ANAEROBIC SEQUENCING BATCH REACTOR
FOR THE TREATMENT OF MUNICIPAL WASTEWATER

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Summary

An anaerobic sequencing batch reactor (AnSBR) was investigated for the treatment of municipal wastewater from a local water reclamation plant.

The study showed that for start-up, the AnSBR required 110d to achieve stable performance at a HRT of 16h compared to only 70d at HRT of 8h. The biomass retention capacity at a start-up HRT of 16h (6,576 mg MLSS/L) was lower than that of 8h (6,933 mg MLSS/L). On the other hand, the TSS (HRT of 16h, 8h - 86%, 57%), VSS (86%, 59%) and tCOD (73%, 45%) removal efficiencies at HRT of 16h were also higher than those of 8h. However, the sCOD removal efficiency was lower at a HRT of 16h (3.6%) than that observed at HRT of 8h (37%) due to the slow growth rate of fermentors and methanogens. The average biogas yield was only 0.97 L/d at a HRT of 16h but 1.7 L/d at a HRT of 8h. The amount of methane gas in the biogas was similar for both HRTs. At 16h, it was 60% and at 8h, it was 62%.

The AnSBR was operated at 3 different HRTs (16, 8 and 6h) and their performances were evaluated. The results showed that the AnSBR was able to retain the largest amount of solids at the HRT of 8h (8,732 mg MLSS/L) because it had a shorter react phase than the HRT of 16h (6,772 mg MLSS/L) and its decant point was higher than that of HRT of 6h (5,873 mg MLSS/L). Meanwhile, a higher HRT led to a higher TSS (HRT of 16h, 8h, 6h – 85%, 60%, 28%), VSS (82%, 70%, 33%), tCOD (74%, 51%, 21%) and sCOD (48%, 47%, 43%) removal efficiencies. The tBOD₅ removal efficiencies were similar at the HRT of 16h and 8h (78%, 82%) but that of 6h was very low (-14%). The sBOD₅ removal efficiency was the lowest (37%) at a HRT of 16h because the growth yield of the fermentors and methanogens were affected by the low organic loading rate. The sBOD₅ removal efficiency was higher at the HRT of 8h (54%) than 6h (47%), which showed that operating the AnSBR at too low a HRT would adversely affect the
performance of the AnSBR. It took nearly 80d for the biogas to reach the maximum 60% methane when operating at a HRT of 16h but only 55d when operating at the HRT of 8 and 6h. Furthermore, at the HRT of 8 and 6h, the maximum methane percentage could reach 70%. Thus, a shorter HRT enabled the reactor to achieve the same quality of biogas in a shorter time and to achieve a biogas with a higher methane percentage. T-RFLP fingerprinting was used to study the microbial community structure in the AnSBR. A change in HRT did not result in significant changes in the bacteria population but there was a distinct shift in the archaea population.

Powdered activated carbon (PAC) was successful in enhancing the performance of the AnSBR. A dosage of 10, 15 and 20% (w/w) was added in the AnSBR operating at HRT of 6h and it was found that there was a large improvement in the suspended solids and organics removal efficiency, amount of methane produced, as well as the consistency of removal efficiency.

The sludge wasted from the AnSBR had a volatile solids reduction of 5.1% when operating at a HRT of 16h and 8.5% to 9% when operating at a HRT of 8 and 6h, with and without PAC. These values met the international standard for assessing sludge biostability which meant that no further treatment was needed before the disposal of the sludge. Microscopic image analysis found that there was a slight increase in the biofloc sizes with increasing organic loading rate, while the addition of PAC in the AnSBR led to a significant increase in the biofloc sizes.

The apparent molecular weight (AMW) distribution of the feed and effluent of the AnSBR showed a bimodal distribution with AMW of greater than 100 kDa and less than 1 kDa. The amount of high-MW fractions (>100 kDa) was higher when operated at a longer HRT. The data also showed that PAC was more successful in removing the high-MW fractions.
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<td>AMW</td>
<td>apparent molecular weight</td>
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<td>AnSBR</td>
<td>anaerobic sequencing batch reactor</td>
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<td>ATP</td>
<td>adenosine tri-phosphate</td>
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<td>AVSR</td>
<td>additional volatile solids reduction</td>
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<td>BOD, BOD$_5$</td>
<td>5-day biochemical oxygen demand</td>
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<td>bp</td>
<td>base pair</td>
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<td>CAS</td>
<td>conventional activated sludge</td>
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<td>COD</td>
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<td>continuous stirred tank reactor</td>
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<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<td>EGSB</td>
<td>expanded granular sludge bed</td>
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<td>emf</td>
<td>electromotive force</td>
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<td>F/M</td>
<td>Food to microorganism</td>
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<td>FVSR</td>
<td>fraction volatile solids reduction</td>
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<td>gel permeation chromatography</td>
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<td>HRT</td>
<td>hydraulic retention time</td>
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<td>kd</td>
<td>decay constant</td>
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<td>kd$_H$</td>
<td>decay constant of heterotrophs</td>
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<td>Kj-N</td>
<td>kjeldahl nitrogen</td>
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<td>Ks</td>
<td>half saturation coefficient</td>
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<td>MBR</td>
<td>membrane bioreactor</td>
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<td>mixed liquor suspended solids</td>
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<td>mixed liquor volatile suspended solids</td>
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<td>MPSA</td>
<td>staged multi-phase anaerobic</td>
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<td>MW</td>
<td>molecular weight</td>
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<td>soluble microbial product</td>
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<td>TSS</td>
<td>total suspended solids</td>
</tr>
<tr>
<td>UASB</td>
<td>upflow anaerobic sludge blanket</td>
</tr>
<tr>
<td>UF</td>
<td>ultrafiltration</td>
</tr>
<tr>
<td>VAR</td>
<td>vector attraction reduction</td>
</tr>
<tr>
<td>VFA</td>
<td>volatile fatty acid</td>
</tr>
<tr>
<td>VSR</td>
<td>volatile solid reduction</td>
</tr>
<tr>
<td>VSS</td>
<td>volatile suspended solids</td>
</tr>
<tr>
<td>WRP</td>
<td>water reclamation plant</td>
</tr>
<tr>
<td>Y</td>
<td>growth yield</td>
</tr>
<tr>
<td>Yₖ</td>
<td>growth yield of heterotrophs</td>
</tr>
<tr>
<td>μ</td>
<td>specific growth rate</td>
</tr>
<tr>
<td>μₘ</td>
<td>max specific growth rate</td>
</tr>
</tbody>
</table>
1.1 Background

There are two major crisis faced by nations worldwide, namely the water and energy crisis.

1.1.1 The Water Crisis

The water crisis is a global issue. Wastewater is generated and dispersed in large amounts such that one out of six people (1.1 billion) has no access to safe drinking water and two out of six people (2.6 billion) lack adequate sanitation (WHO and UNICEF, 2004).

Water is a universal solvent which makes it the most important fluid as well the most easily being contaminated. Although water can be found in a lot of places, only clean and unpolluted water are useful to us. Only 2.5% of the water in the world is freshwater and two-thirds of it is locked in icebergs and glaciers. Of what is left, 20% is in remote areas, and much of the rest is in the wrong place at the wrong time, such as floods and monsoons. As a result, only 0.08% of the water in the world is available for human usage.

Global water consumption rose six-fold between 1900 and 1995 - more than double the rate of population growth - and goes on growing as farming, industry and domestic demand increase. By the year 2020, the World Water Council predicted that 17% more water is needed. This water crisis arises due to 2 main reasons. The first reason is the increase in population (Figure 1.1). The
world population is projected to grow from 6 billion in 1999 to 9 billion by 2042, an increase of 50 percent in 43 years.

![World Population: 1950-2050]

**Figure 1.1 Increase in world population from 1950 to 2050.**

The second reason is a rise in living standards which results in a higher water usage and more water pollution. Water pollution may be due to industrial projects, agricultural runoffs etc.

Governments are becoming increasingly aware of this water shortage problem and are trying to find alternative water sources that will reduce their reliance on rainfall and surface water. For example, Singapore focuses on NEWater and desalinated water as its third and fourth national taps. However, the true solutions to such problems remain a question. Desalination makes sea water available but takes huge quantities of energy and leaves large amount of brine. Similarly, water reuse requires substantial energy.

### 1.1.2 The Energy Crisis

The energy crisis refers to the bottleneck or price increase in the supply of energy resources, including oil, electricity or other natural resources. This is a threat to the economy and can lead to declining economic growth, increasing inflation and rising unemployment.
The world is highly dependent on oil as a source of energy. However, oil depletion is a problem that is inevitable. Alternative sources of oil like tar sands, shale, coal-to-liquids, ethanol and hydrogen proved to be less than satisfactory, because they contribute to global warming and cannot be scaled up on a timely basis. In the meantime, it is speculated that it will take one nuclear power plant every week until 2050 to fill the oil gap. There will be a uranium shortage long before 2050 unless more efficient reactors are used. Solar energy seems to be a viable alternative but it is not always available in all places in sufficient amounts.

The biogas produced by anaerobic treatment of wastewater contains methane which is a hydrocarbon and energy-rich material also found in natural gas. While the amount of methane produced by a wastewater treatment plant may not be enough to replace oil as an energy source, it is certainly worthwhile to tap it to conserve the energy used in wastewater treatment. In addition, there may be excess to feedback this energy to the public.

A report from the US EPA in April 2005 revealed that worldwide methane from wastewater accounts for over 575 million metric tons of carbon dioxide equivalent in 2000. Wastewater is the fifth largest source of anthropogenic methane emissions, contributing approximately 10% of total global methane emissions in 2000. It is easy to imagine the large amount of energy that can be recovered if the methane gas is utilized appropriately. In view that most large-scale municipal wastewater treatment plants in developing and developed countries are aerobic systems right now, a larger amount of this methane gas can be recovered if anaerobic systems are adopted in the future.
1.1.3 Treatment of municipal wastewater

Wastewater is water that has been polluted due to anthropogenic activities. Types of wastewater include domestic, commercial, industrial and agricultural, categorized by their sources as well as type of contaminants and concentration. Municipal wastewater is a mixture of these different types of wastewater. Municipal wastewater has a number of constituents, including pathogens such as bacteria, virus and prions, non-pathogenic bacteria, organics such as faeces, hair, food and fibres, inorganics such as sand, etc.

Due to the imposition of stricter limits of wastewater discharges and the possibility of water reuse, there is a greater demand to treat wastewater efficiently. Many researches were done to design and optimize biological treatment processes. Techniques from the microbiological science, such as DNA fingerprinting, are used to identify the active mass in the biological treatment processes.

Till now, the conventional activated sludge system is the most common method of wastewater treatment for the removal of organics and suspended solids. The system can be designed to perform nutrient removal at the same time. Anaerobic systems have also been commonly used in many places for the treatment of industrial wastewater and sludge digestion.

The wastewater today is continuously changing in quality and quantity. There are also emerging health and environmental concerns, new industrial wastes and new regulations. In the meantime, old wastewater infrastructure needs to be repaired, replaced and its technology updated. Therefore, it is important that new technologies that are more efficient, convenient and environmentally-conscious.
1.2 Objectives

The objectives of this project are:

- To study the feasibility of using anaerobic treatment process to treat raw municipal wastewater obtained from a local water reclamation plant.

- To study the performance of the anaerobic treatment process in terms of effluent quality, suspended solids and organics removal efficiencies, biogas quality and quantity.

- To improve the quality of anaerobic treated effluent to reduce the capacity of aerobic post-treatment processes by powdered activated carbon.

- To study the effect of different operation parameters on the microbial population in the biomass.

1.3 Scope of Work

The project included the design and fabrication of the Anaerobic Sequencing Batch Reactor (AnSBR) system. The system was subjected to hydrotest to ensure construction satisfaction.

For the start-up study, the system was seeded and operated at two different hydraulic retention times (HRT) to determine the effect of organic loading rate on the start-up period required. Sampling was done two to three times per week. The samples, which included feed and effluent, were analyzed based on the following parameters:

- Total and volatile suspended solids
- Chemical oxygen demand
- Biochemical oxygen demand
- Total organic carbon
- Biogas composition
- Volatile organic acids
- Nitrogen and other anions

Operational parameters like pH and volume of biogas produced were also monitored daily. Other tests that were done periodically include molecular weight distribution of the biomass, for the feed and effluent samples and additional volatile solids reduction for the mixed liquor biomass. Biomass samples were also extracted for observation under a light microscope.

After the start-up study, the AnSBR systems were being operated at different HRTs to determine the optimum HRT. Operation parameters will not be changed until a “steady-state” is achieved. This “steady-state” represented the time when the treated effluent is consistent in quality and the volume and composition of the biogas is relatively constant. Similarly, samples were collected two to three times per week and analyzed based on the parameters stated above.

Powdered activated carbon (PAC) was used to improve the performance of the AnSBR system. It was added into the reactors at low HRTs, when the quality of the treated effluent deteriorated. Sampling and analyzes were done continuously.

To further understand the system, biomass were collected periodically for microbiological analysis. Terminal-Restriction Fragment Length Polymerization (T-RFLP) fingerprinting was used to monitor the dynamics of the microbial consortium in the system.
2.1 Anaerobic process for wastewater treatment

2.1.1 Anaerobic microorganisms and their roles

Anaerobic microorganisms are organisms whose respiratory energy is generated using electron acceptors other than oxygen. Some of the electron acceptors used in anaerobic respiration include ferric iron (Fe$^{3+}$), sulphate (SO$_4^{2-}$), carbonate (CO$_3^{2-}$) and certain organic compounds.

Figure 2.1 The electron tower (Madigan and Martinko, 2006).
Compared to the O₂/H₂O redox couple, these acceptors have a larger reduction potential. Due to the positions of these compounds on the electron tower (Figure 2.1), less energy is released when these electron acceptors are used instead of oxygen.

Consortia of microorganisms, mostly bacteria, are involved in the transformation of complex high-molecular-weight organic compounds to methane (equation 2.1).

\[ \text{Organic matter} \rightarrow \text{CH}_4 + \text{CO}_2 + \text{H}_2 + \text{NH}_3 + \text{H}_2\text{S} \quad (2.1) \]

Figure 2.2 shows the metabolic microbial groups involved in an anaerobic treatment of wastewater. Acetate, H₂ and CO₂ from primary fermentations can be directly converted to methane although H₂ and CO₂ can also be consumed by homoacetogens. This figure is true for environments in which sulfate-reducing bacteria play only a minor role, for example, wastewater treatment process.

Bacteria are the dominant microorganisms in an anaerobic treatment system. Large numbers are strict and facultative anaerobic bacteria (e.g. Bacteroides, Bifidobacterium, Clostridium, Lactobacillus, Streptococcus) which perform hydrolysis and fermentation of organic compounds. Microorganisms, including bacteria and archaea, can be categorized into the following four groups.
2.1.1.1 Hydrolytic bacteria

These are anaerobic bacteria which break down complex organic molecules (e.g. proteins, cellulose, lignin, lipids) into soluble monomer molecules such as amino acids, glucose, fatty acids and glycerol.

Eastman and Ferguson (1981) reported that the degradation of particulate organic matter and not the fermentation of the soluble hydrolysis products is rate limiting as they found no accumulation...
of hydrolysis products in their reactor. Hydrolysis reaction is also known to be relatively slow especially when there are high levels of cellulose and lignin in the wastewater.

2.1.1.2 **Fermentative bacteria**

Fermentation is an internally balanced oxidation-reduction process in which the fermentable substrate becomes both oxidized and reduced. To catabolize an organic compound, the fermentative bacteria should at the same time conserve some of the energy released as ATP.

![Diagram of fermentation process](image)

**Figure 2.3 Fermentation process.**

In Figure 2.3, ATP synthesis occurs as a result of substrate-level phosphorylation, which means, a phosphate group gets added to some intermediate in the biochemical pathway and eventually gets transferred to ADP to form ATP. The fermentative bacteria also have to dispose the electrons removed from the electron donor. This is done by the production and excretion of fermentation products generated from the original substrate.

Fermentative acidogenic bacteria refer to acid-forming bacteria (e.g. *Clostridium, Bacteroids, Peptostreptococcus, Eubacterim, and Lactobacillus*). They convert sugars, amino acids and fatty
Acids to organic acids (e.g. acetic, propionic, formic, lactic, butyric or succinic acids), alcohols and ketones (e.g. ethanol, methanol, glycerol, acetone), acetate, CO$_2$ and H$_2$.

2.1.1.3 Acetogenic & homoacetogenic bacteria

Acetogenic bacteria are acetate and hydrogen-producing bacteria which convert fatty acids (e.g. propionic acid and butyric acid) and alcohols into acetate, hydrogen and carbon dioxide. This group includes the syntrophs like *Syntrophomonas*, *Sytrphobacter* and *Acetobacter*.

Ethanol, propionic acid and butyric acid are converted to acetic acid by acetogenic bacteria via the reactions shown in Equation 2.2 to 2.4.

\[
\begin{align*}
\text{CH}_3\text{CH}_2\text{OH} + \text{H}_2\text{O} & \rightarrow \text{CH}_3\text{COOH} + 2 \text{H}_2 \quad (2.2) \\
\text{CH}_3\text{CH}_2\text{COOH} + \text{H}_2\text{O} & \rightarrow \text{CH}_3\text{COOH} + \text{CO}_2 + 3 \text{H}_2 \quad (2.3) \\
\text{CH}_3(\text{CH}_2)_2\text{COOH} + 2 \text{H}_2\text{O} & \rightarrow 2 \text{CH}_3\text{COOH} + 2 \text{H}_2 \quad (2.4)
\end{align*}
\]

The production of acetate or certain other fatty acids is energetically advantageous because it allows the organism to make ATP by substrate-level phosphorylation.

Homoacetogens are a group of strictly anaerobic prokaryotes which can, similar to methanogens, use CO$_2$ as an electron acceptor in energy metabolism. CO$_2$ is abundant in anaerobic environment because it is a major product of energy metabolism of chemoorganotrophs. Hydrogen is the major electron donor for both two types of microorganisms.
Homoacetogens are categorized together because of their pathway of CO\(_2\) reduction, i.e. the acetyl-CoA pathway. Acetyl-CoA pathway is not a cycle, it involves the reduction of CO\(_2\) via two linear pathways, one molecule of CO\(_2\) is reduced to the methyl group of acetate and the other is reduced to the carbonyl group. This is an overall energy-conserving reaction thus, homoacetogens can grow at the expense of it. However, additional energy-conserving steps occur because of a sodium motive force established across the cytoplasmic membrane during acetogenesis. This allows for further energy conservation.

### 2.1.1.4 Methanogens

Methanogens are a group of strictly anaerobic *Archaea* which carry out methanogenesis. Methanogenesis is a series of complex reactions which involve novel coenzymes. Similar to acetogenesis, methanogens use CO\(_2\) as the electron acceptor and hydrogen as a major electron donor. However there is a difference in free energy released (Figure 2.4).

![Figure 2.4 Difference between methanogenesis and acetogenesis.](image)

In anaerobic wastewater treatment systems, the methanogens are of specific concern because not only is methanogenesis the terminal step in the biodegradation of organic matter, methanogenesis
also produces methane gas which can be a source of energy. Methanogens show a variety of morphologies and several taxonomic orders were recognized, based on both phenotypic and phylogenetic analyses. Physiologically, methanogens are obligate anaerobes thus anaerobic treatment systems need to be strictly conditioned to culture the methanogens. Only a very few substrates can be used directly by methanogens, e.g. acetate, that is why methanogens must team up with partner organisms which can supply them with it - syntrophs.

Syntrophy is a situation where two different organisms degrade a substance, conserve energy doing it and that neither could degrade the substrates separately. A syntrophic reaction required the production of H$_2$ by one partner linked to H$_2$ consumption by the other, thus also called, interspecies H$_2$ transfer. Figure 2.5 shows the reactions involved in ethanol fermentation to methane and acetate by syntrophic association of an ethanol-oxidizing bacterium and a H$_2$-consuming partner bacterium - a methanogen. The fermenter carries out a reaction that has a positive standard free-energy change.

\[
\text{Ethanol fermentation:} \\
2 \text{CH}_3\text{CH}_2\text{OH} + 2 \text{H}_2\text{O} \rightarrow 4 \text{H}_2 + 2 \text{CH}_3\text{COO}^- + 2\text{H}^+ \\
\Delta G^\circ = + 19.4 \text{kJ/reaction}
\]

\[
\text{Methanogenesis:} \\
4 \text{H}_2 + \text{CO}_2 \rightarrow \text{CH}_4 + 2 \text{H}_2\text{O} \\
\Delta G^\circ = - 130.7 \text{kJ/reaction}
\]

\[
\text{Coupled reaction:} \\
2 \text{CH}_3\text{CH}_2\text{OH} + \text{CO}_2 \rightarrow \text{CH}_4 + 2 \text{CH}_3\text{COO}^- + 2\text{H}^+ \\
\Delta G^\circ = - 111.3 \text{kJ/reaction}
\]

Figure 2.5 Reactions involved in and nature of interspecies hydrogen transfer (Madigan and Martinko, 2006).
However, the H₂ produced by the fermenter can be used as an electron donor for methanogenesis by a methanogen. The overall reaction then becomes exergonic and supports the growth of both partners.

Thus with the right combination of microorganisms, any organic compounds can be converted into methane.

### 2.1.2 History of research and applications

As early as the beginning of the 20th century, there were researches conducted on anaerobic processes to treat wastes. The researches were mainly focused on the use of anaerobic treatment for digestion of sludge. Bach (1931) concluded that anaerobic treatment was applicable only to sludge digestion and not for liquid wastes. It was found that only 50% reduction of solids was possible for sludge digestion even with a long retention time, resulting in a loss of interest in anaerobic systems for wastewater.

Researchers in the early 1950s recognized the necessity to maintain a high biomass concentration for an anaerobic treatment system (Stander, 1950; Stander and Snyder, 1950; Schroepfer et al., 1955; Schroepfer and Zimke, 1959a, b). In 1953, Fullen proposed a treatment system known as anaerobic contact process which was successful.

McCarty (1964) wrote that it was a fallacy to believe anaerobic treatment as an inefficient process. Unsuccessful experience with anaerobic digestion was due more to the nature of the organic material which were not readily biodegradable than the process itself.
Subsequently, there had been a lot of development in anaerobic treatment processes, especially that of “high-rate” reactors that can achieve high solids retention. This increased the efficiency of anaerobic processes and made it possible for the treatment of liquid wastes. However, there is still a common perception that anaerobic processes are unable to achieve efficient organic removal when treating low-strength wastewater (COD less than 1 g/L).

In 1992, an anaerobic sequencing batch reactor with gas recirculation for mixing during the reaction phase was successfully used to treat medium-high strength (1.5 to 2 g COD/L) wastewater (Pfeiffer et al., 1986; Sung and Dague, 1992). However, it was unable to treat low-strength wastewater because the biogas produced is too low to provide adequate agitation. Ndon and Dague (1997) reported the performance of an anaerobic sequencing batch (ASBR) reactor treating low-strength wastewater at different temperature and hydraulic retention time (HRT). It was found that even at the lowest temperature of 15 °C, shortest HRT of 12h and lowest substrate concentration of 400 mg COD/L, the ASBR can achieve over 80% total COD removal. It seemed that anaerobic process for the treatment of low-strength wastewater is possible after all.

2.1.3 Advantages and disadvantages of anaerobic processes

In both developed and developing countries, the conventional wastewater treatment system usually consists of the conventional activated sludge process (CAS), which is an aerobic process. CAS process is energy intensive due to the high aeration requirement and it also produces large quantity of sludge (about 0.4 g dry weight/g COD removed) that has to be treated and disposed of. As a result, the cost of operation and maintenance of a CAS system is considerably high. It was estimated that the cost of aerobic treatment of wastewater is US$50 per inhabitant equivalent per year (Alaerts et al., 1989) while the cost of anaerobic treatment is half of it (Lens and Verstraete,
Anaerobic process thus becomes an attractive alternative for tropical or subtropical countries. The advantages of adopting anaerobic process for treatment include:

1. Biogas (methane, carbon dioxide or hydrogen) can be generated and tapped to recover energy.

2. Low production of biomass per unit of organics removed.

3. No aeration required.

4. Very high active biomass densities (1% to 3%) can be achieved under favorable conditions. This means that volumetric reaction times can be increased, reactor size decreased and the system’s resistance to shock loadings and toxic compounds can be strengthened.

5. Lower requirement for inorganic nutrients, e.g. nitrogen and phosphorus, due to lower biomass yields.

6. Anaerobic systems can be left dormant without feeding for extended periods without severe deterioration in biomass properties. This means that they can be brought back into service at normal treatment efficiency within very short period of time.

Despite the well-known advantages of anaerobic treatment, there are some disadvantages when compared to aerobic treatment.
1. Generally lower substrate removal rate per unit of biomass, typically 1/3 to 1/10 those of aerobic treatment of similar substrate. This is because anaerobic biodegradation of organics is usually incomplete, often leaving as much as 50% of the organic matter unconverted (Chynoweth, 1996).

2. Growth of anaerobic organisms is slow. Hence, anaerobic systems can fail if it is unable to retain its biomass. Low substrate removal rates and low biomass yields result in a significantly longer time for initial system start-up and recovery after an upset (1 to 6 months).

   However, it is also this characteristic that makes anaerobic system advantageous over aerobic systems. Low biomass yields lead to low sludge production rate which would reduce the cost of sludge disposal.

3. High operating temperature required for efficient performance. This limits the application of anaerobic treatment to tropical or sub-tropical regions

4. Under short hydraulic retention times, it is difficult to avoid accumulation of excessive residual organic matter and intermediate products such as volatile fatty acids, especially conventional continuous-flow suspended growth anaerobic reactors.

5. The chemically reduced conditions necessary for anaerobic process produce \( \text{H}_2\text{S} \), mercaptans, organic acids and aldehydes, which are corrosive and toxic to microorganisms in the system. Anaerobically-treated effluents usually still contain a
substantial amount of pathogens, particles, organic and inorganic compounds as well as ammonia, sulfide and phosphate.

6. Sensitive to certain inhibitory and toxic compounds, such as oxidants (O₂, H₂O₂, Cl₂), H₂S, HCN, SO₃⁻ and some aromatics.

7. Wilén et al. (2000) reported anaerobic conditions can cause deflocculation of biomass in the wastewater which only incurred initially in the case of aerobic conditions. This is of a major concern because the quality of effluent is highly dependent on the efficiency of the solid-liquid separation process. Eikelboom and van Buijsen (1983) explained that the growth of anaerobic or facultative anaerobic bacteria between the flocs or the dying of strictly aerobic organisms in the flocs is the cause of deflocculation. Starkey and Karr (1984) suggested that it was due to an inhibition of the eukaryote population or an inhibition of the production of extracellular polymers. Hydrolysis in the EPS matrix takes place under anaerobic conditions, causing the floc matrix to degrade (Rasmussen et al., 1994; Nielsen et al., 1996).

2.1.4 Common applications of anaerobic process

Anaerobic treatment systems were found in a widespread of applications, especially for industrial wastewaters like sugar beet, slaughter house, starch brewery wastewaters, piggery wastewaters etc. The loadings ranged from 1 to 50 kg COD/m³, the temperatures from 10 to 65 °C and HRT from a few hours to a few days (Metcalf and Eddy, 2003).

Lettinga et al. (1997) and Verstraete and Vandevivere (1999) reviewed the new generations of anaerobic treatment system, such as Upflow Anaerobic Sludge Blanket (UASB), Expanded
Granular Sludge Bed (EGSB) and Staged Multi-Phase Anaerobic (MPSA) reactor systems. These systems have a higher efficiency at higher loading rates. In addition, they are applicable for extreme environmental conditions (e.g. low and high temperatures) and to inhibitory compounds. They can even perform anaerobic ammonium oxidation (anammox) and chemical phosphorus precipitation. By integrating these processes with other biological methods (sulphate reduction, micro-aerophilic organisms) and with physical-chemical methods, the cost of treatment of wastewater can be reduced while at the same time valuable components can be recovered for reuse.

The most widely used anaerobic treatment is the UASB, which has been built for the treatment of municipal wastewater in many tropical and sub-tropical regions, e.g. Brazil, Colombia and India, but also in the temperate regions, e.g. Netherlands and North America. These UASBs operated at a hydraulic retention time of 6 to 8h and were able to achieve BOD removal efficiencies of 80% (Mergaert, 1992). In Columbia, a sewage treatment plant consisting of several UASB reactors followed by polishing ponds was commissioned in 1991 (Van Haandel and Catunda, 1997).

2.2 Applicability of anaerobic process for municipal wastewater

To study the applicability of anaerobic process for municipal wastewater, first, the characteristics of municipal wastewater has to be understood. The important parameters which has to be noted include COD, nitrogen, alkalinity & fatty acids, sulfate, suspended solids, flow rate, concentration of chlorinated compounds (Mergaert, 1992), presence of surfactants and size of particles (Tarek, 2001).
2.2.1 COD

Min and Zinder (1989) suggested that there is a threshold concentration of substrate, below which the microorganisms will not be provided with enough energy to support its uptake and metabolism. This threshold concentration determines the outcome of competition for traces of hydrogen and acetate. Table 2.1 shows the threshold concentrations of typical mesophilic methanogens (Westermann et al., 1989).

<table>
<thead>
<tr>
<th>Type of methanogen</th>
<th>Threshold concentration (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanosarcina barkeri 227</td>
<td>1.18</td>
</tr>
<tr>
<td>Methanosarcina mazei S-6</td>
<td>0.396</td>
</tr>
<tr>
<td>Methanothrix spp.</td>
<td>0.069</td>
</tr>
</tbody>
</table>

Municipal wastewater has low organic concentration, typically between 250 and 1000 mg COD/L. With the low range of threshold concentrations, residual volatile fatty acids levels will be considered high compared to the incoming wastewater and thus reflecting a low removal efficiency.

Therefore, unless highly adapted Methanothrix sludges which are thermophilic can be applied, anaerobic treatment seems to be only suitable for relatively concentrated municipal wastewaters (more than 500 mg COD/L).

2.2.2 Nitrogen

Nitrogen refers to Kjeldahl-nitrogen (Kj-N), which is a representation of organic nitrogen and ammonium nitrogen (NH\textsubscript{4}+-N).
The \( \text{NH}_4^+ \)-N in municipal wastewater ranges from 25 to 40 mg/L on average which is not a problem for anaerobic treatment. The typical COD to N ratio for municipal wastewater is 100 is to 10 (Lettinga et al., 1981).

Due to the low biomass yield of anaerobic microorganisms, the nutrient requirement to support them is usually low. The minimum amount of nitrogen necessary for the growth of anaerobic biomass is a COD to N ratio of 100 is to 1.25 (Lettinga et al., 1981). Therefore, nitrogen concentration in municipal wastewater does not pose a problem for anaerobic treatment.

### 2.2.3 Alkalinity & fatty acids

Alkalinity is defined as the acid-neutralizing capacity of water. It exists primarily in the form of biocarbonates which are in equilibrium with the carbon dioxide in the gas at a given pH. This relationship can be expressed as in Equations 2.5 to 2.8.

\[
\text{pH} = pK_{a,1} + \log \frac{50000}{\frac{[\text{CO}_2(g)]}{K_H}}
\]  

(2.5)

where \( K_H = \frac{[\text{CO}_2(g)]}{[\text{H}_2\text{CO}_3^\text{aq}]} = 38 \text{ atm/mol (35 °C)} \)  

(2.6)

\[
K_{a,1} = \frac{[\text{H}^+][\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3^\text{aq}]} = 5 \times 10^{-7} \text{ (35 °C)}
\]  

(2.7)

\[
pK_{a,1} = -\log K_{a,1}
\]  

(2.8)

Relatively low levels of volatile fatty acid (VFA) and the alkalinity in municipal wastewater make it unlikely that there will be any inhibition caused by VFA. The concern is long-chain fatty
acids e.g. soaps (50% inhibition at 500 mg/L, Hanaki et al., 1981) which can occur in domestic sewage. Moreover, higher fatty acids, lipids and triglyceride emulsions degrade very slowly in anaerobic systems and may cause sludge floatation when its concentration exceeds 100 mg/L. Eastman and Ferguson (1981) reported that municipal wastewater can contain up to 100 mg/L of grease and petroleum ether-extractable matter, thus this may be a cause for concern.

### 2.2.4 Sulfate

Sulfate is a preferred electron acceptor compared to other anoxic electron acceptors. In addition, Widdel (1988) has found that the optimum temperature of sulfate-reducing bacteria is between 30 and 35 °C while the optimum temperature for methane-producing bacteria is between 35 and 40 °C (Huser et al., 1982; Vogels et al., 1988). Thus, treatment at temperature less than 35 °C, the sulfate-reducing bacteria is likely to outcompete the methane-producing bacteria.

This may lead to the production and accumulation of sulfides, primarily the soluble form, H₂S. The critical amount for the inhibition of anaerobic microorganisms activities is 50 mg H₂S/L.

Sulfate levels in domestic waste are relatively low, unlikely to reach the critical value. However, post-treatment becomes a requirement to remove the sulfides formed.

### 2.2.5 Suspended solids

De Baere and Verstraete (1982) wrote that the development of high-rate reactors like the UASB made it possible for low hydraulic retention times and efficient treatment. However they were specifically designed to treat wastes with a low suspended solids concentration, for example, distillery and sugar factory wastewaters. They might not be suitable for municipal wastewater which has a high level of suspended solids. A mathematical model by Rozzi and Verstraete (1981)
was used to estimate the loading rates of suspended solids that anaerobic systems can tolerate. They concluded that anaerobic upflow treatment, which allow short retention times, is not applicable to wastewater which has a high amount of suspended solids, unless the suspended solids has been solubilized e.g. by heat treatment. The amount of particulate COD to soluble COD in the influent water should not exceed a ratio of VSS/COD of 0.1 (Figure 2.6). De Smedt et al. (2002) and Aiyuk et al. (2004) also reported that too high solids content in an anaerobic digester compromises reactor performance and hence granulation.

![Figure 2.6 Theoretical maximum loading and hydrolysis rates vs S\textsubscript{a}/S\textsubscript{b} for a maximum VSS concentration of (S\textsubscript{a}) 10 kg. L\textsubscript{tot} is the loading rate applicable (kg COD/m\textsuperscript{3}.d), LS\textsubscript{a} is the suspended solids lading rate (kg VSS/m\textsuperscript{3}.r.d), S\textsubscript{a} is suspended solids (kg VSS/m\textsuperscript{3}), S\textsubscript{b} is soluble and colloidal solids (kg sCOD/m\textsuperscript{3})](image)

(De Baere and Verstraete (1982))

It was thus suggested that municipal wastewater should pass through a primary sedimentation tank before the anaerobic treatment. However, if a primary sedimentation tank, which occupies a large land area, is still required for anaerobic treatment, there is one less incentive of replacing the aerobic treatment with an anaerobic one. Therefore, an anaerobic treatment system which is able
to tolerate a high level of suspended solids, and in turn eliminate the necessity of a primary sedimentation tank, is desirable.

### 2.2.6 Flow rate of the wastewater

It is well known that municipal wastewater has large fluctuations in organic matter, suspended solids and flow rate. Biochemical oxygen demand, chemical oxygen demand and suspended solids concentration may range with a factor of 2 to 10 in half an hour to a few hours (Alaerts et al., 1989).

There are 2 types of flow variations:

i. Daily variations

Concentration and flow variations may change significantly during the course of a day (Figure 2.7) (Metcalf & Eddy, 2003). BOD generally follows the flow pattern, with a lag of several hours. The peak BOD concentration often occurs in the evening.

![Figure 2.7 Typical hourly variations in flow and strength of domestic wastewater.](image-url)
ii. **Seasonal variations**

For domestic wastewater, neglecting infiltration, the flow rate will vary but not the unit (per capita) loadings and strength throughout the year. The total mass of BOD and TSS will increase directly with the population served.

Infiltration tends to decrease the BOD and TSS concentrations, depending on the characteristics of the water. In cases when the groundwater contains high levels of dissolved constituents, the concentrations of some inorganic constituents may increase. Derycke and Verstraete (1986) also found that there is 2.5 to 3 times more organics, in terms of concentration, in the dry season compared to the wet season.

Thus, any anaerobic system treating municipal wastewater should be at least capable of taking variations in flowrate of a factor of 2 to 3.

### 2.2.7 **Temperature of wastewater**

Microorganisms in anaerobic systems, especially the methanogens, perform only in a specific range of temperature. The optimal temperature for *Methanothrix soengenii*, *Methanosarcina* and most other methanogens is between 35 and 40°C (Figure 2.8).

From Figure 2.8, it can be seen that methanogenic activity at below 10 °C is only a few percent of that at 35 °C. This makes anaerobic treatment feasible only in tropical and sub-tropical regions, such as Singapore.
2.2.8 Concentration of chlorinated compounds

Domestic wastewater may contain dry cleaning products or cleaners with organic solvents which has chlorinated compounds. Most of the chlorinated compounds are toxic and can seriously hamper anaerobic treatment, even at concentrations as low as 1 mg/L (Lettinga et al., 1981). However, the anaerobic process is also known to be able to remove chlorinated organics which aerobic process cannot.

2.2.9 Presence of surfactants

Municipal wastewater contains a certain amount of surfactants due to detergent from domestic households. Surfactants are known to adsorb at both solid/liquid and liquid/air interfaces and will affect the anaerobic biodegradability of particles. They can emulsify poorly soluble hydrophobic compounds in water and improve the accessibility of these substrates to microorganisms (Rouse et al., 1994). However, the emulsifying effect might prevent the physical removal of the particles. Wagener and Schink (1987) and Rouse et al. (1994) concluded that surfactants inhibit anaerobic biodegradation of organic compounds.
Boller (1993), on the study of Zürich City wastewater, reported that the surfactant concentration was 17 to 22 mg/L and the anionic and non-ionic surfactants make up 91 to 94%.

Elmitwalli et al. (2001), on the study of biodegradability and change of physical characteristics of particles during anaerobic digestion of domestic sewage, found that these surface-active components were not biodegraded during digestion, indicated by the development of surface tension.

2.2.10 Size of particles

The size of particles in domestic sewage affects both biological and physical processes (Levine et al., 1985). For larger particles, gravitational and drag forces predominate over colloidal forces (van der Waals attraction and electrostatic repulsion), while for smaller particles (less than a few µm), colloidal forces are more predominant (Gregory, 1993).

Elmitwalli et al. (2001) found that the maximum conversion to methane at 30 °C was the highest (86%) for the colloidal fraction, the next is suspended fraction (78%) and the lowest is dissolved fraction (62%).

2.3 Sequencing batch reactors

2.3.1 Concepts of a sequencing batch reactor

A batch reactor is characterized such that there is neither continuous flow of wastewater entering nor leaving the reactor (i.e. flow enters, is treated, discharged and the cycle repeats). The content is completely mixed (Metcalf & Eddy, 2003). It is significantly different from the commonly used continuously stirred tank reactor (CSTR) systems where it is assumed that complete mixing occurs instantaneously and uniformly throughout the reactor as inflow and outflow takes place.
simultaneously. Figure 2.9 shows the fundamental difference of the 2 systems by definition sketches.

![Diagram showing difference between a batch reactor and a continuous-flow stirred tank reactor (CSTR)](image)

Figure 2.9 Difference between a batch reactor and a continuous-flow stirred tank reactor (CSTR).

A sequencing batch reactor (SBR) provides for time sequencing of operations which include equalization, biological conversion, sedimentation and clarification all in one complete cycle. The SBR process has four main phases, i.e. fill, react, settle and decant. A fifth optional phase is the idle phase, which may or may not be incorporated into a system (Figure 2.10).

![Diagram showing different phases of a batch reactor in one operating cycle](image)

Figure 2.10 Different phases of a batch reactor in one operating cycle.
i. Fill

The wastewater that is to be treated can be fed into the system through several methods.

- Organic contact and biological reactions are minimized by feeding in the wastewater at any rate in a quiescent manner near the liquid surface until the tank is full.
- Wastewater is fed at a low rate with mixing to allow reaction to begin as soon as Fill phase starts. Thus, substrate concentration is still held relatively low.
- Wastewater is fed at a rate equal to the effluent discharge rate which means the system acts as an equalization tank.
- Wastewater is added as a batch dump inflow or any other desired inflow rate and accompanying mixing method to meet the specific treatment objectives.

After the Fill phase, any variations in the wastewater influent no longer have any effect on the treatment processes taking place inside the reactor except to limit or extend the total time allowed for them to take place.

Typically, an anaerobic sequencing batch reactor is operated with a fast fill, leading to a low fill time to cycle time ratio. This operating strategy provides a high initial substrate concentration. This will enable zero order kinetics with respect to the organic acids that form, which may lead to an acid formation problem. However, this phenomenon is more severe if a high strength wastewater is being treated.

ii. React

React phase follows the Fill phase. This is the main period when biodegradation takes place. Mixing is provided to ensure sufficient contact of the microorganisms with the substrate. Organics in the wastewater can be acclimatized by exposing them to high
substrate levels for a short period of time and low levels for a longer period of time. Similarly, it can be done by maintaining a relatively low substrate level during most of the Fill and React phase.

High substrate concentration in the reactor in the beginning of the react phase allows a high food-to-microorganisms (F/M) ratio, which means the rate of substrate uptake is high.

iii. Settle

In the settle phase, solid-liquid separation is allowed to take place by gravitational force. Biogas attached to or entrapped by biological solids can also be separated and collected.

After the React phase, substrate concentration in the reactor is low, meaning that the F/M ratio is low. A low F/M is known to improve the settling properties of biomass. High settling velocities of the biomass in the SBR is expected. Heavy flocs of diameter more than 1 mm can sweep down aggregates of smaller flocs. These heavy flocs are able to form due to the operation regime of the SBR. The gentle stirring of the mixed liquor supports flocculation and during the Settle phase, quiescent conditions are provided to aid in settling.

iv. Decant

The treated effluent is withdrawn from the system from above the sludge blanket. It is usually done at a slow rate to minimize disturbance of the settled solids.

A SBR is also different from other fill and draw systems. It is filled and drawn within a defined period of time so variations in the influent of the treatment plant has no effect on the process after...
the fill phase of the particular cycle has ended. The cycle is continuously repeated in a defined and regulated variation of process conditions.

2.3.2 Advantages and disadvantages of a batch system

The SBR technology is regarded as one of the important methods to gain control over structure and functions of the microbial community in a reactor exposed to varying influent conditions.

Firstly, it has to be understood that the concentration of contaminants in wastewater naturally varies with time or space, thus feed wastewater has a potentially unsteady-state behavior. However, conventional systems for wastewater treatment are all unrealistically designed to operate as steady-state systems. This is because it was always assumed that steady-state conditions were needed for effluent concentrations to be kept constant and within the permitted limits. The incorporation of an equalization tank was thought to be able to dampen the impact of the system’s unsteady behavior but it is not able to equalize variations in mass flowrates. Therefore these systems, instead of being operated as a steady-state system it is designed for, become uncontrolled unsteady-state systems. These uncontrolled unsteady-state systems strain to meet the steady-state demands.

In practice, the factors known to be effective in controlling the structure and function of microbial aggregates (e.g. activated sludges, biofilms) are difficult to maintain in continuous flow systems. In such cases, the growth rate differentials needed to mitigate the impact of the forcing function associated with the mass flow rate of the contaminants are not sufficiently strong. The frequency and amplitude of the changes needed to control variations in the rate functions cannot be implemented because the reactor is designed for maximum influent loading so that the discharge limits can be met during peak loading periods which happened only occasionally. As a result, the
biological system is subjected to sub-optimal control conditions most of the time even though it is a controlled unsteady-state system.

Batch system, on the other hand, is a controlled unsteady-state system. Batch systems were first used in activated sludge processes as a mean of controlling filamentous organisms. Frequent shifting of activated sludge between aerobic, anoxic and anaerobic zones allows establishment of microbial communities capable of executing nitrification, denitrification and enhanced biological phosphate uptake. It appears that short-term unsteady-state conditions, if properly selected and controlled, are an effective tool to maintain long-term quasi-steady-state conditions. Factors known to be effective in controlling the structure and function of microbial aggregates are difficult to maintain in continuous flow systems. As continuous flow systems are mainly designed for maximum loading, such biological systems often operate at sub-optimal control conditions although they are controlled unsteady-state. A batch reactor is able to mitigate these shortcomings of a continuous flow reactor. The batch reactor is able to vary its effective volume by time.

From a microbiological point of view, the key characteristic of SBR technology is the change between feast and famine in a cycle. Interactions between different microorganisms are optimized in such fluctuating conditions especially for rich, diverse and effective microbial population. These microorganisms are trained to utilize even the smallest amount of nutrients and cope with changing conditions on various time levels. Under different conditions, different groups of organisms will be switched on or off. Once the system is well established, it will be more robust and be able to dampen influent fluctuations. Therefore, SBR technology has an obvious advantage over continuous flow systems in the long run.
The SBR technology also gives the benefits of being flexible. By controlling operational parameters, the SBR has the ability to apply environmental pressure on a microbial consortium. During start-up, the environmental pressure applied will enrich for a given consortium. After enrichment, further changes in the operational conditions will cause either changes in the physiological state of the organisms or changes in the reactor products, causing a shift in the microbial population. This enables operators to control the performance of the system by just changing the operational parameters.

2.4 Powdered activated carbon

The application of PAC to wastewater has been documented since 1970s. As early as 1972, Robertaccio et al. has presented a study of treatment of organic chemical plant wastewater with the du Pont PACT process. The PACT, Powdered Activated Carbon Treatment, is a process by which PAC is added to an activated sludge system. Subsequently, several reports on this method have been published (Robertaccio, 1973, 1978, 1979) on treatment of acidic, highly colored, highly variable wastewater containing heavy metals, and biodegradable as well as non-degradable organics.

It has been proven that the use of PAC can improve the performance of the conventional activated sludge process in terms of

2. Higher chemical oxygen demand and refractory organics removal.
3. Able to stand shock loadings and toxic upsets.
4. Improved sludge settling and dewatering.
5. Reduced foaming in aeration reactor.
6. Effluent has lower toxicity to fish.
Using PAC is very cost effective especially when a high sludge age ($\geq 50$ days) is employed (Grieves et al., 1978) because PAC does not have to be replaced frequently. It also reduces the problem of poor sludge settleability and effluent washouts in the conventional activated sludge systems. Therefore, the amount of sludge to be wasted is reduced, making the process more economical.

PAC also offers the advantage of providing fresh carbon continuously since it is fed as a new product and is not recycled through the treatment process. Since PAC is added to the plant dynamically as a feed chemical, it can be used as when it is required.

2.4.1 Activated carbon as an adsorbent

Activated carbon is a type of adsorbents used to accumulate substances in a solution onto a suitable interface. It is often used as a polishing step in wastewater treatment after the normal biological treatment. There are, in principal, many types of absorbents (for example, synthetic polymeric and silica-based adsorbents) but activated carbon is most commonly used due to its low cost.

![Figure 2.11 Powdered activated carbon.](image)

Activated carbon is prepared by making a char from materials such as almond, woods, bone and coal. This is a pyrolysis process whereby these materials are heated to a red heat (less than 700 °C) to drive off the hydrocarbons. Then the char particles are exposed to oxidizing gases such as
Steam and CO\textsubscript{2} at high temperatures of 800 to 900 °C. These gases develop a porous structure and thus the large surface area. Different sizes of activated carbon have different adsorption capacity. The PAC typically has a diameter of less than 0.074 mm (200 sieve). The characteristics of PAC are summarized in Table 2.2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Typical values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total surface area</td>
<td>m\textsuperscript{2}/g</td>
<td>800-1800</td>
</tr>
<tr>
<td>Bulk density</td>
<td>kg/m\textsuperscript{3}</td>
<td>360-740</td>
</tr>
<tr>
<td>Particle density, wetted in water</td>
<td>kg/L</td>
<td>1.3-1.4</td>
</tr>
<tr>
<td>Particle size range</td>
<td>µm</td>
<td>5-50</td>
</tr>
<tr>
<td>Mean pore radius</td>
<td>Å</td>
<td>20-40</td>
</tr>
<tr>
<td>Iodine number</td>
<td></td>
<td>800-1200</td>
</tr>
<tr>
<td>Abrasion number</td>
<td>minimum</td>
<td>70-80</td>
</tr>
<tr>
<td>Ash</td>
<td>%</td>
<td>≤6</td>
</tr>
<tr>
<td>Moisture as packed</td>
<td>%</td>
<td>3-10</td>
</tr>
</tbody>
</table>

### 2.4.2 The adsorption process

Adsorption is a phenomenon whereby a solid surface (adsorbent) is exposed to a certain adsorbate, the adsorbate molecules are adsorbed onto the surfaces of adsorbent. The adsorption process can be simplified into three main steps - macrotransport, microtransport and sorption. Macrotransport is the diffusion of the organic material from the bulk water to the liquid/solid interface by advection and diffusion. Microtransport is the diffusion of organic material through the absorbents’ macropore system to the micropores. The surface area of the macro and mesopores is usually considered negligible compared to that of the micropores. IUPAC classifies porosities of activated carbon (Sing et al., 1985) as follow

- Micropores - width less than 2 nm
• Mesopores - width between 2 and 50 nm
• Macropores - width greater than 50 nm

The solid adsorbents have two important properties, namely

• extremely large surface area to volume ratio
• a preferential affinity for certain constituents in the liquid phase

The kinetics of adsorption of PAC are of crucial importance and typical contact times are in the order of 1h. The homogeneous surface diffusion model (HSDM) has been successfully applied for the prediction of the kinetics of adsorption of a range of compounds into activated carbon (Najm et al., 1991, Knappe, 1996, Sontheimer et al., 1988). The model predicts the diffusion of a molecule from the external surface of the adsorbent particle, along pore surfaces, to the adsorption site. The other three mass transfer steps taking place during adsorption, transfer from bulk liquid to surface film surrounding the particle, transfer through this surface film, and the adsorption step, are not considered rate limiting in this model. The PAC particles are considered to be spherical and of homogeneous structure, and Fick’s first law of diffusion is applied for the calculation of the adsorbent surface concentration as a function of the radial position within the particle. The change in bulk liquid-phase concentration with time is then calculated using a mathematical model that is appropriate for the configuration of the system.

2.4.3 Effect of PAC on biological activity

It is recognized that providing a solid surface for a microorganism makes it largely different from when it is in the bulk liquid. This is in view of pH, ionic strength and concentration of organics. The availability of a solid surface results in a sorptive interaction between microorganisms and the solid, and thus there is a stimulation of biological activity.
Many literatures have stated that there is a synergistic effect when biological activity is combined with activated carbon.

One of the reasons given was that PAC was able to adsorb the toxic substances which inhibit biological activities. PAC is known to stabilize the activated sludge system against shock loads and toxic upsets. Robertaccio (1979) showed that the PACT system can withstand various toxic attacks, including the highly adsorbable trichlorophenol while a similarly spiked activated sludge system failed to. Apparently, the PAC particles are predominantly physically associated with the floc. Once the toxic is absorbed, the inhibitory effects on the biological microorganisms greatly diminished. This resulted in a treated effluent which has a more stable quality.

Although BOD removal is found to have increased with the addition of PAC, there is no change in the oxygen uptake rate (Robertaccio, 1972). As BOD tests usually measure biodegradable substances that are weakly adsorbable, it showed that PAC did not actually adsorbed the organics. Instead, PAC adsorbed the inhibitory substances and in turn enhanced the performance of the biological microorganisms.

Zobell (1937) found that bacteria preferred to reside on solid surfaces rather than remain free in the bulk solution. He speculated that the solid surfaces have the effect of concentrating food and extracellular enzymes in the environment of microorganisms. It was also concluded that both the food and the bacteria should be adsorbed on the solid surface for stimulation to take place, especially in an environment when proteins or other biopolymers serve as a food source. The concentrating of extracellular enzymes is significant to the overall biological activity.
In summary, there are two main ways by which PAC is able to stimulate biological activity. Firstly, it is through the adsorption of toxic and inhibitory substances. Secondly, it is due to the adsorption of the food source onto the solid surfaces, resulting in the concentration of food for the organisms, especially in conditions

2.4.4 Soluble microbial compounds

2.4.4.1 Definition of soluble microbial products

One important reason of using PAC in treatment systems is to control the soluble microbial compounds in the system. Therefore, it is important to study them and their effect on wastewater treatment systems.

The term, Soluble Microbial Products (SMPs), is used to represent a group of organic compounds that are released into the bulk solution due to substrate metabolism (usually with biomass growth) and biomass endogeneous decay. The recognition of this group in wastewater is important in understanding the models of wastewater treatment. Before this, designs based on Monod model, which predicted that the effluent concentration of the rate limiting substrate should be independent of the influent substrate concentration, showed a deviation from the real performance. Thus it was speculated that SMPs had a greater influence on wastewater treatment characteristics than previously thought.

Kuo (1993) listed the factors which cause SMP production (Table 2.3):
<table>
<thead>
<tr>
<th>Factor</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1  Concentration equilibrium</td>
<td>Harold, 1972; Christensen, 1975; Payne, 1976</td>
</tr>
<tr>
<td>2  Starvation</td>
<td>Dawes and Ribbins, 1964; Burleigh and Dawes, 1967; Boylen and Ensign, 1970</td>
</tr>
<tr>
<td>3  Presence of energy source</td>
<td>Saier et al., 1975; Neijssel and Temperst, 1976; Thompson, 1976</td>
</tr>
<tr>
<td>4  Substrate-accelerated death</td>
<td>Postgate and Hunter, 1964; Dawes and Ribbins, 1964; Strange and Dark, 1965; Pirt, 1975</td>
</tr>
</tbody>
</table>
2.4.4.2 Molecular weight distribution of SMPs

Molecular weight (MW) distribution is a common way of characterizing SMPs. It is useful in determining the type of process and removal technology that is applicable to deal with the SMPs. There are two main methods of obtaining the molecular weight distribution; one by gel permeation chromatography, GPC, to obtain a continuous distribution and second by UF membranes in stirred cells to obtain a discrete distribution.
It is important to recognize the limitations of these methods. In a GPC analysis, it was found that some constituents pass through the column more rapidly than the calibrated standards because of ion exclusion or complex formation. Some other constituents may pass through the column less rapidly than it should due to adsorption or electrostatic interaction with the column packing. This would lead to inaccurate results. It is thus important to ensure that there is no chemical interaction between the column packing, the solvent or eluent or the organic compounds. In GPC analysis, usually a concentration of the samples is done by freeze-drying or evaporation. This inevitably changes the sizes of the constituents and again, results in inaccurate data.

The limitations of using UF membranes in stirred cells is mainly due to the uncertainties caused by membrane pore size distribution, water temperature, cell pressure, solution pH, ionic strength, as well as the organic constituents’ molecule size, shape and affinity for the membrane materials (Logan and Jiang, 1990).

Duncan and Stuckey (1999) have summarized the findings on molecular weight (MW) distribution of SMPs.

- Compounds found in biological effluents have a wide spectrum of MW, from less than 0.5 to more than 50 kDa.
- A greater amount of the compounds in biological effluents have high MW.
- Raw wastewater has a non-normal MW distribution skewed towards very low MW (<0.5 kDa) while effluents has bimodal patterns.
- Operating conditions can significantly affect the MW distribution, for example, a high SRT will result in more high-MW compounds.
- The type of substrate used in the treatment system can significantly affect the MW distribution.
2.4.4.3 Chelating properties of SMPs

There are a number of chelating functional groups found in SMPs, such as carboxylates, hydroxyls, sulphydryls (–SH), phenols and amines. These compounds act as ligands and complex with metals commonly found in wastewaters, for example, copper, iron, lead nickel and zinc. These metals, when exist in high concentrations in wastewater, are toxic for biological treatment systems. On the other hand, insufficient quantities of these metals also pose a nutrition deficient problem. The chelators are able to reduce the toxicity of these metals but also make them less available to the bacteria as micronutrients. Anaerobic microorganisms are known to produce nickel-chelating SMP which the amount is able to mitigate a relatively high level of nickel toxicity (Kuo and Parkin, 1996).

2.4.4.4 Toxicity of SMPs

The SMP may actually cause the treated-effluent to be more toxic than the organic components in the influent. Secondary effluent was detected to show a greater mutagenic response than primary effluent. In addition, some SMP was found to inhibit nitrification (Chudoba, 1985a). The toxicity problem becomes more prominent with higher strength wastewater. Treatment of such toxic aerobic system effluents was proposed using GAC which can effectively adsorb SMP of high MWs.

2.4.4.5 SMP effect on process performance

Other than exhibiting chelating and toxic properties, SMPs have other effects on treatment processes. Washington et al. (1970) observed that an accumulation of SMP in a cultivation medium result in reduction in specific respiration. These SMPs also have a negative effect on the settling and flocculating characteristics of activated sludge.
2.4.4.6 SMPs in anaerobic systems

Kuo et al. (1996) found that the normalized production of SMP is lower in anaerobic systems fed on glucose and phenol. Germirli et al. (1993) also showed that residual COD concentration was substantially lower for anaerobic treatment processes compared to single-stage aerobic processes. The amount of refractory compounds released from aerobically degraded biomass is also higher than that produced by anaerobically degraded biomass. Kim et al. (1990) found that effluent from an anaerobic treatment of wastewater has less adsorbable than those from an anaerobic/aerobic effluent and an aerobic effluent. It was also reported that low MW SMPs (MW < 1 kDa) from an anaerobic treatment system is the most difficult to adsorb (Barker et al., 1999).
3.1 System set-up

The lab-scale AnSBR set-up consisted of one 200 L raw sewage tank, one 50 L raw sewage transfer tank, two 22.5 L AnSBR reactor, two 22.5 L effluent tank and two 16 L gas collectors. A photo of the set-up is shown in Figure 3.1.

Figure 3.1 Photo of AnSBR set-up
The 200 L raw sewage tank consisted of a plastic drum (Figure 3.2). A mechanical agitator was mounted on top of the drum. The stirrer rotated at a speed that kept the contents of the tank homogenous at all times.

Figure 3.2 Raw sewage tank

In addition to the raw sewage tank, there was also a raw sewage transfer tank, which is of a smaller volume, to heat up the raw wastewater to the required 30 °C before being fed to the AnSBR reactors. The 50 L raw sewage transfer tank consisted of a plastic bin (Figure 3.3). A mechanical agitator was also mounted on top of the bin to stir the contents. A temperature sensor and heater were submerged into the tank for temperature control.

Figure 3.3 Raw sewage transfer tank and temperature controller
The AnSBR reactors (Figure 3.4) were made using clear acrylic plates and cylinders, and assembled using chloroform. In addition to the holes drilled for the bolting of the cap to the cylinder, there were four other holes for the stirrer, pH sensor, pH dosing line and the biogas line respectively. The stirrer was connected to an external motor for agitation of the reactor contents during the react phase.

Figure 3.4 AnSBR reactor

The pH sensor provision was a hole which enabled the sensor to be lowered into the reactor. After the sensor was installed, the provision was sealed up with a rubber cork and silicon. The sensor was then connected to the pH meter and controller while the pH dosing line was for addition of sodium carbonate when required. The biogas line was connected to the biogas collector.

The fill point was at the bottom of the reactor. The decant point of the reactor was located depending on the HRT the reactor. To enable the reactor to be operated at different HRTs, four decant points were built, namely at 450, 338, 225 and 113 mm from the inner bottom of the reactor.

The top cap was secured to the cylinder or body of the reactor using 8 sets of bolts and nuts arranged symmetrically. The bolts and nuts were tightened carefully to make sure the reactor was air-tight but not too tight as to cause cracks to the acrylic material. The dimensions and details of the reactor construction are illustrated in Figure 3.5.
The effluent tank was also built using acrylic plates. It had a square base of 270 mm and height 400 mm. The effective volume was 22.5 L, which was similar to the volume of one AnSBR reactor.

All the pumps used were Cole-Parmer Masterflex peristaltic pumps.
After the AnSBR system was fully set up, a hydrotest using tap water was performed to ensure there was no leakage. The system was also test-run using tap water to ensure that the Programmable Logic Controller (PLC) was programmed correctly.

### 3.2 Process Flow

The feed wastewater was municipal wastewater collected before the primary clarifiers at the Ulu Pandan Water Reclamation Plant. The feed wastewater was stored in the raw sewage tank equipped with a top-down mechanical stirrer and is subsequently pumped into the raw sewage transfer tank using two raw sewage transfer pumps. The raw sewage transfer tank had a mechanical stirrer and a temperature controller to maintain the temperature of the feed wastewater at 30.0 °C. Then the fill pump was used to transfer the feed wastewater into the AnSBR reactor during the Fill phase. During the Decant phase, the decant pump would be activated to draw the treated wastewater into the effluent tank. Biogas produced in the reactor was channeled into a gas collector.

The schematic diagram of the AnSBR treatment units are illustrated in Figure 3.8.
Figure 3.8 Schematic diagram of AnSBR
3.3 Operating conditions

The AnSBR system was operated and controlled mainly by the PLC. There were 4 phases, fill, react, settle and decant. The equipment were activated or deactivated at the relevant phases (Table 3.1).

<table>
<thead>
<tr>
<th>Phase</th>
<th>Fill Pump</th>
<th>Decant Pump</th>
<th>Stirrer</th>
<th>pH Controller</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fill</td>
<td>Activated</td>
<td>Deactivated</td>
<td>Activated</td>
<td>Deactivated</td>
</tr>
<tr>
<td>React</td>
<td>Deactivated</td>
<td>Activated</td>
<td>Activated</td>
<td>Deactivated</td>
</tr>
<tr>
<td>Settle</td>
<td>Deactivated</td>
<td>Deactivated</td>
<td>Activated</td>
<td>Deactivated</td>
</tr>
<tr>
<td>Decant</td>
<td>Deactivated</td>
<td>Deactivated</td>
<td>Deactivated</td>
<td>Deactivated</td>
</tr>
</tbody>
</table>

According to the objectives of this study, the AnSBR was operated at 3 HRTs, 16, 8 and 6h. Several considerations were taken into account for the operating conditions.

The effective volume of the reactor itself was 22.5 L. To utilize the full capacity of the reactor, the effective volume of each cycle was kept at 22.5 L. At different HRTs, the effective volume remained constant, thus, the variable was the flowrate.

The number of cycles per day also changes according to the required flowrate. A reasonable number of cycles was chosen to make sure the fill/decant volume were not less than half the effective volume of the reactor. This is to ensure that the solids retained (MLVSS) was sufficient to maintain a substantial F/M ratio in the reactor.
The operating protocol was adjusted based on these parameters. The main change was in the duration of the react phase which was done through programming the PLC. Another change was the flowrate of the fill and decant pumps. This was done by calibrating the pumps using wastewater to the required flowrate. The operating parameters are summarized in Table 3.2.

Table 3.2 Operating parameters at different HRTs.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AnSBR 1</th>
<th>AnSBR 2</th>
<th>AnSBR 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRT</td>
<td>16 h</td>
<td>8 h</td>
<td>6 h</td>
</tr>
<tr>
<td>Effective volume</td>
<td>22.5 L</td>
<td>22.5 L</td>
<td>22.5 L</td>
</tr>
<tr>
<td>Flowrate</td>
<td>33.75 L/d</td>
<td>67.5 L/d</td>
<td>90 L/d</td>
</tr>
<tr>
<td>No. of cycles per day</td>
<td>4</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Fill/Decant volume per cycle</td>
<td>8.4 L</td>
<td>8.4 L</td>
<td>11.25 L</td>
</tr>
<tr>
<td>Operating protocol</td>
<td>Fill 15 min React 270 min Settle 60 min Decant 15 min</td>
<td>Fill 15 min React 90 min Settle 60 min Decant 15 min</td>
<td>Fill 15 min React 90 min Settle 60 min Decant 15 min</td>
</tr>
</tbody>
</table>

3.4 **Seeding procedure**

Digester sludge from the anaerobic digester of Ulu Pandan Water Reclamation Plant was used as the seeding sludge. After collection, the sludge was maintained at room temperature of about 30 °C and transferred into the reactors within 4h of collection.

The AnSBR system was checked to be well-connected and air-tight before the seeding was carried out. The air inside the gas collector was also expelled using nitrogen gas as it will not affect the anaerobic process. The seeding sludge was first filtered with a 1.18 mm sieve before transferring into the reactors. When sieving, care had to be taken to minimize contact between the sludge and atmosphere.
After the sieving, the sludge was fed into the reactor by using the fill pump, and with the stirrer on. As the sludge filled up the reactor, the nitrogen gas from the reactor was transferred into the gas collectors. The gas was then expelled from the collectors periodically.

The reactor was filled with 22.5 L (effective capacity of the reactor) of sludge. The reactor and gas collectors were then purged with pure nitrogen gas to rid of any residual air.

A sample of the seed sludge was extracted from the reactor after stirring for a few minutes. It had a MLSS 14300 mg/L and MLVSS 9700 mg/L. Then, the seed sludge was allowed to reside in the reactor for 24h with stirring before the first cycle was started.

3.5 Tests & Analysis

3.5.1 Physical & aggregate properties

3.5.1.1 Total suspended solids

Total suspended solids (TSS) refer to the portion retained by a filter of pore size 0.45 µm. The determination of total suspended solids concentration was done in accordance to the standard methods described by APHA et al. (1998), in Part 2540D. Well-mixed samples were filtered though a weighed filter paper and the residue obtained was dried in an oven at 103 to 105 ºC. The increase in the weight of the filter paper represented the total suspended solids mass. The total suspended concentration was calculated by dividing this mass by the volume of samples used initially.
Samples were measured as soon as possible after sampling. If this was not permitted, samples were refrigerated at 4 °C to minimize microbiological decomposition, and measured within 24h. Samples were brought to room temperature before analysis.

3.5.1.2 Volatile suspended solids
The determination of volatile suspended solids concentration was done in accordance to the standard methods described by APHA et al. (1998), in Part 2540E. The residue from the total suspended solids test was ignited to constant weight in a furnace at 550 °C. The weight lost on ignition was the volatile solids.

Negative errors in volatile solids might result from a loss of volatile matter during drying. Samples with low volatile solids concentration might be subjected to considerable error. Thus, for effluent quality, the volatile suspended solids concentration data was supported by total organic carbon data.

3.5.1.3 Sludge volume index
The sludge volume index (SVI) is the volume in milliliters occupied by 1 g of a suspension after 30 min settling. It is used to monitor the settling characteristics of biomass, which is useful in routine process control. The determination of sludge volume index was done in accordance to the standard methods described by APHA et al. (1998), in Part 2710D.

3.5.2 Aggregate organic constituents
Chemical oxygen demand and total organic carbon are used to assess the total amount of organics present. The measurement of biochemical oxygen demand represents the biodegradable organics present. The analysis of organics is important in assessing the concentration and general
composition of organic matter in wastewaters and treated effluents. It is also important in
determining the treatment efficiency of the process.

Samples were measured as soon as possible after sampling. If this was not permitted, samples
were refrigerated at 4 °C to minimize microbiological decomposition, and measured within 24h.
Samples were brought to room temperature before analysis.

3.5.2.1 Chemical oxygen demand

Chemical oxygen demand is a measure of the oxygen equivalent of the organics content that is
susceptible to oxidation by a strong chemical oxidant. COD can be related to BOD, organic
carbon or organic matter empirically. The dichromate reflux method was chosen because of
superior oxidizing ability, applicability to wastewater and ease of manipulation. The oxidation of
most organic compounds was 95 to 100% of the theoretical value. The closed reflux method was
more economical than the open reflux method in the use of metallic salt reagents but the samples
have to be homogenous.

The determination of COD was done in accordance to the Closed Reflux, Titrimetric method
described by APHA et al. (1998), in Part 5520C. The culture tubes and caps were soaked in 5%
acid (HCl) before use. A sample (2.5 mL) was added into a culture tube with 1.5 mL of Digestion
solution, 3.5 mL of sulfuric acid reagent. Cap the tube and invert it several times to mix
completely. The tube was then placed in a block digester which is preheated to 150 °C and reflux
for 2h. The tube was cooled to room temperature before adding 2 to 3 drops of ferroin indicator
and titrating with 0.1 M FAS with stirring. The end point was a sharp change to reddish brown. A
blank and a standard were also prepared for reference and calculations.
Volatile organic compounds were more completely oxidized because of longer contact with the oxidant. It is thus important to inspect the tube caps of the culture tubes for breaks.

3.5.2.2 Biochemical oxygen demand

5-day Biochemical oxygen demand, BOD$_5$, measures the amount of oxygen utilized during a 5 days period for the biochemical degradation of organic material (carbonaceous demand) and the amount of oxygen used to oxidize inorganic material such as sulfides and ferrous iron. It may also measure the amount of oxygen used to oxidize reduced forms of nitrogen (nitrogenous demand) unless their oxidation is prevented by an inhibitor.

There are a number of factors which affect the accuracy of BOD$_5$ test data, for example, the amount of soluble and particulate organics, the amount of settleable and floatable solids, and even the amount of mixing applied. It is a concern that nitrogenous demand interferes with the BOD$_5$ and affects the accuracy of the data obtained. Usually, an inhibiting chemical is used to reduce this error. Considering that nitrification reaction is minimum in anaerobic process, the inhibiting chemical is not introduced in this context.

The BOD$_5$ concentration in the raw wastewater typically exceeds the dissolved oxygen available in an air-saturated sample. Therefore, it was necessary to dilute the sample before incubation to bring the oxygen demand and supply into appropriate balance. The dilution water used was added with nitrogen, phosphorus, trace metals and buffered to ensure the pH is maintained at a level suitable for bacterial growth. The water used was distilled water.

The determination of BOD$_5$ was done in accordance to the 5-Day BOD Test described by APHA et al. (1998), in Part 5210B. First, the sample was put into a 250-mL airtight glass bottle, with the
appropriate dilution. The bottle was filled until overflowing and incubated at 20 °C for 5 days. The dissolved oxygen was measured before and after the incubation. As degradation of the samples might take place significantly after sampling, samples were measured as soon as possible, within 2h of collection. If this was not permitted, samples are refrigerated below 4 °C to minimize microbiological decomposition, and measured within 24h. Samples were warmed to room temperature before analysis.

3.5.2.3 Total organic carbon

The organic carbon in wastewater composed of a variety of organic compounds in various oxidation states. The carbon in wastewater can be categorized into several fractions:

<table>
<thead>
<tr>
<th>Carbon fraction</th>
<th>Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inorganic carbon</td>
<td>IC, Carbonate, bicarbonate, dissolved CO₂</td>
</tr>
<tr>
<td>Total organic carbon</td>
<td>TOC, All carbon atoms covalently bonded in organic molecules</td>
</tr>
<tr>
<td>Dissolved organic carbon</td>
<td>DOC, Fraction of TOC that passes through a 0.45 μm filter</td>
</tr>
<tr>
<td>Particulate organic carbon / non dissolved organic carbon</td>
<td>POC, Fraction of TOC that is retained by a 0.45 μm filter</td>
</tr>
<tr>
<td>Volatile organic carbon/purgeable</td>
<td>VOC, Fraction of TOC removed from an aqueous solution by gas stripping under specified conditions</td>
</tr>
<tr>
<td>Carbon fraction</td>
<td>Components</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>-------------------------------------------------</td>
</tr>
<tr>
<td>Non purgeable organic carbon</td>
<td>NPOC - Fraction of TOC not removed by gas stripping</td>
</tr>
</tbody>
</table>

TOC test was able to account for the organic fraction which did not respond to the BOD₅ or COD test because TOC was independent of the oxidation state of the organic matter and did not include other organically bound elements, such as nitrogen and hydrogen, and inorganics. The accuracy of TOC results could be enhanced by eliminating the interference by IC. To do this, samples were acidified to pH less than 2 to convert IC to CO₂. The CO₂ is then removed by purging the sample with a purified gas. This purging also removed POC, thus the real measurement is that of NPOC.

The determination of BOD₅ was done in accordance to the Combustion-infrared method described by APHA et al. (1998), in Part 5310B. The method was suitable for samples with TOC ≥ 1 mg/L.

### 3.5.3 Biogas composition

The gas produced by anaerobic process included methane, carbon dioxide, hydrogen and nitrogen gas. It was also saturated by water vapor. The main concern was the relative proportion of these components.

The determination of the biogas composition was done in accordance to the Gas Chromatographic method described by APHA et al. (1998), in Part 2720C. The equipment used was a gas chromatograph (Shimadzu GC-17A, Japan) equipped with a thermal conductivity detector and a 2 m x 1/8 in stainless steel Porapa Q 80/100 mesh column.
3.5.4 Volatile organic acids

The determination of the volatile organic acids presence was done in accordance to the Purge and Trap Packed-Column Gas Chromatographic/ Mass Spectrometric method I described by APHA et al. (1998), in Part 6210B.

This method was applicable to most wastewaters to determine the amount of purgeable organic compounds. The volatile organic compounds were transferred from the aqueous phase by bubbling an inert gas through the wastewater sample contained in a purging chamber at ambient temperature. The vapor was swept through a sorbent trap to collect the organics. After that, the trap was heated and back flushed with the same inert gas to desorb the compounds onto a gas chromatographic column. The gas chromatograph was temperature-programmed to separate the compounds. The detector was a mass spectrometer.

The largest concern with this method was the impurities in the purge gas and organic compounds outgassing from the plumbing ahead of the trap account. Therefore, it was important to prepare blanks for monitoring. However, any data was not corrected through blanks; the blanks were strictly only for monitoring of accuracy. Contamination by carryover was relatively common. The analyzing equipment was thus rinsed several times and checked with pre-known standards regularly.

3.5.5 pH value

The monitoring of pH value was important to the process as microbiological behavior and many water chemical reactions are affected significantly by pH. The pH could also be used as an indication for the alkalinity and carbon dioxide concentration.
The determination of the pH value is done in accordance to the Electrometric Method described by APHA et al. (1998), in Part 4500-H B.

3.5.6 Microscopic images

The mixed liquor biomass in the AnSBR was extracted from the reactors during the react phase. The biomass was allowed to settle a measuring cylinder for 10 min, then the supernatant was discarded. The settled biomass was resuspended and a small volume was placed onto a pertridish. It was then put under the microscope for observation. The microscope system consisted of a stereoscope (Leica Model MZ6) with an image capturing device (Color Video Camera JVC Model TK-C1380 and AC Adaptor JVC Model AA-P700). The images captured were analyzed using the software, *Leica Qwin* (for Windows 95 and Windows NT).

3.5.7 Molecular weight distribution of dissolved organic matter

The molecular weight distribution of dissolved organic matter (DOC) is important to understand wastewater treatment processes. For example, lower molecular weight organics were removed more efficiently than very high molecular weight organics by granular activated carbon (El-Rehaili and Weber, 1987).

A discrete distribution of the molecular weight distribution of DOC was determined using ultrafiltration (UF) membranes in stirred cells. Samples were processed in parallel (Figure 3.9) through an array of pressurized stirred cells with UF membranes of nominal molecular weight cutoffs of 1, 10 and 100 kDa. The size distribution was calculated as a difference in mass concentration between permeates from the respective membranes.
Samples were pre-filtered with 0.45 µm filters. Ultrafiltration experiments were performed at room temperature in 200 mL stirred cells (Figure 3.10) (Amicon Corp., Model 8200, US) with Millipore ultrafiltration membrane discs (Amicon Corp., YM series, US). The membranes chosen were of molecular cut-offs of 1 kDa (YM-1), 10 kDa (YM-10) and 100 kDa (YM-100). The permeates were collected and measured with a Shimadzu TOC-500 total organic carbon analyzer within 12h.

Figure 3.10 Stirred cell for obtaining molecular weight distribution.

60
3.5.8 Biostability of biomass

The biostability of the reactor’s biomass was assessed according to that specified by the Water Environment Research Foundation (2002). The additional volatile solids reduction (AVSR) test protocol is listed in the White House Document (USEPA, 1992).

The assessment was done based on volatile solids reduction (VSR) criteria. VSR had been the primary measurement of the degree of stabilization achieved in anaerobic sludge digestion. Farrell (1980) recommended a level of 38% VSR as achievable and indicative of stabilization. It was later decided that the criteria be reduced to 17% AVSR during bench-scale anaerobic batch digestion for 40 additional days at 30 to 40 °C (Switzenbaum et al., 1997).

The 100-mL Erlenmeyer flasks were used as reaction vessels for the AVSR bench-scale test. Each flask was sealed after filling biomass and a plastic tubing connected each flask to a manifold. The manifold was then connected to a water-sealed bubbler to prevent air from entering the flasks. It was important that the bubbler be placed below the flasks to avoid backflow of air or water into the manifold and flasks. A set-up of the test is shown in Figure 3.11.

![AVSR test set-up](image)

Figure 3.11 AVSR test set-up
Ten replicates of the samples were measured for total solids (TS) and volatile solids (VS) (APHA, 1998) concentration for each batch of sludge collected. Five of them were measured after 20 days and the rest were measured after 40 days. The determination of TS concentration was done in accordance to the standard methods described by APHA et al. (1998), in Part 2540A. Well-mixed samples were dried in an oven at 103 to 105 °C. The increase in the weight of the filter paper represented the total suspended solids mass. The total suspended concentration was calculated by dividing this mass by the volume of samples used initially. The determination of VS concentration was done in accordance to the standard methods described by APHA et al. (1998), in Part 2540A. The residue from the TS test was ignited to constant weight in a furnace at 550 °C. The weight lost on ignition was the volatile solids.

The results were calculated as

\[
FVSR = \frac{VS_{feed} - VS_{40}}{VS_{feed} - (VS_{feed} \times VS_{40})}
\]

(3.1)

Where FVSR = fraction volatile solids reduction
VS\text{feed} = volatile solids fraction of feed solids
VS\text{40} = volatile solids fraction after 40 days of bench-scale digestion

3.5.9 Microbiological analysis

3.5.9.1 DNA extraction

Genomic DNA from the mixed liquor sludge of the reactor was extracted using a chemical lysis and freeze and thaw method.
Mixed liquor biomass (about 1 mL) was collected and added with 600 µL of extraction buffer (Table 3.5), 60 µL of lysozyme (10 mg/mL) and 6 µL of acromopeptidase (1 mg/mL). The mixture was incubated at 37 °C for 30 minutes.

<table>
<thead>
<tr>
<th>Extraction buffer contents*</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris-HCl</td>
<td>0.1 M</td>
</tr>
<tr>
<td>EDTA</td>
<td>0.1 M</td>
</tr>
<tr>
<td>sucrose</td>
<td>0.75 M</td>
</tr>
</tbody>
</table>

*Adjusted to pH 7.5

The mixture was then added with 3 µL of protinase K (20 mg/mL) and 60 µL of SDS (10%) and incubated at 37 °C for at least 2 hours.

The mixture was subjected to freeze and thaw conditions at -80 °C (10 min) and 65 °C (3 min) for at least 3 cycles. Then 60 µL of CTAB (10%) and 84 µL of NaCl (5 M) were added for incubation to take place at 60 °C for 30 minutes.

Purification of the DNA was done using phenol, chloroform and IAA. Then the DNA was precipitated out using isopropanol or cold ethanol and incubating at -20 °C overnight. The DNA pellet obtained was air dried and dissolved again with pure water.

3.5.9.2 Polymerase chain reaction

After the DNA was extracted and purified, polymerase chain reaction (PCR) was done to amplify the 16S rRNA genes. PCR was done using TaKaRa Ex Taq (TaKaRa Bio Inc.), cool start method,
and strictly following the manufacturer’s recommendations. The primers used for PCR are the eubacterial universal primers 47f (5’-Cy5-CYTAACACATGCAAGTCG-3’) and 927r (5’-ACCGCTTTGTGCAGGGCCC-3’) (Amann et al., 1995) and archaea-specific primers 344f (5’-Cy5-ACGG GAGCAGCAGGCAG-3’) and 915r (5’-GTGCTCCCCGCAATTCC-3’) (Hendrik and Muyzer, 2001).

The primers were fluorescent-labeled to facilitate Terminal Restriction Fragment Length Polymorphism (T-RFLP) fingerprinting subsequently, which was carried out based on the protocol described by Liu et al. (1997).

The PCR products were purified using the QIAquick PCR purification kit (Qiagen), following the manufacturer’s recommendations, before performing T-RFLP.

3.5.9.3 Terminal Restriction Length Polymorphism (T-RFLP)

The purified PCR products were digested with three tetrameric restriction nucleases, MspI, AluI and RsaI. All digestions were carried out for at least 3h at 37 °C, according to the manufacturer’s instructions. The digested products were denatured at 65 °C for 10 min.

The enzyme-digested products were added with sample loading solution, CEQ DNA size standard kit-600, well mixed and loaded into the CEQ 8000 automated sequencer (Beckman Coulter) at 55 °C and 4.8 kV for 2h. The CEQ 8000-genetic analysis system software (Beckman Coulter) was then used to analyze the lengths of the fluorescents with internal standards.
4.1 Start-up study of AnSBR

It is well known that the start-up of anaerobic systems can be relatively long compared to aerobic systems because of the slow growth rate of anaerobic microorganisms. Thus this factor becomes an important consideration when adopting an anaerobic system for wastewater treatment. Once the start-up phase is successful, an anaerobic system can be left dormant for extended periods without severe deterioration in biomass properties since it is rather robust. Therefore, it is critical that the start-up be monitored closely.

A long HRT is hypothesized to be beneficial for the start-up period because it might indirectly help to increase the SRT of the system by preventing biomass washout and retaining the biomass in the reactor. Ndon and Dague (1997) showed that anaerobic systems with short HRT have the following problems:

- high organic loading resulting in the domination of microorganisms which are more dispersed;
- microorganisms have a longer settling velocity which resulting in poor solid-liquid separation; and
- high hydraulic loading which caused higher biomass loss in the effluent.

The start-up period of the AnSBR represented the duration of operation starting from D1 until the time when the performance of the AnSBR was relatively stable. The performance was assessed through suspended solids removal, organics removal as well as the quality and quantity of the
biogas produced. The performance of the AnSBR was considered to be stable when minimum fluctuation was observed in the removal efficiency even with variations in the quality of the feed wastewater.

Two HRTs were studied for the start-up of the AnSBR, namely, 16 and 8h. The AnSBR at their respective HRTs were operated continuously until the performance of the reactors stabilized. The start-up period when operating at a higher HRT of 16h was 110d when the sCOD removal efficiency stabilized while the start-up period at HRT of 8h was only 70d and the limiting factor was the methane production rate.

### 4.1.1 MLSS & MLVSS at start-up

The MLSS and MLVSS concentrations in the AnSBRs were monitored because they represented the amount of biomass in the reactor. The biomass level retained in the reactor is closely related to the food to microorganism (F/M) ratio as it affects the settleability of the biomass. In turn, the quality of the effluent is highly dependent on the extent of solids liquid separation. Thus the ability of AnSBR to retain biomass will ultimately affect the effluent quality. An F/M ratio of 0.1 to 1 g COD / g MLVSS · d for efficient treatment has been recommended (Ndon and Dague, 1997).

Table 4.1 showed a summary of the MLSS and MLVSS concentrations during the start-up period while Figure 4.2 illustrated the daily trend of the MLSS and MLVSS concentrations.

<table>
<thead>
<tr>
<th>HRT</th>
<th>n</th>
<th>MLSS (mg/L) min</th>
<th>MLSS (mg/L) max</th>
<th>MLSS (mg/L) average</th>
<th>MLVSS (mg/L) min</th>
<th>MLVSS (mg/L) max</th>
<th>MLVSS (mg/L) average</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 h</td>
<td>30</td>
<td>5080</td>
<td>10033</td>
<td>6576 ± 884</td>
<td>3410</td>
<td>6433</td>
<td>4473 ± 545</td>
</tr>
<tr>
<td>8 h</td>
<td>14</td>
<td>5100</td>
<td>8240</td>
<td>6933 ± 946</td>
<td>3620</td>
<td>5820</td>
<td>4871 ± 687</td>
</tr>
</tbody>
</table>

Anaerobic Sequencing Batch Reactor for the Treatment of Municipal Wastewater
Chapter 4 Results & Discussion
The MLSS and MLVSS concentration were slightly higher for the AnSBR operated at a start-up HRT of 8h compared to that at HRT of 16h. At a HRT of 16h, the average organic loading rate was 0.67 g COD/L.d, while at HRT of 8h, it was 1.25 g COD/L.d (Table 4.1). The organic loading rate at HRT of 8h was nearly twice of that at HRT of 16h. Two contradicting effects were expected. Firstly, the biomass concentration in the AnSBR with a higher organic loading rate was expected to be higher due to higher biomass production. Secondly, more washouts may occur due to a large amount of dispersed microorganisms at a higher organic loading rate. This would result in a decrease in the biomass concentration in the AnSBR. From the data collected, it was shown that the MLSS and MLVSS concentration was observed to be higher at a higher organic loading rate (i.e., HRT of 8h). Therefore, the effect of the higher biomass production was larger than the effect brought upon by the dispersed microorganism when operating at a higher organic loading rate. One of the concerns of high organic loading rate was the inability of the AnSBR to retain the
The biomass concentration in the AnSBR was similar at the HRT of 16 and 8h largely due to the same amount of effluent being decanted out of the reactor at the end of each cycle. Although at HRT of 16h, the AnSBR was operating at 4 cycles per day while at HRT of 8h, it was operating at 8 cycles per day, the volume of fill and decant per cycle was both 8.4 L (Chapter 3.3). This showed that the settling velocity of the solids in the reactor was similar at both HRTs because the time allocated for settling phase was the same.

The MLVSS to MLSS ratio was relatively higher at a higher organic loading rate (Table 4.2). A higher MLVSS to MLSS ratio reflected that the biodegradable organic portion of the biomass was higher. This could mean that at a lower HRT, the reaction time was shorter, thus a higher amount of organic solids remained un-hydrolyzed.

### 4.1.2 TSS and VSS at start-up

A summary of the TSS concentrations in the feed, effluent and the TSS removal efficiencies for a HRT of 16 and 8h start-up are presented in Table 4.3.
Table 4.3 TSS concentrations of feed, effluent and TSS removal efficiency.

<table>
<thead>
<tr>
<th>HRT</th>
<th>n</th>
<th>Feed SS (mg/L)</th>
<th>Effluent SS (mg/L)</th>
<th>SS removal efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 h</td>
<td>30</td>
<td>294 ~ 783 (423±115)</td>
<td>36 ~ 90 (56±14)</td>
<td>78 ~ 93 (86±3)</td>
</tr>
<tr>
<td>8 h</td>
<td>14</td>
<td>195 ~ 475 (342±85)</td>
<td>30 ~ 267 (142±63)</td>
<td>23 ~ 90 (57±21)</td>
</tr>
</tbody>
</table>

min ~ max (average ± standard deviation)

At a HRT of 16h (Figure 4.2a), solids removal efficiency was relatively high since the first few days of the operation of the AnSBR. By D5, the TSS removal efficiency of 87.7% was achieved. During the start-up period, there were little fluctuations in the effluent TSS concentration regardless of the variations in the feed TSS concentration.

![Graphs showing TSS concentration of feed and effluent and TSS removal efficiency at start-up, (a) HRT 16h; (b) HRT 8h.]

Figure 4.2 TSS concentration of feed and effluent and TSS removal efficiency at start-up, (a) HRT 16h; (b) HRT 8h.

Compared to the start-up at a HRT of 16h, the TSS concentrations of the effluent and the TSS removal efficiency at start-up period of HRT of 8h were fluctuating considerably throughout the entire start-up period (Figure 4.2b). The solids removal efficiency was relatively high on the first
few days of the operation of the AnSBR (about 80%). However, TSS removal performance
started to drop subsequently, and by D21, TSS removal efficiency was only 28%.

Table 4.4 VSS concentrations of feed, effluent and VSS removal efficiency.

<table>
<thead>
<tr>
<th>HRT</th>
<th>n</th>
<th>Feed VSS (mg/L)</th>
<th>Effluent VSS (mg/L)</th>
<th>VSS removal efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 h</td>
<td>30</td>
<td>210 ~ 550 (325±83)</td>
<td>20 ~ 88 (49±13)</td>
<td>74 ~ 94 (86±4)</td>
</tr>
<tr>
<td>8 h</td>
<td>14</td>
<td>170 ~ 440 (268±71)</td>
<td>13 ~ 217 (106±53)</td>
<td>27 ~ 95 (59±21)</td>
</tr>
</tbody>
</table>

A summary of the VSS concentrations of the feed, effluent and the removal efficiencies at the 2
start-up HRTs were presented in Table 4.4. It was apparent that the VSS removal trends were
very similar to that of the TSS. Since the beginning of the HRT of 16h start-up, the VSS
concentrations of the effluent remained relatively stable and low, regardless of fluctuations in the
feed VSS concentration (Figure 4.3a).

During the HRT of 8h start-up period, as shown in Figure 4.3b, the VSS concentration of the
effluent was low during the first 5d of operation. However, there was a deterioration of the
effluent VSS concentration after about 20d. Subsequently, the effluent VSS concentration
improved again after D40. In general, the effluent VSS data fluctuated considerably throughout
the 70d start-up period.
Figure 4.3 VSS concentration of feed and effluent and VSS removal efficiency during start-up, (a) HRT 16h; (b) HRT 8h.

The data showed that both the TSS and VSS concentration of the AnSBR effluent were lower during a higher start-up HRT. At a start-up HRT of 16h, the AnSBR was able to achieve an effluent TSS concentration which was nearly one-third of that of 8h and a VSS concentration which was half of that of 8h. With a higher amount of solids in the effluent at short HRT, the AnSBR might have a poor solids retention capacity.

However, the MLSS and MLVSS data collected (Table 4.1) showed that the MLSS and MLVSS concentrations in the AnSBR were actually higher for the short HRT during start-up. This did not mean there was a contradiction but rather, showed that more dense biomass were retained in the AnSBR at a shorter HRT. High hydraulic loading associated with the short-HRT systems helped to select bioparticles which had better settling characteristics and a higher tendency to bioflocculate. During a short HRT operation, the high hydraulic load caused lighter particles to be washed out of the AnSBR, thereby selecting more rapidly settling particles.
In addition, there was also an important difference in operation between these 2 reactors of different start-up HRT, the SRT. Many literatures have reported a recommended SRT for anaerobic treatment processes (Table 4.5). McCarty (1964) recorded that a minimum SRT of 10 and 15d were required for effective anaerobic treatment of wastewater at 25 and 35 °C respectively. In a tropical climate where the temperature is around 30 °C, an SRT of 10 to 15d was deemed satisfactory.

As the effluent of HRT of 16h start-up has a low solids concentration (~ 56 mg TSS/L), without sludge wasting, the SRT of the system could be more than 50d. Thus a SRT of 30d was chosen and maintained through daily sludge wasting. On the other hand, for HRT of 8h start-up, as the effluent had a relatively higher solids concentration (~ 142 mg TSS/L), a SRT of about 20d could only be achieved even without any sludge wasting. Thus it was decided that no sludge wasting would be carried out for the AnSBR operating at HRT of 8h. Meanwhile, the SRT of both AnSBR operating at different HRT had met the minimum requirements stated in literatures.

Table 4.5 Recommended minimum SRT for specific anaerobic process aim.

<table>
<thead>
<tr>
<th>Process</th>
<th>Required SRT (days)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effective anaerobic treatment of wastewater</td>
<td>20</td>
<td>Metcalf &amp; Eddy, 2003</td>
</tr>
<tr>
<td>Effective anaerobic treatment of wastewater at 25 °C</td>
<td>15</td>
<td>McCarty, 1964</td>
</tr>
<tr>
<td>Effective anaerobic treatment of wastewater at 35 °C</td>
<td>10</td>
<td>McCarty, 1964</td>
</tr>
<tr>
<td>To approach full conversion of solids anaerobically</td>
<td>30</td>
<td>Metcalf &amp; Eddy, 2003</td>
</tr>
<tr>
<td>Methanogenesis at 25 °C</td>
<td>5.9</td>
<td>Lawrence &amp; McCarty, 1970</td>
</tr>
<tr>
<td>Methanogenesis at 35 °C</td>
<td>3.2</td>
<td>Lawrence &amp; McCarty, 1970</td>
</tr>
</tbody>
</table>
4.1.3 COD concentration and removal efficiency at start-up

The tCOD concentrations of the feed and effluent very much mirrored the trends seen in the TSS concentrations. This was because the suspended solids in the feed or effluent contributed substantially to the tCOD measured.

During the HRT of 16h start-up period, the tCOD of the effluent was relatively stable regardless of fluctuations in the feed wastewater (Figure 4.4a). The effluent of HRT of 8h start-up period, on the other hand, was relatively higher in tCOD concentration and fluctuated considerably (Figure 4.4b). This could be quantified by the standard deviation presented in Table 4.6. The tCOD of the effluent for HRT of 16h start-up had a standard deviation of 19 while that of the HRT 8h start-up had a standard deviation of 89. Likewise, for the tCOD removal efficiency, the removal efficiency at HRT of 16h start-up was much higher than that of HRT of 8h start-up and also yielded more consistent results.

Figure 4.4 tCOD concentration of feed and effluent and tCOD removal efficiency at start-up, (a) HRT 16 h; (b) HRT 8 h.
Table 4.6 tCOD concentrations of feed, effluent and the removal efficiency.

<table>
<thead>
<tr>
<th>HRT</th>
<th>n</th>
<th>Feed tCOD (mg/L)</th>
<th>Effluent tCOD (mg/L)</th>
<th>tCOD removal efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 h</td>
<td>30</td>
<td>302 ~ 686 (444±92)</td>
<td>79 ~ 168 (120±19)</td>
<td>65 ~ 80 (73±3)</td>
</tr>
<tr>
<td>8 h</td>
<td>14</td>
<td>213 ~ 579 (418±107)</td>
<td>47 ~ 405 (216±89)</td>
<td>10 ~ 89 (45±23)</td>
</tr>
</tbody>
</table>

min ~ max (average ± standard deviation)

The deterioration of effluent quality in terms of tCOD during HRT of 8h start-up was a result of the biomass washouts as discussed in Section 4.1.2. This was a problem faced by low-HRT systems due to the formation of dispersed microorganisms. In addition, the high hydraulic loading at a short HRT resulted in a greater loss of dispersed microorganisms in the effluent.

Figures 4.5a and b showed the trends in sCOD concentration of the feed and effluent, as well as the sCOD removal efficiency at HRT of 16 and 8h start-up respectively. Results showed that at
HRT of 16h start-up, the sCOD removal performance of the reactor was far from satisfactory. The sCOD removal efficiency could hit as low as -68% during the first 20d of operation. There was a slight improvement in the removal efficiency when sCOD concentration of the feed wastewater increased but the removal efficiency dropped again shortly after. The sCOD removal efficiency at HRT of 8h start-up was higher on comparison. Although it was also fluctuating, it was always positive, unlike that of 16h.

The sCOD concentration of the feed was nearly twice during HRT of 8h start-up (average ~ 57 mg sCOD/L) compared to 16h (average ~ 104 mg sCOD/L) (Table 4.7). This could not be controlled as the wastewater was collected directly from a local wastewater reclamation plant. The sCOD concentration of the effluent for HRT of 16 and 8h was quite similar (average ~ 50 and 56 mg sCOD/L, respectively). This showed that although both AnSBR operating at 16 and 8h HRT were treating wastewater of different sCOD concentrations, the sCOD concentrations of the treated effluent was similar.

Table 4.7 sCOD concentrations of feed, effluent and the removal efficiency.

<table>
<thead>
<tr>
<th>HRT</th>
<th>n</th>
<th>Feed sCOD (mg/L)</th>
<th>Effluent sCOD (mg/L)</th>
<th>sCOD removal efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 h</td>
<td>30</td>
<td>18 ~ 123 (57±22)</td>
<td>26 ~ 85 (50±18)</td>
<td>-68 ~ 93 (5.8±36)</td>
</tr>
<tr>
<td>8 h</td>
<td>14</td>
<td>41 ~ 152 (104±33)</td>
<td>27 ~ 85 (56±15)</td>
<td>-49 ~ 70 (37±35)</td>
</tr>
</tbody>
</table>

min ~ max (average ± standard deviation)

The sCOD concentrations of the feed wastewater were found to be low, at only about 13% of the tCOD concentrations. Therefore the performance of the AnSBR in treating wastewater relied heavily on its ability to convert the organics into soluble portion.
When operating at HRT of 16h start-up, it took 110d before sCOD removal efficiency stabilized. All other performance parameters, for example, TSS and VSS removal efficiency, tCOD removal efficiency and biogas yield and quality, had stabilized and the results were quite consistent. The sCOD removal efficiency became the limiting factor for the AnSBR to achieve successful start-up. This was attributed to the slow growth of anaerobic microorganisms group. Table 4.8 showed the kinetic parameters of the methanogens, fermentors and heterotrophic bacteria.

Table 4.8 Kinetics of anaerobic microorganisms (Metcalf & Eddy, 2003).

<table>
<thead>
<tr>
<th>Group</th>
<th>Yield (g VSS/g COD)</th>
<th>Specific decay rate (g VSS/g VSS•d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanogens</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>Fermentors</td>
<td>0.10</td>
<td>0.04</td>
</tr>
<tr>
<td>Heterotrophs</td>
<td>0.50</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Table 4.8 showed that both the growth yields and decay constants of heterotrophic bacteria are higher than that of methanogens and fermentors. Thus hydrolysis of complex organics into soluble compounds is much faster than the substrate utilization rate of fermentative or methanogenic microorganisms. As at the initial start-up period, heterotrophs in the seed sludge dominated the microbial population in the AnSBR, hydrolysis of organics contributed to a higher amount of COD in the effluent. This explained why sCOD removal efficiencies were very low or even negative during the start-up period.

Methanogenic bacteria are also more susceptible to change in environmental conditions and food load than acidogenic bacteria. Inhibitions may be caused by starvation which will decrease the rate of destruction of VFAs (Pavlostathis and Giraldo-Gomez, 1991, Wu et al.,1995). There may
also be an accumulation of VFAs because acidogenic bacteria has a higher growth yield than methanogenic bacteria (Sawyer et al., 1994). All these factors may inhibit the growth of methanogens and decrease their growth yield further. Therefore, the cultivation of methanogens became a potential challenge to improve the sCOD removal efficiencies as well as the overall performance of the AnSBR.

The sCOD removal efficiencies at HRT of 8h start-up seemed to be much better than that of HRT of 16h start-up. The higher organic loading rate was thus seemed to be more beneficial for the growth of the slow-growing fermentors and methanogens. According to Monod’s equation (4.1), at high substrate concentration, the specific growth rate tends towards the maximum specific growth rate.

\[
\mu = \mu_m \frac{S}{K_s + S}
\]  

(4.1)

Where S = concentration of limiting nutrient  
\(\mu\) = specific growth rate  
\(\mu_m\) = maximum specific growth rate  
\(K_s\) = half saturation coefficient

This trend depicting a higher organic loading rate leading to a higher growth rate of the microorganisms was according to the Monod’s equation. It can thus be inferred that at a shorter HRT, sCOD efficiencies will be able to reach its maximum more quickly. Meanwhile, at long HRT start-up, the limiting factor to assess the duration for the system to achieve successful start-up was expected to be the sCOD removal efficiency. A shorter HRT could then mean a shorter period required for successful start-up. However, it is also known that too low an HRT deteriorates the effluent quality in terms of solids and organics concentration. Therefore, the
possibility of low sCOD removal efficiency delaying successful start-up can be reduced by using a shorter HRT, but the start-up period will still be delayed by other factors such as biogas yield and composition.

4.1.4 Biogas production at start-up

During HRT of 16h start-up, biogas production was only detected after 57d (Figures 4.6a and 4.7a). By the end of the start-up period, the composition of the biogas consisted of 60% methane gas and 2.9% carbon dioxide gas. During HRT of 8h start-up, biogas production was detected after 18d (Figures 4.6b and 4.7b). The composition of the biogas reached 62% methane gas and 4% carbon dioxide gas.

![Figure 4.6 Biogas composition at start-up, (a) HRT 16 h; (b) HRT 8 h.](image-url)
Biogas production is calculated from the stoichiometric breakdown of 1 mole of organic substrate under anaerobic conditions, producing 3 moles of methane and 3 moles of carbon dioxide (Metcalf and Eddy, 1991). According to Malina and Pohland (1992), theoretical calculations of methane percentage in the biogas produced by anaerobic wastewater treatment systems is 65 to 70%. Tan (2001) also reported a biogas composition of 66 to 75% consisted of methane gas. Therefore, the data collected in this study at both HRTs were comparable to the calculated and previously reported data. Hydrogen gas was not detectable at both HRTs.

By the end of the HRT of 16h start-up, the average biogas production rate was 0.97 L/d (Figure 4.7a), while for HRT of 8h start-up, the average biogas production rate was 1.7 L/d (Figure 4.7b). There were significant fluctuations in the amount of biogas produced. A sudden drop in biogas production rate was observed when there was a drop in pH in the reactor. When the pH dropped below 6.8, there was also a brief decrease in the amount of biogas produced. During the start-up
period, it was difficult to maintain the pH level in the reactor due to the varying feed sewage quality and thus resulting in fluctuating biogas production rate.

4.2 Performance of AnSBR at different HRT

The performance of the AnSBR was assessed when it was operating at different HRTs. The comparison was done based on the stable period of operation which was shown in Table 4.9. This meant that the start-up period for the system was excluded from the analysis in this section.

<table>
<thead>
<tr>
<th>Reactor</th>
<th>HRT</th>
<th>Period of operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>AnSBR 1</td>
<td>16 h</td>
<td>D110 to 160</td>
</tr>
<tr>
<td></td>
<td>6 h</td>
<td>D161 to 225</td>
</tr>
<tr>
<td>AnSBR 2</td>
<td>8 h</td>
<td>D71 to 165</td>
</tr>
</tbody>
</table>

For AnSBR 1, after successful start-up at HRT of 16h, which took 110d, the system was considered to enter the stable period of operation. After 160d of operation and data collection, the HRT was reduced to 6h. Therefore the period of operation for HRT of 6h was D161 to 225. For AnSBR 2, the start-up at HRT of 8h took 70d, thus the period of stable operation was from D71 to 250.

4.2.1 MLSS & MLVSS concentrations at HRT of 16, 8 and 6h

The MLSS and MLVSS concentrations in the AnSBR at HRT of 16 and 6h were presented in Figure 4.8. During the HRT of 16h stable operation, the MLSS and MLVSS concentrations were relatively stable (average ~ 6,772 mg MLSS/L and 4,636 mg MLVSS/L) (Table 4.10). On D160, the HRT was reduced to 6h. There was an initial dip in the MLSS and MLVSS concentrations to 4,680 mg MLSS/L and 3,140 mg MLVSS/L for the first 15d. Subsequently, the MLSS and
MLVSS concentrations in the AnSBR increased and became relatively stable at about 5,893 mg MLSS/L and 4,091 mg MLVSS/L.

Table 4.10 MLSS and MLVSS concentrations at different HRTs.

<table>
<thead>
<tr>
<th>HRT</th>
<th>n</th>
<th>MLSS (mg/L)</th>
<th>MLVSS (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>min</td>
<td>max</td>
</tr>
<tr>
<td>16h</td>
<td>12</td>
<td>6367</td>
<td>7333</td>
</tr>
<tr>
<td>8h</td>
<td>17</td>
<td>7560</td>
<td>9800</td>
</tr>
<tr>
<td>6h</td>
<td>10</td>
<td>4640</td>
<td>6640</td>
</tr>
</tbody>
</table>

Figure 4.9 showed the MLSS and MLVSS concentrations in the AnSBR during HRT of 8h operation. The MLSS and MLVSS concentrations during this period were relatively consistent at around 8,732 mg MLSS/L and 6,034 mg MLVSS/L, respectively.
Figure 4.9 MLSS & MLVSS concentration at HRT of 8h.

From the collected data, MLSS and MLVSS concentrations were the highest at HRT of 8h, about 29% and 30%, higher, respectively, than that of HRT of 16h. During operation at HRT of 16h, there was daily sludge wasting to maintain the SRT at 30d. During operation at HRT of 8h, there was no sludge wasting and the calculated SRT was about 15d. A higher SRT was shown to occur during higher MLVSS concentrations (Ndon and Dague, 1997). Therefore, the manipulation of the SRT of the AnSBR system was one of the important reasons which caused the MLSS and MLVSS concentrations to be higher at a lower HRT. Another reason for higher MLSS and MLVSS concentrations at shorter HRT was the shorter duration of the reaction phase. At a HRT of 16h, the reaction phase was longer, giving more time for the complex organic molecules to be converted to soluble monomer molecules by the microorganisms. More suspended solids could be converted into soluble compounds during the longer HRT of 16h, thus reducing the MLSS and MLVSS concentration.
On the contrary, at a lower HRT of 6h, its MLSS and MLVSS concentrations were still lower than that of HRT of 8h. The MLVSS and MLVSS concentrations at HRT of 8h were about 30% and 47% higher, respectively, than that of HRT of 16h. This was because, at a HRT of 6h, the decant point was lower than that of HRT of 8h. For HRT of 6h, the amount of decant was 11.25 L while for HRT of 8h, the amount of decant was only 8.4 L. This meant that the sludge blanket height retained in the reactor after every cycle was much lower at HRT of 6h.

Therefore, the amount of biomass solids which could be retained in the reactors was not only dependent on the HRT of the system but also on the operational conditions of the system, for example, the time allocated for reaction phase as well as the decant volume.

### 4.2.2 TSS and VSS concentrations of feed and effluent and removal efficiency

The TSS concentrations of the feed and effluent as well as the removal efficiencies during HRT of 16 and 6h operation were illustrated in Figure 4.10. During stable HRT of 16h operation, average TSS removal efficiency was relatively high (85%), with small fluctuations (±3%) (Table 4.11). On D160, the HRT was reduced to 6h. There was an immediate deterioration of effluent TSS concentration. On D171, the TSS removal efficiency was -11%. Subsequently, there were huge fluctuations in the TSS removal efficiency, ranging between -11% to 54%.

<table>
<thead>
<tr>
<th>HRT</th>
<th>n</th>
<th>Feed SS (mg/L)</th>
<th>Effluent SS (mg/L)</th>
<th>SS removal efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16h</td>
<td>12</td>
<td>320 ~ 610 (411±75)</td>
<td>40 ~ 80 (61±13)</td>
<td>79 ~ 88 (85±3)</td>
</tr>
<tr>
<td>8h</td>
<td>17</td>
<td>260 ~ 860 (519±182)</td>
<td>15 ~ 535 (190±143)</td>
<td>1 ~ 97 (60±26)</td>
</tr>
<tr>
<td>6h</td>
<td>10</td>
<td>260 ~ 445 (338±56)</td>
<td>180 ~ 350 (238±50)</td>
<td>-11 ~ 54 (28±18)</td>
</tr>
</tbody>
</table>

min ~ max (average ± standard deviation)
Figure 4.10 TSS concentration and removal efficiency at HRT of 16 and 6h.

Figure 4.11 showed the trends in TSS concentrations of the feed, effluent and removal efficiency during HRT of 8h operation. From the graph, it could be observed that there were substantial fluctuations in the TSS removal efficiency (average 60% ± 26%) (Table 4.11). On D98, the TSS removal efficiency was as high as 97% but on D108, it dropped to 4%. On D113, the TSS removal efficiency returned to 78%. This problem was both observed during HRT of 6 and 8h operation. This showed that there was a problem for the AnSBR to achieve consistent TSS removal efficiency at a short HRT.
During stable HRT of 16h operation, the VSS removal efficiency was relatively high and stable (Figure 4.12), in the range of 73% to 88% (Table 4.12). After the HRT was reduced to 6h, there was a sudden decrease in the VSS removal efficiency from 81% to 26%. It hit the lowest at -12% on D171. During HRT of 6h operation, VSS removal efficiency was much lower compared to that of 16h (-12% to 61%) and there were significant fluctuations. These observations were similar to that for TSS.

Table 4.12 VSS concentration of feed, effluent and removal efficiency at different HRT.

<table>
<thead>
<tr>
<th>HRT</th>
<th>n</th>
<th>Feed VSS (mg/L)</th>
<th>Effluent VSS (mg/L)</th>
<th>VSS removal efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 h</td>
<td>12</td>
<td>245 ~ 325 (283±32)</td>
<td>30 ~ 70 (52±11)</td>
<td>73 ~ 88 (82±4)</td>
</tr>
<tr>
<td>8 h</td>
<td>17</td>
<td>200 ~ 700 (424±161)</td>
<td>5 ~ 435 (126±127)</td>
<td>4 ~ 99 (70±25)</td>
</tr>
<tr>
<td>6 h</td>
<td>10</td>
<td>205 ~ 345 (266±44)</td>
<td>113 ~ 263 (175±43)</td>
<td>-12 ~ 61 (33±19)</td>
</tr>
</tbody>
</table>

min ~ max (average ± standard deviation)
Figure 4.12 VSS concentration and removal efficiency at HRT of 16 and 6h.

Figure 4.13 illustrated the trends in VSS concentration of the feed and effluent as well as the VSS removal efficiency during HRT of 8h operation. During HRT of 8h stable operation, fluctuations in the VSS concentration of the effluent and removal efficiency were significant. The VSS concentration of the effluent could be as low as 20 mg VSS/L (D98) and suddenly increase to 435 mg VSS/L just 5d later. Likewise, the VSS removal efficiency could be as high as 99% and could also be as low as 4%.
At a high HRT of 16h, the efficiency of the AnSBR, in term of solids and volatile solids removal, was the highest among the different HRTs investigated. Regardless of the quality of the feed wastewater, the effluent quality remained stable. This showed the reliability of the system at a long HRT.

The TSS and VSS removal efficiencies decreased significantly with a decrease in HRT. In other words, a lower HRT was likely to result in an effluent that had a higher solids concentration. Similarly, Ndon and Dague (1997) also found that high hydraulic loading tends to result in greater loss of dispersed microorganisms in the effluent. A lower solids removal efficiency meant that more solids was washed out during the decant phase, resulting in a lower MLSS and MLVSS level in the reactor. This showed that in a system with low HRT, a low mixed liquor biomass
concentration adversely affected the performance of the system and it resulted in a further loss of biomass.

![Photographs of samples](image)

Figure 4.14 Photographs of samples, (a) feedwater; (b) HRT 16h effluent (sampled on D150 of HRT 16h operation); (c) HRT 6h effluent (sampled on D55 of HRT 6h operation).

From the above data, it was apparent that the quality of the effluent seriously deteriorated when the HRT was shortened from 16 to 6h. Figure 4.14a, b and c show the photographs of the feed water, and effluents of the system operating at HRT of 16 and 6h respectively. There was a large difference in the appearance of the 16-h effluent and 6-h effluent. The amount of solids in the 6-h effluent was very high (180 to 350 mg TSS/L), making it nearly as turbid as the feed water (260 to 445 mg TSS/L).

### 4.2.3 COD concentration of feed and effluent and removal efficiency

Figure 4.15 illustrated the tCOD concentration of the feed, effluent and the COD removal efficiency during HRT of 16 and 6h operation. The tCOD removal efficiency was relatively high since the beginning of HRT of 16h operation, with an average of 74% ± 4% (Table 4.13). There were also no significant fluctuations. After the HRT was shortened to 6h, there was a sharp drop in tCOD removal efficiency, from 71% (D158) to 47% (D163). Subsequently, the tCOD removal efficiency dropped further to as low as -46% on D203.
The tCOD concentration and removal efficiency during HRT of 8h operation was presented in Figure 4.16. Throughout the 160d of HRT of 8h operation, the tCOD concentrations of the feed and effluent as well as the removal efficiency fluctuated considerably. There was no particular trend, suggesting that the effluent tCOD concentration and tCOD removal efficiency of the AnSBR at HRT of 8h were unpredictable.
The sCOD concentrations of the feed and effluent as well as the removal efficiency were presented in Figure 4.17. The sCOD removal efficiency at stable HRT of 16h operation was fluctuating between 22% and 75% (Table 4.14). When the HRT was reduced to 6h, there was a decrease of sCOD removal efficiency from 71% (D158) to 13% (D164). However, on D171, the sCOD removal efficiency returned to 53%. Subsequently, it stayed in the region of 36% to 58%.
Figure 4.17 sCOD concentration and removal efficiency at HRT of 16 and 6h.

Table 4.14 sCOD concentrations of feed, effluent and removal efficiency at different HRT.

<table>
<thead>
<tr>
<th>HRT</th>
<th>n</th>
<th>Feed sCOD (mg/L)</th>
<th>Effluent sCOD (mg/L)</th>
<th>sCOD removal efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 h</td>
<td>12</td>
<td>10 ~ 117 (54±24)</td>
<td>5 ~ 48 (27±10)</td>
<td>22 ~ 75 (48±15)</td>
</tr>
<tr>
<td>8 h</td>
<td>17</td>
<td>45 ~ 218 (101±41)</td>
<td>17 ~ 78 (47±15)</td>
<td>11 ~ 88 (47±21)</td>
</tr>
<tr>
<td>6 h</td>
<td>10</td>
<td>17 ~ 113 (66±22)</td>
<td>7 ~ 61 (39±19)</td>
<td>13 ~ 58 (43±36)</td>
</tr>
</tbody>
</table>

Fluctuations in sCOD removal efficiency during the stable HRT of 8h operation was larger than that of 16 and 6h. The sCOD removal efficiency could be as high as 88% on D98 and could dip as low as 11% on D113 (Figure 4.18). Although the fluctuations were large, unlike tCOD removal efficiency, sCOD removal efficiency remained in the positive region though out the 160d of operation. This showed that negative tCOD removal efficiency was due to the suspended solids wash-outs in the effluent.
Figure 4.18: sCOD concentration and removal efficiency at HRT of 8 h.

It was shown that the tCOD and sCOD removal efficiencies decreased significantly as the HRT decreased. This, again, corresponded to Ndon and Dague (1997) studies which showed that the high hydraulic loading at short HRTs tends to result in greater loss of dispersed microorganisms in the effluent. Table 4.15 showed a summary of results from several lab-scale studies. These studies were done with high-rate anaerobic reactors, including UASB, anaerobic filter, anaerobic expanded bed reactor and anaerobic fluidized bed.

The AnSBR operating at a HRT of 16h had a tCOD removal efficiency of 69 to 83%. This was comparable to the anaerobic filter treating sewage at a HRT of 14h which yielded a COD removal efficiency of 78% (Kobayashi et al., 1983). Sanz and Fdzpolanco (1989) also reported the COD removal efficiency of an anaerobic fluidized bed to be 90% when operating at a HRT of 26h. It
could be concluded that the AnSBR performance in terms of COD removal was on par with other high-rate anaerobic reactors.

Table 4.15 A summary of results of other anaerobic reactors.

<table>
<thead>
<tr>
<th>Reactor</th>
<th>waste</th>
<th>HRT(h)</th>
<th>Temp</th>
<th>Waste strength (mg COD/L)</th>
<th>COD removal efficiency (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>UASB</td>
<td>sewage</td>
<td>2.6 – 7.2</td>
<td>9 - 25</td>
<td>150 - 590</td>
<td>50 - 90</td>
<td>Sanz and Fdz-Polanco, 1989</td>
</tr>
<tr>
<td>Anaerobic filter</td>
<td>sewage</td>
<td>5</td>
<td>25 - 33</td>
<td>166 - 515</td>
<td>71</td>
<td>Raman and Khan, 1978</td>
</tr>
<tr>
<td>Anaerobic filter</td>
<td>sewage</td>
<td>24</td>
<td>20 - 35</td>
<td>288</td>
<td>78</td>
<td>Kobayashi et al., 1983</td>
</tr>
<tr>
<td>Anaerobic expanded bed reactor</td>
<td>Synthetic wastewater</td>
<td>0.33 - 6</td>
<td>10, 20, 30</td>
<td>50 - 600</td>
<td>38 - 90</td>
<td>Switzenbaum and Jewell, 1980</td>
</tr>
<tr>
<td>Anaerobic expanded bed reactor</td>
<td>sewage</td>
<td>0.08 - 3</td>
<td>20</td>
<td>88 - 186</td>
<td>80 (max)</td>
<td>Jewell et al., 1981</td>
</tr>
<tr>
<td>Anaerobic fluidized bed</td>
<td>Synthetic wastewater</td>
<td>1, 1.5</td>
<td>13 - 31</td>
<td>557 – 700</td>
<td>62 – 71</td>
<td>Marango and Campos, 1992</td>
</tr>
<tr>
<td>Anaerobic fluidized bed</td>
<td>sewage</td>
<td>2.7 - 26</td>
<td>10 - 25</td>
<td>150 - 590</td>
<td>60 - 90</td>
<td>Sanz and Fdz-Polanco, 1989</td>
</tr>
<tr>
<td>AnSBR</td>
<td>sewage 16</td>
<td>30</td>
<td>239 – 542</td>
<td>69 – 83</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td></td>
<td>sewage 8</td>
<td>237 - 1163</td>
<td>6 - 90</td>
<td>-46 – 50</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>sewage 6</td>
<td>207 - 587</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The performance of the AnSBR at shorter HRTs of 8 and 6h, compared to the reactors in Table 4.15, might not be as satisfactory in terms of COD removal efficiency. For the AnSBR operating at HRT of 6h, the average tCOD removal efficiency was only 21% while for the anaerobic filter at HRT of 5h, the removal efficiency was about 71% (Raman and Khan, 1978). The performance of the AnSBR also fell short of other high-rate anaerobic reactors, for example, the UASB operating at a HRT of 7.2h, which could yield a COD removal efficiency of about 90% (Sanz and
Fdzpolanco, 1989) and the anaerobic expanded bed reactor treating sewage at HRT of 3h could achieve a COD removal efficiency as high as 80% (Jewell et al., 1981). This showed that the AnSBR had limitations in achieving high organic removal efficiency when treating raw sewage at low HRTs.

However, Ndoun and Dague (1997) has shown that the AnSBR was actually very successful in treating synthetic wastewater, i.e. wastewater which does not contain much suspended solids. Average COD removal efficiencies were more than 80% even at a HRT of 12h. Therefore, the AnSBR would be more efficient in treating wastewater with low solids content.

4.2.4 BOD$_5$ concentration of feed and effluent and removal efficiency

Tables 4.16 and 4.17 showed a summary of the tBOD$_5$ and sBOD$_5$ concentration of the feed, effluent and their removal efficiencies at HRT of 16, 8 and 6h.

<table>
<thead>
<tr>
<th>HRT (h)</th>
<th>n</th>
<th>Feed tBOD (mg/L)</th>
<th>Effluent tBOD (mg/L)</th>
<th>tBOD removal efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 h</td>
<td>7</td>
<td>174 ~ 216 (199±14)</td>
<td>28 ~ 58 (43±10)</td>
<td>71 ~ 84 (78±4)</td>
</tr>
<tr>
<td>8 h</td>
<td>11</td>
<td>105 ~ 514 (242±130)</td>
<td>11 ~ 86 (37±21)</td>
<td>59 ~ 96 (82±9)</td>
</tr>
<tr>
<td>6 h</td>
<td>2</td>
<td>190 ~ 195 (192±3)</td>
<td>150 ~ 290 (220±70)</td>
<td>-49 ~ 21 (-14±35)</td>
</tr>
</tbody>
</table>

Table 4.16 tBOD$_5$ concentration of feed, effluent and removal efficiency at different HRT.

<table>
<thead>
<tr>
<th>HRT (h)</th>
<th>n</th>
<th>Feed sBOD (mg/L)</th>
<th>Effluent sBOD (mg/L)</th>
<th>sBOD removal efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 h</td>
<td>7</td>
<td>12 ~ 16 (13±1)</td>
<td>7 ~ 10 (8±1)</td>
<td>26 ~ 48 (38±9)</td>
</tr>
<tr>
<td>8 h</td>
<td>11</td>
<td>8 ~ 28 (16±5)</td>
<td>4 ~ 11 (7±2)</td>
<td>27~ 82 (54±17)</td>
</tr>
<tr>
<td>6 h</td>
<td>2</td>
<td>12 ~ 15 (13±1)</td>
<td>6 ~ 8 (7±1)</td>
<td>46 ~ 48 (47±1)</td>
</tr>
</tbody>
</table>

Table 4.17 sBOD$_5$ concentration of feed, effluent and removal efficiency at different HRT.
The tBOD$_5$ and sBOD$_5$ concentrations of the AnSBR feed and effluent and their removal efficiencies were shown in Figure 4.19. The tBOD$_5$ of the effluent during a HRT of 16h stable operation was quite stable and relatively low (28 to 58 mg/L). There were also minimum fluctuations in sBOD$_5$ concentration of the effluent (7 to 10 mg/L). The sBOD$_5$ concentration of the effluent was consistently low during stable HRT of 16h operation (7 to 10 mg/L) and the sBOD$_5$ removal efficiency was in the range of 26 to 48%.

When the HRT was reduced to 6h, there was a 5 times increase in the effluent tBOD$_5$ concentration from 55 mg/L (D156) to 290 mg/L (D202). This caused the tBOD$_5$ removal efficiency to drop from 73% to -49%. The tBOD$_5$ concentration of the effluent was very poor throughout the 66d of operation. On the other hand, there was no severe deterioration in the effluent sBOD$_5$ concentration and the sBOD$_5$ removal efficiency was 46 to 48 mg/L.

![Figure 4.19 BOD$_5$ concentrations of feed, effluent and removal efficiency at HRT of 16 and 6h.](image-url)
Figure 4.20 showed the tBOD$_5$ and sBOD$_5$ concentrations of the AnSBR feed and effluent and their removal efficiencies during stable HRT of 8h operation. During this period, the tBOD$_5$ concentration was relatively low (ranging from 11 to 86 mg/L) regardless of vast variations in the feed tBOD$_5$ (ranging from 105 to 514 mg/L). The average tBOD$_5$ removal efficiency was 54% ± 17%.

Figure 4.20 BOD$_5$ concentrations of feed, effluent and removal efficiency at HRT of 8 h.

Comparison of the BOD$_5$ data of the 3 HRTs showed that the tBOD$_5$ removal efficiency of HRT of 16 and 8h were quite similar, at 78% and 82%, but at 6h HRT, negative tBOD$_5$ removal efficiency was obtained, meaning that the tBOD$_5$ of the effluent was higher than that of the feed. This indicated that reducing the HRT lower than 8h was detrimental to the tBOD$_5$ performance of the AnSBR. The sBOD$_5$ removal efficiency was the lowest at HRT of 16h because the growth yield of the fermentors and methanogens were affected by the low organic loading rate. Similarly,
sBOD$_5$ removal efficiency at HRT of 8h was higher than that at 6h. This clearly demonstrated that operating the AnSBR at an HRT less than 8h adversely affect the performance of the AnSBR.

Most literatures studying anaerobic treatment processes did not give BOD$_5$ results as a means of assessing the performance of the systems. This was because anaerobic processes were more commonly used for high-strength wastewater or synthetic wastewater which its concentration of organics can be better represented by COD. Therefore, it was not possible to compare these BOD$_5$ results with past literatures.

Dart (1977) and Logan and Wangenseller (1993) also pointed out several problems inherent to the use of BOD$_5$ in analyzing the performance level of treatment processes. One of them is the high variability of results. It was also possible for results to be inconsistent in the presence of toxic species. However, COD tests did not differentiate between recalcitrant and biologically available organic matter. Therefore, BOD$_5$ data here was used to reinforce the COD results.

<table>
<thead>
<tr>
<th>HRT</th>
<th>Feed/effluent</th>
<th>16 h</th>
<th>8 h</th>
<th>6 h</th>
<th>feed</th>
<th>effluent</th>
<th>feed</th>
<th>effluent</th>
<th>feed</th>
<th>effluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed</td>
<td>199±14</td>
<td>43±10</td>
<td>242±130</td>
<td>137±21</td>
<td>192±3</td>
<td>220±70</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>effluent</td>
<td>381±79</td>
<td>98±24</td>
<td>579±241</td>
<td>270±163</td>
<td>408±94</td>
<td>323±39</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average tBOD$_5$ to tCOD ratio</td>
<td>0.47±0.18</td>
<td>0.45±0.10</td>
<td>0.45±0.22</td>
<td>0.43±0.14</td>
<td>0.47±0.18</td>
<td>0.50±0.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The results of BOD$_5$ were very similar to that of COD. Though, theoretically, there was no specific relationship between BOD$_5$ and COD, it was possible to develop a correlation for a
specific type of waste contaminant in a specific wastewater stream. Aziz and Tebbutt (1979) concluded that it was reasonable to expect a significant correlation between COD and BOD$_5$ for domestic sewage. However, the correlation changes as treatment proceeds and might only be applied with caution after a predetermined degree of treatment or at a specific location. Table 4.21 presented the average tBOD$_5$, tCOD and tBOD$_5$ to tCOD ratio of the feed and effluent at different HRT. The data showed that there was no significant change in the tBOD$_5$ to tCOD ratio between the feed and the effluent, it could be assumed that there was no significant change in the type of contaminants in them.

### 4.2.5 Biogas composition and production rate at HRT of 16, 8 and 6h

For operation at HRT of 16h (Figure 4.21), the composition of methane in the biogas was able to achieve 60%. On D143, the biogas was transferred to a new empty gas collector, thus there seemed to be a dip in the methane composition. The methane composition rose steadily subsequently, reaching a new stable methane composition of 74% at HRT of 6h, while at HRT of 8h (Figure 4.22), 71% of methane gas was obtained.

![Biogas composition at HRT of 16 and 6h.](image-url)
It took nearly 80d for methane composition of the biogas to reach the maximum of 60% compared with 55d to reach the same amount of methane gas composition when operating at HRT of 8 and 6h. Furthermore, at HRT of 8 and 6h, the maximum methane percentage in the biogas could reach 70%. Shorter HRT gave rise to a higher organic loading rate which enabled the reactor to achieve its maximum methane production rate faster and also resulted in biogas that had a higher percentage of methane gas.

Figure 4.23 showed the volume of biogas produced at HRT of 16 and 6h. The biogas production rate at stable HRT of 16h operation was fluctuating substantially between 0.14 and 2.83 L/d. Biogas production readings were taken daily and data has shown that the production rate differed daily. The HRT was reduced to 6h on D160 and there was an increase of biogas production rate.
immediately. From D164 onwards, the biogas production rate hovered around 2.83 L/d and showed a relatively consistent daily yield for nearly 25d.

On D191, there was a sudden decrease in the biogas production rate due to a pH upset in the AnSBR. The pH in the AnSBR increased to 11.2 due to excessive sodium carbonate dosing (nearly 5 L of 0.02 M sodium carbonate was added into the reactor in 2 cycles’ time) because of a malfunction of the pH dosing control. This pH upset did not cause any deterioration in the performance of the reactor in terms of solids and organics removal but it was reflected immediately in biogas production rate. Although the upset was rectified as soon as possible by the addition of a weak acid (0.1 M sulfuric acid) during the react phase to bring the pH back to the range of 6.8 to 7.2, the biogas production did not resume until D194 (0.57 L/d). However, this was still much less than the 2.83 L/d biogas production rate experienced before the upset.
By D201, which was 10d after the upset, the biogas production rate returned to 2.83 L/d. This showed that the AnSBR had the capability to withstand pH upsets and was robust enough to recover within days of upset. An interesting phenomenon was that the biogas production rate did not just return to the level before the upset, it further increased to 3.7 L/d (D208). For the next 18d, the biogas production rate was relatively consistent in the range of 3.4 to 4.0 L/d.

It could be observed that when the HRT of the system was decreased from 16 to 6h, there was an almost immediate increase in the volume of biogas produced per day. This was a different observation from Tan (2001) who reported that shock loadings caused an accumulation of volatile fatty acids which decreased the pH and inhibited methanogenic activity. As the limiting factor in substrate conversion in an anaerobic system was the methane production step, a sudden increase in organic loading may upset this delicate step. Methanogenesis might not be able to match the increase in acidogenesis, resulting in an accumulation of acids. According to Gujer and Zehnder (1983), variations in loading rates usually affect acetate decarboxylation or acetoclastic methanogenesis, thus the anaerobic reactor will operate in the acidic regime.

The amount of biogas produced by the AnSBR was not significantly affected by the increase in organic loading partly because

(1) pH control was incorporated in this study. Sodium carbonate was used to maintain the pH within 6.8 to 7.2,

(2) at long HRT, the wastewater had sufficient alkalinity to buffer the accumulation of acids, and

(3) the biomass was able to acclimatize to the shock loading and increase the methanogenesis rate quickly to overcome the acid production
Figure 4.24 showed the volume of biogas produced during the stable HRT of 8h operation. Unlike the operation at HRT of 6h, the AnSBR operation was relatively smooth and there was a steady increase in the biogas production rate from D70 to 79. Subsequently, the biogas production rate remained in the range of 2.83 to 3.68 L/d. The graph also showed that the biogas production rate was relatively stable during the period of HRT of 8h stable operation.

Figure 4.24 Volume of biogas produced at HRT of 8 h.

One of the main factors affecting the methane gas production rate was the amount of methanogenic bacteria in the reactor. Methanogenic bacteria are relatively less tolerant to changes in environmental conditions and food load than acidogenic bacteria. Inhibitions may be caused by starvation which will decrease the rate of destruction of VFAs and methane producing rate (Pavlostathis and Giraldo-Gomez, 1991, Wu et al., 1995).
Table 4.19 Biogas and methane production rate at different HRTs.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>16 h</th>
<th>8 h</th>
<th>6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biogas production (L/d)</td>
<td>0.14 ~ 2.83</td>
<td>1.42 ~ 3.68</td>
<td>0 ~ 3.97</td>
</tr>
<tr>
<td></td>
<td>(0.99)</td>
<td>(3.21)</td>
<td>(2.74)</td>
</tr>
<tr>
<td>Methane production rate (L/d)</td>
<td>0.09 ~ 1.73</td>
<td>0.88 ~ 2.78</td>
<td>0 ~ 2.83</td>
</tr>
<tr>
<td></td>
<td>(0.52)</td>
<td>(2.25)</td>
<td>(1.29)</td>
</tr>
<tr>
<td>Specific methane production rate (L CH$_4$/ g tCOD removed)</td>
<td>0.005 ~ 0.50</td>
<td>0.012 ~ 0.94</td>
<td>0 ~ 0.37</td>
</tr>
<tr>
<td></td>
<td>(0.06)</td>
<td>(0.11)</td>
<td>(0.17)</td>
</tr>
</tbody>
</table>

Format: min ~ max (average)

The calculated theoretical maximum methane yield is 0.375 L CH$_4$/g tCOD removed (Malina and Pohland, 1992). Table 4.19 showed the biogas and methane production rate at different HRT. The average methane yield of the AnSBR was only 16% of the theoretical maximum yield at HRT of 16h, 29% at HRT of 8h and 45% at HRT of 6h. Earley (1990) reported methane yields of 90 to 100% of the theoretical maximum value on his study on anaerobic sequencing batch reactors. Tan (2001) also reported a methane yield of 89 to 95% of the theoretical maximum yield. Apparently, the methane yield of the AnSBR observed in this study was much lower compared with that reported by previous researchers. However, it has to be noted that their reactors were used to treat high strength industrial wastewater. On the other hand, there were few reports on the methane yield for treatment of low-strength municipal wastewater. It could be concluded that, based on methane yield, the AnSBR was more efficient in treating high strength industrial wastewater than low strength municipal wastewater.

4.2.6 Microbial study using T-RFLP fingerprinting

The DNA was extracted and the 16S rRNA targeted to study the microbial community in the AnSBR mixed liquor at different HRTs. This was aimed to study the dynamics of the microbial community in the AnSBR at different HRT. Figure 4.45 showed the T-RFLP fingerprint profiles at different HRTs using bacteria-specific primers and AluI restriction enzyme. The samples were
from AnSBR 1 on D141 (HRT of 16h), AnSBR 2 on D181 (HRT of 8h) and AnSBR 1 on D220 (HRT of 6h, without PAC dosage). The prominent peaks were identified and the corresponding bp values were also shown in the figures. T-RFLP profiles using bacteria-specific primers showed several peaks. This showed that the bacteria residing in the reactor at HRT of 16, 8, and 6h consisted of quite a variety of species. This did not come as a surprise because the AnSBR treated municipal wastewater which contained a variety of pollutants or organic compounds, unlike some reactors which only treat a specific type of pollutant. Therefore, more than one type of bacteria was needed for efficient treatment in the AnSBR.

Comparing the T-RFLP profiles of HRTs of 16, 8 and 6h, there were many peaks that were present in all three HRTs (104, 120, 191, 192, 194, 201, 243, and 254 bp). This showed that although there were a number of dominant species, these species were the same regardless of the operating HRT. Upon closer examination, there was a slight difference in the abundance of each dominant species at different HRTs. At HRT of 16 and 6h, the highest peak was 191 bp but at HRT of 8h, the 191 bp peak was not as high as 194 bp peak. This might not be due to the HRT but due to a difference in the sludge. The AnSBR was operated at a HRT of 16h and then reduced to HRT of 8h, so both operations used the same batch of seed sludge. The operation of HRT of 8h was done in another AnSBR which had a different batch of seed sludge. Therefore, this could be the reason for the slight difference in the composition of the bacterial community.

There were other slight differences in the peaks but these were due more to the sludge source than the operating HRT. Therefore, it was concluded that a change in HRT did not result in a significant change in the bacterial community structure.
Figure 4.25  T-RFLP fingerprint profile at different HRTs using bacteria-specific primers and Alul enzyme.
Figure 4.26 T-RFLP fingerprint profile at different HRTs using archaea-specific primers and AluI enzyme.
Figure 4.46 showed the T-RFLP fingerprint profiles at different HRTs using archaea-specific primers and AluI restriction enzyme. The samples were also from AnSBR 1 on D141 (HRT of 16h), AnSBR 2 on D181 (HRT of 8h) and AnSBR 1 on D220 (HRT of 6h, without PAC dosage).

Unlike the T-RFLP profiles obtained using bacterial primer, the archaea profiles consisted of only two prominent peaks, showing that there were only 2 species of archaea dominant in the AnSBR at all 3 HRTs. The expected archaea in the AnSBR will be the methanogens. At HRT of 16h, profile showed a 286 bp peak and a 289 bp peak as the major T-RFs in addition to a number of minor T-RFs.

At HRT of 8h, there was a significant shift in the T-RFLP profile. There were still 2 prominent peaks but they were 227 bp and 228 bp. This was similar to the T-RFLP profile of HRT of 6h. This could mean that at HRTs of 8 and 6h, a totally different archaeal population represented by 227 bp and 228 bp T-RFs replaced those present at HRT of 16h which were represented by 286 bp and 289 bp T-RFs. The shift in microbial community was probably due to the different organic loading rate which affected the selection of microbial population.

Correlating this piece of information with the performance of the reactor during the respective period, an induction about the AnSBR can be made. The specific methane production rate at HRT of 8 and 6h was much higher than that of HRT of 16h. This could mean that the methanogens represented by 227 bp and 228 bp were more efficient in converting the substrates into the methane gas than those represented by 286 bp and 289 bp.

It is worthwhile to note that the batch of sludge in the AnSBR operating at HRT of 16 and 6h was the same but there was a complete change in the archaea community as shown by the T-RFLP profiles. The SRT of the reactor at HRT of 16 and 6h differed significantly. At HRT of 16h, the
SRT was 30d, while at HRT of 6h, the HRT was only 6 to 10d. This could mean that the archaeal population represented by 286 bp and 288 bp T-RFs during HRT of 16h were slow growers which could not cope with the short SRT. In their place, the archaeal population represented by 227 bp and 228 bp dominated at HRT of 8 and 6h and these were the relatively more efficient population which corresponded to a higher specific methane production rate. Thus, it could be possibly concluded that this archaeal population represented by 227 bp and 228 bp peaks were the ones responsible for the better biogas performance during short HRTs.

The change in HRT did not result in significant changes in the bacterial population but caused a complete change in the archaeal population. This showed that bacteria had a larger tolerance towards changes in organic loading rate of the system than archaea. It also showed that the archaeal population was quite vulnerable and easily affected by operating conditions, yet they are also the rate limiting factor in the anaerobic process. Therefore, the operating conditions anaerobic processes have to be well-examined to come out with the most optimum ones.

4.2.7 Microscopic study of mixed liquor biomass

The mixed liquor biomass was observed under the microscope to analyze the bioparticle size. Representative microscopic images were taken at the 3 HRTs, 16, 8 and 6h, to track the trend of the biofloc size at different operating conditions (Figure 4.27 to 4.29). The bioflocs were characterized by an agglomeration of biomass that was illuminated under the light microscope to show a black coloration which is typical of anaerobic sludge.

These microscopic studies of the mixed liquor biomass were useful in determining the biofloc size and to investigate the presence of granules in the AnSBR. Images of the biomass were taken and the equivalent diameter of the flocs were calculated using an image analytical software (Leica Qwin).
Figure 4.27 Microscope image of mixed liquor biomass at HRT of 16h.

Figure 4.27 showed the microscope image of the mixed liquor biomass sampled from the reactor on D155 when the AnSBR was operating at a HRT of 16h. There was a huge presence of flocculent biomass and it was difficult to determine the shape and sizes of the biomass. The largest particle in this image was calculated to be of only 0.17 mm in equivalent diameter. Although the MLSS concentration during HRT of 16h was relatively high, it consisted mostly of dispersed flocs.
Figure 4.28 Microscope image of mixed liquor biomass at HRT of 8h.

Figure 4.28 showed the microscope image of the mixed liquor biomass sampled from the reactor on D210 when the AnSBR was operating at a HRT of 8h. The image still showed that a large proportion of the bioflocs were dispersed flocs. The largest particles were about 0.19 mm in equivalent diameter. There was a slight increase in the size of particles and also their numbers. This showed that a lower HRT or higher organic loading rate favored the formation of larger bioflocs. This also explained why the MLSS concentration was higher in the AnSBR during HRT of 8h compared to that of 16h.
Figure 4.29 showed the microscope image of the mixed liquor biomass sampled from the reactor on D220 when the AnSBR was operating at a HRT of 6h. Most of the flocs were still dispersed but there was a greater number of large bioflocs. The average equivalent diameter was about 0.29 ± 0.20 mm. Larger bioflocs were starting to form. They were of irregular and angular shapes.

Table 4.20 Summary of equivalent diameter of bioflocs at different HRT.

<table>
<thead>
<tr>
<th>HRT</th>
<th>16 h</th>
<th>8 h</th>
<th>6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.17 (max)</td>
<td>0.19 (max)</td>
<td>0.29±0.20</td>
</tr>
</tbody>
</table>

*Format: average ± standard deviation*

Table 4.20 showed a summary of the equivalent diameter of bioflocs calculated from microscopic images at different HRT. There was a significant increase in the biofloc size when HRT was 6h. compared to HRT of 16 and 8h. During HRT of 6h operation, a substantial amount of solids washout was observed (average TSS removal efficiency ~28%). These washouts could have resulted in the loss of dispersed bioflocs while the denser and heavier bioflocs were retained in...
the AnSBR. In conclusion, the biofloc size was found to increase with a decrease in HRT and this was likely to be the result of higher TSS loss in the effluent.

4.3 Enhancement of AnSBR performance using PAC

PAC was added into the AnSBR at a mass ratio (mass of PAC to mass of mixed liquor biomass) of 10, 15 and 20% when the AnSBR was operated at an HRT of 6h. The AnSBR was operated continuously for 160d at HRT of 16h. From D161 to 225, the AnSBR was operated at HRT of 6h for 65d. A 10% PAC was introduced into the AnSBR from D226 to 295 (70d) while the HRT was still 6h. The PAC dosage was increased to 15% for the next 70d (D296 to 365) and then to 20% for the last 70d (D366 to 435). The performance of the AnSBR was monitored and analyzed during this period to study the influence of PAC on the AnSBR treatment process.

4.3.1 MLSS and MLVSS before and after PAC addition

Figure 4.30a showed the fluctuation of MLSS and MLVSS concentration in the AnSBR during different PAC dosage. The addition of 10% PAC on D226 resulted in an average MLSS concentration of 6,610 mg/L (Figure 4.30b). This was a 12% increase from the MLSS concentration in the AnSBR when no PAC was added when the PAC dosage was increased to 15%, reduced the MLSS concentration by 13% to 5,774 mg/L but a further increase of PAC dosage to 20% increased the MLSS concentration slightly to 6,172 mg/L.

The MLVSS concentration changes showed a similar trend to that of MLSS. The addition of 10% PAC resulted in a 17% increase in the average MLVSS concentration from 4,091 mg/L (no PAC) to 4,796 mg/L (Figure 4.26b). At the PAC dosage of 15%, the MLVSS concentration reduced by 14% (4,122 mg MLVSS/L) but a further increase of PAC dosage to 20% increased the MLVSS concentration by 7% to 4,406 mg/L.
The results showed that the addition of 10% PAC to the AnSBR increased the MLSS and MLVSS concentrations in the reactor. This was expected as PAC can act as a support medium for biofilm formation. However, it was not clear whether granules or just larger biomass flocs were formed in the AnSBR when PAC was added. It was well known that microorganisms that form granules of 0.5–5 mm diameter exhibit high settling velocity; and thus, resist wash-out from the reactor even at high hydraulic loads. Granules also showed excellent settling properties due to higher buoyant densities (Lens et al., 1998) and because of their large sizes. As solid-liquid separation was a major factor affecting the quality of the effluent, granulation was highly desired. Granules were denser than suspended biomass, so there would be a marked increase in MLSS concentration if they were formed.
Another point to note here was the non volatile suspended solids (NVSS) fraction in the MLSS. If PAC were part of the granulation formed in the AnSBR, the NVSS percentage should increase. Table 4.21 showed a calculation of the NVSS fraction in the AnSBR before PAC was added and at different PAC dosage. It seemed that there was no significant increase in the NVSS fraction in the AnSBR mixed liquor at 10, 15 or 20% PAC dosage. This could suggest that the addition of PAC did not aid in the formation of the highly desirable granules.

<table>
<thead>
<tr>
<th>No PAC</th>
<th>10% PAC</th>
<th>15% PAC</th>
<th>20% PAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLSS (mg/L)</td>
<td>5893</td>
<td>6610</td>
<td>5774</td>
</tr>
<tr>
<td>MLNVSS (mg/L)</td>
<td>1802</td>
<td>1814</td>
<td>1652</td>
</tr>
<tr>
<td>MLNVSS fraction</td>
<td>0.31</td>
<td>0.27</td>
<td>0.29</td>
</tr>
</tbody>
</table>

From Figure 4.30b, at HRT of 6h, the average MLSS and MLVSS concentrations were lower than that at HRT of 16h. This was due to the washout of biomass in response to the shock loading. With the addition of 10% PAC, the average MLSS and MLVSS concentrations increased. This meant that the PAC has indeed increased the compactability of the biomass. However, with 15 and 20% PAC, the average MLSS and MLVSS concentrations dropped. In fact, the average MLSS and MLVSS concentrations during 15% PAC operation were similar to that when no PAC was added. The average MLSS and MLVSS concentration during 20% PAC were only slightly higher than that of no PAC. In addition, it was noted that there were no significant washouts. Thus, it could only be concluded that although the addition of PAC increased the MLSS and MLVSS concentration, an increase in the dosage might not be necessarily beneficial to the treatment process.

Specchia and Gianetto (1982) reported that the presence of PAC reduces the growth of activated
Anaerobic Sequencing Batch Reactor for the Treatment of Municipal Wastewater

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sludge which was concluded using a mathematical modeling of an activated sludge process. If the same theory were to be applied to the AnSBR, the decrease in average MLSS and MLVSS concentrations with the increase in PAC dosage could be explained.

4.3.2 TSS and VSS concentration of feed and effluent

Figure 4.31 showed the fluctuation in TSS concentration in the feed and effluent and the TSS removal efficiency during the different PAC dosage. From the results, it seemed that there were slightly more fluctuations in the TSS removal efficiency at a PAC dosage of 10%. It was also apparent that the TSS concentration of the effluent during 20% PAC dosing was more consistent. When 10% PAC was added, the average TSS removal efficiency of the AnSBR increased by 129%. With 15% PAC, there was a further increase in average TSS removal efficiency from 64 to 67% and with 20% PAC, the average TSS removal efficiency was the highest (70%) (Figure 4.32).

![Figure 4.31 TSS concentration of feed, effluent and the TSS removal efficiency at different PAC dosage.](image)

Figure 4.31 TSS concentration of feed, effluent and the TSS removal efficiency at different PAC dosage.
Figure 4.32 Average TSS and VSS removal efficiency at different operating conditions.

Figure 4.33 showed the VSS concentration of the feed and effluent, and the VSS removal efficiency. With the addition of 10% PAC, there was an immediate improvement in the VSS removal efficiency from 33 (no PAC) to 66%. There were substantial fluctuations in the VSS removal efficiency at 10% PAC dosage as it could range from as high as 86.7% on D261 and as low as 43% on D285. There was significantly less fluctuation with 15% PAC dosage, as the range of VSS removal efficiency was only in the range of 54 to 82%. With 20% PAC, the VSS removal efficiency was relatively stable (63 to 75%).
Figure 4.33 VSS concentration of feed and effluent, and the TSS removal efficiency at different PAC dosage.

Figure 4.32 also showed that there was a large improvement in the TSS and VSS removal efficiencies when PAC was added into system. The improvement in TSS and VSS removal efficiencies could be due to the change in sludge quality in the AnSBR. The sludge quality can change due to two reasons:

1. PAC particles acted as a base for biomass to grow on. Biofilm formation was encouraged and the larger bioparticles did not necessarily qualify as granules but their increase will help to improve sludge settleability.

2. PAC was able to remove SMPs which was known to adversely affect the kinetic activity and the flocculating and settling properties (Chudoba, 1985b). Ultimately, an improvement in the sludge settleability will mean less tendency for the biomass to
washout during the decant phase. This resulted in an effluent that had less TSS and VSS, thus increasing the TSS and VSS removal efficiencies.

4.3.3 tCOD and sCOD concentration of feed and effluent

Figures 4.34 and 4.35 showed the fluctuations in the feed and effluent tCOD and sCOD concentrations and the tCOD removal efficiency when different dosage of PAC was added into the AnSBR. Average tCOD removal efficiencies at different operating conditions were shown on Figure 4.32.

![Figure 4.34 tCOD concentration of feed, effluent and the tCOD removal efficiency at different PAC dosage.](image)

Figure 4.34 tCOD concentration of feed, effluent and the tCOD removal efficiency at different PAC dosage.
During HRT of 6h operation without any PAC addition, the average tCOD removal efficiency was 21%. The addition of 10% PAC at HRT 6h increased the tCOD removal efficiency by more than double, while at 15% PAC and 20% PAC, the tCOD removal provided was nearly 3 times more than the tCOD removal when no PAC was added. It was noted that the tCOD concentration of the feed wastewater suddenly increased to 1,018 mg/L on D326 and 1,163 mg/L on D357 when the PAC dosage was 15%. There was no significant deterioration in the effluent tCOD concentration during this period, showing that with 15% PAC, the AnSBR was able to withstand shock organic loadings even at a low HRT of 6h.

Although the tCOD removal efficiency at HRT 6h with PAC was still unable to match that of HRT of 16h (75% tCOD removal), it was still a huge progress. It was thus reasonable to conclude that PAC aided in the removal of substances which were hard to degrade. A similar result was observed by Specchia and Gianetto (1984) who studied the performance of an activated sludge system and reported that the addition of PAC led to a marked improvement in removal capacity, particularly with respect to organic substances. The COD removal efficiency of their system rose...
from 55.8 to 75.6% when 0.2 g PAC/L feed wastewater was added. This improvement was about 35% and the AnSBR in this study yielded an improvement of 120% with only 10% PAC dosage. Thus the PAC addition in this study was successful in enhancing the COD removal of the AnSBR.

Figure 4.36 showed the sCOD concentrations of the feed and effluent as well as its removal efficiency at different PAC dosage. With 10% PAC dosage, the sCOD removal efficiency fluctuated between 36% (D246) and 74% (D261). With 15% PAC dosage, the range of fluctuation of sCOD removal efficiency was slightly smaller, between 52% (D321) and 79% (D296). With 20% PAC, the sCOD removal efficiency had minimum fluctuations within the range of 53 to 71%. This showed that with higher PAC dosage, the range of fluctuations of the sCOD removal efficiency became smaller, meaning that the reliability of the AnSBR in terms of sCOD performance had improved.

![Figure 4.36 sCOD concentration of feed, effluent and the sCOD removal efficiency at different PAC dosage.](image-url)
There was an increase in the sCOD removal efficiency when PAC was added. With an increase in dosage of PAC, there was an increase in the sCOD removal efficiency. This showed that PAC helped to improve the sCOD of the effluent. It is well known that sCOD in treated effluent consists mainly of SMPs which are difficult to biodegrade. These SMPs may be precursors for THM or chlorinated organics formation (Namkung and Rittmann, 1998) and pose as a problem for downstream processes, especially if water reclamation process were to be incorporated. Activated carbon is recognized as the most effective method for the removal of SMP. Although the common practice is to use GAC to remove SMPs in aerobically-treated effluent, the same results were expected for PAC removal of SMPs from anaerobically-treated effluent. However, very little has been reported regarding the use of activated carbon with an anaerobic process. Thus, this study demonstrated that PAC has the potential of improving the anaerobically treated effluent in terms of tCOD and sCOD removal.

The addition of PAC at HRT of 6h did not result in an improvement on the sCOD removal as high as the improvement on tCOD removal. The addition of 10% PAC only increased the sCOD removal by 26% but increased tCOD removal by 120%. With the addition of 15 and 20% PAC, the sCOD removal improved by more than 40% while the tCOD removal efficiency was more than 3 times of the tCOD removal efficiency when no PAC was added. The tCOD and sCOD data sets presented for HRT of 6h without PAC in Chapter 4.2.3 showed that tCOD removal efficiencies were very poor (21%) but the sCOD removal efficiencies were better (43%). Thus, the main problem with the AnSBR COD performance at HRT of 6h without PAC had more to do with the COD contributed by the suspended solids in the effluent. The improvement of tCOD removal efficiency brought about by the addition of PAC was due to the ability of the PAC to reduce the suspended solids washed out with the decanted effluent. On the other hand, the PAC did not significantly enhance the methanogenic activity in the AnSBR, thus the sCOD removal efficiency improvement was not as significant as that of tCOD removal efficiency.
In addition to the improvement in COD removal efficiency, another significant improvement with PAC addition was the stabilization of COD removal. At 10% PAC dosage, the standard deviation was 32% while at 15 and 20% PAC dosage, it was only 11% and 7% respectively. This was also reflected in Figure 4.29 where more fluctuations in the tCOD removal efficiency were observed at 10% PAC dosage. This showed that the addition of PAC at a higher dosage did not only improve the average tCOD removal efficiency, it also reduced the fluctuations in the tCOD removal efficiency. This meant that with higher PAC dosage, the reliability of the AnSBR improved. This was attributed to the adsorbing capacity of PAC in presence of peak loads and subsequent desorbing. It might also be related to the protection offered by PAC to microorganisms from inhibitory or toxic compounds. The PAC could adsorb these inhibiting or toxic compounds and the biofilm formed on the PAC surface acted as a protection for the microorganism. Munz et al. (2007) also observed that the addition of PAC resulted in a more stable COD removal efficiency during a study on MBR-PAC process. For full-scale application, reduction in the effluent COD variability will be useful to ease the control of the downstream treatment processes or water reuse plans.

4.3.4 tBOD$_5$ and sBOD$_5$ of feed and effluent

Figures 4.37 and 4.38 showed the fluctuation of feed and effluent tBOD$_5$ and sBOD$_5$ concentrations and their removal efficiencies during the different PAC dosage. Average tBOD$_5$ and sBOD$_5$ removal efficiencies at different PAC dosage were shown on Figure 4.36.

During HRT of 16h operation, the average tBOD$_5$ removal efficiency in the reactor was 79% while at HRT of 6h operation, the average tBOD$_5$ removal efficiency was -35%. After 10% PAC was added, the tBOD$_5$ removal efficiency was 0%, with 15% PAC, it was 63% and with 20% PAC, it was 66%.
Figure 4.37 tBOD₅ concentration of feed, effluent and the tBOD₅ removal efficiency at different PAC dosage.

During HRT of 16h operation, the average sBOD₅ removal efficiency in the reactor was 38% while at HRT of 6h operation, the average sBOD₅ removal efficiency was -40%. After 10% PAC was added, the tBOD₅ removal efficiency was 0%, with 15% PAC, it was 66% and with 20% PAC, it was 66%.
Figure 4.38 sBOD\textsubscript{5} concentration of feed, effluent and the sBOD\textsubscript{5} removal efficiency at different PAC dosage.

Figure 4.39 Average tBOD\textsubscript{5} and sBOD\textsubscript{5} removal efficiency at different PAC dosage.

The addition of PAC again resulted in an improvement in the tBOD\textsubscript{5} and sBOD\textsubscript{5} removal efficiencies (Figure 4.39). With an addition of 15 and 20% PAC, the tBOD\textsubscript{5} removal efficiency was nearly as high as that of HRT of 16h. The sBOD\textsubscript{5} removal efficiency was also higher than...
that of HRT of 16h. This showed that PAC helped to remove the hard to biodegrade substances in the system. It might include the SMPs which were known to be biodegradable but difficult to degrade because their kinetics of degradation is a lot slower than simple substrates. Therefore, an increase in BOD$_5$ removal efficiency was witnessed with an increase in PAC dosage.

### 4.3.5 Biogas composition and production rate

Figure 4.40a showed the biogas composition while Figure 4.40b showed the average percentage of methane in the biogas when operating at HRT of 6h and different PAC dosage. There were little changes in the biogas composition at different PAC dosage. The average methane percentage in the biogas was 75% for 10% and 20% PAC, and 76% for 15% PAC. In addition, the standard deviation was very small at all 3 dosagea (1.16 to 1.42).

![Figure 4.40a Composition of biogas at different PAC dosage](image)

![Figure 4.40b Average percentage of methane at different PAC dosage](image)

The volume of biogas produced at different PAC dosage was presented in Figure 4.38a. At each PAC dosage, there were little fluctuations in the volume of biogas produced daily. When the PAC
dosage increased, a slight increase in the volume of biogas produced was observed. Figure 4.41b showed the increase in biogas production rate clearly with the average amount of biogas produced. With 10% PAC added, the biogas yield was 3.71 L/d, with 15% PAC, there was a 6% (3.93 L/d) and with 20% PAC, there was a further increase of 6% (4.18 L/d). The amount of biogas produced increased with the addition of PAC. This could not be due to a higher amount of biomass retained in the AnSBR resulting in more biogas producing microorganisms because there was no increasing trend in the MLSS and MLVSS concentrations in the AnSBR with increasing PAC dosage. Thus, it could be due to the adsorption and inhibition of toxic compounds, like detergents and other chlorinated products found in the municipal wastewater, which can adversely affect the performance of the biogas producing microorganisms.

The results have shown that there was no significant change in the methane percentage in the biogas and only a slight increase in the biogas production rate at different PAC dosage. This

Figure 4.41 (a) Volume of biogas produced at different PAC dosage, (b) Average amount of biogas produced at different PAC dosage.
indicated that higher amount of PAC in the AnSBR did not significantly increase the methane gas production rate as it did with suspended solids and organics removal efficiency. Thus, it was concluded that the addition of PAC could improve the solids-liquid separation efficiency of the AnSBR more than it could increase the biological activity in the AnSBR.

A summary of the biogas production rate and composition was shown in Table 4.22 to compare the biogas production quantity and quality at different operating conditions, namely, HRT of 16, 8 and 6h and PAC dosage of 10, 15 and 20%.

Table 4.22 Biogas and methane gas production rate at different operating conditions.

<table>
<thead>
<tr>
<th>HRT</th>
<th>PAC dosage</th>
<th>16 h</th>
<th>8 h</th>
<th>6 h</th>
<th>6 h</th>
<th>6 h</th>
<th>6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No PAC</td>
<td>0.97</td>
<td>3.21</td>
<td>2.74</td>
<td>3.71</td>
<td>3.93</td>
<td>4.18</td>
</tr>
<tr>
<td>Biogas production (L/d)</td>
<td>No PAC</td>
<td>60</td>
<td>74</td>
<td>71</td>
<td>75</td>
<td>76</td>
<td>75</td>
</tr>
<tr>
<td>% methane gas</td>
<td>10%</td>
<td>2.74</td>
<td>2.78</td>
<td>2.99</td>
<td>3.14</td>
<td>0.45</td>
<td>0.39</td>
</tr>
<tr>
<td>Methane gas production rate (L/d)</td>
<td>15%</td>
<td>3.71</td>
<td>3.93</td>
<td>4.18</td>
<td>0.23</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>Specific methane production rate (L CH₄ / g tCOD removed)</td>
<td>20%</td>
<td>3.93</td>
<td>4.18</td>
<td>0.23</td>
<td>0.23</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The biogas production rate was the highest (4.18 L/d) when the AnSBR was operating at HRT of 6h and 20% PAC dosage. This was expected because the organic loading rate was the highest at HRT of 6h and PAC has slightly improved the biogas production rate by adsorbing the toxic compounds. The methane gas composition in the biogas was quite similar for HRT 8 and 6h (with and without PAC dosage) operation, in the range of 71 to 76%. The methane production rate was calculated from the biogas production rate and methane gas composition. The highest methane
gas production rate was also found at HRT of 6h and 20% PAC. The specific methane production rate was the highest during HRT 6h operation without the addition of any PAC. This was also a calculated value. As the amount of tCOD removed during this operation condition was exceptionally low, the specific methane production rate calculated became very high.

The tCOD removal efficiency was very low during HRT of 6h without the addition of PAC mainly because of the failure of AnSBR to sustain good solids removal at low HRT. It was not conclusive that the methanogenic activity was the highest during this operation regime just by looking at the high specific methane production rate.

4.3.6 Microscopic image study of mixed liquor biomass

Figure 4.42 showed the microscope image of the mixed liquor biomass sampled from the reactor when the AnSBR was operating at a HRT of 6h and with addition of 10% PAC. There were a large number of large flocs and less dispersed flocs. This explained why the MLSS concentration was higher when PAC was added. The average equivalent diameter of the bioflocs was about 1.3 ±0.58 mm.
Figure 4.42 Microscope image of mixed liquor biomass at HRT of 6h with 10% PAC.

Figure 4.43 Microscope image of mixed liquor biomass at HRT of 6h with 15% PAC.
Figures 4.43 and 4.44 showed the microscope image of the mixed liquor biomass sampled from the reactor when the AnSBR was operating at a HRT of 6h and with addition of 15 and 20% PAC respectively. The bioflocs from 15 and 20%PAC were very similar under the microscope. The average equivalent diameters were about 1.4 mm (15% PAC - ±0.44 mm and 20% PAC - ±0.48 mm).

Table 4.23 Equivalent diameter of bioflocs at different operating condition.

<table>
<thead>
<tr>
<th>HRT</th>
<th>6 h</th>
<th>6 h</th>
<th>6 h</th>
<th>6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAC</td>
<td>No PAC</td>
<td>10% PAC</td>
<td>15% PAC</td>
<td>20% PAC</td>
</tr>
<tr>
<td>Equivalent diameter (mm)</td>
<td>0.29±0.20</td>
<td>1.3±0.58</td>
<td>1.4±0.44</td>
<td>1.4±0.48</td>
</tr>
</tbody>
</table>

Table 4.23 showed a summary of the equivalent diameter of the bioflocs at different PAC dosage.

With 10% PAC addition, the average biofloc size was nearly 4.5 times that when no PAC was
added. In Chapter 4.3, it was discussed that there was increase in the average MLSS and MLVSS concentrations in the AnSBR when 10% PAC dosage was administered. This increase in average biofloc size was the likely reason for the increase in average MLSS and MLVSS concentrations. There was also a significant increase in average TSS removal efficiency from 28 (no PAC) to 64% (10% PAC). This also proved that the improvement in average TSS removal efficiency when 10% PAC was added was due to the increase in the average biofloc size. Meanwhile, the average TSS removal efficiency was similar when 15% and 20% PAC was added (67 and 70%, respectively). The average biofloc size was also similar at 15 and 20% PAC dosage.

In conclusion, the microscope images showed that the biofloc size were important reasons for the MLSS and MLVSS concentrations changes at different operating conditions. They were also responsible for the TSS removal performance of the AnSBR. The addition of PAC was able to increase the biofloc sizes and improved the TSS removal efficiencies.

### 4.4 Biostability of sludge in the AnSBR

One of the considerations of using anaerobic treatment for municipal wastewater treatment was that the sludge wasted from the system was comparable to that of an anaerobic digester. If the sludge wasted from the AnSBR were stable enough, there would be no additional processes needed to treat the sludge before disposal. Biosolids were usually stabilized to

- reduce pathogens
- eliminate offensive odors
- inhibit, reduce or eliminate the potential for putrefaction.

These phenomena occur when microorganisms are allowed to flourish in the organic fraction of the sludge. Therefore the biological reduction of the volatile content is essential to prevent this.
The additional volatile solids reduction (AVSR) test has long been the primary measure of the degree of stabilization achieved. Stability assessment for anaerobically digested sludge is based on volatile solids reduction (VSR) criteria from the Part 503 rule for vector attraction reduction (VAR). Jeris et al. (1985) concluded that anaerobically digested sludge could be considered to have been stabilized if the FVSR was less than 17% during bench-scale anaerobic batch digestion for 40 additional days at 30 to 40 °C was achieved. This is the basis of Option 2 for VAR in the Part 503 rule. This criterion is also recommended as a basis for assessing sludge stability (Switzenbaum et al., 1997).

The sludge collected from the anaerobic sludge digester in the Ulu Pandan Water Reclamation Plant acted as a control for this set of data. Mixed liquor sludge was collected during each different operating condition. Figure 4.45 showed the results of the biostability of anaerobic digester sludge and AnSBR sludge from different operating condition. All the sludge samples collected fulfilled the criterion for sludge biostability.
Figure 4.45 Biostability of anaerobic digester sludge and AnSBR sludge from different operating conditions.

The %VSR of the digester sludge was 12.5%. The sludge from AnSBR operating at HRT of 16h had the lowest %VSR of 5.1% while the sludge from the AnSBR operating at HRT of 8, 6h and with PAC addition had similar %VSR ranging from 8.5% to 9%. This could be because the SRT of the system at HRT of 16h was much longer than the SRT of the rest. At HRT of 16h, the SRT of the system was maintained at 30d, while at HRT of 8 and 6h, the SRT was only around 6 to 10d. A higher SRT meant that the biomass was retained in the system for a longer period of time and thus can achieve higher sludge stability. There was, however, no significant difference in the biostability of the sludge when PAC was added.

In conclusion, the sludge wasted from the AnSBR at all the operating conditions here met the international standard proposed to assess the stability of biomass.
4.5 Molecular weight distribution of AnSBR feed and effluent

The results from the ultrafiltration analysis revealed the apparent molecular weight (AMW) distribution. Table 4.24 shows the apparent molecular weight distribution of the AnSBR feed and effluent.

Table 4.24 Apparent molecular weight distribution data of AnSBR feed and effluent at different HRTs.

<table>
<thead>
<tr>
<th>Sample</th>
<th>MW&gt;100kDa</th>
<th>10kDa&lt;MW&lt;100kDa</th>
<th>MW&lt;1kDa</th>
<th>MW&lt;1kDa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw wastewater</td>
<td>3</td>
<td>44.8±0.9</td>
<td>5.2±0.5</td>
<td>1.1±0.05</td>
</tr>
<tr>
<td>HRT of 16h effluent</td>
<td>3</td>
<td>35.6±0.8</td>
<td>4.3±0.05</td>
<td>15.4±1.0</td>
</tr>
<tr>
<td>HRT 8h of effluent</td>
<td>3</td>
<td>23.2±0.2</td>
<td>5.1±0.05</td>
<td>14.6±0.8</td>
</tr>
<tr>
<td>HRT 6h of effluent</td>
<td>3</td>
<td>23.4±0.2</td>
<td>6.0±0.5</td>
<td>17.2±0.8</td>
</tr>
</tbody>
</table>

The data shows that for both the feed and effluent of the AnSBR, the bulk of the soluble residual COD appeared to be less than 1 kDa. This fraction contained the VFAs as well as the products of degradation of high MW materials like amino acids and smaller SMP.

There was also a substantial amount of material that was of MW more than 100 kDa. These were likely to be cell wall fragments, exopolysaccharides, humic acids, nucleic acids and proteins. There are more high-MW fractions (i.e. >100 kDa) in long HRT (16h) effluents than short HRT (6 and 8h) effluents. This could be due to the longer SRT achieved by a longer HRT, thus, it contained a higher proportion of products of cells lysis, such as cell walls which had a high MW.
From the AMW data collected from the AnSBR study, the data clearly showed a bimodal distribution. Majority of the compounds in the AnSBR effluent had AMW of greater than 100 kDa and less than 1 kDa. This was similar to what was found by Barker and Stuckey (1999) who concluded in their review paper that both aerobically- and anaerobically- treated effluents usually displayed a bimodal molecular weight distribution. Among other researches which showed similar results, Kuo and Parkin (1996) tried to find out the molecular weight distribution of anaerobic effluents from anaerobic chemostats. It was found that the molecular weight distribution was very similar to that of aerobic effluents, i.e. bimodal with the majority of SMP having AMW less than 1 kDa or greater than 10 kDa while that of AMW between 1 and 10 kDa was comparatively lower. Schiener et al. (1998) examined the MW distribution of SMP in an ABR and found that it exhibited a bimodal distribution with 30% having MW less than 1 kDa and 25% greater than 100 kDa. This was very similar to what was found for the AnSBR effluents.

The AnSBR feed also observed a bimodal patterns. However, this was not entirely supported by literatures. Parkin and McCarty (1981a) found that 50 to 60% of the soluble organic nitrogen and sCOD in untreated municipal wastewater had AMWs of less than 1.8 kDa as measured by GPC. Barker and Stuckey (1999) concluded in their review paper that reactor influents generally exhibit skewed non-normal MW distributions with a predominance of the very low MW fraction (<0.5 kDa). However, it was not stated clearly what type of influent was referred to and it seemed like they referred to the entire variety of substrate possible, including domestic wastewater, synthetic wastewater, glucose, phenol, sucrose, etc.
Table 4.25 Apparent molecular weight distribution data of AnSBR effluent at different PAC dosage.

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>MW&gt;100kDa % w/w</th>
<th>10kDa&lt;MW&lt;100kDa % w/w</th>
<th>kDa&lt;MW&lt;10kDa % w/w</th>
<th>MW&lt;1kDa % w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRT 6h, no PAC</td>
<td>3</td>
<td>23.4±0.5</td>
<td>6.0±0.05</td>
<td>17.2±1.4</td>
<td>53.4±6.8</td>
</tr>
<tr>
<td>HRT 6h, 10% PAC</td>
<td>3</td>
<td>16.1±0.6</td>
<td>7.2±0.1</td>
<td>18.9±1.9</td>
<td>57.8±10.2</td>
</tr>
<tr>
<td>HRT 6h, 15% PAC</td>
<td>3</td>
<td>16.2±0.6</td>
<td>5.4±0.1</td>
<td>19.2±1.0</td>
<td>59.2±5.5</td>
</tr>
<tr>
<td>HRT 6h, 20% PAC</td>
<td>3</td>
<td>21.5±0.8</td>
<td>3.8±0.1</td>
<td>19.5±1.6</td>
<td>55.2±3.5</td>
</tr>
</tbody>
</table>

Table 4.25 showed that there was a significant decrease in the percentage of organic compounds which are more than 100 kDa when PAC was added into the system. On the other hand, there was a significant increase in the lower MW fraction, i.e. less than 1 kDa. This means that PAC was more successful in removing the high MW fractions than lower MW fractions. Barker et al. (1999) also had the same conclusion whereby low MW (i.e. MW <1 kDa) compounds were more difficult to adsorb than the high MW compounds (i.e. MW>1 kDa).

It is well known that the adsorbabilities of organic compounds onto PAC are dependent mainly on their physiochemical properties. For neutral organic compounds, many efforts have been made to quantitatively describe the adsorbabilities by using several physicochemical parameters (Giusti et al., 1974; Abe et al., 1980, 1981, 1982, 1983; Suzuki and Takeuchi, 1993) and one of the main parameter is molecular weight. The amount and rate of adsorption involve thermodynamic aspects (Chianga et al., 2002) and are functions of the chemical nature of the activated carbon (Chuanga et al., 2003; Karanfil and Kilduff, 1999; Kunio et al., 2001; Othman et al., 2000), which depends on the activation method employed (Cheremisinoff, 1980).
Chapter 5  Conclusions & Recommendations

5.1 Conclusions

The performance of the AnSBR treating municipal wastewater from a local water reclamation plant was studied under two start-up conditions, namely a HRTs of 16 and 8h. The HRT was then reduced to 6h to study its capability to perform under a low HRT. A comparison was done to evaluate the performance of the AnSBR under 3 HRTs, 16, 8 and 6h. Subsequently, powdered activated carbon was added into the AnSBR (HRT of 6h) at a dosage of 10, 15 and 20% to investigate its effect on the performance of the AnSBR. The following are the conclusions drawn from the study.

5.1.1 Start-up study

Using a start-up HRT of 16h, it took 110d for the reactor to achieve stable performance. With start-up HRT of 8h, it only took 70d. The limiting factor for achieving stability at higher HRT was the sCOD removal efficiency while at that lower HRT was the biogas quality and quantity.

It was found that a higher organic loading rate increased the amount of solids retained in the AnSBR. The MLVSS to MLSS ratio also seemed to be relatively higher at a higher organic loading rate, meaning that the biodegradable organic portion of the biomass was also higher.

The suspended solids removal efficiency was higher at HRT of 16 than 8h and this was interrelated to the SRT of the system. The tCOD removal efficiency was also higher and more
consistent when operating at a higher HRT of 16h. This meant that at a higher HRT, the effluent produced had a higher quality. However, the sCOD removal efficiency was relatively low at HRT of 16h compared to that of 8h. This was attributed to the slow growth rate of the fermentors and methanogens compared to other hydrolytic and acidogenic bacteria. Their slow growth rate was further aggravated by the low organic loading rate at high HRTs, resulting in a suboptimal condition for these rate limiting microorganisms to grow.

The higher methane production at a lower HRT also showed that a higher organic loading rate was preferred by the anaerobic microorganisms.

This start-up study has shown that a higher start-up HRT will be able to produce a higher-quality effluent in terms of suspended solids and COD concentration but the biogas production will be slower and less than that of a shorter HRT. This resulted in a prolonged start-up period. Therefore a compromise has to be reached between effluent quality and biogas production to choose a suitable HRT for start-up.

5.1.2 Performance of AnSBR at different HRTs

The amount of solids that were retained in the AnSBR was lower at HRT of 16 than 8h because of the longer duration of the reaction phase. This gave the microorganisms more time to convert the complex organic molecules to soluble monomer molecules. More suspended solids could be converted into soluble compounds during the longer HRT of 16h, thus reducing the MLSS and MLVSS concentrations. However, the amount of solids that were retained in the AnSBR was lower at HRT of 6 than 8h. This contradicted to the reason suggested previously. The lower MLSS and MLVSS at HRT of 6h was because of the lower decant point in the AnSBR reactor. Therefore, the amount of biomass solids which could be retained in the reactors was not only
dependent on the HRT of the system but also on the respective operational conditions of the system.

The suspended solids and organics removal efficiencies were the highest at HRT of 16h. They decreased significantly with a decrease in HRT. A shorter HRT resulted in an increase in the amount of dispersed flocs in the AnSBR, causing a deterioration of the solid-liquid separation efficiency.

A shorter HRT resulted in a higher specific methane production rate. However, the methane yield of the AnSBR was not comparable to that presented by previous researches which treated high strength industrial wastewater. This showed that the AnSBR was more efficient in treating high strength industrial wastewater than low strength municipal wastewater.

T-RFLP profiles showed that a change in HRT did not result in a significant change in the bacterial community structure. There were slight differences in the peaks but these were due more to the sludge source than the operating HRT. The T-RFLP profiles for the archaea consisted of only two prominent peaks, showing that there were only 2 species of archaea dominant in the AnSBR at all 3 HRTs. The change in HRT did not result in significant changes in the bacterial population but caused a complete change in the archaeal population. This showed that bacteria had a larger tolerance towards changes in organic loading rate of the system than archaea.

The microscopic study of the mixed liquor biomass at different HRT showed a slight increase in the biofloc sizes when the HRT was decreased from 16 to 8h. However, the biofloc size during HRT of 6h was significantly bigger than that of HRT of 8h. This was caused by the higher loss of TSS in the effluent during a shorter HRT operation.
5.1.3 Enhancement of AnSBR performance using PAC

The MLSS and MLVSS concentrations in the AnSBR were expected to increase with an increase in the PAC dosage, but results have shown that there was no clear relationship between the two.

There was a large improvement in the TSS and VSS removal efficiencies when PAC was added into system. The improvement in TSS and VSS removal efficiencies could be due to the change in sludge quality in the AnSBR. Larger and denser flocs could be formed and SMPs, which were known to adversely affect the kinetic activity and the flocculating and settling properties, were removed by the PAC. Therefore, there was less tendency for the biomass to experience a washout during the decant phase.

There was also an increase in the tCOD and sCOD removal efficiency when PAC was added. PAC aided in the removal of substances which were hard to degrade. Another significant finding was that PAC resulted in a more stable COD removal efficiency. The PAC could adsorb these inhibiting or toxic compounds and the biofilm formed on the PAC surface acted as a protection for the microorganism. This reduction in the variability of the effluent COD will be very useful, especially for a plant which has further downstream treatment processes or water reuse plans.

The BOD$_5$ removal efficiency was also found to be higher with a higher PAC dosage. BOD$_5$ included the SMPs and was known to be biodegradable but difficult to degrade because their kinetics of degradation is a lot slower than simple substrates. PAC was capable of removing the SMP, thus improving the BOD$_5$ removal efficiency.

The amount of methane gas produced increased with the addition of PAC. This showed that the addition of PAC favored the growth conditions of the methanogens and thus improving their
efficiencies. The amount of methane produced also became more stable as the daily production volume and the biogas and the methane percentage in the biogas was quite consistent.

The PAC has indeed improved the performance of the AnSBR in terms of effluent quality and biogas production rate. It was also found that a PAC dosage of 10% (w/w) was sufficient to improve the performance of the AnSBR and any further increase in dosage had resulted in only slight differences.

The microscopic study of the mixed liquor biomass showed that the addition of PAC resulted in a significant increase in the biofloc sizes. The images also showed that the amount of PAC dosed played little part in affecting the size of the bioflocs.

5.1.4 Biostability of sludge in AnSBR

The sludge wasted from the AnSBR at all the operating conditions here met the international standard (Option 2 for VAR in the Part 503 rule) proposed by Switzenbaum et al.(1997) to assess the stability of biomass. The biostability of the sludge wasted depended mainly on the SRT of the system rather than the HRT. The PAC added did not cause any significant changes in the biostability of the sludge.

5.1.5 Molecular weight distribution of AnSBR feed and effluent

The AMW data showed that both the AnSBR feed and effluent has a bimodal distribution. Majority of the compounds had AMW of greater than 100 kDa and less than 1 kDa. There were also more high-MW fractions (i.e >100 kDa) in long HRT (16h) effluents than short HRT (6 and 8h) effluents because of a longer SRT.
PAC was found to be more successful in removing the high MW fractions than lower MW fractions. This was because there was a significant decrease in the percentage of organic compounds which are more than 100 kDa and a significant increase in the lower MW fraction, i.e., less than 1 kDa, when PAC was added into the system.

5.2 Recommendations

This research study has shown that the AnSBR has the potential of treating municipal wastewater. In the meantime, optimization of the AnSBR system is desired because the performance of the AnSBR was still a far cry from aerobic systems in terms of suspended solids and organics removal. Operation parameters other than HRTs can be studied to improve the AnSBR’s performance. For example, Shizas and Bagley (2002) found that longer fill times for a given cycle time improve the performance of an anaerobic reactor treating a rapidly acidifying substrate such as glucose. They also found that for identical fill-to-cycle time ratios at the same loading rate, shorter cycle times with lower initial substrate concentrations provide improved ASBR performance. Therefore, the AnSBR has the potential of achieving better performance by optimizing such operation parameters.

Considering the quality of the AnSBR-treated effluent, aerobic post treatment is required for effluents to reach discharge standards. There are many literatures studying anaerobic-aerobic systems treating a variety of wastewater type (He et al., 2007; Sánchez et al., 2007; Pophali et al., 2007; Mossvi and Madamwar, 1997). Aerobic post treatment systems are not only required for further suspended solids and organic removal, it is also needed for the removal of nutrients like nitrogen and phosphorus. The challenges in treating anaerobically-treated effluent will be different from treating raw sewage.
As the AnSBR was used to treat raw sewage water, the variations in the incoming wastewater becomes a concern. The flow variation, in particular, was expected to affect the performance of the AnSBR. It is important to study whether the AnSBR will be able to cope with a short-term change in HRT, in terms of the quality of the effluent as well as its consistency.

Shock toxic loadings on the AnSBR may adversely affect the performance. Although the anaerobic treatment process is known to be very robust, once upset, it will take a very long time to recover. Toxic compounds, such as surfactants, detergents, disinfectants and other chlorinated compounds from industrial sources can be occasionally found in municipal wastewater system. A study of the effects of such toxicity on the AnSBR and the AnSBR’s tolerance of these compounds will be beneficial in understanding the risks involved in applying the technology in full-scale context.


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