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Development of bio-physicochemical treatment system for molasses-based wastewater

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DEVELOPMENT OF BIO–PHYSICOCHEMICAL TREATMENT SYSTEM FOR MOLASSES–BASED WASTEWATER

BY

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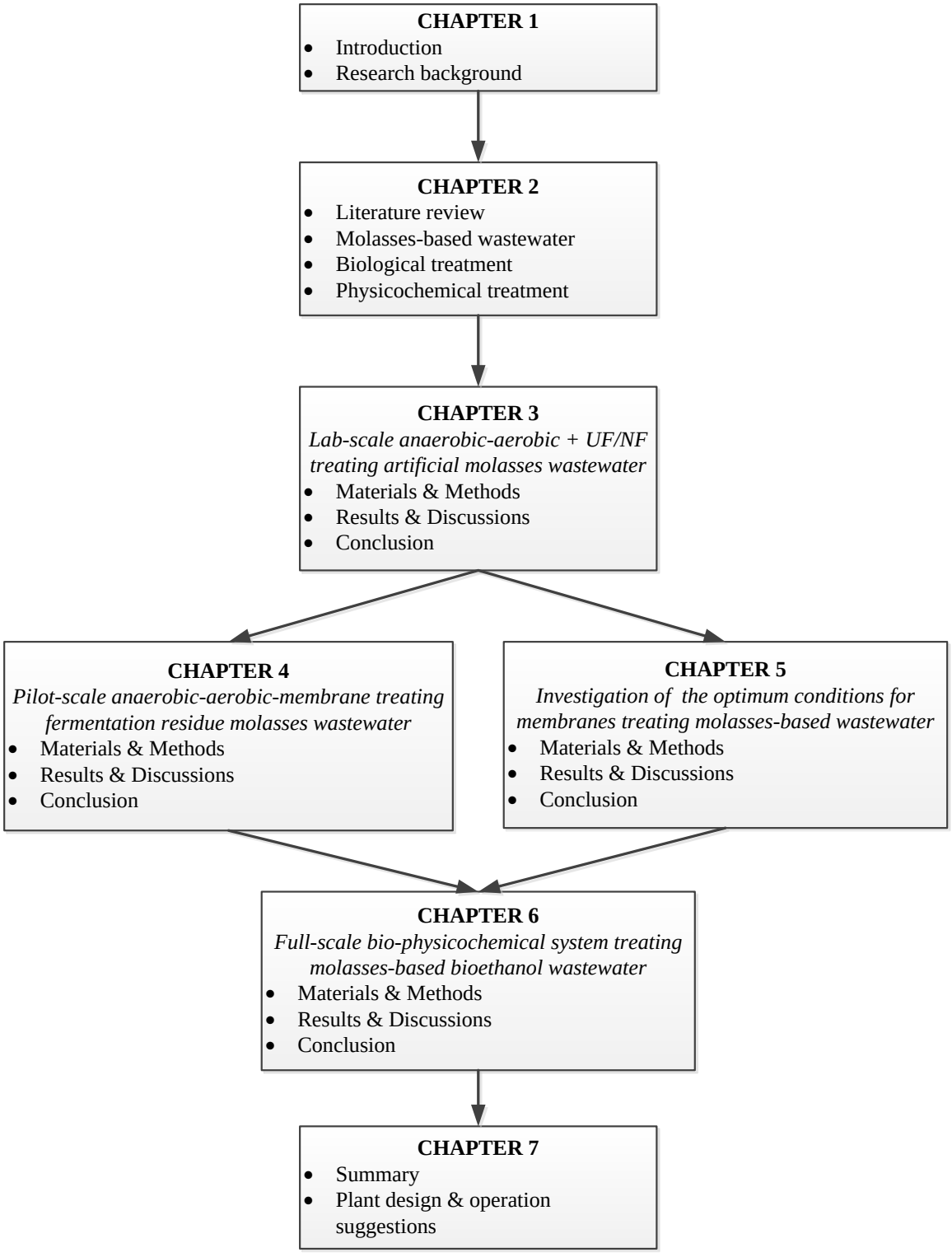
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Chapter 1

Introduction

Recently, production of bioethanol from agricultural by-product such as sugarcane molasses as an alternative source of energy has attracted much attention worldwide due to the high fuel price. In Thailand, one of the largest cane producers, 10%, 20% and 85% of ethanol has been generally blended with gasoline for vehicles. However, in order to produce 1 liter of bioethanol, 10-15 liters of high concentration, low pH, dark brown color spent wash wastewater was also generated. These effluent leads to soil pollution and acidification when used as ferti-irrigation or inappropriately discharge to land, as well as eutrophication and oxygen depletion when release to the water body. The color substance in molasses-based wastewater known as melanoidins is ineffectively degraded and even be increased by biological processes.

Our group has been specifically researching on the anaerobic wastewater processing especially, one of the most efficient anaerobic treatment technology methods so-called up-flow anaerobic sludge blanket (UASB) reactor and aerobic treatment; down-flow hanging sponge (DHS) equipped with filtration technologies. This collaborate project was supported by Mitsui Sugar co.,ltd., Ryuuseki, Muromachi Technos co.,ltd., Sanki Engineering and Nagaoka University of Technology (NUT) under supervisor of Assistant Professor Dr. Masashi HATAMOTO and Professor Dr. Takashi YAMAGUCHI.

Objectives

- The aim of this study was to investigate the possibility of combined lab-scale UASB/DHS processes for treatment of molasses-based wastewater in terms of COD removal rate, volatile fatty acid accumulation, biogas production and composition, profile of solid along the reactor etc.

- To evaluate the feasibility and the stability of a pilot-scale UASB/DHS/ASB following by microfiltration (MF), ultrafiltration (UF), and nanofiltration (NF) treating molasses bioethanol residue wastewater.

- To evaluate the performance and biomolecular analysis of a full-scale combined bio-physicochemical system for molasses-based wastewater treatment.

Chapter 2-4 mainly focus on the performance of the biological processes and possibility of physicochemical applications while chapter 5-6 mainly focus on the operational parameters that affect membranes and performance of the entire full-scale system.

Chapter 2 ***Literature Review*** is a review of the literature and associated theory. It discusses the various biochemical pathways involved in biodegradation of organic compounds by bacteria. It also highlights the different micro-organisms involved in biological degradation and their characteristic features.

Biological wastewater treatment systems discuss the various aerobic and anaerobic reactors and show the advantages and disadvantages of each system. The review also highlights the advantages of anaerobic digestion to developing countries especially the fact that biogas is produced. The biogas can be used to heat the anaerobic system or harnessed to provide heat for cooking, washing or any other work that requires heat. A comprehensive literature review is made of the up-flow anaerobic sludge bed (UASB) reactor and down-flow hanging sponge reactors, and their advantages over other systems are stated.

Physicochemical wastewater treatment systems discuss the various recent physicochemical reactors and demonstrate the characteristics of each system.

Chapter 3 ***Development of Combined Anaerobic–Aerobic System for Treating Industrial Molasses Wastewater*** gives a detailed description of the laboratory equipment used in the study. It also focuses on the various analytical methods undertaken and explains their importance in the evaluation of the systems. The main parameters monitored were the gas production and composition, Volatile Fatty Acid (VFA) and Chemical Oxygen Demand (COD), nutrients, and color that give an early warning of impending failure of the system and relate to the discharge standard.

Chapter 4 ***Pilot-scaled Anaerobic-Aerobic-Membrane System for Molasses Fermentation Residue Wastewater Treatment*** investigates the start-up of the pilot-scale reactors. It follows the stages of each reactor on a basis to show the variations of the parameters under study. It tries to explain the stability of the various parameters in a whole system as a trial before a full-scale operation.

Chapter 5 ***Evaluation of membrane processes for the biological pretreatment of molasses-based alcohol distillery wastewater*** emphasizing on the performance of membrane processes regarding various operating conditions.

Chapter 6 ***Full-scale Bio-Physicochemical System for Molasses-based Wastewater Treatment*** evaluates the practical performance of the system with the actual plant production. Moreover, the analysis of microorganisms in the main biological unit was conducted.

Chapter 7 ***Summary***

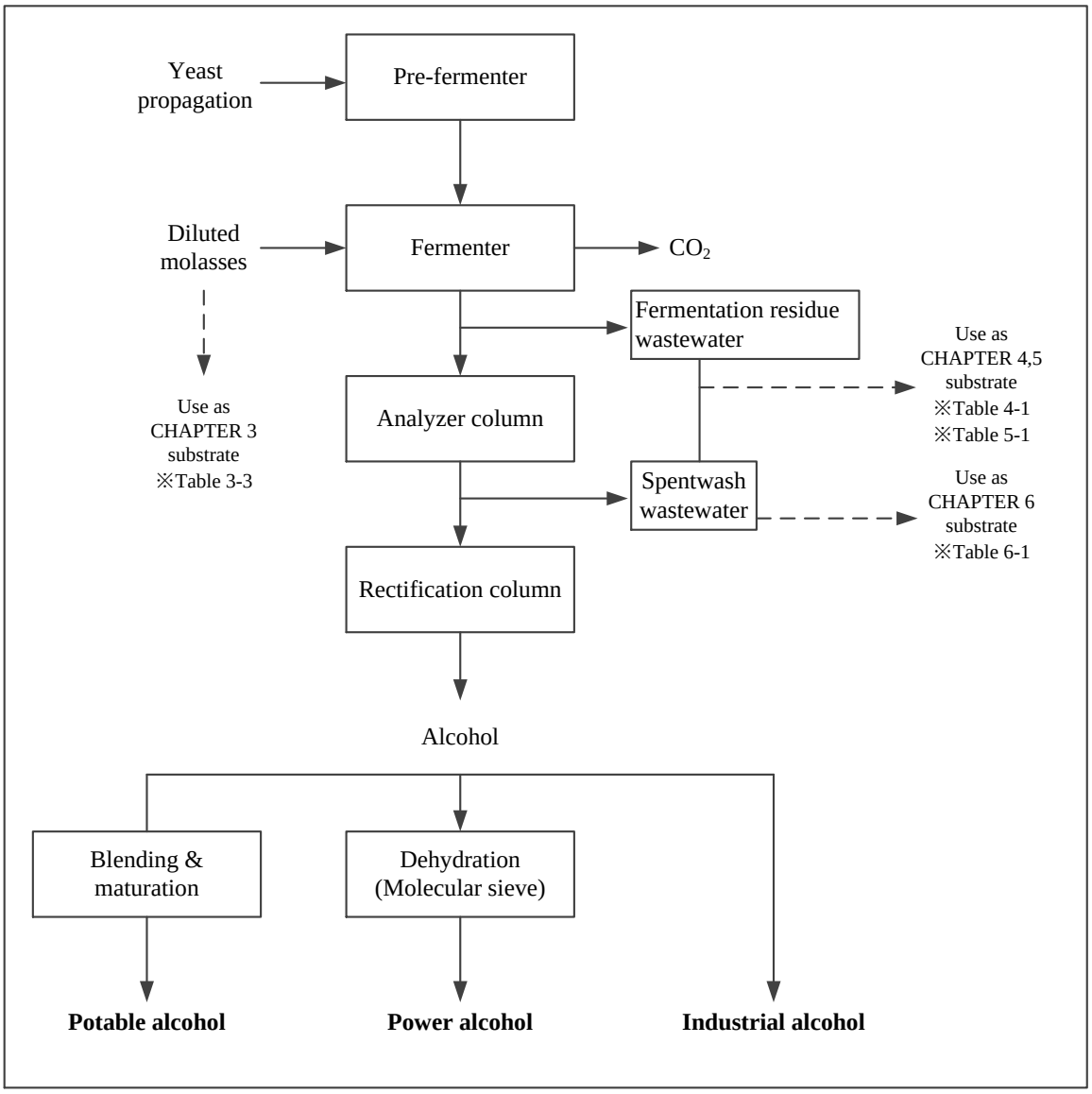
Chapter 2

Literature review

Over a century of industrial wastewater development, environmental engineers and scientists all around the world have made an effort to overcome the difficulties of each unique wastewater treatment. Biological approach began with a long history when population and industrialization were not as immense as today. Biological treatment appears to be a promising technology to reach the concept of Clean Development Mechanism (CDM) defined in the Kyoto Protocol (IPCC, 2007), as methane gas is generated from anaerobic digestion and can be utilized as renewable energy (Metcalf and Eddy, 2004). Anaerobic-aerobic systems have been remarkably employed in industrial and municipal wastewater treatment for many years. While previously most treatment of wastewaters have been carried out in conventional anaerobic-aerobic treatment plants, in recent years, high rate anaerobic-aerobic bioreactors have been increasingly employed for wastewaters with high chemical oxygen demand (COD) (Chan *et al.*, 2009).

2.1 Bioethanol production and molasses-based wastewater

Bioethanol production consists of four main processes; feed preparation, fermentation, distillation, and packaging (**Fig. 2-1**). Firstly, blackstrap molasses, by-product from sugar refinery is diluted into a solution that contains 15-16% of sugar. Then, inoculated with yeast and fermented (Satyawali *et al.*, 2008). During this fermentation process, the high concentration of 80,000~100,000 mg/L fermentation residue wastewater is generated. The fermented mixture then distilled in several columns. The water generated from washing the columns, cooling water, and boiler water is called spentwash which contains COD concentration of 6,000~12,000 mg/L, dark brown color, odor, and low pH (Nandy *et al.*, 2002). The amount and characteristics of molasses-based wastewater are varied depending on the raw material and production process (Pant and Adholeya, 2007).



Modified from Satyawali *et al*., 2008

Fig. 2-1 Molasses-based bioethanol production processes

2.2 Molasses color pigment and decolorization

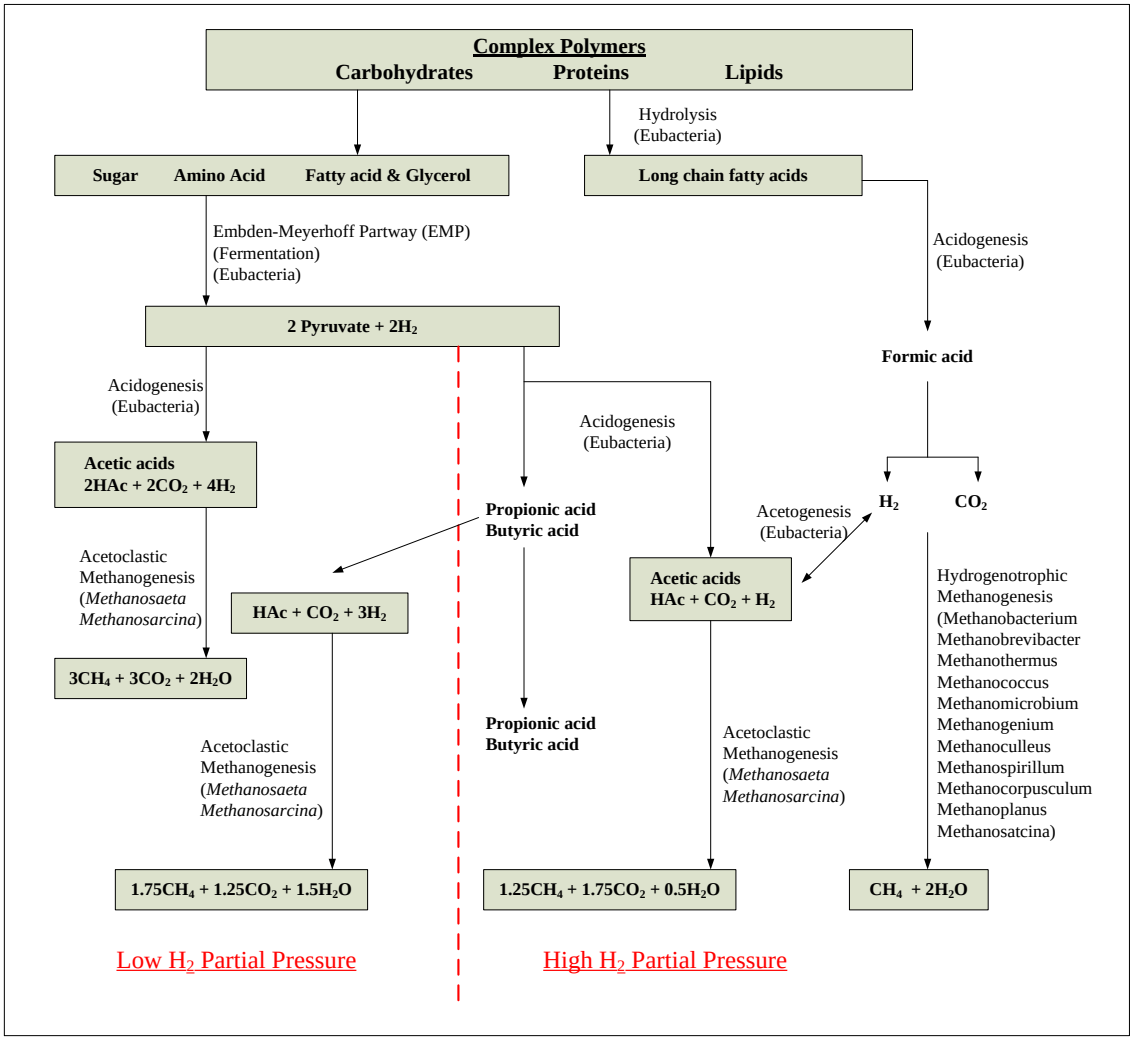
Melanoidins are main color substances that make molasses the dark brown color. Its structure is not yet fully understood, but can be explained as a products of sugar and amino acids by non-enzymatic Maillard reaction between amino and carbonyl groups substances (Chandra *et al.*, 2008). The chemical structures have similar elemental (CHON) compositions, spectroscopic properties, and electrophoretic mobilities at

different pH in both natural and synthetic melanoidins (Migo *et al.*, 1997).

2.3 Molasses-based wastewater treatment

2.3.1 Anaerobic digestion

The rate of engineering and scientific research development in the anaerobic process has been slow with developments in one field having to await developments in another (Mosey, 1982). Anaerobic digestion is a microbial fermentation by which organic matter is converted to carbon dioxide and methane (**Fig. 2-2**). It is a phenomenon, which occurs naturally in river sediments, marshes and the rumen of herbivorous animals. For anaerobic digestion to occur methanogens must be present and there must be little or no oxygen present. And in all these environments these conditions are met and methanogens are present in considerable numbers. The process of anaerobic digestion consists of three discrete stages: hydrolysis, acid formation and methane formation. These three stages normally occur simultaneously in an anaerobic reactor but for convenience they are discussed separately.



Adapted from Sam-Soon (1987)

Fig. 2-2 Diagram showing biochemical partway involving in anaerobic digestion

Usually wastewaters contain lipids, proteins and carbohydrates. Anaerobic digestion was initially applied to complex feedstock, such as municipal wastewater sludge. These contained a wide range of nutrients and alkalinity sources. Molasses-based and sugar wastewater contains readily biodegradable organics and the carriage water has a normal component of inorganic metal ions that are usually found in surface or groundwater. These feedstock contain complex organic material comprising carbohydrates, amino acids or long chain fatty acids. The first stage in anaerobic digestion is the hydrolysis of these complex organic materials into simple organic compounds. This hydrolysis involves the solubilization of the waste particulates (complex organic material) and fermentation into volatile acids (simple organic compounds). The complex organic materials are usually insoluble in water. Hydrolysis not only breaks them into simpler organic molecules but

makes them more soluble in the wastewater. This makes it easier for the acidogens to utilize them.

Acidogenesis The soluble simple organic compounds are generated in the first stage while the acidogenic bacteria (acidogens) utilize those already present in the wastewater. The acidogens transform them into short chain fatty acids (SCFA). The principal SCFAs are acetic acid, butyric acid and propionic acid. Other products of the transformation are carbon dioxide and gaseous hydrogen. The biochemical pathways and end products for acidogenesis depend on:

i. type of feed substrate
ii. hydrogen partial pressure (pH_2)

To fully understand the degradation of simple, soluble organic molecules by acidogens, consider the substrate, glucose. Initially, the glucose is converted to pyruvic acid and then to acetic acid. During this degradation process the partial pressure of hydrogen is a very important parameter as it determines the end product of the process. This is because there is a need to regenerate the NAD^+ for the EMP to remain operative. In order to regenerate NAD^+ there has to be dehydrogenation of the $NADH_2$. This reaction is thermodynamically unfavorable and only becomes thermodynamically favorable when $pH_2 < 10^{-4}$ atm. (Speece, 1983).

At $pH_2 < 10^{-4}$ atm, $NADH_2$ can be dehydrogenated to NAD^+ and H_2. This means the EMP can proceed and glucose is degraded to pyruvic acid and so:

$$2CH_2COCOOH + 2NAD^+ + 2ADP + 2Pi \rightarrow 2CH_3COOH + 2ATP + 2NADH_2$$

$$2NADH_2 \rightarrow 2NAD^+ + H_2$$

Hence under low H_2 partial pressure

$$GLUCOSE \rightarrow ACETATE + CO_2 + H_2$$

$$C_6H_{12}O_6 + H_2O + ADP + 4Pi \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 + 4ATP$$

In anaerobic digestion, the methanogenic and non-methanogenic bacteria normally grow in close association to form a tightly knit symbiotic community of micro-organism which co-operate together to form a self-regulating fermentation which automatically controls its pH values, redox potential and oxygen tension. The acetoclastic methane bacteria (those that produce methane from acetic acid by cleavage) cooperate with the acid-forming bacteria (acidogens) to control the concentration of acetic acid and hence the pH value of fermentation (Mosey, 1982). The growth rate of acetoclastic methane bacteria is relatively slow (minimum doubling times of 2 to 3 days at 35^{o}C) and that of acid forming bacteria is significantly fast (minimum doubling times of 2 to 3 h at 35^{o}C) (Mosey, 1982) This form of control is relatively crude and may lead to acid overload under conditions of shock loads. This is because the slow growing methanogens will be unable to remove the acetic acid that is produced by the faster growing acid formers. Fortunately, the obligate hydrogen utilizing methane bacteria offer a more subtle form of control.

$$PYRUVIC\ ACID + HYDROGEN \rightarrow PROPIONIC\ ACID$$

This reaction is speeded up by the presence of hydrogen, as it is one of the precursors of the end product. Now if the concentration of hydrogen increases the reactions like following equation slow down as they result in the introduction of more hydrogen in the digester gas:

$$GLUCOSE + WATER \rightarrow ACETIC\ ACID + HYDROGEN$$

As a result by controlling these reactions with monitoring the traces of hydrogen in the digester gas, the methanogenic bacteria control the internal metabolism of the acid forming bacteria. When the hydrogen concentration increases the following reaction

$$2CH_3COOH + 2H_2 \rightarrow CH_3CH_2CH_2COOH + H_2O$$

becomes more favorable. This means that the acid load on the system is reduced as one mole of butyric acid is produced instead of two moles of acetic acid

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + CO_2 + H_2$$

This also provides more time for the slow growing acetogens to metabolize the large amounts of acetic acid now present in the digester. This is known as 'acetic acid overload' and the operator does not need to reduce the loading rate to adjust the pH value as the reactor will recover on its own. When the surge loads persist for a long time, the hydrogen partial pressure (pH_2) becomes very high. If $pH_2 > 10^{-4}$ atm then $NADH_2$ cannot be dehydrogenated directly. The organism finds a sink for the electron and H^+ by reducing pyruvate to propionic acid. This triggers a large-scale production of propionic acid. Initially there is the following reaction

$$GLUCOSE + 2NAD^+ + 2ADP + 2Pi \rightarrow 2PYRUVATE + 2\,ATP + 2NADH_2$$
$$C_6H_{12}O_6 + 2NAD^+ + 2ADP + 2Pi \rightarrow 2CH_3COCOOH + 2ATP + 2NADH_2$$

Then when $pH_2 > 10^{-4}$ atm., pyruvate is reduced to propionic acid with dehydrogenation of $NADH_2$:

$$PYRUVATE + 2NADH_2 + ADP + Pi \rightarrow PROPIONATE + 2NAD+ + ATP + WATER$$
$$CH_3COCOOH + 2NADH_2 + ADP + Pi \rightarrow CH_3CH_2COOH + 2NAD^+ + ATP + H_2O$$

So instead of capturing the energy in ATP, use it to dehydrogenate $NADH_2$.

$$GLUCOSE \rightarrow PROPIONATE + ACETATE + CO_2 + H_2$$

$$C_6H_{12}O_6 + 3ADP + 3Pi \rightarrow CH_3CH_2COOH + CH_3COOH + CO_2 + H_2 + 3ATP$$

Then there is reversal of hydrogen production. The metabolism and growth of the acetogenic bacteria, which would, otherwise reverse this process by converting the propionic back into acetate is simultaneously switched off by the accumulated hydrogen in the system. This situation is known as propionic acid overload and the plant operator must act to overcome the overload. This means that if $pH_2 > 10^{-4}$ atm, propionic acid and other SCFAs will accumulate and increase the acidity of the system thereby dropping the pH value. There is the possibility of washing out the small population of acetogenic bacteria before the obligate H_2-utilizing bacteria have time to clear the accumulated hydrogen.

Acetogenesis Acetogenic bacteria play an important role in connecting the process between Acidogenesis and Methanogenesis. Methanogens need specific substrate such as acetic acid, formic acid, hydrogen, methanol and methylamine. Fatty acid that have more than 2 atoms of carbon cannot be directly used as substrate; therefore acetogenic bacteria can degrade 2 atom-carbon or more into carbon dioxide, acetic acid and hydrogen under low H_2 partial pressure of less than $2x10^{-3}$ atm and $9x10^{-3}$ atm for butyrate and propionate degradation, respectively.

Methanogenesis Methane bacteria are fastidious anaerobes having strict requirements for redox potentials and absence of dissolved oxygen. However, in nature, methane bacteria are rarely found on their own. They usually form a tightly knit symbiotic community of micro-organisms which operate together to form a self-regulating fermentation which automatically controls its own pH values, redox potentials and oxygen tension.

2.3.1.1 Anaerobic microorganisms

In natural anaerobic habitats which contain complex organic compounds where light, sulfate, and nitrate are limited, Methanogens are linked to chemo heterotrophic bacteria for the degradation of organic substrates in a four-step process as follows : (1) hydrolysis of polymers by hydrolytic microorganisms, (2) acidogenesis from simple organic compounds by fermentative bacteria, (3) acetogenesis from metabolites of fermentations by homoacetogenic or syntrophic bacteria, (4) methanogenesis by methanogenic archaea from H_2 + CO_2, acetate, simple methylated compounds or alcohol + CO_2 (**Fig. 2-3**). (J.L. Garcia, 2000)

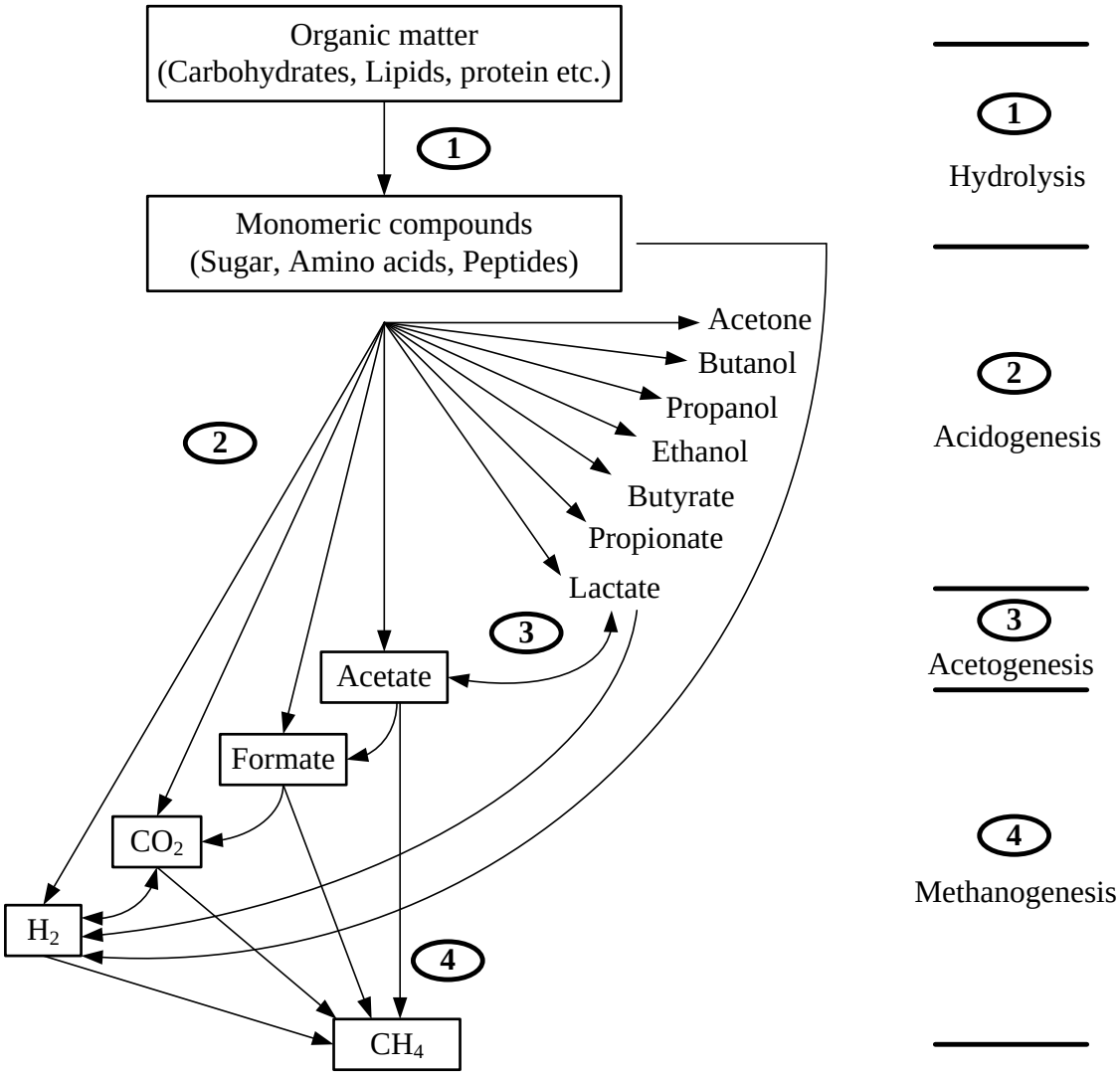


Fig 2-3 Schematic diagram showing anaerobic degradation of organic compound (adapted from Jean-Louis Garcia *et al*, 2000, Madigan, 2003 and R.E. Speece, 1997)

2.3.1.1.1 Acidogenic bacteria

During volatile fatty acid (VFA) formation process or acidogenesis, VFAs are normally produced more by obligate anaerobes, bacteria that die when exposed to atmospheric levels of oxygen, than facultative anaerobes, bacteria that can use oxygen when it is present, due to the numbers of population in the general anaerobic system.
Obligate anaerobes which play an important role in acid formation are *Clostridium*.
For instance, many of the spore-forming anaerobic bacteria (Genus *Clostridium*) ferment amino acids with the production of acetate, ammonia, and hydrogen. Other *Clostridium* species, such as *C.acidi-urici* and *C.purinolyticum*, ferment purines such as xantine or adenine with the formation of acetate, formate, CO_2, and ammonia. The by-products from *Clostridium* varies to its metabolism, it can be butyrate, acetate, CO_2, H_2, ethanol, butanol, acetone etc.; furthermore, some bacteria of Propionibacterium can also produce propionic acid and acetic acid from lactic acid. (Fenchel and Finlay 1995, Madigan et al., 1997)

The soluble simple organic compounds are generated in the first stage while the acidogenic bacteria (acidogens) utilize those already present in the wastewater. The acidogens transform them into short chain fatty acids (SCFA). The principal SCFAs are acetate, butyrate and propionate. Other products of the transformation are carbon dioxide and gaseous hydrogen. The biochemical pathways and end products for acidogenesis depend on:

i. type of feed substrate

ii. hydrogen partial pressure (pH_2)

2.3.1.1.2 Acetogenic bacteria

Since there are many kinds of products from the previous mentioned acid forming bacteria, and some large molecules of organic compound that methanogens cannot consume are still remaining. So that, it is necessary to degrade those molecules into smaller form, easy for methanogens to absorb those nutrients for cell production. Bacteria that degrade large compound of volatile fatty acid into acetate, hydrogen and carbon dioxide can be divided into 2 types as follows:

(1) Homoacetogenic bacteria

This type of bacteria use carbon dioxide as an electron acceptor and produce acetate (Anaerobic Respiration) by biochemical pathway called Acetyl-CoA pathway. These bacteria such as *Acetobacterium woodii* and *Clostridium aceticum* can grow in both autotrophic, capable of synthesizing its own food from simple organic substances, which can use carbon dioxide as electron acceptor and hydrogen as an electron donor to convert carbon dioxide to acetate.

$$2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O \qquad \textit{Autotrophic}$$

And can also grow in heterotrophic condition as the following carbohydrate fermentation equation:

$$C_6H_{12}O_6 \rightarrow 3CH_3COOH \qquad \textit{Heterotrophic}$$

Bacteria that belong to the genus *Clostridium* can be found in both acid forming bacteria group (Acidogenic Bacteria) and Acetate forming bacteria group (Acetogenic Bacteria). These bacteria have various kinds of metabolism as shown in **Table 2-1**.

(2) H_2- producing Acetogenic bacteria

This kind of bacteria uses non-acetate volatile fatty acid or alcohol as substrate

and produce acetate and hydrogen gas which are necessary for methanogens. Therefore, H_2-producing Acetogenic bacteria are very important because they help connecting the activities between Acidogenic bacteria and Methanogenic bacteria. However, these bacteria cannot survive by themselves due to the accumulation of high concentration of hydrogen (high hydrogen partial pressure). Acetogenesis reaction could not be occurred if acetate forming bacteria stopped growing. Therefore, it is necessary to get rid of hydrogen in the first place to make the suitable environment for acetogens then methanogens can afterward take place to consume hydrogen.

Table 2-1 Characteristics of some groups of the genus *Clostridium*[a]

Key characteristics	Other characteristics	Species
I. Ferment carbohydrates		
Cellulose	Fermentation product : acetate, lactate, succinate, ethanol, CO_2, H_2	*C.cellobioparum* *C.thermocellum* *C.butyricum*
Sugar, starch, pectin	Fermentation product : acetone, butanol, ethanol, isopropanol, butyrate, acetate, propionate, succinate, CO_2, H_2, some fixed N_2	*C.acetobutylicum* *C.pastuerianum* *C.perfringens* *C.thermosulfurogenes*
Sugar primarily to acetic acid	Total synthesis of acetate from CO_2; cytochromes present in some species	*C.aceticum* *C.thermoaceticum* *C.formicoaceticum* *C.methylpentosum*
Only pentoses or methylpentoses	Fermentation products : acetate, propionate, *n*-propanol, CO_2, H_2	
II. Ferment amino acids	Fermentation product : acetate, other fatty acids, NH_3, CO_2, somtime H_2 , some also ferment sugar to butyrate and acetate	*C.sporogenes* *C.tetani* *C.botalinum* *C.tetanomorphum*
	Ferments 3-carbon amino acids (ex. alanine) to propionate, acetate and CO_2	*C.propionicum*
III. Ferment carbohydrate or amino acids	Fermentation product from glucose : acetate, formate, small amount of isobutyrate and isovalerate	*C.bifermentans*
IV. Purine fermenters	Ferment Uric acid and other purines, forming acetate, CO_2, NH_3	*C.acidurici*
V. Ethanol Fermentation to fatty acids	Produce butyrate, caproate, and H2; requires acetate as electron accpetor; does not use sugars, amino acids, or purines	*C.kluyveri*

[a] Adapted from Madigan et al. (2000)

In most cases the nature of this kind of bacteria has also been called interspecies H_2 transfer. The H_2 consumer can be any number of organisms. If growth of syntrophic organisms (suppose H_2-acetogenic bacteria and methanogenic bacteria) occurs only when H_2 is removed by a partner organism, the removal itself must obviously affect the energetic of the reaction. H_2-producing fatty acid-oxidizing syntrophic bacteria, secondary fermenters, are the key organisms in the conversion of complex organic materials to methane. For example, *Syntrophomonas wolfei* oxidizes C_4 to C_8 fatty acids yielding acetate, CO_2 and H_2. Other species of *Syntrophomonas* use fatty acids up to C_{18}, including some unsaturated fatty acids. *Syntrophobacter wolonii* specializes in propionate oxidation and generates acetate, CO_2 and H_2, while *Syntrophus gentiane* degrades benzoate to acetate, H_2 and CO_2. (Madigan et al., 2000)

2.3.1.1.3 Methanogenic Bacteria

Anaerobic digestion of organic matter in the environment releases 500-800 million tons of methane per year into the atmosphere and this represents 0.5% of the organic matter derived from photosynthesis (Kirsop, 1984; Sahm, 1984). Methanogenic microorganisms grow slowly in wastewater and their general times range from 3 days at 35^{o}C to as high as 50 days at 10^{o}C.

Methanogens are subdivided into 3 sub-categories;

(1) Obligate Hydrogenotrophic Methanogens

i.e. hydrogen-utilizing chemolithotrophs convert hydrogen and carbon dioxide into methane

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$

The hydrogen-utilizing methanogens help maintain the very low-level partial pressures necessary for the conversion of volatile acids and alcohol to acetate (Speece, 1983).

(2) Obligate Acetotrophic Methanogens

Also called acetoclastic bacteria or acetate-splitting bacteria, which use acetate as energy source and convert it into methane and CO_2 as following equation;

$$CH_3COOH \rightarrow CH_4 + CO_2$$

Acetoclastic bacteria grow much more slowly (generate time = a few days) than acid-forming bacteria (generate time = a few hours). This group comprises two main genera: *Methanosarcina* (Smith and Mah, 1978) and *Methanosaeta* (Huser et al.,1982). During thermophilic digestion of lignocellulosic waste, *Methanosarcina* was the dominant acetotrophic bacteria encountered in the bioreactor. After 4 months, *Methanosarcina* (μ_{max} = 0.3 day^{-1}; K_s = 200mg/L) was displaced by *Methanosaeta* (μ_{max} = 0.1 day^{-1}; K_s = 30mg/L). It was postulated that the competition in favor of Methanosaeta was due to the lower acetate K_s value of this organism (Gujer and Zehnder, 1983; Koster, 1988; Zinder et al., 1984).

(3) Hydrogenotrophic/Acetoclastic Methanogens

Bacteria that can produce methane from both acetate and hydrogen, but normally work better with hydrogen.

Substrate for methanogenesis

Some representative samples of substrate have been shown to be converted to methane by pure culture of methanogens (**Table. 2-2**).

Table 2-2 Substrates converted to Methane by various methanogenic *Archaea*

Carbon dioxide-type substrate	
Carbon dioxide	
$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$	$\Delta G^{0'}$ = -131 kJ/reaction
Formate, $HCOO^-$	
$4HCOO^- + 4H^+ \rightarrow CH_4 + 3CO_2 + 2H_2O$	$\Delta G^{0'}$ = -145 kJ/reaction
Carbon monoxide, CO	
$4CO + 2H_2O \rightarrow CH_4 + 3CO_2$	$\Delta G^{0'}$ = -210 kJ/reaction
Methyl substrate	
Methanol, CH_3OH	
$4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O$	$\Delta G^{0'}$ = -319 kJ/reaction
Methylamine, CH_3NH^+	
$4CH_3NH_3 + 2H_2O \rightarrow 3CH_4 + CO_2 + 4NH_4^+$	$\Delta G^{0'}$ = -230 kJ/reaction
Dimethylamine, $(CH_3)_2NH_2^+$	
$(CH_3)_2NH_2 + 2H_2O \rightarrow 3CH_4 + CO_2 + 2NH_4^+$	$\Delta G^{0'}$ = -230 kJ/reaction
Acetate	
Acetate, CH_3COO^-	
$CH_3COOH + H^+ \rightarrow CH_4 + CO_2$	$\Delta G^{0'}$ = -31 kJ/reaction

(Adapted from Madigan et al., 2000)

The first class includes the important substrate, CO_2, substrate which is reduced to methane using H_2 as electron acceptor:

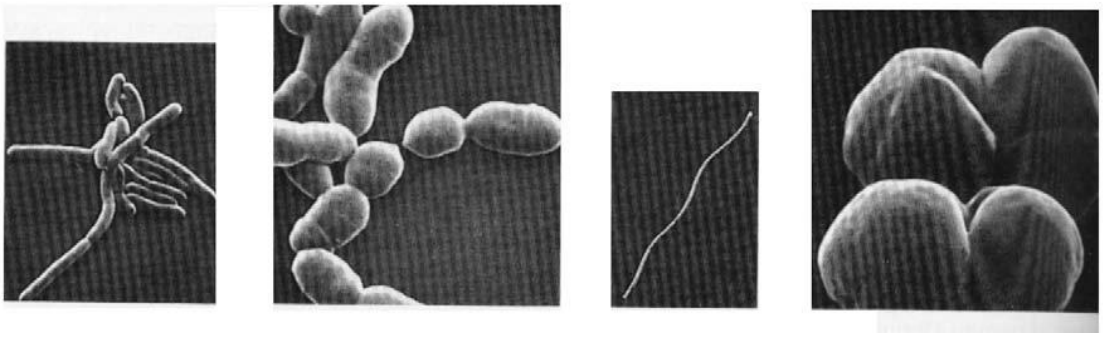
$$\boldsymbol{CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O} \qquad \Delta G^{0'} = -131\text{kJ/reaction}$$

Other substrates in this class include formate and carbon monoxide.

The second classes of methanogenic substrates are methyl group substances (Table 2-2). Using CH_3OH as a model methyl substrate here, the formation of CH_4 can occur in two ways: First, methyl can be reduced using an external electron donor such as H_2 or oxidized to CO_2 in order to generate the electrons needed to reduce other molecules of CH_3OH to CH_4. The final methanogenic process is the cleavage of acetate to CO_2 plus CH_4, called the acetotrophic reaction:

$$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^- \qquad \Delta G^{0'} = -31kJ$$

Only a few methanogens are acetotrophic (**Table 2-3**), methane formation in methanogenic habitats such as sewage sludge have shown that about two-thirds of the methane originated from acetate and one-third from H_2+CO_2.



(a) (b) (c) (d)

Fig. 2-4 Scanning electron micrographs of cell of methanogenic Archaea

(a) Methanobrevibacter ruminantium (b) *Methanobrevibacter arboriphilus*

(c) *Methanospirillum hungatii* (d) *Methanosarcina barkeri*

(Madigan, 2000; photo by Alexander Zehnder)

Several types of methanogens have been shown in **Fig. 2-4** and **Table. 2-3**

Table 2-3 Characteristics of Methanogenic *Archaea*

Genus	Shape	Substrate for methanogenesis
I. Methanobacteriales		
Methanobacterium	Long rods	$H_2 + CO_2$, formate
Methanobrevibacter	Short rods	$H_2 + CO_2$, formate
Methanosphaera	Cocci	Methanol + H_2 (both needed)
Methanothermus	Rods	$H_2 + CO_2$, hyperthermophile
Methanothermobacter	Rods	$H_2 + CO_2$, formate, thermophile
II. Methanococcales		
Methanococcus	Irregular cocci	$H_2 + CO_2$, formate, pyruvate + CO
Methanothermococcus	Cocci	$H_2 + CO_2$, formate
Methanocaldococcus	Cocci	$H_2 + CO_2$
Methanotorris	Cocci	$H_2 + CO_2$
III. Methanomicrobiales		
Methanomicrobium	Short rods	$H_2 + CO_2$, formate
Methanogenium	Irregular cocci	$H_2 + CO_2$, formate
Methanospirillum	Spirilla	$H_2 + CO_2$, formate
Methanoplanus	Plate-shape cell	$H_2 + CO_2$, formate
Methanocorpusculum	Irregular cocci	$H_2 + CO_2$, formate, alcohols
Methanoculeus	Irregular cocci	$H_2 + CO_2$, formate, alcohol
Methanofollis	Irregular cocci	$H_2 + CO_2$, formate
Methanolacinia	Irregular rods	$H_2 + CO_2$, alcohol
IV. Methanomicrobiales		
Methanosarcina	Large irr.cocci in packets	$H_2 + CO_2$, methanol, methylamine acetate
Methanonolobus	Irr. cocci	Methanol, methylamine
Methanohalobium	Irregular cocci	Methanol, methylamine; halophilic
Methanococcoides	Irregular cocci	Methanol, methylamine
Methanohalophillus	Irregular cocci	Methanol, methylamine, methyl sulfides; halophile
Methanosaeta	Long rods	Acetate
Methanosalsum	Irregular cocci	Methanol, methylamine, dimethylsulfide $H_2 + CO_2$; hyperthermophile
V. Methanopyrales		
Methanopyrus	Rods in chain	$H_2 + CO_2$, hyperthermophile

Adapted from Madigan et al. (2000)

2.3.1.2 Environmental factors that affect methanogenesis

2.3.1.2.1 Temperature

Methane production has been documented under a wide range of temperature between 0^{o}C and 97^{o}C. Although psychrophilic methanogenic bacteria have not been isolated, thermophilic strains operating at an optimum range of 50-75^{o}C are found in hot springs. Methanothermus fervidus has been found in a hot spring in Iceland and grows at 63-97^{o}C (Sahm,1984).

In municipal wastewater plants, anaerobic digestion is carried out in the mesophilic range at temperature from 25^{o}C up to 40^{o}C with an optimum at approximately 35^{o}C. Thermophilic digestion operates at temperature range of 50-65^{o}C. It allows higher loading rates and also conductive to greater destruction of pathogens. One drawback is its higher sensitivity to toxicants (Koster,1988).

Because of their slower growth as compared with Acidogenic bacteria, methanogenic bacteria are very sensitive to small changes in temperature. As to utilization of volatile acids by methanogenic bacteria, a decrease in temperature leads to a decrease of the maximum specific growth rate while the half-saturation constant increases (Lawrence and McCarty, 1969). Thus, mesophilic digesters must be designed to operate at temperature between 30^{o}C and 35^{o}C for their optimal functioning (**Fig. 2-5**).

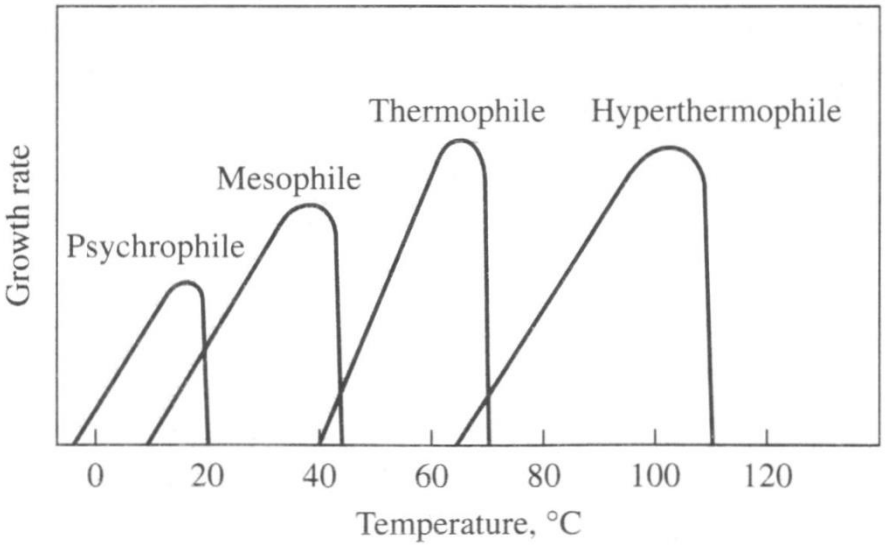


Fig. 2-5 Temperature vs. Growth rate (Speece,1996)

2.3.1.2.2 pH

Most methanogenic bacteria function in a pH range between 6.7 and 7.4, but optimally at pH of 7.0–7.2, and the process may fail if the pH is close to 6.0. Acidogenic bacteria produce organic acids, which lower the pH of the reactor. Under normal condition, this pH reduction is buffered by the bicarbonate that produced by methanogens. Acidity is inhibitorier to methanogens than to Acidogenic bacteria. An increase of volatile acids level therefore serves as early indicator of system down. One method for restoring the pH balance is to increase alkalinity by adding chemicals such as lime, anhydrous ammonia, sodium hydroxide, or sodium bicarbonate.

2.3.1.2.3 Volatile acids

Generally, volatile fatty acids in the anaerobic reactor should be ranged between 20-200 mgHAc/l. The system with exceeded VFA level can be considered that there are the possibilities of low community of methanogens or Acidogenic might be able to produce VFA in an extreme rate. The increasing of VFA indicates that the system is losing balance because the pH level is out of the proper range.

2.3.1.2.4 Hydraulic Retention Time

The hydraulic retention time (HRT), which depends on wastewater characteristics and environmental conditions, must be long enough to allow anaerobic bacteria in digester to degrade the substrate. Digesters base on attached growth have a lower HRT (1-10days) than those based on dispersed growth (10-60days) (Polprasert, 1989). The retention time of mesophilic and thermophilic digesters range between 25 and 35 days but can be lower (Sterritt and Lester, 1988). In laboratory scaled reactors, HRT can be lowered only several hours.

2.3.1.2.5 Nutrient

As with all biological treatment systems, mineral and trace element must be present to satisfy the growth requirements of the microorganisms involved. Many industrial wastewaters, especially those from the chemical industry, may have deficiencies in some required nutrient. Among the inorganic nutrients required for growth are nitrogen and phosphorus. The quantity needed can be determined from estimates of net biological growth. An additional requirement in anaerobic systems is for trace metals, which are needed for activation of key enzymes for methanogenesis.

Table 2-4 is a listing adapted from Speece (1996) of traces metals that have been found to stimulate the anaerobic treatment process. Iron, cobalt, and nickel are known requirements for key enzymes within methane-producing species and must always be present for effective anaerobic treatment. Lack of sufficient trace nutrient may be a cause of failure of anaerobic treatment for many industrial wastewaters. The required concentrations of each differ considerably. Iron often needs to be present as high as 40 mg/l, while 1 mg/l or less of the others is generally sufficient (Rittmann, 2001).

Table 2-4 Nutrient requirements for anaerobic treatment

Element	Requirement mg/g COD	Desired Excess Concentration mg/l	Typical form for addition
Macronutrients			
Nitrogen	5 − 15	50	NH_3, NH_4Cl, NH_4HCO_3
Phosphorus	0.8 − 2.5	10	NaH_2PO_4
Sulfur	1 - 3	5	$MgSO_4 \cdot 7H_2O$
Micronutrients			
Iron	0.03	10	$FeCl_2 \cdot 4H_2O$
Cobalt	0.003	0.02	$CoCl_2 \cdot 2H_2O$
Nickel	0.004	0.02	$NiC_{l2} \cdot 6H_2O$
Zinc	0.02	0.02	$ZnCl_2$
Copper	0.004	0.02	$CuCl2 \cdot 2H_2O$
Manganese	0.004	0.02	$MnCl_2 \cdot 4H_2O$
Molybdenum	0.004	0.05	$NaMoO_4 \cdot 2H_2O$
Selenium	0.004	0.08	Na_2SeO_3
Tungsten	0.004	0.02	$NaWO_4 \cdot 2H_2O$
Boron	0.004	0.02	H_3BO_3
Macronutrients			
Sodium		100 − 200	NaCl, $NaHCO_3$
Potassium		200 − 400	KCl
Calcium		100 − 200	$CaCl_2 \cdot 2H_2O$
Magnesium		75 − 250	$MgCl_2$

Adapted from Speece (1996)

2.3.1.2.6 Toxicants

A wide range of toxicants are responsible for the occasional failure of anaerobic reactors. Inhibition of methanogenesis is generally indicated by reduced methane production and increased concentration of volatile acids. The following are some of the toxicants.

Oxygen Methanogens are obligate anaerobes and are adversely affected by trace levels of oxygen (Oremland, 1988; Roberton and Wolfe, 1970).

Volatile acids If pH is maintained near neutral, volatile acids such as acetate or butyrate appear to be little toxic to methanogenic bacteria. Propionate, however, display toxicity to both acid-forming and methanogenic bacteria.

Long-chain fatty acids The long-chain fatty acids (e.g., caprylic, capric, lauric, myristic, and oleic acids) inhibit the activity of acetoclastic methanogens (e.g., *Methanotrix*) in acetate-fed sludge (Koster and Cramer, 1987).

Heavy metals Heavy metals (e.g., Cu^{2+}, $Pb2^{+}$, $Cd2^{+}$, $Ni2^{+}$, $Zn2^{+}$, Cr^{6+}) found in wastewaters and sludge from industrial sources are inhibitory to anaerobic digestion (Lin, 1992; Mueller and Steiner, 1992). However, some metals (e.g., nickel, cobalt, molybdenum etc.), at trace concentrations, may stimulate methanogenic activity of the bacteria (Murray and van den Berg, 1981; Shonheit *et al.*, 1979; Whitman and Wolfe, 1980).

Feedback inhibition Anaerobic system may also be inhibited by several of the intermediates produced during the process. High concentrations of these intermediates (H_2, volatile fatty acids) are toxic of feedback inhibition (Barnes and Fitzgerald, 1987).
In order to avoid problems discussed above, it has been suggested that two-phase anaerobic digestion systems be used to spatially separate Acidogenic bacteria from methanogenic bacteria (Ghosh and Klass, 1978; Cohen et al., 1980; Pipyn *et al.*,1979). Some of the advantages of the two-phase system enhanced stability and increased resistance to toxicants. A long SRT also allows methanogens to acclimate to toxicants such as ammonia, sulfides and formaldehyde. Thus, anaerobic digestion of industrial wastewater containing toxic chemicals should be undertaken in reactors that allow a long SRT at relatively low HRT (Bhattacharya and Parkin, 1988; Parkin *et al.*, 1983).

2.3.1.3 Anaerobic treatment for molasses-based wastewater

The high organic content of molasses spentwash makes anaerobic treatment attractive in comparison to direct aerobic treatment. Therefore, biomethanation is the primary treatment step and is often followed by two-stage aerobic treatment before discharge into a water body or on land for irrigation (Nandy *et al.*, 2002). Aerobic treatment alone is not feasible due to the high energy consumption for aeration, cooling, etc. Moreover, 50% of the COD is converted to sludge after aerobic treatment (Sennitt, 2005). In contrast, anaerobic treatment converts over half of the effluent COD into biogas (Wilkie *et al.*, 2000). Anaerobic treatment can be successfully operated at high organic loading rates; also, the generated biogas can be utilized for boilers (Nandy *et al.*, 2002). Further, low nutrient requirements and stabilized sludge production are other associated benefits (Jimenez *et al.*, 2004).

Lettinga and co-workers in the Netherlands developed the upflow anaerobic sludge bed reactor in the 1970s. This reactor had no internal packing and yet still incorporated the immobilized cell feature of the anaerobic filters (Speece, 1983). It is the most widely used high rate anaerobic system for anaerobic sewage treatment. The most characteristic device of the UASB reactor is the phase separator. It divides the reactor into a settling zone (upper part) and a digestion zone (lower part). The wastewater is introduced uniformly through the bottom of the reactor. It then passes the sludge bed and enters the settling zone via the aperture between the phase separators. The inclined walls of the phase separator increase the area of the liquid flow in the settling zone as the wastewater approaches the water surface. This decreases the upflow velocity of the liquid as it flows towards the discharge point. This reduced upflow liquid velocity means that sludge drawn into the settling zone can flocculate or settle out. With time the mass of accumulated sludge on the phase separator will exceed the frictional force that keeps it on the inclined surface and it slides back into the digestion zone and reparticipates in the digestion of the incoming wastewater and its organic matter. The presence of the settler on top of the digestion zone enables the system to maintain a large sludge mass in the

UASB. The effluent discharged is relatively free of suspended solids.

The biogas bubbles rise up to the liquid-gas interface under the phase separator. Sludge flocs with adhering gas bubbles may rise up to the interface in the gas collector, but will settle when the gas bubbles are released to the gas phase at the interface. Baffles that are placed under the apertures of the gas collector units operate as gas deflectors and prevent the biogas bubbles from entering the settling zone. This stops them from creating turbulence in the settling zone that would hinder the settling process (Lettinga *et al.*, 1980).

An important feature of the UASB process is that a granular type of sludge develops. These granules are usually 1-5 mm in diameter (Haandel and Lettinga, 1994). The granules have a high density and exceptional mechanical strength. The granules also combine a high settling velocity with a high specific methanogenic activity. The formation of granules is related to the operational conditions prevailing in a UASB. A granular type of sludge develops on mainly soluble types of wastewater. With raw sewage a flocculent sludge develops and the reactors achieve high removal efficiencies. Lettinga and van Haandel (1994) noted that, although, granulation is not a prerequisite for successful anaerobic treatment the use of granular sludge may offer some specific benefits. In a reactor seeded with granular sludge the flocculent sludge from the raw sewage influent settles on top of the better settling granular sludge and is removed from the UASB separately. The UASB has gained precedence over the conventional CMAR. Like all other modern high rate reactors the UASB is able to separate SRT/HRT (Grobicki and Stuckey, 1991) through the use of the sludge blanket that develops as a result of granulation (Lettinga, 1995).

Upflow anaerobic sludge blanket (UASB) reactor is the most popular high rate digester that has been utilized for anaerobic treatment of various types of industrial wastewaters (Akunna and Clark, 2000; Syutsubo *et al.*, 1997). Treatment by a UASB reactor resulted in 75% COD removal in sugarcane molasses spentwash. (Sanchez Riera *et al.*, 1985).

Syutsubo *et al.* (1997) reported that most of the practical UASB systems are operated under mesophilic conditions; however, thermophilic operation results in higher methanogenic activity. Mesophilically grown sludge utilized in thermophilic UASB as a seeding material leads to prompt start up and stable operation with 85% COD removal efficiency at a high loading of 30 kg COD m^{-3} d^{-1}.

Singh *et al.*(2004) summarized the drawbacks of anaerobic lagoons in large space squirement, odor, and groundwater.

Ruiz *et al.* (2002) investigated the treatment of winery wastewater using anaerobic sequencing batch reactor (ASBR) and achieved the COD removal of more than 98% at OLR of 8.6 kg COD m^{-3} $d^{-1}$and HRT of 2.2 days.

Kalyazhnyi et al., (2001) applied the UASB reactors for treating distillery wastewater at psychrophilic condition and resulted in total COD rremoval at 60% in one stage and 70% in two-stage reactor. Kalyazhnyi *et al.*, (2001) also concluded that application of high recycle ratios is necessary for enhancement of UASB pretreatment under psychrophilic conditions.

2.3.1.3.1 Advantages of anaerobic treatment

i. Low production of biological sludge as compared to aerobic systems. In aerobic systems a lot of energy is given out on oxidation of the wastewater to carbon dioxide. As a result there is a lot of energy available for anabolic processes. The absolute quantity in kg of organic matter is low and the dewatering capacity is very high.
ii. High treatment efficiency.
iii. No oxygen requirements.
iv. Methane is produced which is a useful source of energy. The methane is an energy rich end-product because on formation of methane there is very little energy produced. The sludge is generally well stabilized.
v. Low nutrient requirements.
vi. Low capital costs because technically plain and relatively inexpensive reactors are used which can be operated with little consumptive high-grade energy.

vii. Low operating costs.

viii. Anaerobic organisms can be preserved for long periods of time (exceeding one year) without any serious deterioration of their activity (Lettinga, 1995). Other important characteristics of anaerobic sludge generally remain unaffected like the settleability of the sludge.

ix. Very high loading rates can be applied in modern anaerobic wastewater treatment systems. As a result the space requirements of the system are relatively small.

2.3.1.3.2 Disadvantages of anaerobic treatment

i. Low growth rate of microorganisms

ii. Odor production

iii. High buffer requirement for pH control

iv. Poor removal efficiency for low strength wastewater

In some cases, like isolated small island of Miyakojima, while the land requirement and contamination to the groundwater are the main concerns, therefore, conventional wastewater treatment such as anaerobic ponds or facultative ponds are less attractive compare to high rate UASB reactor. However, UASB have some weak points on nutrients removal like nitrogen, phosphorus and sulfide. And can lead to environmental problems like eutrophication when discharged. In some cases of high strength industrial wastewater, UASB cannot fulfill the discharge regulations of organic matters. Moreover, decolorization of molasses wastewater by anaerobic process is negligible. The color removal rate is only 5–10% which is inconsiderable.

2.3.2 Aerobic biological treatment

For the molasses-based wastewater, the anaerobic treated water still remains high concentration, dark color and biological oxygen demand (BOD). Aerobic process generally considered as post treatment after anaerobic stage. Therefore, aerobic treatment can provide rapid oxidation of soluble organic compounds, nitrification of ammonia, and reduction of suspended solids.

The aerobic processes are involving microorganisms that oxidize both dissolved and particulate substances into end products in presence of oxygen. The following equation represented the stoichiometry for aerobic oxidation;

$$\underset{Organic matter}{COHNS} + O_2 + nutrients \xrightarrow{bacteria} CO_2 + H_2O + new\ cells + nutrients + end\ products$$

Many microorganisms are found in aerobic suspended and attached growth treatment processes used for the removal of organic material. Aerobic bacteria found in these processes form biofilms that able to lower wastewater concentration (Metcalf and Eddy, 2004).

The principle advantages of aerobic attached growth process over others are:

i. Less energy required
ii. Simple operation and maintenance
iii. No bulking sludge

Almstrand *et al.* (2011) investigated nitrification potential of nitrifying bacteria in a pilot-scale nitrifying trickling filters (NTFs) fed with full-scale plant wastewater with high and low ammonia concentration. The results showed the stabilization of nitrification potential and the dominant bacteria in the system which are *Nitrosomonas oligotropha.*

2.3.3 Physicochemical treatment

Membrane processes include microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), reverse osmosis (RO), dialysis, electrodialysis (ED). The application of membrane processes are well known for its efficiency. MF Microfiltration (MF) is a process where ideally only suspended solids are rejected, Ultrafiltration (UF) is a process where the high-molecular weight compounds (HMWC), such as protein, and suspended solids are rejected, while all low-molecular weight compounds (LMWC) pass through the membrane freely. There is consequently no rejection of mono- and di-saccharides, salts, amino acids, organics, inorganic acids or sodium hydroxide. NF rejects only ions with more than one negative charge, such as sulfate or phosphate, while passing single charged ions. NF also rejects uncharged, dissolved materials and positively charged ions. The influent to the membrane module is known as the feed stream (feedwater). The liquid that passes through the semipermeable membrane is known as permeate and the liquid containing the retained constituents is known as the concentrate (retentate). The rate at which the permeate flows through the membrane is known as the rate of flux, typically expressed as LMH (Metcalf and Eddy, 2004). The comparison between three membranes are shown in **Table 2-5**.

Table 2-5 Comparison of three membrane processes used in the research

	Nanofiltration	***Ultrafiltration***	***Microfiltration***
Thickness	150μm	150-250μm	10-150μm
Pore size	<0.002μm	0.2-0.02μm	4-0.02μm
Rejection of	HMWC mono-,di- and oligosaccharides polyvalent neg. ions,	Macro molecules, proteins, polysaccharides vira	Particles, clay bacteria
Membrane material	Cellulose acetate Thin film	Ceramic PSO, PVDF, CA Thin film	Ceramic PP, PSO, PVDF
Module	Tubular, spiral wound, plate and frame	Tubular, Hollow fiber, Spiral wound, plate and frame	Tubular, Hollow fiber
Operating pressure	500kPa-3.5MPa	100kPa-1MPa	<200kPa

Modified from Membrane Filtration Handbook

2.3.3.1 Membrane module selection and element design

Spiral wound type

This element was originally designed for desalination, however due to its compactness and low cost made it widely used in other applications such as dairy, pulp and paper, and bioethanol industrial. (**Fig. 2-6**)

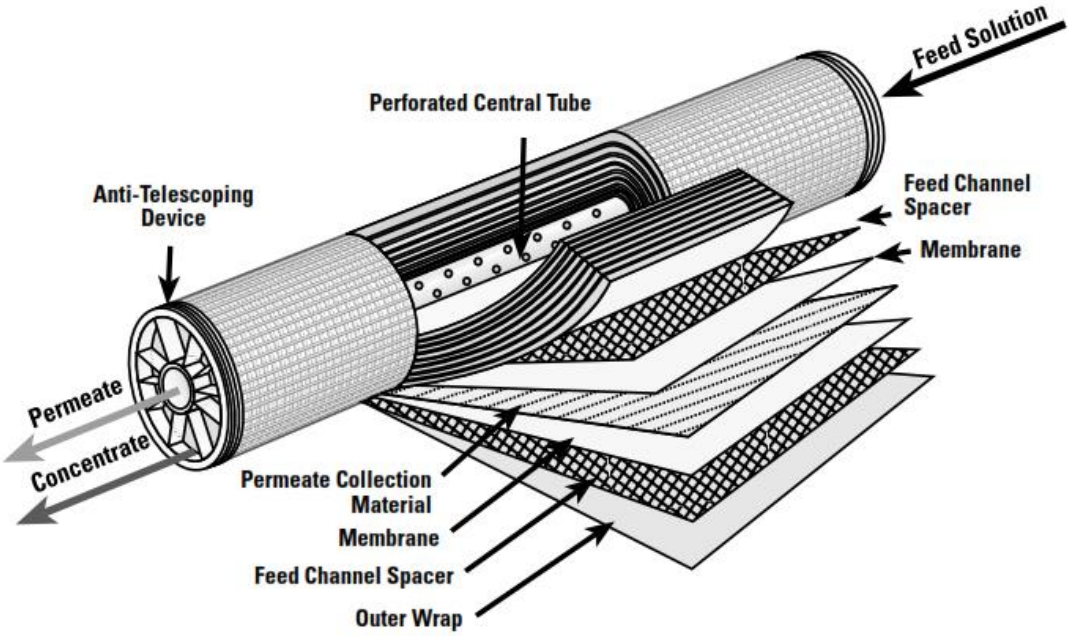


Fig. 2-6 Configuration and of spiral wound element

(Membrane filtration handbook, 2001)

Tubular membranes

Tubular membranes are simple and can tolerate suspended solids, however requiring a lot of space, difficult to change the membranes and consume a lot of energy. (**Fig. 2-7**)

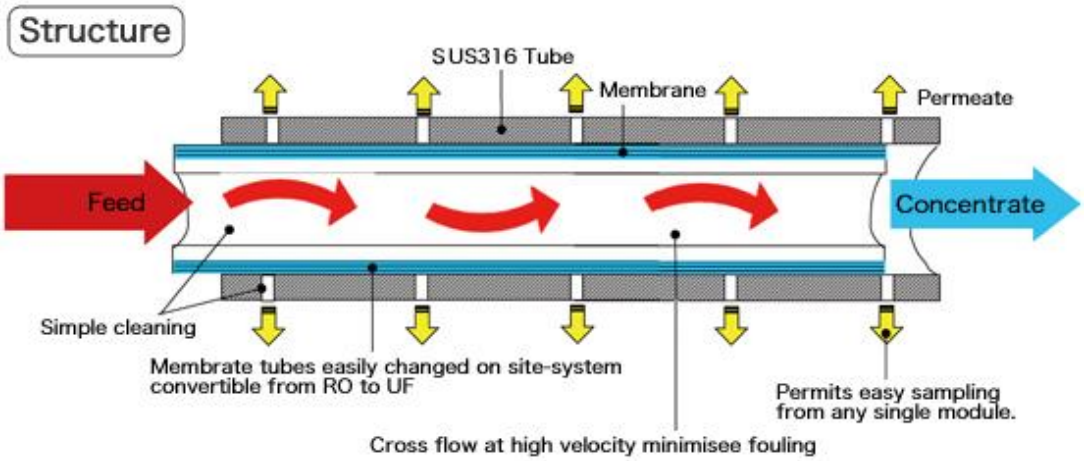


Fig. 2-7 Configuration and mechanism of tubular membrane

(Tsukishima Kankyo Engineering Ltd.)

Flat sheet system

Not very popular recently due to its lacking of development and high system cost. Modern flat sheet membrane are mostly applied for desalination with extreme high pressure of >10 MPa. (**Fig. 2-8**)

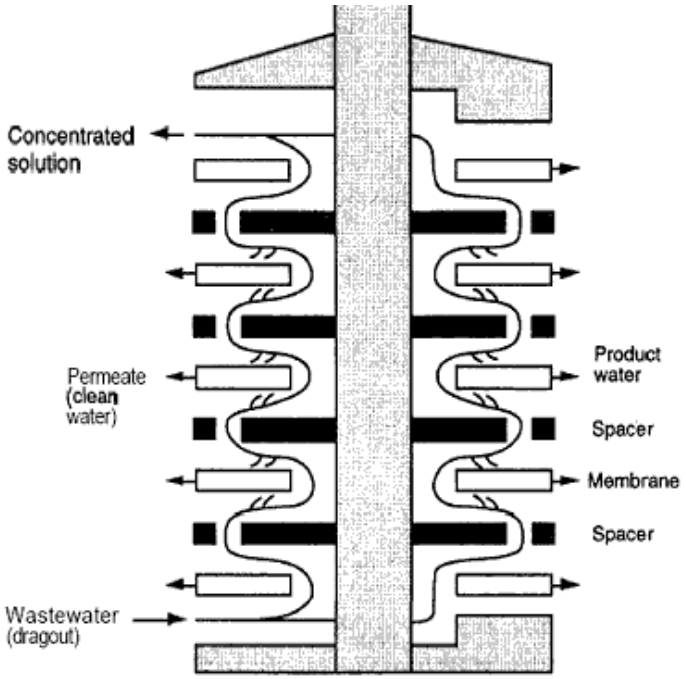


Fig. 2-8 Illustrator of a plate and frame module

(US Environmental Protection Agency, 2005)

Ceramic system

Membranes made from inorganic materials such as alumina, zirconia or glass material. Ceramic membranes has good resistance against heat, corrosion, and solvent and widely used for filtering with long life span (**Fig. 2-9**).

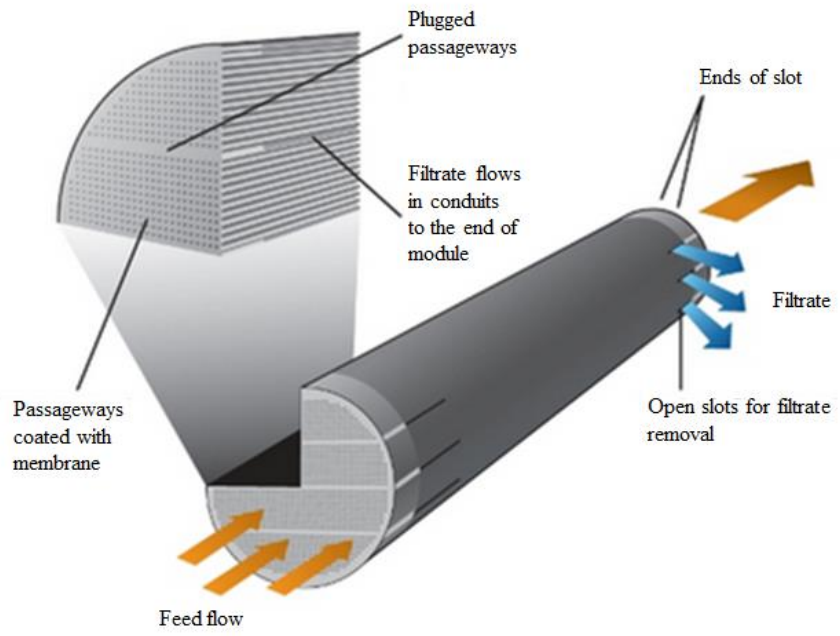


Fig. 2-9 Configuration of ceramic membrane module

(Modified from http://www.veoliawaterst.com/ceramem/en/aboutceramemtechnology.htm)

2.3.3.2 Pretreatment

Pretreatment in membrane filtration is important. The pretreatment principles are as following;

1. Remove suspended solids
2. Remove oxidizer
3. Prevent precipitation

In this research, DHS reactor were applied as suspended solid (SS) remover, however microoraganisms in anaerobic sludge blanket (ASB) reactor may slightly cause SS to be increased.

Color substances occurring in food products may present problems in connection with membrane filtration, with the naturally occurring colored substances being the most

troublesome. The more water soluble a dye stuff is, the less likely is it to cause problems (Wagner, 2001).

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Chapter 3

Development of Combined Anaerobic–Aerobic System for Treating Industrial Molasses Wastewater

3.1 Abstract

High-concentration industrial molasses wastewater treatment was examined using biological reactors coupled with physicochemical filtration membranes. The biological processes combined two mesophilic up-flow anaerobic sludge blanket (UASB) reactors, a multi stage up-flow anaerobic sludge blanket MS-UASB, and a regular UASB for primary anaerobic pre-organic removal, and a down flow hanging sponge (DHS) reactor, equipped with polyurethane sponge media for post-aerobic treatment. Concentrated blackstrap molasses was diluted [12,000~1,500 mg of chemical oxygen demand (COD)/L] with organic loading rate of 4.5~57.7 kgCOD/m^3/d (MS-UASB), 2.3~34.7 kgCOD/m^3/d (UASB), and 0.2~6.0 kgCOD/m^3/d (DHS). A 1:1.3 recirculation ratio within the MS-UASB was evaluated at different influent concentrations for COD, biogas (CH_4) production, and nitrogen, phosphate, and color removal. The average total organic COD removal was over 92% with and without recirculation. A total of 150 NL/d of biogas with 64~75% methane content was collected at the maximum loading rate and influent concentration. Ammonia was reduced from 30 mgN/L to 5 mgN/L in the DHS reactor. The dark influent could not be reduced biologically; however, ultrafiltration and nanofiltration removed 98% of the color.

3.2 Introduction

Molasses, the by-product of sugar refinery process, is considered to be one of the most recalcitrant agricultural products. It is well-known for a high concentration of organic matter and the dark color (Satyawali and Balakrishnan, 2008). Due to the world's rapid consumption of molasses, the large amount of wastewater is also generated during

its manufacturing process (Saha *et al.,* 2005). Since the conventional molasses wastewater treatments including anaerobic lagoons and continuous stirred tank reactors (CSTR) have several major disadvantages such as large space requirement, longer hydraulic retention time (HRT), emission of methane and carbon dioxide as greenhouse gases to the atmosphere (Nandy *et al.,* 2002). So there is the need of such a system which can mitigate these pitfalls. Consequently, the biological anaerobic process such as Up-flow Anaerobic Sludge Blanket (UASB) reactors accompanying with aerobic system has become widely applied for industrial molasses-based distillery wastewater treatment (Mohana *et al.*, 2009),. UASB system has continuously developed and successfully treated alcohol distillery wastewater with the organic loading rate of 20~30 kgCOD/m^3/d under thermophilic condition and produced considerable amount of biogas (Harada *et al.*, 1996). Other mesophilic anaerobic treatments for molasses using reactors are reported to have an average OLR of 12 gCOD/L/d with the average treatment efficiency of removed BOD, COD and methane productivity of 79%, 71% and 3.8 L/L-reactor/d, respectively (Kucivilize, 2004). However, only this system is incapable of treating a highly concentrated wastewater to meet the set up discharge standards. There is the necessity of an aerobic post treatment system to further treat the remaining substances. Thus, aerobic Down-flow Hanging Sponge (DHS) system, firstly designed for application in developing countries proved successfully equipped and is considered for combined operation with UASB (Tandukar *et al.,* 2005). Wilkie *et al*. (2000) also summarized that thermophilic anaerobic digestion of alcohol stillage achieved similar treatment efficiencies and methane yields compared to mesophilic treatment. Furthermore, physiological technology namely Ultra Filtration (UF) and Nano Filtration (NF) spiral-wound membrane were provisionally applied to investigate the feasibility of the hardly degrading dark brown color pigments (Pant and Adholeya, 2007) and recovery of remaining nutrients due to the advantages of membrane that have been recently reported to be prevailing over traditional approach in terms of great organics removal, nutrients removal, and dark color substances (Nataraj *et al.,* 2006). In this study, we evaluated the performance of combined biological and feasibility of the optional physicochemical systems; UASBs/DHS and UF/NF membrane filtration particularly focusing on COD, biogas production, nutrients (N, P),

and color removal.

3.3 Materials and Methods

3.3.1 Molasses wastewater and feed preparation

The substrate used in the study was a dark brown, caramel-like form of blackstrap molasses from sugar refinery in Okinawa. It contains an original COD of 600,000~800,000 mgCOD/L and was diluted with tap water to the setup concentration. Sodium bicarbonate ($NaHCO_3$; 2,500 mg/L) was directly added to the substrate tank as a buffer. The influent was pre-mixed by the raw wastewater from the temperature controlled room at 4^{o}C and pre-heated using water bath to 35~37˚C before feeding into the reactor. **Table 3-1** shows the characteristics of diluted molasses feed.

Table 3-1 Characteristics of the diluted molasses used in the experiment

Wastewater Characteristics	***Unit***	***Value***
COD_{total}	mg/L	1,100~13,000
$COD_{soluble}$	mg/L	1,000~12,000
SS	mg/L	150~600
VSS	mg/L	140~580
TKN	mg-N/L	35~180
PO_4 as P	mg-P/L	15~120
VFAs Acetic acid	mgCOD/L	50~1,240
Propionic acid	mgCOD/L	~370
SO_4 as S	mg/L	70~400
Color	Degree (°)	600~4,000
pH	-	6.5~8.0

3.3.2 Experimental apparatus

Fig. 3-1 shows a schematic of the biological process unit, which was a combination of a multi stage (MS) UASB reactor (working volume: 10 L) (**Fig. 3-2**) followed by regular-type UASB reactor (working volume: 11 L) (**Fig. 3-3**) and DHS (18 -L sponge capacity) (**Fig. 3-4**) reactors. The DHS reactor was filled with ϕ3.5xH3.5 cm polyurethane sponge media attached to the plastic sieve supporter. In the physicochemical process, 4 inches of spiral-wound UF and NF modules were employed separately to treat the effluent from the DHS reactor. UF modules outer vessel was made of fiber reinforced plastics (FRP) and installed with GE thin-film spiral-wound membrane GH8040F1001 which had molecular weight cut off (MWCO) of 2,500 Da, while NF module consisted of 4 inch-Bekaert side port PRO-4-300-SP outer vessel and TORAY spiral-wound SU220 polyamide composite membrane. The microorganism biomass used as seeding for the reactors was digested sludge collected from a local municipal sewage treatment plant mixed with sludge from a non-molasses wastewater treatment reactor.

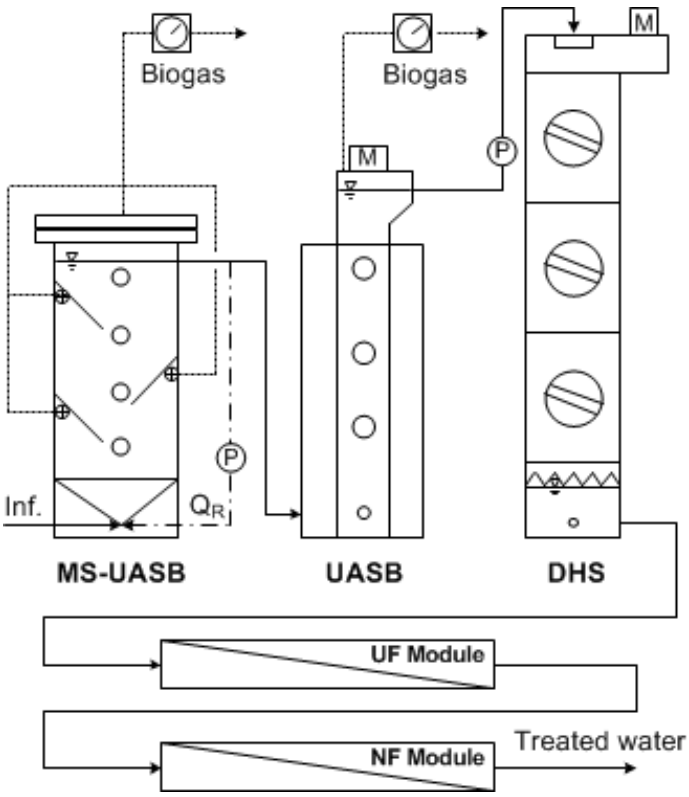

Fig. 3-1 Schematic diagram of combined UASBs/DHS system

3.3.2.1 Multi-stage upflow anaerobic sludge blanket (MS-UASB) reactor

Fig. 3-2 illustrates the schematic diagram of multi-stage UASB reactor. The reactor was made of acrylic sheet and had 8 sampling ports along with the reactor height. The working volume of the reactor is 10 L (the design and volume estimation is described in Chapter 7).

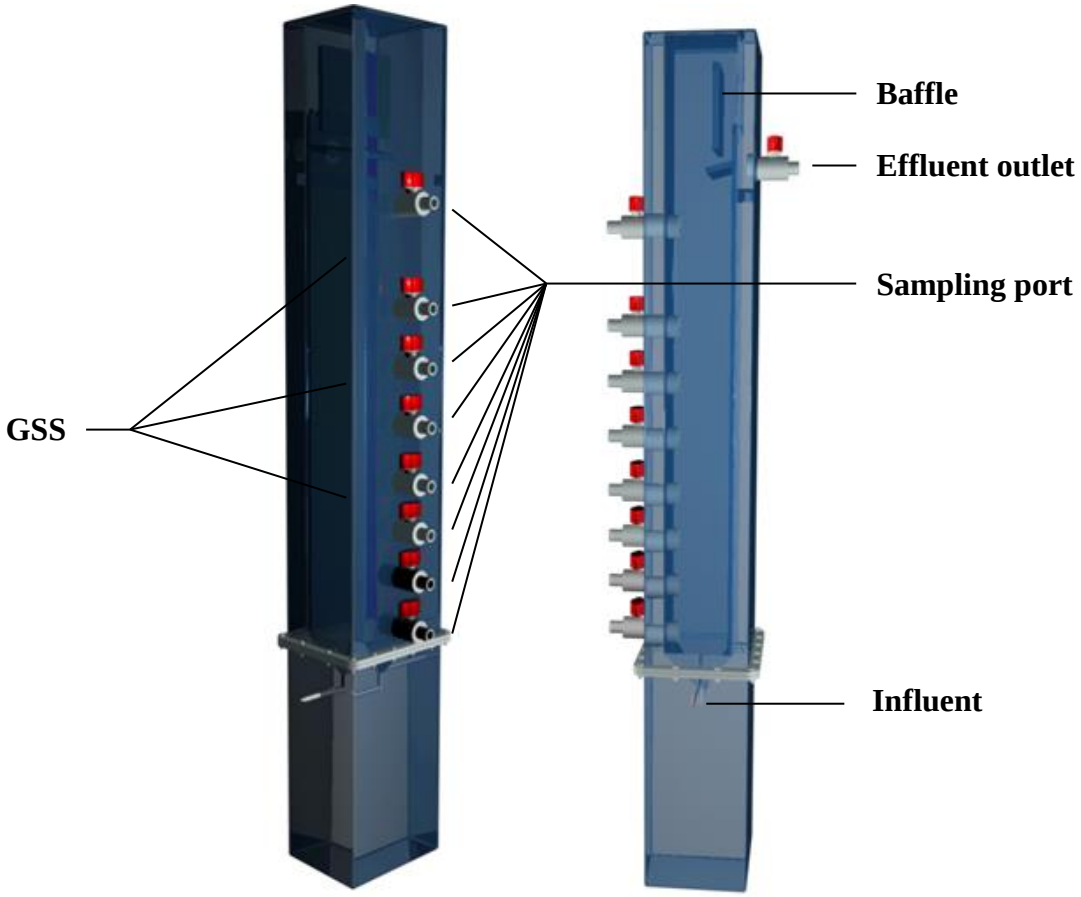

Fig. 3-2 Schematic diagram of multi-staged UASB reactor

3.3.2.2 Upflow anaerobic sludge blanket (UASB) reactor

The reactor (**Fig. 3-3**) was designed and built in accordance with the concept of an upflow anaerobic sludge blanket reactor. The agitator was installed at the upper part of the reactor in order to settle the floating scum and prevent sludge wash out. The working volume was designed at 11 L.

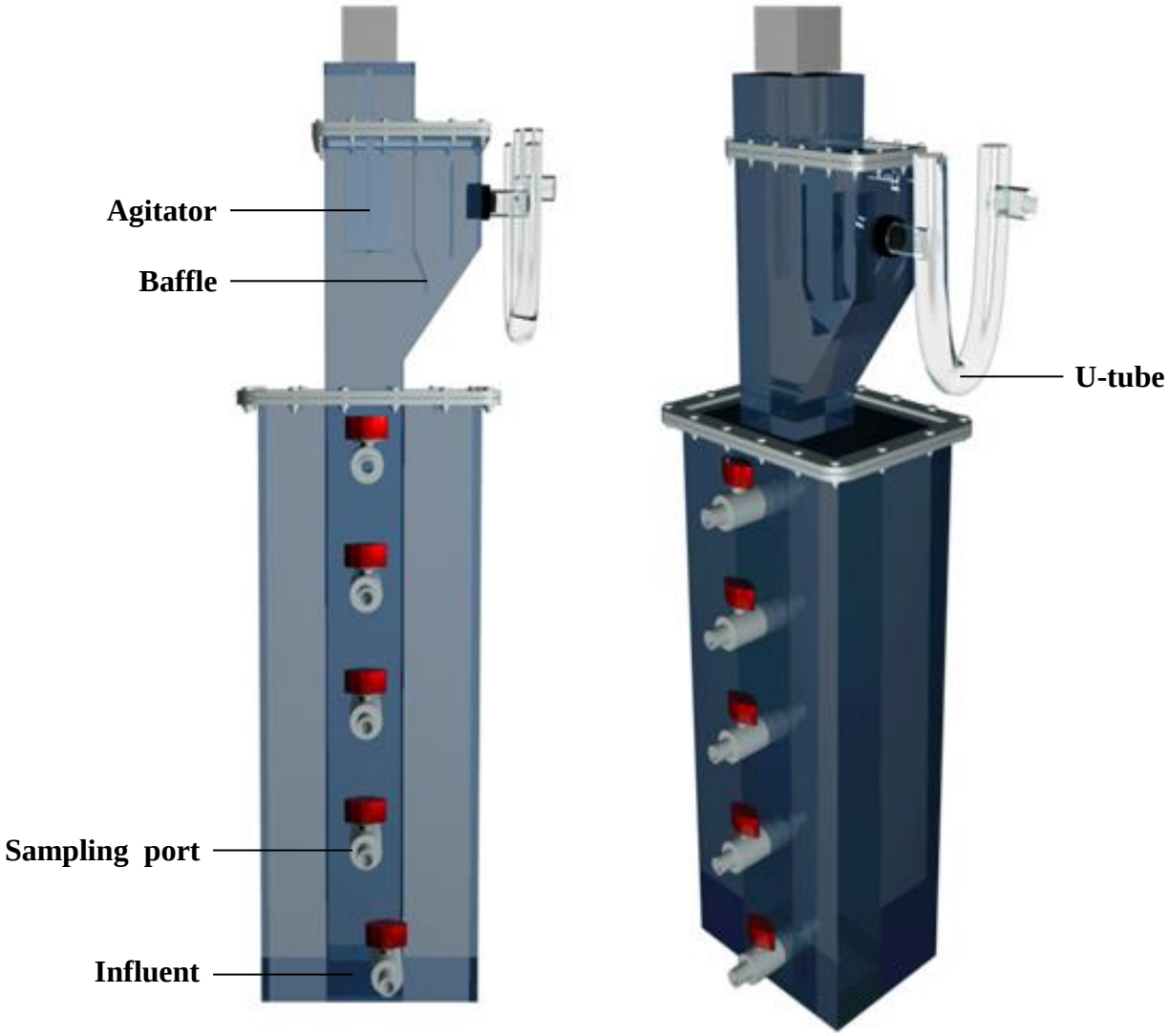

Fig. 3-3 Schematic diagram of regular UASB reactor

3.3.2.3 Down flow hanging sponge (DHS) reactor

The reactor (**Fig. 3-4**) concept is similar to trickling filter treatment. The sponge media was filled up inside the column which divided into 3 compartments. The stream was fed from the upper part and gravitationally flow to the bottom through sponge. The air was supplied into the reactor at the middle compartment.

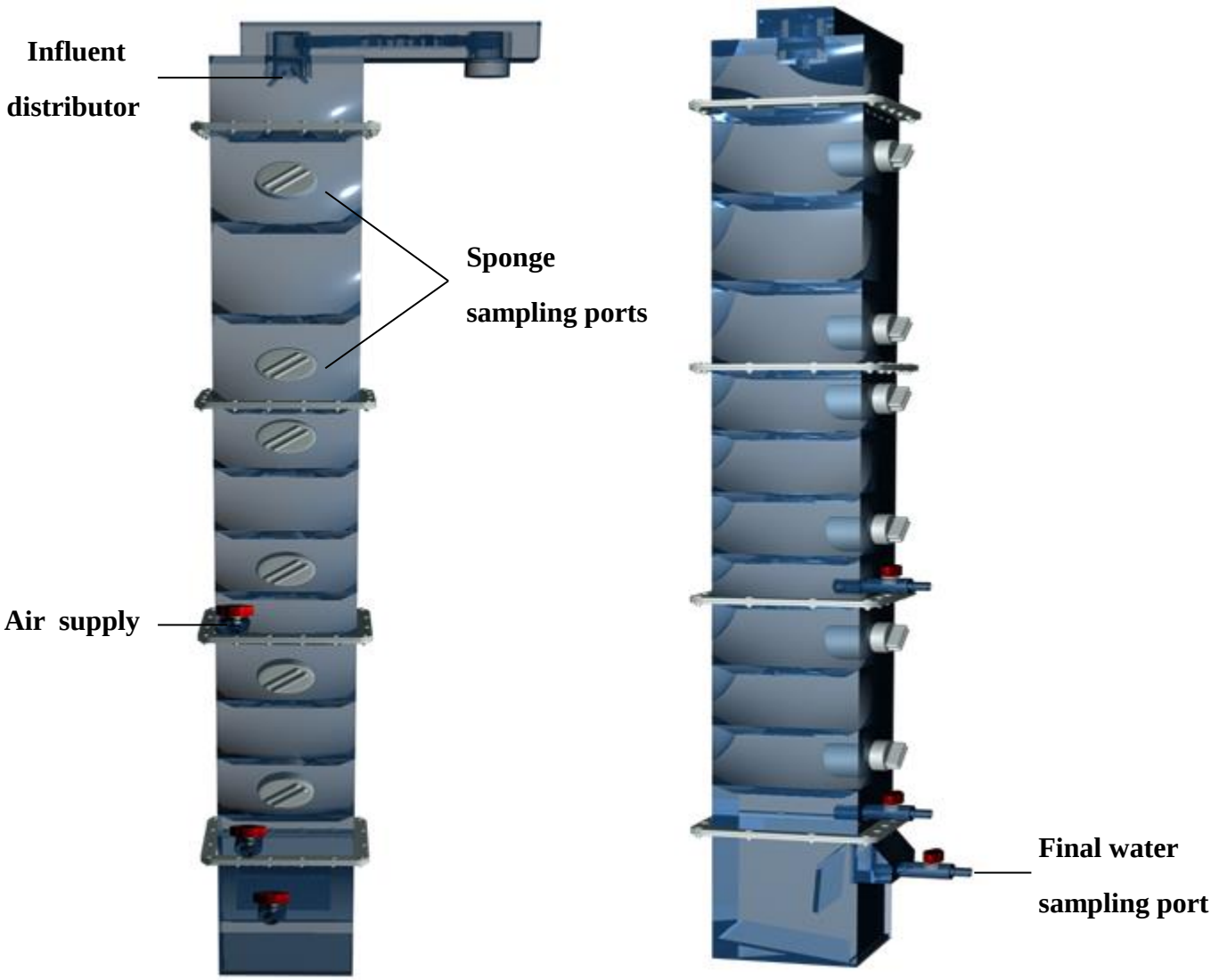

Fig. 3-4 Schematic diagram of DHS reactor

3.3.3 Analytical methods

3.3.3.1 Measurement of temperature inside reactor

The temperature inside MS-UASB and reg-UASB were real time measured and recorded by digital thermometer directly inserted into the middle of the reactor. DHS reactor was operated under ambient condition.

3.3.3.2 Measurement of flow rate from reservoir

The substrate peristalsis pumps were calibrated to ensure the stability of the feed. When the flow rate decreased, the whole transport tubes were purged by tab water to get rid of the slimy scum that attached to the tube inner.

Flow rate can be calculated by the following formula:

$$Flow\ rate\ \left[\frac{mL}{min}\right] = \frac{reactor\ volume\ [L] \times 1{,}000\left[\frac{mL}{L}\right]}{HRT\ [h] \times 60\ \left[\frac{min}{h}\right]}$$

3.3.3.3 Measurement of influent and effluent pH level

Substrate and each reactor's effluents were measured roughly by handy pH meter (HANNA HI 98128 type, pH range 0.00~14.00, temp. range 0.0~60.0^{o}C) at the reactor site when preparing the influent and again precisely measured by digital glass H^{+} electrode pH meter (TOA-DKK, HM-30R). pH meter was calibrated by standard pH solution at 6.89, 4.01 and 9.18. Molasses wastewater usually has acidic pH around 4.5~5.5, $NaHCO_3$ was directly added into the substrate tank and adjusted.

3.3.3.4 Measurement of biogas production and composition

Desulfurizers (Fe_2O_2) which were filled inside the acrylic column had been used to remove H_2S produced from the generated biogas. H_2S removed biogas will pass through the tube to the wet type gas meter (Shinagawa, WS-1A, and Measurable range of 1~600 L/h). The value displayed in the gas meter was recorded about 3~4 times a day. The amount of the gas of generation each day was calculated from the amount of the gas generated within time and the time range during the measurement time.

The gas which produced from the process was measured by Thermal Conductivity Detector (TCD) type gas chromatography (Shimadzu GC-8A).

Gas composition analysis

1. The biogas composition was determined from the gas chromatograms.
2. The volume of biogas was corrected to STP.
3. Biogas samples were analyzed, by gas chromatography, for hydrogen, nitrogen, methane and carbon dioxide.
4. The peak area of each component was recorded.
5. Gas composition percentage can be calculated by the following formula:

$$\textbf{Gas composition}\ (\%) = \frac{\textbf{Standard constant} \times \textbf{area}}{\textbf{sampling vol.}\ [\textbf{mL}]} \times \textbf{100}$$

3.3.3.5 Measurement of Chemical Oxygen Demand (COD)

In this experiment, we applied Close Reflux Method with color comparison with instant HACH dichromate reagent and spectrophotometer for the measurement of COD. The procedures were conducted with HACH Method 8000.

COD Mass Balance

- **COD_{IN}**

Ex. Influent COD = 1,000 mg COD/L

Measured flow rate = 1,400 L/day

$$\therefore \mathbf{1,000}\left[\frac{\boldsymbol{mg\ COD}}{\boldsymbol{L}}\right] \times \frac{[\boldsymbol{g\ COD}]}{\mathbf{1,000}\ [\boldsymbol{mg\ COD}]} \times \mathbf{1,400}\ \left[\frac{\boldsymbol{L}}{\boldsymbol{day}}\right] = \mathbf{1400}\ \left[\frac{\boldsymbol{g\ COD}}{\boldsymbol{day}}\right]$$

- **COD_{OUT}**

Ex. Effluent COD = 400 mg COD/L

Effluent removed = 1,400 L/day

$$\therefore \mathbf{400}\left[\frac{\boldsymbol{mg\ COD}}{\boldsymbol{L}}\right] \times \frac{[\boldsymbol{g\ COD}]}{\mathbf{1,000}\ [\boldsymbol{mg\ COD}]} \times \mathbf{1,400}\ \left[\frac{\boldsymbol{L}}{\boldsymbol{day}}\right] = \mathbf{560}\ \left[\frac{\boldsymbol{g\ COD}}{\boldsymbol{day}}\right]$$

- **$COD_{methane}$**

Fixed constants were set as follows:

Temperature : Varies to the environment Ex. = 25°C = 298 K

ρH_2O : 47mmHg = 0.06184211 atm

R (gas constant) : 82.057 mL·atm/k

Measured pressure: 1.016 atm

Corrected pressure: 1.016 – 0.06184211 = 0.9744 atm

Measured Gas production = 300 L/day

Methane composition: 70%

0.70 x 300 L/day = 210 L/day

PV = nRT

$$\mathbf{n} = \frac{\boldsymbol{PV}}{\boldsymbol{RT}} = \frac{\mathbf{0.9744}\ [\boldsymbol{atm}] \times \mathbf{210}\left[\frac{\boldsymbol{L}}{\boldsymbol{day}}\right] \times \mathbf{1,000}[\boldsymbol{mL}]}{\mathbf{82.057}\left[\frac{\boldsymbol{mL \cdot atm}}{\boldsymbol{K}}\right] \times \mathbf{298}[\boldsymbol{K}]} = \mathbf{8.37}\ \left[\frac{\boldsymbol{moles}}{\boldsymbol{day}}\right]$$

1 mole of CH_4 = 16 g

8.37 moles = 133.89 g

Theoretical COD of 1 g CH_4 is 4 g therefore 89.26 gCH_4 is equal to 535.56 g COD

3.3.3.6 Measurement of Solid contents

Suspended Solids Dried at 103-105^{o}C

$$\frac{\boldsymbol{mg\ SS}}{\boldsymbol{L}} = \frac{(\boldsymbol{A}-\boldsymbol{B})\times \mathbf{1,000}}{\boldsymbol{sample\ volume}\ [\boldsymbol{mL}]}$$

where:

A = weight of dried residue + filter paper [mg]

B = weight of filter paper [mg]

Volatile suspended solids ignited at 550^{o}C

$$\frac{\boldsymbol{mg\ VSS}}{\boldsymbol{L}} = \frac{(\boldsymbol{C}-\boldsymbol{D})\times \mathbf{1,000}}{\boldsymbol{sample\ volume}\ [\boldsymbol{mL}]}$$

where:

C = weight of residue + filter paper after 103–105^{o}C

D = weight of residue + filter paper after ignition at 550^{o}C

3.3.3.7 Measurement of Volatile Fatty Acid (VFA)

In this study, volatile fatty acid (VFAs) were measured by gas chromatography (Shimadzu GC-1700). Firstly, the sample used for VFA analysis were filtered by 0.4 µm (ADVANTEC-GB140) filter paper. To fix dissociated VFA, we added small amount of HCl and adjusted pH to acidity. VFA was finally calculated in accordance with COD equivalent as mg COD/L.

Ex. Acetic acid: CH_3COOH^-

$$CH_3COOH^- + 2O_2 \rightarrow 2CO_2 + 2H_2O$$

O_2 is considered as COD

1 mol of acetic acid is equivalent to 4 mol of oxygen

$\therefore$ 1 mol of acetic acid = 2x16x2 = 64 gCOD

1 molecular weight of CH_3COOH = (12x2) + (1x4) + (16x2) = 60 g

60 g of CH_3COOH = 64 gCOD

$\therefore$ 1 g of CH_3COOH = 64/60 = 1.067 g O_2/g Acetic acid

3.3.3.8 Biodegradability test

This test was conducted in order to investigate the substrate degradability of the seed sludge taken on the day 0 and incubated in 122-mL vials at 35°C. All test sludge were withdrawn from reactor and purged with N_2 gas to maintain anaerobic condition. Test sludge was rinsed with 25-mM phosphate buffer at the test temperature and homogenized (Physcotron, Nichion, Japan). H_2/CO_2 (80:20, v/v), acetate, propionate and diluted molasses were used as substrate.

3.3.3.9 SEM observation of UASB sludge

The observation of retained sludge from UASB reactors by scanning electron microscope (Hitachi, S-4500) were carried out. The sample were filtered using φ0.2 μm pore size hydrophobic PTFE membrane (Advantec T020A025A) followed by 0.9% NaCl washing before fixation. Samples were washed five times with 0.1 M sodium cacodylate buffer [$Na(CH_3)_2\ AsO_2 \cdot 3H_2O$] (pH 5.0‑7.4) and soaked in 2.5% glutaraldehyde for 3 hours. The dehydration by different ethanol concentration and dipping time were conducted (50% 25 min, 70% 20 min, 90% 60 min, 95% 25 min twice, and 100% 30 min 3 times). Finally, samples were soaked in isoamyl acetate for 30 min before drying at critical point using CO_2 liquid with critical point dryer (Hitachi, HCP-2). Dry samples were mounted on specimen stub with graphite and coated with Pt-Pd by ion-sputtering

machine (Hitachi, E-1030) then stored stubs in the desiccator before observation (Kucivilize *et al.*, 2004).

3.4 Result and Discussion

3.4.1 Biological process performance

Figure 3-5 shows the time course of COD removal efficiency in the biological process, which achieved an average organic removal of more than 90% of COD and 95% of BOD. The maximum OLR of 57 kgCOD/m^3/d (MS-UASB), 19 kgCOD/m^3/d (UASB) and 2.1 kgCOD/m^3/d (DHS) corresponded to a maximum influent concentration of 12,000 mgCOD/L with an HRT of 5 h in the MS-UASB, 6 h in the UASB, and 10 h in the DHS. In the membrane process, with 910 mgCOD/L of effluent from the DHS as a feed, the UF and NF membranes achieved final effluent concentrations of 48 mgCOD/L and 18 mg COD/L, respectively.

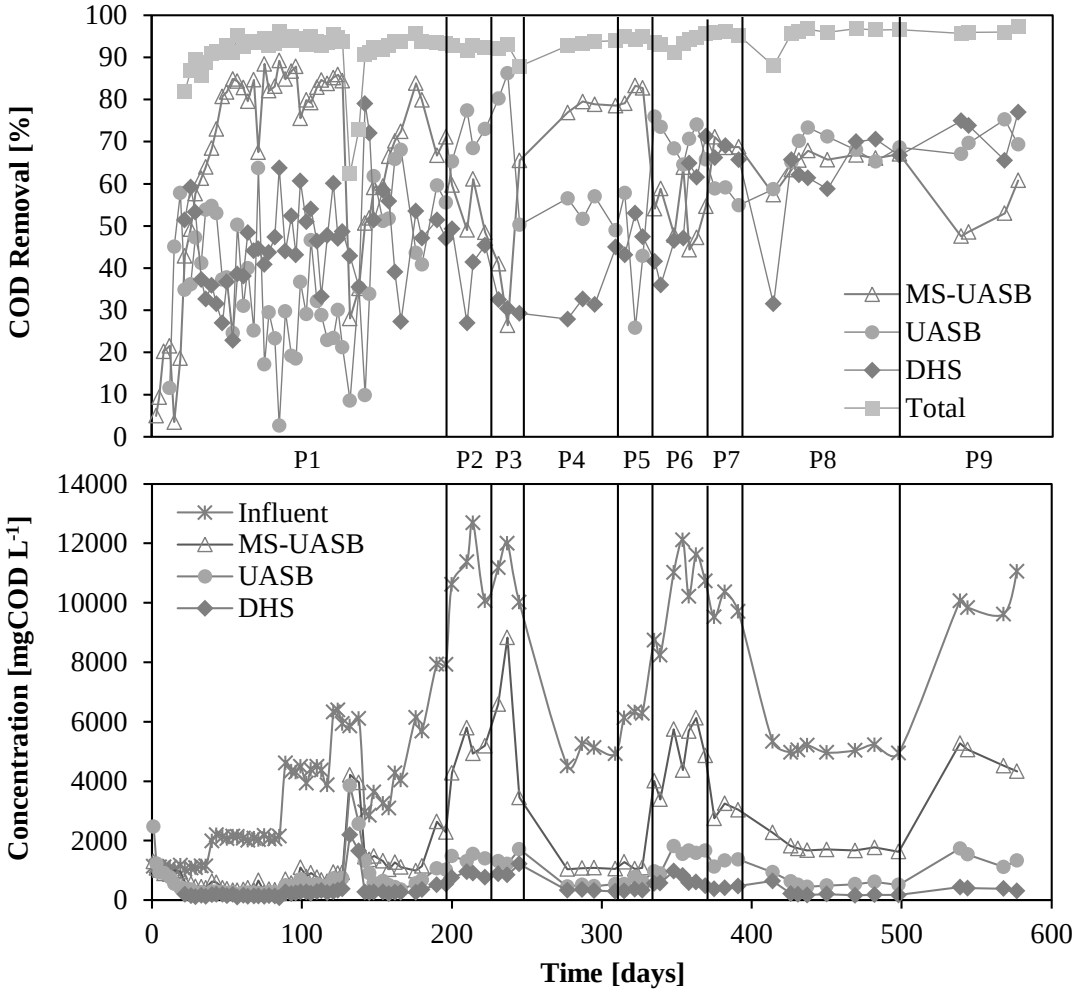

Fig. 3-5 COD removal efficiency and effluent concentration of each reactor

Table 3-2 Operational conditions for the biological processes

Phase no.	Opt.period [day]	COD conc. [Before rec.] [mg·L^{-1}]	Rec.ratio [Rec.Q/Q]	HRT				MS-UASB COD Loading [kgCOD·m^{-3}·d^{-1}]
				Total [h]	MS-UASB [h]	UASB [h]	DHS [h]	
1 ※1	1 - 196	1,000 − 8,000	-	~ 24.1	~ 5.7	~ 6.7	~ 11.9	5 - 36
2 □	197 - 231	10,000	-	21.7	5.3	6.1	10.3	51
3 ■	232 - 245	10,000	1.3	18.7	2.3	6.1	10.3	87
4 ●	246 - 309	5,000	1.3	18.7	2.3	6.1	10.3	29
5 ※2	310 - 339	6,000 − 8,000	1.3	18.7	2.3	6.1	10.3	35 - 60
6 □	340 - 363	10,000	-	21.7	5.3	6.1	10.3	50
7 ☆	364 - 391	10,000	-	43.4	10.6	12.2	20.6	23
8 ○	392 - 498	5,000	-	21.7	5.3	6.1	10.3	23
9 ■	499 - 577	10,000	1.3	18.7	2.3	6.1	10.3	76

※1, ※2 : Start up period and loading up; □○ : Same HRT, Different conc.; ■● : with Recirculation; ☆ : Long HRT, Max.conc.

The operational conditions were divided into nine phases P1-P9 (**Table 3-2**), with P1 as the startup and steady period, and the influent concentration was gradually raised from 1,000 to 8,000 mgCOD/L. On the 140th day, the buffer was reduced (shock loading), and all three reactors showed the same effects from the pH drop, but recuperated in 14 days after the pH was adjusted to normal. During the pH shocking period, acetate accumulated from 15.1 mg/L to 1,043.9 mg/L and propionate from 0 mg/L to 720.2 mg/L, which was assumed to be due to the temporary inhibition of methane-producing bacteria. COD removal by the UASB and DHS reactors appeared to fluctuate; this resulted from the MS-UASB performance when most of the organic compounds were treated in the MS-UASB and the remaining compounds had relatively low concentrations (less than 1,000 mgCOD/L) that could lower the F/M ratio of the system. In P2, the influent concentration was raised to 12,000 mgCOD/L, and the MS-UASB performance deteriorated due to the high OLR after the 200th day, which resulted in the COD removal efficiency decreasing to 60%. However, the UASB and DHS could recover the excessive loading and support a total removal of 90%. When the recirculation ratio was 1:1.3 within the MS-UASB in P3 and the same 10,000 mgCOD/L of influent concentration was applied, COD removal was almost the same, but accumulated propionate decreased from its highest value of 1,120 mg/L in P2 (pH shocks) to 670 mg/L in P3 when recirculated (data not shown). In P4, the influent concentration was reduced to 5,000 mgCOD/L with the same overall HRT to lessen the loading stress and stabilize the system. Consequently, the recovery rate of the MS-UASB again increased to nearly 80%, which led to a decrease of 50~55% in UASB and 30% in DHS removal efficiencies from the 246th day. After system re-stabilization, the loading of each reactor was raised with the recirculation mode, and the performances of all reactors in the second half-period were different. As a result, the MS-UASB appeared to be enfeebled several times by the direct high loadings with concentrations of about 10,000 mgCOD/L and the amount of the granular seed sludge was enlarged and turned into bulking sludge floating out of the reactor. In contrast, the UASB and DHS efficiently recovered untreated organic matter, as revealed in P6. The removal percentages of the UASB and DHS reactors were higher, in contrast to the MS-UASB performance, which gradually decreased and remained in the range of 45~67%.

At the OLR of 30 kg-COD/m^3/d, a substrate concentration of 6,000 mgCOD/L at the HRT of 5 h appeared to be the optimum condition for the MS-UASB to remove 80% of the COD removal percentage in this operation (**Fig. 3-6**).

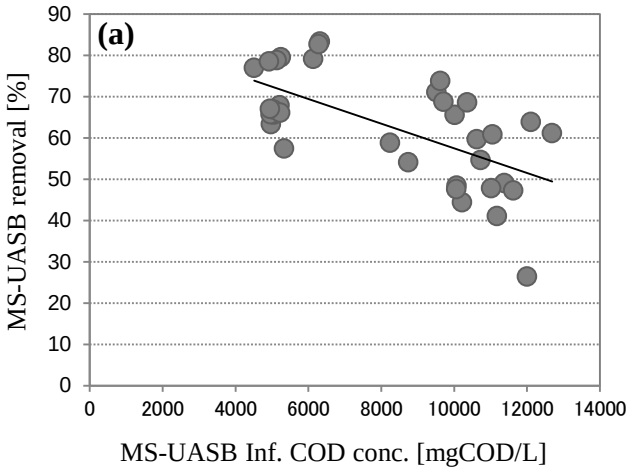

Fig. 3-6a MS-UASB COD % removal vs. influent (inf.) concentration (conc.)

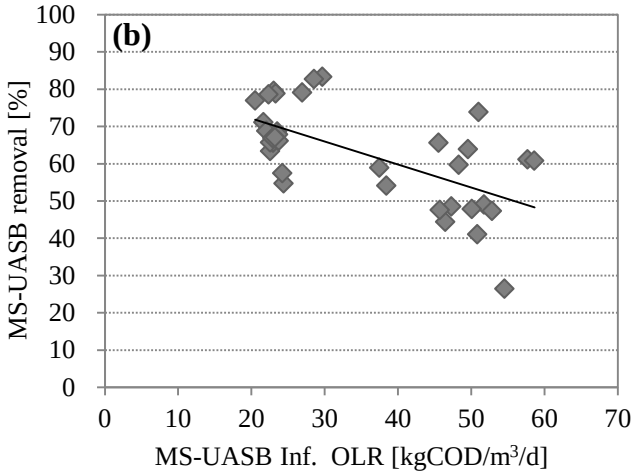

Fig. 3-6b MS-UASB COD % removal vs. influent (inf.) OLR

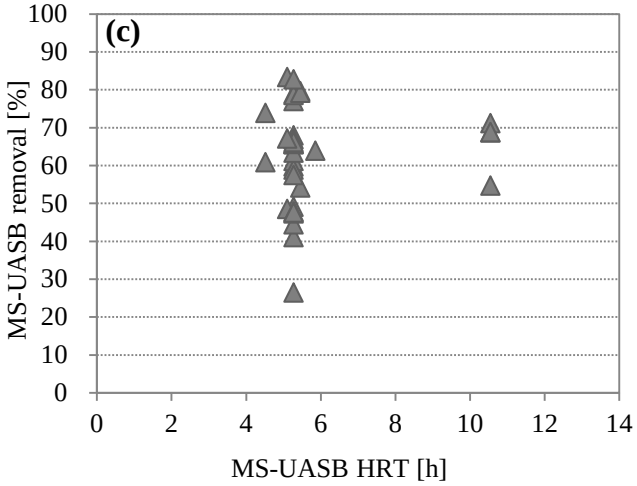

Fig. 3-6c MS-UASB COD % removal vs. influent (inf.) HRT

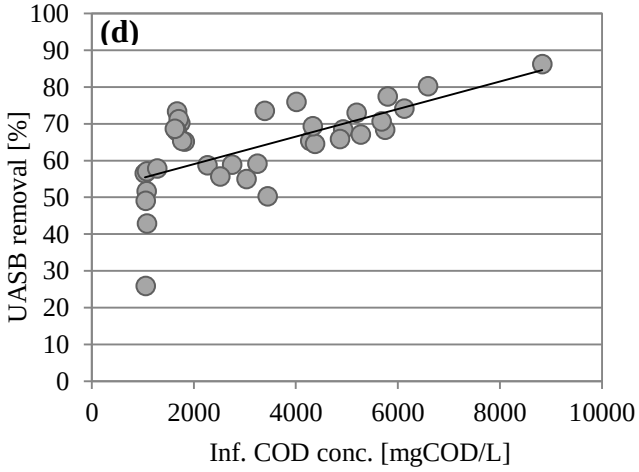

Fig. 3-6d UASB COD % removal vs. influent (inf.) concentration (conc.)

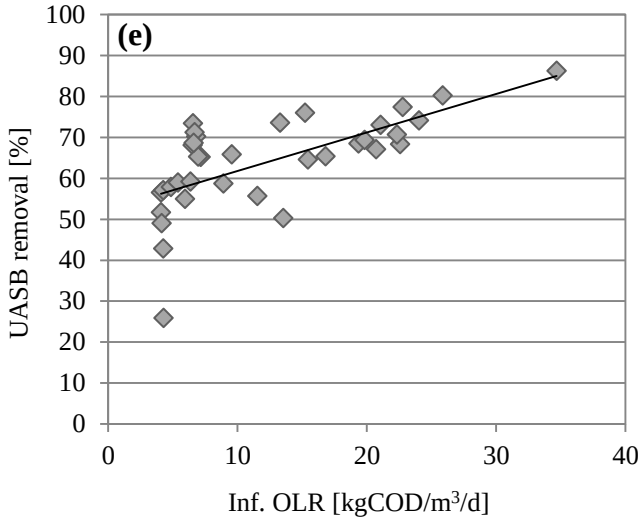

Fig. 3-6e UASB COD % removal vs. influent (inf.) OLR

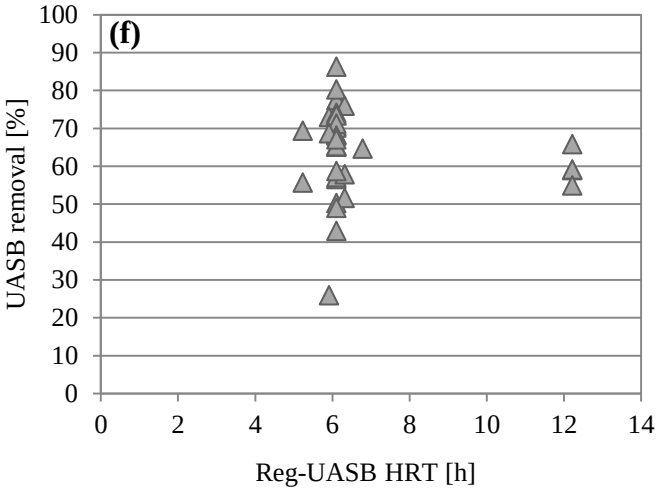

Fig. 3-6f UASB COD % removal vs. influent (inf.) HRT

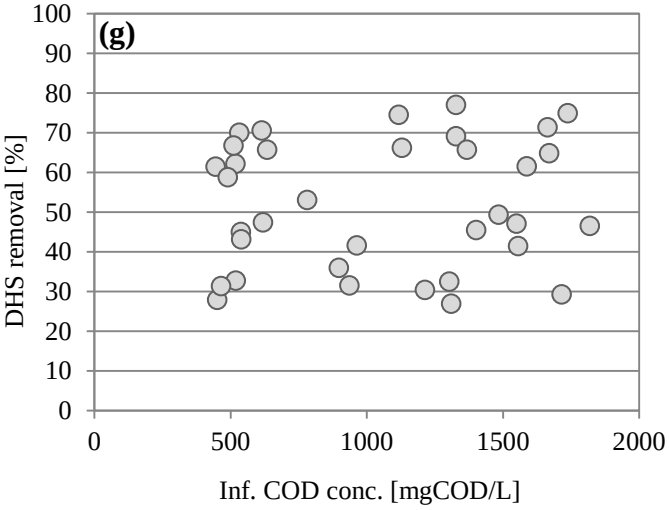

Fig. 3-6g DHS COD % removal vs. influent (inf.) concentration (conc.)

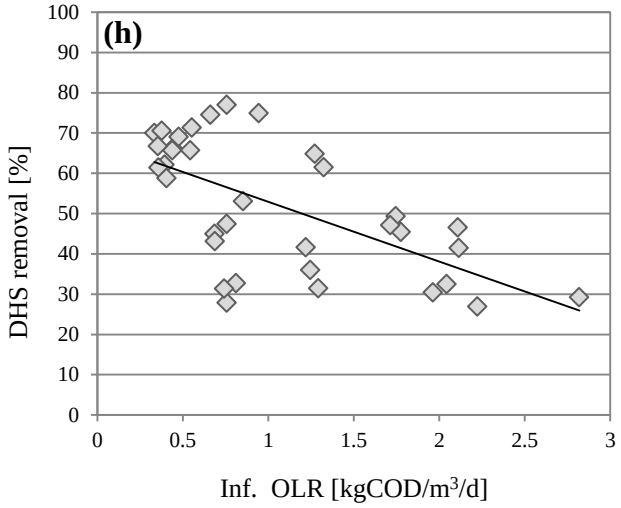

Fig. 3-6h DHS COD removal % vs. influent (inf.) OLR

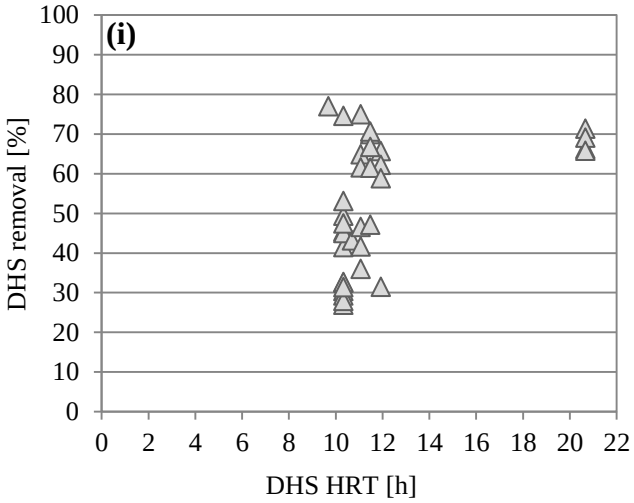

Fig. 3-6i DHS COD removal % vs. influent (inf.) HRT

To further study the reactors' performance, the COD balances of both the UASB and DHS reactors at 5,000 and 10,000 mgCOD/L influent concentrations with and without recirculation were assessed. At a 5000 mgCOD/L influent concentration (**Fig. 3-7**), wastewater was treated and converted by the main MS-UASB unit to methane gas more effectively with recirculation (67%) than the non-recirculation mode (36%) and the percentage removal was 75~80% compared with 65% removal efficiency in the non-recirculation mode. At the higher concentration of 10,000 mgCOD/L (**Fig. 3-8**), recirculation within the MS-UASB slightly increased the methane conversion from 51%

to 61%. This could have resulted from an insufficient recirculation ratio against the high concentration influent.

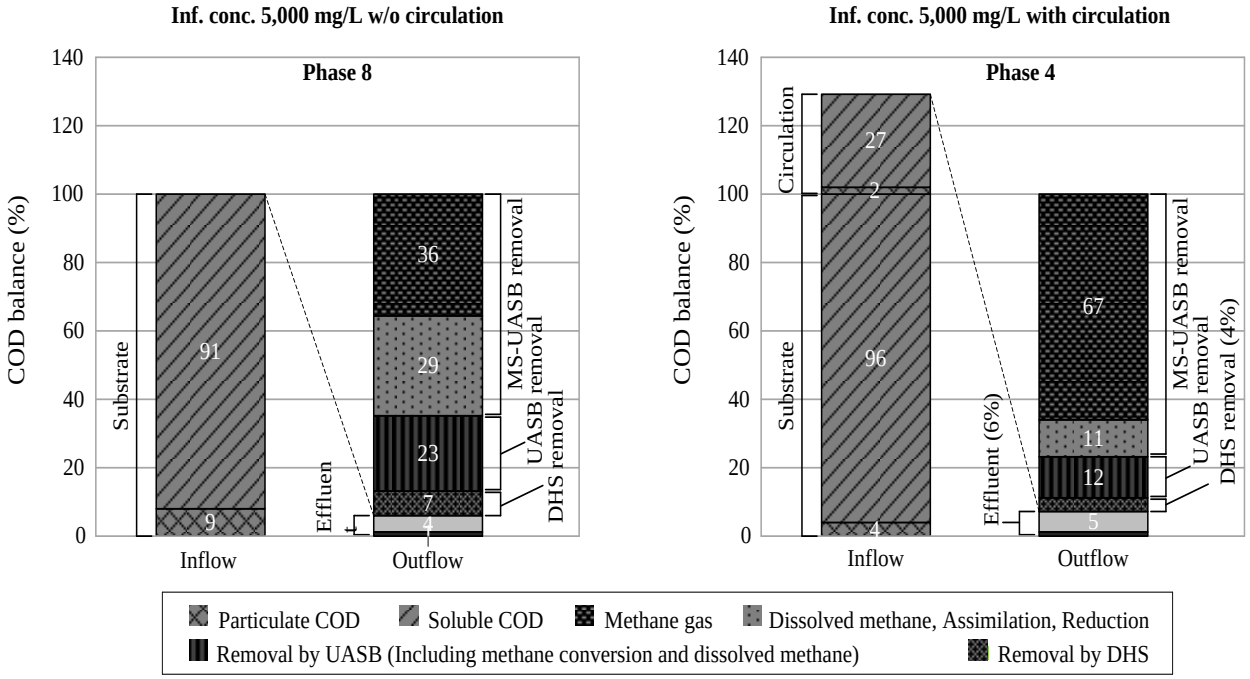

Fig. 3-7 COD balance at 5,000 mg/L concentration

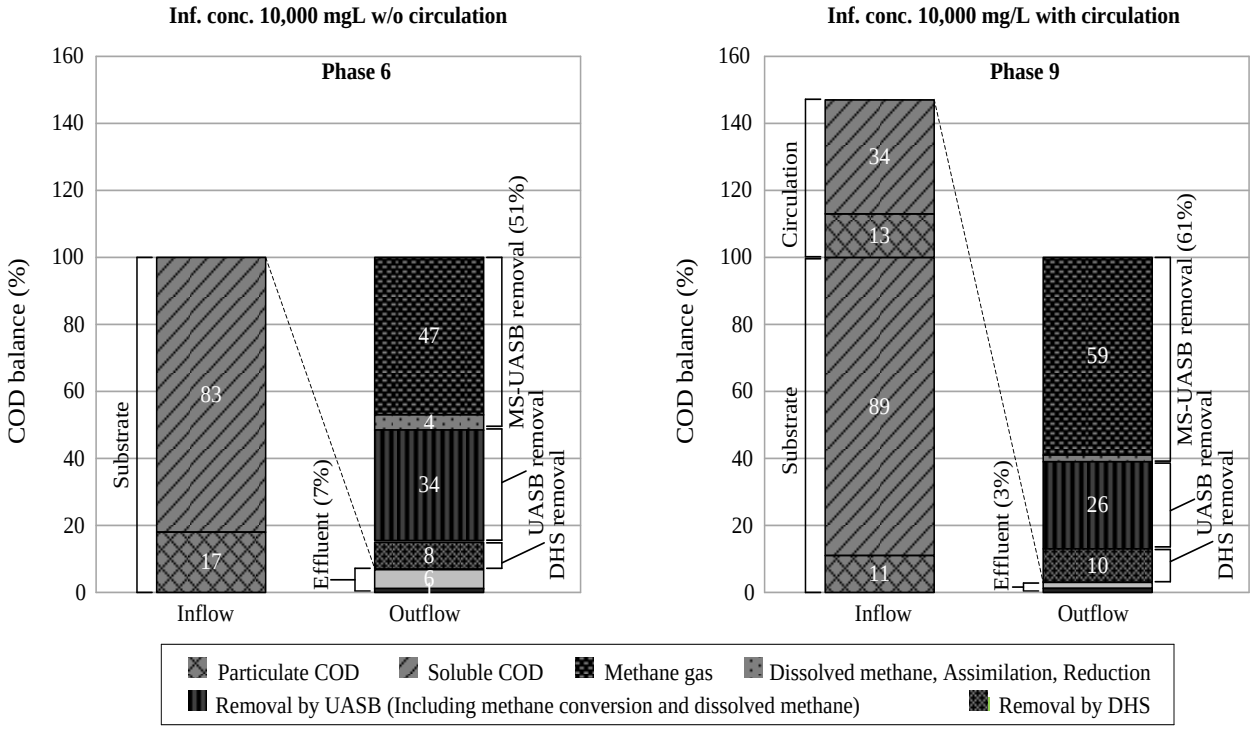

Fig. 3-8 COD balance at 10,000 mg/L concentration

3.4.2 Biodegradability test

This test was performed to evaluate the biodegradability of the wastewater and the adaptability of sludge cultivated on different wastes for molasses wastewater treatment. **Fig. 3-9** indicates that molasses wastewater could be degraded and converted to methane gas with an initial 0.3 gCH_4-COD/gVSS/d compared with other substrates.

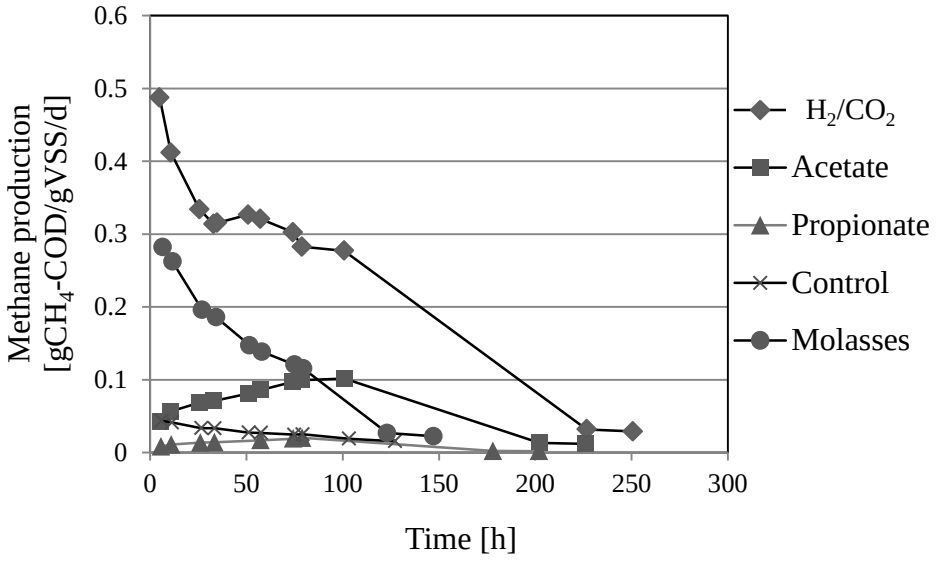

Fig. 3-9 Biodegradability test vs. various substrates

Table 3-3 Summary of pH, COD, color, and nutrients removal of the total system

Subject		COD [mg/L]	BOD [mg/L]	pH	Color [degree]	PO_4 -P	NO_2 -N	NO_3-N [mg/L]	NH_4-N	TKN
Substrate		12,700	5,520	7.1	3,310	119	2	0	1	132
Biological phase	MS-UASB	4,930	1,890	7.8	2,620	258	0	0	27	107
	Reg-UASB	1,160	280	7.9	2,340	111	0	0	30	45
	DHS	911	110	8.8	3,600	48	0	31	5	28
Physiological phase	UF	48	N.D.	8.5	33	12	18	22	9	9
	NF	18	N.D.	8.5	1	6	8	27	1	1
Industrial wastewater Standard		120	160	5.8~8.6	100*	16		120 as TN		

* Standard: not objectionable ; 100 unit is an author's objective value, N.D. : Not Determined

3.4.3 Color and nutrients removal

Anaerobic bacteria are reported to have the ability to decompose the colored substance melanoidin in molasses spent wash (MSW). In fact, in **Fig. 3-10**, MS-UASB and UASB system decolorized approximately 10–15% of the substrate, it was reported that anaerobic bacteria produced some enzymes that could degrade melanoidin substances (Sirianuntapiboon *et al.*, 2004). The color turned almost 50% darker after treatment with the DHS reactor, which could have been due to repolymerization of the pigments. The UF/NF system showed significant removal of the molasses wastewater color, from 3600 color units in the DHS effluent to 1 color unit (colorless; 99% removal). Moreover, the experiments revealed the reduction of PO_4^{3-}-P from 48 mg P L^{-1} to 6 mg P L^{-1} and of total nitrogen from 64 mg N L^{-1} to 37 mg N L^{-1}, which were not removed by biological processes (**Table 3-3**).

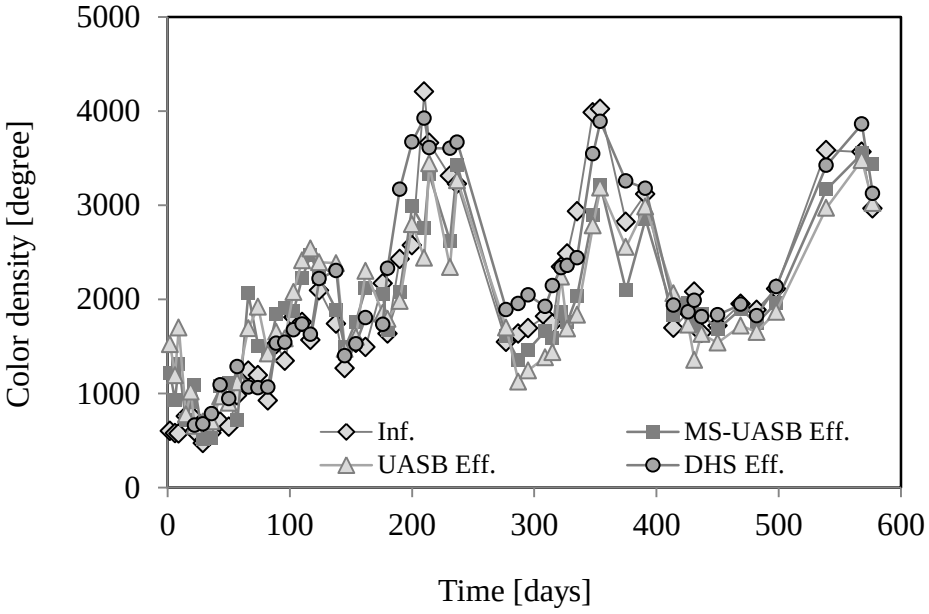

Fig. 3-10 Color profile of biological processes

3.4.4 Microbial observation

Fig. 3-11 shows SEMs of granular sludge taken from the 75th day seed sludge. Clusters of round microorganisms morphologically similar to *Methanosarcina sp.* could be distinguished (**Fig. 3-11a**). These archaea were probably the predominant organisms from the previous treatment environment. In the 175th day sludge sample, when sodium bicarbonate was added to the substrate to adjust the pH (7.5–8.0), *Methanosaeta*-like microorganisms were observed (**Fig. 3-1b**). **Fig. 3-11c** shows filamentous-like microorganisms covering the outer surface of wash-out sludge taken at the 335th day of operation. These microorganisms were possibly related to acid-forming bacteria that caused the biogas to be trapped inside the granular sludge and to flow up along with the stream.

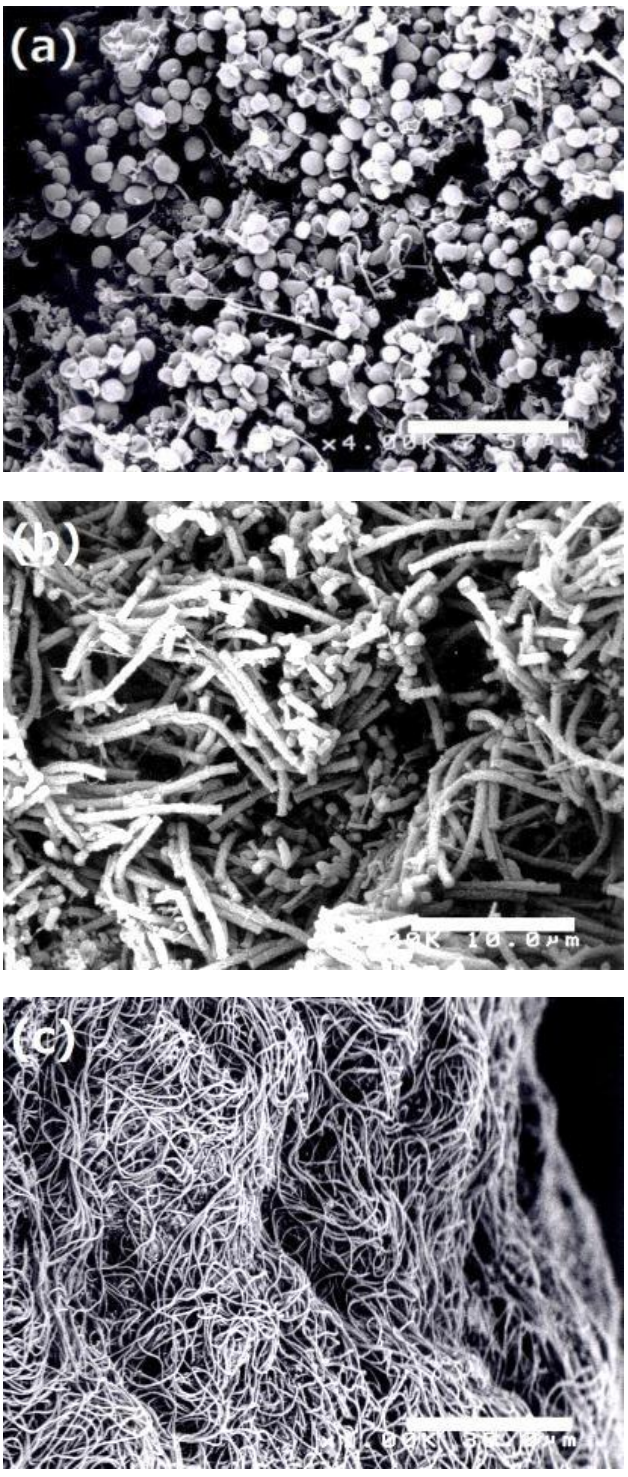

Fig. 3-11 Morphological SEM observations of granular sludge from the MS-UASB reactor: (a) *Methanococcus*-like microorganisms (bar: 7.5 µm); (b) *Methanosaeta*-like microorganisms (bar: 10.0 µm); (c) filamentous microorganisms at the surface of wash-out sludge (bar: 30.0 µm)

3.5 CONCLUSIONS

Over the 500 days of operation, the performance of the UASB/DHS process for the treatment of molasses-based spent wash wastewater was satisfactory, with 90% COD removal efficiency at a total HRT of 16 h and the ability to cover from various critical conditions. The MS-UASB, which performed the main biological treatment, was able to treat MSW wastewater with a concentration of 6000 mgCOD/L and OLR of 20 kgCOD/m^3/d at an HRT of 7 h at optimum stability. The UASB and DHS reactors also played significant roles by effectively backing up the MS-UASB in case of emergency excessive loading at an OLR of 58 kgCOD/m^3/d. The application of the physicochemical membrane (UF/NF) proved to be a pragmatic approach, with 98% COD removal and 99% color removal from the DHS effluent. The typical anaerobic microorganisms reported in the literature could be observed by SEM. *Methanosarcina*-like archaea, *Methanosaeta*-like archaea and filamentous microorganisms were distinguished, but these will require further analysis by microbiological methods, such as 16S rRNA, to elucidate the phylogenetic details. The biological process appeared to be capable of treating the MSW wastewater for organic matter and nitrogen removal with nitrification within the DHS at a total OLR of 15 kgCOD/m^3/d and an HRT of 24 h; whereas the membrane process demonstrated remarkable color and phosphate removal efficiency. Therefore, this combined biological and physicochemical treatment system could achieve optimum performance for meeting the industrial wastewater standard.

3.6 Reference

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Chapter 4

Pilot-scaled Anaerobic-Aerobic-Membrane System for Molasses Fermentation Residue Wastewater Treatment

4.1 Abstract

This paper describes the development of an effective system suitable for trial in a full-scale plant. A series of pilot-scale units consisting of upflow anaerobic sludge blanket (UASB) reactors, downflow hanging sponge (DHS) reactors, and anaerobic sludge blanket (ASB) reactor, followed by microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) membrane units was assembled to conduct concurrent organic matter–nutrient removal and decolorization of molasses fermentation residue wastewater. The bioprocess operated with a total hydraulic retention time of 35 h. The UASB reactors removed 70% of the organic matter at the maximum chemical oxygen demand (COD) volumetric loading of 11.6 kgCOD/m^3/d. More than half (52%) of the raw wastewater organic nitrogen was converted to ammonia by the UASB reactors. The DHS reactors removed 60% of the total nitrogen. Denitrification (64%) occurred in the ASB reactor. MF was applied to remove particulate substances, preventing membrane fouling in the following units. Phosphorus was mainly removed by NF, with 82% rejection. Remarkably, the UF and NF units could achieve 90% and 99% color removal, respectively. No membrane fouling was detected in any of the membrane units along the entire operation.

4.2 Introduction

Bioethanol produced from sugarcane has drawn considerable attraction in the past decade as a renewable source of clean energy to replace fossil fuels. In most sugar- and bioethanol-producing regions, such as India, Thailand, and Brazil, water pollution from agricultural manufacturing is one of the most serious environmental dilemmas. For every liter of bioethanol produced, 8~15 L of wastewater is generated. Three major

wastewaters produced during bioethanol processing are fermentation residue, distillery wastewater, and spent wash wastewater. These wastewaters contain high levels of both organic and inorganic substances. The chemical oxygen demand (COD) of raw residue ranged between 160,000~200,000 mgCOD/L, while the biochemical oxygen demand (BOD) ranged between 90,000~150,000 mg/L. Waste from bioethanol-producing plants has acidic pH, ranging from 4.0~5.0 and contains sugar-degrading microorganisms (yeast). Sugarcane-based bioethanol usually contains potassium and other inorganic compounds, including a dark brown color pigment named melanoidin, which is produced during the Maillard reaction (Plavsic *et al.*, 2006; Kumar and Chandra, 2006). Melanoidin has been reported to be a commercial, nutritional substance that affects food appearance, but it also influences health because it is both a mutagenic and carcinogenic substance (Silvan *et al.*, 2006; Borrelli *et al.*, 2003).

Previously, the Japanese bioethanol industry imported molasses as a raw material from other agricultural countries and manufactured bioethanol in the warm regions of Japan such as Kagoshima and Okinawa prefectures. The conventional treatment processes were mainly based on methane fermentation, which has important disadvantages. For example, the final effluent concentration was occasionally so high that the discharge standard could not be met; the retention time was approximately 10~30 days, which is relatively long; there was a significant energy consumption need after treatment, and others. Other options for disposing of the wastewater, such as ferti-irrigation with the fermentation residue or spent wash also led to the contamination of groundwater, which is the only water source in many areas.

To overcome these aforementioned problems, we developed and evaluated the performance of a combined pilot-scale bioprocess and studied the practicability of physicochemical treatment systems, including upflow anaerobic sludge blanket digestion (UASB), downflow hanging sponge (DHS), and an anaerobic sludge blanket (ASB) reactor for the removal of organic matter and nitrogen. Microfiltration (MF), ultrafiltration (UF), and nanofiltration (NF) membrane units focused on the removal of suspended solid, phosphorus, and color. This pilot-scale plant was able to remove key contaminants from the wastewater and render the effluent acceptable for release to the

environment. We recommend that this design be used as the basis for production scale units to address the problems associated with this waste stream such as high organic-nutrients concentration and recalcitrant color pigment.

4.3 Materials and Methods

4.3.1 Substrate and feed preparation

The substrate used in the study was prepared using the molasses-based residue from a bioethanol fermentation process contained in an open pool. It was mixed with low concentration tank spent wash to a predetermined concentration. The substrate had an initial COD of 160,000~200,000 mgCOD/L. Sodium bicarbonate solution (5N) was automatically added to the pH adjustment tank as a buffer. The influent was pre-mixed by the raw wastewater pre-heated using water bath to 40°C before feeding into the reactor. **Table 4-1** shows the characteristics of raw residue wastewater and spent wash wastewater.

Table 4-1 Characteristics of the molasses-based wastewater

		Fermentation residue	Spentwash
pH	[-]	4.7	4.5
SS	[mg/L]	2,200	1,800
VSS	[mg/L]	1,950	1,460
SS/VSS	[-]	0.87	0.81
CODcr total	[mg COD/L]	210,000	12,000
soluble	[mg COD/L]	170,500	8,200
BOD	[mg/L]	147,000	5,900
TN	[mg-N/L]	4,100	440
TP	[mg-P/L]	152	82
Color	[Degree]	4,200	3,400

4.3.2 Experimental apparatus

Fig. 4-1 shows a schematic of the biological process unit, which consist of a substrate tank, acidification tank, pH adjustment tank, two UASB reactors (working volume: 200 L and 17.6 L) followed by two DHS reactors (sponge volume: 4.7 L each). The DHS reactor was filled with ϕ3.5xH3.5 cm polyurethane sponge media attached to the plastic sieve supporter. The ASB reactor was installed as a final unit of the biological process for the purpose of denitrification. Methyl alcohol (20 mL-MeOH/1L-influent) was added to ASB as carbon source supplement. In the physicochemical process, submerged hollowed type polyvinylidene fluoride (PVDF) with φ0.4μm-pore size MF, 4-inch of spiral-wound UF, and NF modules were employed to treat the effluent from ASB reactor.

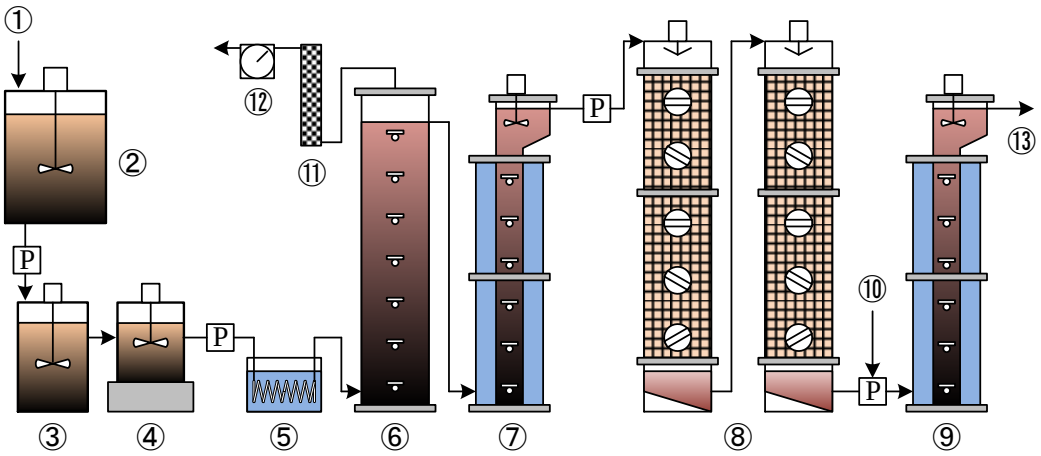


Fig. 4-1 Schematic diagram of combined bioprocess

Biological Process

The pilot-scale biological units were operated under mesophilic conditions (30 ± 5°C) at the Ryuuseki pilot bioethanol plant in Miyakojima Island, Okinawa, Japan. First, the raw fermentation residue retained in an open pool (**Fig. 4-1**-①) was diluted with process water to the designed concentration (**Fig. 4-1**-②). Subsequently, it was passed to a pre-acidification tank (**Fig. 4-1**-③) that made use of an acidogenic environment to enhance the action of acidogens. Sodium bicarbonate solution (5 N) was automatically added to the pH adjustment tank (**Fig. 4-1**-④) as a buffer. The influent was pre-mixed

with the raw wastewater and pre-heated (**Fig. 4-1-⑤**) using a water bath at 40°C before feeding into the reactor. The organic matter was treated using a 200-L UASB reactor (**Fig. 4-1-⑥**) as the main treatment unit, followed by a 17.6-L UASB reactor (**Fig. 4-1-⑦**). Biogas generated from anaerobic processes was trapped by an iron (ferric) oxide desulfurizer (**Fig. 4-1-⑪**) before the volume was measured using a wet gas meter (**Fig. 4-1-⑫**). Two DHS reactors (**Fig. 4-1-⑧**) were applied for BOD, suspended solids, ammonia (NH_4^+), and nitrite (NO_2^-) removal (nitrification). Finally, the wastewater was biologically treated using an 11-L ASB reactor for nitrate (NO_3^-) removal (denitrification) (**Fig. 4-1-⑨**). During this process, methyl alcohol (20 mL MeOH/L influent) was added as a carbon source supplement (**Fig. 4-1-⑩**).

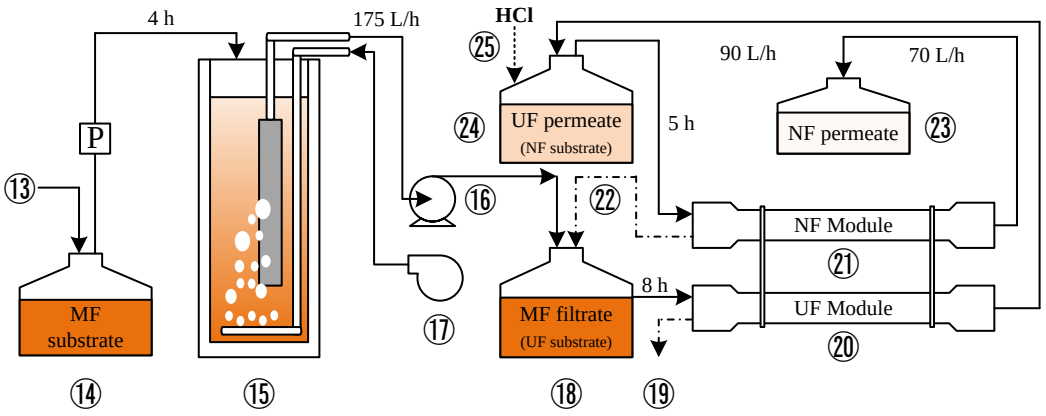


Fig. 4-2 Schematic diagram of physicochemical process

Physicochemical process

Treated water from the biological process (**Fig. 4-2-⑬**) was stored in the MF substrate tank (**Fig. 4-2-⑭**) until the volume reached a minimum of 800 L. The MF module (**Fig. 4-2-⑮**) had an extraction capacity of 175 L/h for each 4-hour operation while an aeration (**Fig. 4-2-⑯**) was applied in order to reduce the biofilm formation on the membrane surface (**Fig. 4-2-⑰**). Because we used the same pump for both the UF (**Fig. 4-2-⑳**) and NF (**Fig. 4-2-㉑**) modules, the experiment was successively performed starting from the UF unit. The UF unit has a permeate capacity of 90 L/h, while a small amount of concentrate was generated at 6 L/h and drained into the residue pool (**Fig. 4-2-⑲**). To prevent scale formation in the NF module, the pH of the UF permeate/NF substrate (**Fig.**

4-2-㉔) was pre-adjusted to 7 by direct addition of concentrated HCl (**Fig. 4-2-㉕**). The NF unit treated the UF permeate and generated a concentrate (**Fig. 4-2-㉒**) that recirculated to the UF substrate tank (**Fig. 4-2-⑱**) at the same rate of 70 L/h.

4.3.3 Analytical methods

Organic matter in the form of COD was determined using a colorimetric method (HACH DR5000 spectrophotometer). Nutrients, i.e., phosphate phosphorus, ammonia nitrogen, nitrite nitrogen, and nitrate nitrogen were analyzed using a colorimetric method (HACH DR5000). Biogas production was measured daily using a wet gas meter (Shinagawa WS-1A; 1–600 L/h), while the composition of the biogas was determined by gas chromatography (Shimadzu GC1700 and GC8A). For color measurement, samples were filtered through a φ0.4-μm pore size filter paper to eliminate turbidity. This enables the measurement of the true color (Nippon Denshoku, NDR2000) using dilution methods (Miyoshi *et al*., 2002). Other analytical parameters were monitored in accordance with the Standard Methods for the Examination of Water and Wastewater (APHA, 2005).

4.3.3.1 Measurement of the effluents temperature

The biological units were continuously operated under control conditions (30±5°C) with preheated water bath for substrate and room temperature control (in winter) while membrane units ran periodically in the open environment. All effluents' temperature was measured at the site by digital thermometer.

4.3.3.2 Measurement of flow rate from reservoir

The substrate peristalsis pumps were calibrated and flushed frequently to ensure the stability of the feed flow rate.

4.3.3.3 Measurement of influent and effluent pH level

Substrate and each reactor's effluents were measured roughly by handy pH meter (Hiroba AS-211, pH range 0.0~14.0) at the reactor site when preparing the influent and again precisely measured by digital glass H^+ electrode pH meter (TOA-DKK, HM-30R). pH meter was calibrated by standard pH solution at 6.89, 4.01 and 9.18. Molasses fermentation residue wastewater usually has acidic pH around 4.0~5.0 (before dilution), $NaHCO_3$ was added into the pH adjustment tank after acidification.

4.3.3.4 Measurement of biogas production and composition

Desulfurizers (Fe_2O_2) which were filled inside the acrylic column had been used to remove H_2S produced from the generated biogas. H_2S removed biogas will pass through the tube to the wet type gas meter (Shinagawa, WS-1A, and Measurable range of 1~600 L/h). The value displayed in the gas meter was recorded twice a day. The amount of the gas of generation each day was calculated from the amount of the gas generated within time and the time range during the measurement time.

Biogas produced from the anaerobic process was measured by thermal conductivity detector (TCD) type gas chromatography (Shimadzu GC-8A).

4.3.3.5 Measurement of Chemical Oxygen Demand (COD)

In this experiment, we applied Close Reflux Method with color comparison with instant HACH dichromate reagent and spectrophotometer for the measurement of COD. The procedures were conducted with HACH Method 8000.

4.3.3.6 Measurement of Nitrogen species

We applied the HACH kit for total nitrogen, nitrite (NO_2^-), nitrate (NO_3^-).

4.3.3.7 Measurement of Phosphorus

We applied the HACH test kit for total phosphate as (PO_4^{3-}).

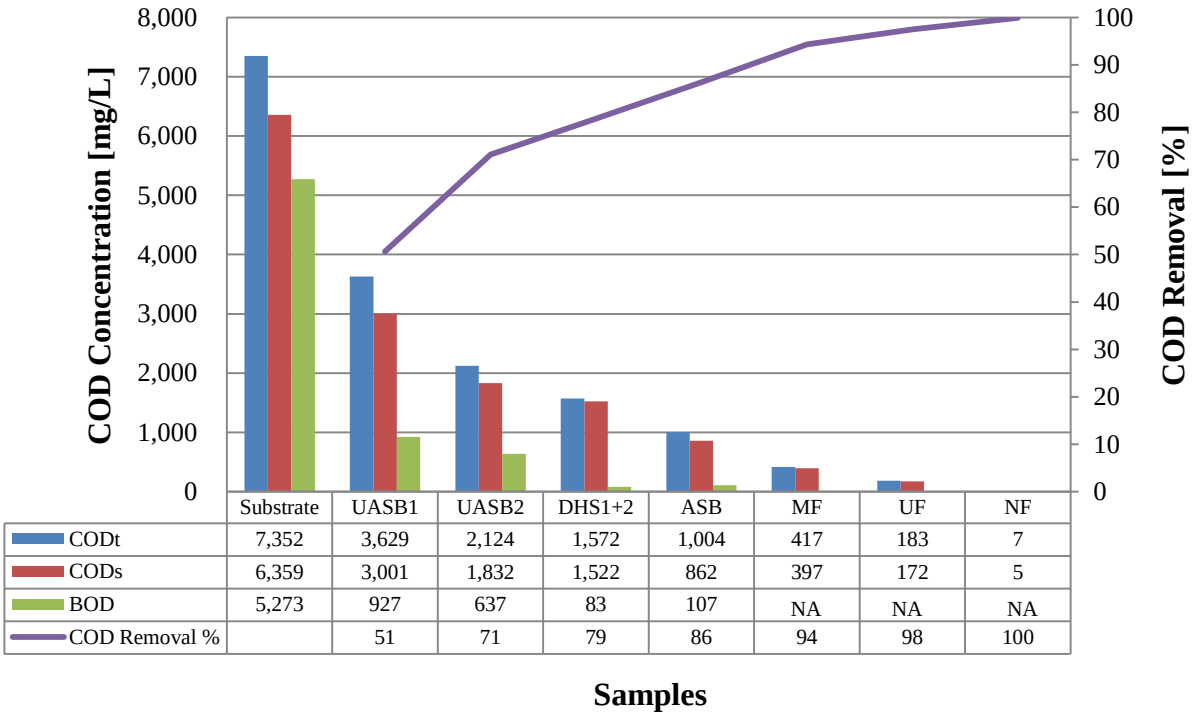
4.3.3.8 Measurement of Color

We measured the sample color using drainage coloration and turbidity meter (NDR2000) with the coloration rage of 0°~10,000° in correlation with coloration (dilution method) and absorbency.

4.4 Result and Discussion

4.4.1 Biological process performance

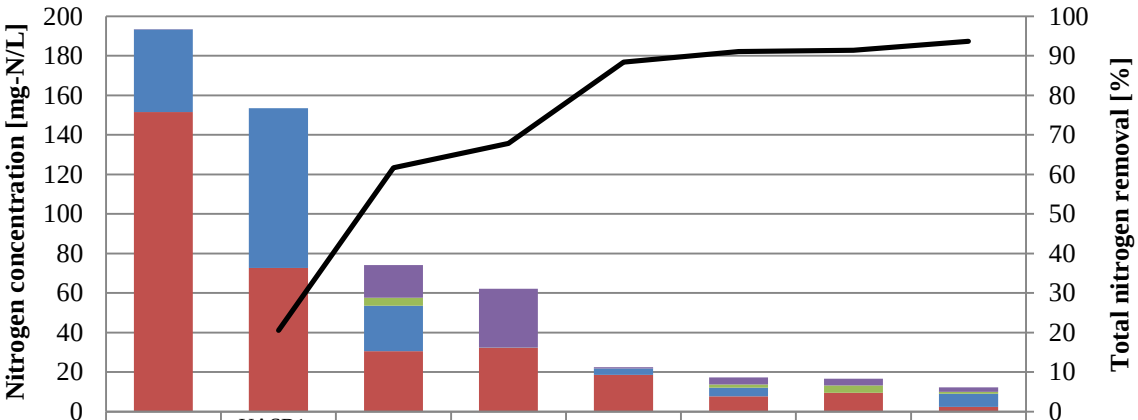
A 200-L UASB reactor was operated at the maximum organic loading rate (OLR) of 8.9 kgCOD/m^3/d (average OLR: 5.9 kgCOD/m^3/d). The volatile fatty acid (VFA) mostly found in the influent wastewater was lactic acid and propionic acid (HPr) in the same proportion. It also included some acetic acid (HAc) and formic acid. The first UASB reactor contained <160 mgCOD/L of VFA, which was mostly HAc and HPr (data not shown). We also observed 10% of total VFA (75%: HAc+HPr) increasing in the acidification reactor. The residue wastewater substrate had an average total COD concentration of 7,400 mg COD/L with 85% of whole biological removal. Some organics decreased in the pre-acidification tank (6–8%) most likely because of some acidification and methanogenesis. The hydraulic retention time of the acidification tank was designed to be 23 h to prevent damage to the UASB sludge (Ahn *et al.*, 2001). The BOD concentration from each effluent reactor increased in accordance with the increase in the COD concentration. BOD_{total} removal was maintained at 75% by the first UASB reactor and <100 mg/L by the second DHS reactor. However, the final biologically treated water BOD increased because of the addition of methanol as an organic source (**Fig. 4-3**) and a rise in sewage sludge from the sludge from the ASB reactor (data not shown).



	Substrate	UASB1	UASB2	DHS1+2	ASB	MF	UF	NF
CODt	7,352	3,629	2,124	1,572	1,004	417	183	7
CODs	6,359	3,001	1,832	1,522	862	397	172	5
BOD	5,273	927	637	83	107	NA	NA	NA
COD Removal %		51	71	79	86	94	98	100

Fig. 4-3 COD removal efficiency and effluent concentration of each reactor

Nitrification by oxidizing ammonia from the UASB reactors (80.9 mg N/L) occurred in the DHS reactors, forming nitrate (16.5 mg N/L by DHS1 and 29.8 mg N/L by DHS2), nitrite (4.1 mg N/L by DHS1), and ammonia (22.9 mg N/L by DHS1). In the ASB reactor, denitrification occurred leading to the conversion of 29.8 mg N/L nitrate in the DHS2 effluent to 0.6 mg N/L. (Fig. 4-4)



	Substrate	UASB1+2	DHS1	DHS2	ASB	MF	UF	NF
Nitrate-N	0.0	0.0	16.5	29.8	0.6	3.5	3.4	2.2
Nitrite-N	0.0	0.0	4.1	0.2	0.0	1.6	3.7	0.9
Ammonia-N	41.7	80.9	22.9	0.0	3.3	4.4	0.0	6.7
Organic-N	151.6	72.6	30.6	32.1	18.5	7.7	9.5	2.4
TN removal %		20.6	61.6	67.9	88.4	91.0	91.4	93.7

Fig. 4-4 Nitrogen balance and removal % by each process

A nitrogen profile along the DHS reactor height taken at the maximum influent COD concentration (12,600 mg COD/L) is shown in Fig. 5. The upper part of the graph represents the top of the DHS1 reactor. The second DHS reactor profile started from 150 cm to 300 cm. At the DHS1 inlet, both nitrite and nitrate were <10 mgN/L; however, we could observe large amount of ammonia (243 mgN/L) in the DHS1 influent. Seventy percent of the nitrate was formed in DHS2, while nitrite did not show any significant changes in either of the DHS reactors (**Fig 4-5**).

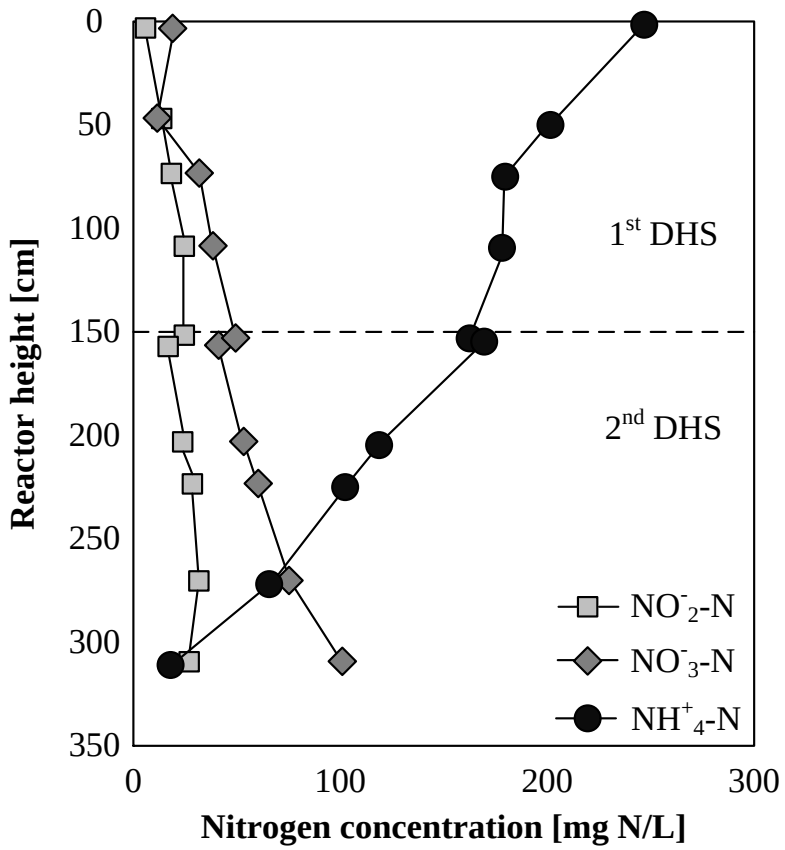


Fig. 4-5 Nitrogen profile of DHS reactors at maximum influent concentration

Fig. 4-6 demonstrates the nitrogen profile of the UASB reactor at maximum influent concentration. The influent data start from the bottom of the graph, and the upper plots represent the nitrogen quality of the treated water. Along the UASB reactor height, nitrite and nitrate were present at low concentrations (<10 mg N/L), while approximately 40 mgN/L of ammonia was present in the UASB reactor.

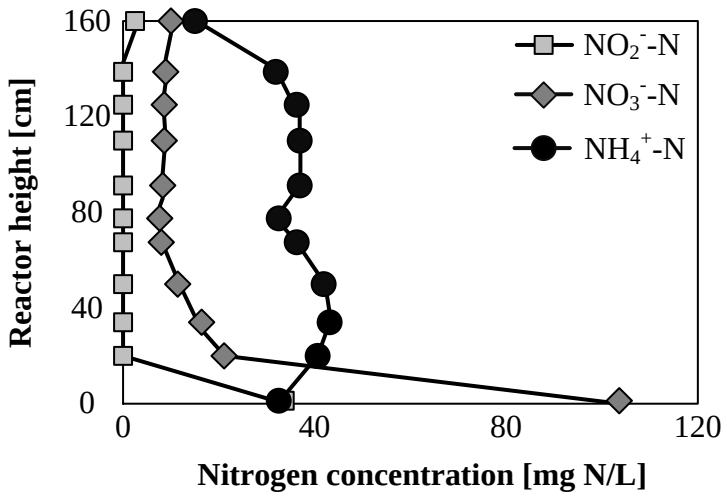


Fig. 4-6 Nitrogen profile of UASB reactor at maximum influent concentration

Fig. 4-7 shows the removal of total phosphorus for each process. Total phosphorus slightly decreased in the UASB reactors from 15.6 mgP/L to 13.6 mgP/L, presumably because of phosphate uptake and release by microorganisms, which require phosphorus for intracellular maintenance and energy production. Meanwhile, the chemical precipitation of PO_4^{3-} with Ca^{2+} which became insoluble hydroxyapatite $\left(Ca_5(PO_4)_3(OH)\right)$ could be occurred since Ca^{2+} was also found in the wastewater (Arvin, 1983). Most of the phosphorus removal occurred in the DHS reactors. The 13.6 mgP/L in the UASB effluent was oxidized to 8.7 mgP/L in DHS1 and 3.9 mgP/L in DHS2. Nevertheless, the ASB reactor did not produce a significant reduction in phosphorus.

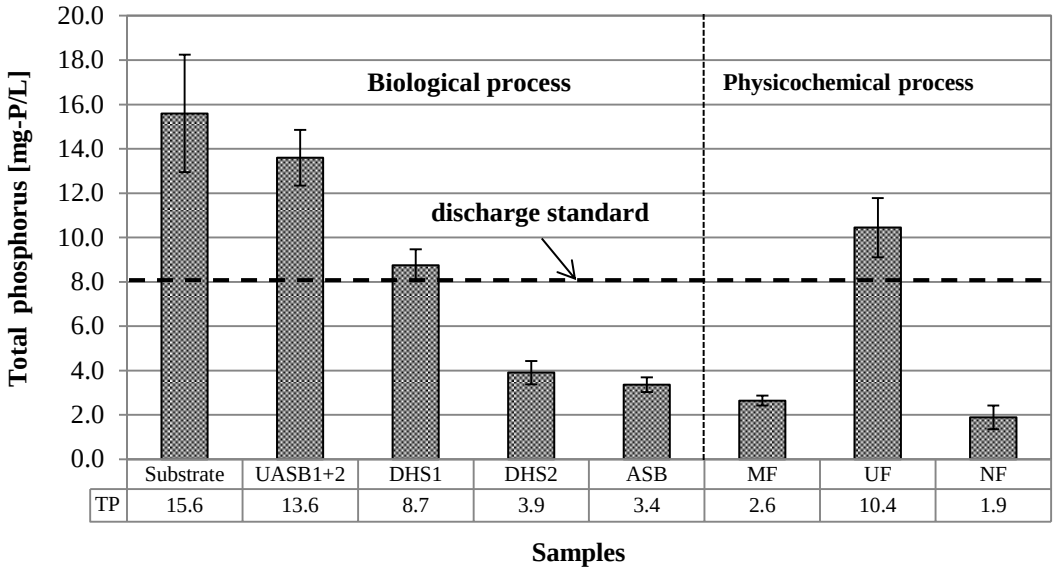
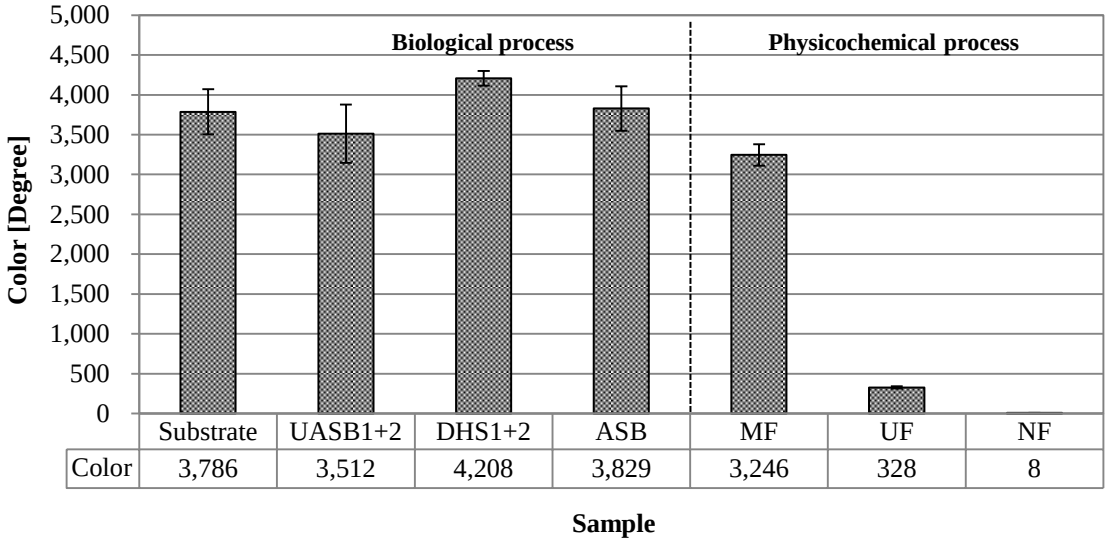


Fig. 4-7 Total phosphorus concentration removal by each process

Fig. 4-8 shows decolorization of molasses spent wash wastewater (MSW) by acetogenic bacteria. These bacteria were able to decolorize the wastewater by 9.75 ± 3.0% (stillage) without nutrient supplementation (Sirianuntapiboon *et al.*, 2004). In our study, the UASB reactors also performed approximately 7% decolorization of a dark brown (3,700°) residue wastewater, changing it to an opaque pale yellow (3,500°) effluent. The DHS reactors did not remove any color. In contrast, the color became 17% darker than the effluent from the UASB reactors and was even darker (4,208°) than the original substrate. Although many aerobic processes have been designed in an attempt to decolorize molasses wastewater using pure cultures of isolated yeast, fungi, and bacteria, the decolorizing rates remain unsatisfactory and are not sufficiently reliable for actual operations.



	Substrate	UASB1+2	DHS1+2	ASB	MF	UF	NF
Color	3,786	3,512	4,208	3,829	3,246	328	8

Fig. 4-8 Color removal by each process

4.4.2 Physicochemical process performance

Fig. 4-3 displays the performance of the membrane filtration systems in reducing organic matter. The MF module used to treat anaerobic wastewater has a physical configuration that is somewhat similar to a membrane bioreactor. It contains microorganisms and particulate matter that have migrated from the previous reactors. The 1,004 mgCOD/L remaining from the biological processes was reduced approximately 60% by filtration through the submerged membrane, leading to an organic matter level of 400 mg COD/L. The MF module appeared to perform better with an organic matter concentration higher than 1,000 mgCOD/L. Membrane fouling, which can be a major drawback, was not detected during the experiment. The subsequent UF module also reduced organic substances by 60%, resulting in a permeate concentration of 180 mg/L. Approximately 6% of the feedwater was rejected to retentate or concentrate, which contain substantial concentrations of materials such as polyphenol and subsequently stored in a separate containment module. Remarkably, the final NF unit could diminish 97% of COD to an almost undetectable concentration of <10 mgCOD/L. However, some of the NF retentate with low COD concentration was rejected to the UF substrate tank to lower the excess discharge volume.

Because the biologically treated effluent was already low in nitrogen concentration, the MF module did not show as much nitrogen removal efficiency as an aerobic DHS reactor treating effluent from UASB reactor even MF in some means functioned similar to DHS reactor. The UF and NF units were able to remove nitrogen at rates of 3.5% and 26.8%, respectively. The lower removal rate in the UF unit may have resulted from the recirculated retentate from the NF unit. The phosphorus removal efficiency of the physicochemical process is shown in **Fig. 4-7**. The NF unit shows better permeability (82% rejection) than the MF and UF units, presumably because of its smaller pore size and pre-pH adjustment. The decolorization yields of the molasses fermentation residue are presented in **Fig. 4-8**. The MF module, which has the same pore size as the φ0.4-μm filter paper, could not successfully remove major color pigments. Nevertheless, the UF and NF units recovered these materials, which resulted in satisfactory decolorization rates of 90% (328°) and 99% (8°), respectively. The UF permeate color was light yellow and the NF permeate was water-like clear.

4.5 CONCLUSION

From these experimental results, we can conclude that

1. The proposed pilot-scale combined system could treat molasses fermentation residue wastewater to successfully remove organic matter, nitrogen, phosphorus, and color. Biological treatment, with a maximum influx total COD of 12,000 mg COD/L, was capable of removing organic matter with 80% average efficiencies.
2. Ammonia generated during the anaerobic process could be completely removed by DHS reactors (nitrification), while nitrate was mostly removed by denitrification in the ASB reactor.
3. Total phosphorus was partially reduced by the UASB reactor possibly by both biological and chemical precipitation and degraded stepwise by the DHS reactors. Finally, the NF module reduced the phosphorus level to <2 mg P/L.
4. More than 90% of the decolorization took place in the UF and NF modules, resulting in a crystal-clear final effluent. The final effluent passed all of the necessary

wastewater discharge standard evaluations and was cleared for release to the environment.

5. In the further study, biomolecular analysis of the main biological treatment unit should be carried out for the suitable microorganism selection to enhance the system performance. Furthermore, the operating and maintenance cost performance of membrane units should be additionally evaluated for the complete system energy balance.

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Chapter 5

Evaluation of membrane processes treating pre-biological treated molasses-based alcohol distillery wastewater

5.1 Abstract

This study evaluated the stability of pilot-scale membrane units for the biological pretreatment of molasses-based wastewater during the start-up period. Essential profiles of all membrane units with the initial setup and operational cost were observed. The initial feed, whose concentration ranged from 5,000 to 12,000 mg/L, was treated in a non-molasses upflow anaerobic sludge blanket reactor as the main biological organic removal unit, followed by an aerobic downflow hanging sponge reactor and an anaerobic sludge bed reactor for nitrification–denitrification. In the final process, membrane unit excluded phosphorus and decolorized dark-colored substances such as melanoidins and phenol. The transmembrane pressure and flux rate, which directly influence the permeability performance at different conditions, were examined to optimize the system and prevent membrane fouling. The operational costs, which mainly include electricity for pumps and meters, chemicals for buffers, and backwashing, were also monitored to provide a cost assessment and inevitable cost reduction.

5.2 Introduction

The manufacture of alcohol using sugarcane molasses is a major source of exports in Asia and South America where sugarcane is cultivated and sugar is produced. However, molasses-based alcohol industries generate vast volumes of waste streams that contain a high concentration of organic compounds and are dark brown in color, which pollute aquatic and terrestrial ecosystems. Many researchers have attempted to develop an efficient treatment system that removes high organic loading, nutrients, and refractory color pigments with stability and cost-effectiveness. Besides the effective biological

treatment that is well-known for highly concentrated industrial distillery wastewater, a physicochemical process such as membrane filtration is one of the most promising options specifically for the remediation of colorant- and nutrient-rich influents because of its dependability. Membrane technologies that are widely used for wastewater treatment include membrane bioreactors (MBRs) for microfiltration (MF), ultrafiltration (UF), and nanofiltration (NF). MBRs were first introduced by the Aqua Renaissance project in 1980 (Nakao, 2010), followed by the Membrane Aqua Century 21 project (MAC21) using an MF/UF membrane for turbidity and pathogen removal, and high-rate MAC21 using an MF/UF/NF membrane for the treatment of organic compounds and wastewater (Itoh, 2000). In the small island nation of Japan, where land is one of the greatest barriers in the field of wastewater treatment, conventional treatments such as anaerobic, aerobic, and facultative ponds are not preferable because they require a significant amount of space and can result in groundwater contamination, overtopping, excessive sludge formation, and odor. To comply with the stringent wastewater discharge standards, three membranes (MF, UF, and NF) were applied as post-treatment for nutrient removal and decolorization. Clech *et al.* investigated the fouling that affected the performance of MBRs for treating wastewater and found that the interactions between the macromolecular and particulate components of the feed can cause unexpected and rapid changes in fouling. Studies of the relationship between pore size and fouling formation have also shown that pore size alone cannot predict hydraulic performance. A recent study by Karode *et al.* showed an approximately 50% reduction in a raw brown sugar solution by polyethersulfone (PES) and mineral membranes with a molecular weight cutoff (MWCO) between 30 and 50 kDa. Decloux *et al.* also suggested that to apply UF for decolorization, a temperature of 60°C, a transmembrane pressure (TMP) of 3 bar, and a cross-flow velocity of 2.5 m/s should be utilized while using a mineral membrane of 15 kDa MWCO. Another study, by Nataraj *et al.*, of a hybrid NF and reverse osmosis (RO) pilot plant used for the color and contaminant removal of distillery spent wash, demonstrated a high rejection of 99.80% for total dissolved solids (TDS), of 99.90% for chemical oxygen demand (COD), and of 99.99% for potassium without any effects from fouling. Galambos *et al.* utilized NF and RO membranes for treatment of high concentrations of food industrial wastewater at

constant temperature and a recycle flow rate. The flux, salt rejection, and COD results revealed that the flux rate was higher with a lower COD concentration and increased with the pressure.

To prevent and alleviate fouling, which is the main problem in this technology, and to improve the performance of membrane treatment, we examined the membrane flux rate in accordance with different runs in order to find the optimal conditions for the MF unit and for the performance stability of the UF and NF units.

5.3 Materials and Methods

5.3.1 Substrate and feed preparation

The wastewater used in this study was molasses-based alcohol residue wastewater from a demonstration plant on Miyako Island, Okinawa prefecture, Japan. The characteristics of the feed are shown in **Table 5-1**. The feed was pretreated by a combination of biological processes including an upflow anaerobic sludge blanket (UASB) reactor, an aerobic downflow hanging sponge (DHS) reactor, and an anaerobic sludge bed (ASB) reactor. The treated effluent still contained high organic matter and suspended particles, minerals and was still dark brown in color (melanoidins and phenol).

Table 5-1 Characteristics of the substrate and discharge standard

Parameters		Alcohol distillery wastewater	Miyakojima discharge standard
pH		4.5	5–9
SS	mg/L	450	<150
COD-Mn	mg/L	4,500	<120
COD-Cr	mg/L	9,000	-
BOD	mg/L	6,300	-
T-P	mg-P/L	18	<8
T-N	mg-N/L	225	<60
Color absorbance*		2–3	<0.1

* Color standard is not specified in discharge regulations. <0.1 is author's defined value

5.3.2 Biological units

5.3.2.1 Upflow anaerobic sludge blanket (UASB)

Two pilot-scale UASB reactors, 200 L and 20.6 L, were applied under mesophilic conditions in order to remove the organic loading of the molasses-based alcohol residual wastewater. The pH of the feed was preadjusted from 4.5~5.3 to 6.8~7.3 before entering the UASB to prevent anaerobic process failure.

5.3.2.2 Downflow hanging sponge (DHS)

Two 27-L DHSs were mainly employed for suspended solids removal and nitrification. The sponge used as media in the experiment is generation 3.2 developed by previous researchers from the same laboratory. The sponge is made of polyurethane and supported by the plastic sieve at the outer surface with dimension of Ø3.3 cm.× 3.3cm. and 45% occupancy ratio.

5.3.2.3 Anaerobic sludge bed (ASB)

A 17.6-L ASB reactor was used as the final biological treatment unit. During this process, methanol was supplemented as a carbon source for denitrification.

5.3.3 Membrane units

The membrane treatment units are schematically shown in **Fig. 5-1** and described below.

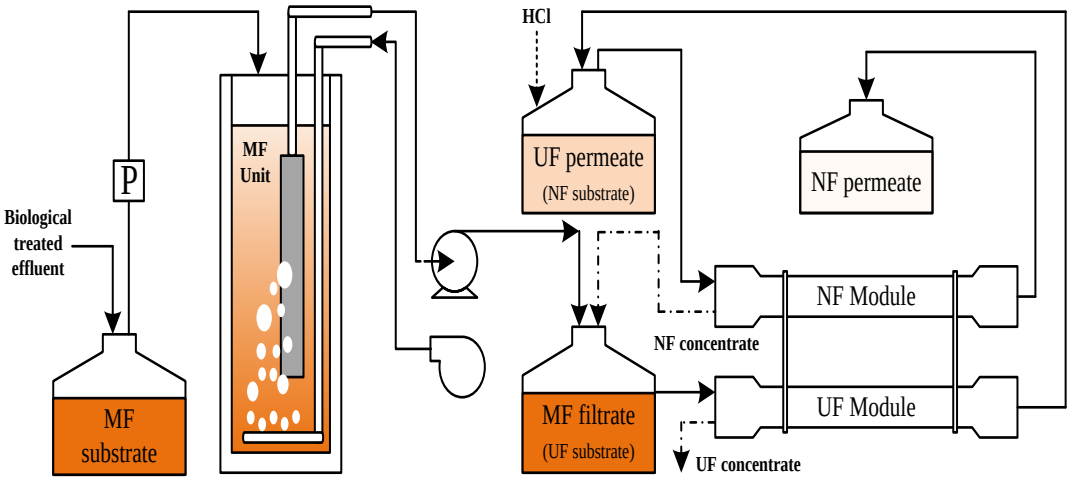

Fig. 5-1: Schematic diagram and flows of membrane treatment units

The design of membrane module and element are based on the water characteristics and also the budget as well. The comparison of the modules and elements are shown in the following tables.

Table 5-2 Comparison of membrane modules

	Spiral wound	**Tubular low price**	**Plate and frame**	**Hollow fiber**	**Ceramic**
Density [m^2/m^3]	high	low	average	Very high	low
Tendency of fouling	average	low	average	medium	Very high
Cleanability	good	good	good	none	good
Variable cost	low	low	average	low	high
Flow demand	medium	medium	medium	low	Very high

* Modified from Membrane Filtration Handbook (2001)

5.3.3.1 Microfiltration (MF) unit

A hollow-type polyvinylidene fluoride (PVDF) membrane with φ0.4-μm pore size and 60 m^2 membrane working area was submerged inside a 750 × 700 × 1400 mm^3 stainless steel tank. The experimental conditions are shown in **Table 5-3** to determine the optimal filtration flow and flux rate, **Table 5-4** to investigate the optimum air supply rate, **Table 5-5** to investigate optimum operation/shutoff timing, and **Table 5-6** to determine optimum performance variations due to membrane area. Moreover, the influence of suspended solids on the TMP was observed.

Table 5-3: Inspection conditions for optimal microfiltration filtration flow and flux rate

Parameters	Run 1	Run 2	Run 3	Run 4	Run 5
Flux [LMH]	17	21	25	29	33
Filtration flow rate [L/h]	200	250	300	350	400
Operational condition (filtration/shutoff time) [min]	7/1	7/1	7/1	7/1	7/1
Air supply rate [NL/min]	209	209	209	209	209
Total operation time [h]	2	2	2	2	0.5

Table 5-4: Inspection conditions for optimal air supply

Parameters	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Flux [LMH]	25	25	25	33	33	33
Filtration flow rate [L/h]	300	300	300	400	400	400
Operational condition (filtration/shutoff time) [min]	7/1	7/1	7/1	7/1	7/1	7/1
Air supply rate [NL/min]	209	300	400	209	300	400
Total operation time [h]	2	2	2	0.5	2	2

Table 5-5: Inspection conditions for optimal filtration/shutoff timing

Parameters	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7	Run 8
Flux [LMH]	25	25	25	25	33	33	33	33
Filtration flow rate [L/h]	300	300	300	300	400	400	400	400
Operational condition (filtration/shutoff time) [min]	7/1	7/2	5/1.4	3.5/1	7/1	7/2	5/1.4	3.5/1
Air supply rate [NL/min]	209	209	209	209	209	209	209	209
Total operation time [h]	2	2	2	2	0.5	2	2	2

Table 5-6: Inspection conditions for optimal microfiltration membrane area vs. performance

Parameters	Run 1	Run 2	Run 3	Run 4
Flux [LMH]	25	33	25	33
Filtration flow rate [L/h]	150	200	300	400
Operational condition (filtration/shutoff time) [min]	7/2	7/7	7/2	7/7
Air supply rate [NL/min]	209	209	209	209
MF membrane area [m^2]	6	6	12	12
Total operation time [h]	2	2	2	2

5.3.3.2 Ultrafiltration (UF) and nanofiltration (NF) units

The spiral-wound polyamide composite membrane was employed for both UF and NF and installed inside 4-in-fiber-reinforced plastic (FRP) modules. Each unit was alternately operated due to the sharing of the same pumping system. Because most of the particles were filtered by the MF unit, fouling was not observed in the short experimental time. However, to prevent minerals from forming scales, which might occur in the NF unit as it has a smaller MWCO range, the pH was preadjusted to 7.0 by adding a small amount of concentrated hydrochloric acid (HCl) into the UF permeate tank (NF substrate tank) before feeding.

5.3.4 Analytical methods

The chemical analysis of the influents and effluents was conducted two times a week. COD was determined by a colorimetric method (HACH DR 5000 spectrophotometer). Nutrients such as phosphate phosphorus, ammonia nitrogen, nitrite nitrogen, and nitrate nitrogen were analyzed by a colorimetric method (HACH DR 5000). For color measurement, samples were filtered through a φ0.4-μm pore size filter paper to eliminate turbidity. This enables measurement of the color absorbance of the samples with a 420-nm wavelength absorption spectrophotometer. Other analytical parameters were monitored in accordance with the Standard Methods for the Examination of Water and Wastewater (APHA). The electricity was monitored by a real-time power control system.

5.4. Results and Discussion

The experiments were conducted on biologically treated molasses-based alcohol wastewater at different operational parameters using MF, UF, and NF to determine the optimal conditions.

5.4.1 Influence of microfiltration rate

The time course of the TMP of a submerged MF under various filtration rates was investigated at constant filtration/shutoff timing, air supply rate, and total operation time (**Table 5-3**). The results are shown in **Fig. 5-2**. The lowest filtration rate of 200 L/h resulted in the lowest TMP of lesser than 5 kPa along the entire operation time (2 h) and gradually increased when the filtration rate was increased. With a 350 L/h filtration rate, the TMP increased from 20 kPa to 30 kPa after 1 h of operation. The TMP rapidly increased from 40 kPa and reached 90 kPa in 30 min when operated at a filtration rate of 400 L/h.

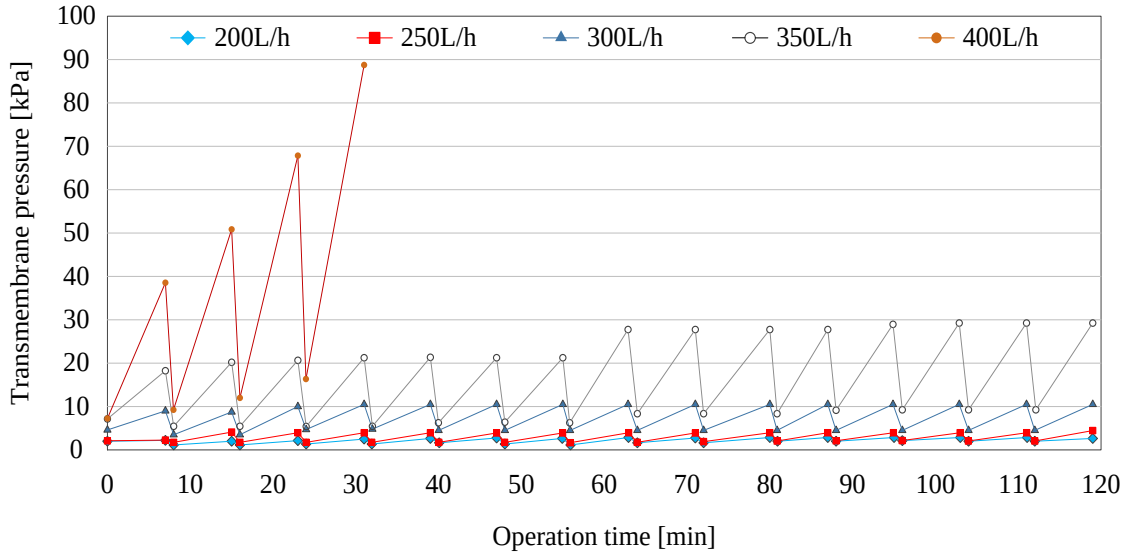

Fig. 5-2 Time course of transmembrane pressure with different filtration rates

5.4.2 Influence of aeration in microfiltration

Aeration was applied to the MF unit during microfiltration to prevent membrane fouling. Therefore, it is important to investigate the optimal aeration rate, because aeration consumes more than 30% of the total energy during filtration (Choi *et al.*, 2009). The experimental conditions are presented in **Table 5-4**. As shown in **Fig. 5-3**, both filtration rates showed the same trend: a higher aeration rate led to a lower TMP. However, the filtration rate of 400 L/h resulted in excessive TMP compared with the 300 L/h filtration rate, especially with the lowest aeration rate of 209 NL/min, which increased TMP to 90 kPa within a 30 min operation.

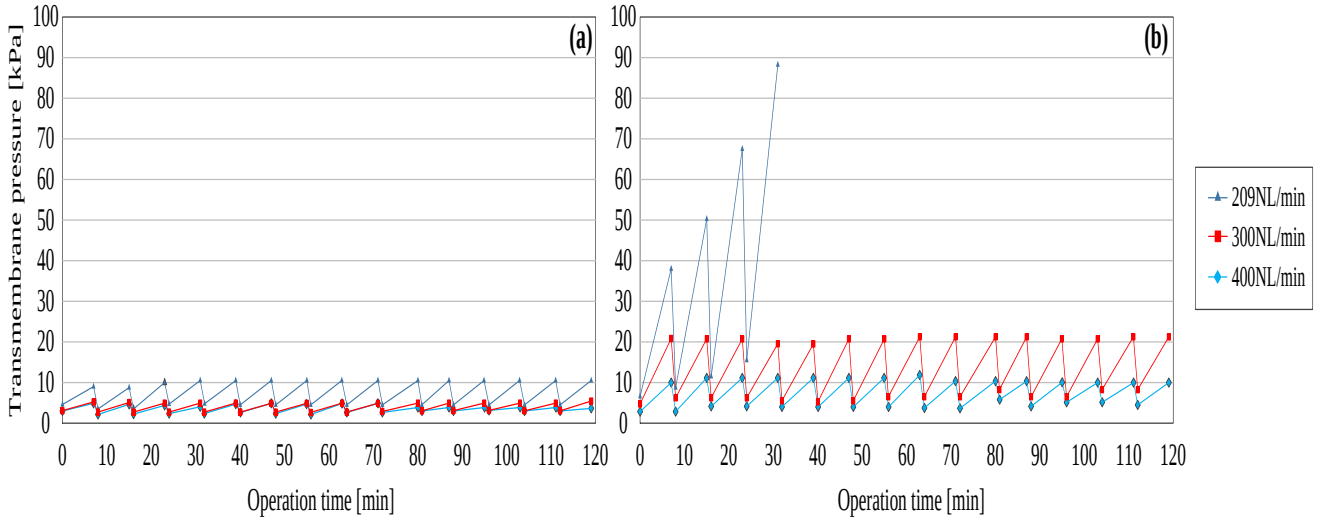

Fig. 5-3 Time course of transmembrane pressure with different aerations

(a) filtration rate, 300 L/h; (b) filtration rate, 400 L/h

5.4.3 Influence of filtration/shutoff timing

For considerations of energy reduction for effective cost performance, the filtration and shutoff timing of the operation were investigated (**Table 5-5**). As shown in **Fig. 5-4(a)** and **Fig. 5-4 (b)**, the time course of TMP demonstrated that less running time and long shutoff time resulted in less TMP difference. In particular, Run 5 (run 7 min/shutoff 1 min) reached the critical level within 30 min, whereas Run 1, which had the same run/shutoff timing, maintained stable TMP at 11 kPa. Runs 2-4 which operated under filtration rate of 300 L/h presented a similar TMP of <11 kPa. However, TMP significantly increased when operated at the filtration rate of 400 L/h compared to filtration rate of 300 L/h (fourfold in Run 7-8 and tenfold in Run 6).

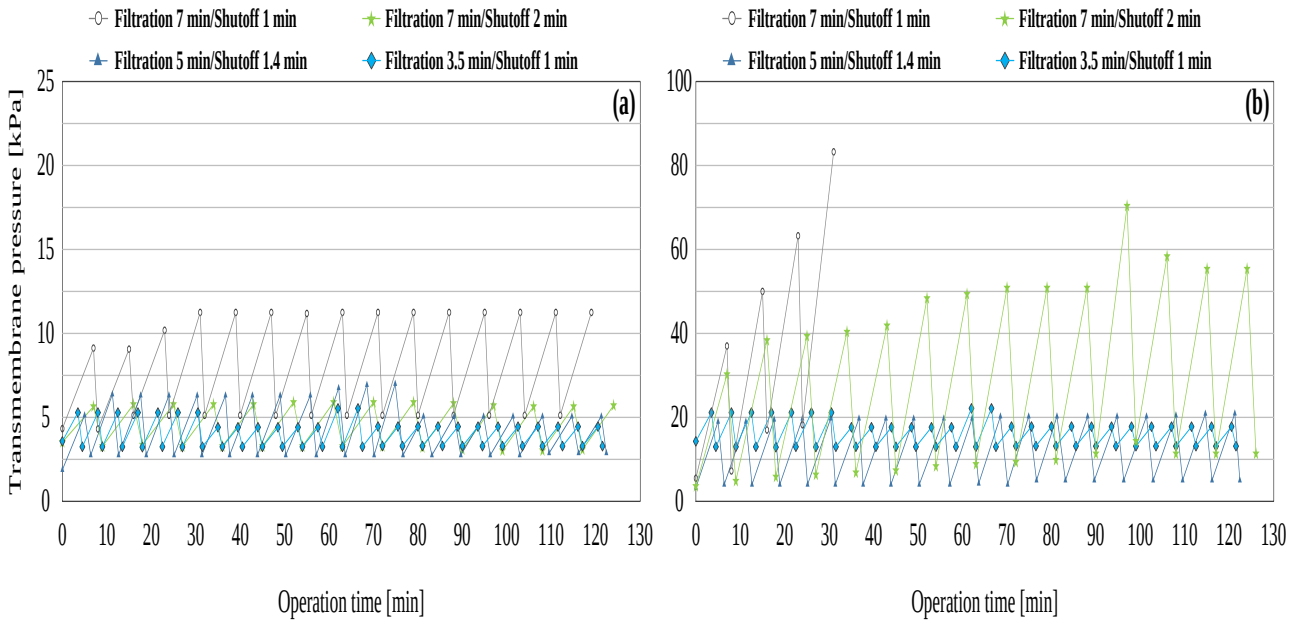

Figure 5-4: Time course of transmembrane pressure with different filtration/shutoff timing. (a) filtration rate, 300 L/h; (b) filtration rate, 400 L/h

5.4.4 Influence of membrane area

The experimental conditions are presented in **Table 5-6**, and the effects of membrane area on the TMP are shown in **Fig. 5-5**. The membrane area did not directly affect the TMP but varied in relation to the flux rate. A smaller membrane area (6 m^2) resulted in different TMPs, 7 kPa (Run 1) and 28 kPa (Run 2). On the other hand, a larger membrane area (12 m^2) also demonstrated analogous results of 7 kPa (Run 3) and 28 kPa (Run 4).

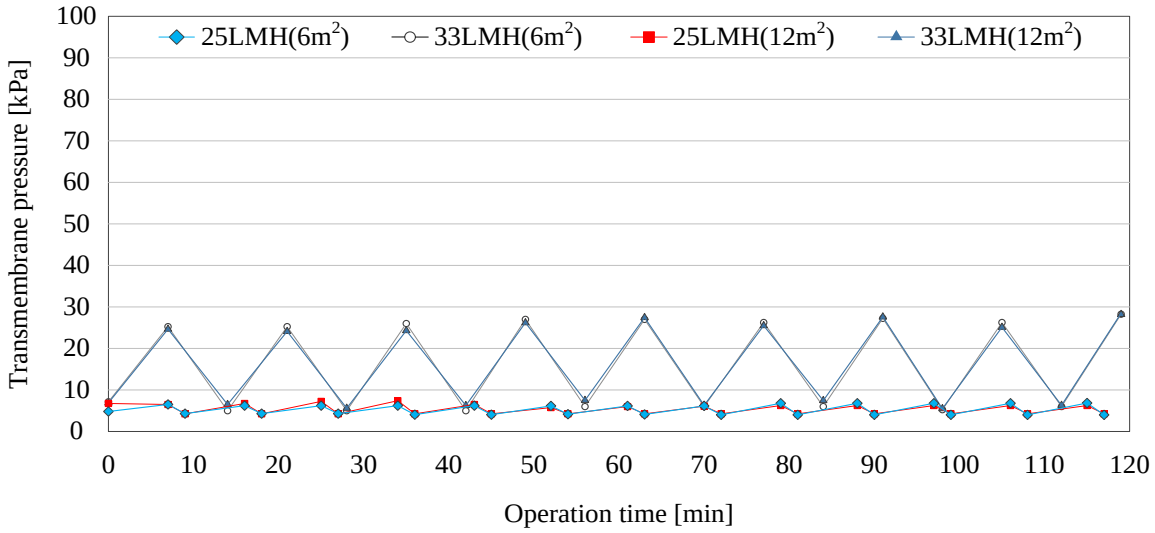

Fig. 5-5: Time course of transmembrane pressure with different membrane areas

5.4.5 Influence of suspended solids on TMP

The influent contained suspended solids (SS) of different concentrations (i.e., 3,000, 1,700, and 1,100 mg/L) in this study (**Fig. 5-6**). The results indicated that SS concentration as high as 3,000 mg/L did not reveal significant differences compared with other concentrations when the flux rate was below 20 LMH. With the flux rate of greater than 20 LMH, the variation began to become distinct. Nevertheless, there were no observable changes between the TMP resulting from 1,100 mg/L and 1,700 mg/L within all flux ranges.

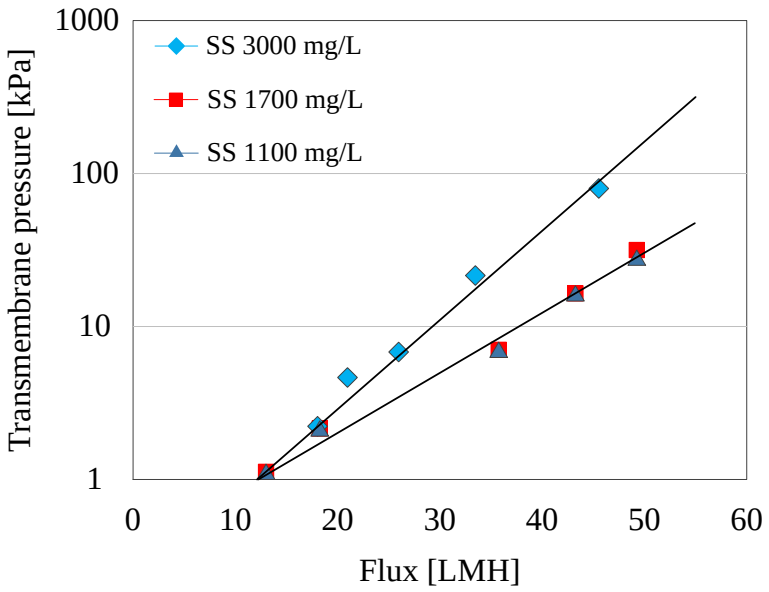

Fig. 5-6: Influence of suspended solids on transmembrane pressure.

5.4.6 Performance of UF under high loading

Table 5-7 demonstrates the performance of the UF unit after several experimental times. The results show that the main dark brown color of the molasses-based alcohol wastewater was satisfactorily removed after 8 h of operation from 6.70 (dark brown color) to 0.253 (pale yellow color). The UF permeate color remained almost the same after 8 h until the end of the experiment (after 113 h).

Table 5-7: Performance of the ultrafiltration unit under high loading

	Inf.	after 8 h		after 41 h		after 73 h		after 113 h	
		perm.	ret.	perm.	ret.	perm.	ret.	perm.	ret.
pH	9.20	9.25	9.21	9.26	9.22	9.24	9.11	9.23	9.15
Conductivity [µS/cm]	7,650	-	-	-	-	-	-	-	-
Absorbance	6.70	0.253	43.3	0.34	54.0	0.329	53.9	0.307	45.7
COD-Cr [mg/L]	1,600	-	-	-	-	-	-	-	-
COD-Mn [mg/L]	1,300	160	8,000	190	9,800	180	8,600	200	8,800

Inf.: Influent, perm.: permeate, ret.: retentate

5.4.7 Performance of nanofiltration unit

Table 5-8 summarizes the treatability of the NF unit during four different periods. The pH and the electrical conductivity of the permeate decreased, which explains the rejection of ions by the membrane. Thus, as the filtration time continues, the organic matter, phosphorus, and color substances in the permeate decrease. In the final treated water, 98% of organic compounds, 98% of phosphorus, and 99% of colored pigments were steadily rejected after 3 h.

Table 5-8: Performance of the nanofiltration unit

	Inf.	after 3 h		after 36 h		after 68 h		after 108 h	
		perm.	ret.	perm.	ret.	perm.	ret.	perm.	ret.
pH	9.19	8.76	9.27	8.58	9.31	8.59	9.32	8.63	9.37
Conductivity [μS/cm]	7,170	5,240	8,780	5,250	9,070	5,270	9,070	5,350	9,220
Absorbance	0.196	0.003	0.384	0.002	0.377	0.002	0.355	0.002	0.351
COD-Cr [mg/L]	140	-	-	-	-	-	-	-	-
COD-Mn [mg/L]	130	3.8	280	2.2	210	2.4	220	2.7	220
P [mg-P/L]*	2.4	<0.05	4.1	<0.05	5.3	<0.05	3.5	<0.05	3.5

Inf.: Influent, perm.: permeate, ret.: retentate

* Phosphorus were measured after 0.5 h, 63 h, 100 h, 150 h, and 200 h, respectively

5.4.8 Material, operation, and maintenance cost estimation

Spiral-wound membrane prices have reportedly declined recently; thus, the installed cost for a plant is approximately $300~$500 US per square meter of membrane, while the replacement element (thin-film NF, polysulfone UF) ranges from $25 to $50 US per square meter of membrane (Wagner, 2001) (these prices vary by country). The reverse osmosis running cost, which is mostly electricity for water treatment, typically ranges between $0.70 and $1.72 US for small-capacity units of 1000~4800 m^3/d (Karagiannis *et al.*, 2008). The proposed system is designed for a maximum capacity of 16 m^3/d. The biological units are operated by using pumps for the wastewater and sodium hydroxide addition. The chemicals prices were estimated from local factory grade sodium hydroxide and hydrochloric acid. The doses were calculated per amount of treated wastewater volume. On the other hand, membrane operation costs are mostly for high-pressure pumps and chemicals used in membrane cleaning. As presented in **Table 5-9**, the treatment per cubic meter of wastewater typically costs around $0.80 US for membrane units and $0.25 US for biological processes.

Table 5-9: Energy consumption of biological and physicochemical processes

	Unit	Biological process	Physicochemical process
Flow rate	m^3/h	0.67	0.59 (permeate)
Energy	kWh/m^3	0.32 (Biogas recovery not included)	2.59
Energy cost*	US$**/$m^3$	0.069 (Biogas recovery not included)	0.57
Chemical cost	US$**/$m^3$	0.18 (NaOH)	0.20 (HCl, NaOH)
Treated water quality	mg-CODcr/L	300~1,800	<50

* Based on the Japanese industrial electricity fee. ** Exchange rate is based on 1 US$ = 102 yen

5.5 Conclusion

We can conclude the following from the profiling of microfiltration (MF) and the performance of ultrafiltration (UF) and nanofiltration (NF):

1. In molasses-based alcohol residue wastewater with maximum SS in the range 1,000~1,700 mg/L, the filtration rate by a hollow-type submerged MF membrane with φ0.4-μm pore size should not exceed 300 L/h.
2. Aeration played an important role in preventing membrane fouling. A higher aeration rate is preferred; however, to conserve energy and thereby lower costs, aeration can be reduced to lesser than 300 NL/h with a filtration flow rate of 300 L/h.
3. To reduce membrane fouling, filtration and shutoff timing of the MF units clearly showed that a longer shutoff time and shorter run time enhance MF performance. The recommended ratio can be 7/1 or 7/2 for filtration/shutoff timing under a 300 L/h filtration rate.
4. Membrane area was directly related to the membrane flux rate, which is defined as flow rate per unit of membrane area. From the perspective of optimal membrane operations, the smallest membrane area that provides the highest flux rate resulted in

the lowest constant pressure.

5. The colored substances visible in the biologically treated molasses-based wastewater were effectively filtered by spiral-wound UF with MWCO of 2,500 Da and completely removed by NF with MWCO ranging between 200 and 1,000 Da.
6. The membrane units have a major drawback in that their consumption of energy is intensive. Consequently, the consideration of other energy recovery treatment systems, such as the anaerobic process, might lessen the total cost.

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Chapter 6

Full-scale Bio-Physicochemical System for Molasses-based Wastewater Treatment

6.1 Abstract

We evaluated the efficacy of a full-scale combined bio-physicochemical system for treating molasses-based bioethanol wastewater in terms of organic substances, nutrient, and dark brown color removal. The main organic removal unit, i.e., the up-flow anaerobic sludge blanket (UASB) reactor, achieved 80.7% removal and 4.3 Nm^3 methane production per cubic meter of wastewater with a hydraulic retention time of 16.7 h. Down-flow hanging sponge (DHS) reactors were important in reducing the biochemical oxygen demand (BOD), and the lowest possible organic waste intake prevented excessive biomass formation. The BOD removal efficiency was 71.2~97.9%. The denitrification up-flow anaerobic fixed bed (UFB) reactor achieved 99.2% total nitrogen removal. Post-physicochemical membrane treatment reduced the total phosphate, color, and remaining organic matter by 90.4%, 99.1%, and 99.8%, respectively. We analyzed the microbial diversity of the sludge from the UASB reactors. *Methanosaeta* was the dominant archaeal genus in the system, followed by *Methanolinea*, *Methanomicrospillum*, *Caldiserica*, *Bacteroidetes*, and *Deltaproteobacteria*.

6.2 Introduction

Recently, the demands and prices of fuel have increased tremendously and molasses-based bioethanol has become an alternative renewable bioenergy source for reducing the use of gasoline. However, this has generated vast amounts of wastewater (molasses spent wash, MSW), which has caused severe environmental problems such as its odor, contamination of groundwater, and depletion of the dissolved oxygen when released to the fresh water (Mohana *et al*., 2009). In Miyakojima, a small island in Okinawa prefecture where brown sugar from sugarcane is the major agricultural product and its by-product, blackstrap molasses, which contains high carbon source, is used as raw material for bioethanol manufacturers and local Awamori (Okinawan alcoholic beverage) distillers. During production, the volume of wastewater discharged after washing the fermentation tanks is about 15 times that of every unit of bioethanol produced (Satyawali *et al*., 2008). Groundwater is the only water source on the island so contamination with pesticides or harmful substances is a concern to the inhabitants and government. Molasses-based wastewater contains a high concentration of organic compounds, nutrients, and dark color pigments. Conventional treatments such as biological processes, including aerated lagoons or ferti-irrigation of cropland, are the most common methods used in most Asian and South American sugar- and ethanol-producing countries. However, these methods lead to the emission of greenhouse gases and have a high area requirement, while they also produce groundwater contamination and odor problems (Nandy *et al*., 2006). To overcome the aforementioned problems, cost-effective and eco-friendly remedies have been widely developed (Wilkie *et al*., 2000). In particular, a high-rate closed biological system has been developed for the treatment of molasses-based bioethanol waste, which consists of an up-flow anaerobic sludge blanket (UASB), down-flow hanging sponge (DHS), and an up-flow anaerobic fixed bed (UFB) followed by a series of microfiltration (MF), ultrafiltration (UF), and nanofiltration (NF) stages (**Fig. 6-1**).

The objective of this study was to evaluate the performance of a full-scale combined biological (UASB+DHS+UFB) system for treating molasses-based bioethanol wastewater in terms of removing the major organic substances, suspended solids (SS), nitrification (in DHS), and denitrification (in UFB), and a physicochemical (MF+UF+NF) treatment system for removing the particles that remained after the UFB (by MF), as well as phosphate (UF and NF) and color (NF). We also observed the recovery of methane for bioenergy and analyzed the predominant microbial diversity that responsible for methanogenesis reactions in different operating conditions to optimize this bioprocesses.

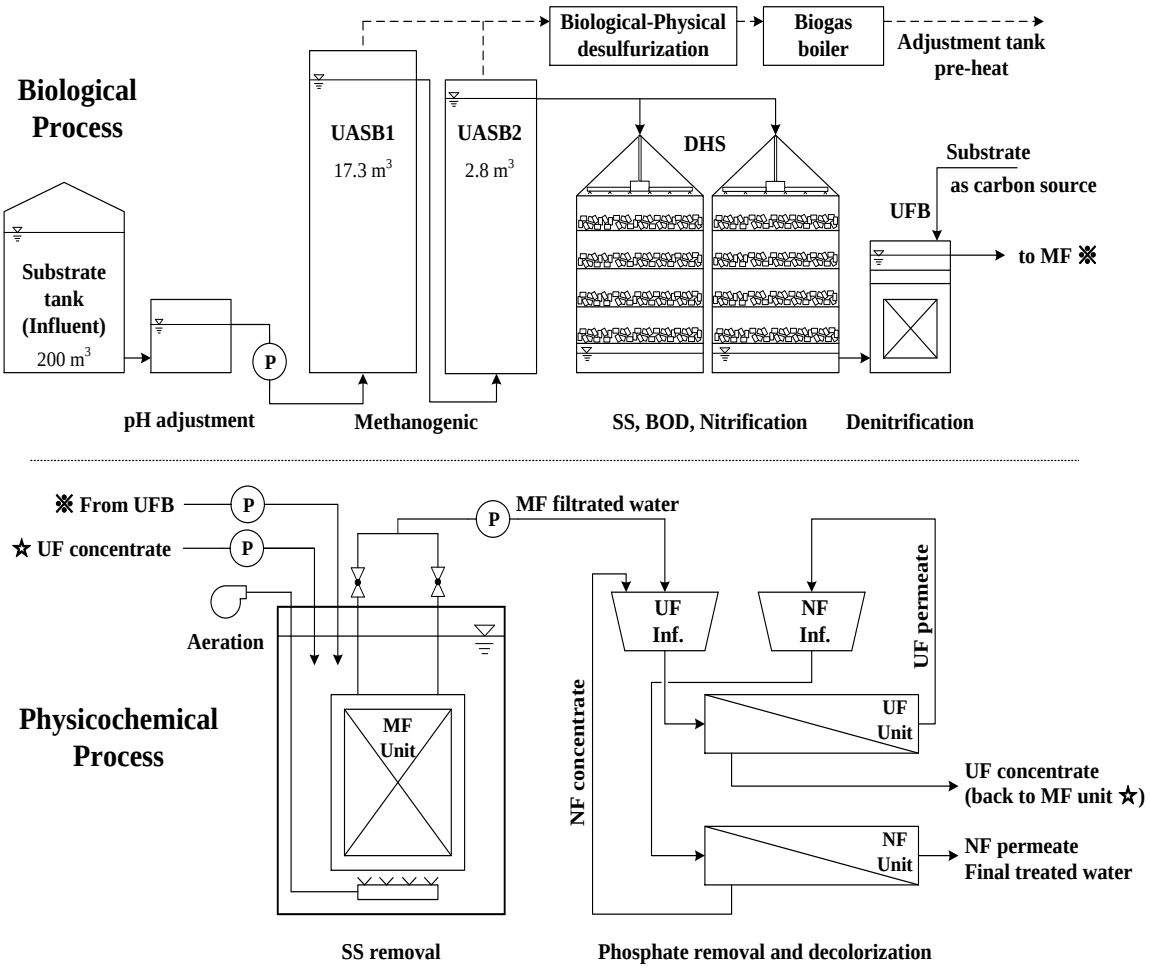


Fig. 6-1 Systematic of the bio-physicochemical processes

Fig. 6-2 Photo of full-scale biological treatment processes

Fig. 6-3 Photo of full-scale physicochemical treatment processes

6.3 Materials and Methods

The wastewater used in this investigation was discharged directly from a local bioethanol plant and stored in a 200-m^3 substrate tank. The raw wastewater was mainly the washing waste from the fermentation tanks and blackstrap molasses containers, which had the following characteristics: chemical oxygen demand (COD) = 4,800~36,000 mg/L, biological oxygen demand (BOD) = 2,600~16,300 mg/L, SS = 200~2,450 mg/L, total nitrogen (TN) = 70~450 mg-N/L, total phosphorus (TP) = 10~40 mg-P/L, sulfate of 120~520 mg-S/L and acidic pH = 3.7~4.9. The UASB reactors were inoculated with sludge from a local beer brewery wastewater treatment plant. The sludge concentrations of the UASBs were 35.5 g-MLVSS/L and 35.3 g-MLVSS/L. The MLVSS/MLSS ratios were 0.87 in UASB1 sludge and 0.84 in UASB2 sludge.

COD was determined using a colorimetric method (HACH DR5000 spectrophotometer). Nutrients, i.e., PO_4^{3-}-P, NH_4^+-N, NO_2^--N, and NO_3^--N, were also analyzed using a colorimetric method (HACH DR5000). Biogas production was monitored automatically by measuring the volume of biogas entering storage while the composition was determined using gas chromatography (Shimadzu GC1700 and GC8A). The samples were filtered through a φ0.4-μm pore size filter paper to eliminate the turbidity, which would enable the measurement of the true color (Nippon Denshoku, NDR2000) using dilution method (Miyoshi *et al*., 2002). Other analytical parameters were monitored in accordance with the Standard Methods for the Examination of Water and Wastewater (APHA, 2005).

The system was operated at full-scale in ambient temperature (18~32°C) conditions. The bio-physicochemical system was located on-site at Ryuuseki Bioethanol Plant, Miyakojima, Okinawa, Japan. The biological process involved a pre-adjustment tank, two UASB reactors, two DHS reactors, and a UFB reactor. The pre-adjustment tank was used to balance the acidic pH and increase the temperature during winter by pre-heating the raw wastewater with a gas boiler, which used the biogas produced by the UASB reactors. The UASB reactors were constructed of plate steel in a cylindrical shape with a working volume of 17.3 m^3 (UASB1) and 2.8 m^3 (UASB2), based on a maximum

influent flow of 15 m^3/d. The design flow per day was calculated using the remaining volume of the generated raw wastewater (**Table 6-1**), which was fed intermittently from 2 m^3/d to 8 m^3/d. In-series UASBs were designed mainly to remove organic substances from the processing manufacturer followed by two DHS reactors, which were mainly applied for BOD, SS, and nitrogen species removal. At the end of the biological process, a UFB was installed to ensure that the denitrification process would occur and nitrate (NO_3) will be reduced before entering the membrane modules. Following the biological process, the effluent was treated with a series of membranes, i.e., the MF, UF, and NF units. MF was a hollow-type 60 m^2 surface area membrane with a 0.4 μm pore size polyvinylidene difluoride (PVDF) membrane, which was equipped with a low pressure suction pump, a 1.6-Nm^3/min air blower, and activated carbon filters. The MF module flow was set at 0.8 m^3/h. Two eight-inch spiral-wound polyamide UF membranes modules with a molecular weight cutoff (MWCO) range of 2500 Da, each with a working surface area of 34.4 m^2, were packed inside separate fiber reinforced plastic (FRP) tubes. The UF permeate flow rate was 0.72 m^3/h and the concentrate flow rate was 0.04 m^3/h, which were maintained throughout the operation. The spiral wound membrane was also applied to the NF module but it had a smaller MWCO range (200 Da), a membrane surface area of 28.0 m^2, and a permeate flow rate of 0.36 m^3/h. To improve the performance of the membrane and prevent fouling problems, we used two types of cleaning once a week, i.e., water flushing and chemical cleaning (100 L of sodium hydroxide solution followed by 100 L of hydrochloric acid solution). The pH was pre-adjusted to 6.5~7.0 by adding concentrated hydrochloric acid before the wastewater feed entered the UF units. Sludge samples were collected from UASB1 and UASB2 on the 200th day of operation. DNA extract from the sludge samples were prepared using FastDNA Spin Kit for soil (MP Biomedicals, Irvine, CA). The 16S rRNA gene sequences were amplified using a One-Shot LA PCR Mix (Takara Bio, Otsu, Japan) with 0.3 μM of each PCR primer. The PCR primer pairs EUB338F (Hatamoto *et al.*, 2007a; Amann *et al.*, 1990; Daims *et al.*, 1999)/Uni1490R (Hatamoto *et al.*, 2007b) and Ar109f (Imachi *et al.*, 2006)/Uni1490R were used for the bacterial and archaeal 16S rRNA genes, respectively. The PCR products were purified with a QIAquick PCR Purification Kit (QIAGEN Inc. Valencia, CA) and subsequently

cloned using a TOPO TA cloning kit (Invitrogen, Carlsbad, USA). The partial 16S rRNA gene sequences were sequenced using Uni907R primer (Hatamoto *et al.*, 2007). The sequencing was performed at Dragon Genomics Center (Takara Bio Inc., Otsu, Japan). Partial 16S rRNA gene sequences with more than 97% identity were grouped in the same Operational Taxonomic Units (OTUs). The phylogenetic affiliations were determined using the NCBI BLAST search tool (BLASTN; http://www.ncbi.nlm.nih.gov/BLAST/), the Ribosomal Database Project (http://rdp.cme.msu.edu), and the ARB program package (http://www.arb-home.de/).

Table 6-1 Operational condition of the biological process

Flow	HRT [d]				Inf. conc.	Inf. TN	Inf. TP	UASB1+2 Loading	DHS loading
[m^3/d]	Total	UASB 1+2	DHS	UFB	[mg COD/L]	[mg N/L]	[mg P/L]	[kg COD/m^3/d]	[kg COD/m^3/d]
2	20.8	5.0	7.1	2.0	15,900 (±9,009)	190 (±107)	26.7 (±7.8)	1.3 (±0.8)	0.3 (±0.13)
4	10.5	2.5	3.6	1.0	8,966 (±3,475)	100 (±70)	12.7 (±2.4)	1.3 (±0.4)	0.4 (±0.07)
6	7.0	1.7	2.4	0.7	8,463 (±981)	124 (±39)	16.5 (±5.8)	2.2 (±0.3)	0.6 (±0.05)
8	5.2	1.2	1.8	0.5	14,522 (±9,101)	199 (±165)	21.8 (±11.4)	4.4 (±2.4)	1.0 (±0.15)

6.4 Results and Discussion

6.4.1 Biological process performance

The full-scale UASB-DHS-UFB system achieved continuous biogas generation and high strength molasses wastewater treatment at the same time. The average COD removal efficiency of each unit during the 200-day operation period is shown in **Fig. 6-4**.

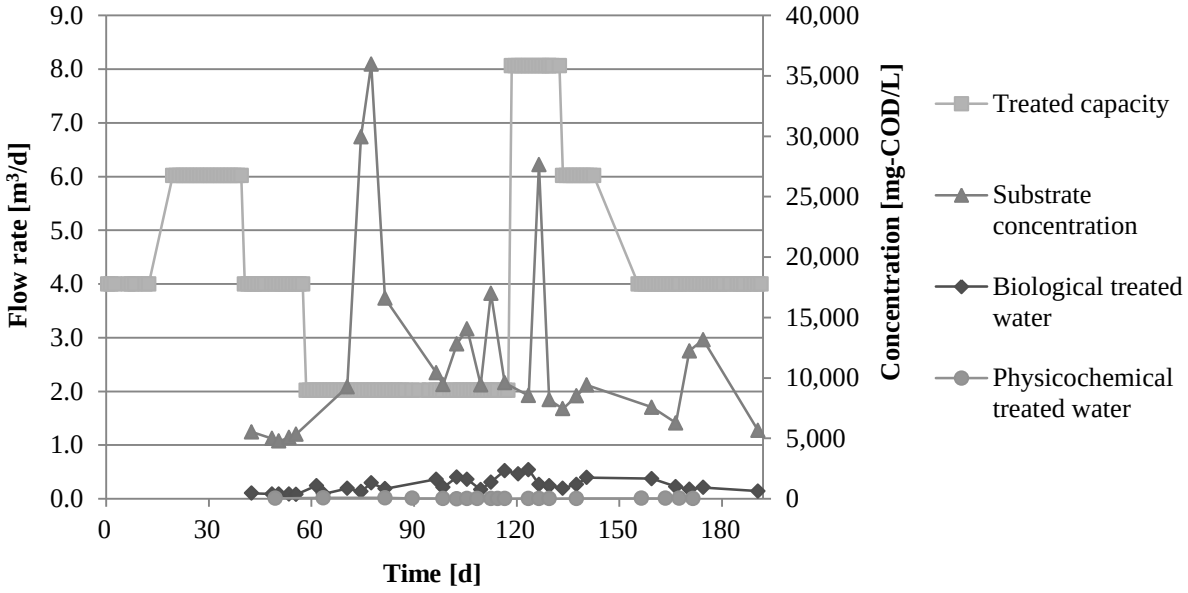
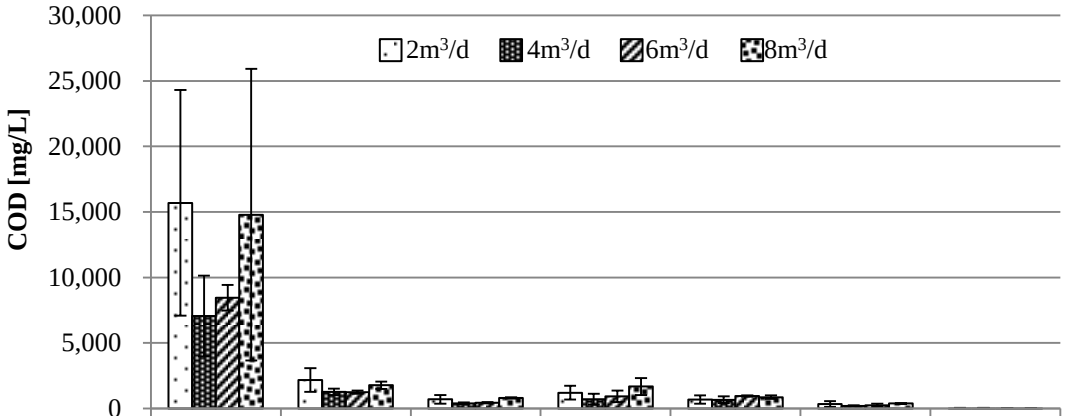


Fig. 6-4 Treated water concentrations of the biological and physicochemical processes

The data indicated that each unit had a significant effect on COD removal during molasses wastewater treatment, although the COD removal effect of each unit was distinct. When the system was operated in the first concentration gradient, the COD removal efficiency of each UASB reactor unit was approximately 60~80%. As the influent into the system increased (because of the periodic production and fermentation tank cleaning conditions), the efficiency of the UASB reactors was enhanced because an adequate carbon source was available for the anaerobic microbes (the COD removal efficiency reached up to 90% with an influent concentration of 35,000 mg-COD/L). Indeed, the COD removal efficiency of the DHS unit did not appear to decrease when the concentration of the treated water was even higher than 2,000 mg-COD/L. The performance of the DHS unit was maintained throughout the entire operation. However,

the UFB effluent concentration was moderately increased after the addition of the carbon source (raw wastewater) before denitrification occurred (**Fig. 6-5**).

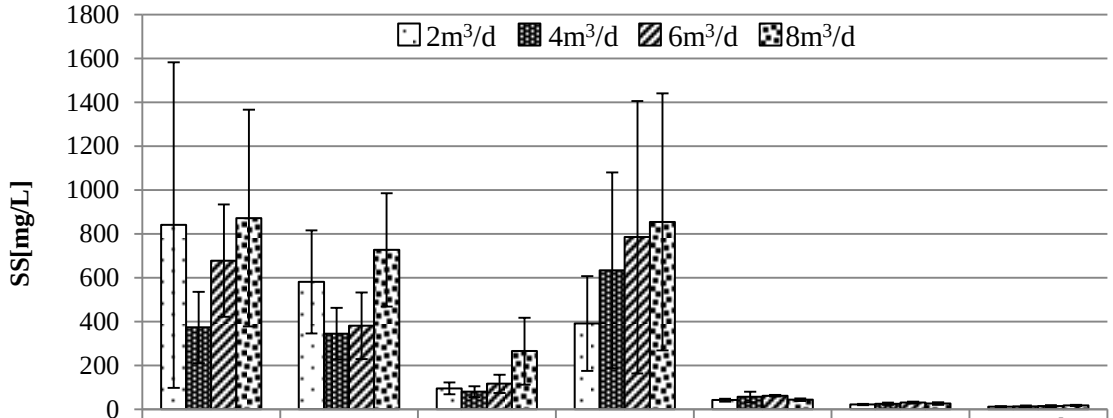


	Substrate	UASB	DHS	UFB	MF	UF	Final treated Water
2m3/d	15,692	2,180	698	1,209	687	342	37
4m3/d	7,065	1,266	399	703	667	201	37
6m3/d	8,463	1,247	446	931	954	246	20
8m3/d	14,793	1,775	801	1,678	861	388	19

Fig. 6-5 Average COD removal by each process

The substrate BOD:COD ratio of approximately 0.6 was because of the molasses-based bioethanol wastewater containing readily soluble biodegradable material, which is reported to have a higher denitrification rate compared with particulate substrates (Griffiths *et al.*, 1994). The use of molasses as a carbon source and denitrification have been studied by several researchers and it was shown that long-chain polysaccharides cannot be readily utilized by denitrifying bacteria so they need to be reduced to monosaccharides such as glucose and fructose (Najafpour and Shan, 2003). The molasses used in this process was fermented into ethanol; it could be presumed that certain amount of the spent wash from the fermentation tank contained the hydrolyzed form of this carbon source, which explained the biodegradability of the substrate. The organic compound in molasses-based bioethanol wastewater was mostly converted to biogas (CH_4) by UASBs in all operating conditions except 8 m^3/d due to the malfunction of the gas meter. Besides biogas, UASBs removal can be assumed as dissolved methane, carbon assimilation by microorganisms and biogas loss by gas meter failure. The

wastewater SS was reduced by 17~44% with a flow of 2, 6, and 8 m^3/d (except 4 m^3/d) using UASB. Most of the SS was absorbed by DHS sponges, which reduced it to approximately 100~250 mg/L, although it increased again to approximately 400~800 mg/L with all flows after treated by UFB because of the anaerobic sludge and additional raw wastewater (**Fig. 6-6**).

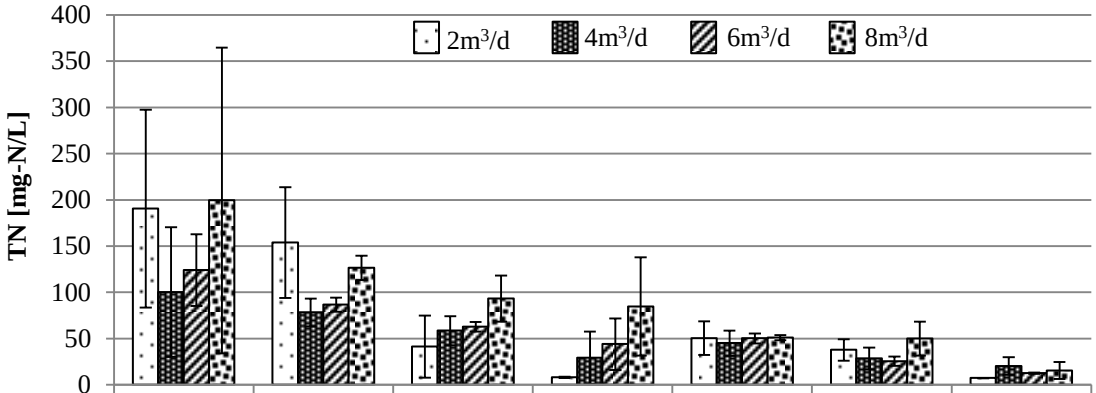


	Substrate	UASB	DHS	UFB	MF	UF	Final treated Water
2m3/d	841	582	95	392	42	21	11
4m3/d	373	345	80	633	56	25	13
6m3/d	678	381	116	785	62	30	14
8m3/d	872	727	265	855	43	26	17

Fig. 6-6 Average suspended solids removal by each process

During the 200 days of operation, 86% of the nitrate was significantly removed by the UFB reactor, i.e., from 55.5 to 7.7 mgN/L. The total nitrogen was reduced by 73% (flow = 2 m^3/d), 25% (flow = 4 m^3/d), 28% (flow = 6 m^3/d) and 24% (flow = 8 m^3/d) reduced by DHS then 80% (flow = 2 m^3/d), 50% (flow = 4 m^3/d), 30% (flow = 6 m^3/d) and 10% (flow = 8 m^3/d) removed in UFB (**Fig. 6-7**). The process was maintained in anaerobic conditions so that the denitrifying organisms could use nitrate instead of dissolved oxygen as an oxygen source during their metabolism and organic substance oxidation. Ammonium was accumulated during anaerobic degradation, which was 87~99% oxidized by the nitrification process in the DHS reactor with all feed conditions. Almost 50% of the substrate and UASB effluent comprised of organic and ammonium forms of nitrogen in oxygen-depleting conditions. During the aerobic stage (DHS), most

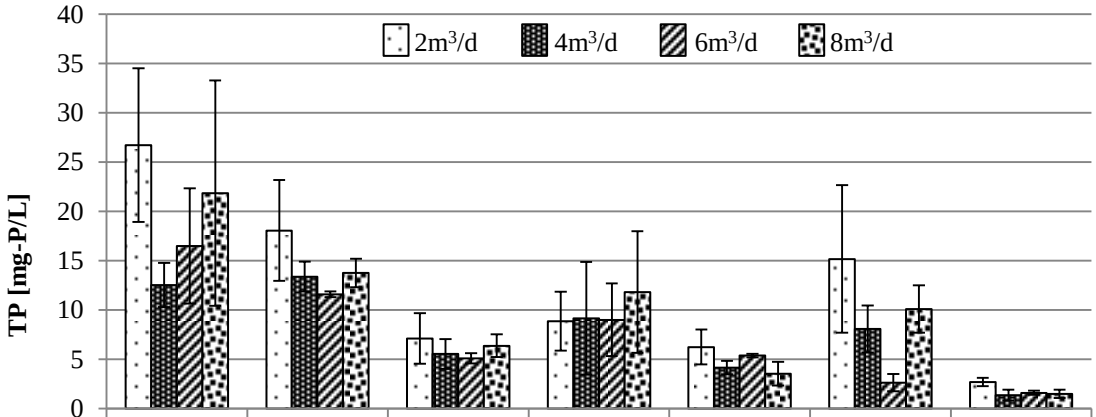
of the ammonium was oxidized to nitrite-N and nitrate-N, whereas the organic form of nitrogen still persisted. After denitrification in the UFB tank, the anaerobic bacteria used nitrate-N for respiration and converted it to the gaseous form of nitrogen, which was used as a carbon source.



	Substrate	UASB	DHS	UFB	MF	UF	Final treated Water
2m3/d	190	154	41	8	50	38	7
4m3/d	100	78	59	29	45	28	20
6m3/d	124	87	63	44	50	26	13
8m3/d	199	127	93	85	51	50	16

Fig. 6-7 Average total nitrogen removal by each process

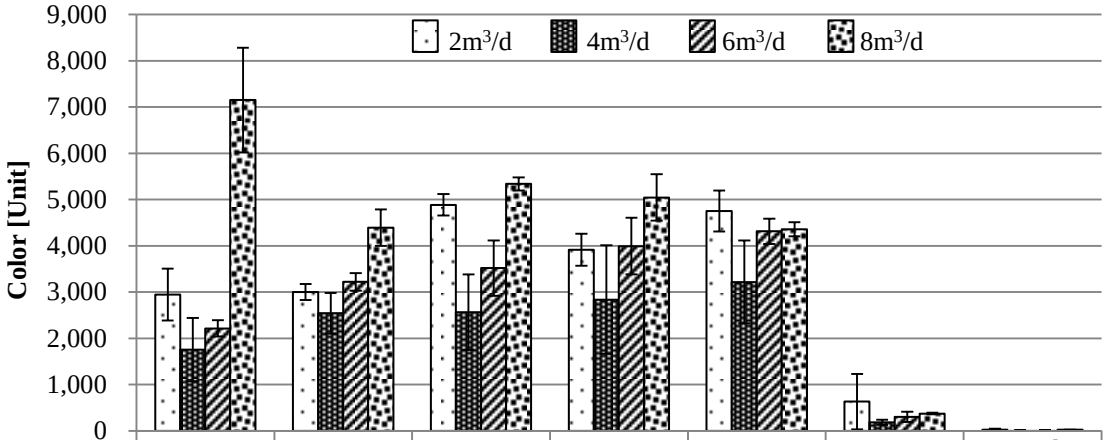
Phosphate was retained by the DHS sponge (DHS-treated water = 6.8 mgP/L; the phosphate level of the UFB-treated water was higher than that of the DHS-treated water because of the addition of raw wastewater) to yield an allowable dischargeable level of <8 mg P/l (**Fig. 6-8**).



	Substrate	UASB	DHS	UFB	MF	UF	Final treated Water
2m3/d	26.7	18.1	7.1	8.9	6.2	15.2	2.7
4m3/d	12.5	13.4	5.5	9.2	4.2	8.1	1.4
6m3/d	16.5	11.6	5.1	9.0	5.4	2.6	1.6
8m3/d	21.8	13.8	6.4	11.8	3.6	10.1	1.5

Fig. 6-8 Average total phosphate removal by each process

The substrate contained dark brown color pigments, which were mostly phenols and high molecular weight (MW) amino–carbonyl melanoidins with an MW value in the range 20~35 kDa. Unfortunately, they remained untreated during the biological process, whereas the UASB reactor appeared to remove color from the molasses-based wastewater (5% to 7%), although the effluent was 26.9% darker after DHS treatment, possibly because of the polymerization of colored substances under aerobic conditions (**Fig. 6-9**).



	Substrate	UASB	DHS	DN	MF	UF	Final treated Water
2m3/d	2,948	3,002	4,887	3,914	4,750	634	26
4m3/d	1,757	2,545	2,568	2,839	3,219	185	9
6m3/d	2,216	3,223	3,520	3,994	4,316	303	14
8m3/d	7,152	4,394	5,340	5,045	4,360	376	25

Fig. 6-9 Average color removal by each process

Biogas with methane contents > 70% was produced by methanogens in UASBs at a production rate of 0.38 Nm^3/m^3-reactor/d which was relatively exceptional for high-rate anaerobic treatment (data not shown).

6.4.2 Physicochemical process performance

The performance of the three membrane filtrations stages was measured in terms of the percent rejection ($\%R_j$) of COD, SS, nitrogen, phosphate, and color. MF showed a potential of rejecting 33.5% of total COD mostly in the form of particulate COD when UF,

which has typical operating range between 0.005 to 0.2 μm, removed 61% of the remaining COD to 275 and 244 mg/L in total COD form. NF showed the advantage of polishing the organic substances up to 32, 25, and 0 mg/L in terms of total COD of the final residue with functional micropores of <2 nm at the pressure range of 0~0.2 MPa (**Fig. 6-5**). MF (filrer pore size = 0.1~5μm) was carried out mainly for the purpose of suspended solid removal. Most of the colloidal particles were filtered by the φ0.4-μm PVDF membrane (similar to the φ0.4-μm pore size of the glass fiber filter used for SS and VSS measurements). The MF module reduced the suspended particles from 496 to 42 mg/L (%R_j = 89.9) and the UFB-treated water was used to feed the MF (**Fig. 6-6**). However, the influent (UFB-treated water) with an average total nitrogen concentration of 43 mg-N/L was not removed during MF, but slightly decreased by UF and NF to the level of 34 and 17 mg-N/L, respectively (**Fig. 6-7**). The ammonia-N, nitrite-N, and nitrate-N were not measured during the physicochemical process, but it was found that the membrane configuration reduced the levels of some ions (NH_4^+-N and NO_3^--N) by NF. Thus, the permeate phosphate (PO_4^{3-}-P) concentration was reduced from 15.2 mg-P/L (UF effluent, 2 m^3/d flow) to 2.7 mg-P/L (NF effluent, 2 m^3/d flow) (**Fig. 6-8**). This showed that the NF membrane was highly efficient in removing trivalent cations including phosphate and certain metals. The MF membrane (MF filtered water: 4,750 units with 2 m^3/d flow; 3219 units with 4 m^3/d flow; 4,316 units with 6 m^3/d flow; and 4,360 units with 8 m^3/d flow) had a micropore size of >50 nm so it could only filter suspended particles and not the colored pigments remaining in the molasses-based effluent from the UFB. However, the color (true color: the color of water which turbidity has been removed) of the permeates from the UF (634 units with 2 m^3/d flow; 185 units with 4 m^3/d flow; 303 units with 6 m^3/d flow; and 376 unit with 8 m^3/d flow) appeared to be transparent light yellow and crystal clear in the NF permeate (26 units with 2 m^3/d flow; 9 units with 4 m^3/d flow; 14 units with 6 m^3/d flow; and 25 units with 8 m^3/d flow) (**Fig. 6-9**).

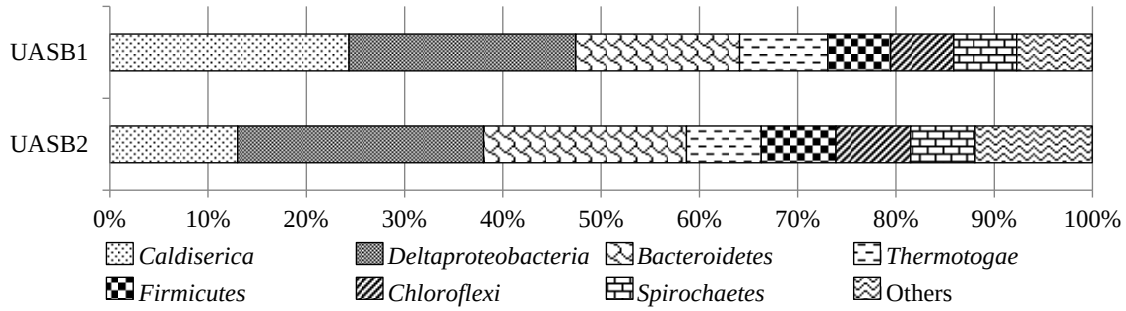
6.4.3 Phylogenetic analysis

We analyzed 78 (UASB1) and 92 (UASB2) bacterial 16S rRNA gene clones. **Fig. 6-10** shows the phylogenetic affiliation of the bacterial clones. Over 50% of the bacterial clones belonged to common dominant bacteria such as *Caldiserica*, *Bacteroidetes*, and *Deltaproteobacteria*, although others such as *Thermotogae*, *Firmicutes*, *Chloroflexi*, and *Spirochaetes* were also found. Most bacteria known from the phylum *Caldiserica* were isolated from a hot spring mat (Skirnisdottir *et al.*, 2000), hydrothermal vent (Inagaki *et al.*, 2006), and anaerobic wastewater treatment reactors (Kaksonen *et al.*, 2004; Chen *et al.*, 2004). The dominant *Caldiserica*-related OTUs (UASB1B_D11 = 16 clones and UASB2B_E12 = 11 clones) were retrieved from the UASBs and they shared 99% sequence similarity with a clone isolated from a muddy hot spring in southwestern Taiwan (FJ638586). *Caldisericum exile* (Mori *et al*., 2009) shared 81% sequence similarity with a known isolate (NR041680). However, there was no significant proof of an important role for the identified microorganisms in both UASBs. Nevertheless, the *Caldiserica*-related clones were the most abundant in UASB1 (**Fig. 6-10**), which suggested that *Caldiserica* had a role in breaking down complex organic compounds (e.g., carbohydrate, protein, and lipids) in this system. Bacteria of the phylum *Bacteroidetes* were also found and they are known to be complex organic compound-degrading anaerobic microorganisms (Kragelund *et al*., 2008). The dominant *Bacteroidetes*-related OTUs (UASB1B_A03 = three clones, UASB1B_E01 = three clones, UASB2B_C06 = four clones, and UASB2B_B10 = three clones) in this system shared high sequence similarity with others collected from anaerobic bioreactors (U81712, FJ228431, DQ661703, and GQ182907). *Firmicutes*-related clones and other complex organic macromolecules-degrading bacteria were less abundant than the *Bacteroidetes*-related clones in both UASBs (**Fig. 6-10**). Thus, it can be assumed that bacteria from the phylum *Bacteroidetes* played a more important role in degrading complex organic compounds in both UASBs compared with *Firmicutes*. The dominant syntrophic bacterial OTUs from the *Deltaproteobacteria* were affiliated to *Syntrophobacter* (UASB1B_G09 = one clone and UASB1B_C10 = three clones), *Syntrophus* (UASB2B_C11 = four clones), and

Syntrophaceae (UASB2B_A03 = three clones). *Syntrophobacter* is known to be a propionate-degrading bacteria (Boone *et al*., 1980; Chen *et al*., 2005; Harmsen *et al*., 1998; Wallrabenstein *et al*., 1995) while *Syntrophaceae* is a long chain fatty acid-degrading bacteria (Jackson *et al*., 1999; Grabowski *et al*., 2005, Hatamoto *et al*., 2007). Moreover, *Syntrophaceae* and *Syntrophus* were reported to have an ability of degrading benzoic acid and butyric acid (Mountfort *et al*., 1984; Jackson *et al*., 1999; Wallrabenstein *et al*., 1995). Anaerobic chemoorganotroph-related clones from the phyla *Thermotoga, Chloroflexi* and *Spirochaetes* were also retrieved, which can biodegrade various organic compounds by fermentation (Balk *et al*., 2002; Yamada *et al*., 2006; Breznak and Warnecke, 2008). The dominant *Thermotogae*-related OTUs (UASB1B_D07 = seven clones and UASB2B_F08 = seven clones) had 100% sequence similarity to an uncultured bacterium retrieved from crude oil-contaminated soil (HQ689254). Despite all cultured members of *Thermotogae* are thermophilic anaerobic bacteria (Nunoura *et al*., 2010). *Thermotogae* 16S rRNA genes have been retrieved from mesophilic anaerobic digesters. Therefore, these OTUs also appeared to be low-temperature-adapted *Thermotogales* (Nesbo *et al*., 2006). In UASB2, the dominant OTU within *Chloroflexi* was *Anaerolineaceae* (UASB2B_F11 = three clones). An *Anaerolineaceae*-related OTU shared 99% sequence similarity with an uncultured bacterial clone retrieved from an anaerobic digester used to treat feedstock (GU389465). *Anaerolineaceae* consisted of only a few isolates and those were filamentous bacteria. In addition, these bacteria may play an important role in granulation in this system (Yamada *et al*., 2005). Other bacterial clones within *Actinobacteria*, *Synergistetes*, *Verrucomicrobia*, *Epsilonproteobacteria*, *Planctomycetes*, Candidate division OP8, Candidate division OP9, and Candidate division WS3 were retrieved from one or both UASBs at low frequencies (**Fig. 6-10**).

In total, 91 archaeal 16S rRNA gene clones were analyzed from UASB2. The acetoclastic methanoarchaeal genus *Methanosaeta* was dominant in granular sludge samples (54 clones), which showed that *Methanosaeta* could overcome *Methanosarcina* (undetected), another major acetotrophic archaean encountered in most anaerobic digesters, in a low acetate environment (Koster *et al*., 1987). We also found 16 clones of *Methanolinea*, which (Imachi *et al*., 2008) has a line-shaped morphotype and utilizes H_2

and formate to produce methane. Other H_2-utilizing *Methanobacterium* clones were also found (ten clones). The remainder were classified as *Thermoplasmata* (three clones), *Methanomethlovorans* (one clone), *Methanospillirum* (one clone), *Thermoprotei* (one clone), and others (six clones).



Others ; Actinobacteria, Synergistetes, Verrucomicrobia, Epsilonproteobacteria, Planctomycetes, Candidate division OP8, Candidate division OP9, Candidate division WS3 and unclassified.

Fig. 6-10 16S rRNA analysis of the microbial diversity in the UASB1 and UASB2 reactors

6.5 Conclusion

1. This study demonstrated that a series of combined biological and physicochemical treatments effective for treating medium to high concentration molasses-based wastewater. The biological UASB-DHS-UFB had an important role as the main organic treatment, especially for reducing COD, SS, BOD, and nitrogen species, while the membranes (MF, UF, and NF) were a functional alternative treatment that enhanced the removal of untreated nutrients and color.
2. In addition, the biogas recovered from UASBs could be used to increase the treatment performance and reduce the operational energy costs.
3. The application of hybrid bio-physicochemical systems could be a pragmatic environment-friendly solution for removing >95% of the overall organic substances, nutrients, and color. Although, the system was started at low organic loading rate of 0.5 kg-COD m3/d, the UASBs was designed for maximum loading rate of 16 kg-COD m3/d at ambient condition.
4. Finally, the molecular microbiological analysis of sludge samples from UASB1 and UASB2 produced similar results because the same 16S rRNA gene sequences of

meso-thermophilic bacteria were the dominant microbial species. In particular, an unusual Caldiserica isolate was detected in both reactors, which is generally found in thermophilic hot spring environments. It is possible that Caldiserica existed along in the seed sludge, which existed prior to the brewery wastewater, and could adapt themselves to the new environment of the molasses-based bioethanol wastewater.

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Chapter 7

System design summary and suggestion

According to long-term experiments of lab-scale, pilot-scale, and full-scale combined bio-physicochemical system for molasses-based wastewater treatment, the summary of the each system performance are summarized as following;

	Influent	**UASB1**	**UASB2**	**DHS**	**ASB**	**MF**	**UF**	**NF Final treated water**	**Standard**
Organic conc. [mg-COD/L]	<12,000	× (70% Removal)	× (65% Removal)	× (50% Removal)	×	△	◎	◎	<120
Temperature [°C]	18~35	◎*1	◎	◎	◎	◎	◎	◎	<40
pH	4.5	7*2	7	8-9	7	9	◎*3	◎	6.5-8.5
SS [mg/L]	>300	×	×	△	×	◎	◎	◎	<200
TN [mg/L]	>250	×	×	△	×	×	△	◎	<120
NH_4 [mg-N/L]	50	×	×	◎	×	×	△	◎	
NO_2 [mg-N/L]	<10	×	◎	△	◎	×	◎	◎	
NO_3 [mg-N/L]	<10	×	◎	△	◎	×	◎	◎	
TP [mg-P/L]	>70	△	×	△	×	×	×	◎	<8
Color [°]	>3,500	×	×	×	×	×	△	◎	<100

*1 Preheated during winter,

*2 Adjusted by adding NaOH before entering UASB1,

*3 Adjust by adding HCl before entering UF to prevent fouling

7.1 Operation design recommendations for anaerobic-aerobic treating molasses wastewater and fermentation residue wastewater

- The configuration and other parameters of the upflow anaerobic sludge blanket (UASB) reactor and downflow hanging sponge (DHS) reactor for molasses wastewater treatment can be designed as following;
  - ✧ *Reactor volume design*

    Lab-scale UASB

    The reactor was designed based on the fundamental approaches of height, possible flow rate and up flow velocity.

    Under the mesophilic condition (35±2°C), assuming lab-scale reactor height: 1 m, one feed preparation capacity: 300 L (maximum flow rate: 50 mL/min), maximum one feed duration: 4 days, up flow velocity: 0.5 m/h, safety factor: 1.5

$$\text{Reactor volume [L]} = \frac{\text{Reactor height [m]}}{\text{up flow velocity } \left[\frac{\text{m}}{\text{h}}\right]} \times \text{flow rate } \left[\frac{\text{L}}{\text{h}}\right] \times \text{safety factor}$$

$$\text{Reactor volume [L]} \approx \frac{1.0\ [\text{m}]}{0.5\ \left[\frac{\text{m}}{\text{h}}\right]} \times 3.0\ \left[\frac{\text{L}}{\text{h}}\right] \times 1.5 \approx 9\ \text{L}$$

    Pilot-scale UASB case

    Under the same mesophilic condition (35±2°C), assuming pilot-scale reactor height: 1.5 m, one feed preparation capacity: 10,000 L (maximum flow rate estimation: 800 L/d), maximum one feed duration: 13 days, up flow velocity: 0.5 m/h, safety factor: 1.5

$$\text{Reactor volume [L]} = \frac{\text{Reactor height [m]}}{\text{up flow velocity } \left[\frac{\text{m}}{\text{h}}\right]} \times \text{flow rate } \left[\frac{\text{L}}{\text{h}}\right] \times \text{safety factor}$$

$$\text{Reactor volume [L]} \approx \frac{2.0\ [m]}{0.5\ \left[\frac{m}{h}\right]} \times 35\ \left[\frac{L}{h}\right] \times 1.5 \approx 200\ \text{L}$$

Lab-scale and Pilot-scale DHS

The concept of the DHS reactor is similar to trickling filter reactor. The sponge media filling inside a column or a tank works as filter, the stream gravitationally flows from the upper part through the sponge to the bottom. The amount of sponges depends on the container (reactor) design. The sponge size is φ3.3 cm. x 3.3 cm., for this two-compartments DHS reactor it requires 165 sponges (4.7 L).

✧ *Pilot-scale membrane operation and maintenance*

Filtration

- The suction pump capacity was set at 200 L/h, however due to 7/1 min (operation/shutoff timing); the actual capacity became 175 L/h. In order to prevent an idle pumping, at least 100 L of substrate must be remained in the MF substrate tank.
- In case of UF and NF module, it is more serious if mistaken operating without wastewater. Therefore, at least 200 L of wastewater must be remained in the substrate tank.
- In order to prevent the formation of scale in NF module which has the smallest pore size, concentrated hydrochloric acid was directly added to NF substrate tank and pH adjusted to 7 (UF permeate pH usually range between 9-11, high pH tends to cause scale formation).

Cleaning

- When ΔP of MF (pressure of the inlet when operating - pressure of the inlet when stopping) increases more than 15 kPa of the first operation day, the chemical (sodium hypochlorite which contains 12% chlorine) cleaning should be applied.
- Chemical cleaning (NaOH and HCl) should be applied immediately when fouling occur. Before cleaning, water flux must be monitored before and after each chemical cleaning.

7.2 Operation design recommendations for anaerobic-aerobic-membrane treating molasses-based bioethanol wastewater

Full-scale UASB

Under the same mesophilic condition (35±2°C), more details on organic loading, pipes' size, pumping capacity etc. should be also considered for the design of the full-scale reactor in order to maintain the stable operation. Assuming that the maximum capacity is 15 m^3/d, HRT: 4 h. The estimation of reactor volume can be also calculated by following formula;

$$\text{Reactor volume } [\text{m}^3] = \text{ HRT [h]} \times \text{flow rate } \left[\frac{\text{m}^3}{\text{d}}\right]$$

$$\text{Reactor volume } [\text{m}^3] \approx 4\ [\text{h}] \times 15\ \left[\frac{\text{m}^3}{\text{d}}\right] \times \frac{1}{24\ [\text{h}]} \approx 3\ \text{m}^3$$

Full-scale DHS

The estimation of the total sponge volume can be calculated based on the following conditions;

1) Influent details

BOD	mg/L	315
SS	mg/L	180
T-N	mg/L	225
Flow rate	m^3/d	15

2) Expected water quality

BOD	mg/L	>100
SS	mg/L	>100
NO_3-N	mg/L	>200

3) Treatment efficiency

BOD loading*	kg/m^3/d	1.2
BOD removal	%	85
SS removal	%	50
Nitrification	%	90

* Based on BOD removal per 1 m^3 of sponge volume

4) Treated water quality

- Effluent BOD

$$315\ \left[\frac{\mathrm{mg}}{\mathrm{L}}\right] \times (100\% - 85\%) = 47.3\ \left[\frac{\mathrm{mg}}{\mathrm{L}}\right]$$

- Effluent SS

$$180\ \left[\frac{\mathrm{mg}}{\mathrm{L}}\right] \times (100\% - 50\%) = 18.0\ \left[\frac{\mathrm{mg}}{\mathrm{L}}\right]$$

- Effluent ammonia

$$225\ \left[\frac{\mathrm{mg}}{\mathrm{L}}\right] \times (100\% - 90\%) = 22.5\ \left[\frac{\mathrm{mg}}{\mathrm{L}}\right]$$

- Effluent nitrate

$$225\ \left[\frac{\mathrm{mg}}{\mathrm{L}}\right] \times 90\% = 202.5\ \left[\frac{\mathrm{mg}}{\mathrm{L}}\right]$$

5) Estimation of sponge volume

- Treated BOD

$$\text{Treated BOD} = (\text{Influent} - \text{Effluent}) \times \text{Flow rate}$$

$$= \left(315\ \left[\frac{\mathrm{gBOD}}{\mathrm{m^3}}\right] - 100\left[\frac{\mathrm{gBOD}}{\mathrm{m^3}}\right]\right) \times 15\ \left[\frac{\mathrm{m^3}}{\mathrm{d}}\right] = 3225\ \left[\frac{\mathrm{gBOD}}{\mathrm{d}}\right]$$

$$= 3.3\ \left[\frac{\mathrm{kgBOD}}{\mathrm{d}}\right]$$

- BOD converted from nitrogen

$$202.5\ \left[\frac{\mathrm{gNO_3}}{\mathrm{m^3}}\right] \times 4.57 \times 15\left[\frac{\mathrm{m^3}}{\mathrm{d}}\right] = 13{,}881\ \left[\frac{\mathrm{gBOD}}{\mathrm{d}}\right] = 13.9\left[\frac{\mathrm{kgBOD}}{\mathrm{d}}\right]$$

- BOD Loading

$$\text{BOD loading} = \text{Treated BOD} + \text{BOD converted from nitrogen}$$

$$\text{BOD loading} = 3.3\ \left[\frac{\mathrm{kgBOD}}{\mathrm{d}}\right] + 13.9\left[\frac{\mathrm{kgBOD}}{\mathrm{d}}\right] = 17.2\left[\frac{\mathrm{kgBOD}}{\mathrm{d}}\right]$$

- Sponge volume

$$\text{Sponge volume} = \frac{17.2\ \left[\frac{\text{kgBOD}}{\text{d}}\right]}{1.2\left[\frac{\text{kg}}{\text{m}^3\text{d}}\right]} = 14.3\text{m}^3 \rightarrow 14.4\text{m}^3$$

Assuming 45% of media density

$$\text{Required sponge volume} = \frac{14.4\ [\text{m}^3]}{45\%} = 32.0\ \text{m}^3(16\text{m}^3 \times 2\ \text{reactors})$$

Each biological system was also designed concerning the wastewater characteristics and estimated removal efficiencies as shown in the following;

	Substrate	UASB1	UASB2	DHSs	UFB
COD [mg/L]	15,000*1	9,000*1	5,400*1	2,000*1	1,200*1
BOD [mg/L]	7,000	4,200	2,000	500	200
TN [mg/L]	300	250	200	150	100
TP [mg/L]	50	40	40	30	40
Color [°]	4,000	3,500	3,500	4,000	3,500

* The COD removal efficiency was estimated at 60% each

✧ *Full-scale membrane operation and maintenance*

The entire system is controlled by digital control panel, however it can be chosen whether fully automatic or manual.

Filtration

- The wastewater must reach the water level conditions which is between H (High) and M (Medium) level in order to automatically operate each system.
- Fouling in UF module has occurred frequently possibly due to the high pH of MF filtrate, therefore concentrated hydrochloric acid solution was prepared with real time pH monitoring equipment and automatically fed until pH become 6.8. The 100 L HCl solution was prepared once a week.

Cleaning

Three types of cleaning (water flushing, acidic, and alkali) were applied for membrane washing procedure.

- Water flushing was applied to wash the particles attached to the surface of the membrane and pipes. MF and UF or MF and NF flushing can be operated together but UF and NF cannot be flushed at the same time.
- Acidic washing solution consisted of 0.03% HCl and 2.0% citric acid solution. This washing was applied to remove the acid soluble substances attaching on the membrane surface.
- Alkali washing solution consisted of 0.1% NaOH solution. This washing was applied to remove the alkali soluble substances attaching on the membrane surface.
- Water rinse and water flux monitoring should be applied after all washing procedures.

✧ Acidic molasses-based wastewater including artificial molasses wastewater, fermentation-spent wash wastewater, and bioethanol spent wash wastewater need to be pH adjusted to 7.0 before feeding to the biological processes.

✧ Air supply in DHS reactors can be calculated based on the following data and reactions assuming continuous fan operating

BOD loading*	kg-BOD/d	17.2
Hydrogen sulfide concentration	mg/L	400
Required air / oxygen needed	m^3/kgBOD	30
Flow rate	m^3/d	15

Hydrogen sulfide conversion

$$H_2S + 2O_2 \rightarrow H_2SO_4$$

$$15\left[\frac{m^3}{d}\right] \times 400\left[\frac{mg}{L}\right] \times \frac{2 \times 32}{34} \times \frac{1000}{10^6} = 12\left[\frac{kgBOD}{d}\right]$$

$$Air\ supply = \frac{\left(17.2\left[\frac{kgBOD}{d}\right] + 12\left[\frac{kgBOD}{d}\right]\right) \times 30\left[\frac{m^3}{kgBOD}\right]}{24\,[h] \times 60[min]}$$

$$= 0.608\left[\frac{m^3}{min}\right] \rightarrow 0.8\left[\frac{m^3}{min}\right]$$

✧ Biological processes require approximately 30~45 days before the

microorganisms can acclimatize to the new environment (wastewater).

- ✧ Sulfate reducing reaction in anaerobic molasses wastewater can be roughly explained by the following formula;

$$SO_4^{2-} + \text{Molasses} \rightarrow HS^- + CO_2 + H_2O$$

Since molasses is complex in chemical compound and vary depending on the material and production. The COD:S ratio can be roughly estimated to 2:1.