# Improvement of Membrane Bioreactor Operations for Color and Oil Removal from Wastewater

（排水からの色度と油分の除去を目指した膜分離活性汚泥法の運転方法の改善）

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# إ هـد ا ء

في البداية، الشكر والحمد لله، جل في علاه، فإليه ينسب الفضل كله في إكمال - والكمال يبقى لله وحده- هذا العمل. وبعد الحمد لله أتوجه إلى البروفيسور الدكتور أوراسي تارو بالشكر والتقدير الذي لن تفيه أي كلمات حقه، فلولا مثابرته ودعمه المستمر ما تم هذا العمل. وبعدها في الشكر موصول إلى فريق لجنة المناقشة في جامعة طوكيو للتكنولوجيا الموقرة.

كل الشكر والتقدير إلى برنامج خادم الحرمين الشريفين الملك عبد الله بن عبد العزيز للإبتعاث الخارجي والمتمثلة بوزارة التعليم العالي و إلى الملحقية الثقافية السعودية في اليابان على الدعم المستمر.

أهدي هذا العمل إلى من كان يضيئون لي الطريق ويساندوني.. إلى النور الذي ينير لي درب النجاح (أبي)، إلى سبب وجودي في الحياة (أمي)، إلى إخوتي أحبابي (نادر، سلمى، عمر) ، إلى صديق الدرب المقرب الذي أجده دائماً شمعة أمل لا تنطفىء (المهندس علاء القاسم)، إلى زوجة المستقبل وأم أولادي، وإلى جميع من دعمني ووثق بقدراتي.

# إ هـد ا ء خـا ص

... وعلمٌ ينتفع به"

أهدي هذا البحث المتواضع

إلى من أتاح الفرصة للأبتعاث ..

إلى من قرب الناس إليه وأولهم أبنائه ..

للذي تمنيت أن يكون معنا يشاركنا ثمرات النجاح ..

إلى من وثق بنا لنبني وطناً بمستقبل أفضل ..

إلى روح ملك الإنسانية المغفور له ..

*خادم الحرمين الشريفين*

*الملك عبد الله بن عبد العزيز آل سعود*

*رحمة الله ..*


## ABBREVIATIONS

BOD biochemical oxygen demand

| | |
|---|---|
| COD | chemical oxygen demand |
| $CaCl_2.2H_2O$ | calcium chloride |
| DO | dissolved oxygen |
| EPS | extracellular polymeric substance |
| FOG | fat, oil and grease |
| FGD | flue gas desulphurization |
| GC/MS | gas chromatography–mass spectrometry |
| HRT | hydraulic retention time |
| $H_2SO_4$ | sulfuric acid |
| MBR | membrane bioreactor |
| MLSS | mixed liquor suspended solids |
| M/F | food-to-microorganism ratio |
| $MgSO_4.7H_2O$ | magnesium sulfate |
| NaCl | sodium chloride |
| NaOH | sodium hydroxide |
| $NH_3$-N | ammoniacal nitrogen |
| $NO_3^-$ | nitrate |
| $NH_3$ | ammonium |
| SRT | sludge retention time |
| SS | suspended solids |
| SMP | soluble microbial products |
| TEP | transparent exopolymer particles |
| TMP | transmembrane pressure |
| TN | total nitrogen |
| WWTP | wastewater treatment plant |

# ABSTRACT

Membrane bioreactor (MBR) process is the technology that has gained a considerable numbers of applications into wastewater treatment processes in recent days. It is a type of modification to conventional activated sludge process under which solid/liquid separation is undertaken through membrane filtration. One of the greater advantages of the MBR process is the operation at a high sludge retention time, which enables keeping in the reactors a variety of microorganism which can extend the removable compounds in biological wastewater treatment. In addition, high effluent water quality without the presence of suspended particles by the introduction of MBR is attractive for the reuse of industrial wastewater.

The characteristics of industrial wastewater are quite different depending on its source. Biomass process including molasses distillation and sulfuric acid hydrolysis often generates wastewater having acidic characteristics. Saline and high-temperature wastewater containing a variety of organic compounds is a difficult target for wastewater treatment. The produced water from oil and gas production activities, shipboard wastewater, and textile wastewater are the examples of this type of wastewater.

The aim of this study is to investigate the performances of membrane bioreactors (MBR) for wastewater treatment under high temperature operation and acidic operation to improve the removal of color and oil from industrial wastewater. The removal of color was focused because the remaining yellow or brown color in treated industrial wastewater usually originates from high molecular weight organic matters which are recalcitrant to biological degradation. Oil was also focused because oil in wastewater often disturbs the treatment of industrial wastewater by forming aggregates especially under low temperature conditions. High temperature operation is preferable to avoid the problems of oil in wastewater.

Few literature can be found for the operation of MBR below pH 3. There are few studies showing the advantage of thermophilic MBR for the treatment of dilute wastewater.

In the first experiment, the advantage of acidic operation below pH of 3, which operation was out of the usually accepted condition for membrane bioreactors (MBRs), was examined targeting the treatment of sulfuric acid hydrolysis wastewater generated in the biomass processing without pH neutralization. Stable operation of both an acidic reactor and a neutral pH reactor was observed for 91 days, though higher trans-membrane pressure was observed for the acidic reactor, which accumulated proteins and polysaccharides in the supernatant. COD removal for the acidic reactor was 48.5% and that for the neutral pH reactor was 63.6% when biologically pretreated molasses wastewater was fed to the reactors. Higher percentage removals of COD (89.0% for the neutral pH reactor and 84.0% for the acidic reactor) were observed, when molasses wastewater (COD 650 mg/L) was directly fed to the reactor because of higher concentration of biologically degradable organic matter in the feed solution. In spite of lower COD removal in the acidic reactor, higher removal of color was observed spectrophotometrically with the low pH operation. Higher color removal in the acidic reactor was due to the enhanced adsorption of colored substances in the acidic environment followed by gradual biological degradation judging from the increased tendency of the removal of color.

The second experiment was targeting for the treatment of saline and high temperature wastewater containing oil and organic matters of different biodegradability. A thermophilic condition (50$^{o}$C) beyond the usual operating condition for MBR was examined to avoid the disturbance for the treatment by oil in wastewater. The performances obtained for 35 days were compared with those of a room-temperature reactor. The removal of COD was comparable for the two reactors. The half-life time of mineral oil ($C_{15}$-$C_{22}$ alkanes) was around 2 hours for the thermophilic reactor, while that of room-temperature reactor was around 3 hours. However, the operation at the high temperature condition decreased the removal of melanoidin color from 58% to 44% compounds. The fouling of the membrane was more severe for the thermophilic reactor. The room-temperature reactor maintained a volume flux of 0.22 m/day, while keeping the volume flux at the same level was difficult for the thermophilic reactor. It was suggested that lower flux operation of the membrane and worse effluent quality have to be considered, if high-temperature operation is required.

These results on MBR operation with extreme conditions showed that the membrane fouling is the most serious problem, though low pH operation is preferable for the color removal and thermophilic operation is preferable to avoid the problems caused by oil in wastewater. Future research for the stable operation will be needed on the mitigation of the accumulation of proteins and polysaccharides in the supernatant of MBRs to realize the operation with the extreme conditions.

# 論文要旨

膜分離バイオリアクター(MBR, membrane bioreactors)は排水処理における適用例が近年増加している。この技術は，標準的な排水処理技術である活性汚泥法に対して，膜による微生物懸濁液の固液分離を付加した技術である。MBR による排水処理の利点として，標準活性汚泥法に比べて，長い汚泥滞留時間で運転が可能である点が挙げられ，そのことによって，多様な微生物を反応タンクに保持することができ，より幅広い物質の分解が可能になることが期待される。さらに，MBR を導入することによって，懸濁物質を全く含まない高品質の処理水を得ることができることから，産業排水の再利用の点でも MBR は魅力的な技術である。

産業排水には，発生源ごとに様々な特徴がある。バイオマスに関連したプロセスでは，糖蜜の蒸溜や硫酸による分解抽出過程で生じる廃水など，極端な酸性廃水が生じる場合がある。また，塩を高濃度に含む高温かつ様々な性質の有機物を含む排水は処理の難しい排水と位置づけられる。こうした塩と難分解性物質を含む処理の難しい排水として，石油や天然ガスの掘削に伴って生じる石油随伴水や船舶のバラスト排水，染色排水がある。

本研究の目的は，色度や油分の除去率を向上させるために，MBR を従来運転可能と考えられていたよりも，低 pH あるいは高温で運転することが可能であるかを調べることである。産業排水中の色度は，高分子の有機化合物によって生じることが多く，一般に生物分解の難しい物質である。また，排水中の油分は低温で処理すると，装置内で固着し生物処理を妨害する。これまで，pH 3 以下の酸性条件で MBR を運転した研究はほとんどない。また，希薄な産業排水に対して，50℃以上の高温処理を試み運転上の利点を報告した研究

はほとんどない。油分含有排水であれば，油分による処理の妨害を避ける点で，高温での運転による利点が生じる可能性がある。

第一の実験として，製糖関係のバイオマス硫酸抽出廃水の処理を念頭に，通常，生物処理で運転される限界を超えて，pH3 程度の酸性条件での MBR の運転を想定した実験をおこなった。酸性条件では上澄水に微生物が生産するタンパク質や多糖類が蓄積し，膜が目詰まりし易く，運転には高い膜操作圧力が必要であった。一方，COD で評価した場合には，酸性条件では中性条件に比べて除去率が低かったが，分光学的測定においてはより高い色度成分の除去率が酸性条件で見られた。酸性条件での高い色度成分の除去率は，着色物質が酸性条件でより汚泥に吸着しやすいためであると考えられた。また，色度除去率の経時変化から，いったん吸着した色度成分はリアクター内に蓄積せず，微生物によってゆっくり分解されたと考えられる。

第二の実験では，石油随伴水処理を念頭に，MBR の高温運転を行った。実験の結果，鉱物性の油の半減期は高温のリアクターでやや短く，油分がリアクター内に蓄積することはなかった。しかし，高温条件下でリアクターを運転した場合，色度成分の除去率は室温条件での結果に比べて低下した。また，膜の目詰まりは高温条件のリアクターでより顕著であった。

これらの結果から，MBR の極端な低 pH 運転には，色度の除去の点で利点があり，高温運転においては，油分による処理の妨害を緩和する効果が見られることがわかった。しかし，膜の目詰まりに対しては，低 pH 運転も高温運転も不利であることがわかった。膜の目詰まりを緩和する具体的な方策は本研究では明らかにされなかったが，MBR 法を産業排

水の処理のために，従来の運転条件の限界を超えて低 pH や高温条件で運転する利点と問題点が本研究によって明らかにされた。

# CHAPTER 1: INTRODUCTION

## 1.1 General Information

### 1.1.1 Water Resource and Wastewater Reclamation

In the near future, the availability of fresh clean water will become limited in wider areas of the world, although at the same time an increasing quantity and quality of water will be required to maintain and support the growing population. Many developing countries of the world already face a shortage of clean drinking water and irrigation water for food production, while in industrialized countries, such as the U.S. and Japan, the quality of available water for public and industrial use will be a larger issue than the quantities.

The process of water treatment that is reliable, effective and cost-efficient in removing a wide range of pollutants is highly needed. The recycling or reuse of wastewater is one way of supplementing available water supplies. The recent developments in membrane technology have made the recycling of wastewater a realistic possibility. The perception of recycled water by the public is less than favorable. In the U.S. the public is generally accepting of the reuse of water for irrigation, but strong opposition of its use for drinking water has been encountered. In areas with greater water scarcity, such as Singapore, the acceptance of recycled water is much greater (Howell, 2004). The additional treatment required for reuse comes at an increased cost, which may not be justified in areas with sufficient water supplies.

### 1.1.2 Industrial Wastewater

Industrial wastewaters have very varied compositions depending on the type of industry and materials processed. Some of these wastewaters contain extremely high organic matter. Because of very high organic concentrations, industrial wastewaters may also be severely nutrients deficient. Unlike domestic wastewater, pH values beyond the range of 6–9 are also frequently encountered. Such wastewaters may also be associated with high concentrations of dissolved metal salts. The flow pattern of industrial wastewater streams can be very different from that of domestic wastewater since the former would be influenced by the nature of the operations within a factory rather than the usual activities encountered in the domestic setting (Biesterfeld *et al*., 2001).

### 1.1.3 Membrane Bioreactor (MBR)

Membrane bioreactor (MBR) process is a kind of technology that has gained increasing applications into the wastewater treatment within the recent times. It is a kind of modification made for the conventional activated sludge process in which case liquid/solid separation is done via filtration through membranes instead of the secondary sedimentation tank (Mittal, 2011).

They have proven to be highly effective in the removal of both inorganic and organic contaminants together with biological entities that arise from wastewater. Although once considered uneconomical, membrane technology costs have decreased by 80% over the past 15 years, making the use of membranes and MBR a viable option for the first time (Layson, 2004).

With new advances in membrane design and technology, the MBR processes appear to have a promising future in industrial wastewater treatments (Cicek *et al*., 1998). In recent years, the annual publication related to MBR technology reached nearly 400 per year and some of them were applied to industrial wastewater treatments. A recent market survey published in Water21 (December 2009) indicated that 566 out of the 800 full-scale MBR plants in operation in Europe are for industrial applications. Although a considerable number of papers have been published, there are still some challenging issues with MBR systems, particularly membrane fouling control. Fouling of the membrane that results into a high consumption of energy and high requirements for expensive cleaning chemicals has always limited the usage of MBR process due to the high cost of operation. Therefore, it is necessary to estimate the cost and the feasibility for the introduction of MBRs in the treatment of industrial wastewater. Although a number of reviews on MBR technology were published in the last few years, most of these reviews focused on municipal wastewater treatment with MBRs (Judd, 2004; Ng and Kim, 2007). Meanwhile, Liao et al., (2006) reviewed anaerobic MBR progress by focusing on applications for treatment of municipal and some industrial wastewaters. Cicek (1998) reviewed the applications of MBR technology for agricultural wastewater treatment. Previous reviews did not cover most of the recent studies regarding various industrial wastewater treatments with MBR systems. Consequently, there is a short of summary of the MBRs for industrial wastewater treatments in the literature. With the rapid development of MBR technology for industrial wastewater treatments, a detailed analysis and review of past academic research progress on industrial wastewater treatments would be valuable.

One of the greater advantages of the process of MBR is based on the fact that it can be operated at a high sludge retention time (SRT) when compared to the conventional activated sludge process. The high SRT operation is favorable for the growth of microorganism that are growing at a slow rate which might also degrade the recalcitrant and the toxic compounds like petroleum hydrocarbons (Kraakman, 2012).

The other advantages that are associated to MBR comprise of high quality effluent free from bacteria and pathogens, plant of a small size, and higher organic loading (Gawad, 2014). Not only a number of successful pilot plants but a number of full scale units are already in use at everywhere in the world. The current existing applications of MBRs comprise of municipal wastewater treatment for relatively small communities, industrial wastewater treatment, and lastly landfill lechate treatment.

Many operational conditions affect MBR performance such as hydraulic residence time (HRT), sludge retention time (SRT), temperature, pH, feed-to-microorganism ratio (F/M), mixed liquor suspended solid (MLSS), aeration and biomass properties. The effect of these parameters on MBR performance and membrane fouling has been the subject of some studies. Among these operation conditions, the values of pH and temperature are the most influential operation conditions since it is directly related to the microorganisms and membranes in reactors.

Thermophilic treatment is attractive for industries producing high-temperature and high organic content wastewaters. Several studies have been conducted on thermophilic MBRs, and MBR has been found as the most reliable system at higher temperature. However, there has to be a trade-off between the cost and the quantity of treated wastewater when appropriate HRT and temperature are being selected.

### 1.2 Study Objectives

The removal of color and oil in wastewater generated from industries is economically difficult. Physical and chemical treatment methods are often suffered from high cost and/or insufficient performance. The residual color and oil sometimes causes a foul smell generating from wastewater (Abeynayaka and Visvanathan, 2011). Therefore, it is important to develop a cost effective method for removing oil and color. Biological treatment is still a good choice for the removal of oil and color, though there is a limitation for the removal of persistent compounds. The application of MBR to the treatment of industrial wastewater may provide a good solution for that.

Biological processes including molasses distillation and sulfuric acid hydrolysis in sugar industry often generate wastewater having acidic characteristics (Satyawali and Balakrishnan, 2008; Onodera *et al.*, 2013). Direct treatment of acidic wastewater without pH neutralization is a target of this study to reduce the use of chemicals in wastewater treatment. Another target of the application of MBR is high-temperature oily wastewater, because oil in wastewater often disturbs the treatment of wastewater by forming aggregates especially under low temperature condition. However, the operation of MBR under high-temperature condition above 50°C or highly acidic condition below pH of 3 is not promising, though the advantage of thermophilic MBR has been shown for high-strength wastewater (Simstich et al., 2012) (Abeynayaka and Visvanathan, 2011).

In this study, MBRs were examined for the treatment of industrial wastewater to extend the range of operational conditions for the acidic range and/or high temperature range, because few literatures can be found for the operation below pH 3 and above 50°C. Effects of high-temperature operation and/or acidic operation on the fouling of the membranes as well as removable range of contaminants were investigated in this study.

### 1.3 Structure of the Dissertation

This paper comprises of 5 main chapters including: Chapter 1 Introduction (general information, study objectives and the structure of the paper), Chapter 2 Literature review (comprising of the review of a number of literatures that are relevant to the removal of color and from wastewater using both conventional methods and membrane bioreactors), Chapter 3 Materials and Methods for the experimental investigations (Experimental set-up , membrane, domestic reactor operation), Chapter 4 Results and discussion and Chapter 5 Conclusion. All these sum up to 5 major chapters are included in the paper.

# CHAPTER 2: LITERATURE REVIEW

## 2.1 Color and Oil in Wastewater

### 2.1.1 Color in Water

There are two definition of color in water; one is "true color" and the other is “apparent color”. True color can only be judged in water from which turbidity has been removed. Apparent color includes not only color due to substances in solution, but also that due to suspended particles.

Suspended material in water bodies may be a result of natural causes and/or human activity. Transparent water with a low accumulation of dissolved materials appears blue and indicates low productivity. Dissolved organic matter, such as humus, peat or decaying plant matter, including biologically treated wastewater, can produce a yellow or brown color. Water rich in phytoplankton and algae usually looks green, reddish or deep yellow water. Soil runoff produces a variety of yellow, red, brown and gray colors.

### 2.1.2 Color of Melanoidins

Molasses, produced from sugar production industry, is widely used in fermentation processes because it still contains organic matter which can be further used by fermentation processes. Anaerobic treatment (biomethanation) is widely applied for the treatment of molasses wastewater (Satyawali and Balakrishnan, 2008; Onodera *et al*., 2013). However, the removal of color in anaerobic treatment is not significant (Satyawali and Balakrishnan, 2008). The main colored constituents of the molasses wastewater are melanoidins (Chandra *et al*., 2008).

Melanoidins are dark brown to black colored natural condensation products of sugars and amino acids. They are produced by non-enzymatic browning reactions known as Maillard reactions (Plavsic *et al*., 2006). Naturally melanoidins are widely distributed in food (Painter, 1998), drinks and widely discharged in huge amount by various agro-based industries especially from distilleries using sugarcane molasses and fermentation industries as environmental pollutants (Kumar and Chandra, 2006; Gagosian and Lee, 1981). The structure of melanoidins is still not completely understood but it is assumed that it does not have a definite structure as its elemental composition and chemical structures largely depend on the nature and molar concentration of parent reacting compounds and reaction conditions as pH, temperature, heating time and solvent system used (Ikan *et al*., 1990; Yaylayan and Kaminsky, 1998). Food and drinks such as bakery products, coffee and beer having brown colored melanoidins exhibited antioxidant, antiallergenic, antimicrobial and cytotoxic properties as in vitro studies have revealed that products from Maillard reaction may offer substantial health promoting effects. (Plavsic *et al*., 2006).

The basic structure of melanoidin is given in Figure 1 (Logan, 2007). Melanoidins have physiologically positive effects such as anti-oxidative activity including strong scavenging activity against reactive oxygen species (Vanhecke *et al*., 2006; Walker and Reamy., 2009). The formation of melanoidins is affected by the reactants and their concentrations, types of catalysts and buffers, reaction temperature, time, pH value, water activity, presence of oxygen and metal ions. During heat treatment, the maillard reaction accompanied by formation of a class of compounds known as maillard products. The reaction proceeds effectively >50 ºC and is favoured at pH 4 to 7 (Azadbakht *et al*., 2005).

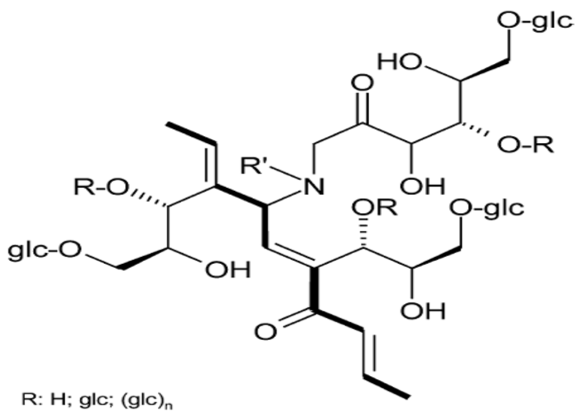



Figure 1: Basic melanoidin structure formed from carbohydrates and amino acid (Logan, 2007).

### 2.1.3 Effect of pH on Removal of Color in Wastewater

Environmental factors like pH, colored substances, aeration and nutrients play vital roles in bacterial removal of the color derived from molasses based wastewater as the metabolism and activity of enzymes are greatly influenced by these environmental factors.

Alkane *et al*. (2006) reported that pH has a crucial role in melanoidins color removal. An increase in pH of medium resulted in less microbial color removal and the increase in color intensity in the effluent. The increase in color may be due to the polymerization of melanoidins (Alkane *et al*., 2006). The decrease in color removal efficiency in highly alkaline pH might be due to the fact that the melanoidins responsible for color were more soluble in the alkaline pH, whereas the melanoidins might be precipitated and removed easily in the acidic pH condition.

### 2.1.4 Sugar Industry Wastewater

Sugar industries are one of the largest agro-based industry. The industry utilized around 1500–2000 L of water and generated about 1000 L of wastewater per ton of can processing (Asaithambi and Matheswaran, 2011). Wastewater mainly comes from floor washing, condensation, leakage, spillage of sugarcane from valve and pipelines, syrup and molasses in different sections. The composition generated from sugar industry has high content of organic material because of the presence of sugar and organic material in the beet or cane. Sugar industry produced untreated effluent of BOD 1700–6600 mg/L, COD 2300–8000 mg/L and total suspended solid 5000 mg/L. Discharge of the effluent without proper treatment can create serious environment problem, therefore, it is need to treat properly before to discharge in water receiving body. Conventional treatment methods used to treat sugar industry wastewater include preliminary filtration of suspended solids, flow and load equalization, biological treatment and sedimentation for sludge removal. Aerated ponds are also candidates for the treatment of sugar industry wastewater but high oxygen consumption limits the process. Some process such as electrochemical oxidation, membrane separation and biochemical oxidation have been reported to treat sugar industry wastewater (Sahu and Chaudhari, 2015).

Aerobic treatment of organic wastewater have been approached as an acceptable process due to its performance for high COD and BOD removal. However all convention available biological process for treatment of sugar industry wastewater may not be feasible and appropriate due to large land requirement as well as high capital of operational cost.

Biological processes including molasses distillation and sulfuric acid hydrolysis in sugar industry often generate wastewater having acidic characteristics (Chandra *et al.*, 2008; Figaro *et al.*, 2009). Direct treatment of acidic wastewater without neutralization is favorable to reduce the use of chemicals in wastewater treatment.

### 2.1.5 Sources of Oil in Wastewater

Fat, oil and grease (FOG) is simply comprise of compounds from glycerol or alcohol with fatty acids which are present in the form of liquid phase in the normal temperature Conditions (Davies *et al*., 2004). Majority of the oil and fat are available in wastewater generated from domestic dwellings and the majority of such oil are considered to be contributed by nuts, meats, margarine, vegetable oils, butter among other fatty/oil contained in food items. Oil in wastewater can also originate from factories, workshops and garages. The other possible sources comprise of road oils, gasoline, kerosene, soaps and so on (Imtiazuddin, 2012). FOG usually creates a kind of thin layer film, which is translucent within the wastewater surface and hence has the possibility of interfering aquatic lives and the WWTPs functioning.

### 2.1.6 Oil and Gas Industry Wastewater

Industrial wastewater have a complex chemical composition and contain organic (fats, lubricants, cutting liquids, heavy hydrocarbons (tars, grease, crude oils and diesel oil), and light hydrocarbons (kerosene, jet fuel and gasoline) (Srinivasan, and Viraraghavan, 2010). and inorganic compounds, with about 20 % all the known chemical elements. Disposal of oily wastewaters into the environment can result in environmental pollutions and serious damages to the ecosystem. In addition, even in the case of very low concentrations in the environment, heavy metals can be accumulated in plants and animal tissue. Further risks to human health may arise, e.g. the risk of skin cancer from skin contact with used motor oils. Although many of these elements are required by living organisms for their normal function, they become toxic effects at high concentrations.

Biological treatment of high-temperature industrial wastewaters and process waters under thermophilic conditions is an attractive alternative in many cases. The minimized need to use heat exchangers renders configuration of the process simpler, i.e. more cost-efficient and reliable. Thermophilic aerobic treatment is particularly suitable for operating as a high concentration wastewater treatment since the degradation rates achieved are higher than they are under mesophilic conditions, which in turn mean more compact reactor configurations (Jahren 1999; LaPara and Alleman 1999). Low sludge yield under thermophilic conditions has obvious benefits due to reduced sludge disposal and handling costs.

### 2.1.7 Saline Wastewater

High Salinity in wastewater often reduces the removal of color and oil in the treatment process. Saline wastewater, which is generated by activities such as fish processing, petroleum, flue gas desulphurization (FGD) and leather industries as well as wastewater after the use of seawater is characterized by the high salinity and nutrient content at the same time. Salinity and nutrient concentrations of different wastewater sources are summarized in the Table 1. It is apparent that the concentrations vary depending on the activities as well as processes conducted in the respective industries. Salinity has a significant chemical and physical effect on the properties of water or wastewater such as solubility of oxygen, pH as well as alkalinity. In MBR treatment of saline wastewater the adhesion of proteins and polysaccharides onto membrane surface is promoted due to the reduction of electric double layer, resulting in severe fouling.

Table 1. Characteristics of saline wastewater.

| Activities | Salt concentration (%) | Ammonia (mg/l) |
|---|---|---|
| Fishery | 0 – 3.5 (%) | 0.039–1940 |
| Tannery | 2.7 (%) | 1200 |
| FGD | 5 (%) | 80 |
| Domestic saline wastewater | 0.55 (%) | 130 |
| Domestic Wastewater | 0.01 (%) | 40 |

Nitrogen removal of saline wastewater is essential to meet wastewater discharge criteria before treated wastewater is guided into a water body. Conventional nitrogen removal processes for protein or ammonia contained in saline wastewater are conducted by nitrification, followed by anoxic denitrification with addition of an external carbon source (Fontenot *et al*., 2007).

Halotolerant or halophilic nitrifiers must be present for the nitrification of saline wastewater. The utilization of halophilic microbial consortia or even of enrichments from non-saline ecosystems like manure, that were adapted to saline conditions, reduces the effect of salt stress on bacterial metabolism (Dincer and Kargi 2001, Antileo *et al*., 2002, Mariangel *et al*., 2008).

## 2.2 Membrane Bioreactor

### 2.2.1 Definition, Configuration and History of Membrane Bioreactor

#### 2.2.1.1 General

Membrane bioreactor (MBR) is a very novel technology among the treatment technologies of wastewater, possessing a number of advantages over the traditionally known conventional activated sludge processes. Membrane bioreactor technology is a type of technology that comprise of membrane separation and biological degradation in wastewater treatment.

Innovations and investigation into the process of MBR in the treatment of wastewater have been intensively conducted within the last few decades (Kundu *et al.*, 2013). For the purpose of meeting the requirement in place for the reuse of wastewater and the kind of strict standards required for effluent in the near future, MBR process application has apparently become an alternative which is very attractive when compared to other conventional forms of water treatment. This is majorly based on its wide range of usability and the characteristics of performance (Wang *et al*., 2014).

### 2.2.1.2 Configuration

Figure 2 shows two configurations that are very different by membrane modules allocation in MBR system (Zhidong, 2010). The first configuration in this case is the cross-flow MBR where the pressurized module of the membrane is separately installed from the tank used in aeration (Zhidong, 2010). The second configuration is submerged MBR under which the membrane get submerged inside the bioreactor and thepermeate is directly suctioned by filtration (Zhidong, 2010). This second configuration (submerged MBR) has been reported by many literatures as superior characteristic to a cross-flow MBR which is externally pressurized with regards to the power consumption and the simplicity of the installation.

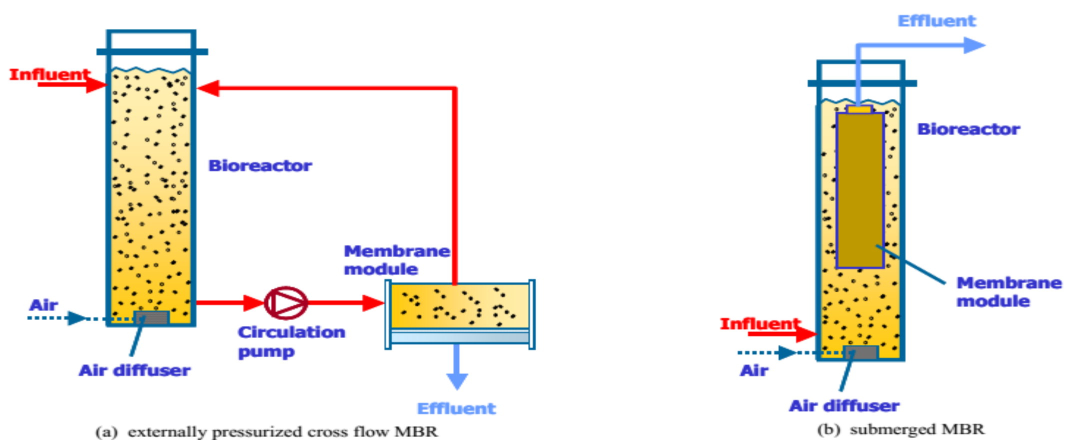



Figure 2: Different configurations of MBR process (Garbhani and Farajnezad, 2012).

#### 2.2.1.3 History

MBR was developed as a combination of the membrane technology and the activated sludge in which the separations of solids was achieved through filtration instead of settling using gravity. The original version as from 1960s did employ the cross-flow configuration (Gawad, 2014). This configuration is still applied for some applications, in spite of a larger energy consumption (Alther, 2001). Since the mid of 1980s, the membrane units became to be submerged directly into the aeration tank leading to a substantial decrease of the required amount of energy from 6 kWh/m$^3$ for the cross-flow type to 1 kWh/m$^3$ for the previous versions of the immersed membranes.

A recent review by Alther (2001) showed that a high growth rate of the number of existing plants and their capacity installed in the market have reached more than 10 % annually within the past decade (Gawad, 2014). At the same time, literatures have reported a significant decline in the cost incurred annually from around \$0.90/m$^3$ one decade ago to more than \$0.08/m$^3$ in 2015 basically as a result of lower cost of membrane together as a result of the increased efficiency of energy to less than 0.4 kWh/m$^3$(Gawad, 2014). While numerous MBR plants are reported to have small capacity and they are likely to be chosen in the case of decentralized treatment, the installed upper limit of the capacity of MBR dramatically expands. Some of the examples of currently existing MBR plants in the world comprise of tertiary treatment at Qinghe (Qinghe Special Steel Corporation disaster), Beijing (400,000 m$^3$/d in 2011), Kaarst in Germany (48,000 m$^3$/d in 2005) and King County in the USA (136,000 m$^3$/d in 2011). Despite these advances in the cost reduction.

MBR is still considered as a new technology that has a very limited design and experience of operation when compared to the activated sludge which was invented for more than a century (Gawad, 2014).

### 2.2.2 Advantages and Disadvantages of Membrane Bioreactor Process

#### 2.2.2.1 Advantages of Membrane Bioreactor Process

MBR for wastewater treatment have always been proved to have a number of advantages when compared to other processes that are conventional biological wastewater treatment (Zhidong, 2010). The major advantages here are high quality of the treated water, the smaller size of the treatment unit, less production of sludge and the flexibility involved in the operation.

The first advantage is the high quality of treated water compared with the conventional activated sludge process in which the quality of effluent is mainly depended on the sludge settling in the sedimentation tank (Zhidong, 2010). In the case of MBR, liquid/solid separation is performed using filtration by membranes (Garbhani and Farajnezad, 2012). The final effluent therefore do not contain suspended matter which enable the direct discharge of the final effluent into the surface water and the reusing of such effluent for the purpose of cooling, flushing of toilet, and/or watering of lawn.

Flexibility in operation is the second advantage that MBR has over other conventional activated sludge process that are used in wastewater treatment (Garbhani and Farajnezad, 2012). The solid retention time (SRT) can be controlled as an operating parameter without dependence on the hydraulic retention time (HRT).

Compact size of the plant is the third advantage of MBR over other conventional methods of wastewater treatment. Due to the fact that the operation of MBR does not depend on the gravity settling of sludge, high concentration of biomass is likely to be maintained up to around 30g/L within the system (Garbhani and Farajnezad, 2012). MBR has an ability of treating wastewater at high volumetric loading rate and it can reduce the size of the existing bioreactor (Garbhani and Farajnezad, 2012). Additionally, the secondary settling tanks, thickener of sludge or further treatment for SS and BOD removal are not very critical in the MBR process, hence the plant become highly compact in size.

Low rate of production of excess sludge constitutes another advantage of MBR process over other wastewater treatment processes. Studies undertaken on MBR reveals that the rate of the production of excess sludge is usually very low (Wang *et al*., 2014). The amount of excess sludge from MBR process is considerably lower than the conventional activated sludge process (Garbhani and Farajnezad, 2012). The low M/F (food-to-microorganism ratio (g BOD/g MVLSS/day) ratio and the longer SRT within the reactor is the main reason for the low excess sludge production in MBR process (Wang *et al.*, 2014).

#### 2.2.2.2 Fouling of Membrane

MBR and activated sludge also differ from each other in one very special aspect. The operation of MBR relies majorly on the ability of membrane module to treat all the flow that are incoming into the plant. If permeability of the membrane is impaired due to some reasons, then it become impossible for the plant to process all the flow volume although the water quality of effluent in the case will still remain high in a consistent way (Abeynayaka and Visvanathan, 2011). This is a contrast with the typical operation of the activated sludge where the hydraulic capacity of the plant is not frequently a problem but the quality of effluent is highly variable. The fouling of membrane and the consequent reduction of the volume flux is the most important problem for the operation of membrane bioreactors (Abeynayaka and Visvanathan, 2011). This problem can lead to be very important in the case of large plants where the safety margin is critically small due to the costs of plant.

A number of studies have always been devoted to the mechanisms and causes of the fouling together with its control (Trivedi and Doare, 2014). It has always been reported that one of the major causes of fouling is the concentration polarization of solid and proteins and polysaccharides in the mixed liquor of activated sludge. The concentration polarization takes place when the forward flux of the solutes become more than the backtransfer away from the membrane (Trivedi and Doare, 2014). The fouling has been reported to be sometimes reversible (by lowering the flux of the membrane) or increasing the intensity of back transfer.

The fouling is likely to be associated with a higher concentration of solids and colloidal matters present in the mixed liquor. A literature illustrates that in the event that the concentration of solids in the solution applied to membrane filtration become more than the threshold, the permeability of the membrane decreases at a rapid rate (Kundu *et al.*, 2013).

The decrease in the permeability is caused by the formation of cake and gel layer at the surface of the membrane built up by the filtration. The decrease in the volume flux is however sometimes reversible and can be controlled by decreasing the mean filtration flux (relaxation of membrane) by increasing backward transport with the operation of an increased aeration (Kundu *et al.*, 2013). In order to increase the backward transport, the cross-flow filtration concept was introduced (Zainal Abidin *et al.*, 2014).

### 2.2.3 Biodegradation and Bacterial Community in MBR

#### 2.2.3.1 Microbial Activity and MBR Operation

In MBRs, microorganisms maintain their growth by oxidation and synthesis as well as endogenous respiration processes using organic/inorganic substances in the wastewaters. Meanwhile, metabolic products excreted from living microorganisms and lysis substances from dead cells are generated. Membranes submerged into reactors inevitably interact with these substances under hydrodynamic conditions. Importantly, once first layer was formed on membrane surfaces by microorganisms and their metabolic matters, further adherence of foulants on membrane surfaces will be controlled by surface properties and structure natures of the initial cake layer. Therefore, the characteristics of microbial flocs and SMP perform key roles on their interactions with membranes in MBRs. Generally, microbial growth and metabolism depend on feed characteristics and imposed environment (e.g., oxygen level, temperature, steady-state/unsteady-state operation). Thus, MBR operating conditions involved in these factors influence the microbial behaviors such as the presence of microbial species, physiological characteristics of microbial flocs, and their metabolic products.

#### 2.2.3.2 Effect of Feed Composition on Bacterial Community in MBR

MBRs have been applied to treat a wide range of industrial and municipal wastewater with variable nutrient inputs (e.g., carbon, nitrogen, and phosphorus contents). Substrate loading and composition are found to be the primary factors influencing bacterial community in MBRs. Wu *et al*. illustrated that bacterial community structure dynamically shifted in different ways under various organic, nitrogen, or phosphorus loadings in MBRs (Wu *et al*., 2011). Ahmed *et al*. reported that when different external carbon sources were provided in MBRs, dominance of α, β, γ-subclass of *Proteobacteria* was dissimilar (Ahmed *et al*., 2008). Concomitantly, the differences in the nutrient sources could influence physiological properties of biomass (e.g., concentration, particle size, viscosity, floc structure) as well as chemical compositions and distributions of EPS in MBRs, which have an effect on membrane fouling profiles (Wu *et al*., 2011; Feng et al., 2012). Wu *et al*. reported that the membrane fouling tendency of biomass in the low-loading MBR (0.57 g COD/L day) was insignificantly different from that in the medium-loading MBR (1.14 g COD/L day), which was apparently lower than that in the high-loading MBR (2.28 g COD/L day). This is attributed to the higher bound EPS contents in the high-loading MBR. On the other hand, the nutrient amount available for bacteria is inversely related to sludge retention time (SRT) employed in MBRs. For example, at the same organic loading, MBRs with a shorter SRT have a higher food to microorganisms (F/M) ratio. A large body of research pointed out that a high F/M ratio in the MBR is beneficial to bacteria for the synthesis of cellular material (including growth of new cells and the production of excreted substances), which as a result aggravates membrane fouling (Wu *et al.*, 2011; Trussell *et al.*, 2006).

#### 2.2.3.3 Effect of Environments on Bacterial Community in MBR

##### 2.2.3.3.1 Oxygen Level

Aerobic growth of microorganisms is strongly dependent on the amount of oxygen available because oxygen is a key terminal electron acceptor to yield energy in their metabolic pathways. In MBRs, imposed dissolved oxygen (DO) level may facilitate propagation of some microbial species, but may disfavor others. Vibration of oxygen amount in a reasonable range (e.g., high DO vs. moderate DO) may not markedly change the microbial community compositions in the MBRs. Almost similar dominant species, for example, *Betaproteobacteria*, *Dechloromonas*, *Rhodocyclus*, *Comanonas*, and *Nitrospira*, are found under such DO conditions. However, lowering DO levels to a threshold (e.g., less than 0.5 mg/L) led to noticeable changes in the microbial community structure (i.e., enhanced denitrifying bacterial growth) and distinct decreases of diversity of predominant microbial populations in MBRs (Gao *et al*., 2011; Tocchi *et al*., 2012). On the other hand, the oxygen level available in MBRs influences microbial metabolisms such as generation, composition, and distribution of EPS (Wu *et al*., 2011; Gao *et al*., 2011). Accordingly, membrane performances associated with microbial behaviors can be greatly affected by DO levels. Gao *et al*. emphasized that insufficient DO amounts in MBRs facilitated EPS production in the mixed liquor and EPS accumulation in the cake layers, which induced higher membrane fouling rates (Gao *et al*., 2011). In other studies, it was observed that lowing DO levels reduced the sizes of microbial flocs, which tend to form dense and compact cake layers on the membranes and give rise to higher resistances (Ma *et al.*, 2006; Jin *et al.*, 2006).

**2.2.3.3.2 Temperature**

In MBRs, microorganisms use their enzymes to hydrolyze and degrade the organic/inorganic matters and the levels of enzyme activities are sensitive to seasonal temperatures. The activities of some enzymes (such as phosphatase and esterase) positively responded to temperature increases in a suitable range, while some enzymes (e.g., glucosidase) may achieve maximum activity at a low temperature when domestic wastewater was treated by the MBR (Molina-Munoz *et al.*, 2010). Reduced enzyme activities lead to less biodegradation of organic substances, resulting in higher concentrations of organic substances retained in the reactors. Meanwhile, environmental temperatures influence microbial growth rate and microbial community compositions in MBRs. Favorable temperatures facilitate propagation of suitable microbes, but unsuitable microbial species may disappear or reduce their quantity in the reactors. In some situations, with temperature changing, almost similar microbial community composition may be present in MBRs, but the microbial diversity developed in a highly dynamic pattern (Calderón *et al.*, 2012; Simstich *et al.*, 2012). Furthermore, temperatures affect not only properties of microbial flocs such as viscosity and size, but also releasing EPS levels.

Miyoshi *et al*. reported that when the temperature decreased from 21.5 to 17.7 °C, almost comparable soluble polysaccharides and protein amounts were observed, while further decreasing the temperature to 12.7 °C significantly induced higher soluble polysaccharides and protein levels in the MBRs (Miyoshi *et al.*, 2009). A similar finding was concluded by Van den Brink *et al*. and his colleagues. Therefore, higher membrane fouling rates were obtained at lower temperatures (Miyoshi *et al.*, 2009; Van den Brink *et al*., 2011).

#### 2.2.3.3.3 Unsteady-State Operation of MBRs

Stable operation of MBRs is desirable in order to maintain steady reactor performance and membrane filtration process. However, in pilot-plants or full-scale MBRs, unsteady states such as seasonal fluctuation of wastewaters, intermittent feeding, shifts in the oxygen supply, pH change, and discontinuous or irregular disposal of waste sludge may happen. Microorganisms in MBRs respond to these variations by developing suitable microbial community or varying their metabolic and synthesis processes to increase their tolerance. Significant bacterial population changes have been observed in the startup period of MBRs when wastewater compositions, organic loadings, and SRTs were varied, even though the stable MBR performances (such as membrane permeability and organic carbon removal rate) were achieved. (Wu *et al*., 2011; Miura *et al*., 2007). On the other hand, a few studies pointed out that unsteady organic loading rates led to higher soluble polysaccharides contents in the reactor, which increased fouling rates. Yogalakshmi and Joseph illustrated that the soluble EPS in the MBRs increased by 22%–66% after transient sodium chloride shock. Wu *et al*. observed that when the levels of soluble polysaccharides and soluble TEP in the MBR unexpectedly and suddenly increased due to pH decrease from ~7.0 to ~3.0, the cleaned membranes tended to be more easily fouled compared to the membranes with the initial cake layers formed in a slow TMP increase stage (Yogalakshmi and Joseph, 2010).

## 2.3 Operational Conditions of MBR for the Removal of Color and Oil

### 2.3.1 Low pH Operation in MBR

Lower pH operation in biological treatment might be beneficial for the removal of color due to higher adsorption nature of melanoidins to solids in lower pH condition (Chandra *et al*., 2008; Figaro *et al*., 2009). In addition, lower pH operation may be a favorable condition for keeping fungi, representative degraders of persistent organic compounds, in the reactors (Hai *et al*., 2009). Biological processes including molasses distillation and sulfuric acid hydrolysis often generate wastewater having acidic characteristics (Satyawali and Balakrishnan, 2008; Sun *et al*., 2013). Treatment of acidic wastewater under acidic conditions would be economically preferable in some applications to reduce the cost of reagents for pH neutralization. Few literatures can be found for the operation of MBR below pH3.

### 2.3.2 Thermophilic Operation in MBR

Saline and high-temperature wastewater containing a variety of organic compounds is a difficult target of wastewater treatment. The produced water from oil and gas production activities often contains salts, oil and hazardous organic compounds (Ahmadun *et al*., 2009). Shipboard wastewater also features high oily and saline concentrations (Di Bella *et al*., 2015). Textile wastewater contains a variety of organic compounds of different biodegradability (De Jager *et al*., 2014). Direct treatment of high-temperature wastewater is attractive, because cooling process is usually required for the treatment of textile wastewater.

Thermophilic aerobic processes have been applied for the treatment of high-strength wastewaters (biodegradable COD 20,000–40,000 mg/L) which make autothermal operation possible without exogenous heat input (Wang *et al*., 2014). Thermophilic aerobic treatment generally has advantages of 3 to 10 times higher biodegradation rates than those of similar mesophilic processes and low sludge yields (LaPara and Alleman, 1999). Thermophilic treatment has been claimed to have the advantage over mesophilic treatment in several aspects, e.g., higher loading rates, faster chemical reaction rates, faster microbial growth rates, lower net sludge yield, increased solubility of organics, increased removal of specific substrates, and increased destruction of pathogens (Brock 1986, Sundaram 1986, Schwarzenbach et al.1993, LaPara and Alleman, 1999, Skjelhaugen 1999, Kosseva 2001, Rozich & Bordacs 2002).

The main drawback of the thermophilic aerobic process is the poor settleability of the sludge (LaPara and Alleman, 1999). Liao *et al.* (2011) investigated the effect of temperature on sludge properties, showing that the high temperature condition was associated with a poorer bioflocculating ability, caused by filamentous bacteria, and higher production of bound extracellular polymeric substances (EPS), especially observed on a high dissolved oxygen concentration condition.

The combination of membrane separation process and thermophilic aerobic process has been studied to overcome poor settleability. In addition, the improvement of effluent water quality by the introduction of membrane bioreactor (MBR) is attractive for the reuse of industrial wastewater. Simstich *et al*., (2012) investigated the application of a thermophilic MBR to the treatment of paper mill deinking wastewater, showing that nutrient supply can be minimized due to a low sludge yield. Abeynayaka and Visvanathan (2011) examined the treatment of molasses-based synthetic wastewater by a thermophilic MBR showing the excessive membrane fouling due to higher proteins and polysaccharides generation within the reactor. They also reported the higher COD removal efficiencies and lower sludge yields in the thermophilic operation. However, few literature has reported the advantage of MBR for dilute high temperature wastewater, because most of the successful applications have been reported for high-strength wastewater.

# CHAPTER 3: MATERIALS AND METHODS

## 3.1 Low pH Operation

### 3.1.1 Reactor Operation

The schematic diagram of MBRs used in this study is shown in Figure 3. Two glass reactors with 5 L volume each were operated simultaneously for 91 days. The pH of the neutral reactor was between 5.5 and 7.0 (typically 6.5), whereas the pH of the acidic reactor was controlled at 3 using a pH controller and hydrochloric acid. The flat sheet membranes with pore size of 0.45 µm, diameter of 142 mm and material of hydrophilic polytetrafluoroethylene, (Millipore Co. Ltd., USA) were used in the MBRs for the separation of sludge and permeate. Transmembrane pressure was measured by pressure gauges. Temperature in the reactors was between 17 to 22°C. The reactors were aerated continuously.

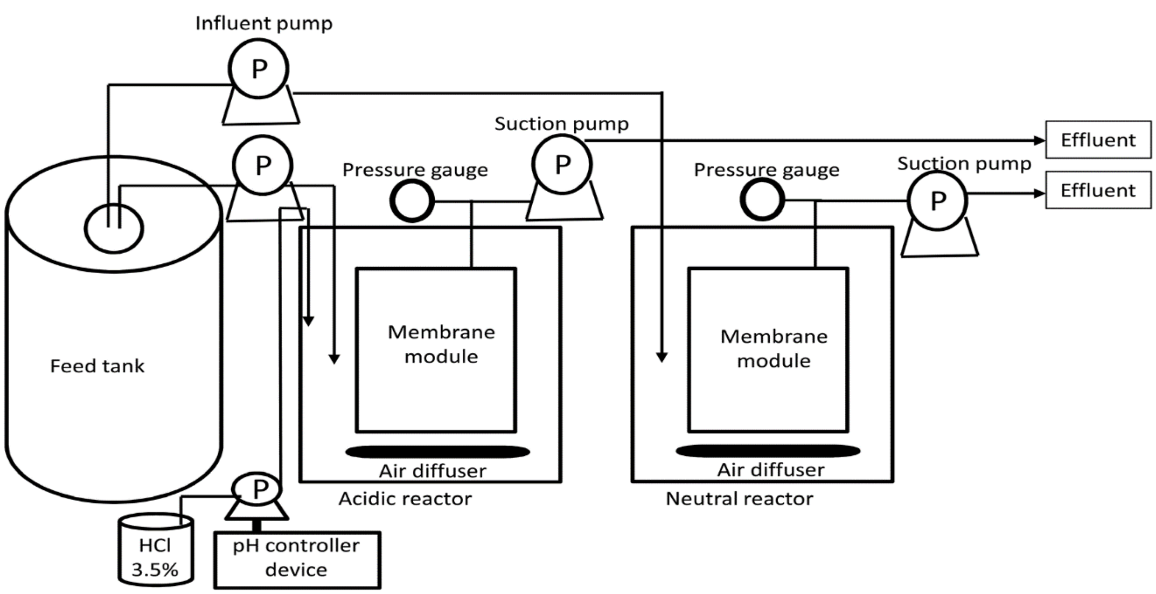



Fig. 3 - Membrane bioreactors used in this study.

### 3.1.2 Feed Solution

The feed solution was prepared by the addition of 1.0 – 1.2 g molasses (Hinode-mitsu, Dai-Nippon Meiji Sugar Co., Ltd., Japan) and 0.05 g urea to 1 L of tap water. The whole experimental period was divided into two periods depending on the process with and without pretreatment. The feed solution was biologically pretreated in the first period of day 1 to day 36, while no pretreatment was applied in the second period of day 37 to day 91. A fixed-bed biological reactor with hydraulic retention time (HRT) of 1.3 days was used for the pretreatment in which COD was reduced approximately from 650 to 250 mg/L. A certain volume (2 L) of the feed solution was added to each reactor three to four times a week, resulting to an average HRT of 4.28 days for the first period and 6.75 days for the second period.

### 3.1.3 Preparation of Sludge

Seed sludge was taken from a wastewater treatment plant at Tokyo University of Technology. Excess sludge was taken out only on the occasions of sampling for the MLSS measurement. The calculated solid retention time (SRT) based on the MLSS sampling frequency was more than 1 year.

## 3.2 Thermophilic Operation

### 3.2.1 Reactor Operation

Figure 4 shows the schematic diagram of MBRs used in this study. Two glass reactors with 6 L volume each were operated simultaneously. One reactor was operated at room temperature between 22 and 29ºC, while the temperature of the other reactor was maintained at 50ºC by silicon rubber heaters. The suction pumps were operated continuously at a flow rate of 540 mL/h (Volume flux: 0.22 m/day). Most of the treated wastewater was returned to the reactor, although 1.5 L/day was wasted to keep the water level in the reactors constant. The amount of oil (mineral oil light white, MP Biomedicals, France) added once a week to each reactor was 0.5 mL to take into account the applications to oil and gas production industry and to the treatment of shipboard wastewater. The average hydraulic retention time (HRT) was 5 days. Flat sheet membranes (surface area: 0.06 $m^2$ (200 mm x 150 mm x two sides) Kubota Corp.) made from chlorinated polyethylene with pore size of 0.4 μm were used in the MBRs for the separation of sludge and permeate. The reactors were operated under aerobic conditions and the dissolved oxygen concentration in the reactors was around 4 mg/L. The air flow rate was 4 L/min for each reactor. Trans-membrane pressure was measured by pressure gauges. The sludge retention time (SRT) calculated from the sampling of mixed liquor was 24 weeks. Except for the sampling, excess sludge was not removed from the reactors. The surface of the membranes was cleaned once in every 12 days of operation using plastic sponges to remove the gel and cake layer.

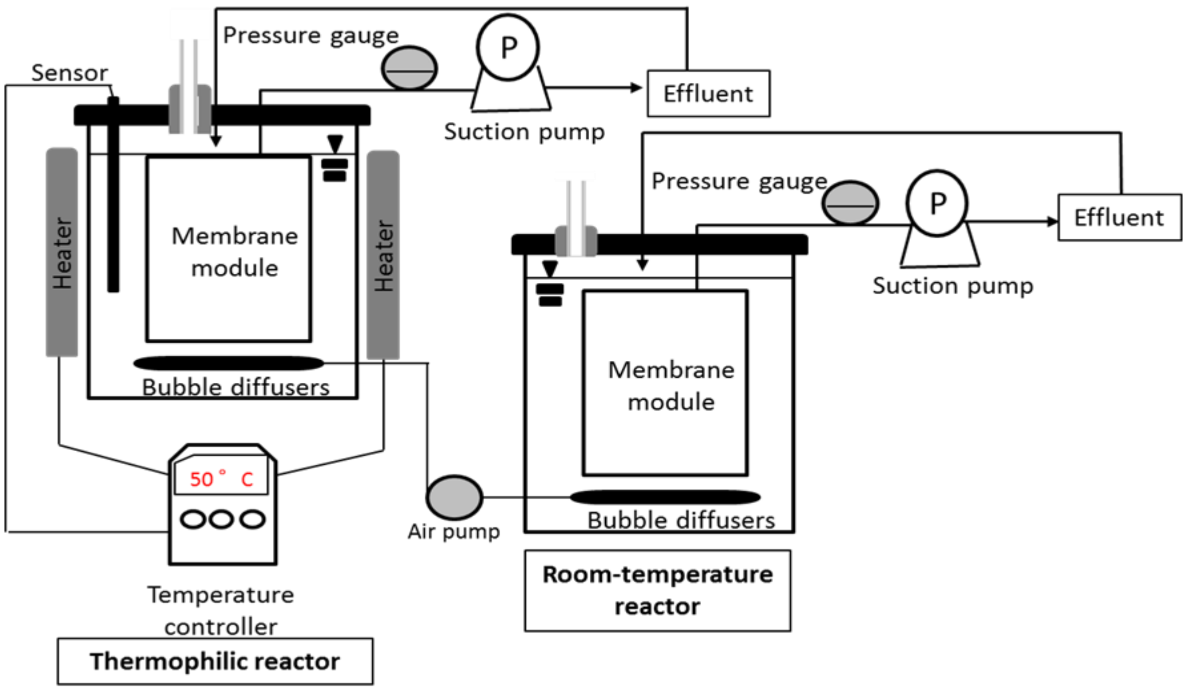



Fig. 4 – Schematic diagram of the membrane bioreactors.

### 3.2.2 Feed Solution

Molasses was used as a carbon source in this experiment because molasses contain a wide range of organic compounds of different biodegradability. Synthetic wastewater (1.5 L) was fed to each of the MBRs containing 3g molasses (Hinode-mitsu, Dai-Nippon Meiji Sugar Co., Ltd.), 0.15g urea, 13.8g sodium chloride (NaCl), 3.15g magnesium sulfate ($MgSO_4.7H_2O$) and 0.75g calcium chloride ($CaCl_2.2H_2O$) everyday except for Saturdays and Sundays. Influent COD fed to the reactor (measured by the Japanese method, in which permanganate was used as the oxidant) was 1000 mg/L, whereas total nitrogen (TN) was 50 mg N/L, almost fully derived from 100 mg/L of urea. The salt concentration of the feed solution was 1.0%.

### 3.2.3 Preparation of Sludge

Seed sludge was taken at a wastewater plant treating wastewater from restaurants, toilets and other sources discharged from Tokyo University of Technology. Initial sludge concentration was around 5000 mg/L. Before starting the regular monitoring, the reactors were operated with the same feeding rate of the same synthetic wastewater and with the same membranes for 30 days to acclimatize the sludge by gradually increasing gradually the temperature of the thermophilic reactor from 40ºC to 50ºC to mitigate the change of temperature. Mixed liquor suspended solids (MLSS) concentration was reduced by the acclimatization from 5000 mg/L to 1700 mg/L for the thermophilic reactor and the reduction was probably due to the inactivation of thermo-sensitive microorganisms, while it was almost constant for the room-temperature reactor.

## 3.3 Water Quality Analysis

### 3.3.1 MLSS

The concentration of sludge (MLSS) was measured by weight after the removal of water by centrifugation (5 minutes, 2000rpm) followed by drying in an oven at 105°C. In the case of the thermophilic operation, the residuals after centrifugation was repeatedly rinsed with pure water to remove high concentration of salts contained in the feed solution.

### 3.3.2 COD

In the measurement of COD for the experiment on the low pH, 2 mL sample was added to the pre-packed potassium dichromate solution before heating for 2hours in an oven. Remaining dichromate in the test tube was quantified by a spectrophotometer (DR-2010, HACH, USA). Detailed monitoring of water quality started on day 20. In the case of thermophilic operation, COD (potassium permanganate method) was measured by analytical kits (Pack test, Kyoritsu chemical-check Lab., Corp.).

### 3.3.3 Color

The dark brown color of the influent, effluent and supernatant of the mixed liquor was measured by a spectrophotometer (UVmini-1240, Shimadzu Corp.) at 475 nm (the peak absorbance of molasses solution containing melanoidin) and 390 nm (generally used wavelength for the color determination). For the analysis of the samples from the acidic reactor, the pH of the solution was adjusted with NaOH at 6 – 7 prior to the measurement of absorbance, because the absorbance was dependent on pH.

### 3.3.4 pH

The pH was analyzed using a pH-meter (SK-620PH) and pH controller (IWAKI PH-70P).

### 3.3.5 Oil and Grease

Oil and grease in the mixed liquor was measured separately for the water phase and for the sludge phase by hexane extraction (Standard Method 5520B, APHA-AWWA-WEF, 2012) before the quantification by gas chromatography mass spectrometry targeting $C_{15}$-$C_{22}$ alkanes, which were the main constituents of the oil added in this study.

#### 3.3.5.1 Preparation of Samples

A 50 ml pure samples acidified by 0.5 ml $H_2SO_4$ (2.5 %) was mixed with 5 ml of hexane around 2 minutes and the hexane layer was collected. 1 µl sample in hexane was injected to GC/MS (GC-2010/Purvum II,Shimadu Co., ltd.). The standard solutions was prepared in the same way by shaking 50 ml pure water, 5 ml of hexane, 0.5 ml $H_2SO_4$ (2.5 %), and 10 µl of mineral oil for 2 minutes.

#### 3.3.5.2 Analytical Condition of GC/MS

The GC used in the analysis of oil was equipped with a column InertCap 5MS／Sil (GL Science Co., ltd), fused silica capillary column (30 m x 0.25 mm i.d., 0.25µm) with the liquid phase of 5% diphenyl (equiv.) - 95% dimethylsilphenylene Siloxane. The column oven temperature was programed as 40 °C (5 min) - 10 °C/min - 270 °C (7 min). The injection temperature of the GC was 280 °C, with the split less injection method (1 min for purge-off time) and the injection volume was 1µL. The carrier gas was He. The detector temperature was 250 °C with the quantification ion mass 99 (m/z).

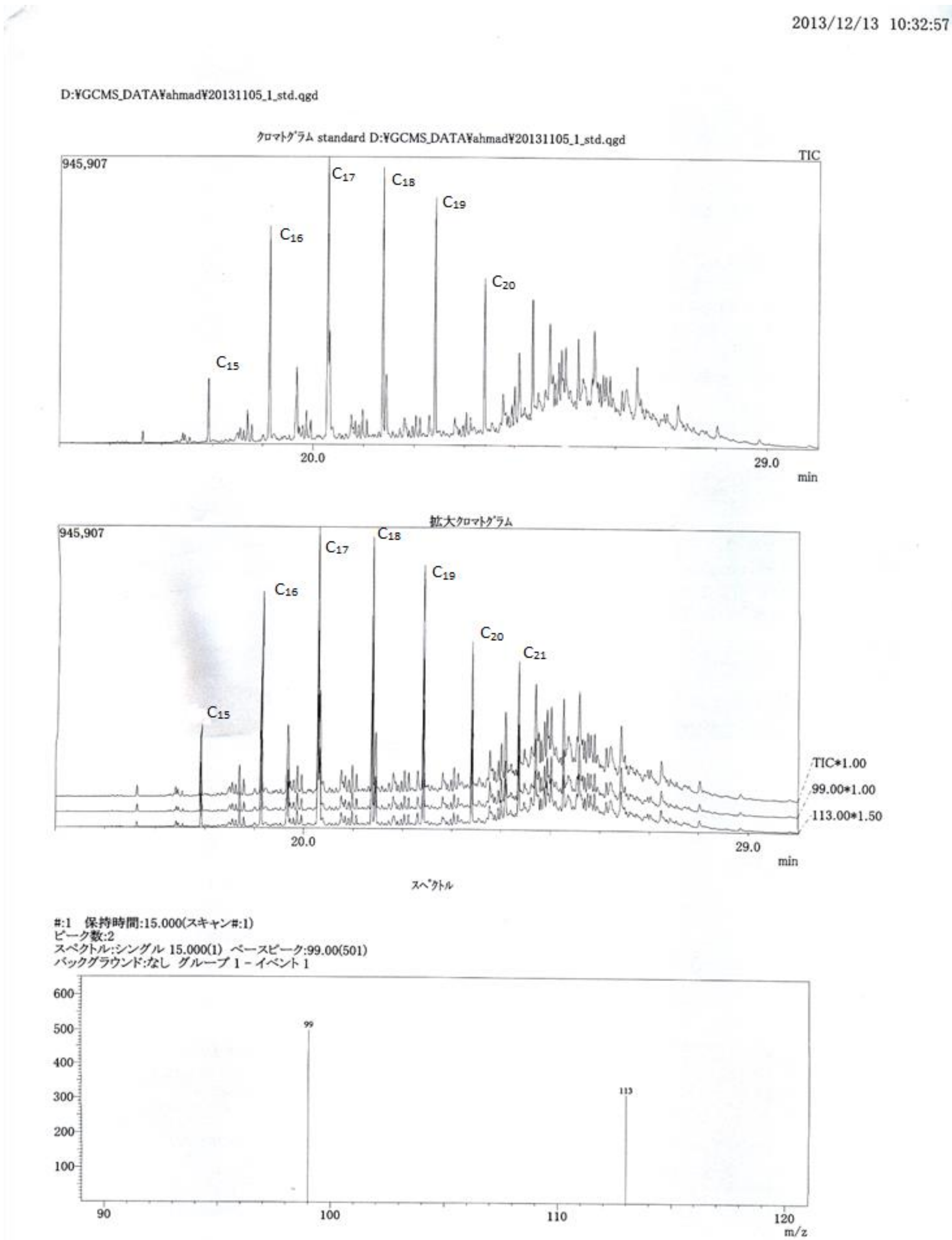



Figure 5: GC/MS Chromatogram of mineral oil used in this study.

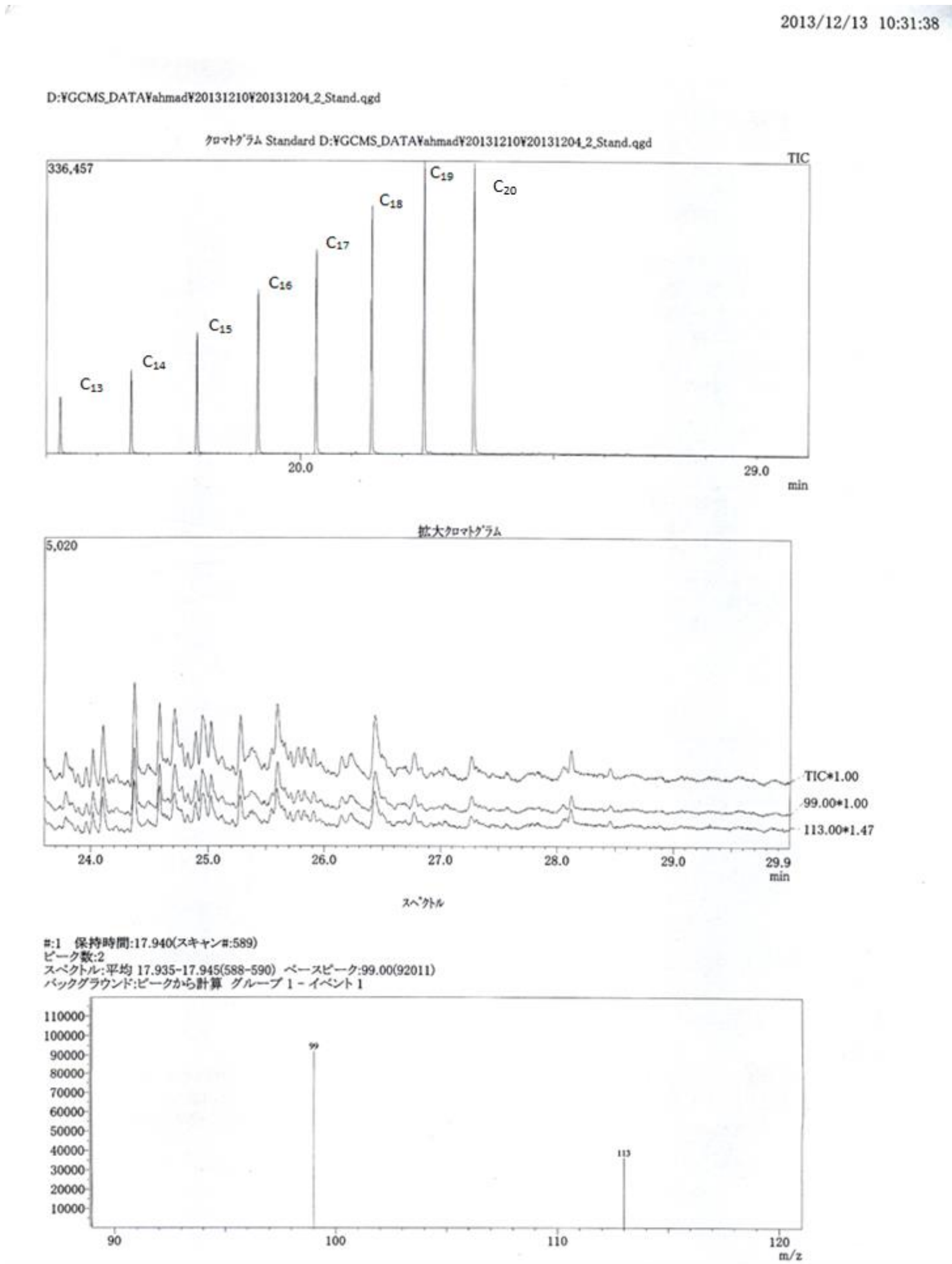



Figure 6: GC/MS Chromatogram of standard solution.

### 3.3.6 Inorganic Nitrogen

The concentration of nitrate ($NO_3^-$) and ammonium ($NH_3$) was measured by analytical kit's (Pack test, Kyoritsu chemical-check Lab., Corp.).

# Chapter 4: RESULTS AND DISCUSSIONS

## 4.1 Low pH Operation

### 4.1.1 Reactor Operation

Two reactors with different pH were operated for 91 days. The change in pH for both reactors is shown (Fig. 7). The pH of the neutral reactor was between 5.5 and 7.0 (typically 6.5), whereas the pH of the acidic reactor was between 2.3 and 3.8 (typically 3.0). The trans-membrane pressures were higher for the low pH reactor due to higher adhesion of proteins and polysaccharides on the membrane surface (Fig. 8) in spite of low volume flux operation below 0.1 m/day. Figure 9 show the stability of temperature in both reactors.

The variations of MLSS in both MBRs during the operation are shown (Fig. 10). The concentration of sludge calculated from the seed sludge concentration at day 0 was 4,520 mg/L in MLSS for both reactors. The initial sludge concentration was maintained between 4,000 to 5,000 mg/L in the acidic reactor except during day 70 – 76 due to the accumulation of the sludge on the membrane surface. In the case of the neutral pH reactor, MLSS reached 7,000 mg/L on day 15 and decreased to the steady-state value of 5,000 mg/L except on day 70 – 76 due to the same reason.

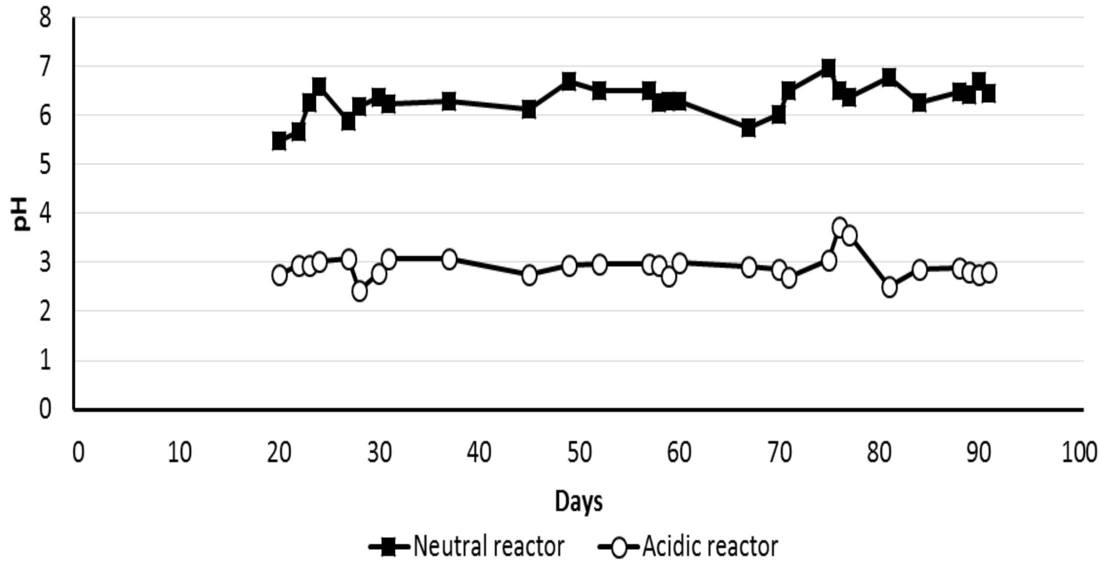



Fig. 7 - Change in pH in the reactors.


Fig. 8 - Change in pressure in the reactors

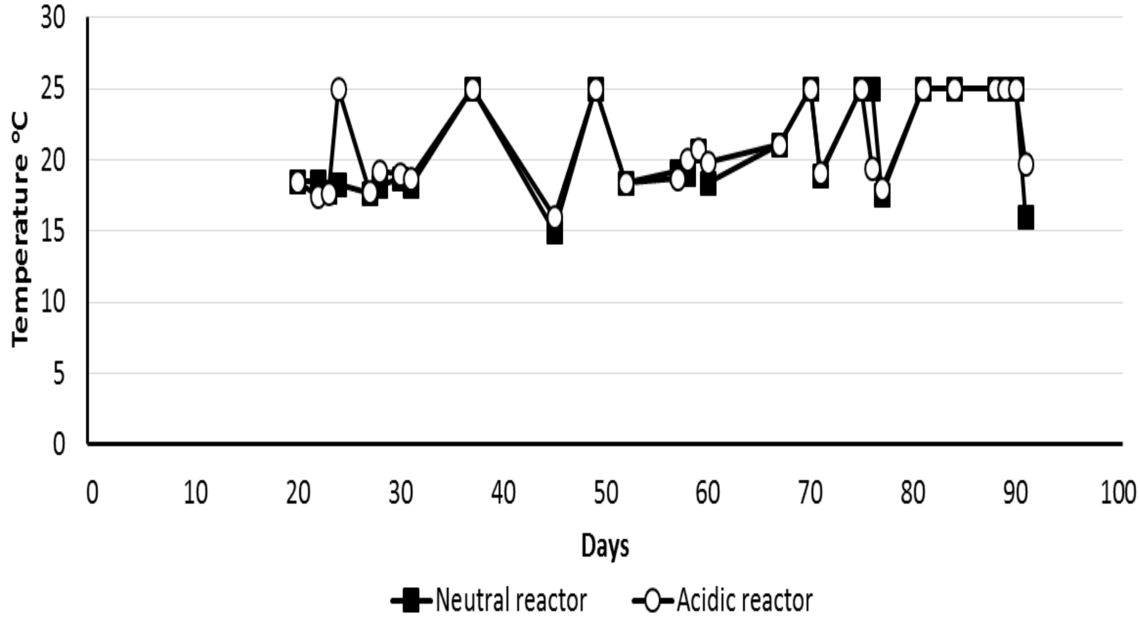



Fig. 9 - Change in temperature in the reactors

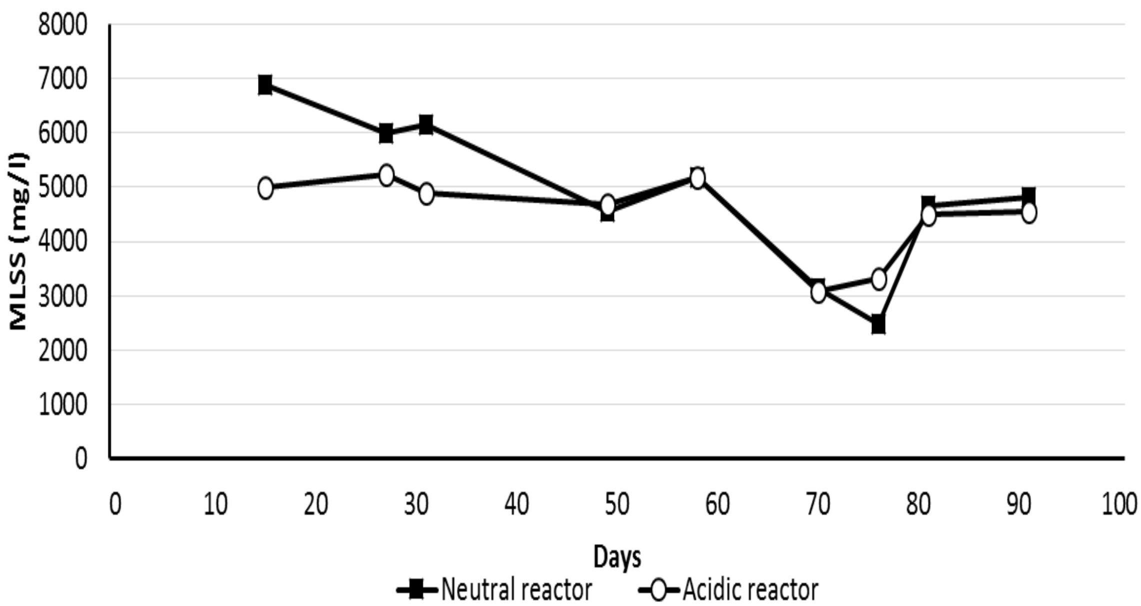



Fig. 10 - Change in MLSS concentration in the reactors.

### 4.1.2 Removal of COD

The results of COD measurements are shown in Figure 11. Influent COD of molasses wastewater was 200 mg/L in the first period and 700 to 1,200 mg/L in the second period. The lower COD in the influent in the first period was due to the pretreatment process where biodegradable organic matter had been removed. A report on MBR treating molasses wastewater demonstrated that the effluent COD was 116 mg/L when molasses wastewater (COD 777 mg/L) was treated by the MBR (Yan *et al*., 2012). Their results on COD were quite similar to our results in the second period, though detailed operational parameters and the composition of the feed solution were different. COD removal in the acidic reactor during the first period was 40% to 56% (average: 48.5%), and the removal in the second period was 76% to 88% (average: 84.0%). In the case of neutral pH reactor, the removal in the first period was 53% to 64% (average: 63.6%) and the removal in the second period was 81% to 92% (average: 89.0%). Lower removal in the first period was caused by the lower residual concentration of biodegradable organic matter in the influent as mentioned earlier. Higher COD removals were obtained for the neutral pH reactor due to higher microbial activity of the reactor. The higher COD (150 - 320 mg/L) was observed for the supernatant of the mixed liquor taken from the acidic reactor (Fig. 11), caused by the higher production of soluble organic matter (proteins and polysaccharides) by the microorganisms in the acidic reactor, though acidic operation showed high COD removals in this study and in a literature (78.6% at pH 3 and 87% at pH 3.5, Sureyya *et al*., 2004).

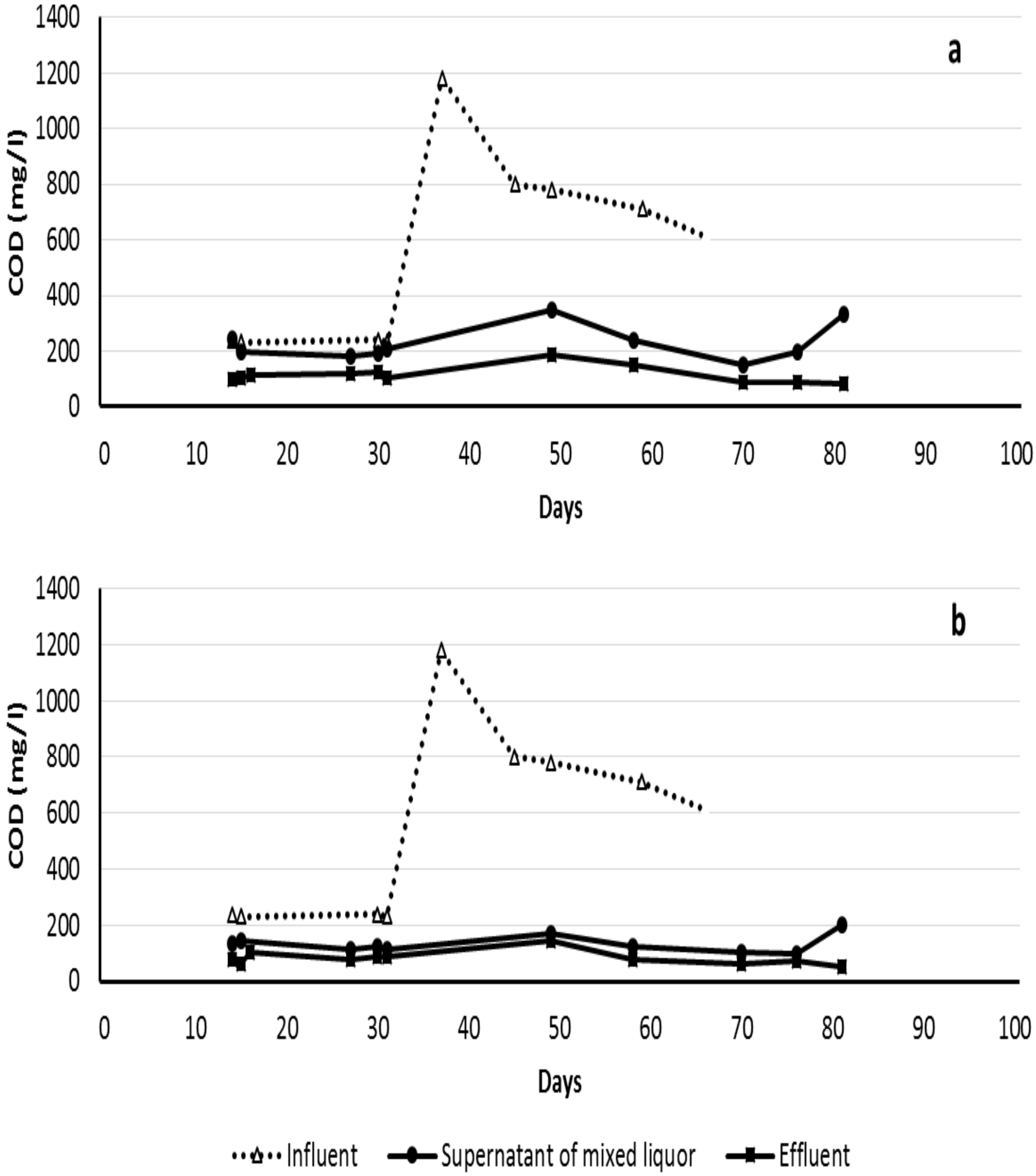



Fig. 11 - COD of influent, effluent and the supernatant of the mixed liquor of activated sludge in (a) the acidic reactor and (b) the neutral reactor.

### 4.1.3 Removal of Color

The pH range can strongly affect the decolorization efficiency and reaction rate. The importance of pH have been stated in literatures (Oliver *et al*. 2000, Gultekin *et al*. 2004, Alaton *et al*. 2002), where the optimal process pH value varies from an alkali condition of pH 11 to an acidic condition of pH 3.

In this study, higher percent removals of color determined spectrophotometrically at 390 nm for the acidic reactor up to 68.1% in the first period and 41% to 60% (average: 51.6%) in the second period were observed (Fig. 12). In the case of the neutral reactor, the removal was 51% to 58% in the first period and 22% to 42% (average: 34.2%) in the second period. The removals of color in the acidic reactor determined spectrophotometrically at 475 nm, which wavelength is usually used for the determination of color in molasses wastewater, were 74.1% in the first period and 41% to 66% (average: 55.8%) in the second period, whereas in the case of the neutral reactor, the removal was 58% to 68% in the first period and 23% to 42% (average: 33.3%) in the second period (Fig. 13). Higher removal in the first period compared with that in the second period was due to higher color intensity in the influent in the first period. The pretreatment process partially degraded the colored compounds. Therefore, the absorbances in the effluents were almost constants throughout the experimental periods.

Higher color removals at lower pH were also reported in the degradation of a kind of dye. Alexandre et al., 2011 studied the effect of pH on the degradation of a kind of dye in the pH range 2 – 8.5. It was observed in all cases that the ratio of degradation increased with decreasing in pH. It was most efficient at pH 2 and very low efficient at neutral or weak alkaline conditions.

Another study (Justina et al., 2009) showed the acidification of the reaction medium substantially increased the rate of the reaction at pH 7. The calculated average reaction rate constant is 10 times higher at pH 7 than at pH 11.4. At pH 3 reaction rate constant is 25 times higher than at pH 11.4. The dependence of degradation rate on pH was strongly dependent on the molecular structures of the target substance for removal.

### 4.1.4 Possible Mechanism for the Removal of Color

The acidic operation of the reactor resulted in lower COD removal and higher color removal. The higher removal of color by the acidic reactor is due to higher adsorption of the colored substances onto the microorganisms inside the reactor in the acidic condition (Chandra *et al.,* 2008).

The removal of color was gradually increased from day 60 to day 91 (Figs. 12 and 13). If the removal of color was due only to adsorption, the removal should have decreased with time elapsed. The long-term stable performance of color removal (or even increased removal with time) suggests that the colored substances were partially degraded by the acclimatized microorganisms in the reactors to a certain extent in the case of both acidic and neutral reactors. In the case of the acidic reactor, it is suggested that the removal from the water phase by adsorption took place and the gradual degradation of adsorbed substances on the microbial surface followed. Enhanced removal caused by the promoted adsorption and degradation by low pH operation in MBR was reported for the removal of pharmaceuticals (Urase *et al.,* 2005). It is suggested that the enhanced removal of color by the acidic operation could be explained by the same adsorption – degradation model, though molecular weights of pharmaceuticals would be far smaller than those of the melanoidins in this study.

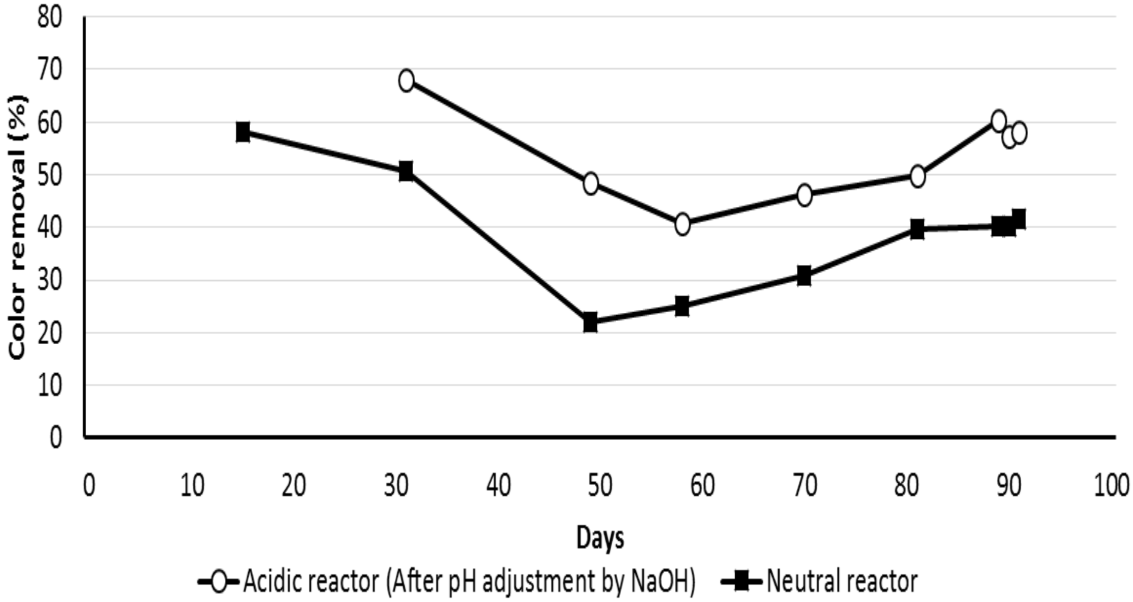



Fig. 12 - Change in the percent removal of color measured at 390 nm.

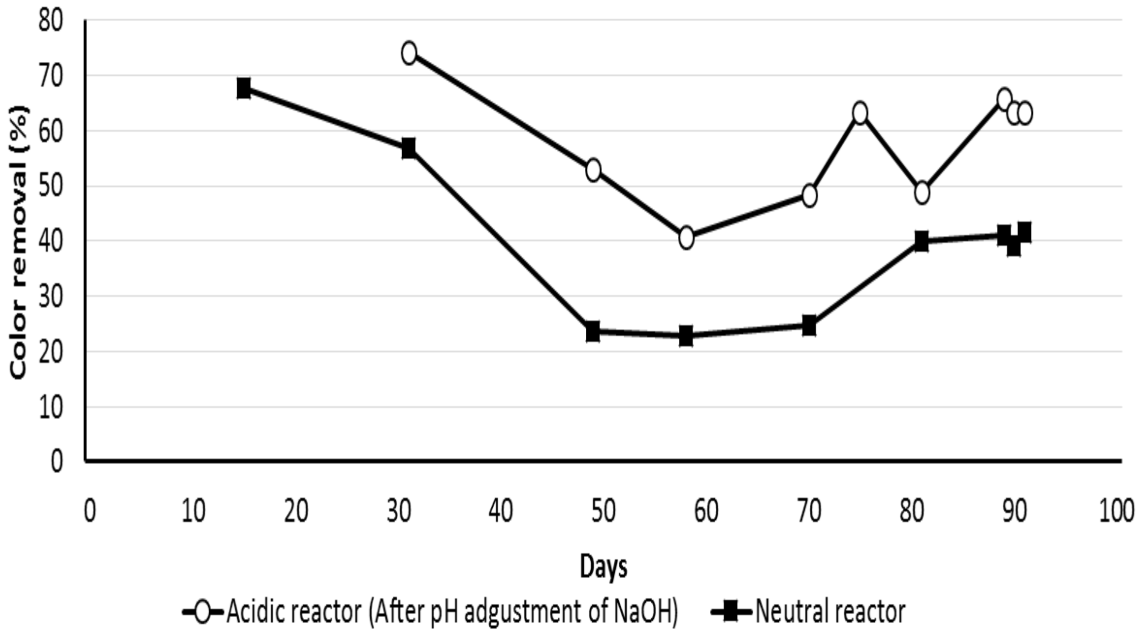



Fig. 13 - Change in the percent removal of color measured at 475 nm.

The density of fungi in the acidic reactor was higher than that in the neutral reactor. A few fungi colonies on $10^4$ dilution plates and no colony on $10^5$ dilution plates were found for the neutral reactor, while a few colonies on $10^7$ dilution plates and no colony on $10^8$ dilution plates were found for the acidic reactor on day 56. Higher density of fungi might have a positive effect on the color removal because certain species of fungi are representative degraders of persistent organic compounds (González *et al.*, 2008). However, the relationship between higher fungi density in the acidic reactor and higher percentage removal of color observed in the acidic reactor was not confirmed because the activities and the species of fungi were not monitored in this study.

### 4.2 Thermophilic Operation

### 4.2.1 Reactor Operation

Two reactors were operated for 35 days in order to study the effect of thermophilic operation on MBR performance. Fig. 14 shows the change in temperature for both reactors. The temperature of the thermophilic reactor was in the range between 47.7 and 50.7◦C (typically 50◦C), while the temperature of the room-temperature reactor was in the range between 22 and 29.2 ◦C (typically 25.0◦C). Figure 15 shows the stability of pH in the both reactors. Figure 16 shows the variations of MLSS in both MBRs during the operation. After starting the regular monitoring on day 1, MLSS of the thermophilic reactor gradually increased to 2200 mg/L. In the case of the room-temperature reactor, MLSS was maintained at around 4500 mg/L except for 2950 mg/L on day 26 due to inadequate mixing in the reactor. The lower equilibrium sludge concentration at thermophilic condition has been reported in several literatures (Surucu, 1975; Lapara and Alleman, 1999; Couillard and Zhu, 1993; Rozich and Colvin, 1997).

In spite of the addition of oil and the high salt concentration in the feed solution, the trans-membrane pressure for the room-temperature reactor was stable is shown in figure 17. More severe fouling of the membrane was observed for the thermophilic reactor. The transmembrane pressure increased rapidly in the case of the thermophilic reactor up to 0.035 MPa on day 12. Due to the surface cleaning of the membrane on day 13, the transmembrane pressure was lowered. However, on day 24 and day 35 the membrane of the thermophilic reactor was again fouled and the trans-membrane reached above 0.065 MPa.

The tendency of easier fouling of the thermophilic reactor was consistent with the previous literature (Abeynayaka and Visvanathan, 2011). It was confirmed that the lower flux operation is needed in the case of thermophilic membrane bioreactor even in the case of the operation of low organic loading as in this study.

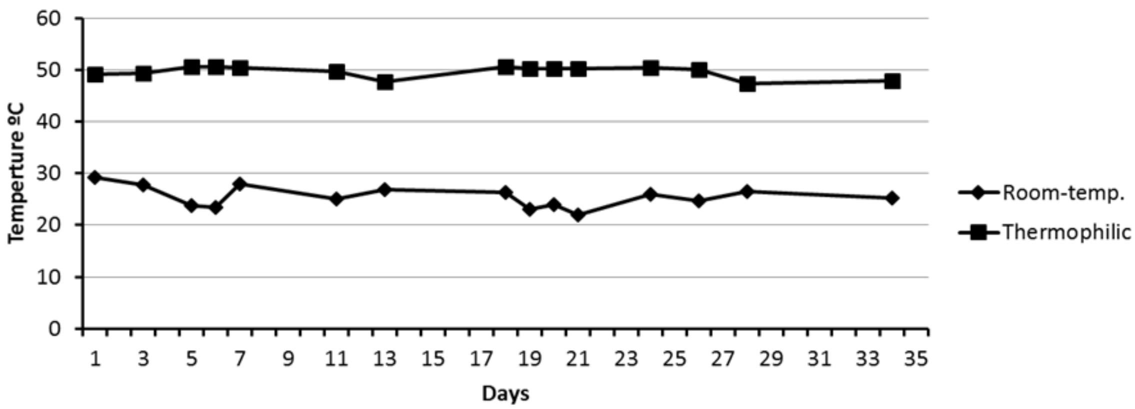



Fig. 14 - Change in temperature in the reactors.

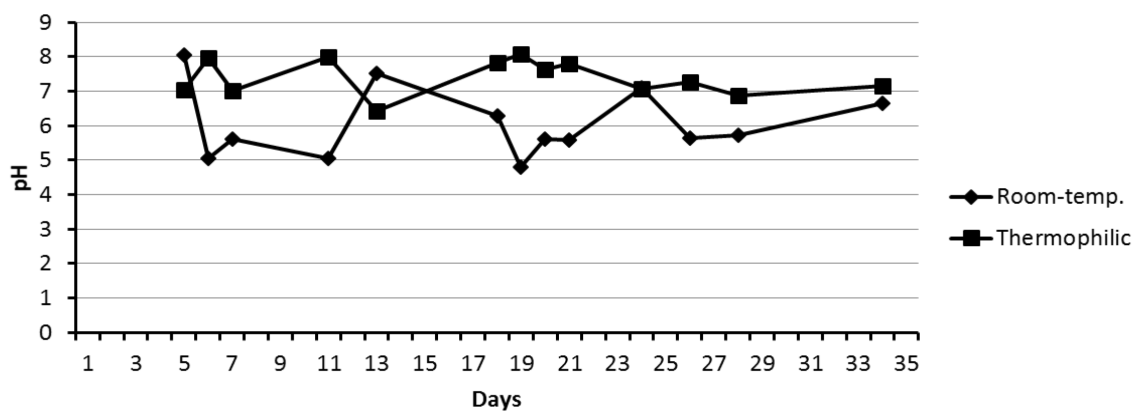



Fig. 15 - Change in pH in the reactors.

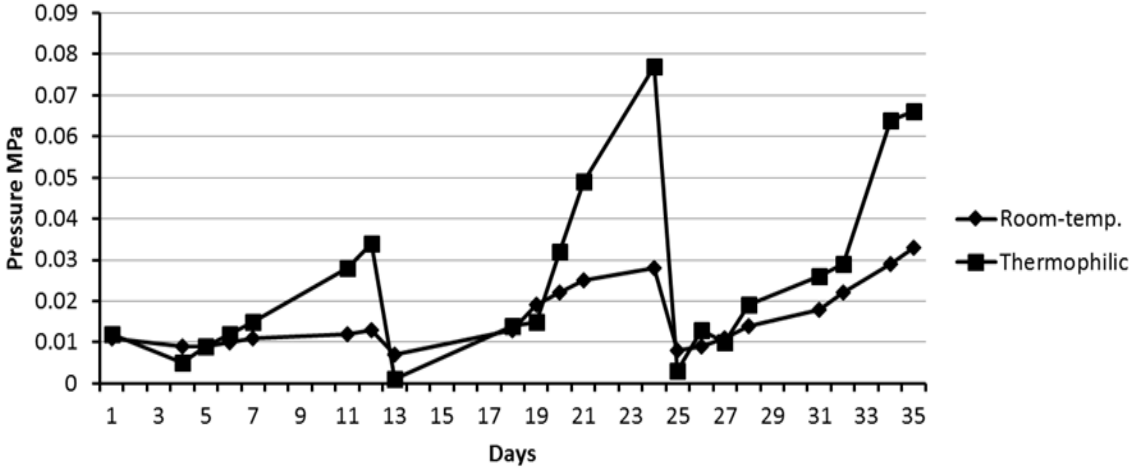



Fig. 16 - Change in MLSS concentration in the reactors.

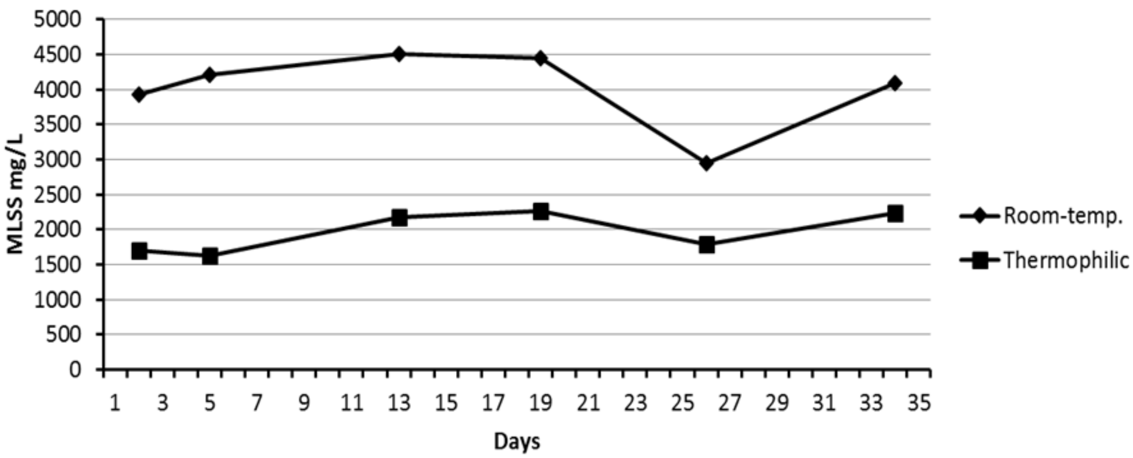



Fig. 17 - Change in pressure in MBR operation.

**4.2.2 Removal of COD**

The results of COD measurements are shown in Fig. 18. The influent COD of molasses wastewater was 1020 mg/L. No significant difference was found in term of COD removal for both reactors. The removal of COD in the thermophilic reactor and the room-temperature reactor was 87% on the average. According to Juhani, 2003 the thermophilic treatment of diluted molasses wastewater gave high (80-90%) COD removals which were the same range of this study. The concentrations of supernatants were in the same ranges as in the effluents, although the accumulation of macromolecules which could not be measured as COD might have taken place in the reactors. Saima et al., 2015 also reported that COD removal under thermophilic operation was comparable with that under room-temperature condition.

A number of aerobic thermophilic wastewater treatment processes treating different wastewaters under high VLRs, low HRTs, and resulting in high COD removals have been reported (Rintala and Lepistö 1993, Ragona and Hall 1998, Becker et al. 1999, Jahren and Ødegaard 2000a, 2000b, Suvilampi et al. 1999, Huuhilo et al. 2002, Jahren et al. 2002, Rozich and Bordacs 2002). Many of these studies have focused on the feasibility of thermophilic aerobic wastewater treatment applied to different industrial or synthetic wastewaters.

The effect of high temperature on the removal efficiencies in MBR was studied by Zhang *et. al.* (2006). The removal efficiency was more than 97% at 35 and 40 °C, while it was 93% at 45 °C. The same researchers reported that the richness in microbial diversity was reduced in high temperature treatment because of the sudden changes in operational conditions. This microbial diversity decay could cause lower removal of pollutants (Tripathi and Grant.1999; LaPara et. al., 2000). In all previous studies, mesophilic activated sludge processes is preferable in terms of COD removal compared with thermophilic processes (Zhang et. al., 2006), though the effect of temperature on COD removal was not seen in our study.

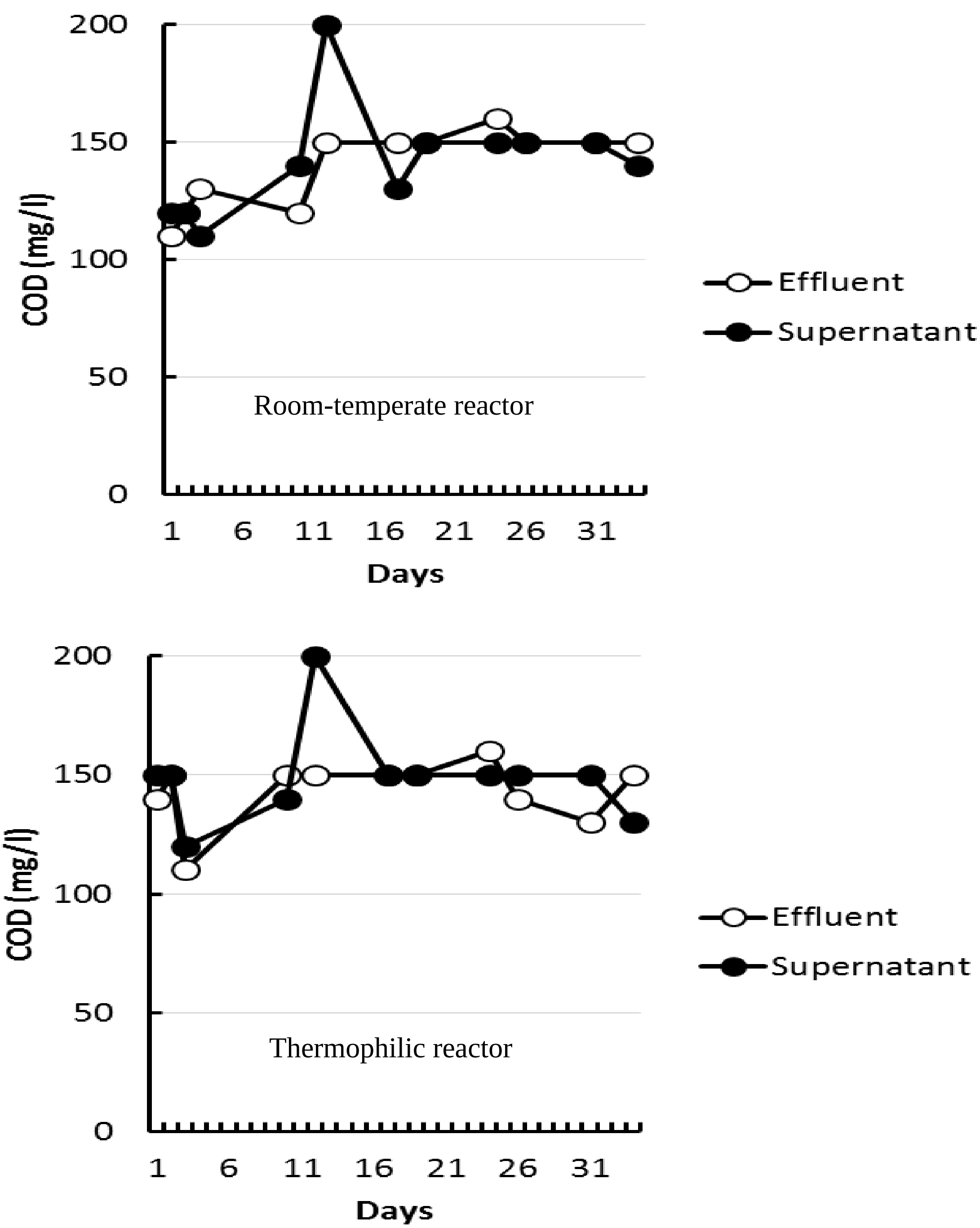



Fig. 18 - COD of effluent and the supernatant of the mixed liquor of activated sludge.

### 4.2.3 Removal of Color

Figure 19 shows the removals of color at 390 nm (generally used wavelength for the color determination) and the removals of color at 475 nm (peak absorbance wavelength for molasses solution containing melanoidin). The removals for the thermophilic reactor at 390nm were in the range between 21 and 28% (average 26%), which were lower than those for the room-temperature reactor ranging 32 to 59% (average 46%). The color removal at 475 nm for the thermophilic reactor was 34 to 62% (average 44%), while the removal for the room-temperature reactor was 41 to 68% (average 58%). Regardless of the wavelength in the measurement, worse color removals were observed for the thermophilic reactor. The variety of consortia of microorganisms was limited in the thermophilic reactor as can be seen from the 50% reduction of MLSS in the acclimatization period. The lack of variety in microorganism would be the reason for the poor color removal in the thermophilic reactor.

The treatment under thermophilic operation narrowed the range of removable organic constituents in molasses solution in this study. As discussed in the section of COD removal, high-temperature operation is generally considered to influence the removal of organic matters for the poor direction. Kambe *et al*., (1999) also pointed out low efficiency of color removal at high temperature and they found that no color removal could be observed under aerobic condition at 55 ºC.

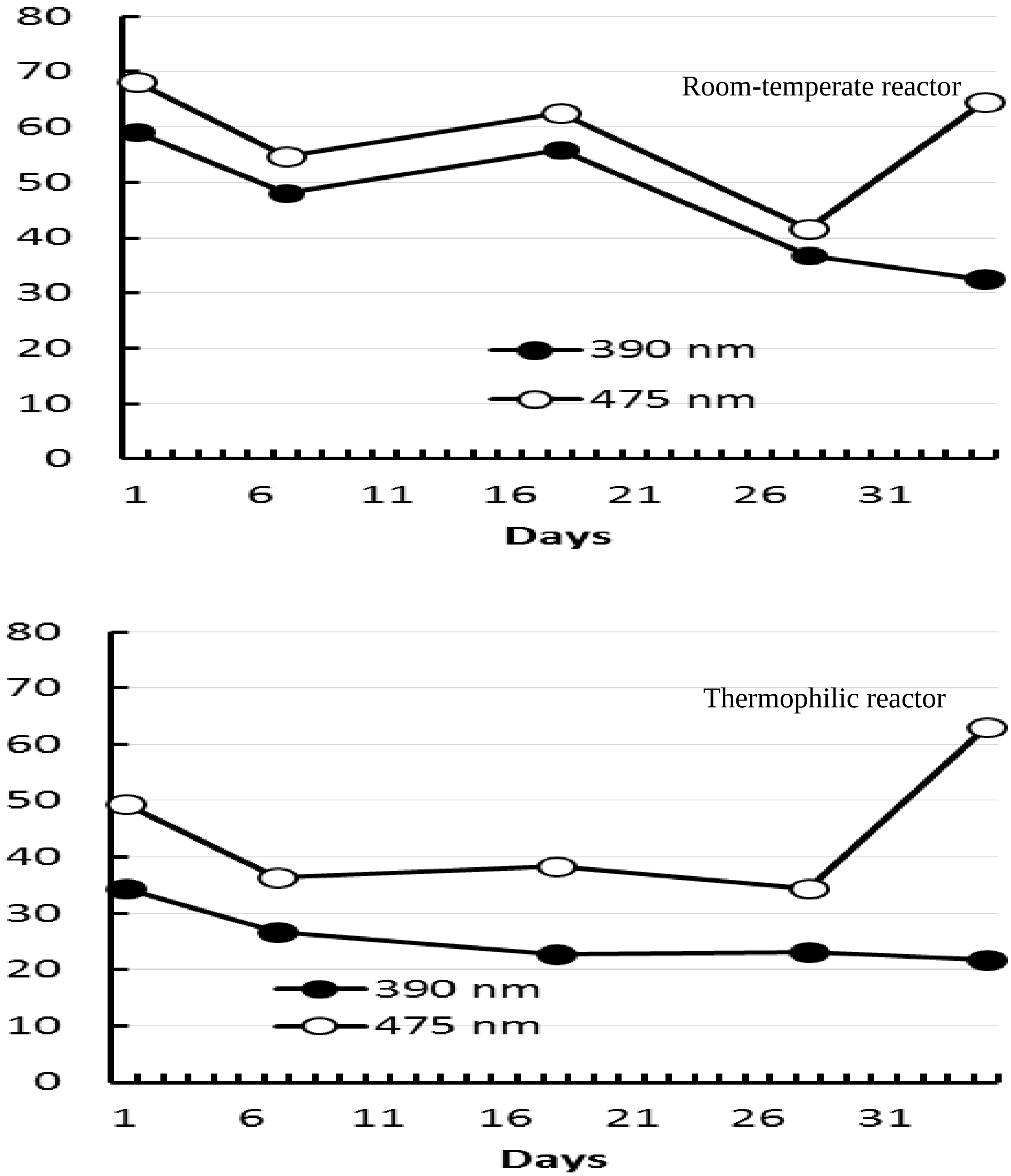



Fig. 19 - Change in the percent removal of color measured at 390 nm and 475 nm.

### 4.2.4 Nitrogen in the Reactor

The average of ammonia nitrogen concentration ($NH_3$-N) in the effluent was 4.5 mgN/L (n=10) for the room-temperature reactor and 6.5 mg/L on the average (n=10) for the thermophilic reactor. Although the accuracy of the results by the simplified method for the measurement of annmonium was not enough for the quantitative discussion, the higher remaining concentration of ammonium in the case of thermophilic condition might be due to the inhibition of nitrification above 40°C, as was reported by Juteau (2006). The difference in remaining ammonium concentration and influent urea concentration (50 mgN/L) might be caused by the air stripping of ammonia (Abeynayaka and Visvanathan, 2011) and a limited contribution of ammonia – oxidizing bacteria in the thermophilic process (Simstich et al., 2012).

### 4.2.5 Removal of Oil in the Reactors

During 35 days of operation, mineral oil was added once a week to make the oil concentration 70 mg/L at the beginning of the week. The bars in Fig. 20 show the averages of 5 times measurements (5 weeks) with activated sludge, while the solid lines in the figures show the changes in the concentration of oil added to the same reactor filled with pure water. The oil concentration rapidly decreased with all of the examined conditions. The half-life was around 3 hours in the room-temperature reactor, while it was around 2 hours in the thermophilic reactor. When oil was added to the mixed liquor of activated sludge, the ratio of the concentration in the sludge phase to the total concentration increased with time especially in the room-temperature reactor.

The contribution of biological reaction to the removal of oil may not be dominant in this experiment because the total oil concentration was decreased with roughly the same half-life-time even without activated sludge, although the contribution of biological removal is suggested in the treatment of shipboard wastewater by a MBR (Di Bella *et al*., 2015). The decreases in oil concentration might be caused mainly by volatilization judging from a high Henry's Law constant of 0.228 atm $m^3$/mol (for C16 alkane, Hazardous Substances Data Bank), although a high log Kow of 8.25 (Hazardous Substances Data Bank) suggests the contribution of adsorption especially in the case of the oil added to pure water. Even though the removal mechanism is not fully clear, the short half-life of hydrocarbon concentration suggests that the MBR can remove mineral oil ($C_{15}$-$C_{22}$ alkanes) at a higher efficiency, if the reactor was operated with an adequate HRT. By heating the reactor, the removal efficiency of oil is considered to be increased. Saima et al., 2015 achieved high oil removal under thermophilic operation MBR, which was consistent with this study.

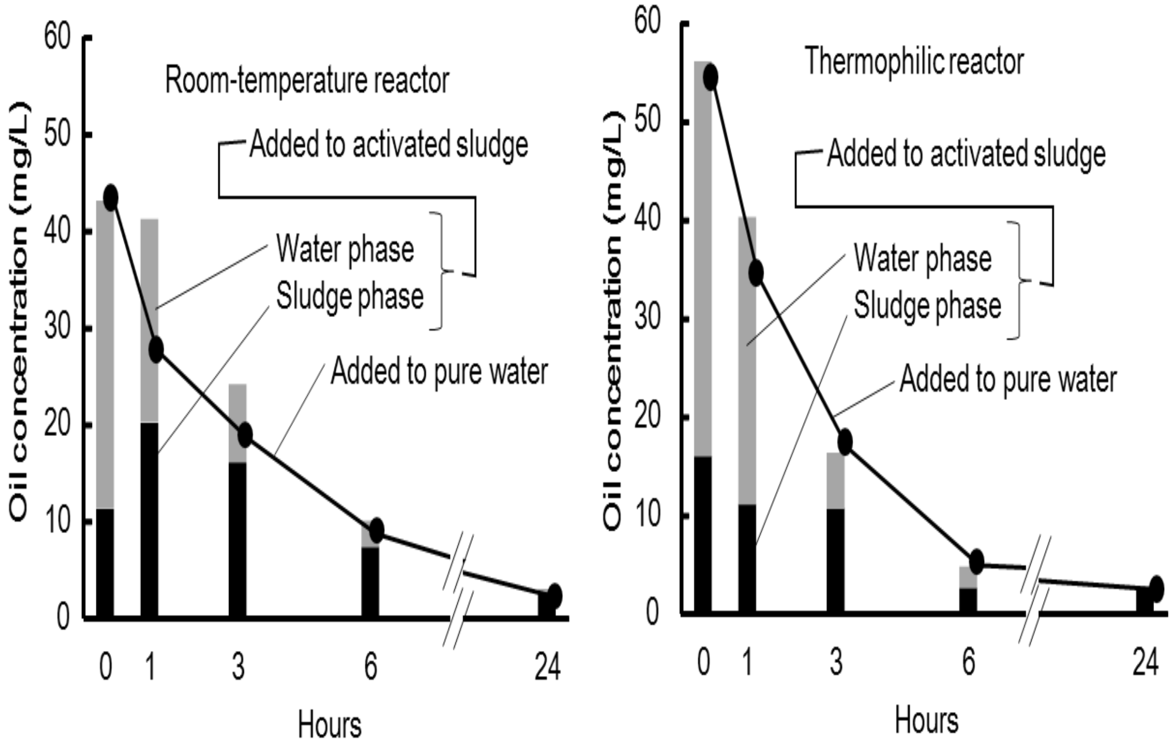



Fig. 20 – Changes in oil concentration during 24 hours after the addition of oil.

# CHAPTER 5: CONCLUSION

Membrane bioreactor (MBR) process is the technology that has gained a considerable numbers of applications into wastewater treatment processes in recent days. One of the greater advantages of the MBR process is the operation at a high sludge retention time, which enables keeping in the reactors a variety of microorganism which can extend the removable compounds in biological wastewater treatment. In addition, high effluent water quality without the presence of suspended particles by the introduction of MBR is attractive for the reuse of industrial wastewater.

Saline and high-temperature wastewater containing a variety of organic compounds is a difficult target for wastewater treatment. The produced water from oil and gas production activities, shipboard wastewater, and textile wastewater are the examples of this type of wastewater.

On the other hand, biomass process including molasses distillation and sulfuric acid hydrolysis often generates wastewater having acidic characteristics. Highly acidic wastewater is another difficult target of treatment.

The aim of this study is to investigate the performances of membrane bioreactors (MBR) for wastewater treatment under high temperature operation and acidic operation to improve the removal of color and oil from industrial wastewater. The removal of color was focused because the remaining yellow or brown color in treated industrial wastewater usually originates from high molecular weight organic matters which are recalcitrant to biological degradation. Oil was also focused because oil in wastewater often disturbs the treatment of industrial wastewater by forming aggregates especially under low temperature conditions. Few literature can be found for the MBR operation below pH 3. Treatment of oily wastewater by MBR above 50°C has hardly been reported, though thermophilic MBR has been studied for many applications.

### 5.1 Low pH Operation

Colored substances contained in molasses wastewater are usually recalcitrant to biological degradation. Lower pH operation in biological treatment might be beneficial for the removal of color due to higher adsorption nature of melanoidins to solids in lower pH condition. In addition, lower pH operation may be a favorable condition for keeping fungi, representative degraders of persistent organic compounds, in the reactors. Biological processes including molasses distillation and sulfuric acid hydrolysis often generate wastewater having acidic characteristics. Treatment of acidic wastewater under acidic conditions would be economically preferable in some cases.

In the first experiment, the advantage of acidic operation below pH of 3, which operation was out of the usually accepted condition for membrane bioreactors (MBRs), was examined targeting the treatment of sulfuric acid hydrolysis wastewater generated in the biomass processing without pH neutralization. Two glass reactors with 5 L volume each, equivalent to an average HRT of 4 to 7 days were operated simultaneously for 91 days. The pH of the neutral reactor was between 5.5 and 7.0 (typically 6.5), whereas the pH of the acidic reactor was controlled at 3 using a pH controller and hydrochloric acid. The flat sheet membranes with pore size of 0.45 μm, diameter of 142 mm and the material of hydrophilic polytetrafluoroethylene, were used in the MBRs for the separation of sludge and permeate. The temperature in the reactors was between 17 to 22°C. The whole experimental period was divided into two periods depending on the process with and without pretreatment consisting of a fixed-bed biological reactor with hydraulic retention time (HRT) of 1.3 days.

As a conclusion, the trans-membrane pressures were higher for the low pH reactor due to membrane fouling caused by the adhesion of microbial products on the membrane surface. COD removal was 48.5% for the acidic reactor and that for the neutral pH reactor was 63.6% when biologically pretreated molasses wastewater was fed to the reactors. Higher removals of COD (89.0% for the neutral pH reactor and 84.0% for the acidic reactor) were observed, when molasses wastewater (COD 650 mg/L) was directly fed to the reactor without pretreatment. In spite of lower COD removal in the acidic reactor, higher color removal was observed spectrophotometrically. Higher color removal in the case of the acidic reactor was probably due to adsorption enhanced by the lower pH operation followed by the gradual biological degradation of persistent colored substances.

## 5.2 Thermophilic Operation

The combination of membrane separation process and thermophilic aerobic process has been studied to overcome the drawback of the poor settleability of sludge in the thermophilic aerobic process. Treating directly high-temperature wastewater is attractive, because cooling process is usually required for the treatment of high-temperature wastewater such as textile wastewater and because oil in wastewater often disturbs the treatment by forming aggregates especially under low temperature conditions. The objective of the second part of the experiment in this study is to compare the performances of MBRs under different temperature conditions to clarify the effect of thermophilic condition on the range of removable contaminants and on the fouling of membranes. This study targeted saline and oily wastewater, which often induce problems in the stable operation of MBRs.

Two glass reactors with 6 L volume each, equivalent to HRT 5 days were operated simultaneously for 35 days. The temperature of the room-temperature reactor was between 22 and 29$^{o}$C, whereas the temperature of the thermophilic reactor was controlled at 50$^{o}$C using a temperature controller. The flat sheet membranes (surface area: 0.06 m2 (200 mm x 150 mm x two sides) Kubota Co.ltd.) made from chlorinated polyethylene with pore size of 0.4 μm were used in the MBRs. The pH in the reactors was between 5 to 8.

The removal of COD was comparable for the two reactors. The half-life time of mineral oil (C15-C22 alkanes) was around 2 hours for the thermophilic reactor, while that of room-temperature reactor was around 3 hours. However, the operation at high temperature condition decreased the removal of dark brown (melanoidin) color from 58% to 44%. The fouling of the membrane was more severe for the thermophilic reactor. The room-temperature reactor maintained a volume flux of 0.22 m/day, while keeping the volume flux at the same level was difficult for the thermophilic reactor. It was suggested that lower flux operation of the membrane and worse effluent quality have to be considered, if high-temperature operation is required.

### 5.3 Recommendations for Future Work

As concluded above, low pH operation has a problem of membrane fouling, though it has a certain advantage on color removal. Thermophilic operation also has a problem of membrane fouling, though it can eliminate the problem caused by oil in wastewater.

It is necessary to find methods for the elimination of membrane fouling in order to operate MBRs in thermophilic or low pH conditions. There are several directions for further research.

1) The identification of microorganisms which release proteins or polysaccharides under thermophilic or low pH operation is of scientific interest.
2) The introduction of pure cultures to the microbial consortia in MBR to reduce fouling can be considered. There are several studies on the control of fouling by the augmentation of microbial consortia in normal operating conditions. It is necessary to investigate bioaugmentation of MBR in extreme conditions like low pH operation and thermophilic operation.
3) More detailed investigation on the effect of salinity is another direction of research, because we did experiments only with wastewater of 1% salinity. The upper limit of salinity for the target wastewater may be 3%, if we consider the case of shipboard wastewater carried by oil tankers.

4) Development of robust membrane which can be regenerated even if membrane fouling takes place under the extreme conditions is important in an engineering sense, if we cannot avoid membrane fouling.

# PUPLICATION

Two papers have been published based on this doctoral dissertation.

- Shahata A., Omata T., Urase T. (2013) Removal of Color from Molasses Wastewater Using Membrane Bioreactor with Acidic Condition. *Journal of Water and Environment Technology*, **11**(6), 539-546.
- Shahata A., Urase T. (2016) Treatment of Saline Wastewater by Thermophilic Membrane Bioreactor. *Journal of Water and Environment Technology*, (Accepted for publication and waiting for printing in vol.14, No.2).

# REFERENCES


- Abeynayaka A. and Visvanathan C. (2011) Performance comparison of mesophilic and thermophilic aerobic sidestream membrane bioreactors treating high strength wastewater. *Bioresource Technology*, **102**(9), 5345-5352.

- Ahmadun F., Pendashteh A., Abdullah L., Biak D. R. A., Madaeni S. S. and Abidin Z. Z. (2009) Review of technologies for oil and gas produced water treatment. *Journal of Hazardous Materials*, **170**, 530-551.
- Ahmed Z., Lim B.R., Cho J., Song K.G., Kim K.P., and Ahn K.H. (2008) Biological nitrogen and phosphorus removal and changes in microbial community structure in a membrane bioreactor: Effect of different carbon sources. *Water Reseach*, **42**, 198–210.
- Alaton I.A., Alcioglu I.A. and Bahnemann D.W. (2002). Advanced oxidation of a reactive dyebath effluent: comparison of O3, H2O2/UV–C and TiO2/UV–A processes. *Water Research*, **36**,1143–1154.
- Alther G. (2001) How to Remove Emulsified Oil from Wastewater with Organoclays. *R & B Ltd*, **14**, 27-84.
- Alkane H.V., Dange M.N., and Selvakumari K. (2006) Optimization of anaerobically digested distillery molasses spent wash decolorization using soil as inoculum in the absence of additional carbon and nitrogen source. *Bioresource Technology*, **97**, 2131-2135.
- Antileo C., Aspe E., Urrutia H., Zaror C., Roeckel M. (2002) Nitrifying biomass

acclimation to high ammonia concentration. *Journal of Environmental Engineering*, **128**, 367–375.

- Asaithambi P. and Matheswaran M., (2011) *Arab. J. Chem.*
- Azadbakht L., Mirmiran P., Esmaillzadeh A., Azizi T., and Azizi F. (2005) Beneficial effects of a Dietary Approaches to Stop Hypertension eating plan on features of the metabolic syndrome. *Diabetes Care* **28**(12), 2823–31.
- Becker P., Köster, D., Popov, M., Markossian, S., Antranikian, G. & Märkl H. (1999) The biodegradation of olive oil and the treatment of lipid-rich wool scouring wastewater under aerobic thermophilic conditions. *Water Research*, **33**,653-660.
- Biesterfeld S., Farmer G., Russell P. and Figueroa, L. (2003). Effect of alkalinity type and concentration on nitrifying biofilm activity. *Water Environ. Res*, **75**(3), 196–204.
- Brock T. (1986) Introduction: An Overview of the Thermophiles. In: Brock TD (Ed), Thermophiles: General, Molecular, and Applied Microbiology. *Wiley Series in Ecological and Applied Microbiology, Wiley-Interscience Publications, Wiley & Sons, New York*,1–16.
- Calderón K., González-Martínez A., Montero-Puente C., Reboleiro-Rivas P., Poyatos J.M., Juárez-Jiménez B., Martínez-Toledo M.V. and Rodelas B. (2012) Bacterial community structure and enzyme activites in a membrane bioreactor (MBR) using pure oxygen as an aerobic source. *Bioresource Technology*, **103**, 87–94.

- Chandra R., Bharagava R. N. and Rai V 2008, Melanoidins as major colourant in sugarcane molasses based distillery effluent and its degradation. *Bioresource Technology*, **99**(11), 4648-4660.
- Cicek N., Franco J.P., Suidan M.T. and Urbain V. (1998). Using a membrane bioreactor to reclaim wastewater. *Journal American Water Works Association*, **90**(11), 105 –113.
- Couillard D. and Zhu S. (1993) Thermophilic Aerobic Process for the Treatment of Slaughterhouse Effluents with Protein Recovery. *Environ. Pollut.*, **79**,121.
- Davies L., Vilhena A., Novais J. and Martins-Dias S (2004), Olive mill wastewater characteristics: modeling and statistical analysis. *Grasasy Aceites*, **55**(3), 178-234.
- De Jager D., Sheldon M.S. and Edwards W. (2014) Colour removal from textile wastewater using a pilot-scale dual-stage MBR and subsequent RO system, *Separation and Purification Technology*, **135**(15), 135-144.
- Di Bella G., Di Prima N., Di Trapani D., Freni G., Gabriella Giustra M., Torregrossa M. and Viviani G. (2015) Performance of membrane bioreactor (MBR) systems for the treatment of shipboard slops: Assessment of hydrocarbon biodegradation and biomass activity under salinity variation, *Journal of Hazardous Materials*, 300, 30, 765-778.
- Dincer A.R., and Kargi F. (2000) Kinetics of sequential nitrification and denitrification processes. *Enzyme and Microbial Technology*, **27**, 37–42.

- Feng S., Zhang N., Liu H., Du X., Liu Y., and Lin H., (2012) The effect of COD/N ratio on process performance and membrane fouling in a submerged bioreactor. *Desalination*, **285**, 232– 238.
- Figaro S., Avril J. P., Brouers F., Ouensanga A. and Gaspard S 2009, Adsorption studies of molasse's wastewaters on activated carbon: Modelling with a new fractal kinetic equation and evaluation of kinetic models. *Journal of Hazardous Materials*, **161**(2-3), 649-656.
- Fontenot Q., Bonvillain C., Kilgen M., and Boopathy R. (2007) Effects of temperature, salinity, and carbon: nitrogen ratio on sequencing batch reactor treating shrimp aquaculture wastewater. *Bioresource Technology*, **98**, 1700–1703.
- Gagosian R.B., and Lee, C., (Book editors: Duursma E.K and Dawson R.) (1981). Process controlling the distribution of biogenic organic compounds in seawater. *Marine organic chemistry*, 91-118.
- Garbhani G, And Farajnezad H. (2012). Coagulation treatment of wastewater in petroleum industry using poly aluminum chloride and ferric chloride. *IJJRRAS*, **13**(1), 306-310.
- González T., Terrón M. C., Yagüe S., Junca H., Carbajo J. M., Zapico E. J., Silva R., Arana-Cuenca A., Téllez A. and González A. E. (2008) Melanoidin-containing wastewaters induce selective laccase gene expression in the white-rot fungus *Trametes* sp. I-62. *Research in Microbiology*, **159**(2), 103-109.

- Gao D.W., Fu Y., Tao Y., Li X.X., Xing M., Gao X.H., and Ren N.Q. (2011) Linking microbial community structure to membrane biofouling associated with varying dissovled oxygen concentrations. *Bioresource Technology*, **102**, 5626–5633.

- Gawad H. (2014), Oil and Grease Removal from Industrial Wastewater Using New Utility Approach. *Journal of Advances in environmental chemistry*, **14**(1), 63-69.

- Gultekin I. and Ince N.H. (2004). Degradation of reactive azo dyes by UV/H2O2: Impact of radical scavengers. *Journal of Environmental Science and Health*, **39**(4), 1069–1081.

- Hai F. I., Yamamoto K., Nakajima F. and Fukushi K. (2009) Factors governing performance of continuous fungal reactor during non-sterile operation – The case of a membrane bioreactor treating textile wastewater. *Chemosphere*, **74**(6), 810-817.

- Howell J. A. (2004) Future of Membranes and Membrane Reactors in Green Technologies and for Water Reuse. *Desalination*., **162**(1).

- Huuhilo T., Suvilampi J., Puro L., Rintala J., Mänttäri M., Nuortila-Jokinen J. and Nyström M. (2002) Internal treatment of pulp and paper mill process waters with a high temperature aerobic biofilm process combined with ultrafiltration and/or nanofiltration. *Paper and Timber*, **84**, 50-53.

- Ikan R., Dorsey T., and Kaplan I.R. (1990). Characterization of natural and synthetic humic substances (melanoidins) by stable carbon and nitrogen isotope measurements and elemental compositions. *Analytica Chimica*, **232**, 11- 18.

- Imtiazuddin. (2012) Pollutants of Wastewater Characteristics in Textile Industries. *Journal of Basic and Applied Sciences*, **4**(1), 34-37.
- Jahren S. and Ødegaard H. (2000a). Thermophilic aerobic treatment of high strength wastewater using the moving bed biofilm reactor. *Environmental Technology*, **21**, 1343-1356.
- Jahren S., Rintala J. and Ødegaard, H. (2002). Aerobic moving bed biofilm reactor treating thermomechanical pulping (TMP) whitewater under thermophilic conditions. *Wat. Res.*, **36**, 1067-1075.
- Jahren S. and Ødegaard H. (2000b). Treatment of thermomechanical pulping (TMP) whitewater in thermophilic (55°C) anaerobic-aerobic moving bed biofilm reactors. *Wat. Sci. Tech*, **40**(8), 81-89.
- Jahren S. (1999 b). Thermophilic treatment of concentrated wastewater using biofilm carriers. *Doctoral Thesis, Norwegian University of Science and Technology, Norway*.
- Jin Y.L., Lee W.N., Lee C.H., Chang I.S., Huang X., and Swaminathan T. (2006) Effect of DO concentration on biofilm structure and membrane filterability in submerged membrane bioreactor. *Water Research*, **40**, 2829–2836.
- Judd S. (2004) A review of fouling of membrane bioreactors in sewage treatment. *Water Science and Technology*, **49**(2), 229-235.
- Juhani S. (2003) Aerobic Wastewater Treatment under High and Varying Temperatures Thermophilic Process Performance and Effluent Quality. Jyvaskyla *Studies in Biological and Environmental Science*, 7-55.

- JustIna R., Mindaugas R.and Harry B. (2009) pH effect on decolorization of raw textile wastewater. *Environment Protection Engineering*, **35**(3), 167-178.
- Juteau P. (2006) Review of the use of aerobic thermophilic bioprocesses for the treatment of swine waste. *Livestock Science*, **102**(3), 187-196.
- Kambe T.N., Shimomura M., Nomura N., Chanpornpong T. and Nakahara T. (1999) Decolourization of molasses wastewater by Bacillus sp.under thermophilic and anaerobic conditions. *Journal Bioscience Bioengineer*, **87**, 119-121.
- Kraakman N. (2012) Biological Odour Control at Wastewater Treatment Facilities. *The Present and the Future*, **104**(2), 67-73.
- Kundu P., and Mishra I., (2013) Removal of emulsified oil from oily wastewater (oil-in-water emulsion) using packed bed of polymeric resin beads. *Separation and Purification Technology*, **118**, 519-529.
- Kumar P., and Chandra R. (2006) Decolourisation and detoxification of synthetic molasses melanoidins by individual and mixed cultures of Bacillus spp. *Bioresource Technology*, **97**(16), 2096-2102.
- LaPara T. and Alleman J. (1999). Review paper; Thermophilic aerobic biological wastewater treatment. *Water Research*, **33**, 895-908.
- LaPara T., Nakatsu C., Pantea L. and Alleman J. 2000. Phylogenetic analysis of bacterial communities in mesophilic and thermophilic bioreactors treating pharmaceutical wastewater. *Appl. Env. Microbiol*, **66**, 3951-3959.

- Layson A. (2004) Impact of Water Technology Developments on Economics of Water Recycling and Reclamation in the Power Industry. *Memcor Technology Centre,* www.usfilter.com.
- Liao B.Q., Kraemer J.T., and Bagley D.M. (2006) Anaerobic membrane bioreactors: applications and research directions. *Crit. Rev. Environ. Sci. Technol,* **36**, 489–530.
- Logan AG. (2007) "DASH Diet: time for a critical appraisal?". Am. J. *Hypertens*, **20**(3), 223–4.
- Ma B.C., Lee Y.N., Park J.S., Lee C.H., Lee S.H., Chang I.S., and Ahn T.S. (2006) Correlation between dissolved oxygen concentration, microbial community and membrane permeability in a membrane bioreactor. *Process Biochemical*, **41**, 1165–1172.
- Mariangel L., Aspe E., Cristina Marti M., and Roeckel M. (2008) The effect of sodium chloride on the denitrification of saline fishery wastewater. *Environment Technology*, **29**, 871–879.
- Miura Y., Hiraiwa M.N., Ito T., Itonaga T.; Watanabe Y., and Okabe S. (2007) Bacterial community structures in MBRs treating municipal wastewater: Relationship between community stability and reactor performance. *Water Research*, **41**, 627–637.
- Miyoshi T., Tsuyuhara T., Ogyu R., Kimura K., and Watanabe Y. (2009) Seasonal variation in membrane fouling in membrane bioreactors (MBRs) treating municipal wastewater. *Water Research*, **43**, 5109–5118.

- Mittal A. (2011) Biological Wastewater Treatment. *Water Today*, **36**, 32-44.
- Painter T.J. (1998) Maillard reaction with special reference to discoveries of preserved foods in Sphagnum-dominated peat bogs. *Carbohydrate* Polymers, **36**, 335-347.
- Molina-Munoz M., Poyatos J.M., Rodelas B., Pozo C., Manzanera M., Hontoria, E., and Gonzalez-Lopez J. (2010) Microbial enzymatic activities in a pilot-scale MBR experimental plant under different working conditions. *Bioresourse Technology*, **101**, 696–704.
- Oliver J.H., Hyunook K. and Pen-Chi, C. (2000). Decolorization of wastewater. *Crit. Rev. Environ. Sci. Technol*. **30**(4), 499– 505.
- Onodera T., Sase S., Choeisai P., Yoochatchaval W., Sumino H., Yamaguchi T., Ebie Y., Xu K., Tomioka N., Mizuochi M. and Syutsubo K. (2013) Development of a treatment system for molasses wastewater: The effects of cation inhibition on the anaerobic degradation process. *Bioresource Technology*, **131**, 295-302.
- Ng A.N.L., and Kim A.S. (2007). A mini-review of modeling studies on membrane bioreactor (MBR) treatment for municipal wastewaters. *Desalination*, **212**, 261–281.
- Painter, T.J. (1998) Carbohydrate polymers in food preservation: an integrated view ofthe Maillard reaction with special reference to discoveries of preserved foods in Sphagnum-dominated peat bogs. *Carbohydrate Polymers*, **36,** 335-347.
- Rintala J. and Lepistö R. (1993) Thermophilic anaerobic-aerobic and aerobic treatment of kraft bleaching effluents. *Wat. Sci. Tech*, **28**(2), 11-16.

- Plavsic M., Cosovic B., and Lee C. (2006) Copper complexing properties of melanoidins and marine humic material. *Science of the Total Environment*, **366**, 310-319.
- Ragona C and Hall E. (1998) Parallel operation of ultrafiltration and aerobic membrane bioreactor treatment systems for mechanical newsprint whitewater at 55°C. *Wat. Sci. Tech*., **38**(4-5), 307-314.
- Rozich A.F. and Colvin R.J. (1997) Design and Operational Consideration for Thermophilic Aerobic Reactors Treating High Strength Wastes and Sludges. *In Proceedings of the 52nd Industrial Waste Conference, Purdue University*, ed. J.E. Alleman, Ann Arbor Press, Ann Arbor, MI, U.S.A.
- Rozich, A. and Bordacs, K. 2002. Use of thermophilic biological aerobic technology for industrial wastewater treatment. *Wat. Sci. Tech.,* **46**(4-5), 83-89.
- Saima F., Beiping Z., Zhenxing Z., Lan G. and Xuechuan Chen (2015). Industrial Wastewater Treatment by Using MBR (Membrane Bioreactor) Review Study. *Journal of Environmental Protection*, **6**, 584-598.
- Satyawali Y. and Balakrishnan M. (2008) Wastewater treatment in molasses-based alcohol distilleries for COD and color removal: A review. *Journal of Environmental Management*, **86**(3), 481-497.
- Simstich B., Beimfohr C., and Horn H. (2012) Lab scale experiments using a submerged MBR under thermophilic aerobic conditions for the treatment of paper mill deinking wastewater. *Bioresource Technology*, **122**, 11- 16.

- Srinivasan A. and Viraraghavan T. (2010) Oil removal from water using biomaterials. *Bioresource technology*, **101**(17), 6594-6600.
- Sureyya M., Deniz K., Tu gba O. (2004) Color and COD removal from wastewater containing Reactive Black 5 using Fenton's oxidation process. *Chemosphere*, **54,** 435–441.
- Surucu G.A. (1975) Thermophilic Aerobic Treatment of High-Strength Wastewaters with Recovery of Protein. Ph.D. Thesis, University of Illinois, Champaign-Urbana.
- Suvilampi J., Huuhilo T., Puro L., Rintala J., Nuortila-Jokinen J. and Nyström, M. (1999) Closing water circuits in pulp and paper mills: internal thermophilic purification using suspended carrier aerobic biofilm process combined with membrane filtration. *4th Int. Conf. on Pulp and Paper Industry*, **14-16**, 605-609.
- Tocchi C., Federici E., Fidati L., Manzi R., Vincigurerra V., and Petruccioli M. (2012) Aerobic treatment of dairy wastewater in an industrial three-reactor plant: Effect of aeration regime on performances and on protozoan and bacterial communities. *Water Research*, **46**, 3334–3344.
- Tripathi Chandra S. and Grant Allen D. (1999). Comparison of mesophilic and thermophilic aerobic biological treatment in sequencing batch reactors treating bleached kraft pulp mill effluent. *Water Res*. **33**(3), 836-846.
- Trivedi R., and Doare D., (2014), A Review on Membrane Bioreactors: An Emerging Technology for Industrial Wastewater Treatment. *International Journal of Emerging Technology and Advanced Engineering*, **12**(4), 198-204.

- Trussell R.S., Merlo R.P., Hermanowicz S.W., and Jenkins D. (2006) The effect of organic loading on process performance and membrane fouling in a submerged membrane bioreactor treating municipal wastewater. *Water Research*, **40**, 2675–2683.
- Urase T., Kagawa C. and Kikuta T. (2005) Factors affecting removal of pharmaceutical substances and estrogens in membrane separation bioreactors. *Desalination*, **178**, 107-113.
- Van den Brink J.C., Satpradit O.A., Van Bentem A. Zwijnenburg A., Temmink H., and Van loosdrecht M.C. (2011) Effect of temperature shocks on membrane fouling in membrane bioreactors. *Water Research*, **45**, 4491–4500.
- Vanhecke T.E., Miller W.M., Franklin BA., Weber J.E., McCullough P.A. (2006) Awareness, knowledge, and perception of heart disease among adolescents. *Eur J Cardiovasc Prev Rehabil*, **13**(5), 718–23.
- Walker C. and Reamy B.V. (2009) Diets for cardiovascular disease prevention: what is the evidence? , *Am Fam Physician*, **79**(7), 571–8.
- Wang H., Yang Y., and Lix. (2014) Removal of Chemical Organics and Chromaticity from Printing and Dyeing Wastewater Using Nanofiltration Membrane. *Asian Journal of Chemistry*, **26**(8), 2318-2320.
- Wu B., Yi S., and Fane A.G., (2011) Microbial community developments and biomass characteristics in membrane bioreactors under different organic loadings. *Bioresour. Technol*, **102**, 6808–6814.

- Yan X., Bilad M. R., Gerards R., Vriens L., Piasecka A. and Vankelecom I. F. J. (2012) Comparison of MBR performance and membrane cleaning in a single-stage activated sludge system and a two-stage anaerobic/aerobic (A/A) system for treating synthetic molasses wastewater. *Journal of Membrane Science*, **394-395**, 49-56.
- Yaylayan V.A., and Kaminsky E. (1998). Isolation and structural analysis of Maillard polymers: Caramel and melanoidin formation in glycine/glucose model system. *Food Chemistry*, **63**, 25-31.
- Yogalakshmi K.N., and Joseph K. (2010) Effect of transient sodium chloride shock loads on the performance of submerged membrane bioreactor. *Bioresource Technology*, **101**, 7054–7061.
- Zainal Abidin M., Mukhtar H., and Shaharun M., (2014), Removal of Amines from Wastewater Using Membrane Separation Processes. *AMM*, **625**, 639-643.
- Zhang S., Yanga F., Liua Y., Zhanga X., Yamadac Y. and Furukawad K. (2006) Performance of a metallic membrane bioreactor treating simulated distillery wastewater at temperatures of 30 to 45°C. *Desalination,* **194(**1–3), 146–155.
- Zhidong L. (2010) Integrated Submerged Membrane Bioreactor Anaerobic/Aerobic (ISMBR-A/O) for Nitrogen and Phosphorus Removal During Oil Refinery Wastewater. *Petroleum Science and Technology*, **28**(3), 286- 293.

# APPENDICES

**Low pH Operation Experiment Raw Data of the Experiment on the Low Operation**

## a) pH, temperature and pressure

| Days | Acidic reactor | | | | Neutral reactor | | |
|---|---|---|---|---|---|---|---|
| | pH controller | pH meter | Temperature °C | Pressure MPa | pH meter | Temperature °C | Pressure MPa |
| 1 | | | | | 6.34 | 19.1 | 0 |
| 2 | | | | | 6.55 | 21.2 | 0.0027 |
| 3 | | | | | 6.65 | 25 | 0.0094 |
| 6 | | | | | 6.77 | 20.1 | 0.0917 |
| 7 | | | | | 6.31 | 19.9 | 0.0948 |
| 8 | | | | | 6.98 | 18.9 | 0.093 |
| 9 | | | | | 6.8 | 18.6 | 0.09 |
| 10 | | | | | 6.34 | 19.4 | 0.0785 |
| 13 | | | | | 6.47 | 18.2 | 0.0751 |
| 14 | | | | | 6.91 | 21.4 | 0.0922 |
| 15 | | | | | 7.01 | 18.7 | 0.0898 |
| 16 | | | | | 6.67 | 25 | 0.0933 |
| 17 | | | | | 6.8 | 25 | 0.0089 |
| 20 | 2.75 | 2.98 | 18.5 | 0.095 | 5.48 | 18.5 | 0.058 |
| 22 | 2.94 | 3.06 | 17.5 | 0.0958 | 5.67 | 18.5 | 0.0872 |
| 23 | 2.94 | 3.21 | 17.7 | 0.0965 | 6.26 | 17.8 | 0.0895 |
| 24 | 3.03 | | 25 | 0.0965 | 6.58 | 18.3 | 0.0895 |
| 27 | 3.08 | | 17.8 | 0.0942 | 5.88 | 17.7 | 0.0902 |
| 28 | 2.44 | 2.83 | 19.2 | 0.0955 | 6.17 | 18.2 | 0.085 |
| 30 | 2.77 | 2.67 | 19 | 0.00957 | 6.36 | 18.7 | 0.0807 |
| 31 | 3.07 | 3.17 | 18.7 | 0.0959 | 6.24 | 18.2 | 0.0778 |
| 37 | 3.07 | 2.89 | 25 | 0.0947 | 6.28 | 25 | 0.0072 |
| 45 | 2.76 | 2.99 | 16 | 0.0947 | 6.14 | 15 | 0.0072 |
| 49 | 2.95 | 3.13 | 25 | 0.038 | 6.7 | 25 | 0.0035 |
| 52 | 2.97 | 3.12 | 18.4 | 0.0188 | 6.5 | 18.4 | 0.0005 |
| 57 | 2.97 | 3.08 | 18.7 | 0.0045 | 6.51 | 19.2 | 0.0008 |
| 58 | 2.95 | 3.11 | 20 | 0.014 | 6.27 | 19 | 0.0124 |
| 59 | 2.72 | 2.82 | 20.8 | 0.0151 | 6.28 | 20.7 | 0.0028 |
| 60 | 2.99 | 3.17 | 19.8 | 0.0151 | 6.29 | 18.4 | 0.0028 |
| 67 | 2.91 | 2.84 | 21.1 | 0.0151 | 5.75 | 21.1 | 0.0028 |
| 70 | 2.86 | 3.04 | 25 | 0.0272 | 6.02 | 25 | 0.008 |
| 71 | 2.69 | 2.8 | 19.1 | 0.0272 | 6.52 | 18.9 | 0.008 |
| 75 | 3.04 | 3.15 | 25 | 0.0272 | 6.96 | 25 | 0.008 |
| 76 | 3.72 | 3.6 | 19.4 | 0.0122 | 6.5 | 25 | 0.007 |
| 77 | 3.56 | | 18 | 0.0122 | 6.36 | 17.6 | 0.007 |
| 81 | 2.52 | 2.83 | 25 | 0.0122 | 6.77 | 25 | 0.007 |
| 84 | 2.86 | 2.99 | 25 | 0.0122 | 6.26 | 25 | 0.007 |
| 88 | 2.89 | 2.96 | 25 | 0.0051 | 6.48 | 25 | 0.0047 |
| 89 | 2.8 | 2.97 | 25 | 0.0218 | 6.42 | 25 | 0.0037 |
| 90 | 2.76 | 2.92 | 25 | 0.0191 | 6.69 | 25 | 0.0077 |
| 91 | 2.82 | 2.96 | 19.7 | 0.0091 | 6.46 | 16 | 0.0067 |

**b) COD**

| Days | COD (mg/l) | | | | |
|---|---|---|---|---|---|
| | Influent | Acidic reactor | | Neutral reactor | |
| | | Supernatant | Effluent | Supernatant | Effluent |
| 1 | | | | | |
| 2 | | | | | |
| 3 | | | | | |
| 6 | | | | | |
| 7 | | | | | |
| 8 | | | | | |
| 9 | | | | | |
| 10 | | | | | |
| 13 | | | | | |
| 14 | | 244 | 100 | 132 | 78 |
| 15 | | 196 | 106 | 146 | 64 |
| 16 | | | 112 | | 104 |
| 17 | | | | | |
| 20 | | | | | |
| 22 | | | | | |
| 23 | | | | | |
| 24 | | | | | |
| 27 | | 180 | 120 | 116 | 80 |
| 28 | | | | | |
| 30 | 240 | 192 | 124 | 126 | 86 |
| 31 | 232 | 208 | 102 | 114 | 88 |
| 37 | 1180 | | | | |
| 45 | 800 | | | | |
| 49 | 780 | 348 | 188 | 168 | 146 |
| 52 | | | | | |
| 57 | | | | | |
| 58 | | 240 | 150 | 124 | 76 |
| 59 | 710 | | | | |
| 60 | | | | | |
| 67 | | | | | |
| 70 | | 148 | 88 | 104 | 64 |
| 71 | | | | | |
| 75 | | | | | |
| 76 | | 196 | 90 | 100 | 70 |
| 77 | | | | | |
| 81 | | 332 | 82 | 204 | 54 |
| 84 | | | | | |
| 88 | | | | | |
| 89 | | | | | |
| 90 | | | | | |
| 91 | | | | | |

## c) Color

| Color removal | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Acidic reactor | | | | | | | |
| | Influent | | Supernatant | | | | Effluent | | | |
| Days | 390 nm | 475 nm | 390 nm | 390 nm NaOH | 475 nm | 475 nm NaOH | 390 nm | 390 nm NaOH | 475 nm | 475 nm NaOH |
| 1 | | | | | | | | | | |
| 2 | | | | | | | | | | |
| 3 | | | | | | | | | | |
| 6 | | | | | | | | | | |
| 7 | | | | | | | | | | |
| 8 | | | | | | | | | | |
| 9 | | | | | | | | | | |
| 10 | | | | | | | | | | |
| 13 | | | | | | | | | | |
| 14 | | 0.1462 | | | 0.2067 | | | | 0.0503 | |
| 15 | 0.3481 | | 0.2645 | | 0.1124 | | 0.1549 | | 0.0463 | |
| 16 | | | | | | | | | | |
| 17 | | | | | | | | | | |
| 20 | | | | | | | | | | |
| 22 | | | | | | | | | | |
| 23 | | | | | | | | | | |
| 24 | | | | | | | | | | |
| 27 | | | | | | | | | | |
| 28 | | | | | | | | | | |
| 30 | | | | | | | | | | |
| 31 | 0.4692 | 0.1915 | | | | | 0.1176 | 0.1495 | 0.0344 | 0.0496 |
| 37 | 0.3132 | 0.1111 | | | | | | | | |
| 45 | 0.3054 | 0.1025 | | | | | | | | |
| 49 | 0.3185 | 0.1093 | 0.3662 | 0.3866 | 0.2098 | 0.2111 | 0.1276 | 0.1638 | 0.0342 | 0.0515 |
| 52 | | | | | | | | | | |
| 57 | 0.3022 | 0.1 | | | | | | | | |
| 58 | | | 0.2249 | 0.257 | 0.1023 | 0.1195 | 0.1332 | 0.1787 | 0.037 | 0.0591 |
| 59 | 0.3153 | 0.1079 | | | | | | | | |
| 60 | 0.3027 | 0.0999 | | | | | | | | |
| 67 | 0.3191 | 0.1108 | | | | | | | | |
| 70 | 0.2999 | 0.0974 | 0.2709 | 0.278 | 0.1437 | 0.1477 | 0.1299 | 0.1615 | 0.0361 | 0.0504 |
| 71 | | | | | | | | | | |
| 75 | 0.3202 | 0.1082 | | | | | 0.1223 | 0.1398 | 0.0332 | 0.0396 |
| 76 | | | | | | | | | | |
| 77 | | | | | | | | | | |
| 81 | | | 0.2003 | 0.248 | 0.1084 | 0.1156 | 0.1086 | 0.1606 | 0.0291 | 0.0552 |
| 84 | | | | | | | | | | |
| 88 | | | | | | | | | | |
| 89 | | | 0.2056 | 0.2129 | 0.0867 | 0.1084 | 0.1023 | 0.1266 | 0.0288 | 0.0371 |
| 90 | | | 0.1682 | 0.2013 | 0.0779 | 0.094 | 0.1077 | 0.1368 | 0.0278 | 0.0396 |
| 91 | | | 0.1847 | 0.2329 | 0.093 | 0.1023 | 0.1106 | 0.134 | 0.0308 | 0.0396 |

## d) color and MLSS

| Color removal | | | | | | | MLSS (mg/l) | |
|---|---|---|---|---|---|---|---|---|
| | | | Neutral reactor | | | | | |
| | Influent | | Supernatant | | Effluent | | | |
| Days | 390 nm | 475 nm | 390 nm | 475 nm | 390 nm | 475 nm | Acidic reactor | Neutral reactor |
| 1 | | | | | | | | |
| 2 | | | | | | | | |
| 3 | | | | | | | | |
| 6 | | | | | | | | |
| 7 | | | | | | | | |
| 8 | | | | | | | | |
| 9 | | | | | | | | |
| 10 | | | | | | | | |
| 13 | | | | | | | | |
| 14 | | 0.1462 | | 0.1672 | | 0.0726 | | |
| 15 | 0.3481 | | 0.4264 | 0.1624 | 0.1462 | 0.0472 | 4990 | 6900 |
| 16 | | | | | | | | |
| 17 | | | | | | | | |
| 20 | | | | | | | | |
| 22 | | | | | | | | |
| 23 | | | | | | | | |
| 24 | | | | | | | | |
| 27 | | | | | | | 5240 | 5990 |
| 28 | | | | | | | | |
| 30 | | | | | | | | |
| 31 | 0.4692 | 0.1915 | | | 0.2314 | 0.0826 | 4900 | 6155 |
| 37 | 0.3132 | 0.1111 | | | | | | |
| 45 | 0.3054 | 0.1025 | | | | | | |
| 49 | 0.3185 | 0.1093 | 0.3147 | 0.1294 | 0.2485 | 0.0835 | 4690 | 4540 |
| 52 | | | | | | | | |
| 57 | 0.3022 | 0.1 | | | | | | |
| 58 | | | 0.2704 | 0.1055 | 0.2266 | 0.0771 | 5180 | 5190 |
| 59 | 0.3153 | 0.1079 | | | | | | |
| 60 | 0.3027 | 0.0999 | | | | | | |
| 67 | 0.3191 | 0.1108 | | | | | | |
| 70 | 0.2999 | 0.0974 | 0.2791 | 0.1187 | 0.2074 | 0.0732 | 3073.3 | 3123.3 |
| 71 | | | | | | | | |
| 75 | 0.3202 | 0.1082 | | | 0.2054 | 0,0686 | | |
| 76 | | | | | | | 3313.3 | 2483.3 |
| 77 | | | | | | | | |
| 81 | | | 0.2323 | 0.0908 | 0.193 | 0.0651 | 4510 | 4653.3 |
| 84 | | | | | | | | |
| 88 | | | | | | | | |
| 89 | | | 0.2349 | 0.079 | 0.1913 | 0.0637 | | |
| 90 | | | 0.251 | 0.0959 | 0.1917 | 0.0658 | | |
| 91 | | | 0.2312 | 0.0874 | 0.1869 | 0.0631 | 4540 | 4820 |

**Raw Data on the Experiment Thermophilic Operation**

**a) pH, temperature and MLSS.**

| Days | Pressure | | Tempreture | | pH | | MLSS | |
|---|---|---|---|---|---|---|---|---|
| | Room-temp. | Thermophilic | Room-temp. | Thermophilic | Room-temp. | Thermophilic | Room-temp. | Thermophilic |
| 1 | 0.011 | 0.012 | 29.2 | 49.1 | | | | |
| 2 | | | | | | | 3930 | 1696.67 |
| 3 | | | 27.8 | 49.3 | | | | |
| 4 | 0.009 | 0.005 | | | | | | |
| 5 | 0.009 | 0.009 | 23.8 | 50.6 | 8.06 | 7.04 | 4220 | 1630 |
| 6 | 0.01 | 0.012 | 23.4 | 50.6 | 5.06 | 7.97 | | |
| 7 | 0.011 | 0.015 | 27.9 | 50.5 | 5.61 | 7.02 | | |
| 8 | | | | | | | | |
| 9 | | | | | | | | |
| 10 | | | | | | | | |
| 11 | 0.012 | 0.028 | 25.1 | 49.7 | 5.05 | 7.99 | | |
| 12 | 0.013 | 0.034 | | | | | | |
| 13 | 0.007 | 0.001 | 26.9 | 47.7 | 7.53 | 6.43 | 4510 | 2180 |
| 14 | | | | | | | | |
| 15 | | | | | | | | |
| 16 | | | | | | | | |
| 17 | | | | | | | | |
| 18 | 0.013 | 0.014 | 26.3 | 50.7 | 6.28 | 7.83 | | |
| 19 | 0.019 | 0.015 | 23 | 50.3 | 4.79 | 8.07 | 4453 | 2273 |
| 20 | 0.022 | 0.032 | 24 | 50.2 | 5.61 | 7.63 | | |
| 21 | 0.025 | 0.049 | 22 | 50.2 | 5.57 | 7.8 | | |
| 22 | | | | | | | | |
| 23 | | | | | | | | |
| 24 | 0.028 | 0.077 | 25.9 | 50.4 | 7.11 | 7.08 | | |
| 25 | 0.008 | 0.003 | | | | | | |
| 26 | 0.009 | 0.013 | 24.8 | 50 | 5.63 | 7.26 | 2953.3 | 1790 |
| 27 | 0.011 | 0.01 | | | | | | |
| 28 | 0.014 | 0.019 | 26.6 | 47.3 | 5.71 | 6.86 | | |
| 29 | | | | | | | | |
| 30 | | | | | | | | |
| 31 | 0.018 | 0.026 | | | | | | |
| 32 | 0.022 | 0.029 | | | | | | |
| 33 | | | | | | | | |
| 34 | 0.029 | 0.064 | 25.2 | 47.9 | 6.64 | 7.15 | 4086.6 | 2233.3 |
| 35 | 0.033 | 0.066 | | | | | | |

**b) COD**

| Days | COD | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Room-temperature | | | | | Thermophilic | | | |
| | Effluent | Removal (%) | Supernatant | Removal (%) | Feed | Effluent | Removal (%) | Supernatant | Removal (%) |
| 1 | | | | | | | | | |
| 2 | 110 | 89 | 120 | 88 | 1000 | 140 | 86 | 150 | 85 |
| 3 | 120 | 88 | 120 | 88 | 0 | 150 | 85 | 150 | 85 |
| 4 | 130 | 87 | 110 | 89 | | 110 | 89 | 120 | 88 |
| 5 | | | | | | | | | |
| 6 | | | | | | | | | |
| 7 | | | | | | | | | |
| 8 | | | | | | | | | |
| 9 | | | | | | | | | |
| 10 | | | | | | | | | |
| 11 | 120 | 88 | 140 | 86 | | 150 | 85 | 140 | 86 |
| 12 | | | | | | | | | |
| 13 | 150 | 85 | 200 | 80 | | 150 | 85 | 200 | 80 |
| 14 | | | | | | | | | |
| 15 | | | | | | | | | |
| 16 | | | | | | | | | |
| 17 | | | | | | | | | |
| 18 | 150 | 85 | 130 | 88 | | 150 | 85 | 150 | 85 |
| 19 | | | | | 1100 | | | | |
| 20 | 150 | 85 | 150 | 88 | | 150 | 85 | 150 | 85 |
| 21 | | | | | | | | | |
| 22 | | | | | | | | | |
| 23 | | | | | | | | | |
| 24 | | | | | | | | | |
| 25 | 160 | 84 | 150 | 85 | | 160 | 84 | 150 | 85 |
| 26 | | | | | | | | | |
| 27 | 150 | 85 | 150 | 85 | | 140 | 86 | 150 | 85 |
| 28 | | | | | | | | | |
| 29 | | | | | | | | | |
| 30 | | | | | | | | | |
| 31 | | | | | | | | | |
| 32 | 150 | 85 | 150 | 85 | | 130 | 87 | 150 | 85 |
| 33 | | | | | | | | | |
| 34 | | | | | | | | | |
| 35 | 150 | 85 | 140 | 86 | | 150 | 85 | 130 | 87 |

### c) Nitrogen compound

| Nitrification | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | NH4 (mg/L) | | | | NO3 (mg/L) | | | | | |
| | Room-temp. | | Thermophilic | | Room-temp. | | Thermophilic | | Room-temp. | Thermophilic |
| Days | Effluent | Supernatant | Effluent | Supernatant | Effluent | Supernatant | Effluent | Supernatant | Nitrification (%) | |
| 1 | 5 | 5 | 10 | 10 | 10 | 10 | 5 | 5 | 66.66666667 | 33.3333333 |
| 2 | | | | | | | | | | |
| 3 | 4 | | 5 | | 13 | | 9 | | 76.47058824 | 64.2857143 |
| 4 | | | | | | | | | | |
| 5 | | | | | | | | | | |
| 6 | | | | | | | | | | |
| 7 | 2 | | 5 | | 10 | | 8 | | 83.33333333 | 61.5384615 |
| 8 | | | | | | | | | | |
| 9 | | | | | | | | | | |
| 10 | | | | | | | | | | |
| 11 | 5 | | 5 | | 20 | | 15 | | 80 | 75 |
| 12 | | | | | | | | | | |
| 13 | 5 | | 10 | | 10 | | 5 | | 66.66666667 | 33.3333333 |
| 14 | | | | | | | | | | |
| 15 | | | | | | | | | | |
| 16 | | | | | | | | | | |
| 17 | | | | | | | | | | |
| 18 | 5 | | 5 | | 20 | | 5 | | 80 | 50 |
| 19 | | | | | | | | | | |
| 20 | 10 | | 10 | | 20 | | 5 | | 66.66666667 | 33.3333333 |
| 21 | | | | | | | | | | |
| 22 | | | | | | | | | | |
| 23 | | | | | | | | | | |
| 24 | | | | | | | | | | |
| 25 | 5 | | 10 | | 10 | | 5 | | 66.66666667 | 33.3333333 |
| 26 | | | | | | | | | | |
| 27 | 2 | | 2 | | 10 | | 2 | | 83.33333333 | 50 |
| 28 | | | | | | | | | | |
| 29 | | | | | | | | | | |
| 30 | | | | | | | | | | |
| 31 | | | | | | | | | | |
| 32 | 2 | | 5 | | 5 | | 5 | | 71.42857143 | 50 |
| 33 | | | | | | | | | | |
| 34 | | | | | | | | | | |
| 35 | 5 | | 5 | | 20 | | 20 | | 80 | 80 |

## d) Color

| Color removal | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Room-temp. | | | | Thermophilic | | | | Color remova (%) | | Color remova (%) | | Feed | |
| | Effluent | | Supernatant | | Effluent | | Supernatant | | Room-temp | Thermophilic | Room-temp | Thermophilic | | |
| Days | 390nm | 475nm | 390nm | 475nm | 390nm | 475nm | 390nm | 475nm | 390 nm | | 475 nm | | 390nm | 475nm |
| 1 | 0.2505 | 0.0714 | 0.4227 | 0.108 | 0.4016 | 0.1134 | 0.5122 | 0.1879 | 59.035159 | 34.32542927 | 68.1534344 | 49.4201606 | | |
| 2 | | | | | | | | | | | | | | |
| 3 | | | | | | | | | | | | | | |
| 4 | | | | | | | | | | | | | | |
| 5 | | | | | | | | | | | | | | |
| 6 | | | | | | | | | | | | | | |
| 7 | 0.317 | 0.1017 | 0.3685 | 0.134 | 0.4484 | 0.1425 | 0.6333 | 0.2974 | 48.160262 | 48.16026165 | 54.6387154 | 36.440678 | | |
| 8 | | | | | | | | | | | | | | |
| 9 | | | | | | | | | | | | | | |
| 10 | | | | | | | | | | | | | | |
| 11 | | | | | | | | | | | | | | |
| 12 | | | | | | | | | | | | | | |
| 13 | | | | | | | | | | | | | | |
| 14 | | | | | | | | | | | | | | |
| 15 | | | | | | | | | | | | | | |
| 16 | | | | | | | | | | | | | | |
| 17 | | | | | | | | | | | | | | |
| 18 | 0.2693 | 0.0839 | 0.3 | 0.097 | 0.4727 | 0.1381 | 0.714 | 0.3184 | 55.960752 | 22.69828291 | 62.5780553 | 38.4032114 | | |
| 19 | | | | | | | | | | | | | 0.6115 | 0.2242 |
| 20 | | | | | | | | | | | | | | |
| 21 | | | | | | | | | | | | | | |
| 22 | | | | | | | | | | | | | | |
| 23 | | | | | | | | | | | | | | |
| 24 | | | | | | | | | | | | | | |
| 25 | | | | | | | | | | | | | | |
| 26 | | | | | | | | | | | | | | |
| 27 | | | | | | | | | | | | | | |
| 28 | 0.387 | 0.1309 | 0.4272 | 0.15 | 0.4697 | 0.1472 | 0.6577 | 0.2721 | 36.713001 | 23.1888798 | 41.6146298 | 34.3443354 | | |
| 29 | | | | | | | | | | | | | | |
| 30 | | | | | | | | | | | | | | |
| 31 | | | | | | | | | | | | | | |
| 32 | | | | | | | | | | | | | | |
| 33 | | | | | | | | | | | | | | |
| 34 | | | | | | | | | | | | | | |
| 35 | 0.413 | 0.0796 | 0.459 | 0.106 | 0.4781 | 0.083 | 0.4781 | 0.083 | 32.461161 | 21.8152085 | 64.4959857 | 62.9794826 | | |

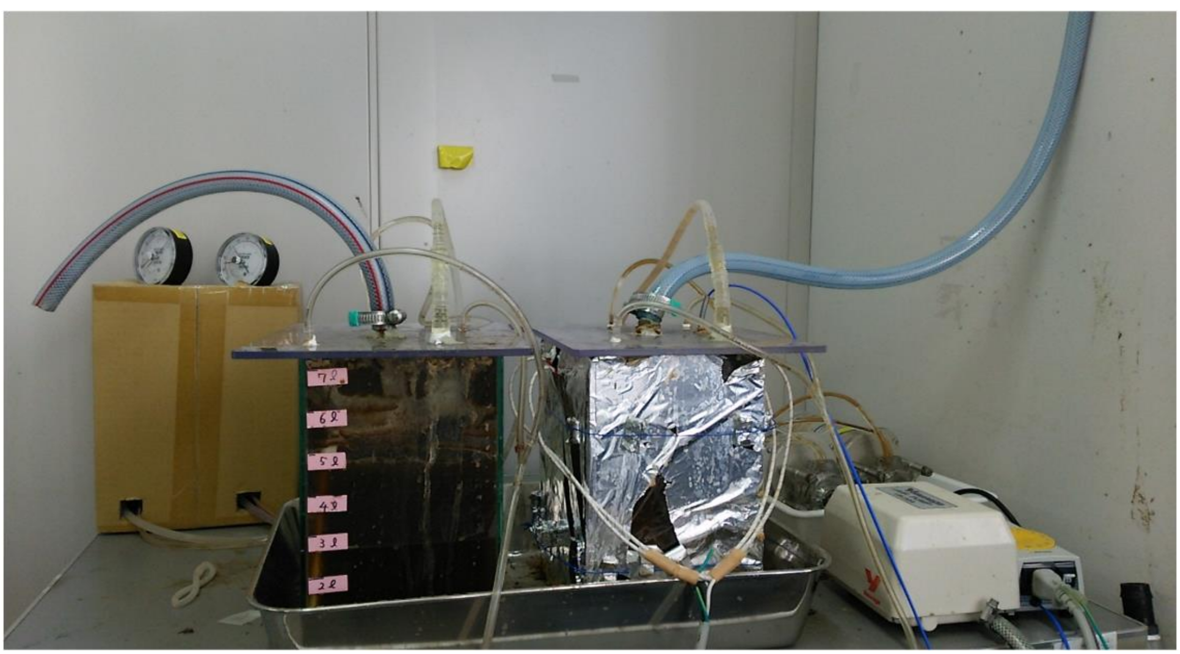
**e) Oil in average.**

| Oil concentration | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Room-temperature | | | | | | | | | | |
| Hours | 0 | | 1 | | 3 | | 6 | | 24 | |
| weeks | Water phase | Sludge phase | Water phase | Sludge phase | Water phase | Sludge phase | Water phase | Sludge phase | Water phase | Sludge phase |
| 1 | 54.9762086 | 19.8612589 | 40.7492719 | 32.5830381 | 17.159269 | 31.86275469 | 8.56706661 | 20.7371985 | 0.81557263 | 8.576337989 |
| 2 | 34.29477088 | 9.39958688 | 21.3798436 | 34.0611815 | 7.71642906 | 21.32469277 | 2.17216226 | 10.6783832 | 0.28326429 | 1.985275107 |
| 3 | 30.67236736 | 8.48834038 | 12.513837 | 12.804027 | 6.88671809 | 9.520595902 | 1.14866898 | 2.23163278 | 0.27884865 | 1.047158696 |
| 4 | 18.47746098 | 3.49369254 | 11.5414214 | 9.20634669 | 2.87991332 | 7.476631865 | 0.23391014 | 0.77970046 | 0.11662562 | 0.540689931 |
| 5 | 20.78161847 | 14.8271988 | 18.795045 | 12.0797882 | 5.34703294 | 10.12089305 | 1.50694668 | 1.94001126 | 0.31801439 | 0.823512404 |
| Average | 31.84048526 | 11.2140155 | 20.9958838 | 20.1468763 | 7.99787247 | 16.06111366 | 2.72575093 | 7.27338524 | 0.36246511 | 2.594594826 |
| Thermophilic | | | | | | | | | | |
| Hours | 0 | | 1 | | 3 | | 6 | | 24 | |
| weeks | Water phase | Sludge phase | Water phase | Sludge phase | Water phase | Sludge phase | Water phase | Sludge phase | Water phase | Sludge phase |
| 1 | 65.88780185 | 31.207474 | 101.57665 | 28.9518496 | 22.8298526 | 44.28125666 | 10.1047769 | 11.4477882 | 0.62870276 | 10.47934078 |
| 2 | 69.83000622 | 19.280903 | 33.6807578 | 16.1484896 | 2.75378888 | 4.612490774 | 0.65031098 | 0.36282236 | 0.26378547 | 0.891566727 |
| 3 | 26.88344689 | 5.50709682 | 8.03407484 | 8.14851995 | 0.57522493 | 0.834437012 | 0.10788397 | 0.09799538 | 0.19392869 | 0.391606609 |
| 4 | 22.95574803 | 19.1296273 | 0.71573817 | 0.59784356 | 1.62238704 | 1.997009318 | 0.13600221 | 0.31378368 | 0.10993898 | 0.226198545 |
| 5 | 15.43178457 | 4.48746947 | 2.41243988 | 1.2235377 | 0.7514705 | 1.296965024 | 0.19329637 | 0.30464206 | 0.1326089 | 0.152787859 |
| Average | 40.19775751 | 15.9225141 | 29.2839322 | 11.0140481 | 5.7065448 | 10.60443176 | 2.23845409 | 2.50540634 | 0.26579296 | 2.428300105 |

# Experiment Pictures

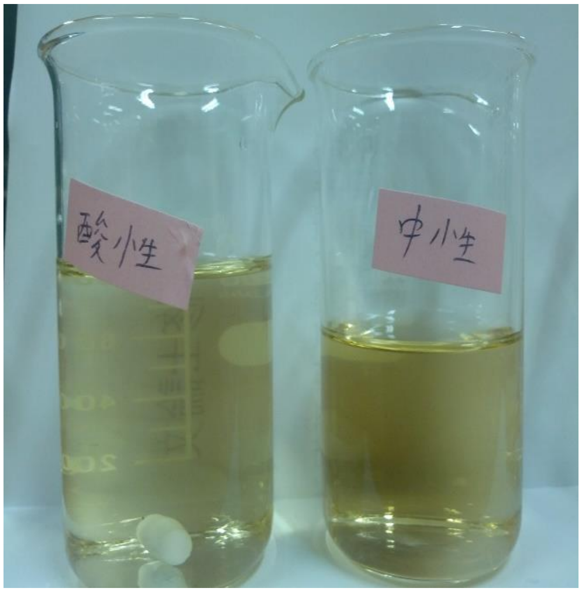
### Low pH Operation Reactor

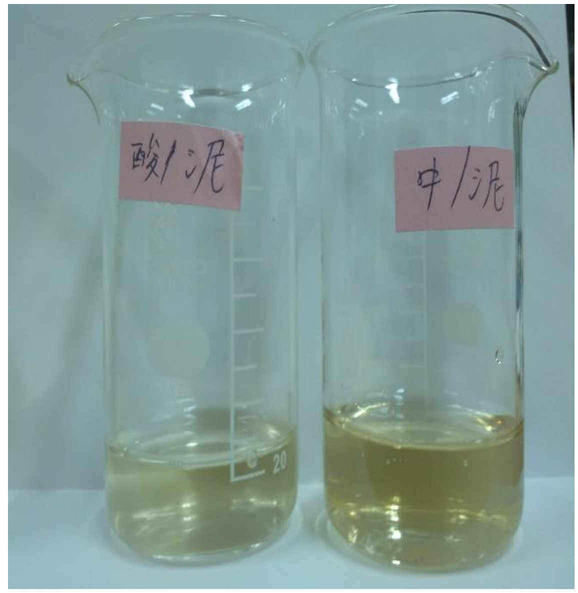
### Thermophilic Operation Reactor



**Color Removal**

**Low pH Operation**




**Thermophilic Operation**