002) also indicated that limitations in the reactor hydrodynamic and the hydrolysis of the particulate fraction in the anaerobic fixed-film reactor were detected at high flow-rates (e.g. 0.013 cm/s) when treating slaughterhouse wastewater. It is generally believed that the hydraulic shock loading disturbs, to some extent, the physical contact between syntrophs and methanogens (Nachaiyasit and Stuckey, 1997; Pozo et al., 2002), leading to an inhibition of the methanogenic bacteria. Under such circumstances, the organic substrate simply flows through the reactor without being metabolized, particularly during transient states of hydraulic shock loadings (Hanakai *et al.,* 1990; Nachaiyasit and Stuckey, 1997; Demirel and Yenigun, 2002; Pozo *et al.,* 2002). A combination of the factors of a low pH environment and a low contact time between anaerobes and substrate resulted from an inadequate mass transfer from the bulk liquid to the attached biofilm. It is therefore suggested that the pathway of converting the acetate to carbon dioxide and methane by *Methanosaeta* spp. and *Methanosarcina* spp. was inhibited, and the reduction of carbon dioxide with hydrogen by *Methanococcus* spp. was also affected within such an unbalanced microbial ecosystem (Figure 7.8).

A high concentration of VFAs accumulates in the effluent when methanogensis is inhibited (Ashely and Hurst, 1981; Sorensen *et al.*, 1981). As a consequence, excess amounts of VFAs accumulating in the effluent were observed in this study. The methane production also declined, ultimately affecting the stability of the system (Figures 7.1). Microbial observations also confirmed that, unlike in conventional suspended-growth reactors, the temporarily inhibited biofilm was not being washed out from the system, and it could rapidly regain activity and re-establish a new balance

of anaerobic bacteria within the ecosystem when favorable conditions were encountered or presented

7.4.2 The Failure of the AF Under Critical Organic Shock Loading

During critical organic shock loading, a low specific methane yield (e.g. 0.05 L/gCOD) and a low methane concentration in the biogas (e.g. 45%) was observed. The failure of the AF, under 16-fold organic shock loadings, was attributed to process souring, resulting in an accumulation of VFAs. The VFAs that accumulated in critical organic shock loading were predominantly acetate and butyrate. Similar observations were also made by other researchers (Liu and Fang, 2002; Baloch and Akunna, 2003). Baloch and Akunna (2003) indicated that microbial ecology in an anaerobic system (e.g. anaerobic granular bed baffled reactor) appeared to favor acetate and butyrate production at high organic loading rates. McCarty and Mosey (1991) indicated that the production of butyrate, in contrast to format, acetate, or propionate, is favored at a low pH environment, because the low pH levels enhance the growth of butyrate-forming bacteria. The average pH value measured at critical organic shock loadings was 5.7. It is suggested that an increased production of butyrate instead of acetate may be a feedback solution adopted by anaerobes against a highly acidic environment. The accumulation of acetate, propionate and butyrate in effluent may also lead to an increase of hydrogen partial pressure, thus limiting the degradation of VFAs (Ferguson and Man, 1983; Huang *et al.*, 2000). It has been reported elsewhere that both acetogenic bacteria (e.g. *Syntrophomonas* spp.) and methane-producing bacteria could not tolerate the presence of high concentrations of acetate (Schink, 1992, Jetten *et al.*, 1992; Dong *et al.*, 1994; Chen and Hashimoto, 1996).

The lowest pH value measured under the critical organic shock loading was 4.1. The decrease in pH value in the AF, resulting from an accumulation of VFAs, further inhibits the activity of anaerobes within the consortium. When the pH value of an anaerobic system is higher than 7.8 or lower than 6.3, methanogensis decreases significantly. Acidogensis is less sensitive to the fluctuation of pH values of the system. As a result, acid fermentation prevails over the methanogenic fermentation, resulting in souring the system. However, the activity of acidogenic bacteria is seriously damaged when the pH value is less than 5 (Zoetmeyer *et al.*, 1982). An anaerobic treatment system maintained with a stable pH value is of paramount importance for its successful operation, because the methanogensis proceeds only at a high rate within a neutral range of pH value, such as 6.6 to 7.6 (McCarty, 1964b; van Haandel and Lettinga, 1994; Zinder, 1994). Clark and Speece (1970) observed that the low pH (e.g. < 6) level of an anaerobic system (e.g. AF) exerts a bacteriostatic effect on acetate-fermenting methanogens.

In contrast, the utilization of VFAs in methanogensis is of paramount importance in respect of the stability of an anaerobic system. The results of the transient response of VFAs show that the very high levels of organic shock loadings introduced to the system inhibit the activities of both methanogenic bacteria and acidogenic bacteria. As a result, the acetoclastic methanogens (e.g. *Methanosaeta and Methanosarcina* spps.) and H_2-utilizing bacteria (e.g. *Methanococcus* spp.) were both affected. However, the magnitude of inhibition on these anaerobes was different, as evidenced by the variation of CO_2 concentrations in the biogas. The CO_2 concentration increased as an increment of the organic loading rate and ranged between 29% and 65% at different organic

shock loadings conditions. The highest CO_2 level measured in the critical organic shock loading was about 65% (Figure 7.4).

Under such circumstances, the supply of VFAs (e.g. acetate and butyrate) exceeded the assimilative capacity of methane-forming bacteria (e.g. *Methanosaeta* and *Methanosarcina* spps.), leading to an accumulation of organic acids measured in the effluent. Combining the factors of a low pH environment and the low assimilation capacity of methane-forming bacteria, it is suggested that the pathways of (i) converting acetate to carbon dioxide and methane by *Methanosaeta* and *Methanosarcina* spps., and (ii) the reduction of carbon dioxide with hydrogen, were inhibited. Figure 7.9 shows the pathway of the anaerobic organic transfer that was inhibited under critical organic shock loading.

The results suggest that the magnitude of inhibition on hydrogen-utilizing bacteria (e.g. *Methanococcus* spp.) was higher than that on acetoclastic methanogenic bacteria. Consequently, excess amounts of carbon dioxide were accumulated, resulting from the inhibition on the reduction of carbon dioxide during critical organic shock loading.

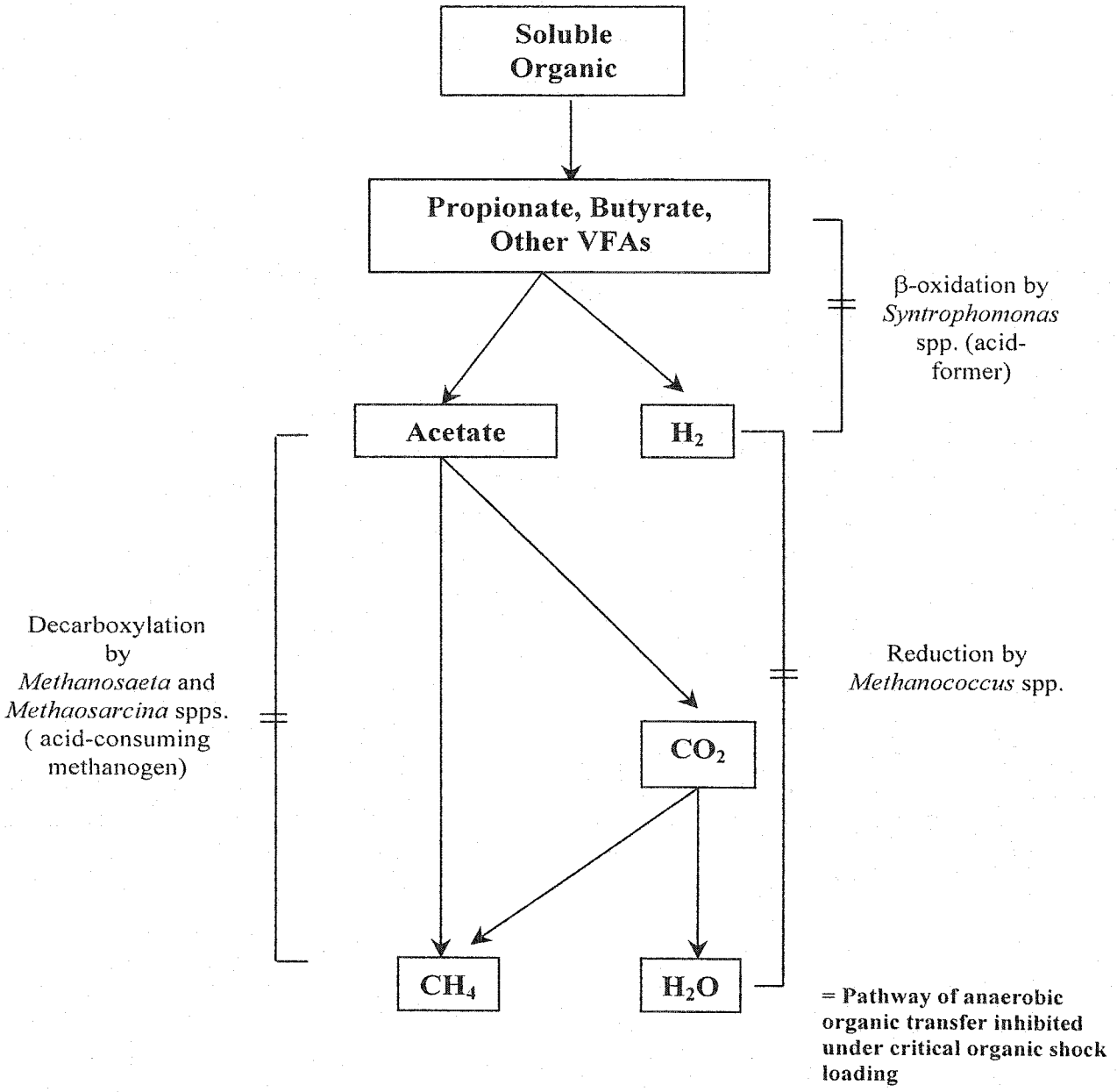


Figure 7.9 Inhibition of Anaerobic Organic Transfer Under Critical Organic Shock Loading

7.5 Summary of Findings

The results of this study confirmed that the individual AF section contributed a stable environment for the immobilized biomass in the AHR, and helped to improve the performance and process stability of the AHR.

Under shock loading conditions, the AF also enabled the AHR more readily to recover from unstable operating conditions or even process failure. The organic load of the individual AF section was progressively increased to 115.2 gCOD/L-dThe total COD removal efficiency was maintained at above 90%. The AF could recover from various shock loadings equivalent to a ten times increase in hydraulic loads and a 16 times increase in organic loads. Combining the mechanisms of organic adsorption and biodegradation rendered the AF more stable under various shock loading conditions. Biogas with an average concentration of 69.0% and 66.8% methane was generated, at a generation rate ranging from 0.28 to 0.32 L/gCOD and 0.12 to 0.22 L/gCOD under hydraulic and organic shock loadings respectively.

Under critical shock loading conditions, significant amounts of VFAs were accumulated in the treated effluent. It is suggested that the increased production of butyrate instead of acetate may be a feedback solution adopted by anaerobes against a high acidity environment. The accumulation of acetate, propionate and butyrate in the effluent may also result in an increment of hydrogen partial pressure, thus limiting the degradation of VFAs. The quantities of VFAs accumulation in organic shock loadings

were higher than those during hydraulic shock loadings. Such a discrepancy may be attributed to the low availability of organics, resulting from a high volumetric flow-rate introduced under various hydraulic shock loading conditions.

The anaerobic pathway of organic degradation, converting acetate to carbon dioxide and methane by *Methanosaeta and Methanosarcina* spps. was prone to inhibition under the critical hydraulic shock environment. The magnitude of inhibition on these two groups of bacteria is presumed to be similar, since no accumulation of carbon dioxide was observed. The concentration of carbon dioxide in the biogas was maintained at low level, resulting from a relative dynamic equilibrium maintained in the AF.

The hydraulic shock loadings disturbed, to some extent, the physical contact between the syntrophs and methanogen, leading to an inhibition on the methanogenic bacteria. This inhibition led to an imbalance of the microbial ecosystem, including the accumulation of VFAs and a decline of methane concentrations in the biogas.

In contrast, the anaerobic pathways of VFAs to acetate, hydrogen and carbon dioxide conversion by (i) *Syntrophomonas* spp., that of acetate to methane and carbon dioxide conversion by (ii) *Methanosaeta* spp., that of butyrate to methane and water conversion and that of carbon dioxide and the reduction of hydrogen mediated by (iii) *Methanococcus* spp. were prone to inhibition under the critical organic shock loading environment. The magnitude of the inhibition on these three groups of bacteria was

found to be different (e.g. iii > ii > i), as evidenced from the concentration of carbon dioxide that was increased as an increment of organic loading.

As a result, the supply of organic acids, mainly acetate and butyrate, exceeded the assimilative capacity of methane-forming bacteria, namely *Methanosaeta* spp., leading to the accumulation of VFAs. The content of methane in the biogas decreased and that of the carbon dioxide increased. Generally, the failure of the AF under various shock loadings was attributed to process souring, as indicated by low methane yields and an accumulation of VFAs.

8. Conclusions

The conclusions of this study are summarized below:

1. This study demonstrated that the AF section of the AHR played a significant role in COD removal, particularly when the AHR was operated under shock loading conditions. The AF not only provided the AHR with a better capability for recovery from various shock load conditions, it also performed as a biofilter, maintaining the temporarily biomass on the supporting medium. The AF is able to prevent a wash out of the inhibited biomass under shock loadings.

2. Results of this study show that the AF section of the AHR was much quicker to recover after introducing the shock loadings than that of the UASB section. Under a transient period of shock loading, high efficiency of treatment was achieved by the AF section of the AHR within a few days. The UASB section, however, normally requires a much longer time for recovery in response to each of the hydraulic shock loadings introduced to the AHR.

3. Under 2, 4 and 5 times hydraulic shock loadings, the efficiency of removal of COD, as achieved by the individual AF section, was temporarily affected. It was reduced from about 98% to around 93 to 90% during the hydraulic shock loading study. The average pH of the treated effluent was found to drop from 7.2 to 6.0 with biogas production also being affected (this dropped from 0.6 L/d to 0.37 L/d). The biogas, with an average methane concentration of 69%, was generated at a rate of between 0.28 and 0.32 L/g COD. The AF was able to

recover from the temporary inhibition due to the shock loadings, and resumed to normal operation within 8 days. However, under a 10 times hydraulic shock loading, the treatment performance deteriorated drastically with significant amounts of volatile fatty acids (VFAs) accumulating in the AF liquor, and a resulting souring of the reactor and system failure. When the HRT was returned to 5 days, the AF recovered within a few days. The ability of the AF to recover from critical hydraulic shock loadings and system failure was attributed to the immobilized-biofilm design, which enabled the temporarily inhibited biofilm to be retained within the AF and to resume its activity when favourable conditions were restored.

4. In the study of the organic shock loading, the efficiency of removal of COD reached 90% during the entire operational period of 150 days. The individual AF possessed an excellent anti-shock loading capacity, which could accommodate a sixteen times normal organic load, and still readily recover from the shock loading. Combining the mechanisms of organic adsorption and biodegradation rendered the AF more stable under the various shock organic loading conditions. The biogas generated, with an average methane concentration of 66.8%, was discharged at a rate of between 0.12 and 0.22 L/g COD. This corresponded to an energy production rate of $2.2*10^5$ kJ/m^3 for the trade wastewater.

5. The anaerobic pathway of the organic degradation, converting acetate to carbon dioxide and methane by *Methanosaeta and Methanosarcina* spps., was prone to inhibition under the critical hydraulic shock loading environment. The degree

of inhibition of these two bacteria was similar, as evidenced by the concentrations of carbon dioxide, which were maintained at a fairly low level. The present results suggest that the hydraulic shocking loading disturbs, to some extent, the physical contract between the syntrophs and methanogen, leading to an inhibition of the methanogenic bacteria, and resulting in an accumulation of VFAs (mainly acetate and propionate) and a decline in the methane concentrations of the biogas.

6. The anaerobic pathways of the conversion of the VFAs to acetate, hydrogen and carbon dioxide by (i) *Syntrophomonas* spp., the conversion of the acetate to methane and carbon dioxide by (ii) *Methanosaeta* spp., the conversion of the butyrate to methane and water and the conversion of the carbon dioxide and reduction of the hydrogen, mediated by (iii) *Methanococcus* spp., were prone to inhibition under the critical organic shock loading environment. The degree of inhibition of these three groups of bacteria was found to be different (e.g. iii > ii > i), as evidenced by the concentration of carbon dioxide that increased incrementally as the organic loading increased. As a result, the supply of organic acids, mainly acetate and butyrate, exceeded the assimilative capacity of the methane-forming bacteria, *Methanosaeta* spp., leading to an accumulation of VFAs. The content of the methane in the biogas decreased and the concentration of carbon dioxide increased. Generally, the failure of the AF under various shock loadings was attributed to process souring, as indicated by the low methane yield and an accumulation of VFAs.

7. In summary, it can be concluded that the AF contributed a stable environment for the immobilized biomass in the AHR, which helped to improve the performance and process stability of the AHR. Under shock loading conditions, the AF also rendered the AHR more able to recover following unstable operating conditions.

9.0 Significance of this Study and Suggestions for Future Research

9.1 Significance of this Study

The results of this study demonstrate that the AF section is one of the key components for the successful operation of an anaerobic treatment system, particularly the AHR, during adverse conditions, namely shock hydraulic and organic loadings in respect of local applications where space and balancing facilities are limited and insufficient.

It has also demonstrated that the AF section of the AHR contributes a stable environment for the immobilized biomass, thus improving the performance and process stability of the system. Under shock loading circumstances, the AF makes it easier for the AHR to recover quickly from unstable operating conditions or even from process failure.

The UASB section of the AHR is the major contributor responsible for the removal of COD during steady state conditions, resulting in the retention of a dense and active biomass population by the sludge blanket in the USAB section. The results of the present study also confirm that the function of the UASB section is dominant under normal stable operation. On the contrary, the AF section is dominant under shock loading conditions.

In addition, the pathways of the inhibition of organic transfer under critical shock hydraulic and organic loadings are also elucidated by the results of this study.

9.2 Suggestions for Future Research

Being a relatively new, complex and non-homogeneous type of reactor, in-depth studies on the AHR are still lacking. It is suggested that a structured mathematical model, based on detailed studies in the hydrodynamics, transport and microbial kinetics of the AHR be conducted in future, to enable a better understanding of the AHR. Such studies also provide data for the design, optimisation, operation, control and prediction of its treatment performance. The resulting structured model should describe the substrate, intermediate and product conversion, the production-consumption imbalance of VFAs, the dynamics of the bacterial population and ratios, and the rates of biochemical reactions as functions of biological responses to environmental conditions, namely hydraulic and organic shock loadings, the substrate concentration, and the presence of inhibitors, pH and temperature.

The structured models should also validate the production of a more optimized and rational design for an AHR in treating varied types of waste water.

Appendices

Publications Arising from this Study

1. Chua H., Yu P.H.F., Hu W.F and **Cheung M.W.L.** (1996) Stability of Anaerobic Biofilter under Critical Hydraulic Shock Loadings. International Association of Water Quality, Presented in the 18th Biennial International Conference, Singapore, 23-28, June 1996.

2. Chua H., Hu W.F., Yu P.H.F. and **Cheung M.W.L.** (1997) Responses of an Anaerobic Fixed-Film Reactor to Hydraulic Shock Loadings. *Bioresource Technology*, 61, 79-83

3. **Cheung M.W.L.** and Chua H. (2000) Responses of a Fixed-Film Anaerobic Bioreactor to Shock Organic Loading in Industry Wastewater Treatment Environment, *J. Institution of Engineers, Singapore*, 30 – 35

4. **Cheung M.W.L.** and Chua H. Roles of the Fixed-Film Anaerobic Filter in an Anaerobic Hybrid Reactor to Shock Loading Environment (to be submitted).

5. **Cheung M.W.L.** and Chua H. Behavior of the Fixed-Film Anaerobic Filter in Anaerobic Hybrid Reactor under various Hydraulic and Organic Shock Loadings (to be submitted).

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