Influence of stressed loading on start-up and granulation on UASB treating food processing wastewater

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INFLUENCE OF STRESSED LOADING ON START-UP AND GRANULATION ON UASB TREATING FOOD PROCESSING WASTEWATER

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School of Civil and Environmental Engineering

A thesis submitted to the Nanyang Technological University
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Abstract

Upflow Anaerobic Sludge Blanket (UASB) process has been proven by many industrial applications to be effective and efficient since it was developed in 1980s. It has been successfully applied to a wide variety of industrial wastewaters offering high organic removal, low sludge production, energy recovery in the form of biogas in addition to low energy consumption. However, major drawbacks of the UASB processes are the long start-up period and the slow development of active biogranules using digester sludge as seed sludge. Several studies have been conducted attempting to accelerate the startup process and granulation. Stress-loading operating condition is one of the ways to accelerate the start-up and granulation. It has been studied on synthetic glucose-based wastewater and proven feasible. In addition to start-up process, little was known on the re-startup of the UASB reactor upon the starvation which often occurred in industrial applications when the reactors were shut down for maintenance. The present study looks into the stress-loading strategy applied on a laboratory-scale UASB system treating a combined industrial food wastewater from over 100 food-processing factories. The re-startup of the UASB reactor upon 3 starvation periods of 5, 10 and 15 days were also studied.

The wastewater characteristics were first studied to investigate the fluctuation of its compositions as the wastewater was a combination of different food-processing wastewaters. The organic concentrations of the wastewater were found to be ranging from 1106 ~ 4060 mg COD/L with an average of 3036 mg COD/L and a daily flow ranging from 240 ~ 849 m³/d with an average of 715 m³/d. Sodium bicarbonate was added to adjust the low pH of the wastewater (4.94 ~ 5.62) to the optimum conditions for UASB operation. Macro-nutrients like nitrogen and phosphorus were present as well as trace amount of micro-nutrients. No inhibitory elements such as arsenic and cadmium of UASB were found in the wastewaters. Other parameters like alkalinity, total and volatile suspended solids and temperature were measured as well.

Two reactors were used to investigate the feasibility of accelerating the startup and granulation processes. One of the reactors, R1, acting as a control, was run using the conventional startup protocol which conventional start-up protocol for UASB steps up the OLR of the reactor slowly upon a steady state in terms of removal efficiency and biogas production is obtained and while the other reactor, R2, looked into the stress-loading strategy with organic loading increased to the next stage when the reactor had achieved 75% in COD removal efficiency.

The results obtained showed that the stressed loading method was able to accelerate the start-up duration by up to 48% in time without affecting the ultimate loading capacity. The stressed reactor, R2 had granules formed 12 days faster than the control reactor, R1.

The stress applied to R2 caused the granules formed to be with smaller mean size diameter of 1.92 mm at its peak compared to the control, which had a peak of 2.76 mm. The granules in R2 also had poorer settleability than R1 which might be due to fluffier granules developed by the stress applied. The granules of R2 also had lower strength than R1 when subjected to abrasion. But the stress applied to R2 had caused the granules to develop higher methanogenic activity than R1.

Stressed loading had shortened the start-up duration of UASB treating food industrial wastewater. The granules developed under stress, though developed faster and with higher methanogenic activity, they compromised some of their physical properties like size, settleability and strength.

Treatment processes inevitably need to shut down for maintenance or other reasons. Effects on restart-up and granule properties undergoing starvation were studied. Three reactors, R5, R10 and R15, operating at a steady-state load of 10.4 g COD/L.d, were starved for 5, 10 and 15 days, respectively. Results showed that R5 and R10 required less than 1 day to reach its previous performance and required a few days to return gradually to its original performance. But R15 required 4 days in order to achieve its original COD removal efficiency. Granules properties like VSS, SVI and mean granule diameter

showed no significant changes. SMA increased significantly once operation resumed and returns to its previous values -15 days after restart. Granule strength showed decrease of 1%, 6% and 12% for R5, R10 and R15 respectively. The results indicate that starvation period of up to 15 days had no essential loss on reactor performance and granule properties after restart and for starvation period of more than 15 days, reactor performance will take more time to recuperate and granule strength will deteriorate.

CHAPTER 1

INTRODUCTION

1.1 Background

Environmental issues have drawn concern of general public when many realized the importance of environment protection. People are demanding better living environment for themselves and their future generations in addition to preventing hazards or pollutions caused by human being's ignorance from affecting them. Human activities have been causing many pollutions and harms to the environment like water pollution in the rivers, ozone depletion and nuclear hazards. With improving knowledge, man has been trying to control the destructions to the natural environment.

Proper discharging of wastewater can prevent water hazards from happening. Therefore wastewater has to be treated prior to its discharge to the nature. Many authorities in different countries have set stringent standards for all discharge of wastewater to public sewers or back to the nature. Many establishments have been paying high tariffs to discharge their wastewaters which increases their production cost inevitably. Therefore, these establishments have resort to pre-treat their wastewaters prior to discharge.

Different types of wastewater require different treatment systems. In biological treatment of wastewater, anaerobic processes have been widely used for treatment of high strength organic wastewater due to its advantages of low operating cost and energy recovery in the form of biogas. Especially with many successful full-scaled applications, anaerobic processes have gained increasing creditability.

The upflow anaerobic sludge blanket (UASB) reactor, developed by Lettinga and co-workers in 1980, is one of the most efficient anaerobic treatment systems. It is able to retain high biomass concentration without any support media despite the high upflow velocity compared to other anaerobic systems.

Biogranulation has been proven to be a key factor for highly efficient anaerobic treatment of industrial and municipal wastewater. Granulation is the coagulation of the non-discrete biomass into a well-defined dense pellet having high settling ability and high tolerance to high organic loading. It enables the reactors to perform to its optimum with its high settleability and activity of granules.

Granulation is a complicated process. It has been noted that granulation is resulted from physiochemical and microbiological interactions. There are many factors affecting the granulation process including temperature, characteristics of the seed sludge, characteristics of the wastewater, pH and alkalinity, presence of essential nutrients and loading and operation conditions. Though it has been proven that granulation is one of the key for successful high-loading anaerobic treatment, an undesirable drawback is the long start-up period required to develop granular sludge from digester sludge as its seed. Much attention has been given to the remediation of this drawback as well as biogranulation and also to extend the applications of the UASB reactors.

Other than varying the physical parameters of the operating conditions, start-up strategy is also important to its acceleration. It has been proven that optimum stress-loaded operating conditions are beneficial to the acceleration and granulation treating synthetic protein-carbohydrate wastewater (Yang, 2002). However, there are still many uncertainties as far as real industrial wastewater is concern.

The ability for the granular sludge of UASB reactor to be preserved under starvation for a long duration without affecting its activity significantly is another advantage of the UASB reactor system. However, information on the granule

property changes during the starvation period as well as the response to restart is not readily available. Even though there were many experience in the full-scale plants about the starvation, results and details were not properly recorded and published.

In many applications of biological industrial wastewater treatment, apart from promoting a cleaner environment, it is also to save high amount of tariff paid to discharge untreated effluent into public sewers. Time is always a cost factor in business world. The ability to shorten the start-up time of a treatment system means ability to save cost. Often, there will also be a maintenance period whereby there will be no production of wastewater. This will mean that there will be no feed for the biological system. Very little was told about the effects of starvation over different periods on the biological system like UASB.

This study was initiated to examine the stress-loaded strategy upon the start-up and granulation of UASB reactor treating industrial food wastewater. It also examined the granule property changes during the starvation period and the response of the UASB reactors to restart.

1.2 Objectives

The specific objectives of this study are:

1. To evaluate the feasibility of accelerated start-up of UASB reactor treating wastewater from food industry using stressed-operation strategy.
2. To examine the development of biogranules and their characteristics from the stressed operation.
3. To examine the characteristic change of granules upon starvation
4. To examine the restart-up of a UASB reactor after different starvation periods.

1.3 Scope of work

The characteristics of the wastewater generated from food industry were examined. These parameters examined were flow rate, pH, alkalinity, total suspended solids (TSS), volatile suspended solids (VSS), biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), total organic carbon (TOC), nutrients for bacteria growth and heavy metals.

Two reactors were operated. One acted as a control unit while the other was run with stress-loading. Reactor performance indicators like pH, alkalinity, effluent suspended solids, volatile fatty acids (VFA) concentrations and COD removal efficiency were tested for routine maintenance and monitoring. Biomass or granules characteristics parameters like specific methanogenic activity (SMA), sludge volume index (SVI), particle/granule size and distribution, granule strength and volatile fatty-acids (VFA) concentration were also measured at each steady state. The results obtained during the operation were used to analyze the effects of stress-loading on granulation of UASB reactor treating food industry wastewater.

Three reactors were operated under the same organic loading rate (OLR) in examining responses to restart. Restart-up durations were investigated on 3 different starvation periods. Routine maintenance and monitoring parameters were measured accordingly. Granule properties were also measured before and after the starvation periods.

CHAPTER 2

LITERATURE REVIEW

Most pollution occurs due to improper treatment of discharged wastewater as well as the ignorance of human beings. As standard of living gets higher and people get more knowledgeable, stringent regulatory requirements are enforced by respective authorities. There are many ways of treating wastewater, including physical and chemical methods, but no single method can be used effectively for all the different types of wastewater.

Biological wastewater treatment systems including aerobic, anaerobic or combination of both, are widely recognized to be able to treat a wide variety of wastewater to the most stringent requirements. Anaerobic processes have several favorable features such as low operation cost, biogas yield, low energy consumption and etc, which have been gaining increasing credibility from successful industrial applications. With these successful applications, it is agreeable that anaerobic treatment is a well recognized and mature wastewater treatment technology. In this chapter of review, past studies on the UASB process, granulation and factors affecting its performance are discussed.

2.1 UASB PROCESS AND GRANULATION

2.1.1 UASB process

Upflow anaerobic sludge blanket (UASB) has been developed since 1980 by Lettinga and his co-workers. It was an attempt to overcome problems encountered by the contact reactor then by combining the contact reactor and the clarifier of the anaerobic process in one chamber. Since the first feasible study by Lettinga and co-workers, the UASB system has gained widespread acceptance and has been successfully applied to treat a wide variety of industrial wastewaters.

The UASB design consists of two main zones: the reactor zone and the clarifier zone as shown in Figure 2.1. Wastewater is pumped from the bottom of the reactor and flows upwards through a layer of sludge blanket which is the reaction zone. The wastewater is degraded by the anaerobic bacteria as it passes through the sludge blanket. In the upper clarifier zone, biogas produced by the reaction is collected in an inverted funnel (the 3 phase separator) while the effluent flows over the funnel and out of the reactor with the biomass settles back to the sludge blanket.

Figure 2.1: UASB reactor

2.1.1.1 Factors affecting UASB performance

A successful UASB system depends on many factors. One study stated that the success of the high-efficient UASB process relies on the formation of active and settleable sludge granules (Fang et al., 1994). Other factors include wastewater characteristics, organic concentration of the wastewater, selection of inoculum material, necessary nutrients like nitrogen and phosphorus, and environmental conditions.

As granulation is considered as one of the most important factors in determining the success of the system, factors affecting the granule formation and microbial characteristics of the granule are important concerns to address.

2.1.1.2 Start-up of UASB

The start-up period is defined as the duration required from the initial seeding of the reactor system to the designated organic loading rate. The start-up period of UASB is the most important period throughout the operation of the UASB. In a way, it dictates the success and the efficiency of the system. Start-up of UASB is more than any routine job which requires experience and might not even result in expected success. It is affected by many factors including wastewater compositions and strength, the presence of the required nutrients, the pH value of the wastewater, the presence of inhibitory substances, the organic loading rate, hydraulic retention time, mixing of liquid, volume and activity of the inoculum and reactor's configurations geometry and size. These parameters stay in strong interaction and any one of them might cause the failure to the whole system.

Very often, failure of the UASB start-up for high-efficient system is coupled with a deficient formation of granular or flocculated sludge. Many studies have shown that granules formation is one of the key factors to successful high-efficient UASB system (Fang et al., 1994), but sufficient knowledge on the granule formation has not been attained. Several factors that complicate the UASB start-

up are the dominating bacteria groups, the growth rate of the methanogenic species, the dominating shapes of the methanogenic species (rod, cocci, sarcina), the biomass yield coefficient, half velocity constant (K_s) of the microorganisms and the ability to excrete polysaccharides (glycocalyx) (Weiland and Rozzi, 1991).

Other than the factors mentioned above, strategy of the start-up process also contributes greatly to the success. Several strategies have been tried in starting-up anaerobic reactors continuously including the maximum substrates conversion efficiency, maximum loading rate (Albagnac, 1989; Yen and Shieh, 1993) and in between, which is an appropriate loading rate with a designed degree of organic removal (Lettinga et al., 1985).

Most common and conservative strategy is the maximum substrates conversion efficiency, which means that the raising of organic loading in small increment only when the substrate conversion efficiency is high or when the conversion is high with stable total volatile fatty acids (VFA) in the effluent. This conservative strategy favors the growth and predominance of higher acetate affinity Methanosaeta and concomitant higher conversion of substrates (Takashima, 1987). This will also minimize the possibility of pH depression even with poorly buffered feeds (Switzenbaum et al., 1987). This strategy is highly recommended for start-up with an unacclimatized seed and a complex substrate. However, a major drawback of this strategy is that it requires longer time for start-up.

Another more aggressive strategy is called the maximum loading rate which raises the loading rate stepwise even while the effluent volatile fatty acids (VFA) are high. This strategy favors growth and predominance of lower acetate affinity Methanosarcina. It was found that VFA concentrations lower than 200 mg/L were favorable for Methanosaeta spp. (Morvai et al., 1992). When the VFA concentration was higher, it led to predominance of Methanosarcina over Methanosaeta. It was also reported that the acetate concentration lower than 200 mg/L provides a desirable condition for the growth of Methanosaeta rather than

Methanosarcina (Hulshoff, 1989; Gujer and Zehnder, 1983). This start-up strategy is rather risky and could possibly upset the system if there is no competent online monitoring and control of the mixed liquid pH and the organic loading rate (Yan, 1996).

2.1.1.3 Approaches to rapid start-up

Since start-up is one of the major drawbacks of the anaerobic system, many studies have attempted to accelerate this process. Physical-chemical and biological factors promoting the acceleration have been extensively documented.

One successful example of an aggressive strategy for starting-up a fixed-film reactor was a laboratory scale study on bacterial attachment (Aivasidis et al., 1988). In this study, accelerated start-up was achieved through optimum selection stress by coupling a porous media (synthetic glass sponge rasching rings). This porous material provided superior initial attachment with selective pressure for adhering microorganisms by a dilution rate higher than the washout rate. The reactor was seeded with pre-acclimatized mixed culture and fed with an acetate substrate. The loading rate was raised whenever feasible based on the pH value and biogas production. This strategy, selected for bacteria with superior adhering capability, had achieved a loading rate of 160 g COD/L.d in less than a month.

Rapid start-up of thermophilic UASB reactors was also achieved at process temperature 46, 55 and 64°C using mesophilic granular sludge as seed and fatty volatile acids mixtures as feed (Lier et al., 1992). The start-up of the thermophilic was brought about by simply increasing the temperature of mesophilic UASB reactors in a single step. Stable thermophilic methanogenesis was achieved within a period of one to two weeks depending on the temperature it was risen to. Another study on the thermophilic UASB reactor using the mesophilic granular sludge as seed had shown to be advantageous for rapid and stable start-up of a thermophilic UASB reactor comparing with the use of suspended sludge from a thermophilic anaerobic digester (Syutsubo et al., 1997).

2.1.2 Granulation

2.1.2.1 Anaerobic granulation

Since the uncover of the importance of anaerobic granulation, numerous studies were done attempting to understand the phenomenon of the formation of microbial aggregates and their characteristics. Formation of the microbial aggregates or settleable biogranules is significant to biological wastewater treatment systems where microorganisms have to be separated from the effluent. Dispersed individual bacteria are stable in solution and extremely difficult to settle down due to their tiny size (most bacteria cells are $0.5 \sim 3 \mu\text{m}$), weight (slightly heavier than water specific gravity – $1.02 \sim 1.06$) and negatively charged nature (Bitton, 1994). Granulation has not only significantly enhanced the settleability of the microorganisms, it also improves the physiological conditions enabling them to interact better, especially syntrophs in the anaerobic system (McCarty and Smith, 1986). In other anaerobic biological processes like methanofication, acidification and denitrification, granulation has also been reported in their studies (Lettings et al., 1980; Jhung and Choi, 1995).

In mesophilic ($30\sim 38^{\circ}\text{C}$) granule formation, different granule structures can be cultivated on different wastewaters and under different start-up conditions. Filamentous type granules, developed on mainly VFA feed, tends to be 5 mm in size and mechanically fragile (Hulshoff Pol et al., 1984). They are reported to contain inert material and are dominated by highly filamentous form of *Methanosaeta*, presumed to be *M. soehngenii*. When fed with sugar beet or potato-processing wastewaters, more robust, rod type granules are formed. There is no detectable inert carriers and is dominated by *M. soehngenii*-like species, but in a much shorter chain-length of up to 3 mm in size (Adebowale, 1990).

Thermophilic ($49\sim 57^{\circ}\text{C}$) granules consisting mainly of thermophilic *Methanosarcina* spp. are distinctively different from mesophilic granules. They are generally smaller in size. Successful formation of very small thermophilic

granules (0.2 mm) from a mixture feed of acetic, propionic and lactic acids has been reported (Endo and Tohay, 1988). Larger aggregates (3.0 mm) have also been obtained in chemostat studies of acetate enrichments (Bochem et al., 1982). These granules are consisted of densely packed methanosarcina clusters surrounding a more loosely-packed central area, which contained at least two non-methanogenic species.

The growth rates and the granule cultivated vary widely with different substrate concentration feed. Large Methanosaete-like species (saete granules) were cultivated with 1000 to 5000 mg COD/L influent, and small Methanosarcina-like species (sarcina granules) were cultivated with 10000 mg COD/L influent. The saete granules with median diameters of 2.5 ~ 3.4 mm exhibited better settleability, higher substrate affinity and slightly higher bioactivity than the 0.54 mm sarcina granules (Tay and Yan, 1996). Although it is generally accepted that Methanosaete is critical for granular formation, size and form of granules are not necessarily a function of ratio Methanosaete:Methanosarcina. It is probably issues of growth versus abrasion are more important, especially in a non-acidified wastewater where the overwhelming majority of the cell yield is due to fermentative bacteria.

2.1.2.2 Granulation in UASB system

Although not all UASB systems develop granules, it is a process when a non-discreet flocculent biomass develops into discreet well-defined pellets, or granules. It could be seen as a conglomeration of microorganisms and this conglomeration can be classified as flocs, pellets or granules (Dolfing, 1987).

- Flocs are conglomerates with a loose structure. After settling, a layer of flocs formed a macroscopic layer;
- Pellets are conglomerates with a denser structure than flocs. After settling, they are still visible as separate entities;

- Granules are dense pellets; they have granular appearance and their shapes do not rely on the presence of water. They can withstand a certain amount of pressure.

But if an appropriately high loading rate is not imposed finally, microbial granulation will be retarded and hindered, and sometimes even no granulation will occur (Hulshoff Pol et al., 1987; Manjunath et al., 1989). Granular biomass serves several advantages over flocculent biomass. They have higher settling ability, which allow themselves to retain in the reactor more efficiently, and higher activity rate which can biodegrade waste at a higher rate (Lettinga et al, 1980; Hulshoff Pol et al., 1983; Dubourguier et al., 1988a).

Granulation has been successful on treatment of a wide variety of food or organic synthetic wastewater as well as industrial food wastewater as shown in Table 2.1.

Table 2.1: Successful granulation observed

Synthetic food/organic wastewaters	Reference
Ethanol and propionate	Zeeuw, 1984
	Dolfing, 1987
Acetate	Zeeuw, 1984
	Harada et al., 1988
Mixed volatile fatty acids	Zeeuw, 1984
	Brummeler et al., 1985
	Harada et al., 1988
Mixed sucrose and glucose	Ross and Smollen, 1981
	Wiegant and de Man, 1986
	Harada et al., 1988
Glucose plus peptone and meat extract	Fang and Chui, 1993
Gelatin	Schulze et al., 1988
Industrial food wastewaters	
Sugar beet wastewater	Lettinga et al., 1980; 1984 and 1985
Potato starch wastewater	Paris et al., 1987
Citric wastewater	Wu et al., 1987
Brewery wastewater	Novaes, 1988
	Fang et al., 1990
	Yan and Tay, 1994
Starch wastewater	Wu et al., 1985
Distillery wastewater or molasses	Adebowate and Kiff, 1988
	Harada et al., 1988
	Xiushan et al., 1988
Whey	Clark, 1988
	Killilia and Colleran, 1988
Apple juicing wastewater	Dold et al., 1987
Clam processing wastewater	Boardman et al., 1995

Granulation process has been investigated using UASB reactor under mesophilic conditions (Hulshoff Pol et al., 1982; 1983; Hulshoff Pol, 1989; Zeeuw, 1984; Brummeler et al., 1985; Wu, 1984; Wu et al., 1985; 1986; 1987; Manjunath et al., 1989). It was reported that when digested sewage sludge was used as inoculum and a VFA mixture (acetate plus propionate) was used as feed, three different phases was distinguished during the granulation process (Hulshoff Pol et al., 1982; 1983). These phases were characterized by the sludge bed concentration profiles of reactors and the nature of the sludge.

- Phase 1: the sludge bed expanded and the sludge concentration in the sludge bed increased.
- Phase 2: the sludge concentration in the bed continued to increase and the wash-out of light fraction of sludge started and consequently the total amount of sludge in the reactor dropped to the minimum level. At the same instances, granules were formed in the sludge bed.
- Phase 3: the growth of granule exceeded the wash-out rate of the sludge and therefore, the total sludge concentration in the reactor increased again.

It was also found that the amount of sludge being washed-out and level of sludge remaining in the reactor depended heavily on the density of the inoculated sludge (Zeeuw, 1984; 1988).

In addition, it was reported that adding a small amount of granules to non-granular sludge was still needed to stimulate the granulation process (Hulshoff Pol et al., 1983; Alibhai and Forster, 1986). This could be viewed as adding an inoculum of microorganisms which are responsible for granulation.

2.1.2.3 Granulation in other systems

Granulation is observed in the operation of internal circulation (IC) reactors. IC reactors are able to operate at a higher volumetric loading rate than UASB reactors, resulted in increased gas production as well as shear rates in the reactors. In IC reactors, the liquid upflow velocity is much higher (about 8 ~ 20 times higher) than the UASB reactors, yet granules still develop successfully (Pereboom and Vereijken, 1994). The IC reactor design is able to prevent the wash-out of granules as it prevents high turbulence in the final three-phase separator. The larger granule size on the IC reactor results from the increased solid wash-out as fines causing a lower sluicing rate. However, the higher sludge loading rates and subsequent higher growth rates in the IC reactors resulted in the development of granules of lower strength (Pereboom et al., 1994).

Granules are also formed in gas-lift reactors. Studies showed that pumice particles, like biofilms, and carrier-free aggregates, were observed. The structures of the aggregates are pH dependent. At pH 7, the aggregates have a hairy structure, but at lower pH, the structure becomes smooth or at higher pH, the hairy structure of the aggregates is more irregular. The biomass of the aggregates was predominantly *Desulfovibrio* sp. and *Acetobacterium* sp. and was rather heterogeneously distributed throughout the aggregates at all pH values (Houten et al., 1996). Presence of CO₂ led to formation of layered biomass structure in which the *Acetobacterium* sp. was predominantly at the outside of the aggregates and the *Desulfovibrio* sp. was located at the inner part of the aggregates.

In upflow anaerobic filters, the biomass retention is accomplished by immobilizing microorganisms by a process of spontaneous aggregation of the bacteria to the granular sludge within the interstitial void spaces. In this operation, granulation had also been observed (Tay et al., 1996; Tay and Show, 1998; Show and Tay, 1999).

2.1.2.4 Granule characteristics

- Physical and chemical characteristics

Good settleability of the UASB granules is very important for an efficient and effective UASB system. It depends greatly on the granule size and density (Grotenhuis et al., 1991). Though reported values of the settling velocity of granular sludge are in range of 18 ~ 100 m/h, typical values are between 18 and 50 m/h (Dolfing et al., 1985c; Hulshoff et al., 1986; Beeftink et al., 1988; Andras et al., 1989). A relationship between the minimum granule diameter given a density which have a settling velocity of 20 m/h was established as shown in Table 2.2 (Schmidt and Ahring, 1996). With this relation, whether a granular sludge has been developed can be determined.

Table 2.2: Minimum diameter of granule settling at 20 m/h given its density

Density (mg/mL)	Diameter (mm) at 37 °C
1010	1.2
1020	0.8
1030	0.6
1040	0.5
1050	0.4
1060	0.4
1070	0.3
1080	0.3
1090	0.3
1100	0.3
1200	0.2
1300	0.2
1400	0.1
1500	0.1

According to Stoke’s law of terminal velocity, settling velocity of a spherical object has a linear relationship with the square of its diameter. It was reported that there is a linear relationship between the settling velocity and the size of the granule (Satoshi et al., 1991). The possible difference between Stoke’s Law and the findings of Satoshi and his co-workers is the assumption of a solid spherical object in Stoke’s Law while granules are permeable. However, another study observed that the settling velocity decreases with increasing size when the diameter of the granules reaches 2.8 mm (Wu, 1999). It could be due to the hollow core of the large granules that caused the decrease in density. Fluffiness is also a factor to the settling ability of granules. Filaments extending from the outer surface of the granule will resist the settling of the granule and trap tiny gas bubbles which cause sludge flotation. It is desirable to have compact and smooth granules. Upflow velocity and substrate compositions are probably some factors contributing to the fluffiness of granules.

The specific density of UASB granules varies from 1.00 ~ 1.07 (Dolfing et al., 1985a; Holshoff Pol, 1989). Several factors including the starting inoculum material, feed composition, pH, organic loading rate and VFA concentration are

able to affect the specific density of the granules formed. Many studies have tried to establish a relationship on the specific density and the size of the granules. However, contradicting conclusions were made. Roam (1989) reported that the specific density of the granule is independent of its size. However, another reported that granule density diminishes as the diameter of the granules increases. The explanation given was because there is a substrate insufficiency or depletion in the core of large granules, arising from the mass transfer limitations, resulting the inactivation and autolysis of the inner cells and consequently low cell density areas or even hollow core (Beefink and Staugaard, 1986; Guiot et al., 1992; Alphenaar et al., 1993).

The size of the granules varies greatly from 0.1 mm to 8 mm in laboratory scale reactors, though more often is about 0.5 mm to 5 mm. In industrial or commercial UASB installations, the granules grown are not larger than 3 mm. This might be due to severe shear stress caused by large height of commercial installations (5~7 m) compared to that of laboratory UASB reactors (0.5 ~ 2 m). All factors affecting granulation do play a part in affecting the granule size and distribution. However, the more important factors seem to be temperature, substrate feed characteristics, hydraulic retention time, organic loading rate and the microbial populations cultivated. Several reports had shown that the granulation of Methanosaeta can occur relatively much faster. Granule of diameter 1 mm can be observed within a month of operation (Switzenbaum et al., 1987). Granules containing more of the filamentous Methanosaeta tend to grow to a larger size than granules mainly containing rod-type methanosaeta (Hulshoff et al., 1987).

Sludge Volume Index (SVI) is the most common parameters to quantify the settling characteristics of suspended-growth activated-sludge solids due to its simple test procedures. The SVI of granules is usually low ranging from 5 to 30 mL/g SS (Lettinga et al., 1980; Ross, 1984; Grotenhuis et al., 1991).

The strength of the granule is defined as the resistance of the granule against disintegration during operation (Ghangrekar et al., 1996). It is affected by the

microbial compositions of the granules. It was reported that Methanosaeta forms a network which might stabilize the granule structure (Fukuzaki et al., 1991). In addition, Methanosaeta granules in UASB reactors are able to withstand variations in operating parameters, both physically and biologically, without significant deterioration in its characteristics and the reactors' performance.

The mineral composition of granules varies widely with different elements and different sources of granules. A wide range of nutrients is required for growth of microorganisms. The inorganic material or ash content of granules varies from 10 to 90% of its dry weight. A correlation of the density and ash content has been found, showing that an increase in density can be explained by an increase in ash contents (Fukuzaki et al., 1995).

The main components of the inorganic materials are sodium, calcium, iron and potassium. Many heavy metals influencing the granules' activity as component of essential enzymes in numerous anaerobic reactors include Fe, Co, Ni, Cu, Zn and Mo. Iron is seemed to be involved in the energy metabolism as a cytochrome and ferredoxin in the methylotrophic methanogens, and also responsible for the black color of the granules (Shen et al., 1993). However, high concentrations of heavy metals can inhibit acidogenic or/and methanogenic activities (Pavlostathis et al. 1996; Lin and Chen, 1997).

- Metabolic activity

Metabolic activity can be expressed in terms of volatile suspended solids (VSS), most probable number (MPN), specific methanogenic activity (SMA), enzymes etc. However, SMA is currently used most extensively to quantify maximum potential substrate utilization rate of a biomass (Lettinga et al., 1980; 1984 ; 1985; Valcke and Verstraete, 1983; Zeeuw, 1984; Dolfig and Blomen, 1985b; Reynolds and Colleran, 1987; Speece, 1988; Colleran et al., 1992).

SMA is affected by temperature, wastewater characteristics and also the bacterial populations. It varies from 0.04 ~ 1.2 L CH₄/g VSS.d, but most granules have SMA of 0.2 ~ 0.8 L CH₄/g VSS.d. However, thermophilic Methanosaete granules cultivated on acetate and butyrate feed at 55°C have a SMA of 2.9 L CH₄/g VSS.d (Wiegant and de Man, 1986). Granules cultivated on domestic sewage are much lower in SMA, generally between 0.02 ~ 0.08 L CH₄/g VSS.d. A comparison of SMA is compiled and shown in Table 2.3.

Table 2.3: A comparison of Specific Methanogenic Activity

Feed Source	Scale	Temp (°C)	SMA (L CH ₄ /g VSS.d)	References
Acetate	Lab	35	0.83	Harada et al., 1988
Acetate	Lab	55	1.7~2.9	Wiegant and de Man, 1986
Brewery	Lab	35	0.8	Wu et al., 1985
				Wu, W.M, 1991
Citrate	Lab	35	0.3	Wu et al., 1987
Potato	Lab	35	0.4~0.6	Hulshoff Pol et al., 1983
				Lettinga et al., 1984
Sugar beet	Full	30	0.5	Patte and Versprille,1980
Sucrose	Lab	30	0.4	Sierra-Alvarez et al., 1988
VFA mixtures	Lab	30	0.5~0.7	Brummeler et al., 1985
VFA mixtures	Lab	30	0.9	Hulshoff Pol et al., 1983
VFA mixtures	Lab	35	2.8~3.2	Wu, W. M, 1991
				Thiele et al., 1990
Yeast	Full	30~35	0.3~0.4	Hulshoff Pol et al., 1983
				Lettinga et al., 1984

- Microbial composition and granule structures

Methanogens, particularly acetate utilizers, were found to be the major microbial group present in all granules examined. It was reported that, for granules

developed on a VFA mixture, the level of hydrolytic-fermentative bacteria was quite low while the predominant groups were still methanogens (Hickey et al., 1991).

Microbial structure also plays an important part in the granule characteristics. It can mediate the sludge granulation process. Both *Methanosaeta* and *Methanosarcina* play important roles in the formation of UASB granules. The filamentous *methanosaeta* forms a network capable of stabilizing the overall structure and colonizing the *Methanosarcina* clumps to act as microbial nuclei in the initial granule development (Fukuzaki et al., 1991; Sanchez et al., 1994).

It has been reported that granule structure improved the syntrophic cooperation between bacteria species resulted in better mass transfer in anaerobic degradation (Schink, B. 1997). The flux of hydrogen between the hydrogen-forming fermenter and the hydrogen-consuming methanogen is inversely proportional to the distance between the two (Schink, B and R.K Thauer).

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) are used extensively to identify qualitatively the microbial composition and structure of granules. *Methanosaeta*-type acetate utilizers (bamboo-shape) are normally the predominant microorganism observed. Exceptions like *sarcina*-type methanogens to be found as predominant were also observed (Dolfing and Bloemen, 1985; Hulshoff Pol et al., 1983; Lettinga et al., 1984; 1985; Wu et al., 1985; 1987; Wiegant and de Man, 1986). SEM observations indicate that *Methanosaeta*-like bacteria form a network and a bundle which may stabilize the overall structure of the granules (Dolfing, 1987; Dubourguier et al., 1988; Hulshoff Pol et al., 1983; Wu, 1984). It was also found that over 30% of the total cells in a granule belong to *Methanosaeta* spp, which could be explained, as 60 ~ 70% of the total methane production is through acetate conversion.

In previous studies, the granule developed on VFA mixture (acetate, butyrate and propionate) under mesophilic temperature conditions can be classified into three

distinct types according to the predominant acetate utilizing methanogens observed using SEM (Hulshoff Pol et al., 1983; Lettinga et al., 1984; Zeeuw, 1984; 1988):

- “Rod-type” granules, which are mainly composed of rod-shaped bacteria in fragments of about 4 to 5 cells resembling Methanosaeta;
- “Filament-type” granules, which consist predominantly of long multicellular rod-shaped bacteria;
- “Sarcina-type” granules, which develop when a high concentration of acetic acid is maintained in the reactor.

A wider diversity of methanogenic sub-populations was observed as the complexity of wastewater increases. At least four distinct microcolonies were observed in granules treating brewery wastewater (Wu, 1991). One of them was composed of Methanosaeta-like rods only, while the others consisted of H_2 - CO_2 utilizing Methanobacterium-like rods juxtaposition with three different rod-shaped syntrophs (Hickey et al., 1991).

2.2 TREATMENT OF INDUSTRIAL FOOD WASTEWATER

Different qualities of wastewater are being discharged by food processing industry due to the different raw materials, processing methods and types of products involved. Therefore, specific nature of the wastewater such as its content, concentration and quantity produced in a food industry will vary to its site activities. In general, these wastewaters contain high suspended solids concentrations and organic pollutants expressed as BOD or COD concentration.

According to the relevant authorities in different countries, effluent discharged into the public sewers is guided by regulations set. In Singapore, the Trade Effluent Regulations state that tariff will be charged if effluent quality exceeds the BOD_5 and suspended solids limit of 400 mg/L each. In order to avoid high tariff

charges, food processing companies have to pre-treat their effluent prior to discharge.

2.2.1 Industrial food wastewater treatment methods

Many food-processing industries have been evaluating new technologies for improving wastewater treatment efficiency. Different treatment methods work differently even when treating the same wastewater. Treatment methods range from physical to chemical or to biological or even combinations of few different technologies in order to treat the wastewater. Therefore, many considerations, like treatment efficiency, available technology, and cost are made before installing any treatment methods.

Hydrothermal processing systems are used for the reduction of BOD and nutrients in food processing wastewaters. These technologies are particularly suited for the highly BOD-loaded effluent and for effluent containing suspended solids. The process operates in a hot-liquid water environment using pressurized reactor systems. It includes catalytic gasification systems that convert the organic material into medium-Btu fuel gas (Elliott and Hart, 1995). In this process, the organics react with water to produce methane and carbon dioxide gas, which are easily separated from the water for reuse or recycle. Nitrogen in the wastewater remains as residual ammonia after the organic processing. For this purpose, a nitrogen removal system has been developed where residual ammonia and nitrates are removed from the product water by non-catalytic processing. Reduced and oxidized nitrogen is balanced in the reactor and the product is nitrogen gas (Sealock et al., 1996).

Membrane filtration applications have been used in the food industry for a variety of wastewater treatment. Reverse osmosis (RO) has been installed at a dairy cheese plant to reduce BOD in the wastewater so as to allow direct discharge of the filtered water into a nearby creek (Anon, 1993). The reduction of BOD was from 35-40 mg/L down to 8-9 mg/L. The feed was evaporator condensate from a

cheese-whey concentrator and was essentially 50% of the plant's discharged water. Ultrafiltration (UF) systems can separate SS and microbes, with capability to regularly achieve greater than 90% reduction in BOD and less than 5 mg/L residue solids. Membrane-treated water is suitable for reuse as demonstrated at many full-scale applications, and although more expensive to purchase and install, it offers a saving in operating costs in the long run.

Dissolved air flotation (DAF) could be a viable method for the treatment of concentrated fish farming wastewater (Jokela et al., 2001). The possible advantages include the use of high hydraulic and solids loadings, relatively small floor space requirements, good separation efficiency, aerobic process conditions, removal of microorganisms and thick floated sludge (Viitasaari et al., 1995). The wastewater treatment is concentrated on suspended solids (SS) removal with efficiency judge according to phosphorous removal. The main purpose of SS removal is to remove nutrients bound to the solids. Treatment is done in two steps: concentration of the very diluted wastewater and subsequent treatment of the concentrated wastewater.

Ozonation of organics in water is a very complex technology involving mass transfer processes and a variety of possible chemical reactions. Use of ozone to reduce the level of phenolic compounds in food processing wastewater appeared as an interesting technology due to the high reactivity of these organics with ozone, especially at high pH (Hoigne, 1982). In addition to reducing organic matter content, it increases the biodegradability of the treated wastewater (Beltran et al., 1997). Thus, ozone might be used as a pretreatment for biological digestion.

Biological treatment uses microorganisms to remove organic solids and nutrients (nitrogen and phosphorus) from wastewaters. It is generally considered to be more cost effective in treating high organic strength wastewaters, such as food processing wastewater, as compared with physical and chemical treatment methods.

An emerging area of biotreatment involves the use of thermophilic microorganisms to treat the wastewaters under more severe conditions. The advanced fluidized composting (AFC) technology, involving aerobic thermophilic bacteria and an intermediate chemical treatment step, is an example. This technology is advertised for use with high-strength wastewaters and capable of 90% COD reductions with sludge generation of about one-tenth that of conventional biotechnology. It operates at rates 10 times faster than conventional activated sludge systems (Rozich and Colvin, 1996).

Significant developments in the last decade have been made with denitrification, particularly for nitrogen and phosphorus removal from wastewater (Lopes et al., 2001). Biological nitrogen removal is relatively well understood, whereas knowledge concerning the mechanism of bio-removal of phosphorus and the organisms responsible remain ill defined. Research has been conducted recently in the area of autotrophic denitrification using hydrogen or elemental sulfur as substrate (Johns, 1995).

Aerobic technologies are used including trickling filter, activated sludge, rotating biological contactors, oxidation ditch, sequencing batch reactor and even controlled wetlands. Anaerobic technologies currently available are high-rate systems including continuous-flow stirred tank reactor, contact reactor, upflow anaerobic sludge blanket, anaerobic filter (upflow and downflow), expanded or fluidized bed and two-stage systems that separate the acid-forming and the methane-forming phases of the anaerobic process. In many cases, aerobic and anaerobic processes are combined in one treatment system (Austermann-Haun et al., 1999; Oliva et al., 1995). Anaerobic treatment is used for removing higher concentration of organic matter, and aerobic treatment is used for the lower concentration or as a polishing step to further remove residual organic matter and nutrients from the wastewater.

In a market study on the anaerobic wastewater treatment systems, over 750 anaerobic systems were installed over the last 2 decades (Zakiah et al, 2003).

Lusk (1996) estimated 44% of international industrial anaerobic wastewater treatment plants are located in Europe and 14% were located in North America. In North America, more than 70% of its anaerobic systems were used for food-processing wastewaters (Zakiah et al, 2003). One of the reasons where food-processing wastewaters are suitable for anaerobic treatment process was because they rarely contained significant amount of toxicants or inhibitory compounds in their composition (Foresti, 1990). Upflow Anaerobic Sludge Blanket (UASB) process has been proven by many industrial applications to be effective and efficient since it was developed in 1980s. It has been successfully applied to a wide variety of industrial wastewaters, including the food industry as shown in Table 2.4. Despite the many applications of UASB system to treat a variety of food wastewater, there is a lack of studies conducted on treatment of a combination of food wastewater. The present study involved laboratory-scale UASB reactors for the treatment of combined food-processing wastewater produced from over 100 industrial food-processing factories.

Table 2.4 - UASB process used in treating different industrial wastewaters			
Type of Wastewater	Max. Loading Rate (g/L.d)	COD Removal (%)	References
Sugar beet factory	30 – 32	75	Lettinga and Hulshoff, 1991
Potato starch production	30	75	Zueew, 1998
Vegetable canning	10 – 20	60 – 80	Zueew, 1998
Yeast factory	14	70	Zueew, 1998
Dairy factory	15	80	Hulshoff and Lettinga, 1986
Slaughter house	10	55	Zueew, 1988

CHAPTER 3

METHODS AND MATERIALS

Experimental setup and analytical methods

3.1 EXPERIMENTAL DESIGN

The entire study was separated into 3 phases, as follows;

Phase 1: Industrial food wastewater characterization

Phase 2: Effects of conventional and stressed-loading methods on granulation and start-up.

Phase 3: Effects of starvation on granule properties and restart-up

3.2 PHASE 1: INDUSTRIAL FOOD WASTEWATER CHARACTERIZATION

3.2.1 Wastewater sampling frequency and procedures

In JTC food factories building where the wastewater was collected, not all the tenants are in operation at the same time. Therefore, to obtain a representative wastewater sample, sampling schedule and frequencies for this study were set as follows:

Day samples were collected twice weekly on Tuesday or Wednesday, and on Friday at 1100 hr. Night samples were also collected at 2300 hr on the night before the day samples were collected. The wastewater samples were tested within 2 hours after collection to prevent premature degradation. Samples were preserved for tests that could not be carried out immediately.

3.2.2 Wastewater characteristics tests

The industrial food wastewater was tested for the various parameters as listed in Table 3.1.

Table 3.1: Parameters examination methods

Parameters	Examination Methods
pH & temperature	Horiba Portable pH meter
Suspended solids	Standard Methods 2540D
Total organic carbon	Total Organic Carbon Analyser, Shimadzu TOC-VCHS
Chemical oxygen demand	Standard Methods 5220C
Biochemical oxygen demand	Standard Methods 5210B
Phosphorus	Inductively Coupled Plasma
Nitrogen (TKN)	Lachaf Instrument
Volatile fatty acids	Perkin Elmer HPLC
Alkalinity	Standard Methods 2320B
Heavy metals content	Inductively Coupled Plasma

3.3 PHASE 2: EFFECTS OF CONVENTIONAL AND STRESSED-LOADING METHODS ON GRANULATION AND START-UP

3.3.1 Experimental procedure

The second phase of the experiment investigated the effects on UASB start-up and granulation in treating the food industry wastewater at an average design organic loading rate (OLR) of 17.3 g COD/L.d using conventional and stressed-loading methods. The OLR was calculated as the ratio of the COD concentration of the wastewater to its hydraulic retention time. As the COD concentration of the wastewater was fluctuating, the hydraulic retention time (HRT) was used as a control parameter. The average COD concentration of the wastewater found in

phase 1 was used to calculate the average OLR in this phase of study. Hydraulic retention time (HRT) was calculated as the ratio of the effective volume of reactor to the influent flowrate. Tracer study was not conducted as theoretical calculation has been well accepted for UASB process.

Two UASB reactors were set up for phase 2 of this study. One was started-up with conventional start-up strategy and the other was setup with stressed-loading strategy.

In the conventional method, the initial OLR was set low so as to allow the seed sludge to acclimatize to the substrate feed. Upon reaching a steady-state performance, which is determined by its steady biogas production and consistent COD removal efficiency over a period of 3 to 4 days, the OLR then increased to the next stage until it reached the design loading.

In the stressed-loading method, OLR was increased to the next stage before reaching its steady-state. Stressed-loading in this study was referring to the creation of an adverse condition for the microorganism by the organic loading strategy. Previous studies had shown that suitable amount of stress can accelerate the start-up duration. Yang (2002) used COD removal efficiency as an indication to determine the stress on the reactors. The study reported that the OLR was increased to the next stage when COD removal efficiency attained 75% or 85%.

As previous studies were mainly based on synthetic wastewater, the influent COD concentrations and nutrients were controlled. In the present study, the COD concentrations, as well as other characteristics of the combined food wastewater, varied and fluctuated. In determining the stress level and considering the fact that it was difficult to reach a high COD removal rate, a 75% COD removal efficiency was used as an indicator for stepping up of OLR.

Due to the fluctuation of the influent COD concentration, an average influent COD concentration of 2.167 g COD/L was obtained through the wastewater

characterization phase. Using this estimation, OLR was controlled and designed using the hydraulic retention time (HRT). Hydraulic retention time (HRT) was calculated as the ratio of the effective volume of reactor to the influent flowrate. Tracer study was not conducted because the theoretical calculation has been well accepted for UASB process. The design step-up stages are presented in Table 3.2.

Table 3.2: Organic loading stages

Stage	Ave. COD concentration (g/L)	HRT (hr)	OLR (kg COD/m ³ .d)
1	2.167	20	2.6
2	2.167	10	5.2
3	2.167	7	7.5
4	2.167	5	10.4
5	2.167	4	13.0
6	2.167	3	17.3

During the operation of the reactors in Phase 2, parameters measured were categorized into two groups: routine tests and special tests. For maintenance and monitoring of the reactors, routine tests for parameters like biogas production and composition, alkalinity and pH value of the influent and effluent, effluent suspended solids concentration and volatile fatty acids (VFA) concentration were carried out. Upon reaching steady-state operation, special tests for granule characteristics such as sludge volume index (SVI), scanning electron microscopy (SEM), specific methanogenic activity (SMA) and granule size and strength were measured. Tests frequencies of the parameters are listed in Table 3.3.

Table 3.3: Parametric Test Frequency

Parameters	Frequencies
Influent and effluent pH	Daily
Biogas production	Daily
Biogas composition	Twice weekly
Influent and effluent COD concentration	Daily
Influent and effluent alkalinity	Twice weekly
Influent and effluent SS	Twice weekly
Influent and effluent VSS	Twice weekly
System SS	Weekly
System VSS	Weekly
Influent and effluent heavy metals content	weekly
System and effluent VFA	weekly
Granule strength	upon steady-state
Particle/granule size	upon steady-state
SMA	upon steady-state
SVI	upon steady-state
SEM	upon steady-state

3.3.2 Experimental setup

The UASB reactor system works in a way that when the influent wastewater flows through the sludge blanket, the organic compounds are removed. Biogas produced at the same time improves the contact between the sludge and the feed. It is then captured by the three-phase (gas, solids, and liquid) separator and channeled to the gas meter.

Two plexiglass upflow anaerobic sludge blanket (UASB) reactors were used in this study. These reactors were constructed with identical physical features and configurations. The internal diameter of the reactors was 100 mm and the height

was 680 mm with a working volume of 4.4 L. There were sampling ports located along the height of the reactors at an interval of 100 mm. Schematic diagram of the experiment setup is shown in Figure 3.1 .

Figure 3.1: Schematic diagram of experimental setup

The reactors were housed in a walk-in temperature-control room set at $35 \pm 1^\circ\text{C}$. The industrial food wastewater collected from site was stored in a chiller set at 4°C to prevent premature degradation. The wastewater was then pumped into the reactors' inlet by peristaltic pumps (Cole-Parmer, MasterFlex ® L/STM). The influent tube, connecting the wastewater tank to the reactors inlet, was long enough to ensure the wastewater had been warmed to about 35°C prior its entry to the reactors to simulate the real situation. The biogas produced by the reactors was first passed through a water trap before entering into the wet gas meters (Ritter TG 05) to prevent severe corrosion or any other damages to the gas meters.

3.4 PHASE 3: EFFECTS OF STARVATION ON GRANULE PROPERTIES AND RESTART-UP

3.4.1 Experiment procedures

Phase 3 of the experiment investigates the effects on the granule properties and restart-up of UASB subjected to different starvation periods. As there are times when the factories are shut down for maintenance, effects on the granules and performance of the UASB are required to be understood.

Another three UASB reactors were set up like the previous phase with the same type and quantity of seed and operating conditions. All three reactors were started-up in parallel using conventional start-up method mentioned in Chapter 3.3.1 from OLRs of 2.6, 5.2, 7.5 g COD/L.d to their designated OLR of 10.4 g COD/L.d, corresponding to a HRT of 5 hr. HRT of 5 hrs was selected because, in phase 2, both R1 and R2 had attained this ultimate loading with a removal efficiency of 77% which was considered reasonably well.

All the three reactors, R5, R10 and R15, were ensured in the steady-state and properties of the granules like SS, VSS, SVI, granule strength, granule size and SMA, and reactors performance with respect to COD removal efficiency, gas production, gas composition, SS and VSS of effluent and system VFA, before the starvation commence. Three starvation periods of 5, 10 and 15 days were studied. After the starvation periods, the reactors were loaded immediately to its previous OLR. Properties of the granule were tested every 5 days during the starvation periods and also after the recovery of the reactors performance. Reactors performance was monitored closely after the starvation periods to investigate the effects of the starvation.

3.5 SEED SLUDGE

Selection of suitable inoculum material contributes greatly to the success of the start-up of UASB. It is preferable to inoculate the UASB with microorganisms that are able to degrade organics compounds which is similar to the targeted wastewater. Thus, the time taken for the seed sludge to acclimatize will be shortened.

Digested sewage sludge was selected as it was considered an adequate seed material due to its relatively high methanogenic activity, and a diverse microorganism ecosystem that is suitable for the treatment of many kinds of wastewater. Both reactors were inoculated with 3 L (about 70%) of digested sludge each, obtained from an anaerobic digester of Jurong Water Reclamation Plant treating industrial and domestic sewage. The characteristics and elements of the seed sludge are shown in Tables 3.4 and 3.5.

Table 3.4: Characteristics of seed sludge

Suspended solids (SS)	24.75 g/L
Volatile suspended solids (VSS)	15.19 g/L
Sludge volume index (SVI)	61.4 mL/g SS
Specific methanogenic activity (SMA)	0.26 L CH ₄ /g VSS.d
Median particle size	135.6 μm

Table 3.5: Metals content in seed sludge expressed in % dry sludge solids

Elements	Concentration (%)
Phosphorus	0.110
Calcium	0.051
Potassium	0.034
Magnesium	0.003
Sodium	0.154
Aluminium	0.038
Copper	0.054
Iron	0.123
Manganese	0.001
Molybdenum	Nil
Nickel	0.013
Zinc	0.041

3.6 ANALYTICAL METHODS

3.6.1 pH

pH is one of the most crucial monitoring parameters especially in the sensitive start-up period. It was measured using a pH meter (BECKMAN Φ 71) equipped with a glass electrode. Its normal range of accuracy was 0.01 pH unit. Samples' pH was measured immediately so as to prevent or reduce the error due to the escape of dissolved carbon dioxide.

For a more representative data, the wastewater pH was measured on site during collection using a portable pH meter (Horiba Portable pH meter).

3.6.2 Alkalinity

Alkalinity provides the system a buffer to maintain the pH to its desired range. Thus, the amount of alkalinity in the influent and the effluent serves as an indication of the stability of the pH in the reactor. Alkalinity was determined according to the Standard Methods (APHA 1998) Section 2320B via a potentiometric titration to pH value of 4.3 ~ 4.5.

100ml of wastewater sample was used in each test. It was titrated with H_2SO_4 solution of 1 Normality until the pH value of the wastewater sample, measured using a pH meter, reaches pH value of 4.5. A magnetic stirrer was used to ensure complete mixing. The alkalinity of the wastewater was then calculated from the amount of acid used.

3.6.3 Chemical oxygen demand (COD)

Both the wastewater and the effluent of the UASB were tested for their respective COD values as they were used as an indication of the reactor performance in terms of COD removal. The method used in this project was Closed Flux, Titrimetric method of the Standard Method (APHA,1998) Section 5220 C. Samples were diluted to the sensitive range of the method chosen which was less than 400 COD mg O_2/L . Reagents used in the test were standard potassium dichromate digestion solution, sulfuric acid reagent, ferrion indicator solution and standard ferrous ammonium sulfate titrant. Blanks and standards are tested with the samples to correct for the COD in the dilution water and to correct for the reagent inconsistencies, respectively. All samples, blanks and standards were tested in triplicate to minimize experimental errors. Samples that cannot be analyzed immediately were preserved by concentrated H_2SO_4 acidification to a pH below 2 and stored at 3 ~ 5°C.

3.6.4 5-day biochemical oxygen demand (BOD₅)

Both the wastewater and the effluent of the UASB reactors were tested for their 5-day BOD. BOD₅ was used as an indication of wastewater quality and the reactor performance. The method used was the standard 5-Day BOD Test of the Standard Methods (APHA,1998) Section 5210B. For food industry wastewater, it would be more appropriate to judge the quality by BOD₅ value. But, as the duration of the test takes 5 days, it would not be a good monitoring parameter. Therefore, a co-relationship between the BOD₅ and the COD was established and using COD value as the indicator instead.

In establishing of the co-relationship, all blanks and samples were tested in triplicate to minimize experimental errors.

3.6.5 Total organic carbon (TOC)

The total organic carbon of the wastewater and the effluent of the UASB were tested. The test was carried out by the Total Organic Carbon Analyser, Shimadzu TOC-VCHS. Samples were filtered with Whatman 0.45 µm cellulose nitrate membrane filters.

3.6.6 Volatile fatty acids (VFAs)

Concentrations of the total VFAs as well as the components of formic acid, acetic acid, propanoic acid, butyric acid, valeric acid, isobutyric acid and isocaproic acid were measured by high performance liquid chromatograph (HPLC) (Perkin Elmer, Series 200). All samples were filtered by Whatman 0.2 µm cellulose nitrate membrane filters.

The HPLC is a physical technique in which a sample dissolved in liquid is injected into a column packed with small particles and is separated into its constituent components. One or more solvent reservoirs hold vacuum degassed

mobile phase, which was pumped through the column system. A pulse damper used to regulate the flow rate, and an analytical column was used to remove particulates and contaminants from the solvent. The sample was introduced into the system with an autosampler and as it was pushed through the analytical column by the solvent, separation of constituent and components of fatty acids occurs. A suitable detector for the species of interest was used at the column exit, and the response was stored, integrated, and recorded by the data station. The column used was Polypore H (220 × 4.6 mm Dimensions) and the detector was UV at 210 nm. A 0.005 N H₂SO₄ was chosen as the mobile phase and the flow rate is 0.15 mL/min

3.6.7 Gas production and analysis

Biogas production of the reactors was recorded by a wet gas meter (Ritter TG 05) each. Biogas composition was analyzed by a gas chromatograph analyzer (HP 5890 A-GC) using a 2-m long and 3-mm-ID packed column (Haye-Sep Q, 80/100 mesh) and a thermal conductivity detector with a temperature of 200°C. Helium was used as the carrier gas in the gas chromatograph operation with a flow rate of 30 mL/min. Gas samples were extracted from the reactors using a micro-syringe and were injected into the gas chromatography immediately after sampling.

3.6.8 Suspended solids and volatile suspended solids (SS/VSS)

Biomass retention is very important for a successful reactor operation. Biomass inventory was evaluated from the SS and VSS concentrations of the sludge and the effluent. The methods used to analyze SS and VSS are described in the Standard Methods (APHA,1998) section 2540 D and 2540 E. Samples were residue collected over filtration using Whatman glass microfiber GF/C with pore size 1.2 µm. Sample volume was varied to obtain about 10 to 200 mg of residue. All tests were done in triplicate.

3.6.9 Heavy metals contents

Heavy metals content including Na, Fe, Ca, Mg and K of the wastewater and the effluent of the UASB were tested with inductively coupled plasma (ICP) described in the Standard Methods (APHA,1998) section 3120. Samples were filtered with Whatman 0.45 μm cellulose nitrate membrane filters and preserved with 2 to 3 drops of nitric acid and were kept in fridge below 4 °C.

3.6.10 Phosphorus and nitrogen

Phosphorus was measured using ICP described in the Standard Methods (APHA,1998) section 3120. Samples were filtered with Whatman 0.45 μm cellulose nitrate membrane filters and preserved with 2 to 3 drops of nitric acid and were kept in fridge below 4 °C.

Nitrogen was measured as ammonia, nitrite and nitrate. Ammonia was measured by flow injection analysis method (QuikChem Method 10-107-06-1-I, Lachat Instruments, Inc.). This method is based on the Berthelot reaction. Ammonia reacts with alkaline phenol, then with sodium hypochlorite to form indophenol blue. Sodium nitroprusside is added to enhance sensitivity. The absorbance of the reaction product is measured at 630 nm, and is directly proportional to the original ammonia concentration in the sample.

Nitrite and nitrate nitrogen were also determined by the flow injection analysis method. Nitrate is quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column. The nitrite (reduced nitrate plus original nitrite) is then determined by diazotizing with sulphanilamide followed by coupling with N-(1-naphthyl) ethylenediamine dihydrochloride. The resulting water-soluble dye has a magenta colour which is read at 520 nm. Nitrite alone can be determined by removing the cadmium column.

3.6.11 Particle/granule size

The sludge samples, taken from a sampling port 15 cm from the bottom of the reactor, were measured for its bioparticles/granules sizes using laser particle size analyzer system (Malvern MasterSizer Series 2000). The analysis was based on the principle of laser diffraction and the range of measurable particles of the MasterSizer was 0.5 ~ 1880 μm .

While using the MasterSizer, sample cell was filled with distilled water and it was stirred magnetically. It was first analyzed for the background information. A small amount of sludge, eg. a few drops, was added into the continuously-stirred cell. The bioparticles being analyzed were kept suspended and evenly distributed with an adjustable stirring speed. An appropriate stirring speed was desired as too slow a speed could not give a evenly distributed sample, while too fast a speed might broke up the bioparticles. The instrument first checked the sample concentration in the cell and then automatically analyzed the bioparticle size by volume.

As granules grew in size beyond the limits of the MasterSizer, an Image Pro Analyzer was used to measure the size of the granules greater than 0.2 mm. The granules were placed under a microscope and images were taken. The images were then analyzed using software to determine the size and other physical properties of the granules.

3.6.12 Sludge volume index (SVI)

One of the most common parameters used to quantify the settling characteristic of sludge is the sludge volume index. It is due to its simplicity of which the test is performed. SVI was measured according to the Standard Methods (APHA,1998) Section 2710 D. Suspended solids of the sludge was first determined and with the volume of the well-mixed sludge settled for 30 min. The lower the SVI indicates

the better settlability of the suspended solids. The SVI can be calculated from the following formula:-

$$\text{SVI} = \text{settled sludge volume (mL/L)} \times 1000 / \text{suspended solids (mg/L)}$$

3.6.13 Settling velocity

Settling velocity of the bioparticles was determined by a method proposed by a team of researchers (Thaveesri et al., 1994). Bioparticles were transferred to a 1.25- m high glass cylinder (diameter 4 cm) filled with water. Static settling velocity was measured by the time taken for half of the total biomass volume to move downwards and settled at the bottom of the cylinder.

3.6.14 Granule strength

Strength of the granule is defined as the resistance of the granule against disintegration during operation (Ghangrekar et al., 1996). Granule strength is expressed in term of integrity coefficient (%), which is defined as the ratio of the residual granule VSS and the total VSS. A higher integrity coefficient implies higher granule strength. Though this measurement does not indicate the granule absolute shear strength, it represents the strength of the granule against abrasion and shear, which the granule often experience during the normal operation of the reactor.

Sludge sample, taken from a sampling port 15 cm from the bottom of the reactor, was diluted 10 times with tap water as the sludge sample was too viscous to shake and generate the abrasion and shear effects required on the granules. 25 mL of the diluted sample was used for the test. The sample was placed on a shaker at a speed of 200 rpm for 5 min, subjecting the sample to abrasion and shear. The sample was then allowed to settle and granules settled within 2 min in a column were collected for VSS test. The VSS of the two portions of the sample, the settled granules and the suspended solids, could be measured respectively. The

strength of the granule was determined as the ratio of the residual granule VSS and the total VSS.

3.6.15 Microbial compositions

A scanning electron microscopy (SEM) (Stereoscan 420, Leica Cambridge Instrument) was used to study the microbial compositions of the granules. The samples were fixed overnight in 0.1 M phosphate buffer (pH = 7.0) containing 2.5% glutaraldehyde, and dehydrated with a graded series of ethanol (50%, 70%, 85%, 95% and 100%) solutions. Subsequently, the sample was dried by critical point dryer or freezer dryer and sputter-coated with gold at 20 mA in a high vacuum (2.8×10^{-6} Torr) and low temperature (-170°C) cryochamber for 90 sec. The pretreated sample was directly viewed and observed under using SEM.

3.6.16 Specific methanogenic activity (SMA)

In an effective biological wastewater treatment, an active biomass is an important contributing factor. VSS concentration, in some cases, has been used as an indication of biomass activity which may not be a good indicator for system performance. In this study, a more direct indication of the biomass activity based on a specific methanogenic activity (SMA) analysis was used. It was used to determine the specific methane production rate per unit of sludge present. A combined substrate containing glucose, peptone, and meat extracts was used. The content of the substrate used was shown below. No buffering of SMA assay was provided. A 250 mL Kimax flask was used in this study. The flask containing the biomass and the substrate was placed on a platform shaker at 20 rpm in a water bath of controlled temperature at $35 \pm 1^\circ\text{C}$ in order to enhance the contact between the biomass and the culture. The headspace was purged with nitrogen gas. Biogas produced from the incubation was passed through a concentrated NaOH (2 N) solution to remove the CO_2 produced. The methane gas volume was measured by an auto-pipette with a minimum scale of 0.1 mL.

The test was based on the assumption that the methanogenic biomass yield is minimal for a consistent amount of incubated biomass during the incubation period of about 10 to 12 hours. It was also assumed that a zero order substrate utilization rate exist under high substrate concentration. Zero order utilization rates ensured that the methanogenesis is independent of the substrate concentration so that a constant gas yield can occur throughout the incubation period. Actual food wastewater was not used because of its low substrate concentration. In SMA test, substrate should not be a limiting factor. A synthetic substrate containing a good mixed of glucose, peptone and meat extracts was used as it was highly biodegradable.

In the 250 mL Kimax flask, about 100 mL of the adjusted effluent was added anaerobically to the flask, then 25 ~ 50 mL (0.5 ~ 1.0 g VSS) of a sludge sample, taken from a sampling port 15 cm from the bottom of the reactor, 7.5 ~ 12.5 mL (0.75 ~ 1.25 g COD) of the concentrated substrate which compositions are listed in Table 3.6 and finally refilled the adjusted effluent again to a predefined level. During the preparation and the transferring of the effluent and sludge sample, special care have to be taken to avoid exposure of both effluent and sludge to air as possible. Buffer capacity was provided by adding sodium bicarbonate to maintain a pH of 6.5 ~ 7.2 in each test. An experimental setup is shown in Figure 3.2.

Figure 3.2: Experimental setup for specific methanogenic activity

Table 3.6: Synthetic substrate compositions

Based on COD concentration of 2.5 g/L	
Carbon source	
Peptone	500
Glucose	1700
Meat extract	350
Macro-nutrients	
CaCl ₂ ·H ₂ O	24
MgSO ₄ ·7H ₂ O	27
NH ₄ Cl	400
FeSO ₄ ·7H ₂ O	20
KH ₂ PO ₄	100
Micro-nutrients	
H ₃ BO ₃	0.05
ZnCl ₂	0.05
CuCl ₂	0.05
MnSO ₄ ·H ₂ O	0.05
(NH ₄) ₆ Mo ₇ O ₂₄ ·4H ₂ O	0.05
AlCl ₃	0.05
CoCl ₂ ·6H ₂ O	0.05
NiCl ₂	0.05
Conc.HCl (36%)	1 mL
Alkalinity buffer	
NaHCO ₃	1500

CHAPTER 4

RESULTS AND DISCUSSION

4.1 INDUSTRIAL FOOD WASTEWATER CHARACTERIZATION

Understanding the wastewater characteristics can aid in the design of the treatment system as well as to avoid unnecessary upset in the reactor.

Several characteristics of the wastewater are very important in order to design the start-up strategy, nutrients requirement, design loading and entire treatment design. These parameters include the organics concentration, nutrients concentration, total solids and others.

The wastewater used in this study was collected from the JTC Woodland Food Industry in Singapore. This food industry houses more than 100 food processing factories. The wastewaters produced from all these factories are channeled to a centralized point where they go through a grit screening and dissolved air floatation (DAF) pretreatment. Particles of more than 5 mm are trapped in the screen, and the DAF pretreatment basically removes the fats, oils and greases in the wastewater. The wastewater used in this study was collected from the effluent of the DAF treatment before discharge to public sewer. A schematic diagram is depicted in Figure 4.1.

Figure 4.1: Schematic diagram of wastewater pretreatment at site

4.1.1 Wastewater flow

The wastewater flow was studied in order to design an adequate treatment system. Without prior knowledge of the flow pattern and peak rates, the design might not be adequate.

The flow of the wastewater was recorded hourly by a flow meter (Figure 4.1) and tabulated from a flow meter installed after the DAF system over the study period of 5 months from 14th April'02 to 15th September'02. The daily flow fluctuated over a wide range, from 240 m³/d to 849 m³/d, with an average flow of 715 m³/d (Figure 4.2). The lowest peak occurred during the maintenance of the plant resulting in zero flow from 0800 hr to 1900 hr on 10th July'02. The next two lowest flows occurred on Sundays when very few factories were in operation. The maximum flow and other high flow days were likely due to increase in production to meet the demand for certain festive seasons. The wastewater flow fluctuation gave a standard deviation of 82.3 m³/d. The hourly flow varies from 0 m³/hr which meant that no wastewater was generated during the hours in midnight, to 50 m³/hr and averaging at 30 m³/hr.

Figure 4.2: Daily wastewater flow rate

4.1.2 Total suspended solids (TSS) and volatile suspended solids (VSS)

Solids content in wastewater is also a major concern in environmental engineering due to its potential contamination by the amount of suspended solids. Stringent criteria are stipulated by the related authority to maintain a high standard of effluent quality prior discharge. In Singapore, the discharge limit for suspended solids set by the Ministry of Environment and Water Resource (MEWR) is 400 mg/L.

The TSS of the wastewater was found to be above the MEWR limit at all time ranging from 404 mg/L to 1247 mg/L with an average of 854 mg/L and standard deviation of 246 mg/L as shown in Figure 4.3. Therefore this wastewater posed a potential of contamination if discharged untreated.

Figure 4.3: Total suspended solids and volatile suspended solids of wastewater

The volatile suspended solids (VSS) in the wastewater could be used as an indication of organic content of the wastewater. In this wastewater, the VSS content, shown in Figure 4.3, was very high ranging from 392 mg/L to 1188 mg/L, which were about 88% to 99% of TSS. From the high VSS content, the suspended solids of the wastewater were considered to be highly biodegradable, which was probably due to the nature of food processing wastewater.

When the TSS concentration was too high, it could be harmful to the UASB reactor. High concentration of suspended solids flowing into the UASB reactor could cause the biomass in the reactor being replaced by the suspended solids.

A simple primary settling tank was installed to remove the TSS from the wastewater before entering the UASB. It obtained a removal efficiency of 73% and with an averaged treated effluent TSS concentration of 248 mg/L which satisfied the MEWR limit (Figure 4.4). The treated effluent after the settling tank was used as the substrate in the UASB studies.

Figure 4.4: Efficiency of primary settling tank

4.1.3 Temperature

The temperature of the wastewater was measured during the collection of the wastewater samples. It varied from 29.5°C to 32.7°C which fell within the mesophilic range (20°C to 45°C). The fluctuation of the temperature was considered low as it only changed for about 2°C. High fluctuation of temperature might disturb the growth of the bacteria especially changes from one range to another like from mesophilic to thermophilic.

4.1.4 pH value and alkalinity

pH, which is a measure of the hydrogen-ion concentration, is an important quality parameter of wastewater. The concentration range suitable for the existence of most biological life is quite narrow and critical. Wastewater with high concentration of hydrogen-ion is difficult to treat by biological means if it is not adjusted.

Anaerobic treatment proceeds well at system pH varying from 6.6 to 7.6 with an optimum range from 7.0 to 7.2 (Bhatti et al., 1996). The efficiency declines rapidly at pH below 6.2. Maintaining an optimum system pH is especially crucial during the sensitive start-up period. A pH value below 6.5 can increase the length of start-up considerably (Young, 1980). Anaerobic systems are able to tolerate higher pH fluctuations only after steady-state is attained. To maintain the pH in the desired range, an alkalinity buffer is essential. Alkalinity is derived from the breakdown of organics and is present primarily in the form of bicarbonate, which is in equilibrium with the carbon dioxide in the gas at a given pH.

Weekly samples were taken for more than 5 months. The pH value was taken on site with a portable pH meter. The pH values, shown in Figure 4.5, measured over the study period ranged from 4.94 ~ 5.62 which were lower than the desired value of 6.8 ~ 7.2. The pH of the wastewater had a mean value of 5.16 and a standard deviation of 0.16, indicating a low fluctuation in pH.

The alkalinity of the wastewater measured was ranged from 45 mg CaCO_3/L to 95 mg CaCO_3/L . The wastewater pH and alkalinity were rather low for an efficient and effective operation of a UASB treatment system. Adjustment of the pH and alkalinity was required for the UASB system to work successfully. To maintain a pH value of between 6.6 and 7.4, an alkalinity of 1000 ~ 5000 mg CaCO_3/L was required. Sodium bicarbonate, NaHCO_3 , was added both to adjust the pH to the desired range between 6.8 ~ 7.2 and the alkalinity to about 2000 mg CaCO_3/L . An amount of 3.0 g of NaHCO_3 was required to adjust the pH of 1 L of wastewater to the requirement, which also giving an adequate alkalinity buffer (approximately 2100 mg CaCO_3/L) for the system.

Figure 4.5: pH value of wastewater

4.1.5 5-days biochemical oxygen demand (BOD₅)

This determination involves the measurement of the dissolved oxygen used by microorganisms in the biochemical oxidation of organic matters. BOD is also widely used as a parameter in the wastewater discharge standards.

The standard of discharge varies from place to place. In Singapore, the discharge limit for public sewer is set at 400 mg BOD₅ /L. As shown in Figure 4.6, the wastewater fluctuated quite significantly from as high as 4262 mg/L to as low as 1203 mg/L which exceeded the discharge limit set. The average BOD₅ reading for the wastewater measured was 2134 mg/L with a standard deviation of 807 mg/L.

Figure 4.6: BOD₅ of wastewater

4.1.6 Chemical oxygen demand (COD)

The wastewater discharge standard for public sewer set for COD by MEWR in Singapore is 600 mg/L. In Figure 4.7, the COD concentration of the wastewater tested ranged from 1961 mg/L to 4060 mg/L exceeding the MEWR limit. The average COD concentration of the wastewater was 3063 mg/L and a standard deviation of 668 mg/L.

Figure 4.7: COD of wastewater

4.1.7 Total organic carbon (TOC)

The TOC test is measured by injecting a known amount of sample into a high temperature furnace, oxidizing the sample into carbon dioxide in presence of a catalyst then measures by an infrared analyzer. One of the major advantages of this test is the short time requirement which making it popular.

The measured TOC in the wastewater, ranged from 516 mg/L to 1149 mg/L, as shown in Figure 4.8. The wastewater has an average concentration of 714 mg/L with a lowest standard deviation of 152 mg/L.

Figure 4.8: TOC of wastewater

4.1.8 Correlations between BOD₅, COD and TOC

For many types of wastewater including the wastewater in this study, it is possible to establish a correlation between the three parameters namely BOD, COD and TOC. One of the main advantages in establishing the relationship is reduction of time and effort required by using the TOC or COD values to estimate the BOD₅ value which requires 5 days. This enables almost instant monitoring of the system and allows immediate response if there is any system upset. Their correlations and R-square value of fitness are shown in Table 4.1.

Table 4.1: BOD, COD and TOC correlation

Ratio	Correlations	R-square
BOD : COD	$BOD=0.7115COD$	0.7448
BOD : TOC	$BOD=2.047TOC$	0.7485
TOC : COD	$TOC=0.3796COD$	0.8479

The correlation between BOD₅ and COD was established over the study period of 5 months. The correlation between BOD and COD was fitted into a linear line

with R-squared value of 0.74 which was considered well-fitted statistically. The ratio obtained from the correlation was expressed as $\text{BOD}:\text{COD} = 0.7115 : 1$. This ratio of $\text{BOD}:\text{COD}$ was considered high compared to other wastewaters. It was probably due to the fact that the food wastewater compositions were relatively consistence.

As TOC test required the sample to be filtered, filtered sample was used for BOD_5 as well for its correlation to achieve a better comparison. The ratio of $\text{BOD}:\text{TOC}$ was found to be 2.047:1, having a R-square value of 0.6987 which was considered satisfactory.

Correlation between COD and TOC gave the best, having a R-square value of 0.85. Similarly, filtered samples were used for TOC and COD to obtain a closer comparison. The ratio of $\text{TOC}:\text{COD}$ was found to be 0.3796:1.

The correlation test of TOC, COD and BOD_5 of the wastewater showed that they can be correlated with a R-square value of more than 0.7. It means that, by using TOC or COD values, BOD_5 of the wastewater can be estimated. This helps to avoid the long waiting period of 5 days and enables almost instant monitoring of the treatment system.

The effluent COD and BOD_5 of the primary settling tank were measured as well. It was realized that the BOD_5 of the wastewater dropped by about 24% while the COD of the wastewater dropped about 29% compared with the wastewater prior to the primary settling tank, as shown in Figure 4.9 and Figure 4.10 respectively. These results showed that the suspended solids of the wastewater contributed significantly to the COD and BOD_5 concentrations of the wastewater. Furthermore, the correlation of the COD and the BOD_5 of the primary settling tank effluent compared with that of the wastewater prior to it has an improved R-square value of 0.811 as shown in Figure 4.11. The ratio of BOD_5 to COD has also changed from 0.7115:1 to 0.7448:1. This showed that the primary settling

tank improved the biodegradability of the wastewater by removing more of the non-biodegradable suspended solids.

Figure 4.9: BOD removal by primary settling tank

Figure 4.10: COD removal by primary settling tank

Figure 4.11: Correlation of BOD and COD of wastewater

4.1.9 Wastewater characteristics in the night

In order to obtain a better understanding of certain characteristics of the wastewater, night samples were collected and analyzed. It was found that the BOD, COD, TOC and TSS values are generally lower compared to daytime values. Though these values are significantly lower than the daytime values, they are still above the MEWR discharge limits like 400 mg BOD₅/L and 400 mg SS/L. since the night time wastewater characteristics are generally lower, treatment of the wastewater is designed according to the characteristics of wastewater in the daytime.

4.1.10 Essential nutrients (nitrogen and phosphorus)

Nitrogen and phosphorus are two of the most important nutrients for growth of bacteria. Phosphorus was found with a concentration ranged from 1.71 mg/L to 14.31 mg/L with an average value of 11.75 mg/L, while nitrogen, in the form of ammonia, was found ranging from 3.5 mg/L to 135.4 mg/L with an average of

108.4 mg/L. Speece and McCarty (1964) had shown that the minimum theoretical COD:N:P ratio for sufficient nutrients may be assumed to be 350:7:1. Using the average values of the COD, N and P concentrations, the ratio of COD:N:P of the wastewater is estimated as 184:9:1. Therefore it shows that this wastewater has more than sufficient nutrients, which allows the bacteria in the UASB reactor to function and grow well, for the amount of COD to be treated.

4.1.11 Heavy metals

Certain types of heavy metals could be beneficial to the growth of the bacteria as they can serve as nutrients. For example, it was reported that Fe^{2+} , Ca^{2+} , Mg^{2+} and Ni^{2+} content assisted in granulation in UASB system (Speece and McCarty, 1964; Thauer, 1981; Murray and van den Berg, 1981; Lettinga et al., 1980;) . On the contrary, high concentration of heavy metals was toxic to biological treatment. Therefore, determining the heavy metals presence and concentration was crucial.

The wastewater was tested for common metals found in the wastewater. The results are presented in Table 4.2.

Table 4.2: Heavy metals content of wastewater

Elements	Range of concentration (ppm)
Na	223.6 ~ 393.8
Ca	5.179 ~ 62.48
Mg	1.508 ~ 15.58
Fe	0.2691 ~ 2.416
K	60.15 ~ 185.9
Cr	Not detected
Co	Not detected
Cd	Not detected
As	Not detected
Al	0.2164 ~ 0.4552
Cu	0.0178 ~ 0.0832
Mn	0.0077 ~ 0.0092
Mo	0.0000 ~ 0.0330
Ni	0.1119 ~ 0.1813
Zn	0.1223 ~ 0.1843

The results obtained were satisfactory. There was no metal of extreme high concentration. High concentrations of Ni, Cu and Zn will inhibit acidogenic and/or methanogenic activity (Pavlostathis et al., 1996; Lin and Chen, 1997). Inhibitory metals like cadmium and arsenic were absent from the wastewater. In addition, trace amount of divalent cat-ions were present in the wastewater which was reported to have positive effects on granulation (Speece and McCarty, 1964; Thauer, 1981; Murray and van den Berg, 1981; Lettinga et al., 1980; Berg van den et al., 1980).

4.2 EFFECTS OF CONVENTIONAL AND STRESSED-LOADING METHODS ON GRANULATION AND START-UP

4.2.1 Monitoring of the reactor systems

In the operation of UASB system, close and careful monitoring is essential, especially in the sensitive start-up period when a little non-compliance to the preset operating conditions might upset the system and cause a significant prolong of the start-up period.

4.2.1.1 pH and alkalinity

pH of the influent, R1 effluent and R2 effluent are shown in Figure 4.12. The pH of the influent was maintained at between 6.6 and 7.4, which was essential for UASB system to proceed well (Bhatti et al., 1996). The pH of the effluent fluctuated between 6.94 and 7.74 for R1 (control) while R2 fluctuated between 6.62 and 7.48. From the pH values, there was of no signs of system upset since the start of reactors. This could be attributed to adequate alkalinity provided and an excess alkalinity found in the reactors' effluent. As shown in Figure 4.13, the average effluent alkalinity of R1 and R2 were 2466 mg CaCO₃/L and 2416 mg CaCO₃/L which were higher than the average influent alkalinity of 2084 mg CaCO₃/L.

Figure 4.12: Influent and effluent pH

Figure 4.13: Influent and effluent alkalinity

4.2.1.2 Gas production and composition

Biogas production is one of the important parameters to evaluate the activity rate and stability of the anaerobic processes. In this study, the biogas production was one of the parameters used to indicate the attainment of steady-state in reactor performance.

Gas production generally increases with the OLR of the reactors. The gas production increased sharply when the reactor was just stepped up in OLR. The gas production fell a little after the next few days for R1 but fell sharply for R2 (shown in Figures 4.14 and 4.15). This might be due to the instability of the system caused by the stress applied to the reactors, especially to R2. The fall in gas production might also be due to the wash-out of finer biomass resulting in the reduction of overall biomass concentration. The gas production picked up sharply again when it acclimatized to the new loading rate. For the same OLR, the gas production in R1 was higher than R2. This might be due to the higher conversion efficiency of COD to methane in R1 as longer time was used to stabilize the performance.

During anaerobic digestion, CO_2 and CH_4 are produced. In R1, methane content in the biogas produced from the digestion ranged from 58% to 72% while 60% to 72% in R2 (Figures 4.15 and 4.16). From the biogas production, methane content, organic loading rate and COD removal, the COD-methane balance was calculated to monitor the performance of the reactor. Based on a theoretical amount of 0.395 L methane produced per gram of COD removed using synthetic glucose-wastewater (Tay et al., 1996), R1 had a balance of 0.300 L CH_4/g COD removed while R2 had 0.291 L CH_4/g COD removed, which were both satisfactory comparing to the theoretical value. The difference between the theoretical biogas yield and the actual gas production was caused by the cell yields of different trophic groups in anaerobic food chain. Portion of the organics has been used for biomass growth instead of converting to methane.

Figure 4.14: Gas production and methane content of R1

Figure 4.15: Gas production and methane content of R2

4.2.1.3 Effluent suspended solids (SS)

Biomass wash-out is an inevitable process. After inoculation of the seed sludge, upflow velocity of the wastewater in the reactor creates a form of selective pressure upon the biomass, causing the lighter and finer particles or biomass to wash-out together with its effluent. Other than the influence of the upflow velocity, other factors like system upset resulting in the disintegration of the biomass also cause the biomass to be washed-out. Therefore, by monitoring the effluent suspended solids, it also gives indications to the system stability.

From the data collected as shown in Figure 4.16, biomass wash-out was observed during first few days after new step-up of organic loading rate and also during sudden increase in organic loading rates which were due to the high influent organic concentration. Effluent suspended solids of R1 showed 5 peaks on the 2nd, 45th, 76th, 128th and 177th day. In R2 effluent, 4 peaks were shown, during the 2nd, 27th day, 48nd, 66th and 116th day. For both reactors, wash-outs were noticed right after inoculation which was on the 2nd day. Lighter particles or biomass were washed-out by the upflow force. Equilibrium was later attained, shown by a stable amount of SS wash-out. When the organic loading rate was increased by a step-up in hydraulic loading, the upflow velocity increased, thus caused the lighter particles or biomass to be washed-out again. After all the lighter particles and biomass were washed-out, a new equilibrium was attained. Therefore, peaks were seen on days after the change of hydraulic loading or the HRT. Tremendous wash-out in R2 was observed on the 116th and 118th days. SS of the effluent reached a very high amount of 1076 and 798 mg/L respectively. This might have caused the failure of R2 as the performance of R2 deteriorated rapidly since then.

Figure 4.16: Effluent suspended solids concentration

4.2.1.4 Volatile fatty acids (VFA)

Volatile fatty acids are one of the most common inhibitors of anaerobic system as they are generated within as intermediates in the anaerobic decomposition of organics. System pH has great influence to the inhibition of VFA. Toxicity to the system is usually exhibited at low pH values but not at neutral pH.

VFA also serves as an indication of a balanced reaction within the anaerobic system. A low VFA concentration indicates that the methanogenic reaction is not the rate-limiting step in the anaerobic decomposition.

VFA concentrations in the two reactors had shown correspondence with the removal efficiency of the treatment system. High VFA concentrations were observed whenever a higher organic loading rate was newly applied or the influent concentration had suddenly fluctuated high. At steady-state or at high

COD removal efficiency, the VFA concentrations were low in value and only low molecular volatile acids like acetic acid were observed.

As shown in Figures 4.17 and 4.18, VFAs in R1 were found to be generally lower than R2 throughout the different HRTs of 20, 10 and 7 hr. This might be due to the stress loading applied to R2 which caused the VFA concentration to increase before the VFA conversion decreased further or stabilized. At OLR of 13.0 g COD/L.d when both R1 and R2 failed, R1 showed an increasing accumulation of VFA which might be the cause of the failure as there was no sign of biomass wash-out while R2 experienced massive wash-out when its OLR was adjusted to 13.0 g COD/L.d which might highly be its cause of failure.

Figure 4.17: Volatile fatty acids concentrations in R1

Figure 4.18: Volatile fatty acids concentrations in R2

4.2.2 Start-up duration

The start-up of anaerobic biological process is commonly defined as reactor operation from the initial seeding to a specific OLR for wastewater treatment. A significant benefit of rapid granulation in UASB or any other anaerobic system is reduced operating time to reach the designated OLR. Short start-ups render anaerobic processes more attractive through time and cost saving.

The duration taken for R1 to attain steady-state performance and for R2 to reach the specified 75% COD removal efficiency under different OLRs are shown in Table 4.3.

At OLR 2.6 g COD/L.d, R1 took 36 days to attain steady-state in operation while R2 took 25 days to attain 75% COD removal efficiency. At the next OLR of 5.2 g COD/L.d, R1 took total of 65 days to attain steady-state in operation while R2 took only 42 days to reach 75% COD removal efficiency. Comparing the time

needed ranging from 36 to 171 days for the control R1 to operate from OLR 2.6 to 10.4 g COD/L.d, R2 which stressed at 75% COD removal efficiency took a markedly reduced time ranging from 25 to 114 days to operate from OLR 2.6 to 10.4 g COD/L.d. It was shown that R2 achieved the same OLRs at a shorter period than the control R1.

The start-up time was obviously reduced in R2 in which stressed-loading was applied. Under such operating mode, the start-up time would be significantly accelerated by 30 to 49%.

Table 4.3: Table of start-up durations

Ave OLR (g COD/L.d)	HRT (hr)	R1- startup duration (day)	R2- startup duration (day)	time difference (day)	% of time saved
2.6	20	36	25	11	30.6
5.2	10	65	42	23	35.4
7.5	7	119	61	58	48.7
10.4	5	171	114	57	33.3

From the experiment, it has shown that the stressed strategy could be used to start-up the UASB reactor up to 10.4 g COD/L.d treating food industrial wastewater with a reduced duration. COD removal efficiency of at least 75% was obtained with granules first observed on the 36th day of operation. Details were discussed at the later sections.

4.2.3 COD removal

The COD removal efficiency for R1 seemed to improve from first steady-state to second steady-state as shown in Figures 4.20. In the first steady-state at 2.6 g COD/L.d, the removal efficiency had only reached 85.7% but it reached 90.7% in the second steady-state at 5.2 g COD/L.d. The COD removal efficiency had dropped after every stepwise increased in organic loading due to the organic shock the system received and the active biomass wash-out. Figures 4.19 ~ 4.22

had shown that R1 responded less drastically than R2 when in the second organic loading stage. COD removal efficiency of R1 had dropped from 85.8% to 70.8% in the second loading stage while COD removal efficiency of R2 had dropped from 80.1% to 44.5%. This could indicate that R2, being stressed, had a lower stability than R1.

In the third organic loading stage of 7.5 g COD/L.d, COD removal efficiency of R2 had dropped even more drastically to as low as 21.2% and COD removal efficiency for R1 had also dropped to 23.4%. This might probably due to the sudden increase in influent COD concentration. The average influent concentration was about 2167 mg COD/L but on the 66th day, it received a COD concentration of 3642 mg COD/L which was about 80% higher than usual. This had given additional 'shock' to the reactors which were detrimental to the activity of the biomass, especially during the sensitive startup.

In the fourth organic loading stage of 10.4 g COD/L.d, the COD removal efficiency of R1 at steady-state was only at about 77% which was as high as R2 just before it was stepped up to the next stage. This might have indicated the ultimate loading it can reached with a 77% COD removal efficiency, any further increase in organic loading, the removal efficiencies will be compensated. That will mean the discharge to the public sewer having a high risk of exceeding its discharge limits.

In the fifth organic loading stage of 13.0 g COD/L.d, both R1 and R2 failed. COD removal efficiency in R1 dropped slightly and maintained for about 13 days before deteriorating signs started to surface. This might be due to the accumulation of VFAs which inhibited the system. As shown in Figure 4.18, VFAs in R1 started to shoot up after this increase in organic loading. For R2, COD removal efficiency started to fall each day after the organic loading had increased to 13.0 g COD/L.d. But after 7 days, the COD removal efficiency plunged to 31% and failed. This might be due to the massive wash-outs occurred after the increase in organic loading which is shown previously in Figure 4.17.

The massive loss of biomass might be the cause of the failure of R2. VFAs concentration after this increase of organic loading showed no sign of inhibition as the concentration remained low after the plunge in COD removal efficiency.

In all cases, COD removal efficiencies were inevitably reduced when OLR was just increased. A sudden increase in OLR caused shock to the biomass in the reactors. As the HRT was decreased with the increase of OLR, higher upflow velocity had caused some biomass to be washed out. With the combination of these factors, COD removal efficiency was affected and dropped. Another factor which contributed to the shock of the biomass was the fluctuation of the COD concentration in the industrial food wastewater. An example was that the influent COD concentration rose to 3642 mg/L just when R1 increased its OLR to 7.5 g COD/L.d. This had caused a great organic shock to R1 as the actual OLR shot to 12.6 g COD/L.d on that particular day resulting in consistently low COD removal efficiencies which required a longer time for it to recover.

Figure 4.19: COD concentration of R1

Figure 4.20: COD removal efficiency of R1

Figure 4.21: COD concentration of R2

Figure 4.22: COD removal efficiency of R2

4.2.4 Effects of stressed-loadings on granulation

4.2.4.1 Effects of stressed-loadings on granule formation and particle size

Particle size is one of the most important parameters to characterize the progression of sludge granulation. Figure 4.23 shows the results of the particles size development in both reactors.

Granulation is defined here as biomass of diameter larger than 0.5 mm. In the present study, granulation was deemed to begin when biological solids having diameter larger than 0.5 mm were measured (Lettinga et al, 1987). Granule samples for testing were obtained from sampling ports 15 cm from the bottom of the reactors. Granules were first observed in R2 on the 36th day of operation. In the control reactor, R1, granules larger than 0.5 mm were only found after the 48th day of operation. Granulation in both reactors took place in the same OLR of 5.2

g COD/L.d. But in terms of time taken, the stressed reactor R2 had taken 12 less days (25%) to form granules of mean diameter of 0.5 mm.

Though granulation was able to take place earlier in the stressed reactor R2, the mean diameter of the granules had peaked at 1.95 mm on the 70th day of operation and started to decrease gradually. In the control reactor R1, the granules were formed later, but the mean diameter of the granules managed to peak at 2.76 mm on the 176th day of operation. The mean diameter of the granules in R2 peaked when HRT was at 5 hr or OLR was at 10.4 g COD/L.d, while for the control reactor R1, its mean diameter of the granules peaked when HRT was at 4 hr or OLR was 13.0 g COD/L.d and it still showed no signs of decreasing even till the reactor failed.

From this observation, R2 showed that stress-loading could accelerate the forming of granules but it restricted the growth in size at HRT of 5 hr. The control reactor R1 had out-performed the stressed reactor R2 in terms of granule size after 112 days when R1 was at HRT of 7 hr or OLR of 7.5 g COD/L.d while R2 was at HRT of 5 hr or OLR of 10.4 g COD/L.d. The additional selection pressure applied on R2 has shown that it formed granules faster, but it had also restricted the peak size of the granules. It might be due to additional shear pressure applied on the granules which forbid further growth in size as the upflow velocity increased along with the increase of organic loading. The size of granules in R2 even decreased as the abrasive force increased with the organic loading.

Figure 4.23: Mean granule diameter

4.2.4.2 Effect of stressed-loading on biomass retained

The biomass retained in the reactor was indicated by the concentration of suspended solids in the system. The suspended solids in both reactors shown in Figure 4.24, indicated a growth of biomass under high HRT of 20, 10 and 7 hr or low OLRs of 2.6, 5.2 and 7.5 g COD/L.d. Suspended solids in R1 increased from 24.45 g/L to 31.10 g/L while suspended solids in R2 increased to 31.35 g/L. The increase of the suspended solid concentration was likely due to enhanced bacterial growth under moderately increased OLRs and that biomass growth rate exceeded the rate of wash-out. R2 showed a higher growth compared to R1 over the first three OLRs. Suspended solids in R2 started to decrease at the HRT of 5 hr or OLR of 10.4 g COD/L.d. This was probably due to the high upflow velocity under this OLR causing the wash-out rates to exceed the growth rate of the biomass which had a smaller mean diameter. Suspended solids in R1 continued to increase to 32.50 g/L at HRT of 5 hr or OLR of 10.4 g COD/L.d. It might be due to the larger in size of the granules in R1 which better withstood the high upflow velocity that allowed the wash-out rate to minimize.

Excessive levels of suspended solids in the reactor inhibit granulation process (Lettinga et al, 1980), impair the methanogenic activity of the sludge (Sayed, 1987) and may cause a sudden washout of the sludge bed (Lettinga et al, 1980). However, none of these adverse effects was observed in the operation of R1 and R2.

Figure 4.24: Suspended solids in reactors

4.2.4.3 Sludge volume index (SVI)

SVI is an important parameter indicating sludge settleability. A reduction in SVI value indicates improved settleability of sludge. Figure 4.25 shows the SVI values of biomass of both R1 and R2. SVI values of R2 fell from 65.7 to 49.7 mL/g SS which was 24% drop, while SVI values of R1 fell by 43% from 65.7 to 37.5 mL/g SS when the HRT decreased from 20 to 5 hr or the OLR increased from 2.6 to 10.4 g COD/L.d. Both SVI values of the biomass in R1 and R2 decreased as the OLR increased indicating that settleability was improved over the increased of OLR. From the SVI values, R1 was able to withstand higher hydraulic upflow velocity in terms of biomass wash-out than R2.

As granules form, the upflow velocity and the shear pressure applied on the granules cause them to coagulate due to the abrasive force between the granules which therefore resulting in the improved settleability. SVI value of the biomass of the stressed reactor though decreased, but it did not decrease as much as the control reactor, R1. It might be the additional abrasive force that caused less dense sludge or granules to be formed resulting in lower settleability of the sludge.

Figure 4.25: SVI values

4.2.4.4 Specific methanogenic activity (SMA)

High methanogenic activity is one of the characteristics of granular sludge. SMA allows one to understand the removal rate per unit of VSS per day in an UASB reactor operation. This helps to estimate the amount of active biomass in the reactor corresponding to the actual COD removal or vice versa. The methanogenic activity of the biomass was measured at every loading before moving to the next higher loading, which meant steady-state for R1 and COD removal efficiency of 75% for R2. The SMA results are shown in Figure 4.26. In this study, all the SMA results were in the range of 0.26 ~0.66 L CH₄/g VSS.d.

The results were in agreement with the values reported by Schmidt and Ahring (1996) in the range of 0.20 ~ 0.79 L CH₄/g VSS.d.

The SMA of both reactors increased rapidly immediately after start-up. The rapid increase of the SMA may have resulted from both rapid multiplication of bacteria and increase of enzymatic activity of the cells due to bacteria adaptation to the environment. SMA in R2 was higher in R1 in each OLR from 2.6 to 10.4 g COD/L.d. This was likely due to the fact that stress-loading promoted bacterial activity in the reactor. Though granules in R2 had shown smaller in size when both R1 and R2 were at OLR of 10.4 g COD/L.d, they had higher SMA than R1. From OLR of 2.6 to 10.4 g COD/L.d, SMA values of the granules from control reactor increased from 0.26 to 0.62 L CH₄/g VSS.d over the OLR of 2.6 to 10.4 g COD/L.d while that of stressed reactor increased from 0.26 to 0.66 L CH₄/g VSS.d. Higher permeability of the granules of smaller size aids in the bacterial activity. As the size of granules increases, substrate might not be able to permeate through the granule which resulted in dead cell in the core of the granules. Therefore, with the same amount of biomass, smaller granules might show higher activity rate due to higher permeability and contact surface area. But as the reactors failed at OLR of 13.0 g COD/L.d, the SMA of both reactors fell.

Figure 4.26: Specific methanogenic activity

4.2.4.5 Granule strength

The granule strength is expressed in terms of the integrity coefficient (%), which is defined as the granule resistance against disintegration during operation. The integrity coefficients for granules cultivated under different organic loading rates are depicted in Figure 4.27. Granule strength was measured when the biomass reached a size of 0.5 mm in diameter which occurred after the HRT reaching 10 hr or the OLR of 5.2 g COD/L.d. For all OLRs, granules in R1 showed a higher and more stable granule strength of above 80% than granules in R2. Granule strength in R2 a demonstrated decreasing trend as the OLR increased. The stressed loading might had caused a fluffier granule to form, thus the strength of the granule decreased from 76.4 to 69.6% as the OLR increased from 5.2 to 10.4 g COD/L.d. At OLR 13 g COD/L.d when both reactors failed, granule strength of R1 and R2 fell sharply to 66.7% and 45.2% respectively. As the decrease in strength in R2 granules and the increase in strength in R1 granules throughout the operation were not significant, the difference in strength between the granules developed in R1 and R2 was not substantial enough to observe the effects of additional loading stress.

Figure 4.27: Granule strength

4.2.4.6 Microbial morphology

Figures 4.28a~e present the scanning electron microscope (SEM) micrographs of the seed sludge, a typical granule, granule of R1 at OLR of 10.4 g COD/L.d, and granule of R2 at OLR of 10.4 g COD/L.d.

From the micrograph of the seed sludge (Figure 4.28a), it shows diversified types of morphology range from coccus and sarcina to filamentous which could be methanococcus, methanosacina and methanosaete respectively. This proved that the digested sludge chosen as seed has a diverse microorganism ecosystem which is suitable to treat different kinds of wastewater. Figure 4.28b shows a typical spherical granule in UASB which were formed in both R1 and R2.

From Figures 4.28c~f, both R1 and R2 consisted mainly of Methanosaete identified with filamentous type. It had been reported that filamentous Methanosaete spp. plays an important role in the formation of granules (Hulshoff Pol et al., 1983). Filamentous granules were found to be predominant in R1 and R2, but filamentous in R1 seemed to be more closely packed than R2. This might

explain the higher strength and better settleability of the granules of R1 than R2. Apparently *Methanosaeta* spp. played an important role in granule strength by forming a network which might stabilize the overall structure of the granule (Hulshoff Pol et al., 1983). This is further substantiated by Yan (1996) who observed that UASB reactors with more *Methanosaeta* were able to withstand variations in operating parameters, both physically and biologically.

From the images of the granules in Figures 4.28g and 4.28h, granules formed under stressed loading were more loosely packed with filamentous methanosaeta which results in the poorer settleability and strength. The loosely packed structure might have caused the granules to have better contact and permeability with the wastewater which results in the higher SMA.

Figure 4.28a: Micrograph of seed sludge

Figure 4.28b: Micrograph of typical granule

Figure 4.28c: Micrograph of R1

Figure 4.28d: Micrograph of R1 (enlarged)

Figure 4.28e: Micrograph of R2

Figure 4.28f: Micrograph of R2 (enlarged)

Figure 4.28g:

Enlarged image of granule from R2

Figure 4.28h:

Enlarged image of granule from R1

4.3 EFFECTS OF STARVATION ON GRANULE PROPERTIES AND RESTART-UP.

The effects of starvation on removal efficiency, granule properties and restart-up duration are to be understood as short term starvation is common, for example; treatment process is shut down for maintenance, long holiday break when factories are not operating and so on. Three different durations of starvation period of 5, 10 and 15 days were tested on reactors R5, R10 and R15 respectively. Starvation period was chosen to illustrate the short maintenance period of most wastewater treatment facilities in industry. Some factories do operate seasonally, but most operate all year round except for one to two weeks shut down for

maintenance. The study aimed at examining the effects on the granule properties and restart-up duration which could be useful to most industries.

4.3.1 Gas production and composition

During starvation, biogas was observed to be produced by the reactors at a very low rate. As soon as the reactors underwent starvation, gas production decreased rapidly. Within 48 hours, the gas production dropped to a very low amount of less than 0.5 L/d from about 13 to 15 L/d.

For R5, gas production almost resumed immediately when the wastewater was fed after 5 days (Figure 4.29). Hourly gas production was measured and it was used to project the daily gas production as a comparison to the measurements before starvation. Within 5 hours, the projected daily gas production had risen to 16.6 L/d, higher than the period before starvation. The projected daily gas production stabilized over the next day and gas production returned to its normal rate from 13 to 15 L/d.

Figure 4.29: R5 Cumulative gas production and projected daily gas production

For R10, gas production increased immediately when wastewater was fed (Figure 4.30). Gas production of R10 responded slightly slower than R5. At the 6th hour after operation was resumed, its projected daily gas production only rose to about 8.3 L/d, but had risen to 16.9 and 18.4 L/d at the 22nd and 46th hour. Similar to R5, R10 stabilized itself after about 3 days and resumed its normal gas production rate of 13 to 15 L/d.

Figure 4.30: R10 Cumulative gas production and projected daily gas production

For R15, though gas production increased immediately after wastewater was fed, gas production was the slowest to pick up (Figure 4.31). Gas production in R15 also followed similar trend as R5 and R10, the projected daily gas production had peaked at the 53rd hour with 19.7 L/d, but stabilized at 13 to 15 L/d after 5 days.

Figure 4.31: R15 Cumulative gas production and projected daily gas production

It was observed that when the biomass in the reactor experienced a sudden drop in organic loading, the biomass in the reactors would continue to consume on the remaining organic matters available in the reactors. As there was a sudden reduction of organic matters, gas production decreased sharply within 48 hrs until insignificant gas was produced.

For R5, the biomass experienced a starvation state in the next 72 hrs. Gas production recovered within an hour when the reactor resumed to a normal loading. The projected daily gas production increased sharply just hours after loading and returned to its usual daily gas production after about 24 hrs. It has shown that the biomass in the reactor going through starvation consumed organics at a higher rate as indicated by an increase in activity indicated by SMA test when the organic loading was resumed. Another possible contributing factor to the sudden increase of gas production is that, during the starvation, gas produced by the biomass was trapped below the sludge blanket until the substrate was fed

again, and the hydraulic action due to the feed flow released the trapped gas, generating a false phenomenon of increased gas production.

For R10 and R15, the biomass in the reactors experienced longer periods of starvation which might have led to inactivation of the biomass. When wastewater feeding resumed after 10 days and 15 days, gas production took a longer time than R5 to recover. The gas production was slower than what experienced in R5 initially, but it resumed to higher peaks. R15 took the longest time of 53 hrs, to reach its peak while R10 took about 46 hrs.

In conclusion, based on the gas production of the biomass, a short period of starvation of less than 15 days, would not have detrimental effects on the performance of the reactor system. The restart period could be well within two hours under a starvation of 5 days or less, and within about 2 to 3 days under a starvation period of 15 days or less.

4.3.2 COD removal efficiency

Before the 3 reactors, R5, R10 and R15, were starved, they were at steady-state with COD removal efficiencies of 76~79%, 77~79% and 80~83% respectively. The COD removal of the 3 reactors is shown in Figures 4.32, 4.33 and 4.34.

Upon the re-start of the reactors, the COD removal efficiency for R5 reached as high as 82.9% on the following day. It even reached 88.2% COD removal on the second day and slowly fell to its original range before starvation of 76~79% on the 6th day.

Figure 4.32: COD removal of R5

For R10, COD removal efficiency also reached a high removal efficiency of 82.7% on the first day after re-start of the reactor. It continued to increase until it peaked on the third day with 89.2% COD removal. Then it returned to its original range of values of 77~79% on the 7th day.

Figure 4.33: COD removal of R10

For R15, COD removal efficiency did not respond as quickly as R5 and R10. COD removal only reached 75% on the first day after re-start of the reactor. But the COD removal efficiency continued to improve and peaked on the 4th day with 90% COD removal. It returned to its original range 80~83% of COD removal efficiency on the 10th day.

Figure 4.34: COD removal of R15

The increase of COD removal when wastewater feeding was just resumed might be due to the demand of substrate by the biomass also demonstrated in the increased SMA values. The COD removal efficiencies had shown that starvation periods of 5 and 10 days required less than 1 day to resume their previous COD removal performance while for starvation of 15 days, the reactor required 3 days to attain its previous COD removal performance. They even performed better for the first few days and returned to their original ranges of performance. These results have indicated that the treatment can resume its efficiency within a single day for starvation period of 5 and 10 days and after 3 days for starvation period of 15 days.

4.3.3 VFAs concentration

Results of VFAs concentrations before and after the starvation are shown in Table 4.4. The low VFA concentrations before the starvation were comparable to Figure 4.17 instead of Figure 4.18 as these reactors are started-up using the conventional start-up strategy. The table shows that the VFA concentration of the reactors dropped significantly to 7.4, 2.6 and 1.9 mg/L for R5, R10 and R15 respectively. As VFAs are the intermediates of the conversion of organic compounds to methane in anaerobic digestion, no input of wastewater into the reactor and the continuous conversion of VFAs to biogas had caused the VFAs in the reactor to drop. It means that microbial processes in anaerobic digestion like acidogenesis, acetogenesis, homoacetogenesis and methanogenesis were limited by the reduction of organics supplied from the wastewater.

Table 4.4: VFA concentrations before and after starvation

	VFA before starvation (mg/L)	VFA after starvation (mg/L)
R5	35.0	7.4
R10	27.0	2.6
R15	19.9	1.9

4.3.4 VSS concentration

VSS can be used as an indication of the biomass concentration. Table 4.5 shows that the VSS concentration of the 3 reactors has no significant changes over the starvation periods. VSS concentration after the starvation remained a range from 29 to 32 g/L. This might be due to the lack of food for biomass growth as well as the starvation periods are too short to see any decrease in biomass.

Table 4.5: VSS concentrations before and after starvation

	VSS before starvation (g/L)	VSS after starvation (g/L)
R5	30.7	29.4
R10	31.7	31.3
R15	29.9	30.4

4.3.5 Granule size

Granule size before and after the starvation period was studied. Results from Table 4.6 have shown that there was no significant effect on the size of granules over the 3 starvation periods. As the granules were stagnant inside the reactor during the starvation period, there was no physical force to cause a change in the size of granules, nor there was much growth with the limited food, mean granule diameter remained with no significant changes.

Table 4.6: Granule size before and after starvation

	Mean diameter before starvation (mm)	Mean diameter after starvation (mm)
R5	2.109	1.976
R10	1.984	1.957
R15	2.144	2.076

4.3.6 SVI

From the results shown in Table 4.7, there is no significant effect on SVI by three durations of starvation. As settleability depends very much on granules permeability, shape and density, starvation does not alter any of these properties of the granules, especially for only 15 days.

Table 4.7: SVI before and after starvation

	SVI before starvation	SVI after starvation
R5	39.5	39.9
R10	38.8	38.7
R15	38.9	39.6

4.3.7 SMA

SMA was tested before the starvation, right after the starvation periods and 15 days after re-start when the reactors had stabilized. Biomass of the reactors, R5, R10 and R15, showed tremendous increase in SMA after the starvation periods.

Table 4.8 shows that the increase in SMA values just after the starvation corresponded with the starvation period. But soon after the reactors stabilized, SMA returns to its original values before starvation. This increase in SMA explains the initial improvement of the gas production and the COD removal efficiency which were discussed earlier. When the biomass was starved, methanogenic activity was suppressed. In R5, R10 and R15, methanogenic activity resumed responsively once wastewater was fed. The SMA revived to higher values once contacted with 'food'. The increased biomass activity was likely due to availability of energy from the food supply.

Table 4.8: Effects on SMA subjected to starvation

	SMA before starvation (L CH₄/g VSS.d)	SMA just after starvation (L CH₄/g VSS.d)	SMA after starvation (L CH₄/g VSS.d)
R5	0.57	0.70	0.59
R10	0.55	0.74	0.56
R15	0.56	0.75	0.58

4.3.8 Granule strength

Granule strength test prior and after the starvation was conducted. The results in Table 4.9 show that the starvation has some effects on the granule strength. R5 and R10 did not show significant fall in granule strength, but R15 showed a drop 12.1%. It showed that starvation of more than 10 days was detrimental to granule strength and the longer the starvation, the higher the fall in strength.

Table 4.9: Granule strength before and after starvation

	Granule strength before starvation (%)	Granule strength after starvation (%)
R5	80.1	79.6
R10	85.3	79.5
R15	87.4	75.3

CHAPTER 5

CONCLUSION

Wastewater characterization

The industrial food wastewater was characterized using various standard methods. Characterization results are as follow:

- The wastewater's TSS concentration ranged from 196 ~ 1247 mg/L could be reduced to less than 400 mg/L by a primary settling tank of hydraulic retention time of 45 minutes.
- The wastewater has a range of BOD₅ and COD values of 1027 ~ 4262 mg/L and 1961 ~ 4060 mg/L respectively. It has an average BOD₅ value of 2134 mg/L and an average COD of 3063 mg/L.
- Both COD and BOD₅ concentrations had been reduced by 29% and 24% respectively by the primary settling tank.
- The pH of the wastewater was adjusted from 4.94 ~ 5.62 to between 6.8 ~ 7.2 by dosing of NaHCO₃, which at the same time increased the alkalinity of the wastewater from 45 ~ 95 mg CaCO₃/L to about 2000 ~ 2500 mg CaCO₃/L.
- With the knowledge of the flow rate and pattern, as well as the average COD concentration of the primary settling tank effluent, an estimate of the design organic loading rate for the UASB could be arrived.
- Other parameters like high temperature of 29.5 ~ 32.7°C, presence of nutrients like nitrogen and phosphorus and absence of inhibitory elements had contributed to the successful operation of the UASB process.

Effects of conventional and stressed-loading methods on granulation and start-up.

- Start-up and loading capacity

Based on the experimental results obtained, start-up durations of the stressed reactor for operation from HRT at 20 to 5 hr were able to be shortened by 11 to 57 days corresponding to 30% and 48% start-up shortening compared to the control reactor. Both the control and the stressed reactors had reached an ultimate loading capacity of 10.4 g COD/L.d. This result indicates that the start-up of reactors treating industrial food wastewater could be significantly accelerated under stressed loading conditions. The results also show that applying stress in starting-up of the reactors does not reduce the reactor loading capacity.

- Granule development

Under stress-loading conditions, the sludge particles began to form granules earlier as granules were observed after 36 days of operation in the stressed reactor, R2, while granules were only observed in control reactor, R1, after 48 days of operation. This demonstrated that development of granule could be accelerated with the unconventional approach of stress-loading by 33%. Though stress-loading developed granules faster, it restricted the growth of size which peaked at 1.95 mm occurring at HRT of 7 hr, while granules of R1 grew up to the size of 2.76 mm occurring at the HRT of 5 hr.

- Granule characteristics

With the results obtained, stress-loading showed benefits on the acceleration of start-up and granulation which compromised slightly on certain granules properties like settleability and strength but was able to increase the microbial activity of the biomass.

The SVI values of the granules from the stressed reactor for all 4 loading stages of HRT of 20, 10, 7 and 5 hr were higher than that of the control reactor. The SVI values of granules of the stressed reactors fell by 24% from 65.7 mL/g SS to 49.7 mL/g SS while that of the control reactor fell by 43% from 65.7 mL/g SS to 37.5 mL/g SS.

The strength of the granules of the control reactor remained consistently above 80% while that of the stressed reactor fell from 76.4% to 69.6% when the OLR increased from 5.2 to 10.4 g COD/L.d. This result shows that the stress applied during start-up has detrimental effects on granule strength.

Granules of the stressed reactor showed superiority over granules of the control reactor in their SMA over all the organic loadings. SMA of granules of control and stressed reactors increased tremendously from 0.26 to 0.49 and 0.53 L CH₄/g VSS.d respectively. From OLR of 2.6 to 10.4 L CH₄/g VSS.d, SMA values of the granules from control reactor increased from 0.26 to 0.62 L CH₄/g VSS.d over the OLR of 2.6 to 10.4 g COD/L.d while that of stressed reactor increased from 0.26 to 0.66 L CH₄/g VSS.d.

The study concluded that stressed-loading was able to accelerate the start-up of UASB reactor by up to 48% saving in time for an OLR of up to 10.4 g COD/L.d. This operating approach is significant for industrial application as saving of reactor startup time could result in saving of cost. However, the stressed loading start-up strategy may affect the development of granules. The granules in the stressed loading reactor were able to develop faster initially, but the granules were unable to develop to the size as those in the conventional loading reactor. Despite this, the stressed reactor maintained its performance with higher activity rate of the granules.

The failure of R2 was highly due to the loss of biomass after the OLR increased to 13.0 g COD/L.d shown by a high washout of suspended solids for a week. The COD removal performance of R2 fell sharply after being operated for 8 days at

13.0 g COD/L.d. The failure of R1 was not mainly due to the loss of biomass. The washout was not significant as indicated by the effluent suspended solids. The COD removal was stable until it started to deteriorate after about 12 days of operation after OLR increased to 13.0 g COD/L.d. The VFA concentrations began to accumulate which was a sign of organics overloading.

Therefore, it can be concluded that stressed loading strategy is able to shorten the start-up duration which could be time and cost-saving. It is also able to develop granules faster and have high biomass activity rate than the conventional strategy. However, granules formed under the stressed loading strategy are smaller in size which could cause the reactor to fail by biomass washout, while the reactor operated under normal conditions to fail by organics overloading.

Effects of starvation on granule properties and restart-up

As there are times when plants are shut down for maintenance, effects on the granules and restart-up of three starvation periods were studied.

- **Restart-up performance**

During starvation, biogas production decreased immediately and sharply to values less than 0.1 L/d once wastewater feeding stopped. Upon restart, gas production picked up immediately. The gas composition remained as normal with methane content stayed within 60 ~ 70%.

The COD removal of reactors, which were starved for 5 days and 10 days respectively, resumed to their previous efficiencies within a day of operation and peaked on the second and third day with 88.2 and 89.2% respectively. The COD removal of both reactors then gradually returned to their original efficiencies of 76 ~ 79% and 77 ~ 79% on the 6th and 7th day respectively. For reactor which was starved for 15 days, COD removal picked up slightly slower. One day after the restart, the COD removal efficiency was only 75% compared to its original

efficiency of 80 ~ 83% before starvation. Unlike the other 2 reactors, which were starved for 5 and 10 days respectively, the reactor which was starved for 15 days only achieved above its original removal efficiencies after 3 days with 90% which was also the peak after restart.

- Granule properties

Granules properties like VSS, mean granule diameter, and SVI did not change significantly over the three starvation periods. Whereas SMA of the granules from reactors which were starved for 5, 10 and 15 days increased tremendously to 0.70, 0.74 and 0.75 L CH₄/g VSS.d respectively. The difference between the three temporarily increased SMA values was not significant. But the increases in SMA of the three reactors were temporary as they dropped to their original values of 0.59, 0.56 and 0.58 L CH₄/g VSS.d respectively after restart for 15 days. Granule strength of the reactors which were starved for 5 and 10 days showed 1% and 6% deterioration over the starvation periods 5 and 10 days respectively, while deterioration of granule strength of reactors which was starved for 15 days decreased by 12%.

Based on the experimental results, it can be concluded that stressed-loading was able to accelerate the start-up time and granule development significantly. However, granules in the stressed reactor became more active as measured by the improved specific methanogenic activity results although the size and strength of the granules decreased.

It can also be concluded that reactors which were starved for 5, 10 and 15 days responded rapidly to restart and encountered essentially no significant loss in COD performance, gas production and granule properties. Granule properties were generally maintained during the three starvation periods, and this could explain the ability of the reactors to re-start responsively. From the study, starvation period of up to 15 days indicated no significant harmful effects on the reactors.

It is known that slow start-up is one of the major drawbacks of the upflow anaerobic sludge blanket reactor. However, from this study, the acceleration of start-up by stressed-loading method has made the already established upflow anaerobic sludge blanket reactor more attractive as time would be saved up to 48%. In addition, the upflow anaerobic sludge blanket reactors can be shut down up to 15 days and restarted spontaneously without significant harmful effects on the reactors. This could not be done with either conventional digesters or anaerobic activated sludge processes, which requires a somewhat continuous feed for satisfactory operation (Young, 1980). This is extremely useful for treatment plants which require to be shut down occasionally either due to maintenance or emergency.

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